

Title	Design and process parameter evaluation of bilayer capsule systems for targeted ileal drug delivery
Authors	Millet, Elisa;Vandenbroucke, Sofie S. T.;O'Shea, Joseph P.;Griffin, Brendan T.;Jannin, Vincent
Publication date	2026-07-04
Original Citation	Millet, E, Vandenbroucke, S S T, O'Shea, J P, Griffin, B T & Jannin, V 2026, 'Design and process parameter evaluation of bilayer capsule systems for targeted ileal drug delivery', European Journal of Pharmaceutical Sciences, vol. 224, 107601, pp. 1-13. https://doi.org/10.1016/j.ejps.2026.107601
Type of publication	Article (peer-reviewed)
Link to publisher's version	https://www.scopus.com/pages/publications/105043940673 - 10.1016/j.ejps.2026.107601
Rights	© 2026, the Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/). - https://creativecommons.org/licenses/by/4.0/
Download date	2026-09-21 08:12:27
Item downloaded from	https://hdl.handle.net/10468/19064



UCC

University College Cork, Ireland
Coláiste na hOllscoile Corcaigh



Design and process parameter evaluation of bilayer capsule systems for targeted ileal drug delivery

Elisa Millet^a, Sofie S.T. Vandenbroucke^b, Joseph P O'Shea^c, Brendan T Griffin^c, Vincent Jannin^{a,*}

^a Capsugel France SAS, Colmar, France

^b Capsugel Belgium NV, Bornem, Belgium

^c School of Pharmacy, University College Cork, College Road, Cork, Ireland

ARTICLE INFO

Keywords:

Bilayer capsule
Drug delivery
Ileal targeting
Ethylcellulose
Sodium alginate
Delayed release

ABSTRACT

The dynamic and transitory nature of the distal small intestine makes targeted drug delivery to this region particularly challenging. This study aimed to develop a bilayer capsule system for site-specific drug release in the terminal ileum. The capsules consisted of an inner water-soluble layer based on hydroxypropyl methylcellulose (HPMC) and pullulan, combined with an outer ethylcellulose layer incorporating sodium alginate as a pore-forming agent.

The influence of alginate concentration and curing conditions (temperature and relative humidity) on capsule structure and performance was systematically investigated. Thermal analysis by differential scanning calorimetry guided plasticizer selection and curing parameters. Capsule morphology and surface properties were characterized by scanning electron microscopy and roughness analysis, while functional performance was evaluated through disintegration, water ingress, and dissolution studies under sequential pH conditions simulating the gastrointestinal tract.

Alginate concentration significantly influenced surface morphology and permeability of the outer layer, whereas curing conditions modulated film cohesion and porosity. Lead formulations (4–6% alginate, cured at 70 °C and 40% RH) exhibited low water ingress under acidic conditions, maintained capsule integrity, and enabled delayed release at pH 7.4. Compared with reference enteric capsules (Capsugel® Enprotect® capsules), these systems showed a delayed release profile, with potential for targeting of the distal ileum.

Overall, this study highlights the critical role of formulation and process parameters in controlling capsule microstructure and provides a robust platform for site-specific oral drug delivery to the distal small intestine.

1. Introduction

Oral drug delivery remains the most widely preferred route of administration due to its convenience, patient acceptability and cost-effectiveness (Begum et al., 2018). Over the past decades, significant advances have been made in the development of modified-release systems designed to control drug release kinetics and enable site-specific delivery within the gastrointestinal (GI) tract. In particular, considerable research effort has focused on regional targeting strategies, especially for colonic drug delivery, where physiological triggers such as pH variation, transit time, and microbial activity can be effectively exploited (Kotla et al., 2019; Ferraro et al., 2024).

In contrast, reliable targeting of the distal small intestine, and

specifically the terminal ileum, remains more challenging. This region presents a dynamic and transient physiological environment characterized by relatively short and variable residence times, progressively increasing pH, and a gradual rise in microbial density compared to the proximal small intestine. While several delivery platforms have demonstrated partial success in reaching the ileal region, achieving consistent and reproducible drug release specifically at this site remains difficult (Huntsman et al., 2021). As a result, the terminal ileum remains comparatively less well addressed than the colon in the context of oral modified-release systems.

Nevertheless, the terminal ileum constitutes a highly relevant therapeutic target. It is a primary site of inflammation in Crohn's disease and represents a key absorption region for certain drugs and emerging

* Corresponding author.

E-mail address: vincent.jannin@lonza.com (V. Jannin).

<https://doi.org/10.1016/j.ejps.2026.107601>

Received 22 May 2026; Received in revised form 16 June 2026; Accepted 2 July 2026

Available online 4 July 2026

0928-0987/© 2026 The Author(s). Published by Elsevier B.V. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>).

therapeutic modalities, including biologics and nucleic acid-based therapies. Targeted delivery to this region offers the potential to enhance local efficacy while minimizing systemic exposure and off-target effects. These considerations highlight the need for dosage forms capable of withstanding gastric and proximal intestinal conditions while enabling controlled and predictable drug release in the distal small intestine.

Conventional hard capsules, typically fabricated from gelatin or cellulose-derived polymers, are primarily designed as immediate-release systems that rapidly hydrate and disintegrate in the gastric environment. While suitable for conventional oral dosing, this inherent behavior often leads to premature drug release and insufficient protection of acid-labile or sensitive APIs before reaching their intended site of action. As a result, unmodified capsule shells are not well adapted for applications requiring site-specific or regional delivery along the gastrointestinal (GI) tract (Hoffmann et al., 2025). To overcome these limitations, modern capsule design increasingly relies on the use of functional polymers and tailored architectures capable of delaying disintegration and enabling controlled or targeted drug release (Millet et al., 2025).

Among capsule-forming polymers, hydroxypropyl methylcellulose (HPMC) is widely used due to its favorable film-forming properties, mechanical robustness, and compatibility with a broad range of active pharmaceutical ingredients (Millet et al., 2025). Pullulan, a neutral polysaccharide, also exhibits excellent film-forming properties and has been explored as a capsule material due to its mechanical strength and biocompatibility. Importantly, both HPMC and pullulan can contribute to hydration-driven disintegration, while pullulan may additionally be susceptible to enzymatic degradation under intestinal conditions (Aghajannataj Ahangarkola et al., 2024).

For delayed-release applications, ethyl cellulose (EC), a water-insoluble polymer, is commonly used to form diffusion-controlling barriers that limit premature drug release (Giakoumis et al., 2026). In such systems, permeability is typically tuned through the incorporation of water-soluble or swellable excipients acting as pore formers. Sodium alginate, a hydrophilic and biodegradable polysaccharide is particularly relevant in this context, as it can modulate film permeability through hydration, swelling, and potential enzymatic degradation within the intestinal environment (Tønnesen and Karlsen, 2002).

Alginate is a natural polysaccharide susceptible to degradation by microbiota-derived enzymes, primarily alginate lyases belonging to different polysaccharide lyase (PL) families (e.g., PL6 and PL17), which cleave mannuronate and guluronate residues through a β -elimination mechanism (Rønne et al., 2023, 2024). These enzymes are produced by specific members of the gut microbiota, particularly *Bacteroides* species, which harbor dedicated polysaccharide utilization loci enabling the depolymerization and fermentation of alginate (Fu et al., 2023; Mathieu et al., 2018). Although alginate degradation is generally considered to occur predominantly in the colon due to the higher microbial density and enzymatic activity, recent studies have demonstrated that the ileal microbiota also exhibits a fibrolytic potential, including the presence of carbohydrate-active enzymes involved in the degradation of complex polysaccharides (Patrascu et al., 2017). Therefore, the onset of alginate degradation may already occur in the distal ileum, although to a lesser extent and depending on the local microbial composition and activity.

The combination of EC and alginate therefore provides a means to engineer a functional layer with controlled permeability that evolves in response to both physicochemical conditions and microbiota-derived enzymatic activity along the gastrointestinal tract.

In this context, bilayer capsule shell systems represent a promising approach to decouple the structural and functional roles of the capsule shell (Millet et al., 2025). The inner layer can be designed to ensure mechanical integrity and controlled disintegration, while the outer layer provides a protective barrier against gastric and proximal intestinal conditions. However, most commercially available or reported capsule systems rely predominantly on pH-dependent polymer dissolution,

typically triggering release in the jejunum or early ileum (e.g., at pH ~6.5–6.8). Such systems may not provide sufficient delay to consistently reach the terminal ileum.

The present work proposes a bilayer capsule design specifically engineered to achieve delayed release in the distal ileum through a combination of diffusion-controlled and environmentally responsive mechanisms. The outer layer, composed of ethylcellulose and sodium alginate, acts as a semi-permeable barrier, where EC provides hydrophobicity and structural resistance, while alginate progressively increases permeability through hydration and pore formation at elevated pH. In addition, the susceptibility of alginate to microbiota-derived enzymatic degradation provides a further level of responsiveness that may contribute to region-specific permeability in the distal small intestine. In contrast to purely pH-triggered systems, this approach introduces a time-dependent diffusion barrier that delays drug release beyond the proximal small intestine.

The inner layer, based on HPMC or HPMC/pullulan blends, provides structural support and governs disintegration once the outer barrier is sufficiently weakened. The inclusion of pullulan introduces the potential for enhanced hydration and enzymatic susceptibility, which may further contribute to capsule opening in the distal small intestine, where microbial activity increases relative to the proximal intestine. Together, the combination of a diffusion-limiting outer layer and a responsive inner structural layer provides a mechanistic basis for targeting drug release to the terminal ileum rather than the colon.

The objective of this study was therefore to design and characterize bilayer capsule shells fabricated exclusively by a double dip molding process, a comparatively simple, robust, and industrially scalable manufacturing approach enabling precise modulation of capsule shell architecture while maintaining process reproducibility (Millet et al., 2025). Capsules were manufactured in size #1, a format recently shown to empty efficiently from the porcine stomach and therefore particularly relevant for future translational studies in a porcine model (Hoffmann et al., 2025). The systems were designed to enable site-specific drug release in the distal small intestine through the combined effects of formulation composition and processing conditions. In particular, the influence of sodium alginate concentration and curing parameters (temperature and relative humidity) on capsule microstructure, surface properties, permeability, and in vitro performance was systematically investigated. This study was conceived as a proof-of-concept approach aimed at screening key formulation and processing parameters in order to establish structure–function relationships and identify lead capsule prototypes. These systems are intended for subsequent evaluation under biorelevant conditions and, ultimately, for further translational assessment in physiologically relevant models. This work therefore provides a rational framework for the iterative development of capsule-based systems for ileum-targeted oral drug delivery.

