ORAL DRUG DELIVERY SYSTEMS BASED ON POLYSACCHARIDES FOR COLON TARGETING

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Faculté des Sciences Pharmaceutiques et Biologiques
Ecole Doctorale Biologie Santé

DOCTORAL THESIS

A thesis submitted in fulfillment of the requirements
for the degree of Doctor of Philosophy (PhD)
in the

Advanced drug delivery systems - INSERM U1008
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Acknowledgements

Who would have thought that I would move…
…far from Paris, the city I was so attached to…
…for a PhD, a possibility that I never considered before.
Even I would not.
Back from Bolivia with a heart as heavy as my backpack, January 2022 marked the start of a piece of work I would be passionate about.
In fact, when I began to apply myself to the topic of tablets coating, I lost track of time.
I now feel as if I have emerged from a tunnel in which I have been for one year and a half.

Dr Youness Karrout: it would be impossible to forget your incredible availability and reactivity when needed. I do feel grateful and lucky to have benefited from your permanent presence and skills during this whole project. Again, thank you for opening your doors to me in November 2019. Being my doctoral supervisor led me to surpass myself even in the toughest moments.

Prof. Marie-Bénédicte Romond: as a President of the jury for both my PharmD and my PhD, I can ensure that your presence was more than appreciated. The way you animated, framed and taught things is my best recording of these important moments.

Prof. Lahiani-Skiba and Dr Hamdi-Degobert: as thesis reporters, I was touched by the time you invested to read my manuscript. I measure the task and the time it may have involved. Thanks for your presence at my PhD defense in which I could feel comfortable and myself, while learning from your relevant advices and knowledge.

Prof. Juergen and Prof. Florence Siepmann: let me thank you for your pleasant and nice presences for all these years. The way you managed this laboratory, always trying to have a positive mindset, was really appreciated.
My research project was hard and yet intellectually satisfying.
Thanks to life for this intense and meaningful period of my short existence.

Cable car in La Paz, Bolivia.

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Abbreviations

5-ASA: 5-aminosalicylic acid
AEs: Adverse events
API: Active Pharmaceutical Ingredient
ASL: Aqueous Shellac Solution
BCS: Biopharmaceutical Classification System
BID: *Bis in die* (twice a day)
CD: Crohn's disease
CL: Coating level
Cmax: Maximal concentration
DPM: Dips *per* minute
EC: Ethylcellulose
GLY: Glycerol
U.S. FDA: U.S. Food and Drug Administration
HPLC: High Performance Liquid Chromatography
HPMC: Hydroxypropylmethylcellulose
IBD: Inflammatory bowel disease
GIT: Gastrointestinal tract
GRAS: Generally Recognized As Safe
HCl: Hydrochloric acid
IP: Intellectual property
LHRH: Luteinizing Hormone Realeasing Hormone
mAb: Monoclonal antibody
MW: Molecular weight
NAACCR: North American Association of Central Cancer Registries
PBS: Phosphate buffer saline
Ph. Eur.: European Pharmacopoeia
PEs: Permeation enhancers
PPs: Proteins and peptides
RPM: Rotations *per* minute
SCFAs: Short-chain fatty acids
SCF: Simulated colonic fluid
TEC: Triethyl citrate
Da: Dalton
UC: Ulcerative colitis
USP: United States Pharmacopoeia
UV: Ultraviolet
vs: *Versus*

I. INTRODUCTION

I. Introduction

I.1 General

I.1.1 Inflammatory bowel disease and their epidemiology

10 million people worldwide, over 1.5 million in North America and 2 million in Europe [1]. Those are the numbers of people affected by inflammatory bowel disease (IBD) in each region quoted, respectively. Including both Crohn's disease (CD) and ulcerative colitis (UC), inflammatory bowel disease has emerged as a public health challenge worldwide in the past decades [2]. IBD are characterized by moderate to severe symptoms, and have in common relapsing-remitting cycles of mucosal inflammation [3]. Often diagnosed between 15 and 35 years old, they are a matter of industrial countries. Within the populations of North America, Australia and Europe, the prevalence of IBD now exceeds 0.3 % [4]. In France, 273100 people were concerned by IBD in 2019.

In western countries, IBD involve morbidity, mortality, and significant costs to the health-care systems. The increasing incidence of inflammatory bowel disease in recently industrialized countries could be the sign of an emerging epidemic of the disease out of the western world. In the past century, the incidence of inflammatory bowel disease has grown, then stagnated in the western parts of the world, whereas the other countries seem to be in the first stage of this sequence.

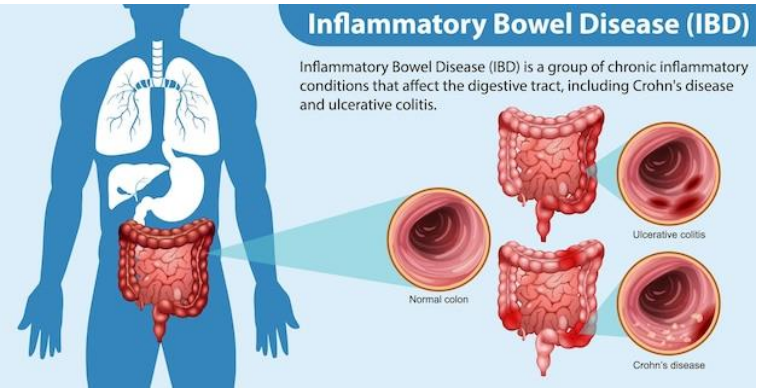


Figure 1: Inflammatory bowel disease – UC and CD.
Image taken from free images (https://fr.freepik.com/).

First described by Burril Bernard Crohn in 1932, Crohn's disease can affect all the regions of the gastrointestinal tract (GIT), from the mouth to the anus, though it mainly impacts the ileum and the large bowel (colon). The lesions are transmural: mucosa, submucosa and serosa are harmed.

I. INTRODUCTION

Recognized in 1875, ulcerative colitis (UC) only concerns the large bowel and the rectum. The small intestine is never impacted. The main part of UC is distal (60 %) and affects the rectum, but also both rectum and sigmoid colon.

The rest of UC can either be pancolonic (attacking the whole colon and rectum, 15 % of the total UC) or under intermediate forms (between distal and pancolonic, counting for 25 % of UC). Only the superficial part of mucosa is affected. Basic symptoms consist of abdominal pains, diarrheas, but can lead to severe complications such as stenosis, intestinal perforation, or cancer.

To date, there is no cure for IBD. These affections only benefit from symptomatic medical care *via* treatments for the maintenance of remission episodes. Glucocorticoids are a choice for acute exacerbations of UC and CD, but their long-term use can lead to serious systemic side effects, which can be a cause of bad compliance. Other strategies, with oral aminosalicylates (5-ASA), antibiotics (metronidazole) or parenteral immunosuppressive agents (azathioprine) can help to maintain remission. Over 90 % of patients receive a 5-ASA within the first year of diagnosis, with between 60 % and 90 % continuing their use up to 15 years [5]. It is of utmost importance to note that 70 % of CD patients will undergo, at least, one surgical intervention in their lifetime. As for UC patients, up to a third of them will require surgery after 30 years of disease [6]. Defined as such, colon targeting is a technique for the delivery of pharmacologically active substances in a selective and effective manner to a pre-identified targeted location at therapeutic concentrations, while reducing adverse effects [7]. Research in this field has been motivated by the concern of better treating local affections of the colon such as inflammatory bowel disease, irritable bowel syndrome, diverticulitis, carcinoma, colonic dysmotility and parasitic diseases [8].

Beyond treating these kind of affections, it can be interesting for the oral administration of drugs that are fragile in the upper gastrointestinal tract (due to the gastric environment) [9–12]. This could be particularly advantageous for the medicines based on proteins and peptides, as proteolytic activity in the distal part of the GIT (colon) is low [13, 14]. Local drug delivery seems to be a relevant compromise to improve therapeutic efficacy and to lower serious systemic side effects. It must be emphasized that oral route is the most common route of administration due to its well-established acceptability, cost-effectiveness, manufacturing advantages and drug stability. In addition, oral dosage forms provide better compliance, greater convenience, lowered chance of needle stick injuries and cross-infections [15]. Owing to these advantages, oral dosage forms still account for the majority of the market [16].

I.1.2 Colon cancer

Colorectal cancer is the third most prevalent type of malignant neoplasm in the world, the fourth-largest cause of cancer-related death, and the leading cause of gastrointestinal cancer [17].

Each year, 1.36 million patients are newly diagnosed, with more than 500,000 deaths and 40 % of cancer cases diagnosed annually. 90 % of diagnosed patients are over 50 years old, with a median of 64 years old, but the disease is more aggressive in young subjects. Table 1 shows an estimate of the proportion of digestive cancers from the rates incidence data collected between 1998 and 2012, reported by the North American Association of Central Cancer Registries (NAACCR).

TABLE 1

Percentage of digestive cancer cases based on 1998–2012 incidence data reported by the North American Association of Central Cancer Registries

Cancer	% Estimated cases	% Estimated deaths
Digestive system	100	100
Esophagus	5.54554	10.2529
Stomach	8.64789	7.0117
Small intestine	3.30896	0.86911
Colon	31.2432	32.144
Rectum	12.862	0
Anus, anal canal and anorectum	2.64979	0.70574
Liver and intrahepatic bile duct	12.8652	17.7547
Gallbladder and other biliary	3.74512	2.42436
Pancreas	17.404	27.3018
Other digestive organs	1.72827	1.53565

Table 1: Estimation of digestive cancers and their localization between 1998 and 2012. Table taken from NAACCR data online. Accessible on https://www.naaccr.org/.

Treatments for colon cancer include surgery, cryosurgery, radiation therapy, and targeted therapy.

Surgery remains the first indication, with a 50 % cure rate, but post-operative relapses often lead to death. Also, most cytotoxic drugs do not differentiate between healthy cells and cancerous cells, causing systemic toxicity and numerous adverse events. These observations focus on investing in the development of new anticancer drugs. Despite the active search of new chemical entities [18], another alternative is the repositioning of the non-anticancer marketed drug for their anticancer activity. Repositioning is interesting as it is less complex than a new drug discovery cycle. The main challenge is the frequent absence of appropriate physicochemical properties of drugs, being a limiting step in new anticancer drug

development [19]. Providing anti-tumor properties and used to treat advanced colorectal carcinogenesis in humans, indomethacin is an example of these candidates. The main difficulty for effective colonic drug delivery is protecting the drug from premature dissolution when pH < 6.8. This hurdle needs to be solved *via* a strategy able to improve local drug release and to maximize the therapeutic effect.

Colon targeting would be an opportunity to address this issue.

Estimated New Cases*

Males			Females		
Prostate	241,740	29%	Breast	226,870	29%
Lung & bronchus	116,470	14%	Lung & bronchus	109,690	14%
Colon & rectum	73,420	9%	Colon & rectum	70,040	9%
Urinary bladder	55,600	7%	Uterine corpus	47,130	6%
Melanoma of the skin	44,250	5%	Thyroid	43,210	5%
Kidney & renal pelvis	40,250	5%	Melanoma of the skin	32,000	4%
Non-Hodgkin lymphoma	38,160	4%	Non-Hodgkin lymphoma	31,970	4%
Oral cavity & pharynx	28,540	3%	Kidney & renal pelvis	24,520	3%
Leukemia	26,830	3%	Ovary	22,280	3%
Pancreas	22,090	3%	Pancreas	21,830	3%
All Sites	**848,170**	**100%**	**All Sites**	**790,740**	**100%**

Estimated Deaths

Males			Females		
Lung & bronchus	87,750	29%	Lung & bronchus	72,590	26%
Prostate	28,170	9%	Breast	39,510	14%
Colon & rectum	26,470	9%	Colon & rectum	25,220	9%
Pancreas	18,850	6%	Pancreas	18,540	7%
Liver & intrahepatic bile duct	13,980	5%	Ovary	15,500	6%
Leukemia	13,500	4%	Leukemia	10,040	4%
Esophagus	12,040	4%	Non-Hodgkin lymphoma	8,620	3%
Urinary bladder	10,510	3%	Uterine Corpus	8,010	3%
Non-Hodgkin lymphoma	10,320	3%	Liver & intrahepatic bile duct	6,570	2%
Kidney & renal pelvis	8,650	3%	Brain & other nervous system	5,980	2%
All Sites	**301,820**	**100%**	**All Sites**	**275,370**	**100%**

Figure 2: Ten Dominant Cancer for the Estimated New Cancer Cases and Deaths by Sex, United States, 2012. *Estimates are rounded to the nearest 10 and exclude basal and squamous cell skin cancers and *in situ* carcinoma except urinary bladder. Figure from Siegel et al. (2012, https://doi.org/10.3322/caac.20138).

I.1.3 Other pathologies in the realm of colon targeting: the power of chronotherapy

Chronotherapy consists in fitting with the natural rhythms of the body for a better drug pharmacokinetic [20]. The colon represents an ideal site of delivery for chronotherapy, as targeted delayed drug delivery systems can release the drug during the night or early in the morning, when many symptoms peak. For instance, asthma is one of the diseases evolving along circadian rhythm. The risk of bronchospasm increases at night owing to circadian up-

regulation of inflammatory response and oxidative stress [21]. Beyond improving patients' symptoms, colon targeting with nocturnal release of anti-asthmatic drugs would also improve their sleep quality, providing numbers of physical, psychological, and social benefits. In a same way, rheumatoid arthritis, whose symptoms follow about a 24-hour rhythm with highest stiffness, occasions movement difficulty and pain early in the morning [22]. This pathology is likely to interest chronotherapy concept. Likewise, oncology has somewhat embraced chronotherapy [23]. Chest pain, angina, and hypertension are examples of cardiovascular disorders that have a definite circadian rhythm. Studies in the epidemiology have shown that there is an increased risk of angina, myocardial infarction, and stroke in the morning. [24]. Figure 3 presents the various pathologies in connection with circadian cycles.

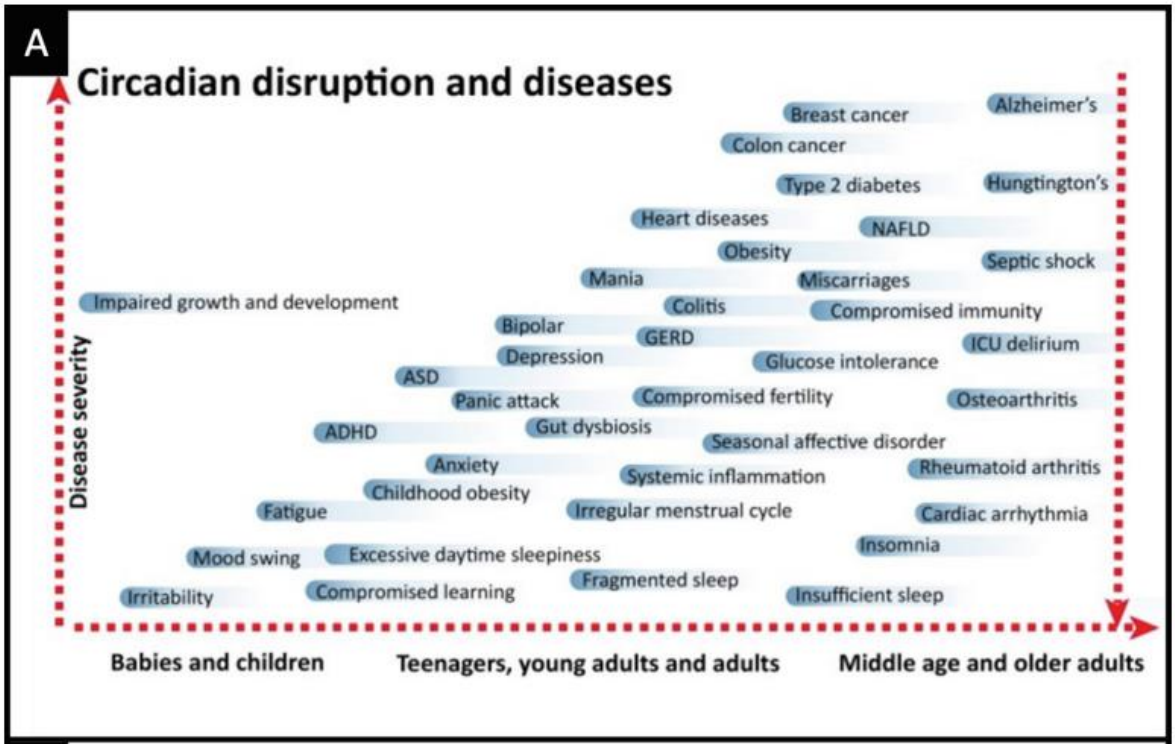


Figure 3: Circadian disruption and diseases. Figure taken to Sulli et al. (2018, https://doi.org/10.1016/j.tips.2018.07.003).

I.1.4 Strategies for colon targeting

Current strategies rely on *) prodrug-based formulations, **) pH-sensitive drug delivery, ***) time-dependent systems, and ****) microbiota sensitive systems.

I.1.4.1 pH-dependent systems

The pH-dependent approach exploits the innate pH variations all along the GIT. Indeed, the pH of the GIT shows inter- and intra-individual changes and is very affected by food intake. While

the stomach region has the lowest pH ranges (pH 0.4 - 4.0 at fasted state, and pH 2.0 - 4.5 at fed state) [25], pH starts to climb in the proximal regions of the duodenum (pH 5.0 - 7.0) [26]. From the jejunum (pH 6.6 ± 0.5) to the ileum, GI pH attains 7.5 ± 0.5. Within the colon, pH lowers to 6.4 ± 0.6 in the cecum before progressively increasing at the end of the GI tract to 7.0 ± 0.7 in the rectum [27].

The pH fluctuations of the GI tract can be used for targeted drug delivery. The latter can be obtained by coating forms with an enteric polymer able to disintegrate or dissolve, depending on the GI pH.

The pH-dependent approach for colon targeted drug delivery is clinically relevant. Numerous products are marketed. Some of them are indicated for treating IBD (*e.g.* Asacol®, Salofalk® and Lialda®, containing mesalamine, or Budenofalk®, containing budesonide). Despite the successfulness of classic pH-dependent polymers, their efficacy in colon-targeting has shown significant patient variability, due to changes in pH (feed/fast state condition, healthy *versus* disease conditions), fluctuations in gastric emptying *etc*. Several publications have noted that Eudragit S coated pills dissolved incorrectly in the intestinal environment [28]. Relying on the only dynamic pH dependence may not pledge enough targetability.