2. Materials and methods

2.1. Materials

The inner layer of the capsule was made with pullulan sourced from Hayashibara (Okayama, Japan), and hydroxypropyl methylcellulose (HPMC, Tylopor® 65SH-5) provided by Shin-Etsu (Wiesbaden, Germany).

The outer layer of the double layer capsule was made with an ethylcellulose aqueous dispersion supplied by Ashland (Aquarius® Control ECD, Waalwijk, Netherlands) and sodium alginate purchased from IFF (Vormedal, Norway). For the plasticizers screening dibutyl sebacate (DBS) obtained from Merck KGaA (Darmstadt, Germany), triethyl citrate (TEC) and triethyl 2-acetyl citrate (ATEC) supplied by Sigma Aldrich (Saint Louis, MO, USA) were selected. Polysorbate 80 (PS80) was supplied by BTC (Paris, France).

Then for the disintegration and water ingress, acidic media and

phosphate buffer were prepared with fuming hydrochloric acid (37%), disodium hydrogen phosphate dodecahydrate ($\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) and potassium dihydrogen phosphate (KH_2PO_4) obtained from Merck KGaA. Citric acid monohydrate was purchased from Thermo Fisher Scientific (Waltham, MA, USA). Sodium chloride (NaCl) was purchased from Sigma-Aldrich (Burlington, MA, USA). Capsules were filled with thymol blue (Merck KGaA) as a colorant and either lactose (Pharmatose® 200 M, DFE Pharma, Germany) for disintegration studies or mannitol (Pearlitol® 200 SD, Roquette, France) for water ingress experiments. And for dissolution analytical-grade trisodium phosphate dodecahydrate, hydrochloric acid (2 N and 37%) and anhydrous caffeine (ReagentPlus®, 100.0%) obtained from Merck KGaA were used and UPLC/MS-CC/SFC-grade methanol was purchased from Biosolve Chimie SARL (Dieuze, France). Glacial acetic acid (analytical reagent grade, $\geq 99.7\%$) was sourced from Fisher Chemical (Loughborough, United Kingdom) and sodium hydroxide (5 N) was obtained from Honeywell (Seelze, Germany).

2.2. Methods

2.2.1. Design and development of bilayer capsule shell

2.2.1.1. Film casting. Dried films were prepared using an automatic film deposition system (EURAMECA, France). The polymeric formulations (ethylcellulose/alginate with or without plasticizer) were prepared in water and transferred into the reservoir of the deposition system. Film thickness was controlled by adjusting the slit opening of the coating head according to the viscosity of the formulation (Fig. 1).

During deposition, glass plates were conveyed beneath the reservoir using a piston-driven system, allowing uniform spreading of the formulation at a defined thickness. Following casting, films were dried in an oven at 60°C for 30 min to allow water evaporation and initial film formation.

After drying, films were carefully removed from the glass substrate. As aqueous polymer dispersions may exhibit incomplete particle coalescence after drying, an additional curing step was applied. Curing was performed at 70°C under controlled relative humidity conditions (approximately 5%, 40%, or 75% RH) in climatic chambers. This step promotes polymer chain mobility and interdiffusion, improving film homogeneity and mechanical integrity.

2.2.1.2. Plasticizer selection and thermal analysis. The first step was to choose the best plasticizer. The plasticizer was used first to decrease the glass transition (T_g) of the formulation. Based on the Hildebrand solubility parameter (δ_{tot}), which reflects the cohesive energy density and thus the affinity between materials, three plasticizers were selected to span a relevant compatibility range with ethylcellulose. Dibutyl sebacate (DBS), a hydrophobic plasticizer, exhibits a δ_{tot} of $18 \text{ MPa}^{1/2}$ (Brandrup et al., 1999). Acetyl triethyl citrate (ATEC), slightly hydrophilic and only sparingly soluble in water (1 in 140), has a δ_{tot} of $19 \text{ MPa}^{1/2}$ while triethyl citrate (TEC), which is water-soluble, presents a δ_{tot} of $20.4 \text{ MPa}^{1/2}$ (Sawada et al., 2015). These values were chosen to bracket the solubility parameter of ethylcellulose; $\delta_{\text{tot}} = 20.4 \text{ MPa}^{1/2}$ (Alke et al., 2026), thereby enabling the evaluation of how

plasticizer–polymer affinity influences capsule shell formation and performance.

Films were produced with 20% (w/w) of plasticizer and are compared to films without plasticizers. They were cut into samples of approximately 10 mg, accurately weighed, and sealed in aluminum pans; an empty pan was used as reference.

Analyses were performed under a nitrogen atmosphere (50 mL/min). Samples were first heated from 25°C to 60°C at $10^\circ\text{C}/\text{min}$ and subsequently cooled to remove residual moisture without altering polymer structure. A second heating cycle was then conducted from 0°C to 150°C at $30^\circ\text{C}/\text{min}$ to capture relevant thermal transitions. The glass transition temperature (T_g) and other thermal events were determined from the second heating cycle.

2.2.2. Capsule shell fabrication using the double-dipping process

Bilayer capsule shells (size 1) were fabricated using a double-dipping process adapted from conventional hard capsule dip-molding. Polymer solutions or dispersions were prepared in water, homogenized, and degassed to remove entrapped air. The viscosity of each formulation was adjusted to ensure appropriate coating thickness and uniformity.

Stainless steel pins were first dipped into the inner layer formulation, composed of hydroxypropyl methylcellulose (HPMC) and, for selected formulations, pullulan (90%:10% w/w). After withdrawal, the pins were rotated to ensure uniform coating and then dried at 60°C for 1 h under ambient humidity conditions to remove water and initiate film formation.

The inner structural layer accounted for approximately 70% of the total capsule shell mass. A second dipping step was subsequently performed using the outer functional layer dispersion, composed of ethylcellulose and sodium alginate (3%, 6%, or 9%), representing approximately 30% of the final shell mass (Table 1). This sequential process enabled the formation of a bilayer structure with distinct functional properties (Millet et al., 2025).

After drying, the capsule shells were stripped from the pins, trimmed to uniform size, and assembled in a pre-lock position (Lafargue, 2020; Jones et al., 2017). The final capsule mass was approximately 95 ± 5 mg.

A final curing step, distinct from drying, was applied after complete capsule formation. Capsules were cured at 70°C under controlled relative humidity conditions (approximately 5%, 40%, or 75% RH) to promote polymer coalescence, enhance interlayer adhesion, and modulate film permeability.

Table 1

Composition of the capsule outer layer (dry basis).

Product	Dry quantity (%w/w)
Ethylcellulose	74.6
Sodium alginate	4.0
Plasticizer (DBS, ATEC or TEC)	20.0
Polysorbate 80	1.4
TOTAL	100.0

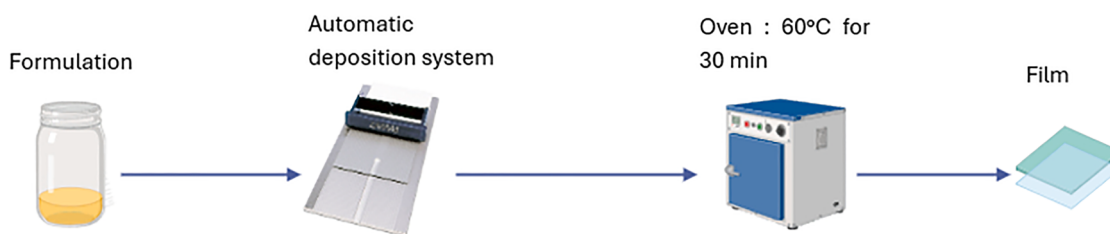


Fig. 1. Schematic representation of the film casting process.

2.2.3. Capsules characterization

2.2.3.1. Capsule dimensions and bilayer structural characterization

2.2.3.1.1. X-Ray imaging. High resolution X-ray imaging was used to visualize the layer organization of the bilayer capsules. Images were acquired with a Skyscan 1272 (Bruker, Belgium) using a microfocus X-ray source at a voltage of 60 kV and current of 100 μ A, no filter and a magnification of 1.5 μ m pixel size. The individual layer thickness was estimated using the original ImageJ software (Schneider et al., 2012).

2.2.3.1.2. Diameter measurement. Silhouette images were taken of the capsule's cap and body with a TM-X5040 (Keyence NV, Belgium). Using the TM-X Navigator software, the circumscribe and inscribe diameter of the cap or body was determined, from which the average diameter was calculated. The mean of the uncured average diameter was used as reference point to calculate the delta diameter for each measurement.

2.2.3.2. Morphological analysis (SEM/EDX and XRF). Capsule microstructure was examined by scanning electron microscopy (SEM) after curing. Transverse sections were prepared using Castroviejo scissors, mounted on SEM stubs, and sputter-coated with gold to ensure electrical conductivity. Imaging was performed using a FlexSEM 1000 II microscope (Hitachi, Japan) to assess layer organization, thickness, and surface morphology.

Alginate distribution within the functional layer was investigated by energy-dispersive X-ray spectroscopy (EDX) using sodium (Na) mapping as a marker element associated with alginate. For elemental analysis, samples were carbon-coated prior to EDX measurements to enable accurate detection of sodium. In addition, X-ray fluorescence (XRF) analysis (Thermo Scientific™ ARL™ QUANT'X, Czech Republic) was performed to quantify sodium content.

2.2.3.3. Surface roughness. Surface roughness was measured with a MarSurf SD26 instrument (Mahr Metrology, Germany). Parameters determined included average roughness (Ra), valley depth (Rv), and peak density per millimeter (R Pc).

2.2.4. Capsule performance evaluation

2.2.4.1. Disintegration studies. Disintegration testing was performed using standardized pharmacopeial media adapted for prototypes screening. Capsules were filled with approximately 0.4 g lactose containing 0.1% red carrot extract as a visual marker.

Disintegration was assessed using a DT2 disintegration tester (Sotax, Aesch, Switzerland) with six replicates per formulation ($n = 6$), in accordance with the European Pharmacopoeia method: 2 h in 0.1 M HCl (gastric phase), followed by 1 h in phosphate buffer pH 6.8 (EP, proximal small intestinal phase) with a visual checkpoint after 30 min and 60 min, and subsequently up to 1 h in phosphate buffer pH 7.4 (EP, ileal phase) using discs with constant observation for 1 h.

2.2.4.2. Water ingress studies. Water uptake was assessed under simulated gastric conditions. Capsules were filled with mannitol powder containing 0.1% (w/w) thymol blue as a visual tracer. Each capsule was individually weighed prior to immersion in 0.1 N hydrochloric acid (HCl) at 37 °C for 2 h. After, immersion, capsules were then gently blotted with absorbent paper to remove excess surface liquid, opened using a scalpel, and the internal powder recovered and weighed. Loss on drying (LOD) was calculated according to Eq. (1).

$$LOD (\%) = \frac{\text{Initial mass} - \text{Final mass}}{\text{Initial mass}} \times 100 \quad (1)$$

The water ingress method provides a comparative assessment of capsule permeability but does not directly reflect API stability or in vivo performance.

2.2.4.3. Dissolution testing. In vitro dissolution of bilayer capsules was performed using a type II paddle apparatus (Sotax AT Xtend, Aesch, Switzerland) at 100 rpm and 37.0 ± 0.5 °C with a total volume at 1000 mL. Capsules were filled with 280 ± 10 mg of caffeine, used as a model drug for dissolution studies. Capsugel® Enprotect® capsules (Capsugel, France) served as reference for enteric capsule targeting the jejunum / early ileum regions with proven performance in humans in fasted and postprandial state (Rump et al., 2022; Grimm et al., 2023).

The dissolution method comprises three successive phases:

- Gastric phase: 0.1 N HCl for 2 h to confirm capsule shell resistance against premature release in acidic conditions.
- Jejunal phase: Adjustment of pH to reach 6.8 for 1 h (samples at 30 and 60 minutes) to assess capsule integrity under proximal small intestinal pH.
- Ileal phase: Adjustment with NaOH to obtain a pH at 7.4 then the test is run for 2 h (samples every 5 min) to evaluate drug release.

Samples were analyzed using a reversed-phase HPLC on an Alliance HPLC system Waters™ (Milford MA, USA) on a C18 microsphere column (100×4.60 mm, 3 μ m) (Agilent, Santa Clara CA, USA). 20 μ L aliquot of each sample was injected into the column. The mobile phase consisted of 69.0% (v/v), 29.5% of methanol and 1.5% of acetic acid, delivered at a flow rate of 0.65 mL/min. Detection was performed at 273 nm. Caffeine served as a model API, with calibration standards from 0.05 to 472.80 μ g/mL. Retention time was 2.013 min, with excellent linearity ($R^2 > 0.99$) and a lower limit of detection (LLOD) of 0.05 μ g/mL.

2.2.5. Statistical analysis

Statistical analyses were performed using GraphPad Prism 11. Data were first assessed for normality. When normality assumptions were met, a two-way ANOVA was performed to evaluate the effects of the studied factors, followed by Tukey's multiple comparison test to identify significant differences between groups. When normality was not met, a non-parametric Kruskal-Wallis followed by a Dunn's test was applied. This approach allows control of Type I error when comparing multiple groups. Statistical significance was defined as $p < 0.05$.

3. Results

The overall objective of this study was to gain a mechanistic understanding of how formulation composition and processing parameters can be leveraged to engineer bilayer capsule shells for targeted intestinal delivery. By systematically investigating the influence of polymer selection, alginate concentration, and curing conditions, the study aimed to establish structure–function relationships linking capsule shell microstructure, barrier properties, and in vitro performance.

3.1. Plasticizer selection and thermal analysis

Film formation of the EC–alginate layer was first evaluated under different plasticization and curing conditions to ensure consistent and reproducible film development. The resulting films exhibited thicknesses in the range of approximately 60–80 μ m, depending on formulation and processing conditions, and were used for subsequent characterization.

Figure S11 presents the DSC thermograms of EC films with and without plasticizers. Both triethyl citrate (TEC) and dibutyl sebacate (DBS) effectively plasticized EC, as evidenced by a decrease in the glass transition temperature (T_g) from ~ 76 °C to ~ 51 °C and ~ 41 °C, respectively. In contrast, acetyl triethyl citrate (ATEC) showed no significant plasticizing effect, with no clear reduction in T_g observed.

Based on its superior plasticizing efficiency, DBS was selected for the remainder of the study. The curing temperature was set at 70 °C, in line with literature reports recommending curing of aqueous ethylcellulose dispersions at elevated temperatures between 60 and 80 °C to promote

effective film formation and polymer coalescence (Körber et al., 2010).

3.2. Capsules characterization

3.2.1. Capsule dimensions and bilayer structural characterization

Capsule shell weight and thickness were measured to evaluate the influence of formulation and processing parameters on structural properties (Table SI1 and SI2). A representative image of a bilayer capsule (size 1) in closed state, showing proper cap–body alignment and locking, is presented in Figure SI2.

3.2.1.1. X-ray imaging. X-ray imaging enabled clear differentiation of the two capsule layers. As shown in Fig. 2, the inner layer, composed of HPMC and pullulan, appeared darker and accounted for approximately 66% of the total shell thickness, whereas the outer layer, composed of ethylcellulose and alginate, appeared lighter and represented approximately 34% of the shell thickness. The boundary between the layers was continuous, indicating good compatibility and strong adhesion at the interface.

3.2.1.2. Diameter measurement. The change in diameter (delta diameter), relative to the mean diameter of uncured capsules, is presented in Fig. 3 for both the cap and body. A decrease of delta diameter was observed following curing treatment for all conditions, with the highest reduction in diameter observed under 75% RH conditions. In general, higher curing humidity is expected to promote a decrease in delta diameter due to water-induced plasticization, which enhances polymer chain mobility and facilitates structural relaxation and densification.

3.2.2. Morphological analysis

3.2.2.1. Observation of capsules surfaces and cross-sections with different curing. Scanning electron microscopy (SEM) of capsule cross-sections revealed clear morphological differences in the outer layers depending on curing conditions (Fig. 4). Curing at 70 °C under dry conditions (5% RH) produced a loosely packed and stratified structure, indicative of weak polymer cohesion and limited interchain interactions. In contrast, curing at 70 °C and 40% RH generated a dense, continuous, and well-consolidated bilayer, with minimal porosity, reflecting enhanced polymer relaxation and improved mechanical integrity. Conversely, curing at 70 °C and 75% RH resulted in a highly porous and irregular film, with visible delamination likely caused by excessive swelling and over-plasticization of the alginate network during humid curing.

SEM observations revealed notable differences between cross-sectional morphology and surface morphology as a function of curing relative humidity (Fig. 4). In the cross-sections, increasing relative humidity progressively promoted polymer swelling and pore formation, with dry-cured capsules exhibiting a poorly cohesive internal structure, 40% RH capsules displaying a denser and more continuous matrix, and 75% RH capsules showing a highly porous and swollen organization. In contrast, the corresponding surface morphologies did not follow the same trend. Dry-cured capsules presented relatively smooth and

compact surfaces despite their heterogeneous internal structure, suggesting that rapid dehydration mainly affected internal polymer organization. Capsules cured at 40% RH exhibited a more textured and irregular surface, while those cured at 75% RH showed pronounced surface roughness and heterogeneity, consistent with increased polymer chain mobility and relaxation at the interface under high humidity conditions.

3.2.2.2. Quantification of sodium alginate by XRF. The sodium content quantified by XRF increased proportionally with the alginate concentration in the outer layer (Figure SI3). Capsules containing 4%, 6%, and 9% alginate exhibited Na levels of approximately 2200 ppm, 4600 ppm, and 8300 ppm, respectively, while no sodium was detected in capsules without the alginate-containing outer layer.

3.2.2.3. Observation of sodium alginate on capsules by EDX. Energy-dispersive X-ray (EDX) elemental mapping images are presented in Fig. 5. The purple colored dots represent the distribution of sodium across the surface of the bilayer capsules surface. In general, a greater sodium presence is observed at higher alginate concentrations, reflected by the increased intensity and coverage of the purple signal. Overall, these findings are consistent with the XRF results, which also showed an increase in sodium signal intensity with increasing alginate content. Importantly, EDX mapping demonstrated a homogeneous distribution of sodium across the capsule surface, with no evidence of localized colored aggregates.

3.2.3. Surface roughness

Fig. 6 summarizes the surface roughness parameters (R_p , R_v , and R_a), expressed as mean values with corresponding ranges, as a function of sodium alginate concentration (4%, 6%, and 9%) and curing conditions.

For all roughness parameters, a global trend was observed with 4% alginate generally exhibiting the highest values, followed by 9% and then 6% alginate formulations. Regarding R_a , non-cured capsules systematically displayed the highest values, indicating that the curing process globally reduced average surface roughness regardless of alginate concentration. This suggests that curing promotes partial surface consolidation and relaxation of the polymer network.

At 4% alginate, capsule surfaces exhibited the highest peak density (R_p) together with elevated R_a values, reflecting a finely textured surface composed of numerous surface asperities. Although R_v values remained globally higher at this concentration, a significant effect of curing was only observed for the 70 °C–75% RH condition, which showed values comparable to the non-cured capsules. These results indicate that at low alginate concentration, surface morphology remains highly sensitive to curing-induced dehydration and polymer rearrangement.

Increasing alginate concentration to 6% resulted in a marked decrease in R_p compared with 4% alginate formulations, with several conditions showing statistically significant differences. This decrease indicates a reduction in peak density and the formation of a more

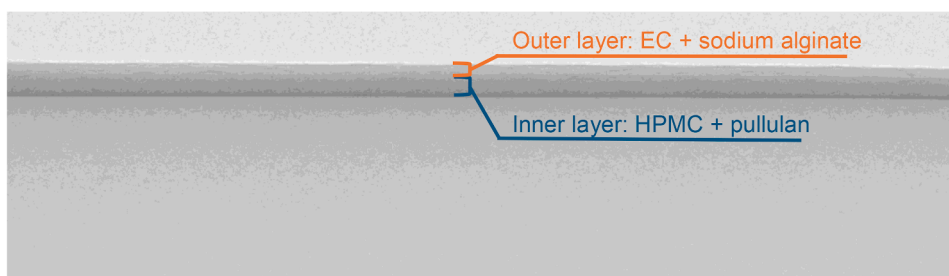


Fig. 2. X-ray image of the capsule shell clearly displaying the two distinct layers.