I.1.4.2 Time-dependent systems

Time-dependent methods seek to achieve colon-specific targeting by utilizing the lag time between dosage form administration and colonic arrival. Such an example is Pentasa®, a formulation based on ethyl cellulose-coated microgranules, which slowly dissolves all over the duodenum, the ileum, and the colon. Whole gut transit, on the other hand, is subjected to many variables, with movement through areas occurring at irregular intervals. Moreover, various factors influence intestinal motility and transit. Biological sex is one such example. Females have clearly delayed stomach emptying and longer intestinal and colonic transit durations than males [29, 30]. On the other hand, small intestinal transit time (represented by simulated intestinal fluid) is 20 % longer in IBD patients, with a median value of 4 h. It is important to know that, irrespective of healthy or IBD state, this transit time can exceed 6 h [31]. The time-based formulation strategy for colon-targeted drug delivery is based on the mean GI transit times. Despite the considerable variation and irregularity of residence time in the stomach, time-dependent systems should be entirely intact when in the stomach region. The reliance on predictable GI transit is the fundamental constraint of time-dependent colonic delivery techniques. As previously stated, GI transit is very varied across and among individuals, making reliable prediction challenging, particularly in illness situations. Given these considerations, time-dependent delivery alone is not a suitable strategy and may cause treatment failure.

I.1.4.3 Microbiota-sensitive systems

The gut contains the main part of the 100 trillion bacteria in the human microbiome, with the colon having the greatest concentration of 10^{12} bacteria *per* gram of feces, and over 500 bacterial species [32]. These microorganisms have enormous metabolic power that can be used to deliver drugs. Microbiota-dependent methods for targeted medication delivery to the colon have received a lot of attention [33]. Certain polymers are indigestible in the proximal intestine but preferentially metabolized by colonic bacteria, making them appealing coverings for colonic release dosage forms [34]. To date, two types of polymers have been investigated for use: azo-polymers and polysaccharides [35]. Polysaccharides can be fermented by colonic bacteria through enzymatic reactions to lactate and short-chain fatty acids (SCFAs) [36]. Despite the fact that individual microbiome compositions differ, some functions like polysaccharide digestion are similar in the majority of the population due to significant functional redundancy among microbiota, making polysaccharides largely reliable materials for colonic drug delivery [37]. Microbiota-sensitive systems are known to be the most promising and effective ones [38, 39]. Because of the carcinogenic risk of azo-polymers and the requirement of organic solvents in their manufacture, further usage in humans has been no longer used [40]. As a result, polysaccharide-based formulations are relevant enablers of microbiota-sensitive systems for colonic drug release. Pectin, starch, alginate, gums, amylose, chitosan, dextran, chondroitin sulfate, inulin, β-cyclodextrin, and galactomannan are examples of naturally occurring polysaccharides used for colon targeting [41]. To overcome the limitations of single pH or time-dependent approaches, a double stimulation can be proposed, combining both aforementioned strategies:

1. pH- and time-dependent combinations
2. Time- and microbiota-dependent combinations
3. pH- and microbiota-dependent combinations

By introducing a backup or supplemental trigger in targeted formulations, it is possible to improve the likelihood of drug release in the colon [14].

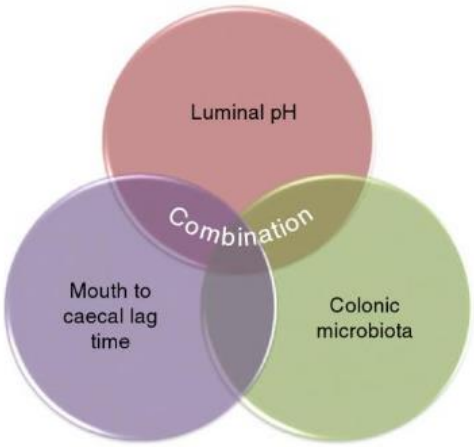


Figure taken from the book *Controlled Release in Oral Drug Delivery*, Clive G Wilson, Patrick J Crowley (Eds.). Springer US (2011). Serie: Advances in Delivery Science and Technology. ISBN: 978-1-4614-1003-4.

The following figure represents a timeline of different drug delivery systems within the history:

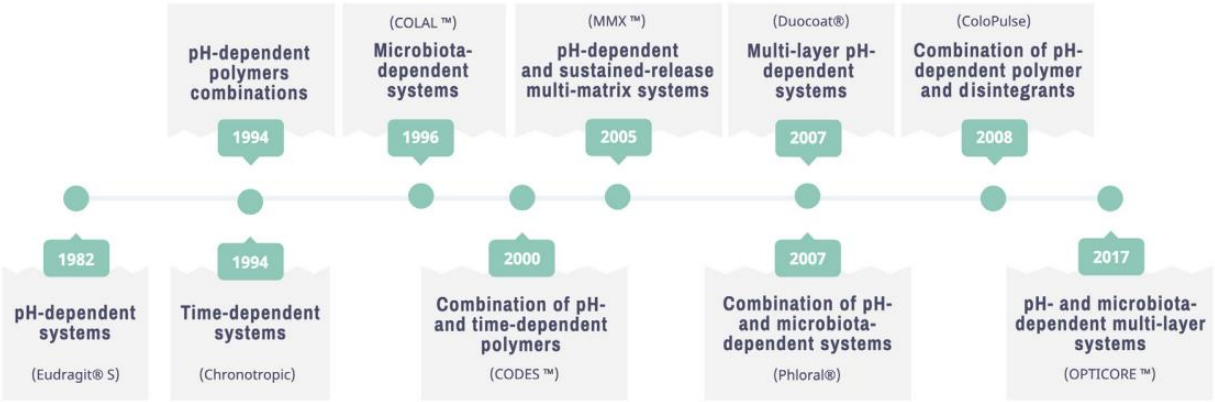


Figure 4: Timeline with different colonic drug delivery systems along years. Figure taken from Awad et al. (2022, [14]).

I.2 A few patents and technologies for colon targeting

The following section will describe some available technologies patented and exploited, with or without marketed forms of IBD treatments.

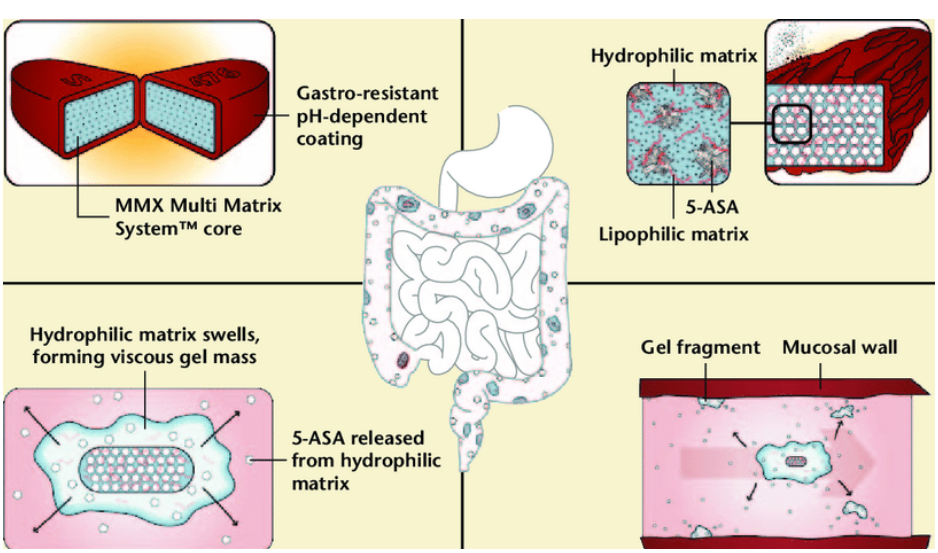
I.2.1 Microbiota-dependent systems: COLAL™ system

Referring to the fresco above, COLAL™ appeared in 1996 as a microbiota-sensitive technology. COLAL-PRED®, as a treatment for UC, is a proprietary gastrointestinal product developed by Alizyme Therapeutics Ltd. It arose from combining Alizyme's proprietary colonic drug delivery system, COLAL™, with an approved generic steroid (prednisolone sodium metasulfobenzoate). This system, based on amylose mixed with the water-insoluble polymer ethylcellulose, was successfully used in the colonic delivery of prednisolone for treating inflammatory bowel disease and reducing undesirable side effects associated with the use of systemic steroids [42]. Amylose serves as food source for colonic bacteria. Presented as tablet, the combination of colon-specific polysaccharide and water insoluble polymer achieved consistent colonic targeting with a wide variety of drug molecules [43]. It was noticed that COLAL-PRED® formulation displayed the following benefits: equivalent efficacy to prednisolone; elimination of systemic steroidal side effects; treatment of the entire colon; once-daily oral administration. 4 weeks of intensive therapy and three weeks of maintenance dosing protocol showed a relief of patients' symptoms. 33 patients with mild-to-moderate ulcerative colitis attended a Phase II clinical trial using COLAL-PRED®; a clinical response was observed in 63 % of patients in the 60 mg dose group and 35 % of patients in the 40 mg dose group. Importantly, orally administered prednisolone did not show any steroid-related adverse effects or changes in cortisol levels [44]. Likewise, phase III clinical trials (2007, [45]) in UC patients indicated that this formulation achieved superior safety records, even though it did not show better clinical efficacy. As a result of these clinical trials, it was demonstrated that neither pathophysiological and histological changes in the colonic mucosa nor alterations in mucosa-associated microflora in UC patients had effect on the *in vivo* performance of COLAL™ technology [44]. Despite its advantages, this system wasn't commercialized.

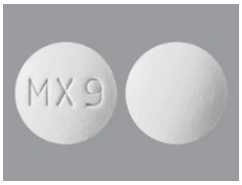
I.2.2 pH and time-dependent combinations

I.2.2.1 Multi Matrix System (MMX®)

The Multi Matrix System (or MMX®) combines a pH-triggered mechanism with a time-controlled release method [46]. The medicine, such as mesalazine (Lialda® in France, Mezavant® in the USA) or budesonide (Cortiment® in France, Uceris® in the USA), is placed into small lipophilic matrices within a larger hydrophilic matrix. This "dual" matrix system is encased in an enteric film coating (*e.g.*, based on Eudragit L and S). The "dual matrix" is predicted to slow medication release down after the enteric coating dissolves at neutral pH.
The lipophilic matrix permits the release of the API at a controlled rate throughout the colon [47].



a)



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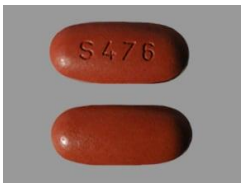


Figure 5: Figure taken from Baker et al. (2006).
a) Uceris® tablets. Source: Uceris tablet illustration
b) Lialda® tablets. Source: Lialda tablet illustration

Lialda was FDA-approved for the induction of remission in UC in January of 2007, based on results from two phase III clinical trials (2.4 g/day and 4.8 g/day *per os* Lialda). These data demonstrated that Lialda was effective for the induction of remission in mild-to-moderate UC patients compared to placebo after eight weeks of treatment [48]. It was then approved for maintenance of remission in 2011 [49].

Mezavant® and Cortiment® are french marketed products relying on this technology. MMX® technology allows for a reduction of the daily intake of tablets from patients in non-acute phase to about two tablets a day. In acute phases of UC, three to four tablets a day are taken. This reduction in daily tablets uptake, as compared with typical mesalazine oral administration, results in an increase in medication adherence [48].

Multi Matrix System ®

Mesalamine: Lialda®, USA (Approval on January 16, 2007) [1]
Mezavant®, France (Approval on March 26, 2007) [2]

Budenoside: Uceris, USA (Approval on March 2013) [3]
Cortiment®, France (Approval on June 23, 2016) [4]

[1] Development timeline for Lialda [50]
[2] Mézavant HAS [51]
[3] Budenoside MMX FDA approval [52]
[4] Cortiment HAS [53]

Over the previous several years, MMX® system has been extensively exploited in the realms of inflammatory and infectious disorders localized in the colon. Namely, MMX® mesalamine, budesonide and parnaparin formulations have been evaluated in patients with ulcerative colitis, and as mentioned previously, the first two have attained global registration for treating this disease. On the other hand, positive trials are being conducted on MMX®-rifamycin for the treatment of bacterial colonic infections, such as traveler's diarrhea. MMX® technology is proving to be a very reliable formula to treat different colonic illnesses. Apart from specific colonic delivery, this efficacy has also been reported as for its capacity to function at a single daily dosage, supporting patients' compliance. The effective delivery of the active drug to the site of interest in the colon goes hand in hand with low rates of systemic absorption and reduced risks of adverse events (AEs) [54]. The following figure represents the pharmaceutical projects involving MMX® technology, as well as the medicines yet on the market.

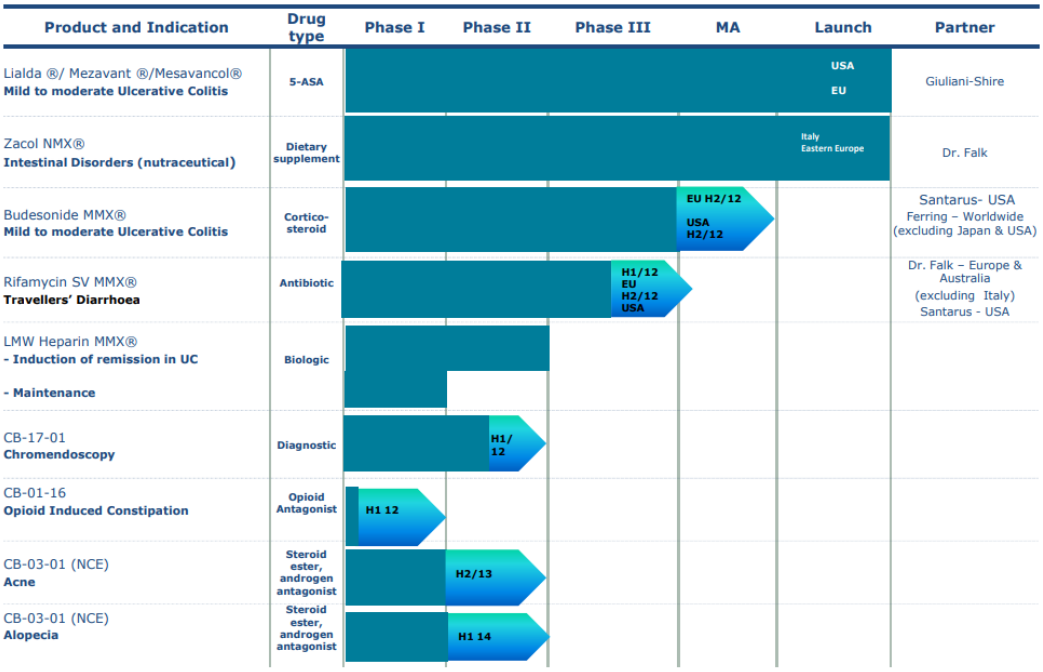


Figure 6: Pipeline projects for different medicines and marketed drugs using MMX® technology. Figure taken from Cosmo Pharma (consulted in August 2022).

I.2.2.2 Ethyl Cellulose Matrix (ECX®)

Another example is the Ethylcellulose Matrix (or ECX™) found in the multi-particulate dose product Entocort™ EC. To treat CD and UC, the system is meant to deliver the corticosteroid budesonide to the ileo-colonic area. This system is a hard gelatin capsule carrying 3 mg of the medication in pellets form. The pellets have an inert saccharose core coated with an insoluble **E**thyl**C**ellulose matri**X** inner layer and a Eudragit L100-55 outer layer [55].

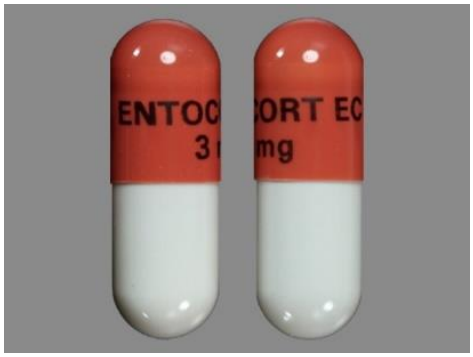


Figure 7: Entocort capsules.
Source: Entocort capsules illustration

I.2.3 pH- and microbiota-sensitive combinations

I.2.3.1 CODES™

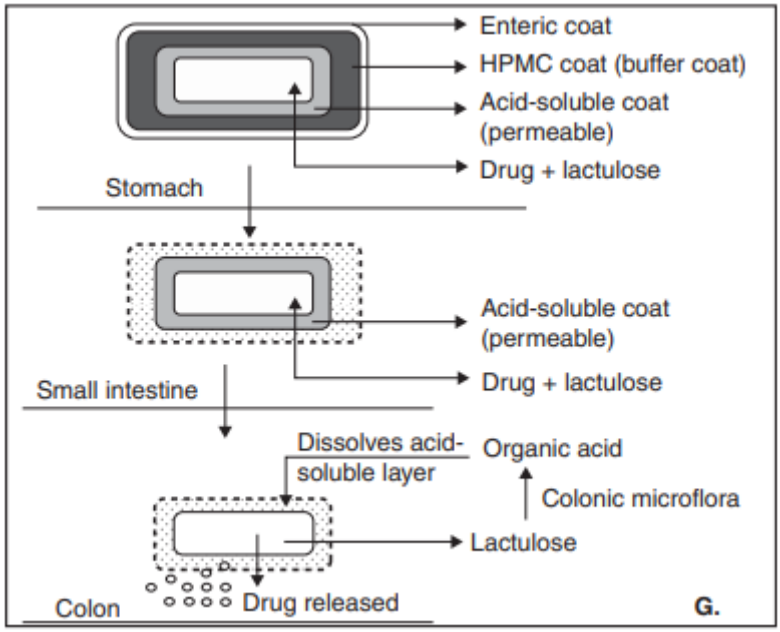


Figure 8: Schematic diagram of CODES™. Figure taken to Patel et al. (2011, https://doi.org/10.1517/17425247.2011.597739).

CODES™ is a multilayer formulation consisting in a lactulose core surrounded by Eudragit® E (immediate release polymer, soluble up to pH 5.0), the latter coated with Eudragit® L (dissolve at pH > 6, enteric polymer). After ingestion, the first Eudragit® L coating protects the tablet from the unfavorable acidic conditions of the stomach, then dissolves when entering the small intestine. The acid-soluble polymeric coat prevents the release in the alkaline medium of the

small intestine. Upon arrival in the colon, the colonic microflora enters the core tablet through the channels present on the acid coat, ferments the lactulose, and generates an acid environment sufficient to dissolve Eudragit E coating. The fermentation produces SCFAs that induce the dissolution of the acid-sensitive Eudragit® E layer, exposing the tablet core and, thus, driving drug release [56, 57]. The CODES™ is basically formulated as a disintegrating and erodible dosage form. The study of the influence of food in both fed and fasted states could be realized and led to delayed gastric emptying time and ileocecal junction arrival times of the formulation. Yet, this did not adversely affect the disintegration profile of the tablet with the contents of the core still being delivered to the colon. The colon specificity of drug release has been confirmed in healthy human volunteers using γ-scintigraphy imaging. This system would be widely applicable for the colonic delivery of various kinds of drugs including proteins and peptides [57].