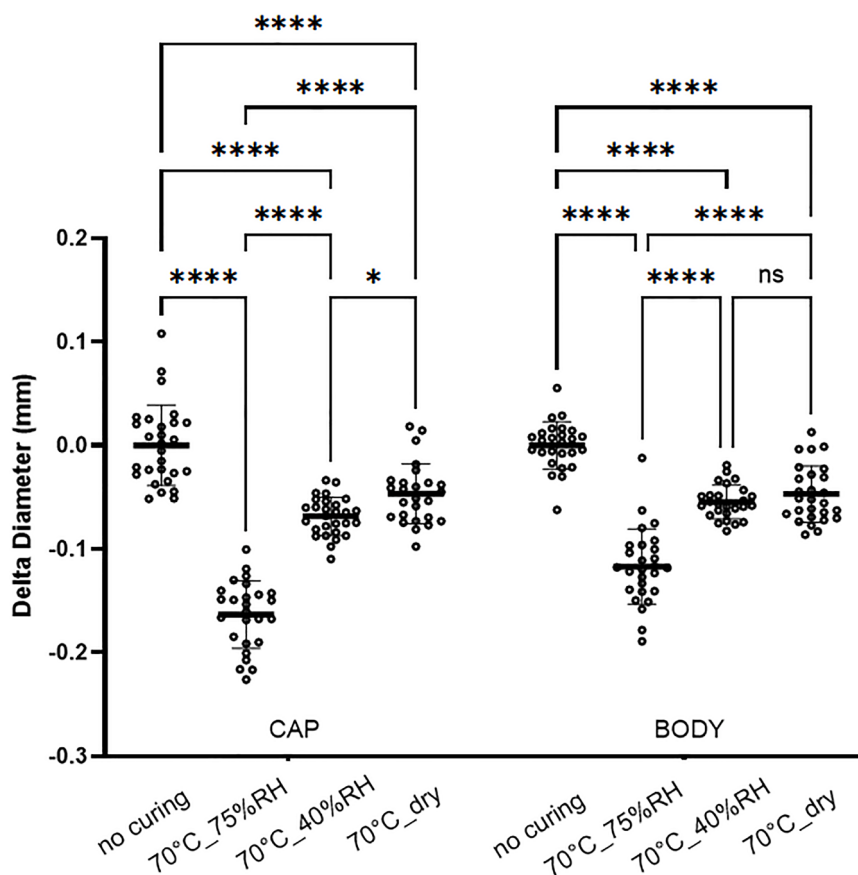


Fig. 3. Delta diameter of cap (left) and body (right) compared to the mean diameter of the uncured capsules. Statistical significance is indicated as follows: * $p < 0.05$, and **** $p < 0.0001$.

homogeneous and consolidated surface structure. R_a values were also lower than at 4% alginate, confirming reduced overall roughness. Despite this general trend, curing conditions had limited influence within the 6% alginate population, suggesting that the polymer network became less responsive to curing-induced structural reorganization.

At 9% alginate, roughness parameters remained intermediate between 4% and 6% alginate systems. $R_{p,c}$ values stayed relatively stable across curing conditions, with no significant differences observed within this population. Similarly, R_a values remained lower than for non-cured systems and showed limited sensitivity to curing. These observations suggest that at high alginate content, the surface architecture becomes more stable and less affected by curing conditions due to increased polymer entanglement and network density.

Overall, curing conditions influenced surface roughness mainly by reducing R_a values, while alginate concentration remained the primary factor governing peak density and surface organization.

3.3. Capsule performance evaluation

3.3.1. Disintegration studies

Disintegration studies aimed to identify key parameters influencing release from bilayer capsules (Fig. 7). Three variables were evaluated: (i) inner layer composition (HPMC vs. HPMC/pullulan), (ii) sodium alginate concentration in the outer layer, and (iii) curing conditions.

Fig. 7A presents the disintegration results for capsules comprising HPMC/pullulan inner layers. All capsule prototypes demonstrated resistance to 0.1 N HCl for 2 h, indicating effective gastric resistance. Upon transition to a pH representative of the upper small intestine, the behavior of capsules prototypes varied depending on composition and curing conditions. Prototypes prepared with 4% alginate across all

curing and those containing 6% alginate at 75% relative humidity, remained intact after one hour of exposure. In contrast, capsules formulated with 9% sodium alginate and with 6% alginate cured at 70 °C–40% RH disintegrated in under 1 hour at pH 6.8.

At pH conditions representative of the distal small intestine (pH 7.4), further differences between the prototypes became evident. Capsules containing 4% or 6% alginate, cured at 75% relative humidity, emerged as the most promising candidates for targeted ileal release, as they released their contents within 15 min at pH 7.4.

Fig. 7B shows disintegration results for capsules with HPMC-only inner layers. All prototype capsules exhibited resistance to 0.1 N HCl for up to 2 h, indicating effective gastric protection. Similar to results observed with for the HPMC/pullulan inner layer prototypes, An outer layer comprising 4% alginate (across all curing conditions) and 6% alginate (for oven-dried and 75% RH conditions) also demonstrated sufficient resistance during exposure for over 1 hour at pH 6.8. These findings suggest that the release characteristics at pH 6.8 are primarily influenced by the composition of the outer layer.

At pH 7.4, all prototype capsules remained intact for >20 min, confirming sustained resistance under distal intestinal conditions. Comparison of the inner layer compositions suggested that capsules containing HPMC alone generally exhibited longer disintegration times than those containing HPMC/pullulan, indicating that pullulan incorporation promoted faster disintegration at this pH.

3.3.2. Water ingress studies

Water ingress studies were conducted to assess capsule impermeability under simulated gastric conditions, as excessive water uptake may compromise payload stability and delayed-release performance. A loss on drying (LOD) $\leq 10\%$ was defined as the acceptance criterion,

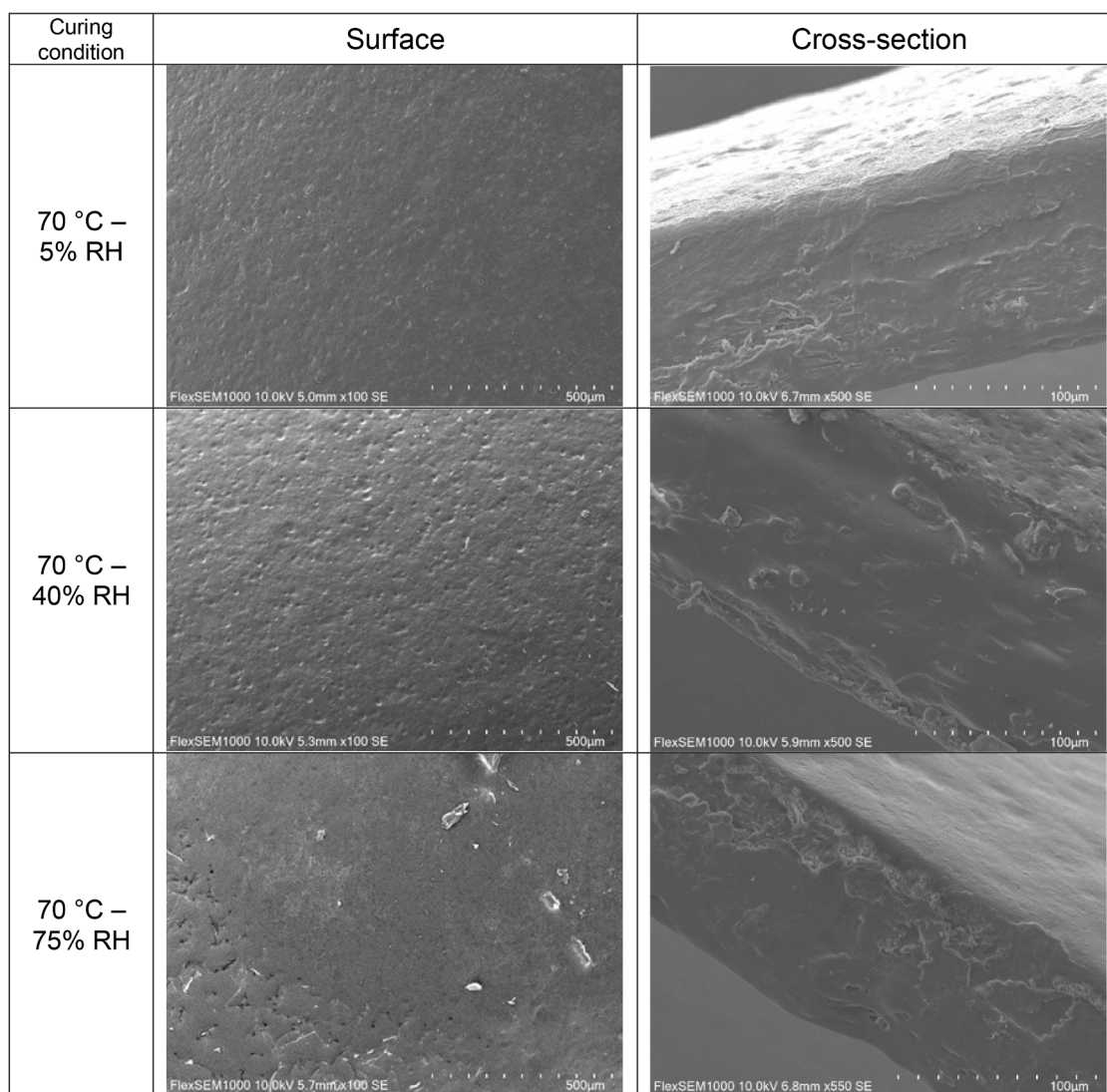


Fig. 4. SEM analysis of capsule shell surfaces and cross-sections after curing at 70 °C with different relative humidities (5%, 40%, and 75% RH).

based on reference performance of Capsugel® Enprotect® capsules (double layer capsules made with HPMC and HPMC-AS) LOD which is equals to $6.3 \pm 3.5\%$.

Fig. 8B shows the results for capsules with HPMC-only inner layers. Non-cured formulations containing 4% alginate exhibited lower water ingress than capsules cured at 70 °C–75% RH, suggesting a detrimental effect of high-humidity curing on barrier properties. Among 4% alginate formulations, curing at 70 °C–5% RH and 70 °C–40% RH resulted in significantly lower LOD values and improved resistance to water penetration compared with Capsugel® Enprotect® capsules. A similar trend was observed for capsules containing 6% alginate.

Fig. 8A shows the permeability results for capsules incorporating an HPMC/pullulan inner layer. Prototypes containing 4% or 6% alginate cured at 70 °C–40% RH exhibited low permeability, confirming that this curing condition effectively stabilize the bilayer structure. In contrast, two formulations showed insufficient resistance to acidic conditions: capsules cured at 70 °C–40% RH with an HPMC inner layer only, and capsules cured at 70 °C under dry conditions with an HPMC/pullulan inner layer. Both opened prematurely during acidic exposure and were therefore excluded from further evaluation. This behavior contrasts with the stable performance of the corresponding 4% and 6% alginate prototypes, highlighting the strong influence of both the inner-layer composition and the curing environment on capsule integrity.

Overall, these findings demonstrate that both alginate concentration and curing humidity strongly influence capsule impermeability, with moderate to low relative humidity during curing favoring formation of a denser and more effective barrier against water ingress.

3.3.3. Dissolution testing

During the acidic phase (0–120 min), caffeine release remained close to 0% for most prototypes, confirming their delayed release performance. In Fig. 9A, which presents capsules containing an HPMC/pullulan inner layer, only the formulation containing 9% sodium alginate and cured at 75% RH showed a substantial loss of acid resistance, with approximately 50% caffeine release during the gastric stage. Overall, the incorporation of pullulan appeared to reduce gastric resistance compared with HPMC-only formulations. These observations are consistent with the permeability trends previously identified through surface analysis, water ingress, and disintegration studies.

During the pH 6.8 phase (120–180 min), the influence of alginate concentration became more apparent for HPMC/pullulan-based capsules (Fig. 9A). Formulations containing 9% sodium alginate exhibited the highest permeability, with caffeine release exceeding 50%, whereas intermediate release profiles were observed for 6% alginate formulations (~20%), and minimal release for 4% alginate systems. Although curing conditions affected the dissolution profiles, no consistent trend

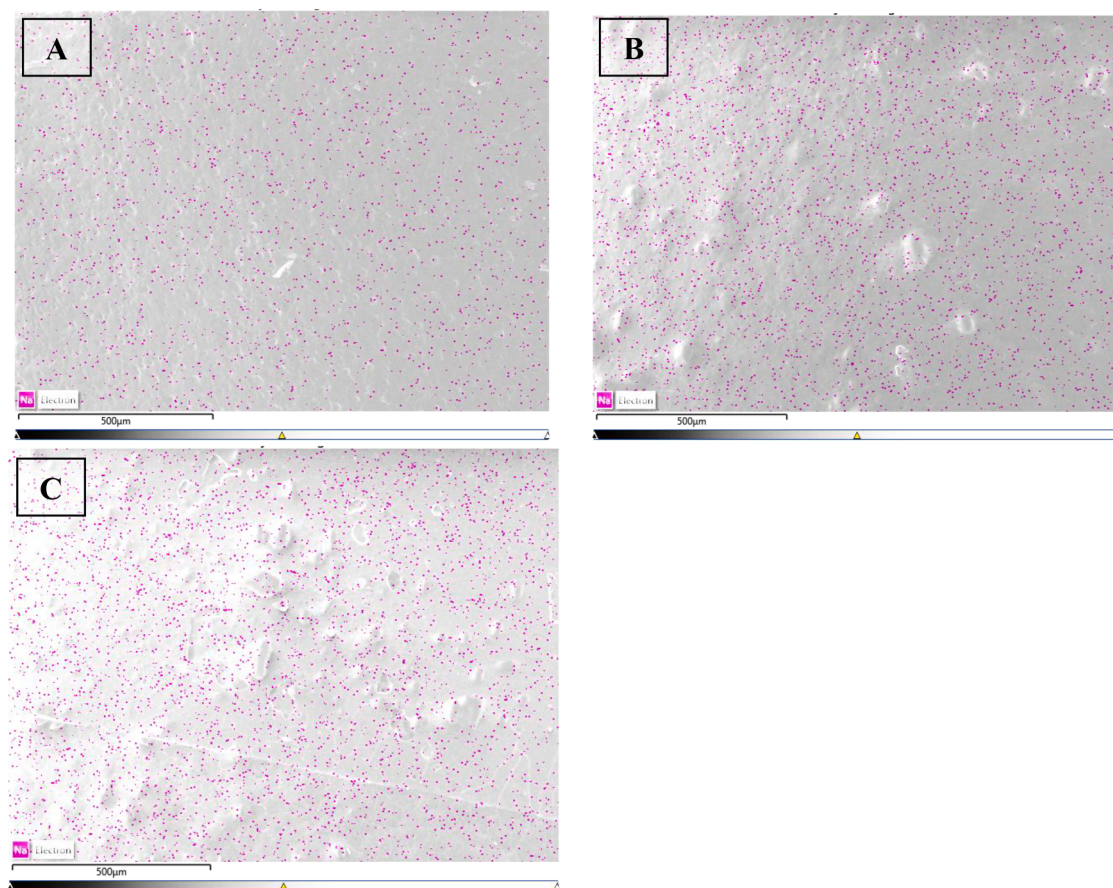


Fig. 5. Energy Dispersive X-ray Spectrometer analysis on bilayer capsules with an outer layer composed of ethyl cellulose and A) 4% of sodium alginate B) 6% of sodium alginate and C) 9% of sodium alginate. Sodium is marked as purple dots on images.

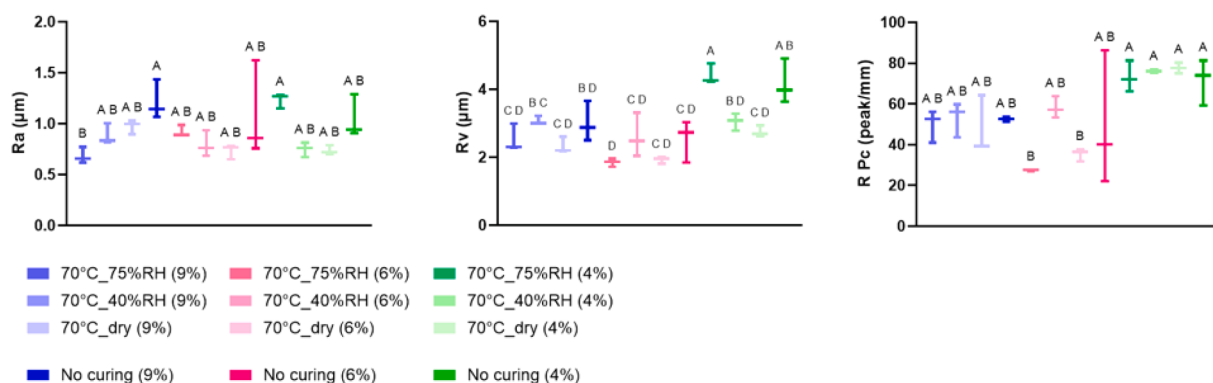


Fig. 6. Effect of the sodium alginate concentration and curing conditions on surface roughness parameters. Data represent mean $\pm$ SD, N = 6. Statistical significance marked by letter is represented with $p < 0.05$.

associated with relative humidity could be clearly established, limiting mechanistic interpretation of its influence at this stage.

In the final pH 7.4 phase, rapid release was again observed for formulations containing 9% sodium alginate, particularly for oven-cured capsules. In contrast, capsules featuring 4% sodium alginate exhibited limited release throughout the three-stage dissolution process, rendering them unsuitable for effective distal ileum targeting. Overall, HPMC/pullulan systems could therefore be classified into three categories according to alginate concentration: high release for 9% alginate, intermediate release for 6% alginate, and minimal release for 4% alginate formulations.

In the case of capsules containing HPMC alone as the inner layer

(Fig. 9B), improved gastric resistance was observed overall, with caffeine release remaining below 5% during the acidic phase for most formulations. Similar trends to those observed for HPMC/pullulan systems were identified during the intestinal stages, with the fastest release obtained for 9% alginate formulations, followed by 6% and then 4% alginate systems. However, overall release remained lower than for HPMC/pullulan capsules, with only the formulation cured at 40% RH exceeding 50% caffeine release at pH 7.4, while most others remained below 20%.