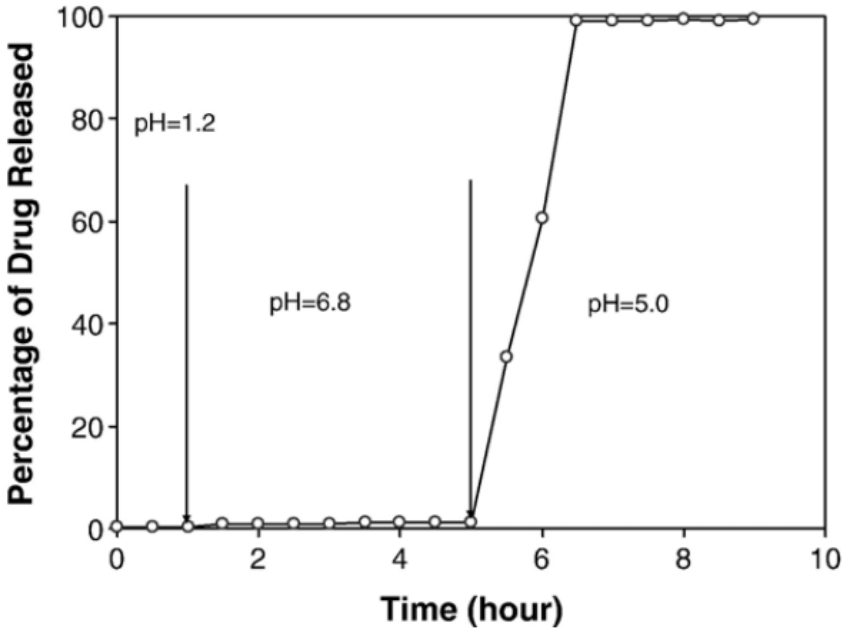
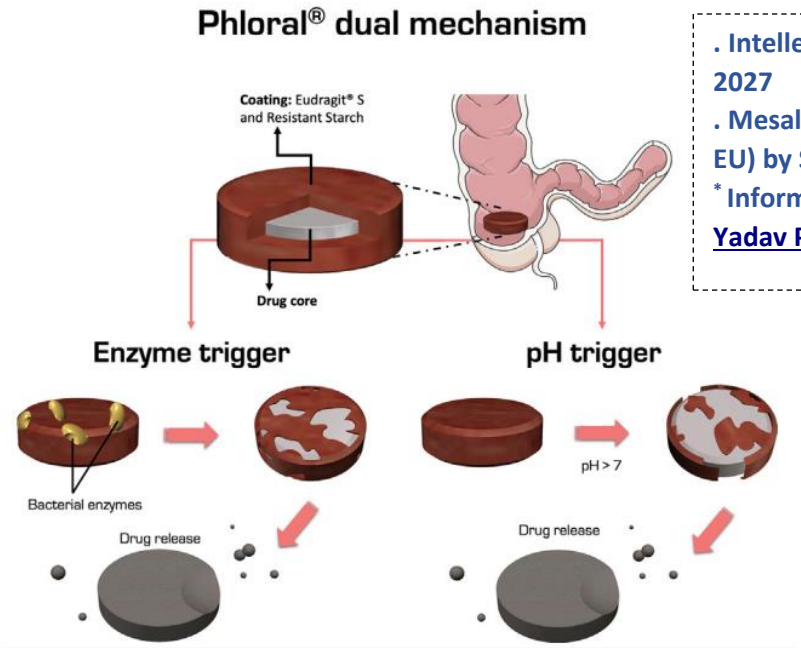


Figure 9: *In vitro* drug release profile of CODES™ tablets in different buffers. Figure taken from Yang (2008, [58]).

I.2.3.2 Phloral® coating



. Intellectual property (IP) protection till 2027
. Mesalazine product on the market (in EU) by Swiss licensee Tillotts Pharma *
* Informations from Intract Pharma: Vipul Yadav Presentation

Figure 10: Composition and mode of action of the Phloral® coating. Figure reused from Awad et al. (2022, [14]).

First successfully marketed combination technology, Phloral® is a combined film coating made up of bacteria-activated (resistant starch) and pH-activated (Eudragit® S) components, patented and available only from Intract Pharma. The starch is not digested by mammalian amylase enzymes secreted by the pancreas, but by colonic bacterial enzymes, which makes the technology useful in colonic drug targeting. Phloral® is the world's only dual-triggered coating technology, capable of overcoming the constraints of conventional polymer coatings by demonstrating precise, fail-safe release in the colon in both healthy and sick states. The independent but complementary triggers of a bacterially sensitive component within a pH-responsive polymer are effective and operate as failsafe mechanisms for one another in drug delivery. Phloral® technology was created at University College London. It has demonstrated enough potential to be utilized in the colon for either local therapy or systemic treatment (*e.g.* IBD) due to its high site-specificity in all feeding situations (fasted, pre-fed, and fed states) [59]. Apart from IBD, these tablets were successful in fecal microbiota transplantation therapy in patients with recurrent *Clostridium difficile* infection [60].

I.2.3.3 Opticore™

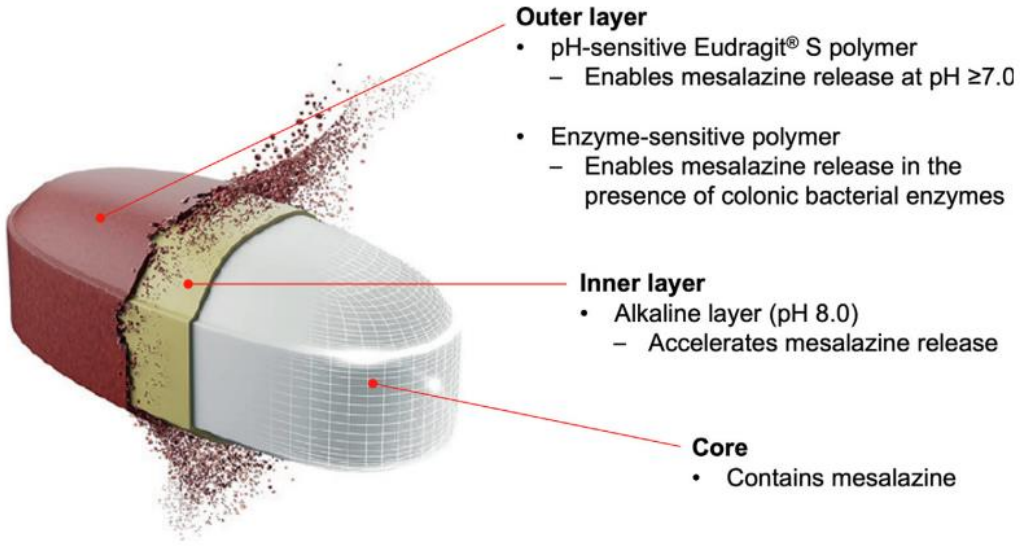
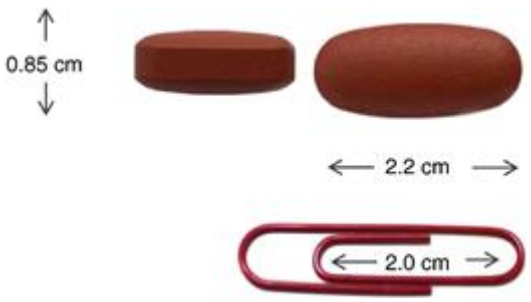


Figure 11: Illustration of Asacol™ 1600 mg marketed drug product and its OPTICORE™ coating system. Figure reused from Awad et al. (2022, [14]).

OPTICORE™, a dual-responsive device used to successfully administer 5-ASA to the colon for the treatment of IBD [61], is a more recently marketed technology. The word OPTICORE™ refers to **OPTI**mised **CO**lonic **RE**lease, which explains the aim of this formulation. To improve colonic drug distribution, OPTICORE™ combines an inner alkaline coat (containing a neutral enteric polymer such as Eudragit S and a buffering salt) with an external Phloral® coating. The pH, buffer capacity, buffer salt concentration, ionic strength, and viscosity of the colonic fluid all influence medication release upon the OPTICORE™ system. The coating promotes a quick drug release within the ileal and colonic region, where fluid is more abundant than in the colonic distal regions. As the outer coat dissolves or undergoes fermentation by bacteria, fluid penetrates the formulation while developing pores in the coating, causing the inner coat to dissolve.



At the inner surface of the Phloral® coating layer, this creates an environment with higher pH, buffer capacity, and ionic strength. As a result, the Eudragit S in the Phloral® coat ionizes and dissolves quickly, accelerating drug release. The inner layer underneath the outer layer contributes to a quicker release of drug from OPTICORE™ coated tablets in buffer mimicking the luminal milieu of the terminal segment of ileum (Krebs buffer pH 7.4), as compared to enteric coatings for colon targeting found in state-of-the-art (Eudragit® S, (Evonik) dissolution above pH 7) [62].

Asacol™ 1600 mg, a 5-ASA medication based on OPTICORE™ technology, recently completed Phase III clinical trials. It is now available in Europe [63] in multiple markets, as following brands: Asacol™ 1600, Yaldigo™ 1600, Asacolon™ 1600, and Octasa™ 1600. The drug product developed based on OPTICORE™ technology are owned by Tillotts Pharma.

More than 300 people work at Tillotts Pharma, a global specialized pharmaceutical company, in Switzerland and other countries. Through its affiliates in Europe and a global network of partners with a gastrointestinal specialty, it promotes its own medications—such as Asacol™ and Entocort™—as well as in-licensed drugs in about 65 countries. Since 2009, Tillotts has been associated with the Japanese Zeria Group (more informations on Tillotts Pharma).

I.2.3.4 Soteria®

Thanks to a ground-breaking new technology called Soteria®, patients can now consume bio-pharmaceuticals, including monoclonal antibodies, as an easy-to-take, secure tablet or capsule. Soteria® acts, first, by protecting the drug from the harsh environments of the stomach and small intestine, and by releasing the compound in the colon. The dual-action enhancer in the core protects the drug from enzymatic degradation whilst simultaneously enhancing its uptake into the colon tissue. From here, the drug can engage local targets, or enter the systemic circulation to enact its therapeutic effects. Soteria® allows for infliximab to be administered orally, creating a more targeted IBD treatment [64]. The oral infliximab product was admitted by the UK regulatory body (Medicine and Healthcare products Regulatory Agency) to progress to Phase 1b/2a clinical trials in IBD patients, during the second half of 2021, without any demand for further preclinical research or clinical safety study. Figure 12 illustrates the enhanced infliximab stability in the human colon.

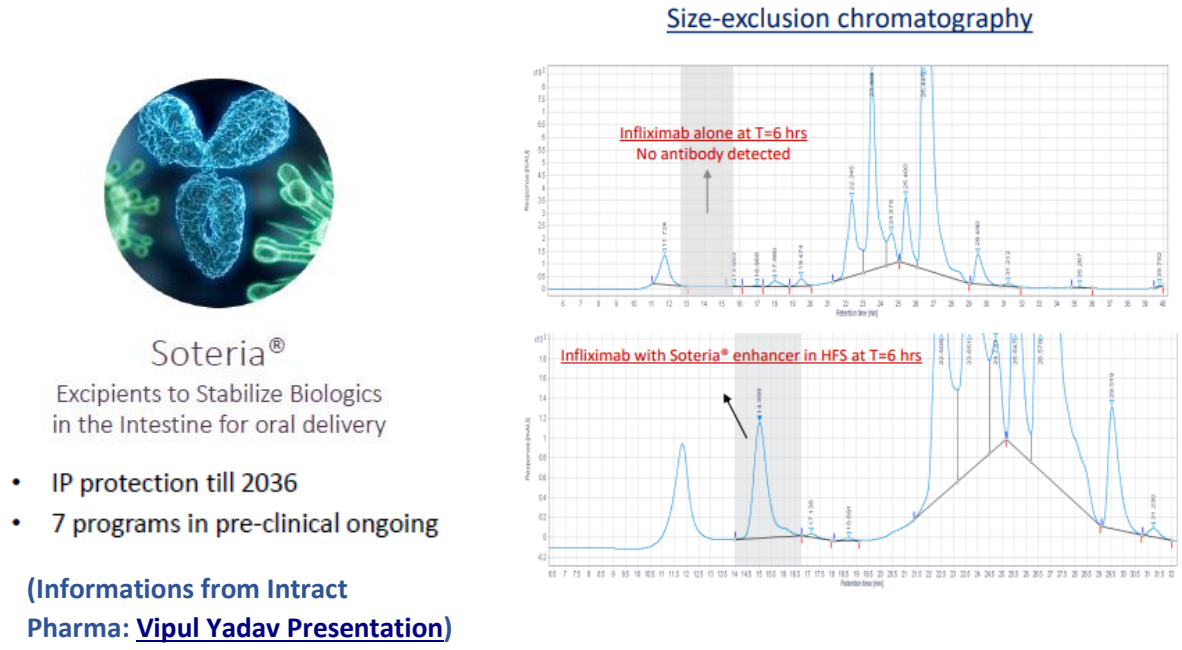


Figure 12: Soteria® characteristics (left) and improved stability of infliximab in human colon thanks to Soteria® technology (right). Figures reused from Yadav et al. (2019, [47]).

In a more synthetic way, figure A_1 (in appendix) ([65]) recaps some of the aforementioned systems and their characteristics.

The following table (table 2) represents the marketed products for colonic disorders.

Table I. Currently Marketed Formulations

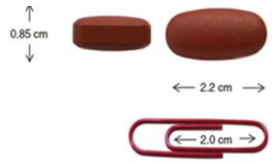
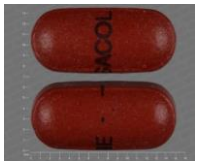
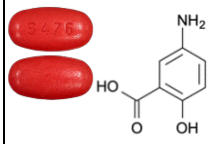
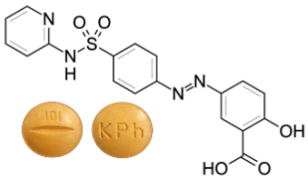
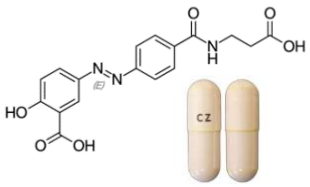
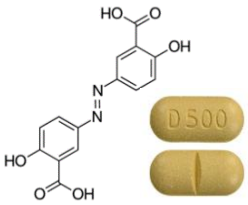
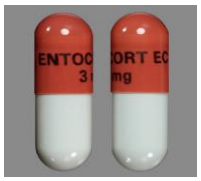
Colon disease/disorder	Drugs	Delivery system
Inflammatory bowel disease	Mesalazine	
Ulcerative colitis	- Asacol®	DR tablets
Crohn's disease	- Pentasa®	TR capsules
	Sulfasalazine	
	- Azulfidine EN-tabs®	DR tablets
	Prednisone	
	- Rayos®	DR tablets
	Budesonide	
	-MMX®	Multi-matrix tablets
	- Uceris®	ER tablets
	- Clipper®	Gastro-resistant prolonged-release tablets
	Prednisolone (Colal-Pred®)	Oral colon-targeted pellets
	Metronidazole (Flagyl® ER)	ER tablets
	Azathioprine (Azasan®)	IR tablets
	Mercaptopurine (Purinethol®)	IR tablets
	Cyclosporine (Gengraf®)	IR capsules, oral solution
Diverticulosis and diverticulitis	Methylcellulose (Citrucel®)	Oral powder, IR tablet
	Psyllium (Metamucil®)	Oral powder, IR capsule
	Mesalazine (Asacol®)	DR tablets
	Rifaximin (Xifaxan®)	IR tablets
Colonic amoebiasis	Doxycycline (Doryx®)	DR tablets
	Metronidazole (Flagyl® ER)	ER tablets
Irritable bowel syndrome	Methylcellulose (Citrucel®)	Oral powder, IR tablets
	Psyllium (Metamucil®)	Oral powder, IR capsules
	Loperamide (Imodium®)	IR capsules
	Dicyclomine (Bentyl®)	IR capsules, IR tablets
	Hyoscyamine (Levbid®)	ER tablets
	Lubiprostone (Amitiza®)	Soft gelatin IR capsule
	Linaclotide (Linzess®)	IR capsules
	Rifaximin (Xifaxan®)	IR tablets
	Amitriptyline (Elavil®)	IR tablets

IR immediate release, *ER* extended release, *DR* delayed release, *TR* timed release

Table 2: Marketed products for colon disorders. Figure taken to Amidon et al. (2015, [194]).

Some of these marketed products are represented in the following table, namely those as first-line treatments, *i.e.* non-steroidal and steroidal anti-inflammatory drugs.

*

5-ASA based products	Approvals
 Asacol™ 1600 mg  Pentasa® capsule 250 mg  Asacol® 400 mg  Apriso® capsule 375 mg	**Mesalamine / Mesalazine** (*mm/dd/yyyy*) **Asacol®**: 01/31/1992 (tablets 400 mg) [66] **Pentasa®**: 05/10/1993 (capsules 500 mg, 250 mg) **Pentasa®**: 06/18/1990 (tablets) 500 mg) [67]
5-ASA prodrugs	
 5-ASA  Sulfasalazine  Balsalazide  Olsalazine	**Sulfasalazine**: 06/20/1950 [68] **Balsalazide**: 07/18/2000 [69] **Olsalazine**: 07/31/1990 [70] **Sulfasalazine**: 03/31/1992 [71] **Balsalazide**: Non marketed in France [71] **Olsalazine**: 09/26/1990 (250 mg capsules) [72] 07/25/1995 (500 mg tablets)
Corticosteroids	
 Entocort® 3 mg capsule  Uceris® 9mg – USA	**Entocort EC®**: 10/02/2001 (capsules 3 mg) [73] **Budénoside MMX®**: Uceris®, March 2013 [74] **Rayos®** : 07/26/2012 [75] **Entocort®**: 07/31/1996 (capsules 3 mg) [76] **Budenoside MMX®**: Cortiment®, 06/23/2016 [77]
*** Table created by Samuel STRICH in September 2023.**	

I.3 Oral biotherapies

As mentioned in chapter I.1.1, the oral route is the most employed route for drug administration, and it can be used either for systemic or local (gastrointestinal diseases) drug delivery [78]. Formulations can be made to improve drug delivery specifically in the upper or lower GIT. With a total surface area of about 200 m^2, the intestinal epithelium offers a vast target.

The last two decades have seen a sudden rise in the approval and prescription of peptide- and protein-based marketed products. Indeed, adalimumab and pembrolizumab, being both monoclonal antibodies, accounted for the top two medications sold worldwide in 2020. This underlines the proportion of this therapeutic class [79]. The global market worth of biopharmaceuticals was estimated at $192.5 billion in 2020, with expected growth to $326.3 billion by 2026 [80]. In a same way, biologics, encompassing nucleotides, proteins, and peptides (PPs), accounted for about 30 % of all drugs approved by the U.S. Food and Drug Administration (FDA) between 2015 and 2018. In addition, more than 90 % of the recently approved biologics were monoclonal antibodies (mAbs) based drugs [81].

This increasing interest about peptide-based therapeutics originates from the enhanced selectivity of molecules, but also their potency and efficacy, compared to small molecule drugs. The overall good safety profiles with peptides also contribute to this interest [82].

The main advantage of biological agents is their ability to maintain remission and to achieve mucosal healing without using corticosteroids [83]. Nonetheless, almost the totality of peptide therapeutics are formulated for parenteral administration, to avoid their degradation in the upper GI tract [78], with approximately 75 % of them given as injectable [82]. Injections often require a health professional, weighting even more on healthcare systems and the quantity of visits patients must attend.

However, after parenteral administration, the delivery to the receptor may still need to overcome significant hurdles, related to their short plasma half-life and poor serum stability. Rapid metabolism or clearance from the circulation can require injections several times daily, which results in low-adherence rates and poor quality-of-life for patients [84].

For a variety of small molecules, published oral dose formulations with controlled release have been developed for the colon and the lower portion of the small intestine to treat disorders related to the region; however, this has not yet been accomplished for macromolecules [85].

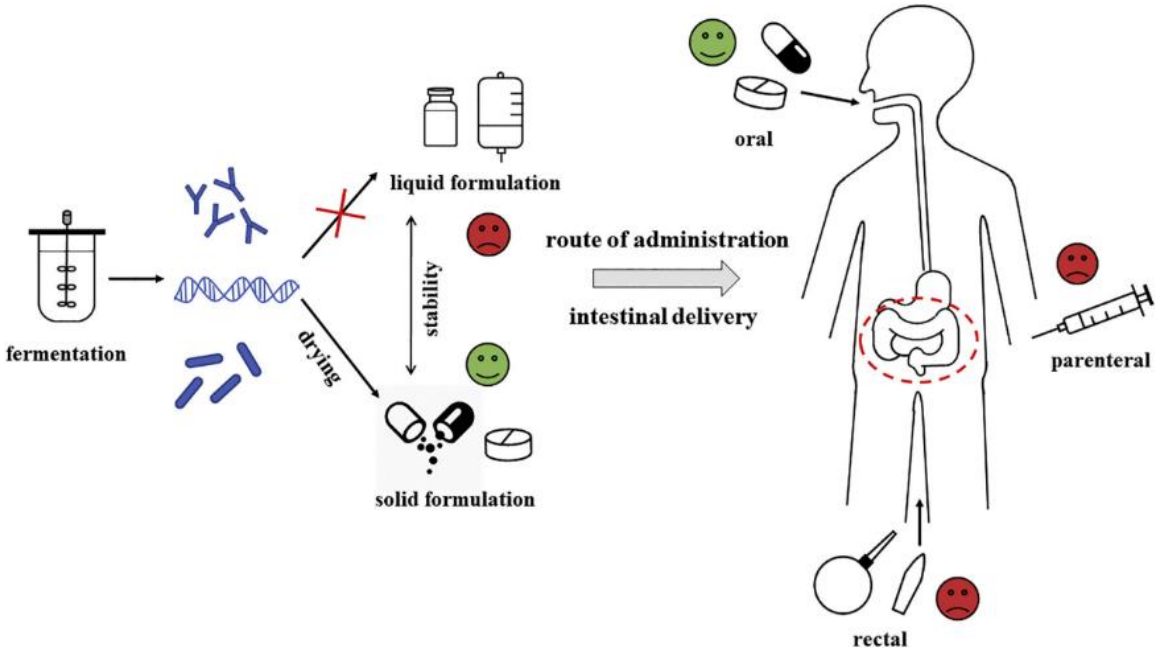
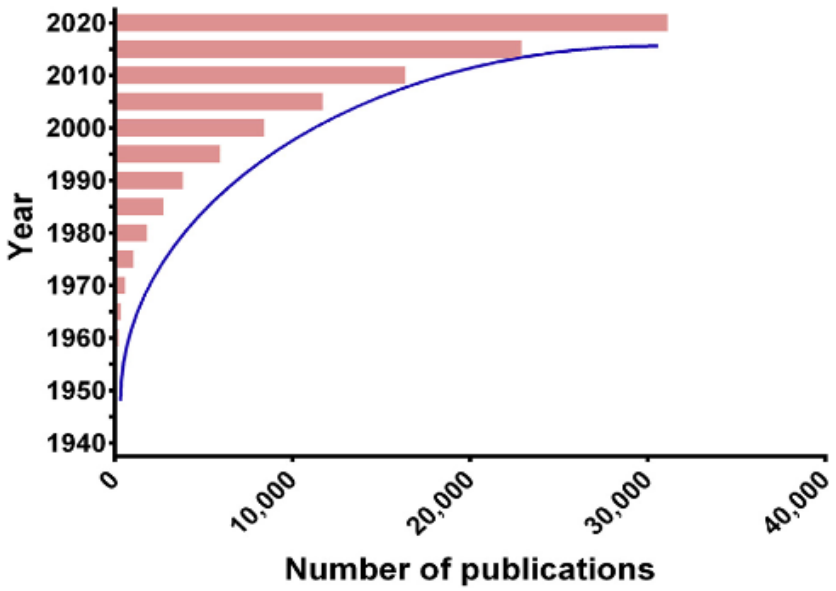


Figure 13: Formulation and delivery routes of biopharmaceuticals for intestinal targeting. Figure taken from Vass et al. (2019, [11]).

Whilst there are a few examples of colonic formulated mAbs reaching clinical trials stage, such as oral infliximab for IBD treatment, colonic peptide delivery persists as a relatively untapped opportunity [86].



Number of publications along decades about orally administered PPs. Research was conducted in Web of Science to Nov. 9th, 2020, formulating « oral AND (absorption OR delivery) AND (protein* OR peptide*) ». Figure taken to Zhu et al. (2021, [87]).

Academic efforts are focused on developing some novel technologies to improve the oral absorption of PPs [88].

To improve oral administration of peptides, current strategies rely on enzyme inhibitors, permeation enhancers (PEs), nanotechnology, multi-particulate systems, targeted particulates, nanotechnology colonic delivery approaches, or else peptide modification [89–91]. The following figure shows the milestones in the development of oral delivery of protein- and peptide-based delivery systems.

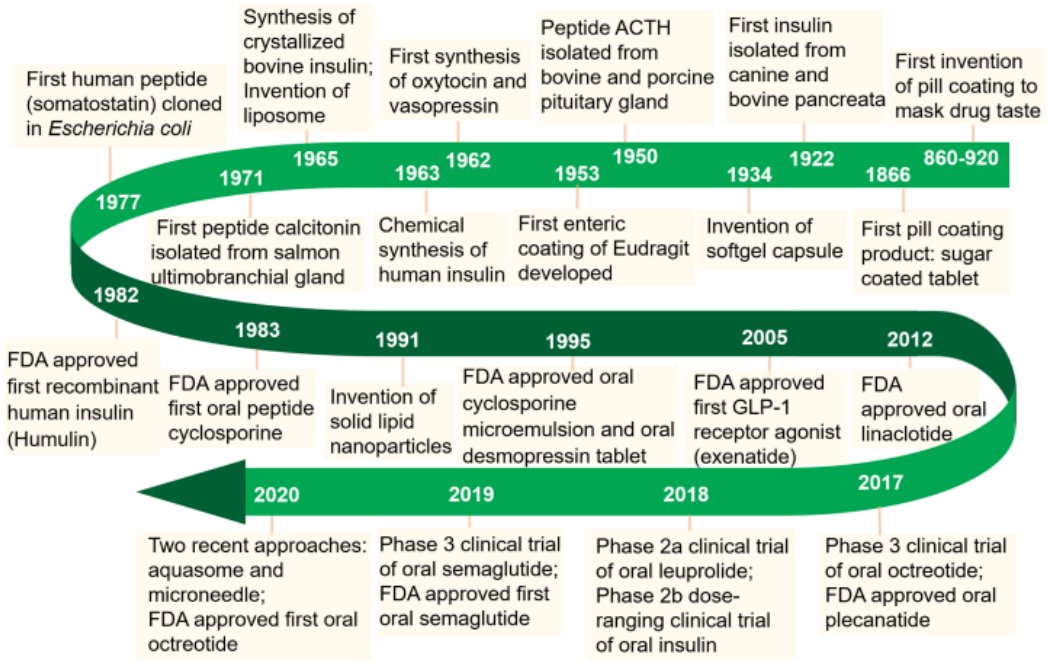


Figure 14: Landmarks in the development of oral proteins and peptides delivery systems. Figure taken from Chen et al. (2022, [92]).

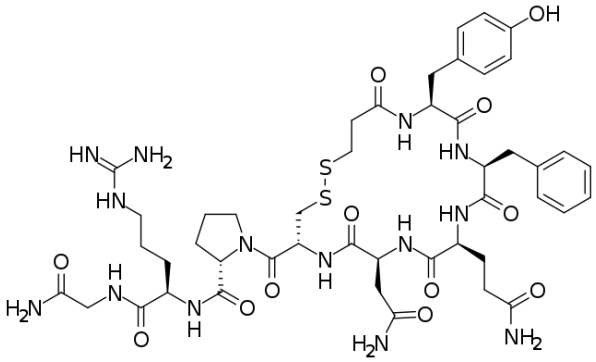
In 1990, Sandimmune was approved by FDA as first oral solid dosage form of cyclosporin A, which is a cyclic peptide (Novartis AG, Switzerland). After 5 years, the improved formulation of cyclosporin A, Neoral®, was developed by Novartis and approved [87, 93].

Figure 15: Sandimmune capsules images. Left : 25 mg, right: 100 mg.
FDA approval: March 2, 1990. Applicant: Novartis.
Images from drugs.com - Sandimmune.

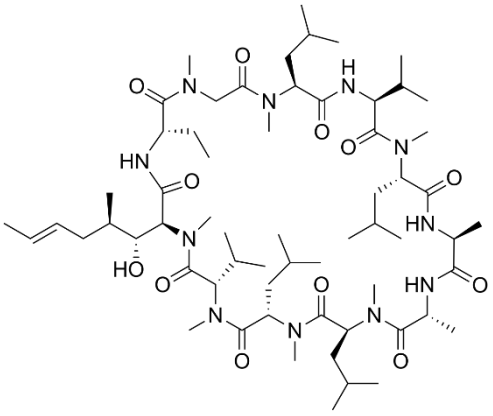
Considering the vast obstacles before achieving systemic peptide absorption after oral administration, the ideal candidates should have high potency and wide margin of safety. In spite of this, insulin is still one of the main contender for oral delivery [94]. Apart from oral delivery of PPs, there has been increasing interests in targeting PPs drugs to the colon thanks to evidence for quite low proteolysis levels in this area compared to the small intestine [95–97]. Some studies suggest that colonic proteolysis would be 20–60 times lower than that of the ileum [95]**.** In fact, the large intestinal milieu has shown lower proteolytic activity than the small intestine of brushtail possums and rats [98–100]. Degradation of LHRH [101, 102], glatiramer acetate [103], and desmopressin [104], was lower in colonic fluids and mucosa compared to those of small intestine. These results indicate that the large intestine suits better for oral peptide and protein drug delivery than the small intestine. Less enzymatic barrier to peptides, such as degradation proteases, are present in colonic enterocyte membranes in comparison to those of the small intestine [105]. Thus, peptides susceptible to proteolysis degradation in the small intestine may potentially be delivered for local administration or systemic absorption from the colon. The thickness of mucus, pH, surface area, and dissolving capability, are further regional differences that could impact the colonic ability to absorb peptides [106]. Also, there is proof that the apex of colonic membrane is more sensitive to PEs than the small intestine [107, 108].

I.3.1 Marketed oral PPs

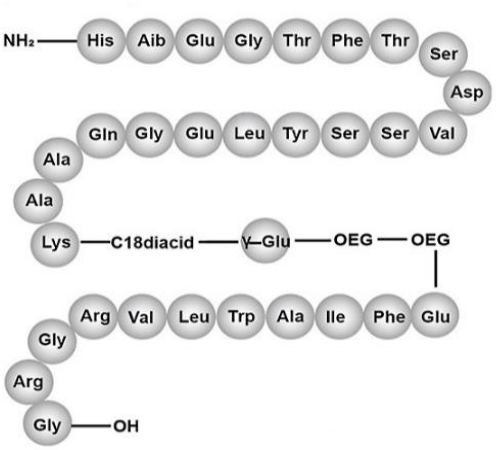
In accordance with figure 14, a reference from October 2022 [109] reported only 5 FDA-approved oral peptide products to date: Cyclosporin (1990), Desmopressin (1995), Semaglutide (2019), Octreotide (2020), Voclosporin (2021).



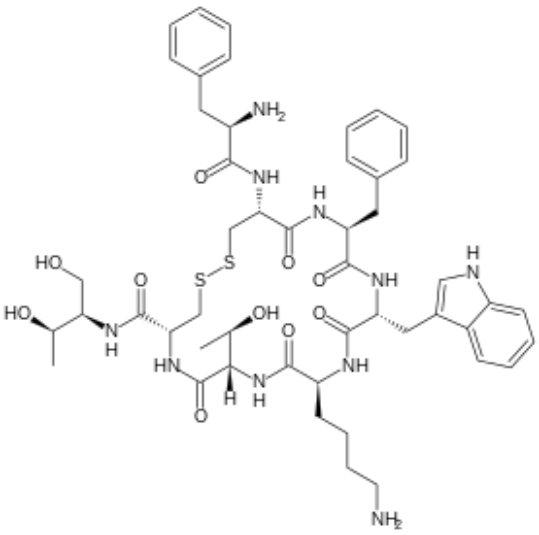
Desmopressin
9 amino acids; MW: 1,069



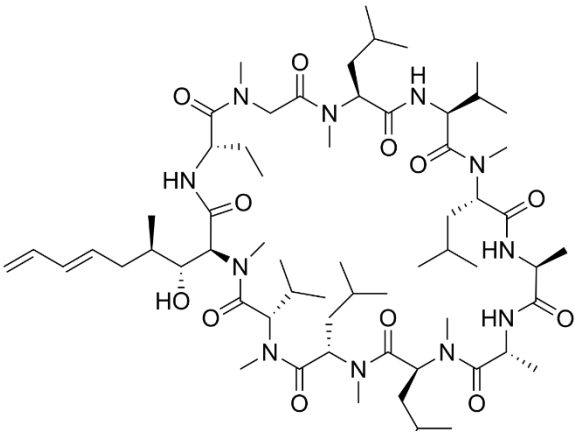
Cyclosporin
11 amino acids; cyclic peptide;
MW: 1,202



Semaglutide
31 amino acids; MW: 4,113



Octreotide
8 amino acids; MW: 1,019



Voclosporin
11 amino acids; MW: 1,214

Table 3 shows the commercialized oral administered peptides on the global markets (all laboratories confounded).

Table 5 Oral products of PPs on markets.

PPs	Trade name	Technology	Indication	Company
Cyclosporin A	Neoral®/Sandimmune®	SNEDDS	Immunosuppression; systemic delivery	Novatis AG (Switzerland)
Desmopressin acetate	DDAVP®	Chemical modification	Central diabetes insipidus; systemic delivery	Ferring Pharmaceuticals (Switzerland)
Octreotide	Mycapssa®	Enteric coating; permeation enhancer	Long-term maintenance treatment in acromegaly patients; systemic delivery	Chiasma (USA)
Semaglutide	Rybelsus®	Permeation enhancer	Type 2 diabetes mellitus; systemic delivery	Novo Nordisk (Denmark)
Taltirelin hydrate	Ceredist®/Ceredist OD®	Chemical modification	Spinocerebellar degeneration; systemic delivery	Mitsubishi Tanabe Pharma Co. (Japan)
Linaclotide	Linzess®	Acts locally	Irritable bowel syndrome, chronic idiopathic constipation; local delivery	Actavis, Inc. (USA)
Vancomycin	Vancocin®	Acts locally	Infection	ANI Pharmaceuticals, Inc (USA)
Colistin sulfate	Koolistin®	Acts locally	Infection	Biocon Ltd. (India)
Tyrothricin	Lozenges®	Acts locally on the throat	Pharyngitis	The Boots Company PLC (UK)
Pancrelipase	Creon®	Delayed release; acts locally	Exocrine pancreatic insufficiency	AbbVie Inc. (USA)
Tilactase	Lacteeze®	Chewable tablets; acts locally	Lactose intolerance	Lacteeze (USA)
Sacrosidase	Sucraid®	Oral solutions; acts locally	Congenital sucrase-isomaltase deficiency	QOL Medical, LLC (USA)
Diamine oxidase	DAOSiN®	Acts locally	Histamine intolerance	SCiOTEC (Austria)

Table 3: Oral products of PPs on markets. Table obtained from a reference of 2021 ([87]).

Among these oral proteins, 3 are intended for intestinal delivery [110]:

- Linaclotide (Linzess®, Ironwood, MA, USA), approved in 2012 for the treatment of irritable bowel syndrome associated with constipation.

- Vancomycin. This one was first introduced to the market as injectable by Eli Lilly (IN, USA) in the 1950's, as a result of a rise in staphylococcal resistance to penicillin [111]. Vancocin® HCl is not well absorbed *per os* owing to its large molecular weight and hydrophilicity. This antibiotic is only indicated by that route for pseudomembranous colitis [112], and few attempts have been made to develop alternative oral vancomycin formulations. [113].

- Colistin. Intended in last resort for the treatment of multidrug-resistant bacterial infections [114].

Please note that oral enzymes intended to restaure gastrointestinal metabolic deficiencies such as Pancrelipase (Créon®), Tilactase (Lacteeze®), Sacrosidase (Sucraid®), Diamine oxidase (DAOSiN®) are mostly considered as dietary supplements [87]. As for the three aforementioned colonic acting peptides, the latter do not have bioavailability. This might explain why they are not mentioned among the few FDA-approved peptides.

Linaclotide (Linzess® in USA, Constella® in Europe)

Vancomycin (Vancocin®)

I.3.2 Macromolecules in the pipelines of preclinical and clinical development

Table 4 stands for the clinical development status of oral macromolecules intended for colonic delivery.