Based on the desired performance profile, namely minimal release under gastric conditions, limited release at pH 6.8, and enhanced release at pH 7.4, the 9%/75% RH and 6%/40% RH formulations appeared to

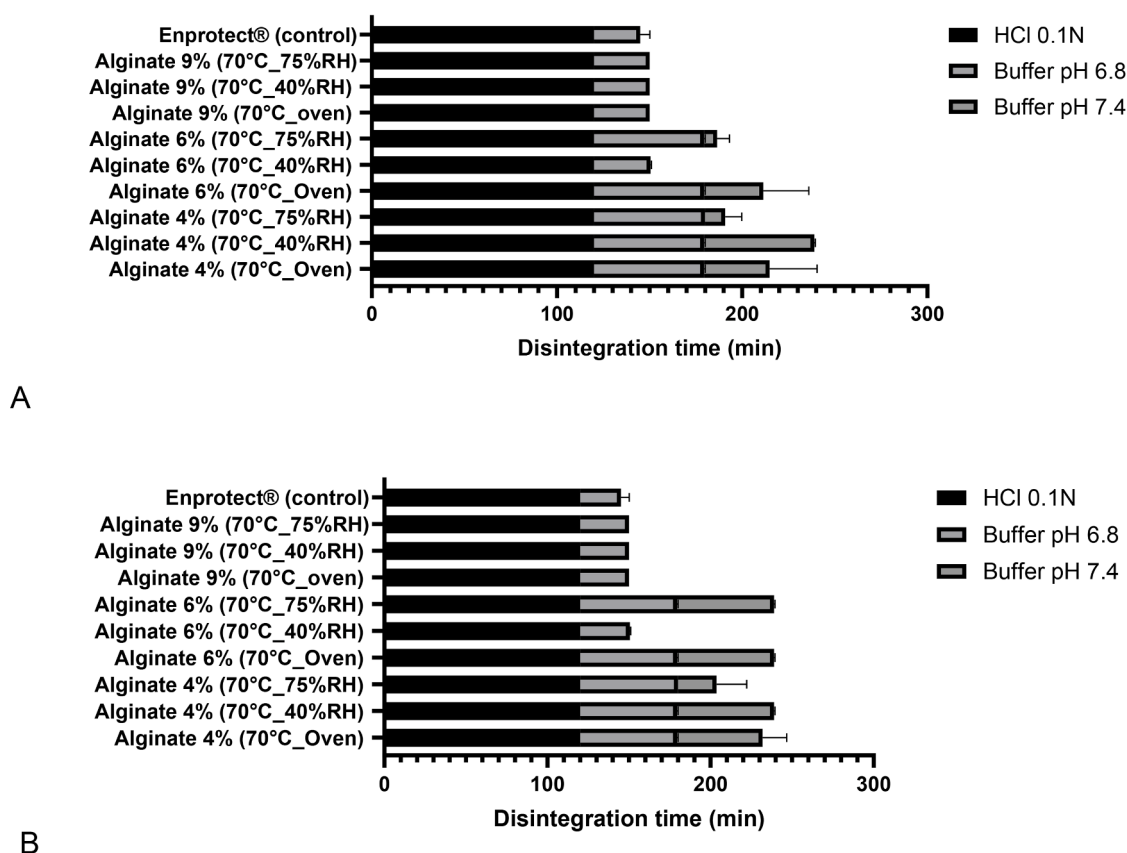


Fig. 7. Disintegration test on double layer capsule HPMC with Pullulan (A) or without pullulan (B)/ EC and alginate with a successive pH exposure in HCl 0.1 N then transfer in pH 6.8 then in pH 7.4; capsules filled lactose monohydrate. Data represent mean $\pm$ SD, N = 6.

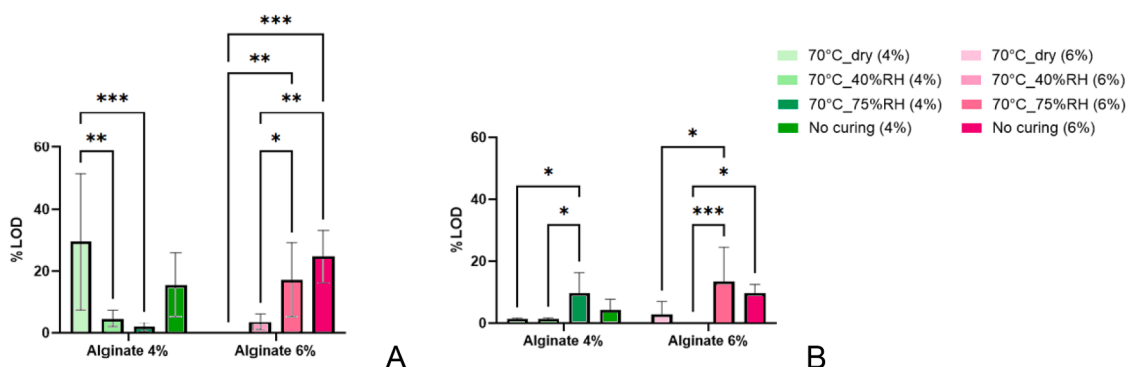


Fig. 8. Water ingress on double layer capsule A: results on inner layer composed of HPMC pullulan B: results on inner layer composed of HPMC only; capsules filled mannitol and thymol blue. Data represent mean $\pm$ SD, N = 6. Statistical significance is indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

provide the most balanced behavior.

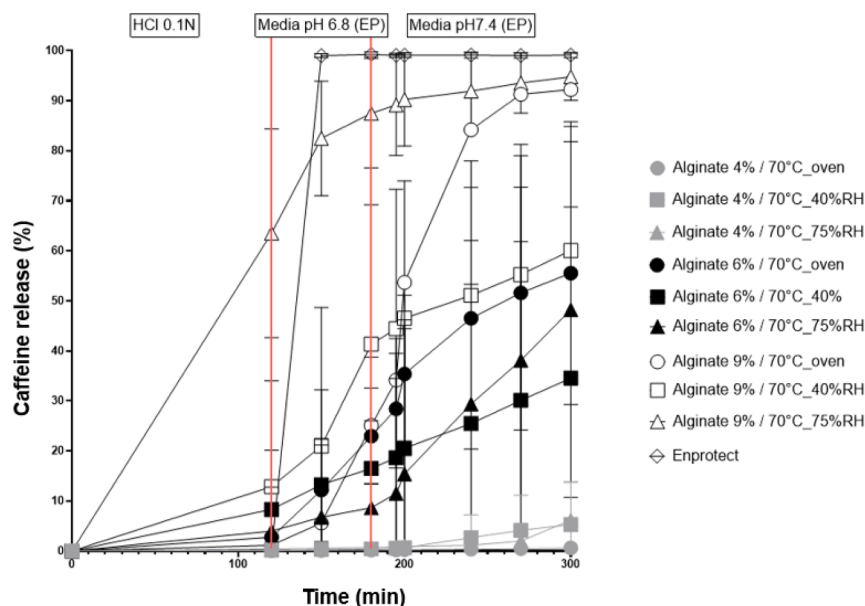
4. Discussion

Attaining reproducible, site-selective drug release within the distal small intestine and ileocecal junction—continues to represent a critical challenge in the development of targeted delivery systems for the localized treatment of pathologies in these regions (Millet et al., 2025). To address this, a bilayer delayed release capsule was developed, comprising an inner layer of HPMC or HPMC/pullulan and an outer layer of ethylcellulose and alginate. This combination of cellulosic ethers, neutral polysaccharides, and anionic alginate was strategically selected to yield a mechanically robust and acid-resistant shell capable of delaying drug release until the targeted intestinal region is reached.

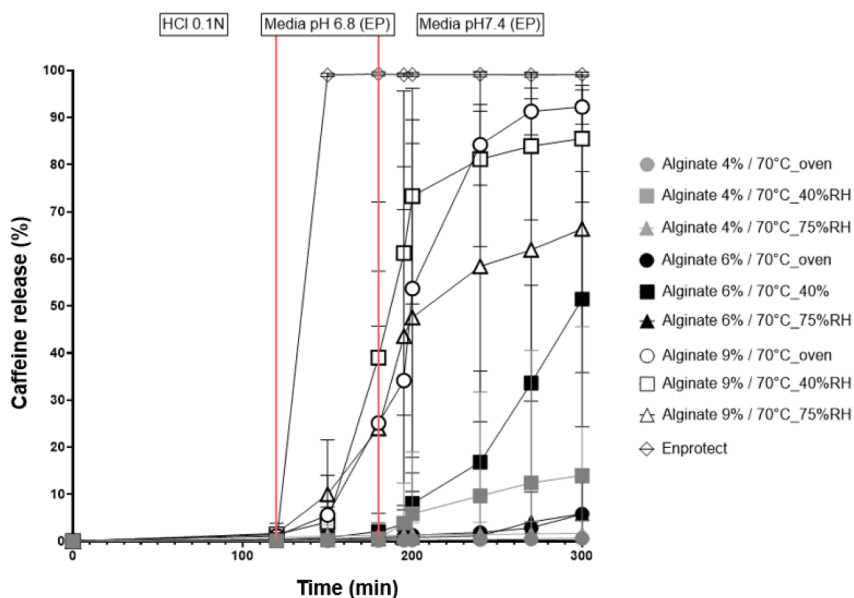
Consequently, elucidating the influence of polymer composition and curing conditions on capsule microstructure and functional performance is critical for the rational design and development of next-generation ileum-targeted delivery systems.

This study further demonstrates that bilayer capsule systems can be rationally engineered to achieve delayed drug release compatible with targeting of the distal small intestine. By combining a diffusion-controlling ethylcellulose–alginate outer layer with a responsive HPMC-based inner layer, the system enables modulation of permeability, lag time, and disintegration behavior. These findings highlight the capacity of this bilayer design to integrate multiple release mechanisms within a single dosage form.

Overall, the performance of the novel bilayer capsules was influenced by the interplay between the functional ethylcellulose–alginate



A



B

Fig. 9. Dissolution on double layer capsule A: results on inner layer composed of HPMC and pullulan B: results on inner layer composed of HPMC only; capsules filled mannitol and thymol blue. Data represent mean $\pm$ SD, N = 6.

outer layer and the structurally supportive HPMC based inner layer. The outer layer dictated resistance under gastric and proximal intestinal conditions, with alginate concentration and curing parameters modulating permeability through pore development and film consolidation. As this barrier diminished at neutral pH, the inner-layer composition became the primary determinant of disintegration behavior and drug release. Specifically, HPMC conferred structural integrity, whereas pullulan enhanced hydration-induced polymer relaxation, resulting in more rapid capsule opening. Ileal-targeted performance was most favorable at 4–6% alginate combined with moderate-humidity curing, enabling effective protection in the upper gastrointestinal tract alongside a controlled increase in permeability at pH 7.4. These findings underscore the importance of integrating a tunable outer barrier with a responsive inner structural matrix, rather than relying solely on outer-layer functionality for effective ileal targeting.

A key outcome of this work is that capsule performance is governed by the combined effect of formulation parameters, particularly alginate content and polymer selection, and processing conditions such as curing under controlled humidity. These factors directly influence barrier integrity, permeability, and disintegration behavior, enabling controlled and tunable release profiles.

Curing was shown to be a key processing parameter influencing the functional performance of the bilayer capsules. Although plasticization at 70 °C ensured sufficient ethylcellulose chain mobility, curing relative humidity (RH) largely governed the reorganization of the ethylcellulose–alginate outer layer during thermal treatment. SEM analysis revealed that curing humidity affected surface morphology and cross-sectional organization differently. Increasing RH progressively promoted polymer swelling and porosity within the cross-sections, with capsules cured at 75% RH displaying swollen and partially delaminated

internal structures, consistent with enhanced water sorption and increased polymer chain mobility (Magramane et al., 2025; Barham et al., 2015). Similar moisture-induced structural rearrangements have previously been reported in ethylcellulose-based systems, where excess water disrupts polymer packing and limits proper film consolidation during curing (Yang et al., 2016; Ouyang and den Mooter, 2025).