AVX-470, developed by Avaxia Biologics Inc. (http://avaxiabiologics.com) is a milk-derived antibody with natural capacity to survive in the GIT and passed Phase I for UC treatment in 2013.

The company identified the structural basis for milk-derived antibodies to withstand the GIT and transferred the key attributes to a recombinant platform. Their first designed candidate was Avaximab-TNF, an oral anti-TNF antibody for the treatment of IBD. Avaximab-TNF carried out the optimized features of stability against intestinal digestion, minimal systemic exposure, penetration into inflamed gut mucosa and potential ability for local stimulation of effector functions [110]. Across all AVX-470 doses, patients exhibited a clinical response in 25.9 % of cases *versus* 11.1 % with placebo. The best outcomes were observed in the group consuming 3.5 grams daily, related to proximal colon endoscopic improvement. In a span of 28 days, none of the 37 patients experienced any significant adverse effects [115].

Clinical development status of selected orally-delivered macromolecules for pathologies expressed (at least in part) in the colon.

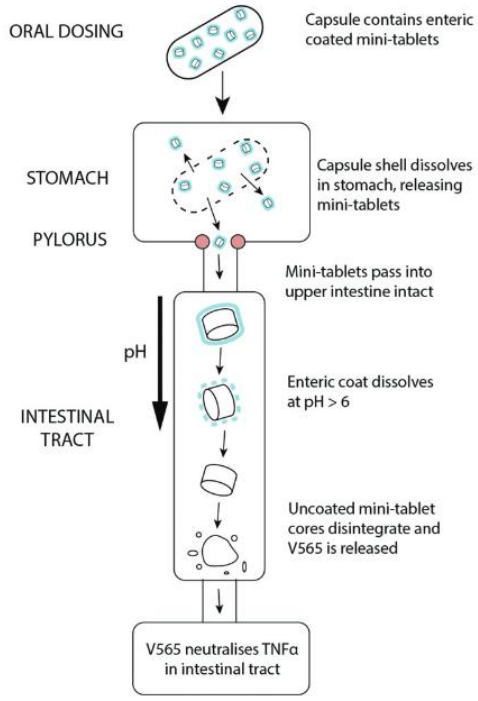
Name (MW, Da)	Company	Formulation	Indication (stage completed)
Linaclotide, Linzess® (1525)	Allergan/Ironwood	Cyclic guanylate cyclase peptide agonist tablet	IBS-C, (market)
Plecanatide, Trulance® (1682)	Synergy	Cyclic guanylate cyclase peptide agonist peptide tablet	IBS-C, CIC (market)
Doclanatide (1682)	Synergy	Cyclic amino acid substituted guanylate cyclase peptide agonist tablet	OIC (Phase II); UC (Phase I)
Vancomycin, Vancocin® (1449)	Generic (e.g. ViroPharma)	Cyclic modified macrolide peptide; capsule or tablet	CDAD; *Staphylococcal* enterocolitis including MRSA (market)
Fidaxomycin, Dificid® (1058)	Merck	Macrocycle macrolide, tablet	CDAD (market)
Surtomycin (1681 Da)	Merck	Cyclic lipopeptide, over-encapsulated tablet	CDAD (Phase III, discontinued in 2017)
LFF-571 (1367 Da)	Novartis	Semi-synthetic thiopeptide, capsule	CDAD (Phase II)
AVX-470, Avaximab™-TNF, (160–900 kDa)	Circle33 LLC (Avaxia Biologics)	Polyclonal bovine antibody, enteric-coated capsule	UC (Phase 1B, terminated 2017)
V565 (12–15 kDa)	VHsquared	Protease-resistant anti-TNF-α domain antibody capsule	CD (Phase II Harbour Study, initiated in 2017)
Mongersen, GED-0301 (6952 Da)	Celgene	21-mer antisense oligonucleotide capsule targeting *Smad7*, mRNA	CD (Phase II, terminated 2017)
Alicaforsen (6368 Da)	Atlantic Healthcare	20-mer antisense oligonucleotide capsule targeting ICAM-1 and TLR-9 mRNA	Pouchitis (Phase II (enema), entering Phase 1 (tablet)

Sources (as of May 2016): PharmaCircle™ business reports, ClinicalTrials.gov; Press Releases. Abbreviations: MW = molecular weight; IBS-C: irritable bowel syndrome-with constipation; CIC: chronic idiopathic constipation; OIC: opioid-induced constipation; UC: ulcerative colitis; CD: Crohn's disease; CDAD: *Clostridium difficile*-associated diarrhoea; TLR-9: *toll*-like receptor-9.

Table 4: Clinical development stage of orally administered macromolecules intended to treat pathologies affecting (at least partly) the colon. Table taken from Bak et al. (2018, [85]).

An alternative technology has been presented by VHsquared (www.vhsquared.com), under the name of Vorabodies. A Vorabody is an oral domain antibody engineered to enhance intestinal protease resistance. Their leading compound is V565, an oral anti-TNFα Vorabody, capable of inhibiting inflammation in UC patients [116]. The final oral dosage form comprised a capsule containing enteric-coated 3 mm minitablets loaded with V565. In the stomach, the capsule dissolves and releases the minitablets, which travel along the intestine and release the Vorabody at pH > 6. The authors reported very low systemic exposure of the drug in healthy monkeys. The US10633438B2 patent (property of Crowe *et al.*) was published on April 28th 2020 [117].

The local and systemic pharmacokinetics in four UC patients were examined in a phase I clinical research. Hydroxypropyl methylcellulose (HPMC) capsules were used to encase the Eudragit-coated V565. Overall, this investigation demonstrated that oral-delivered active V565 can bind to V565 TNF-α producing cells in UC lesions, and can reach significant quantities at inflamed regions [118]. In another phase I clinical study, 47 CD patients participated in a different phase I clinical research to examine safety and tolerability of oral V565. This candidate engaged Phase II clinical trials in 2016 (NCT02976129) [119].



Dissolution of V565 mini-tablets in the GIT. Upon oral administration, the capsule coating dissolves in the stomach and releases enteric-coated mini-tablets containing V565. These forms withstand low pH and cross the gastric pylorus. When the pH increases within the intestine, the enteric coating dissolves and releases active V565 due to the disintegrating cores. In IBD patients, the mAb will get into the inflamed *lamina propria*, where intestinal epithelium is harmed and will neutralize TNFα.

Figure obtained from Crowe et al. (2019, https://doi.org/10.1080/03639045.2018.1542708).

As for Mongersen (table 4, before last line), this oligonucleotide was developed in a modified-release tablet conceived to release the API firstly into the lumen of the terminal ileum and right colon. This is achieved *via* the pH-dependent coating of the tablet, composed of methacrylic acid–ethyl acrylate copolymers. Unfortunately, a phase III study showed no benefit over placebo, though phase II study displayed effective clinical remission in patients with active Crohn's disease [120].

However, only four formulations of oral biologics (AVX-570, V565, AG011, and Mongersen) transited into clinical study, and none of them was approved [115].

Figure 16 displays the PPs drugs in the project pipelines of Intract Pharma (*cf.* Phloral®) for the treatment of IBD and intestinal inflammation.

Candidate	Partner	Feasibility	Preclinical	IND Enabling	Phase 1
Epithelial Integrin β-1 Peptide	NIBEC	Ulcerative Colitis			
Fusion Protein (Undisclosed target)	elasmogen	Ulcerative Colitis			
Monoclonal Antibody (Undisclosed Target)	Big Pharma	IBD			
Monoclonal Antibody (Undisclosed Target)	百奥泰 BIO-THERA	Ulcerative Colitis			
Monoclonal Antibody (Undisclosed Target)	FERRING PHARMACEUTICALS	GI Inflammation			
Nanobody (Undisclosed Target)	Big Pharma	Undisclosed			

Figure 16: Projects in progress with PPs drugs for the treatment of IBD and intestinal disorders on Intract Pharma's initiative [121].

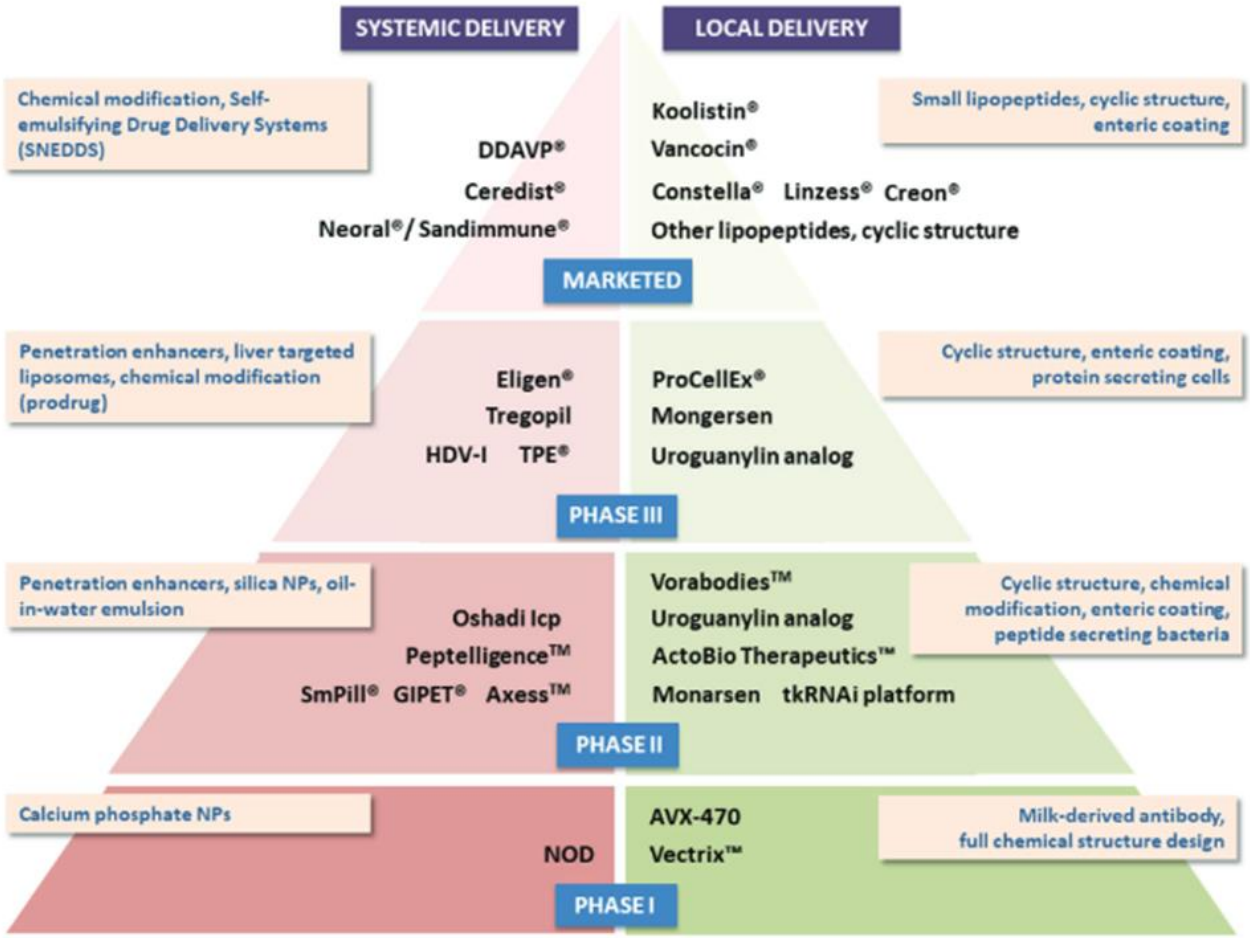


Figure 17: Oral drug delivery technologies for biologics marketed or in clinical trials. Figure reused from Durán-Lobato et al. (2020, https://doi.org/10.1002/adma.201901935).

I.3.3 More recently: Protagonist Therapeutics Inc. and Janssen

In April 2022, an article from Protagonist therapeutics Inc. (Newark, California, USA) ([122]) dealt with IDEAL clinical trial, a randomized, double-blinded, placebo-controlled, multicenter Phase 2 study. The aim was to assess both safety and efficacy of PN-943, a gut-restricted, alpha-4-beta-7-integrin antagonist taken *via* oral route. In this trial, a randomization was carried out for 159 patients subjected to moderate-to-severe active UC. They were proposed either twice daily (also called "*bis in die"*) 450 mg or 150 mg PN-943, or placebo, for 12 weeks. Subsequent analysis permitted outcome measures.

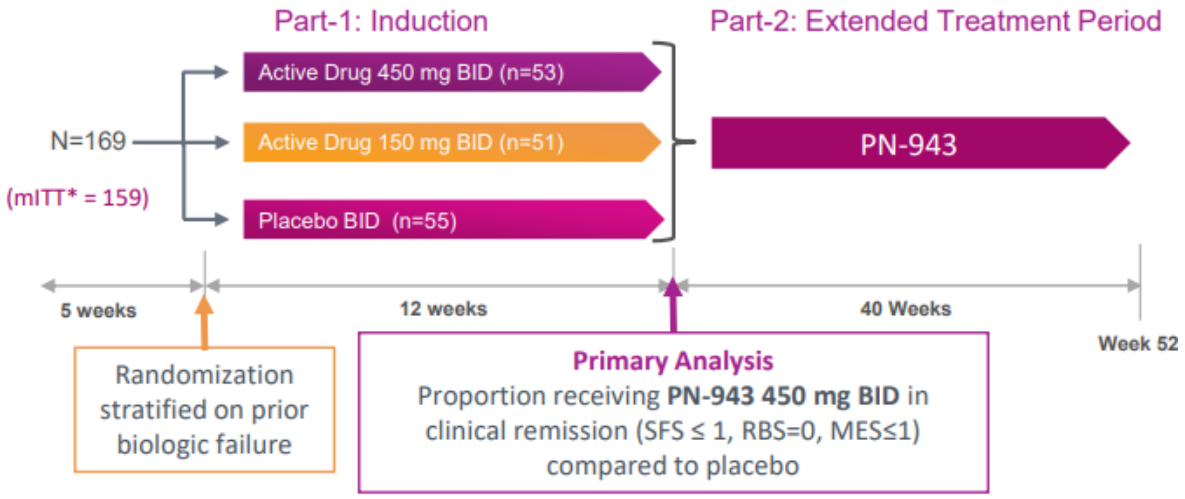


Figure taken to the IDEAL Study Group publication, presented at the Digestive Disease Week 2022 - May 21-24 | San Diego, CA.
File available on the following link: https://www.protagonist-inc.com/publications/a-phase-2-randomized-double-blind-placebocontrolled-multi-center-study-to-evaluate-the-safety-and-efficacy-of-the-oral-gut-restricted-47-integrin-peptide-antagonist-pn-943 from https://www.protagonist-inc.com website.

PN-943 was used in a gut-restricted approach clinically validated with first generation compound (PTG-100). PN-943 showed ~3x more potency in pre-clinical studies & Phase 1 normal healthy volunteer study versus the 1st generation candidate PTG-100 [123]. PTG-100 demonstrated indications of therapeutic effectiveness in a Phase 2a UC study [124]. Clinical remission in 27.5 % of PN-943 150 mg *bis in die* (abbreviated as "BID") group *versus* 14.5 % in placebo was observed (13 % Δ, nominal p = 0.08) for modified intention-to-treat analysis (mITT).

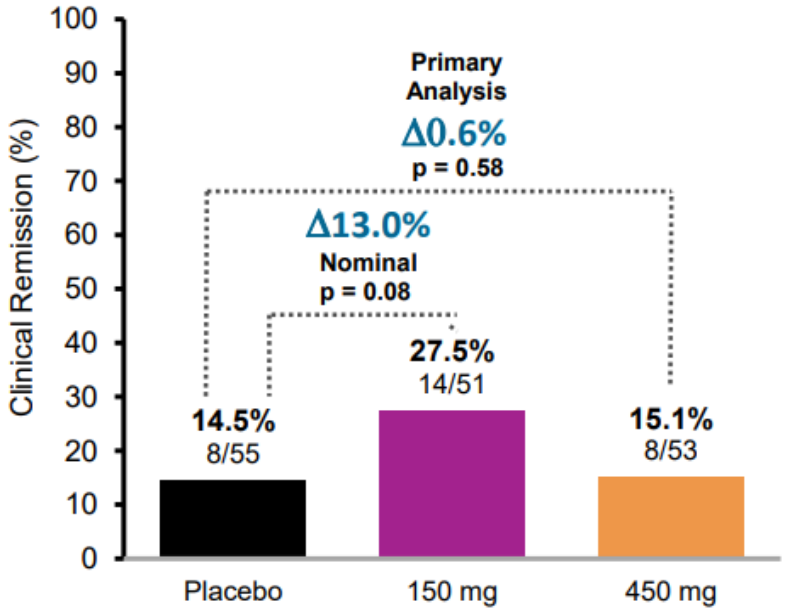


Figure 18: Clinical Remission at Week 12 – mITT. Figure taken to the IDEAL Study Group publication presented at the Digestive Disease Week 2022 - May 21-24 | San Diego, CA. Complete name of the publication: A Phase 2 randomized, double-blind, placebocontrolled, multi-center study to evaluate the safety and efficacy of the oral, gut-restricted α4β7 integrin peptide antagonist PN-943. Available on https://www.protagonist-inc.com/ website.

There was a consistency with efficacy across multiple key secondary endpoints, including improvement or histologic remission, and endoscopic remission at 150 mg BID dose. A 40-week extended treatment period (Part 2) was ongoing.

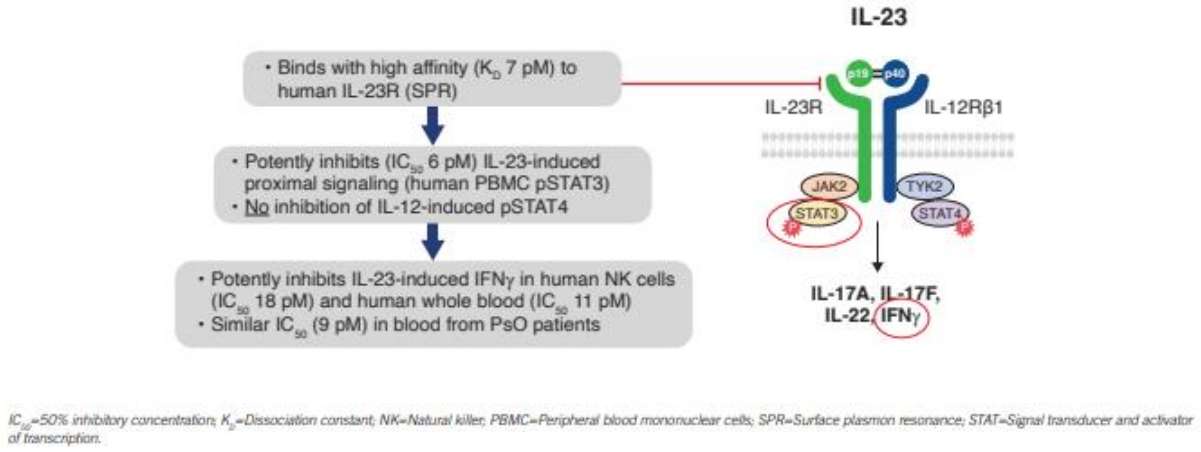
The IDEAL study supported further development of PN-943 in UC registrational trials.

April 2022 signed the conclusion of the IDEAL Phase 2 study for PN-943 in moderate-to-severe UC. The PN-943 150 milligram dose was advanced as a result of the Phase 2 findings. Hence, 150-milligram dose of PN-943 was further registered into a phase 3 study. The registrational study for the Phase 3 was being planned in April 2022 [122].

On the other hand, Protagonist has allowed Janssen to exclusively research, develop and market IL-23 receptor antagonists *per os* by exploiting the Company's intellectual property. Current development efforts concentrate on PN-235, found out by Protagonist, and developed with Janssen's collaboration. In early 2022 started FRONTIER 1, a Phase 2b randomized multicenter placebo-controlled trial with dose-ranging design to assess the safety and effectiveness of PN-235 to treat moderate-to-severe plaque psoriasis. The Company was targeting ulcerative colitis as the initial indication.

Figure 19: Product pipelines from Protagonist and Janssen in the realm of inflammatory & immunomodulatory diseases. Scheme accessible on Protagonist product pipeline.

Human genetic relationships and anti-IL-23 mAbs effectiveness highlight the significance of the IL-23 pathway in psoriasis (PsO), psoriatic arthritis (PsA), CD, and UC. Currently, no oral medicine can selectively target this pathway. To give patients more options for treatment, they created an oral therapeutic peptide, JNJ-77242113, blocking IL-23 by specifically targeting IL-23R.

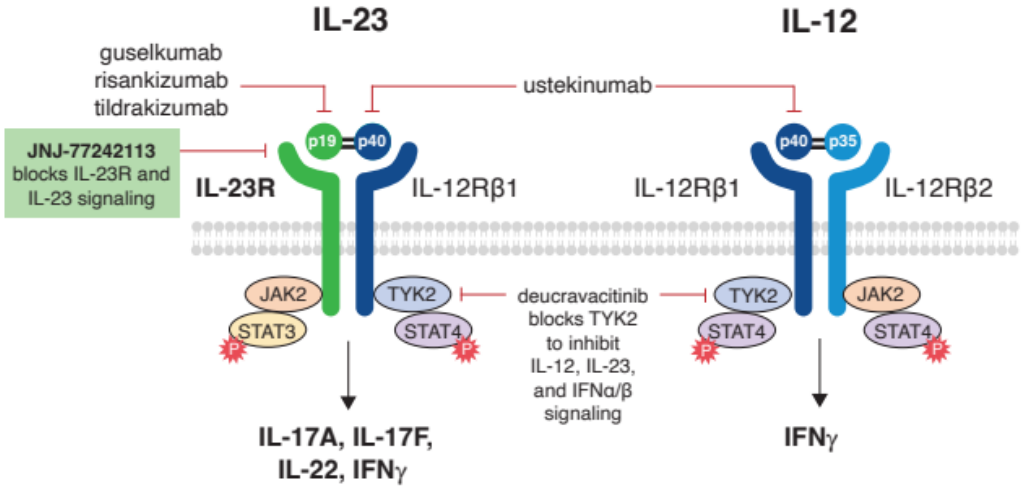


IC_{50}=50% inhibitory concentration; K_D=Dissociation constant; NK=Natural killer; PBMC=Peripheral blood mononuclear cells; SPR=Surface plasmon resonance; STAT=Signal transducer and activator of transcription.

Fourie et al., ISID Meeting; May 10-13, 2023; Tokyo, Japan [125].

This pathway is involved in PsO, PsA, CD and UC.

Competitive peptide antagonist JNJ-77242113 selectively and very potently blocks IL-23 proximal signaling as well as downstream cytokine production. It binds to IL-23R with great affinity. In a Phase 1 human investigation, preclinical results were effectively translated and systemic reduction of *ex vivo* IL-23-induced IFNγ production in blood was noted. Phase 2 dose ranging trial in individuals with PsO getting from moderate-to-severe form was completed in February 2023. All these data can be further read on International Society for Infectious Diseases Meeting, May 10-13, 2023; Tokyo, Japan [125].



Fourie et al., ISID Meeting; May 10-13, 2023; Tokyo, Japan [125].

The following table, obtained from David Brayden's work (2022, [109]), tries to provide an update on the true clinical development status for the delivery of macromolecules to regions of the GI tracts. Several sources among which ClinicalTrials.gov, AdisInsight, PubMed, and company websites, were perused.

Clinical development of macromolecules in oral dosage forms for local delivery in the GI tract.

Class	*Name (Trade name)*	*GI target*	*Indication*	*Structure*	*Key clinical trials and dosing details*
Peptides	Linaclotide (LINZESS®, Ironwood Pharma) MW: 1527 Da.	GC-C agonist	IBS-C; CC	CCEYCCNPACTGCY; Synthetic STa, guanylin, and uroguanylin hybrid.	Approved 2012; generic linaclotide approved in 2021 (Mylan Pharma). NCT00765882 completed Phase III trial for CIC. Dose: 145-290 µg QD.
	Peclanatide (TRULANCE®; Salix Pharma). MW: 1682 Da.	GC-C agonist	IBS-C; CIC	NDECELCVNVACTGCL; analogue of uroguanylin.	Approved 2017. NCT01982240 Phase III trial completed for CIC. Dose: 3-6 mg QD.
	Dolcanatide, SP-333, (Synergy Pharma/Bausch Health) MW: 1682 Da.	Stable GC-C agonist	UC, colorectal cancer	L-Asn^{1} and L-Leu^{16} in peclanatide are replaced by D-analogues.	NCT03300570 Phase I trial for colorectal cancer. Dose: 27mg QD
	PTG-100 (Protagonist Therapeutics)	T cell $\alpha_4\beta_7$ integrin antagonist	UC, CD	See USP 9,809,623	NCT02895100 Phase IIa PROPEL trial for UC (terminated). Dose: 150-900mg QD.
	PN-943 (Protagonist)	More potent $\alpha_4\beta_7$ integrin antagonist than PTG-100	UC, CD	See USP 9,809,623	NCT04504383 Phase II IDEAL trial in UC patients reported top line data in 2022. Dose: 150 and 450mg BID.

	PN-235 (JNJ77242113) (Protagonist and Janssen)	IL-23 receptor antagonist presented in enteric tablets	UC, CD, psoriasis	Not available	NCT05357755 Phase II SUMMIT trial for psoriasis (recruiting). Dose: Delayed release tablet QD.
	PL-8177 (Palatin Technologies)	Colonic MC1R agonist	UC, uveitis	Not available	NCT05466890 Phase IIa trial in UC patients (not yet recruiting). Dose: QD
Fusion proteins	AMT-101 (Applied Molecular Therapeutics)	*Cholix* derivative fused to human IL-10 presented in enteric capsules and targeted at IL-10 receptors in colonic lamina propria.	UC, pouchitis	Refs 27-29.	NCT04583358 Phase IIa trial (LOMBARD) in UC patients missed primary endpoints in 2022.Dose: QD.
	OPRX-106 (Protalix Biotherapeutics)	Dimeric soluble fusion protein of the human TNFR and Fc fragment of IgG protected by plant cellulose coatings.	UC	Not available	NCT02768974 Phase II trial in UC patients completed in 2017, with no further updates. Dose: 2mg or 8mg QD.

Antibodies	AVX-570 (Avaxia Biologics, Circle 33)	GI-stable polyclonal anti-TNF chimeric human antibody.	UC	Not available	NCT01759056 Phase I trial in UC patients (last update 2014). Dose: 0.2 g, 1.6 g, or 3.5 g QD.
	V565 ((VHSquared). MW: 12,600 da.	Variable heavy chain antibody against TNF presented in enteric capsules.	UC, CD	115 amino acids	NCT02976129 Phase II trial in CD patients completed in 2019 but missed primary endpoints. Dose: TID.
	OKT-3 (muromonab; Ortho Biotech). 146,190 Da.	Anti-human IgG2a mAb against CD3 on T cells	UC	https://go.drugbank.com/drugs/DB00075	NCT01287195 Phase II trial in UC patients. Latest update in 2019. See ref. 48. Dose: 1-2mg QD.
Enzymes	ALLN-346 (Allena Pharma)	Stable engineered urate oxidase presented in capsules.	Hyperuricemia, gout, and chronic kidney disease (CKD).	Not available.	NCT04987242 Phase II trial in patients with hyperuricaemia and CKD. Dose: 9-15 capsules QD.
Antisense	Mongersen (GED-0301) Bristol-Myers-Squibb)	Over-expressed SMAD-7 mRNA in terminal ileal and colonic epithelia; presented in a	CD	21-base single strand phosphorothioate with the sequence: 5′-GTC GCC CCT TCT CCC CGC AGC-3′.	NCT02596893 Phase III trial in CD patients. Terminated in 2017. Dose: 160 mg QD for 12weeks followed by alternate day or QD dosing of 40mg or 160mg.

		pH-dependent coated tablet.			
	Alicaforsen (ISIS 2302; Ionis, Atlantic Pharma). MW: 6368 Da.	ICAM-1 mRNA over-expressed in inflamed colonic epithelia. Presented as a rectal gel.	UC, pouchitis	20-base targeted 2'-H ASO with a sequence of: GCCCAAGCTGGCATCCGTCA.	NCT02525523 Phase II trial of a daily enema in pouchitis patients. Reported in 2020. Dose: 240mg enema QD.
	AZD8233 (ION-863633; AstraZeneca)	PCSK9 mRNA over-expressed. Asialoprotein receptors in the liver are targeted. Presented in a tablet with sodium caprate.	Atherosclerotic cardiovascular disease.	16-nucleotide oligomer with a conjugated GalNAc ligand (Ref 64).	NCT04641299 Phase II trial in patients with dislipidemia tested only by the sub-cutanous route of delivery. Study completed in 2022. Dose: low, medium, high doses at Days 1, 8, 29, 57.
Miscellaneous	AG011 (ActoGeniX)	Genetically modified *Lactococcus lactis* expression of human IL-10 in enteric capsules	UC, CD	Not available.	NCT00729872 Phase II trial in UC patients completed in 2009. Dose: Capsules at three dose levels BID, combined with Enema at three dose levels, QD.

MW: molecular weight; UC: ulcerative colitis; CD: Crohn's disease; IBS-C: irritable bowel syndrome-constipated; CC: chronic constipation; CIC: chronic idiopathic constipation; STa: E. coli (Escherichia coli) heat stable enterotoxin; GC-C: guanylate cyclase-C; MC1R: melanocortin 42 receptor-1 selective agonist; TNFR: tumour necrosis factor receptor; ICAM-1: intercellular adhesion molecule-1; GalNAc: Nacetylgalactosamine; PCSK9: proprotein convertase subtilisin/kexin type 9 (PCSK9); NCT sources: www.clinicaltrials.gov. QD: Daily dosing; BID: Twice daily dosing; TID: thrice daily dosing.

Table recapping the clinical development of macromolecules in oral dosage forms for local delivery in the GI tract. Extracted from Brayden et al. (2022, [109]).

Colon targeting *via* the oral route has displayed relevant properties such as increased patients' compliance, reduced adverse events, and improved drug concentration in inflamed regions. The oral delivery systems used now for biologics in IBD therapy are implemented for two fundamental requirements: safe transition of biologics throughout the GIT and reduced systemic exposure while maintaining therapeutic efficacy [115].

The next section will aim to present the main biomaterial used for this thesis, with good enteric properties for colon targeting.

I.4 Presentation of shellac

The word “lac”, originated from the Sanskrit word “Laksha”, meaning a hundred thousand, refers to many insects that cover twigs of host trees and are involved in its production [126, 127]. Lac is the only insect originated natural resin obtained from lac insects, mainly Kerria spp. (Family-Tachardiidae, order-Homoptera).

Shellac, a refined lac product which received GRAS (Generally Recognized As Safe) status by the U.S. FDA [128], is a pH-dependent polymer. Shellac is a resin containing long chain polyesters with inter- and intra-esters bounds of polyhydroxycarboxylic acids, among which some are aliphatic long-chain hydroxy acids, and other are sesquiterpene acids [129]. It has a molecular weight of about 1006 g/mole, dissolves at pH 7.0, and is completely soluble at pH 7.4.

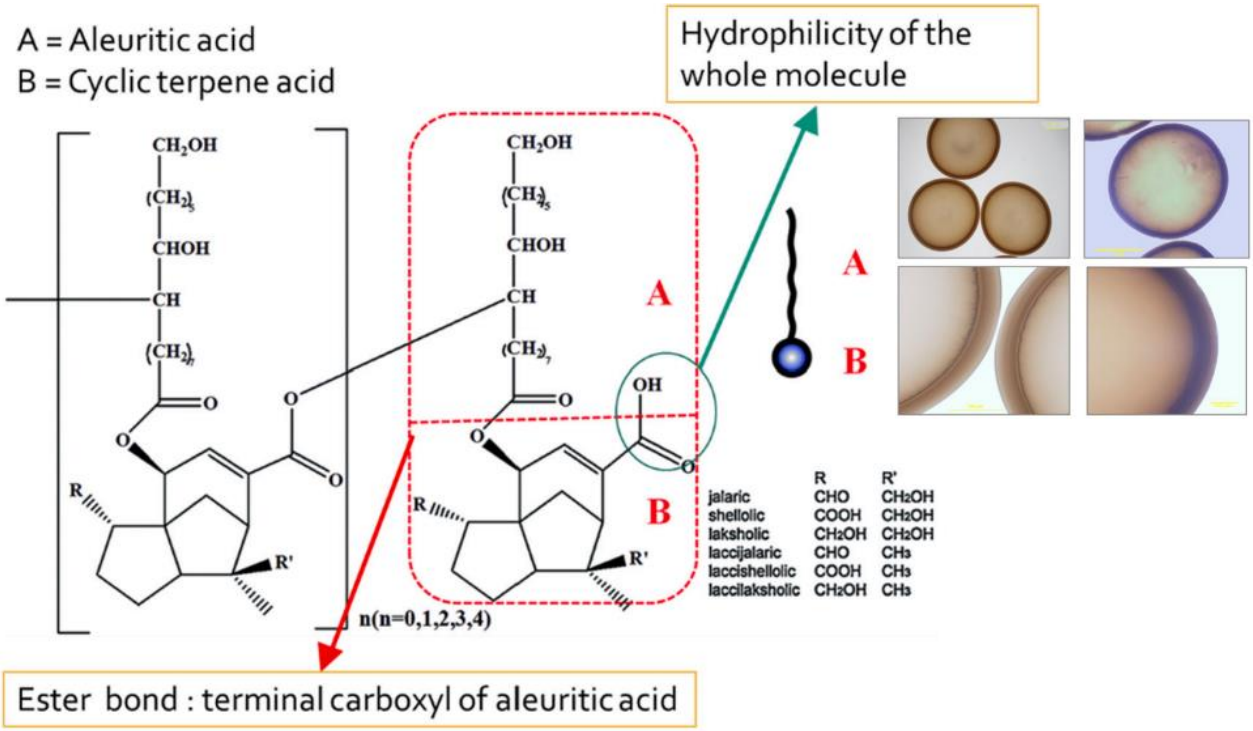
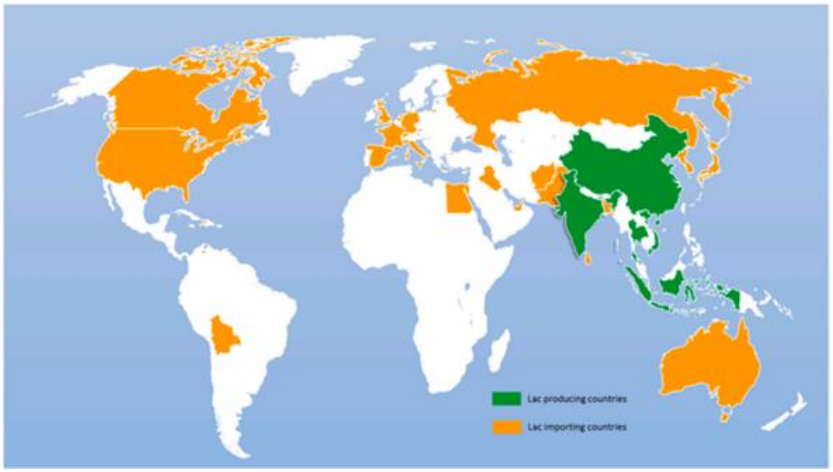


Figure 20: On the left: chemical structure of the typical building block of shellac. Figure reused from Thombare et al. (2022, [127]). On the right: microscopic images of capsules coated by shellac. Photos reused from Ben Messaoud et al. (2016, [130]).

A typical unit of shellac (Figure 20) is supposed to have a whole five hydroxyl groups, one free carboxyl group, three ester groups, a single partially hidden aldehydic group, and an unsaturation with a double bond in one place. These functional groups are chemically bound together with ester, acylal, acetal and ether linkages [131]. The material is amphiphilic in nature, where hydrophilicity is imparted by the free carboxylic part of the sesquiterpene acids, and

aliphatic long chain hydroxy acids are responsible for its hydrophobicity. Owing to its unique properties, it finds extensive applications in food [132] and pharmaceutical industries [133–135]. Shellac presents excellent filmogenicity as well as binding properties. Associated to its biocompatible nature, these features make it an appropriate coating agent [136].



Countries most involved in lac production and importation in the world. Figure taken to Thombare et al. (2022, [127]).