In contrast, profilometry analysis showed that curing globally reduced surface roughness parameters compared with non-cured capsules, particularly for Ra values. Surface morphology was primarily governed by alginate concentration, while curing mainly modulated roughness amplitude rather than the overall structural organization. At low alginate concentration (4%), curing effects were more pronounced, indicating that thin polymer networks remained highly sensitive to dehydration and polymer rearrangement dynamics. Conversely, formulations containing 6% and 9% alginate exhibited more stable roughness profiles with limited sensitivity to curing conditions, suggesting the formation of denser and more entangled polymer networks less prone to structural reorganization.

Collectively, these findings demonstrate that curing humidity governs both internal film organization and surface morphology, although through distinct mechanisms. High humidity promotes internal swelling and porosity due to increased polymer mobility, whereas curing simultaneously reduces average surface roughness through partial surface consolidation. These structural modifications are expected to directly influence capsule permeability, mechanical integrity, water ingress, and ultimately dissolution behavior, highlighting curing RH as a critical parameter in governing the performance of bilayer capsule.

Elemental analyses supported the compositional integrity of the ethylcellulose–alginate outer layer. XRF analysis showed that increasing alginate concentration in the coating formulation resulted in higher sodium content, while EDX mapping confirmed a homogeneous distribution of sodium throughout the layer, indicating uniform dispersion of alginate within the ethylcellulose matrix. More specifically, the sodium content quantified by XRF increased proportionally with alginate concentration, with approximately 2200 ppm measured at 4% alginate, increasing to ~4600 ppm and ~8300 ppm for 6% and 9% alginate, respectively. As alginate was introduced exclusively in its sodium form and no other sodium-containing excipients were present, the sodium signal can be considered a reliable marker of alginate incorporation. This interpretation is consistent with the established role of sodium as the native counterion of alginate carboxylate groups (Draget et al., 2005; Grant et al., 1973).

It should be noted that sodium distribution may not strictly reflect the spatial organization of alginate within the matrix, due to potential ion dissociation during processing; therefore, elemental analysis should be interpreted as an indirect indicator of polymer incorporation rather than a direct measure of polymer microstructure.

This behavior aligns with the well-established film-forming and pore-forming properties of alginate in composite polymer systems (Jain and Bar-Shalom, 2014; Sriamornsak et al., 2007). Consistent with its role as a hydrophilic polysaccharide, higher alginate levels led to increased caffeine release under dissolution conditions, reflecting the expected modulation of permeability associated with controlled incorporation of alginate into the functional layer.

Importantly, the incorporation of alginate introduces not only physicochemical responsiveness (hydration and swelling) but also potential responsiveness to microbiota-derived enzymatic activity. This feature extends the design beyond conventional pH-triggered systems by integrating a diffusion-controlled barrier with a secondary, environmentally responsive mechanism that may contribute to region-specific permeability in the distal small intestine.

Several strategies have been developed to achieve site-specific drug delivery in the gastrointestinal tract, including pH-dependent systems, time-controlled devices, and enzyme-responsive formulations (Lee et al., 2020; Mishra et al., 2026). pH-dependent systems, such as those based on methacrylate copolymers, are widely used but are sensitive to

variability in gastrointestinal pH, which may result in premature or delayed drug release (Choudhary et al., 2020). Similarly, time-dependent approaches rely on predictable transit kinetics, but inter-individual variability in gastrointestinal transit time can reduce targeting precision (Lee et al., 2020). Enzyme-triggered systems based on polysaccharides offer improved specificity through microbial degradation; however, their performance strongly depends on microbiota composition and enzymatic activity, which are typically higher and more consistent in the colon than in the distal small intestine (Li et al., 2026; Dong et al., 2026).

Clinically validated formulations such as budesonide-based systems (e.g., Tarpeyo®/Nefecon) demonstrate the feasibility of targeting drug release to the distal ileum, primarily through pH-dependent coatings combined with controlled release matrices (Barratt et al., 2024). While these systems have demonstrated therapeutic efficacy, their performance may still be influenced by physiological variability in pH and transit conditions, which can affect the consistency of the release location (Mishra et al., 2026).

In comparison, the bilayer capsule system developed in this work integrates multiple release mechanisms, combining a diffusion-controlled outer barrier with pH-dependent and potentially microbiota-responsive components. This multi-mechanism approach may provide improved robustness compared with single-trigger systems by reducing reliance on a single physiological parameter. However, it should be noted that microbiota-mediated contributions in the ileum are expected to be more limited and variable than in the colon, which may influence the predictability of this mechanism.

Overall, this strategy represents a complementary approach to existing technologies, aiming to extend delayed-release performance toward the distal small intestine while balancing the limitations associated with pH-, time-, or microbiota-driven systems alone.

Notably, the selected prototypes exhibited a more delayed release profile compared to commercial Capsugel® Enprotect® capsules, which are primarily designed to release their contents in the proximal to mid-small intestine. In contrast, the present bilayer systems demonstrated limited release at pH 6.8 and increased release at pH 7.4, suggesting a shift toward more distal regions of the small intestine. This behavior is attributed to the combined effect of a diffusion-controlled ethylcellulose outer layer and a tunable alginate-mediated permeability, enabling a prolonged lag phase compared to predominantly pH-triggered systems.

While these findings indicate the potential of the bilayer capsule design to extend release toward the terminal ileum, it is important to emphasize that this study is based solely on *in vitro* data obtained under simplified conditions. In particular, the dissolution experiments were conducted in the absence of digestive components such as pancreatin, bile salts, and brush-border enzymes, which may significantly influence capsule disintegration and permeability *in vivo*. This effect may be especially relevant for polysaccharide-based components such as alginate and pullulan, which are susceptible to enzymatic degradation.

Therefore, no direct extrapolation to *in vivo* performance can be made at this stage. Future work will focus on evaluating the selected lead formulations under more physiologically relevant conditions, including enzyme-mediated dissolution studies and *in vitro* models simulating both healthy and Crohn's disease environments, followed by *in vivo* validation to confirm ileal targeting performance.

Overall, this work establishes a structure–function framework linking formulation and processing parameters to capsule performance and identifies promising bilayer configurations for further investigation as targeted delivery systems for the distal small intestine.

5. Conclusion

This study demonstrates that bilayer capsule systems can be rationally engineered to achieve delayed drug release compatible with targeting of the distal small intestine. By combining a diffusion-controlling ethylcellulose–alginate outer layer with a responsive HPMC-based inner

layer, the system enables modulation of permeability, lag time, and disintegration behavior.

A key finding is that capsule performance is governed by the combined effect of formulation parameters, particularly alginate content and polymer selection, and processing conditions such as curing under controlled humidity. These parameters were shown to directly influence barrier integrity and permeability, enabling controlled and tunable release profiles.

Importantly, the proposed design extends beyond conventional pH-triggered systems by incorporating a diffusion-controlled mechanism with potential microbiota-responsive contributions, thereby offering a strategy to shift drug release toward more distal regions of the small intestine.

Overall, this proof-of-concept work establishes a clear structure–function framework for the design of bilayer capsule systems and identifies promising prototypes for further evaluation under biorelevant and in vivo conditions, supporting the development of targeted oral drug delivery approaches for the distal ileum.

Funding

Funding for this publication is provided under the GENEGUT (<https://genegut.eu/>) project funded by the European Commission as a Horizon Europe Research and Innovation Action (RIA) under the call tools and technologies for a healthy society (2021) (HORIZON—HLTH-2021-TOOL-06, GA 101,057,491).

AI use statement

During the preparation of this work, the author(s) used ChatGPT (OpenAI) in order to assist with language refinement. After using this tool, the author(s) reviewed and edited the content as needed and take full responsibility for the content of the publication.

CRedit authorship contribution statement

Elisa Millet: Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Sofie S.T. Vandenbroucke:** Writing – review & editing, Resources. **Joseph P O'Shea:** Writing – review & editing. **Brendan T Griffin:** Writing – review & editing. **Vincent Jannin:** Writing – review & editing, Supervision, Funding acquisition.

Declaration of competing interest

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

Brendan T. Griffin, Joseph P. O'Shea, Elisa Millet and Vincent Jannin report financial support was provided under the GENEGUT project funded by the European Commission as a Horizon Europe Research and Innovation Action (RIA).

Vincent Jannin, Sofie Vandenbroucke and Elisa Millet report a relationship with Lonza-Capsugel that includes: employment.

Brendan T. Griffin and Joseph P. O'Shea report a relationship with University College Cork that includes: employment.

There are no other known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.ejps.2026.107601](https://doi.org/10.1016/j.ejps.2026.107601).

Data availability

Data will be made available on request.