As compared with uncoated amidated pectin beads, Oehme *et al.* demonstrated that shellac coating could perform fast release in the colon and reduce the total anthocyanin release from the beads in simulated gastric juice from 75 % to 18 % [137]. The unique property of shellac, being insoluble in water at neutral to acidic pH and soluble in alkaline pH, makes it an ideal pharmaceutical coating material intended for colon targeted release. In particular, the carboxyl groups in shellac provide its weak acidity with a pKa in the range 5.6−7.0. After it enters the digestive tract, its dissolution can only occur in the weak alkaline surroundings (pH = 7.4) found in the colon. Therefore, shellac-based delivery systems are well suited for colon-targeted delivery [138], and shellac as an enteric coating is often used to transport drugs and nutritional supplements to the colon [139]. One of the most recent advancements in this sector is the development of shellac-coated sustained-release pellet formulation. Farag *et al*. made double-layered pellets with a constant outer shellac coating and a varying sub-coat. Each sub-coat was either calcium chloride, Eudragit E® or citric acid [136]. The application of modifying sub-coats was a successful mean to achieve sustained release. As for its moisture protection potential, it was found that shellac coated tablets containing acetylsalicylic acid required a lower coating level than common cellulose derivatives, for similar drug release kinetics [140]. Shellac manifests better performance over synthetic polymers when exploited as a single-layer enteric coating. In a study, Sinha *et al*. compared four types of enteric polymers, namely

Eudragit S-100, ethylcellulose, cellulose acetate phthalate, and shellac. They coated lactose-based indomethacin tablets and carried out dissolution tests under the simulated stomach and small intestine conditions.

Results indicated that the dosage form coated with 3 % shellac (m/m) was clearly advantageous for colon-specific delivery [141].

Being a wax matrix, the role of shellac in extended drug release is important. However, due to its slow dissolution in the intestinal segment, drug release can be excessively slow. The need to adjust the hydrophilicity of shellac-based delivery systems is predominant to obtain the desired drug release profile. For now, the primary application of shellac is as an enteric coating for the delivery of drugs. Only a couple of studies have stated the delivery of probiotics [142], which deserves further attention in IBD, as probiotics are known for their excellent influence upon the regulation of intestinal flora and homeostasis [143].

Herein, aqueous shellac solution based on ammonium (Swanlac® ASL10) was used. It ensured product stability (better resistance against ageing and self-esterification phenomena) and offered advantages: ready for use and weakly viscous. Using theophylline tablets, this commercial shellac solution has already proved its enteric release capabilities, and provided aesthetic, durable and stable coatings [144].

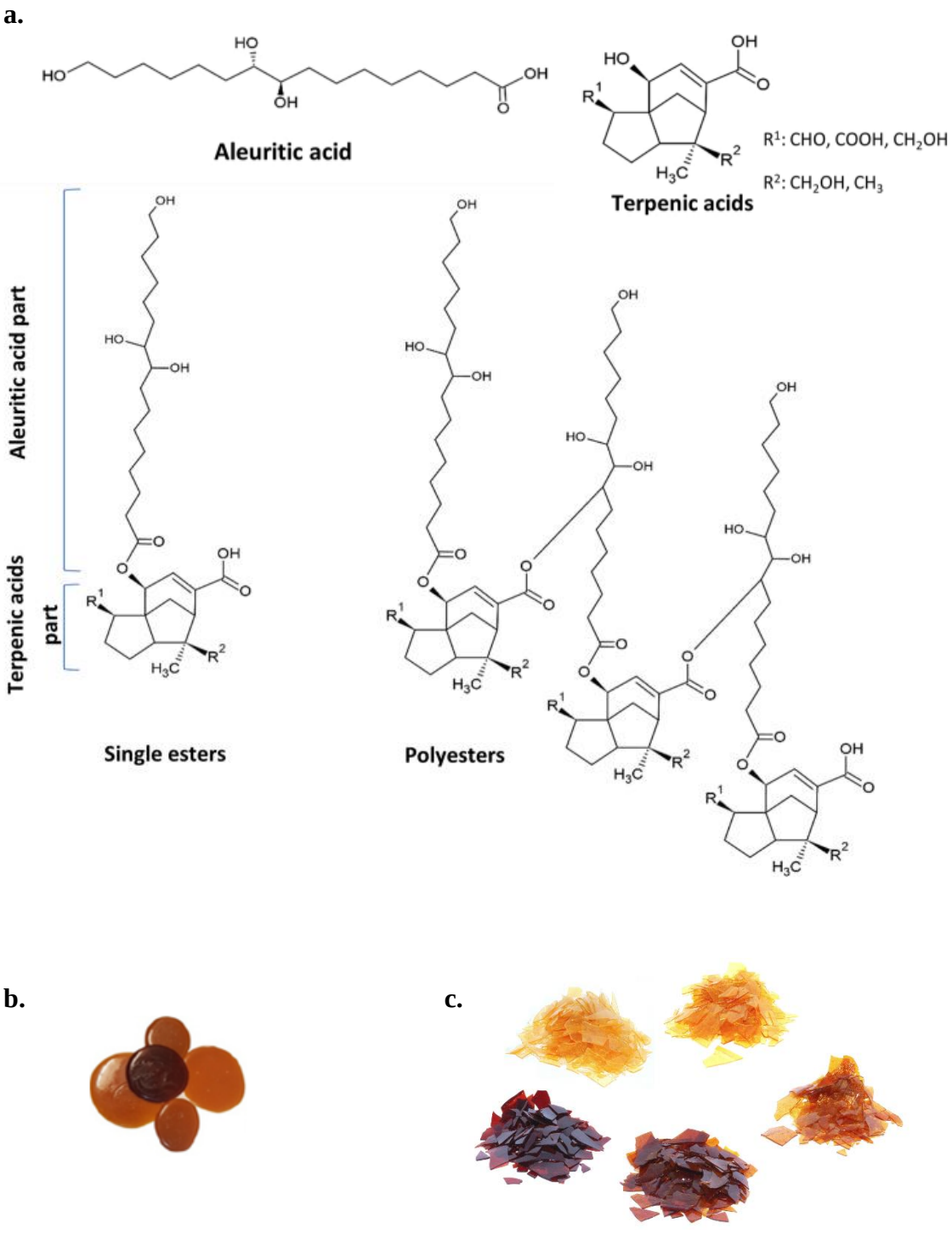


Figure 21: Molecular polymeric structure of shellac (a), button lac (b) and shellac flakes (c). Figure 21a. reused from Bar et al. (2021, [145]).

I.5 Aim of thesis

The major purpose of this work was to identify innovative polymeric film coatings allowing for colon targeted drug delivery *via* a microbial sensitivity. Once a novel film coating was found, we aimed to develop oral dosage forms prepared by direct compression and further coated thanks to pan coating technology. These types of formulations are of paramount importance, as conventional forms intended to treat IBD lack efficacy, due to premature drug release. In addition to IBD, colon targeted drug delivery systems could be useful for other intestinal affections such as parasitosis, colonic cancer, or even pathologies aligned with circadian rhythm.

The strategy consisted in associating a natural compound (mainly polysaccharides), serving as a substrate of microflora for colonic specificity, with a permanent hydrophobic thermoplastic polymer (ethylcellulose, written as "EC"). The latter helps to control the water uptake of the system. Finding an optimal balance between both permitted to protect the drug in the upper GIT and to ensure microbially triggered drug release in the colon, thanks to the metabolic activity of microbiota.

An association was particularly kept in this study: the mix of ethylcellulose and shellac. This basic and invariant coating formulation was assessed *in vitro* and optimized to get an appropriate kinetic drug release profile within the entire GIT.

Numerous objectives were to be undertaken:

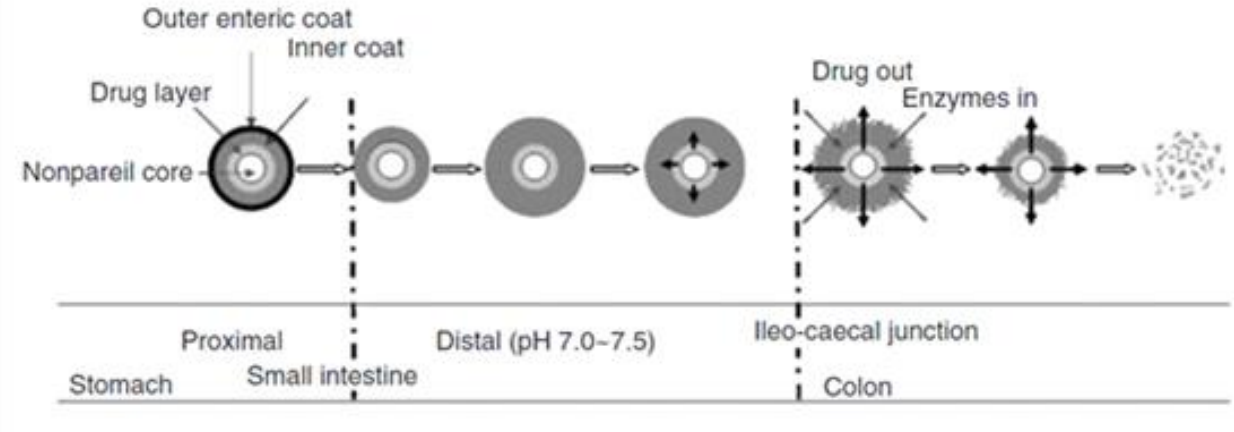
- First, a screening step, with the preparation and physicochemical characterization of several polymeric films associating EC and one polysaccharide or natural compound. The interest was to determine the ones that most protected drug in the upper GIT.

- The manufacture of uncoated and coated mini-tablets for colonic drug delivery and the set of the pan coater process parameters.

- The *in vitro* drug release study of mini-tablets for 32 h incubation assays. Afterwards, the identification of a basic and promising batch that may be able to fulfill our criteria.

- The elucidation and optimization of the process parameters for a resistant tablet coating and the troubleshooting of tablet coating defect.

In vitro drug release from theophylline-containing tablets was quantified in different conditions, among which the exposure to faeces from IBD patients to be closer to pathophysiological conditions.



Colon targeting process. Figure taken to Ji et al. (2009).

II. MATERIALS AND METHODS

II. Materials and methods

II.1 Materials

II.1.1 Polymer aqueous dispersions

Ethylcellulose dispersion (Aquacoat ECD 30, DuPont Nutrition USA, Wilmington, DE, USA), aqueous shellac ammonium salt solution (Swanlac® ASL 10, A.F. Suter, Witham, United Kingdom).

II.1.2 Polysaccharide powders

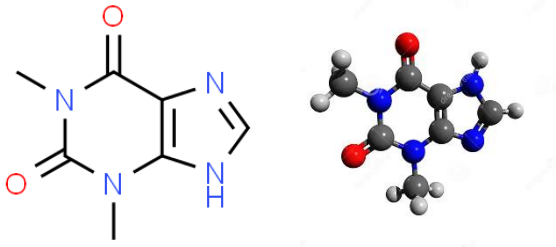
Inulin (Orafti® HP, Beneo, Mannheim, Germany), maltodextrin (Glucidex Maltodextrin 19, Roquette, Lestrem, France), alginates (Aquateric N100, DuPont, Wilmington, Delaware, USA), locust bean gum (Cesagum, Tate & Lyle, Villeneuve d'Asq, France), arabic gum, pullulan sample (DKSH, Miribel, France), pregelatinized maize starch (C*PharmGel 03406, Cargill, Gent, Belgium), pectin (Herbstreith & Fox, Neuenbürg, Germany), maize dextrin (Nutriose® FM06 Roquette, Lestrem, France), pregelatinized potato starch (Prejel PA5PH, Pharma Excipients International AG, Beim Bahnhof 5, Steinhausen, Switzerland).

II.1.3 Plasticizers

Triethyl citrate (TEC, Alfa Aesar, Karlsruhe, Germany), glycerol (Alfa Aesar, Karlsruhe, Germany).

II.1.4 Drug powders and dosage forms

Theophylline anhydrous powder (BASF, Ludwigshafen, Germany).

Drug	Anhydrous theophylline
Chemical structure	
Chemical formula	C7H8N4O2
Molecular weight	180.17 g/mol
Solubility (in water at 25 °C)	7.36 g/L

pKa	8.81
Log P	- 0.02

According to the biopharmaceutical classification system (BCS), theophylline is a Class I drug [146]. The aqueous solubility and relatively small molecular weight suggest this drug as an ideal model drug to test the enteric coating efficiency *in vitro*.

Mini-tablet cores containing theophylline: Microcrystalline cellulose (Avicel PH 102, FMC Biopolymer, Philadelphia, USA), magnesium stearate (Merck, Saint-Quentin-Fallavier, France), theophylline anhydrous powder (BASF, Ludwigshafen, Germany).

Mini-tablet cores without theophylline: Placebo mini-tablets (Chemische Fabrik, Budenheim, Germany).

II.1.5 Preparation of simulated digestive media

Simulated gastric and intestinal fluids: Hydrochloric acid pH 1.2 (0.1 M HCl), phosphate buffer pH 6.8 (PBS 6.8).

Simulated colonic fluid: Culture medium inoculated with and without fresh fecal samples from patients (with given consent).

Culture medium: Extracts from beef and tryptone (Pancreatic digest of casein, Becton Dickinson, Sparks, USA), yeast extract (Oxoid, Hants, United-Kingdom), sodium chloride (J. T. Baker, Deventer, Netherlands), L-cysteine hydrochloride hydrate (Acros Organics, Geel, Belgium), Ringer solution (Merck, Darmstadt, Germany).

II.1.6 HPLC-UV analysis

Mobile phase: Acetonitrile (VWR, Fontenay-sous-Bois, France), phosphate buffer pH 6.8 (PBS 6.8).

Column: C18 column (Gemini® 5 µm C18 110 Å, 100 mm x 4.6 mm; Phenomenex, Le Pecq, France).

II.2 Preparation of dosage forms

II.2.1 Polymeric films

Polymeric films represented the coating of the future oral dosage form and were designed with its same composition. These films were prepared by mixing ethylcellulose (EC) with one natural compound (polysaccharide mainly) at different blend ratios: (90:10); (80:20); (70:30); (60:40).

The blend ratios expressed the proportion of the dry mass of ethylcellulose with respect to the dry mass of the second fraction. For instance, 90:10 ratio meant 90 % ethylcellulose associated with 10 % of natural compound in the film. Please note that blend ratios can be equally written with ":" as with "-" (90:10 or 90-10).

First, ethylcellulose was mixed with 25 % triethyl citrate (abbreviated as "TEC", referring to ethylcellulose mass at dry state) for 24 h, as a plasticization step. Then, dissolved polysaccharide was added for 3 h to get a homogeneous dispersion. 1 % of theophylline anhydrous powder (w/w, referring to the total dry mass of the film) was added to the dispersion and mixed for 2 h. Please note that the drug was dissolved molecularly into the dispersion (solubility in H_2O: 7.36 g/L at 25 °C [147]). After mixing, the dispersion was poured into Teflon molds and dried in an oven at 60 °C for 24 h. Films were cut into pieces and their thicknesses were measured using a thickness gauge (MiniTest 600, ElektroPhysik, Köln, Germany). Samples were measured in triplicate. Thicknesses were standardized around 600 µm.

II.2.2 Manufacture of mini-tablets

II.2.2.1 Mini-tablet cores

i. Definition and advantages of mini-tablets

Pharmaceutical mini-tablets are tablets with a diameter between 1–4 mm. Mini-tablets can be single or multiple unit dosage forms, easy to manufacture [148].

Mini-tablets provide all the advantages of conventional tablets: stability, dosage uniformity and predictable performance. Moreover, mini-tablets offer some exclusive advantages such as (1) help for swallowability in elderly and pediatric populations, (2) dosage adaptability (dosage titration in little increments), (3) set dosage combinations for several drugs (various mini-tablet products with a single drug are delivered in the right amounts), (4) greater than liquids (*e.g.*, dosage stability and accuracy) and particulates/granules (*e.g.*, uniformity and accuracy of dose, exposure dangers), and (5) more accurate dosing than adult tablet splits [149].

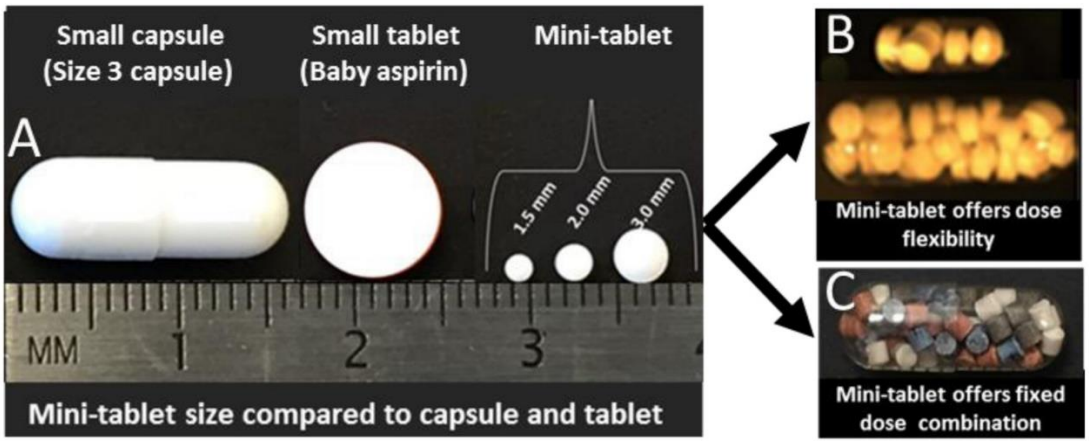


Figure 22: Illustrations of mini-tablets. Figure reused from Mitra et al. (2017, [149]). Please note that our tablets measured 5 mm but were still considered as mini-tablets.

ii. Preparation of mini-tablet cores

Non-coated mini-tablets were prepared as follows: microcrystalline cellulose (MCC) was mixed with 10 % theophylline anhydrous powder for 10 minutes, then lubricated with 1 % magnesium stearate for 5 more minutes using a 3D powder blender mixer at 20 rotations *per* minute (rpm) (Turbula T2C, Willy A. Bachofen, Basel, Switzerland). Mini-tablets were obtained after compression of the powder thanks to an automatic single-punch tablet machine (Korsch, EKO/DMS, Berlin, Germany). Placebo mini-tablets from Chemische Fabrik (Budenheim, Germany) were added to these newly obtained tablets to get the right batch mass for coating process (400 g). Tablets diameter was 5 mm.

Figure 24: Single-punch tablet machine to get tablet cores (Korsch, EKO/DMS, Berlin, Germany).