References

- Aghajannataj Ahangarkola, F., Najafpour-Darzi, G., Ramezani Kalmer, R., 2024. Towards a novel pullulan and pullulan-enteric hard capsules: study of drug release and crucial capsule features. *J. Drug Deliv. Sci. Technol.* 92, 105297. <https://doi.org/10.1016/j.jddst.2023.105297>.
- Alke, B., Gabirondo, E., Alves, V.D., Crespo, J.G., Brazinha, C., Tomé, L.C., 2026. Tailoring the properties of ethyl cellulose membranes using hydrophobic deep eutectic solvents. *ACS. Sustain. Chem. Eng.* 14, 7262–7274. <https://doi.org/10.1021/acssuschemeng.5c13894>.
- Barham, A.S., Tewes, F., Healy, A.M., 2015. Moisture diffusion and permeability characteristics of hydroxypropylmethylcellulose and hard gelatin capsules. *Int. J. Pharm.* 478, 796–803. <https://doi.org/10.1016/j.ijpharm.2014.12.029>.
- Barratt, J., Kristensen, J., Pedersen, C., Jerling, M., 2024. Insights on Nefecon®, a targeted-release formulation of budesonide and its selective immunomodulatory effects in patients with IgA nephropathy. *Drug. Devel. Ther.* 18, 3415–3428. <https://doi.org/10.2147/DDDT.S383138>.
- Begum, S.G., Hasmitha, Y., Reddy, U.G., Deepa, M., Reddy, K.S., Susmitha, R., 2018. A review on manufacturing and evaluation of capsules. *World J. Pharm. Sci.* 98–105. <https://wjpsonline.com/index.php/wjps/article/view/review-manufacturing-evaluation-capsules>.
- Brandrup, J., Immergut, E.H., Grulke, E.A., 1999. *Polymer Handbook*. Polymer Handbook. A Wiley Interscience Publication.
- Choudhary, L., Jain, A., Agarwal, D., 2020. Colon-targeted oral drug delivery systems: a review.
- Dong, H., Zhou, A., Zeng, J., Zhang, N., Xi, H., Huang, Y., Pan, X., Xie, J., Xie, L., 2026. Polysaccharides from medicine and food homology species used in China: a comprehensive review of extraction, bioactivities structure-activity relationship and applications. *Food Biosci.* 79, 108638. <https://doi.org/10.1016/j.fbio.2026.108638>.
- Drage, K., Smidsrød, O., Skjåk-Bræk, G., 2005. Alginates from Algae. *Biopolymers Online*. <https://doi.org/10.1002/3527600035.bpol6008>.
- Ferraro, F., Sonleitner, L.M., Neut, C., Mahieux, S., Verin, J., Siepmann, J., Siepmann, F., 2024. Colon targeting in rats, dogs and IBD patients with species-independent film coatings. *Int. J. Pharm.* X 7, 100233. <https://doi.org/10.1016/j.ijpx.2024.100233>.
- Fu, T., Wang, Y., Ma, M., Dai, W., Pan, L., Shang, Q., Yu, G., 2023. Isolation of alginate-degrading bacteria from the Human gut microbiota and discovery of *Bacteroides xylanisolvens* AY11-1 as a novel anti-colitis probiotic bacterium. *Nutrients* 15, 1352. <https://doi.org/10.3390/nu15061352>.
- Giakoumis, T., O'Shea, J.P., Clarke, G., Griffin, B.T., 2026. Assessment of pectin-ethyl cellulose coated pellets for bacterially enhanced colonic drug delivery in progressively complex in vitro and in vivo models. *Int. J. Pharm.* 690, 126521. <https://doi.org/10.1016/j.ijpharm.2025.126521>.
- Grant, G.T., Morris, E.R., Rees, D.A., Smith, P.J.C., Thom, D., 1973. Biological interactions between polysaccharides and divalent cations: the egg-box model. *FEBS Lett.* 32, 195–198. [https://doi.org/10.1016/0014-5793\(73\)80770-7](https://doi.org/10.1016/0014-5793(73)80770-7).
- Grimm, M., Rump, A., Kromrey, M.-L., Morof, F., Dumont, C., Jannin, V., Tzvetkov, M.V., Weitschies, W., 2023. In vivo evaluation of a gastro-resistant Enproct® capsule under postprandial conditions. *Pharmaceutics* 15, 2576. <https://doi.org/10.3390/pharmaceutics15112576>.
- Hoffmann, S.V., Griffin, B.T., Jannin, V., O'Shea, J.P., 2025. Evaluating drug absorption from enteric capsules in pigs: impact of capsule size on gastric emptying. *Eur. J. Pharm. Sci.* 213, 107237. <https://doi.org/10.1016/j.ejps.2025.107237>.
- Huntsman, M., Lee, S.N., Stylli, J., Stork, C., Shimizu, J., Quintana, N., Singh, S., Wahl, C., Chuang, E., 2021. Development of a novel drug delivery system to deliver drugs directly to the colonic mucosa, resulting in improved efficacy and reduced systemic exposure for the treatment of ulcerative colitis. *Crohn's Colitis* 360 (3), otab045. <https://doi.org/10.1093/crocol/otab045>.
- Jain, D., Bar-Shalom, D., 2014. Alginate drug delivery systems: application in context of pharmaceutical and biomedical research. *Drug Dev. Ind. Pharm.* 40, 1576–1584. <https://doi.org/10.3109/03639045.2014.917657>.
- Jones, B.E., Podczeczek, F., Lukas, P., 2017. *Capsule Shell Manufacture*. Pharmaceutical Dosage Forms. CRC Press.
- Körber, M., Hoffart, V., Walther, M., Macrae, R.J., Bodmeier, R., 2010. Effect of unconventional curing conditions and storage on pellets coated with Aquacoat ECD. *Drug Dev. Ind. Pharm.* 36, 190–199. <https://doi.org/10.3109/03639040902882314>.
- Kotla, N.G., Rana, S., Sivaraman, G., Sunnapati, O., Vemula, P.K., Pandit, A., Rochev, Y., 2019. Bioresponsive drug delivery systems in intestinal inflammation: state-of-the-art and future perspectives. *Adv. Drug Deliv. Rev.* 146, 248–266. <https://doi.org/10.1016/j.addr.2018.06.021>.
- Lafargue, D., 2020. Etude Et Elaboration de Films à Base De Polysaccharides Pour La Fabrication De Gélules Pharmaceutiques (Thesis: Génie des Procédés). University of Nantes. <https://hal.science/tel-02823382/>.
- Lee, S.H., Bajracharya, R., Min, J.Y., Han, J.-W., Park, B.J., Han, H.-K., 2020. Strategic approaches for colon targeted drug delivery: an overview of recent advancements. *Pharmaceutics* 12, 68. <https://doi.org/10.3390/pharmaceutics12010068>.
- Li, J., Yu, X., Huang, D., 2026. Enzyme-responsive polymeric drug delivery systems for the treatment of inflammatory bowel diseases: a review. *Polym. (Basel)* 18, 1146. <https://doi.org/10.3390/polym18101146>.
- Magramane, S., Kállai-Szabó, N., Farkas, D., Süvegh, K., Zekó, R., Antal, I., 2025. Comparative evaluation of gelatin and HPMC inhalation capsule shells exposed to simulated humidity conditions. *Pharmaceutics* 17, 877. <https://doi.org/10.3390/pharmaceutics17070877>.
- Mathieu, S., Touvrey-Loidice, M., Poulet, L., Drouillard, S., Vincentelli, R., Henrissat, B., Skjåk-Bræk, G., Helbert, W., 2018. Ancient acquisition of “alginate utilization loci”

- by human gut microbiota. *Sci. Rep.* 8, 8075. <https://doi.org/10.1038/s41598-018-26104-1>.
- Millet, E., O'Shea, J.P., Griffin, B.T., Dumont, C., Jannin, V., 2025. Next generation capsules: Emerging technologies in capsule fabrication and targeted oral drug delivery. *Eur. J. Pharm. Sci.* 214, 107277. <https://doi.org/10.1016/j.ejps.2025.107277>.
- Mishra, A.S., Ghosh, B., Malliappan, S.P., Dutta, G., Vasanthan, M., 2026. Formulation approaches for colon-specific drug delivery: conventional to nanocarrier systems. *RSC. Adv.* 16, 10022–10059. <https://doi.org/10.1039/D5RA05194K>.
- Ouyang, J., den Mooter, G.V., 2025. Unraveling the role of pectin biodegradability and blend composition on the permeability of ethylcellulose-based blend films designed for colon targeting. *Int. J. Pharm.* 675, 125538. <https://doi.org/10.1016/j.ijpharm.2025.125538>.
- Patrascu, O., Béguet-Crespel, F., Marinelli, L., Le Chatelier, E., Abraham, A.-L., Leclerc, M., Klopp, C., Terrapon, N., Henrissat, B., Blottière, H.M., Doré, J., Béramaillet, C., 2017. A fibrolytic potential in the human ileum mucosal microbiota revealed by functional metagenomic. *Sci. Rep.* 7, 40248. <https://doi.org/10.1038/srep40248>.
- Rønne, M.E., Dybdahl Andersen, C., Teze, D., Petersen, A.B., Fredslund, F., Stender, E.G. P., Chaberski, E.K., Holck, J., Aachmann, F.L., Welner, D.H., Svensson, B., 2024. Action and cooperation in alginate degradation by three enzymes from the human gut bacterium *Bacteroides eggerthii* DSM 20697. *J. Biol. Chem.* 300, 107596. <https://doi.org/10.1016/j.jbc.2024.107596>.
- Rønne, M.E., Madsen, M., Tandrup, T., Wilkens, C., Svensson, B., 2023. Gut bacterial alginate degrading enzymes. *Essays Biochem.* 67, 387–398. <https://doi.org/10.1042/EBC20220123>.
- Rump, A., Kromrey, M.-L., Scheuch, E., Jannin, V., Rehenbrock, L., Tzvetkov, M.V., Weitschies, W., Grimm, M., 2022. In vivo evaluation of a gastro-resistant HPMC-based “Next Generation Enteric” capsule. *Pharmaceutics* 14, 1999. <https://doi.org/10.3390/pharmaceutics14101999>.
- Sawada, S., Ursino, C., Galiano, F., Simone, S., Drioli, E., Figoli, A., 2015. Effect of citrate-based non-toxic solvents on poly(vinylidene fluoride) membrane preparation via thermally induced phase separation. *J. Memb. Sci.* 493, 232–242. <https://doi.org/10.1016/j.memsci.2015.07.003>.
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675. <https://doi.org/10.1038/nmeth.2089>.
- Sriamornsak, P., Thirawong, N., Korkerd, K., 2007. Swelling, erosion and release behavior of alginate-based matrix tablets. *Eur. J. Pharm. Biopharm. : Off. J. Arbeitsgemeinschaft Pharm. Verfahrenstechnik E.V.* 66, 435–450. <https://doi.org/10.1016/j.ejpb.2006.12.003>.
- Tønnesen, H.H., Karlsen, J., 2002. Alginate in drug delivery systems. *Drug Dev. Ind. Pharm.* 28, 621–630. <https://doi.org/10.1081/DDC-120003853>.
- Yang, Q., Ma, Y., Zhu, J., 2016. Sustained drug release from electrostatic powder coated tablets with ultrafine ethylcellulose powders. *Adv. Powder Technol.* 27, 2145–2152. <https://doi.org/10.1016/j.apt.2016.07.027>.