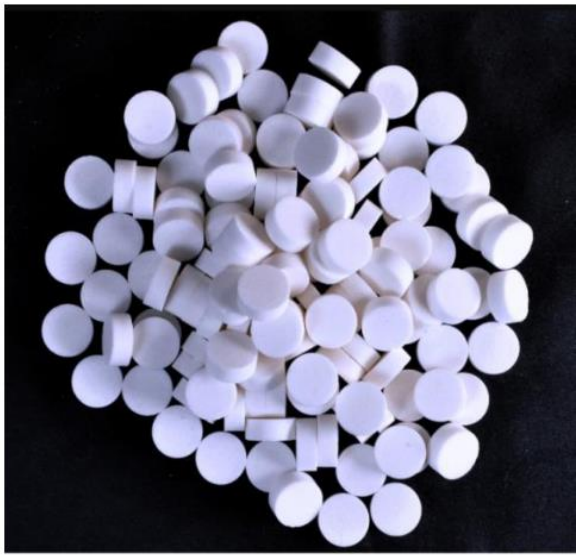


Figure 23: Example of mini-tablets. Figure taken from Priyanka et al. (2018, https://doi.org/10.22270/jddt.v8i6.2060).

iii. Quality control of mini-tablet cores

The quality control of mini-tablet cores was performed according to European Pharmacopoeia (Ph. Eur.) 11.1 (section 2.9: "Pharmaceutical technical procedures"). This control involved the following criteria: uniformity of mass, uniformity of drug content, hardness, friability, disintegration of tablets, *in vitro* dissolution test, tablets height and diameter.

Among 20 mini-tablets, the mean mass should not get beyond a 10 % standard deviation value. UV-spectrophotometry (UV-1650 PC, Shimadzu, Kyoto, Japan) was used at a wavelength of 275 nm to determine the drug content. The mean drug content value should range from 85 to 115 %.

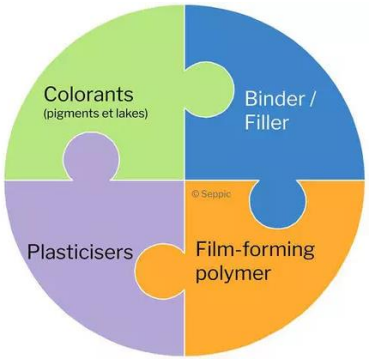
Gravimetry was used to determine the friability of mini-tablets, and should be inferior to 1 %.

Theophylline release from the mini-tablets was measured using the USP II dissolution apparatus (paddle method, 80 rpm, 37 °C) in 300 mL phosphate buffer pH 6.8 (Sotax, Basel, Switzerland). At predetermined time points, 3 mL samples were withdrawn and analyzed spectrophotometrically (UV-1650 PC, Shimadzu, Kyoto, Japan) to quantify their drug content (λ=275 nm). All experiments were fulfilled in sextuplicate.

II.2.2.2 Coating process

i. Generalities about coating process

Tablet coating is a process that consists in the application of a polymer homogeneously to the surface of a galenic formulation. Contemporary tablet coatings are mainly films, flexible materials that can be applied onto a variety of dosage forms (tablets, capsules, pellets, drug crystals, and granules). Tablet coating is a complex process and it implies several parameters: spray pattern, nozzle spacing, drop size...and many other parameters, not related to spray matter. In order to get a uniform film coating, all those variables must be accurately controlled [150].



The Solidlab 1 laboratory machine processes batch sizes from 0.05 to 1 kilogram and is the equivalent for the highest performance in the smallest space.

ii. The many benefits of film coating [151]

Above the aesthetic dimension, the coating film has a main impact on the functional properties and therapeutic adherence of tablets:

- It protects the tablets from light, moisture and oxidation, extending thus their shelf life. As an example, Seppic has created Sepifilm™ LP to protect the active substances sensitive to humidity.
- It ameliorates the look of the tablets, particularly if the API or nutraceutical active ingredient is colored or displays an unequal color. When colored, coating films can serve for brand recognition, or to differentiate several medications taken by the patient.
- It smoothens the surface of tablets and simplify the ingestion.
- It permits a better taste and hides the odors due to the active pharmaceutical and nutraceutical substance.
- It can provide controlled release properties. Using particular polymers can delay drug delivery or target different segments of the gastrointestinal tract, relying on the pH.

iii. Presentation of pan coater

The following pictures represent the Solidlab 1 pan coater and its main characteristics.

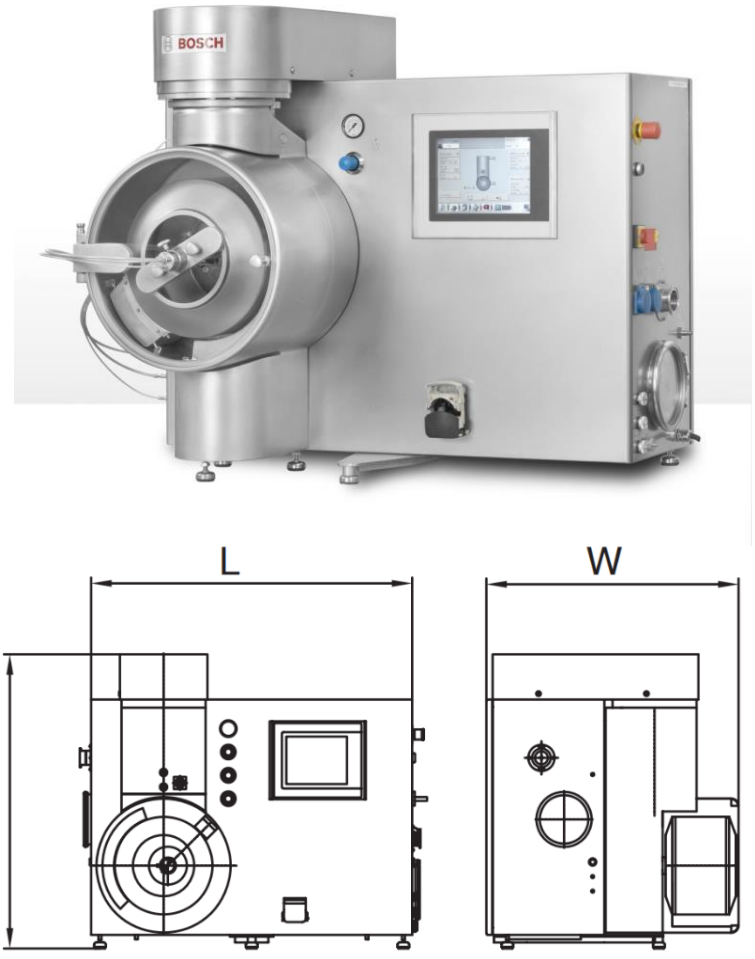


Figure 26: Spray nozzle and its body.

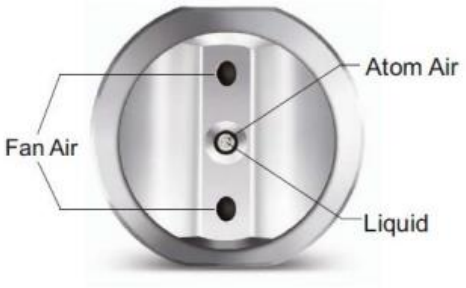


Figure 25: Shaping/fan/pattern air system disposition. Picture reused from Nyamweya et al. (2019).

Figure 26 represents the nozzle and its body, responsible for the spraying of the formulation onto tablets bed. **Atomizing air pressure** refers to the pressure into the central channel, enabling the atomization of polymer particles (see "Atom air" zone on figure 25). **Shaping air** (also called fan air or pattern air) **pressure** refers to lateral air flows modulating the width and shape of the sprayed polymer (see "Fan air" zone on figure 25). This requirement is essential to cover the whole tablets bed and ensures that every dosage form is equally exposed to the formulation. Shaping air pressure is always inferior or equal to atomizing air pressure.

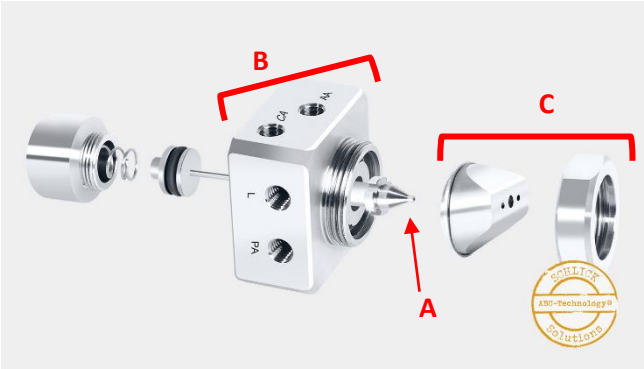


Figure 27: The different parts of the nozzle (A), its body (B) and its head (C). Figure reused from Düsen-Schlick GmbH [83].

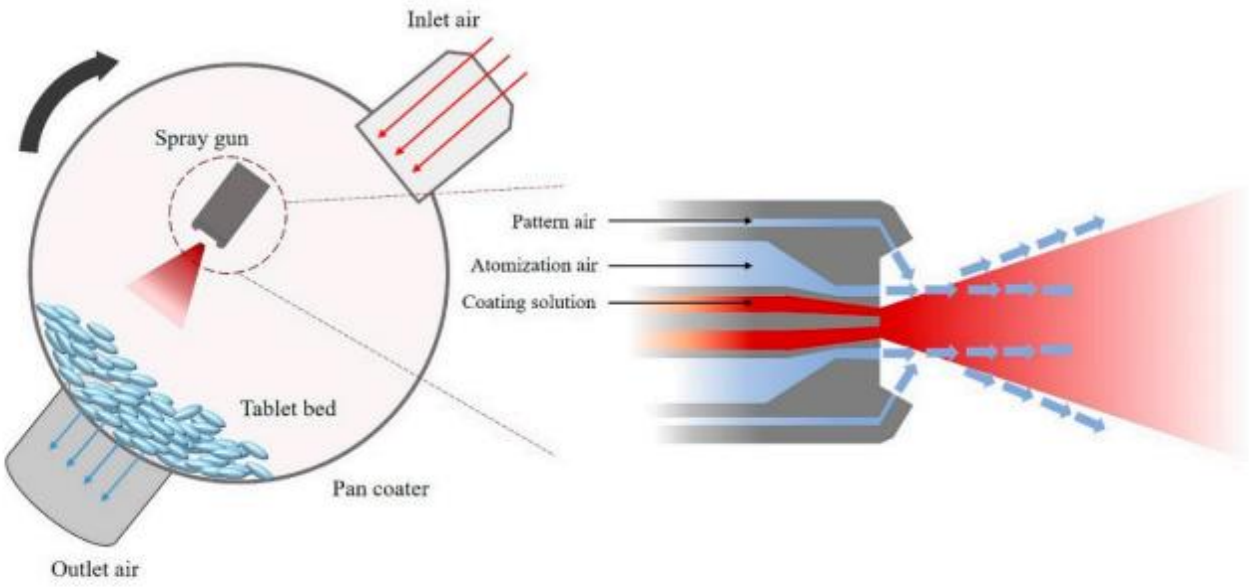


Figure 28: Simplified illustration of a coating pan. Inlet air (red arrows) is distributed into the pan and extracted as outlet air. Figure from Ki-Soo et al. (2020, https://doi.org/10.3390/pharmaceutics12090853).

Among the other parameters, **inlet air system** is the air flow circulating inside the machine and redistributed throughout the pan. The temperature obtained in the pan is lower and corresponds to the **outlet air system**. Inlet air temperature is a setpoint, whereas outlet air temperature is a function. Both are related and changing inlet air system will have an impact upon outlet air system.

The coating process leads to a thin layer around the tablet, containing the polymer. This layer, with a thickness from 20 to 100 µm, accounts for about 1 to 10 % of the initial weight of the tablet. This process enables the formulation to be applied onto the tablet core in the coating turbine.

After water evaporation, each of the polymer particles gather and, thanks to higher plasticizer quantity, they coalesce upon contact and form a homogeneous film coating layer.

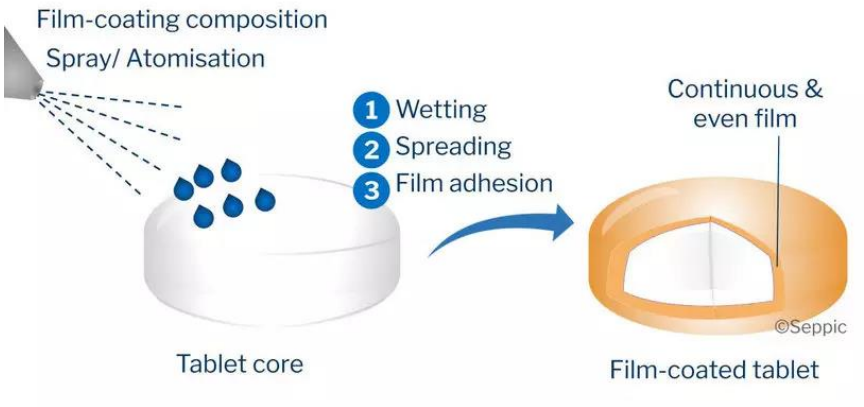
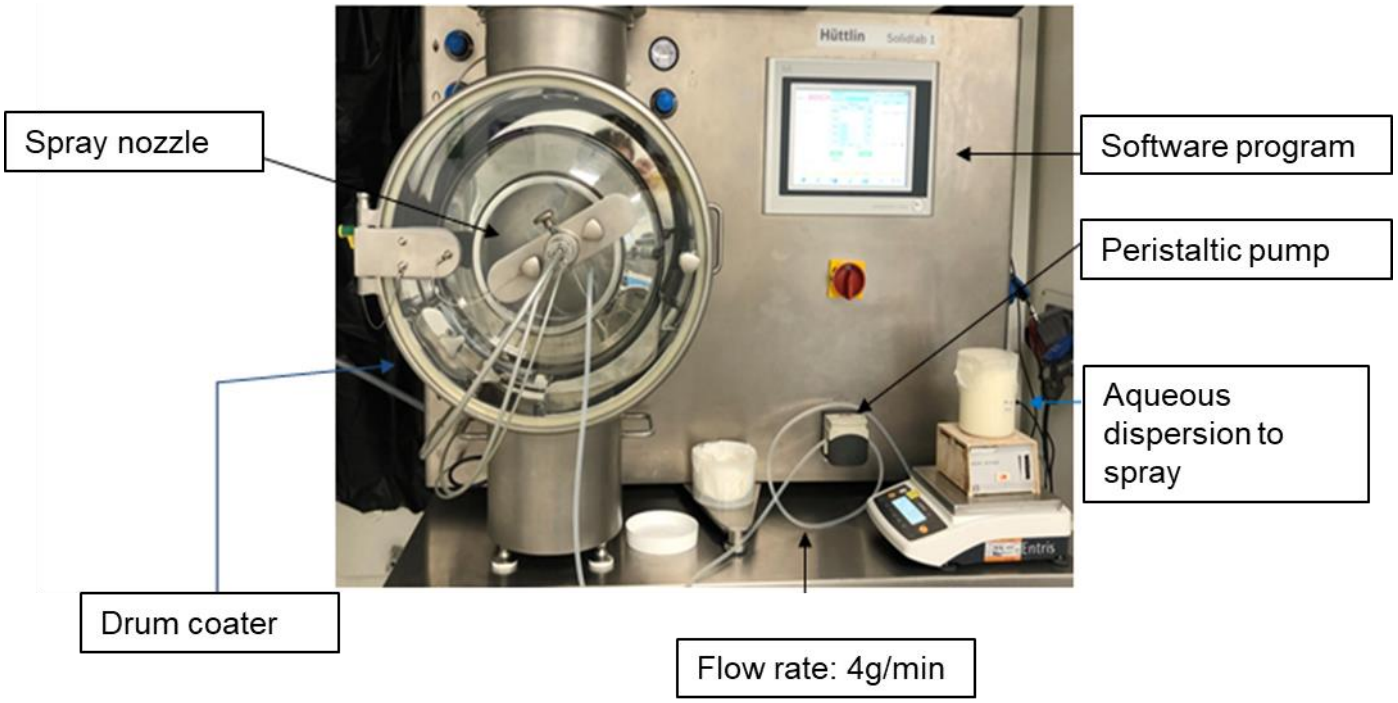


Figure 29: Coating process showing polymer particles coalescence around the tablet core. Figure taken from SEPPIC [151].

Tablet cores were coated using a drum coater module (Solidlab 1 Hüttlin, Syntegon Technology, Waiblingen, Germany) with a 0.5 mm nozzle. Films are frequently applied *via* spray coating to achieve the mechanical properties associated with thin films.



Mini-tablets coating was carried out using following parameters:

- 4 g/min spray rate
- 60 °C inlet air temperature
- 40 °C outlet air temperature
- 1 bar atomizing air pressure
- 0.5 bar shaping air pressure
- rotation speed was set at 25 rotations *per* minute.

The dispersion aimed to be sprayed was a blend of ethylcellulose and aqueous shellac ammonium salt solution, with and without the addition of 5 % and 10 % inulin. 80:20; 75:25; and 60:40 blend ratios were exploited. Different coating levels, ranging from 10 % to 35 %, were carried out, depending on blend ratio. Coated mini-tablets were cured (= post-thermal treatment) in an oven at 60 °C for 24 h. These conditions were reported in previous studies as appropriate to ensure polymeric coalescence and optimal film formation for ethylcellulose-based systems [152, 153]. Please note that coating levels represent the weight gain after coating, calculated with the initial tablet weight.

II.2.3 Physico-chemical characterization

II.2.3.1 Optical macroscopy

Macroscopic pictures of coated mini-tablets were taken with a stereoscopic microscope SMZ-U (Nikon, Minato-Ku, Tokyo, Japan). Pictures were taken at t=0 h, t=8 h and t=24 h after incubation of mini-tablets into simulated digestive (= gastric, intestinal and colonic) fluids.

II.2.3.2 Water content and dry mass

Water content and dry mass loss rates of polymeric films were measured gravimetrically upon incubation in (i) simulated gastric fluid (2 h 0.1 M HCl pH 1.2) and (ii) simulated intestinal fluid (6 h phosphate buffer pH 6.8).

Samples were put into flasks (1 sample *per* flask), containing 100 mL 0.1 M HCl or phosphate buffer pH 6.8 and stirred at 80 rpm (using a horizontal shaker at 37 °C, GFL 3033, Gesellschaft fuer Labortechnik, Burgwedel, Germany). Samples were weighed (wet mass) with precision and dried at 60 °C (dry mass) to constant weight. Water uptake and dry mass loss (%) were calculated as follows, at predetermined time points:

$$\text{Water content}\,(\%)\,(t) = \frac{\text{wet mass}\,(t) - \text{dry mass}\,(t)}{\text{wet mass}\,(t)} \times 100\% \quad (1)$$

$$\text{Dry film mass}\,(\%)\,(t) = \frac{\text{dry mass}\,(t)}{\text{dry mass}\,(t=0)} \times 100\,\%. \quad (2)$$

II.2.3.3 *In vitro* drug release from polymeric films and coated mini-tablets

i. Upper GIT - Upon exposure to simulated gastric and intestinal fluids

Polymeric films were incubated in flasks (1 sample *per* flask) containing 100 mL 0.1 M HCl

and stirred at 80 rotations *per* minute (using a horizontal shaker at 37 °C, GFL 3033, Gesellschaft Für Labortechnik, Burgwedel, Germany). After 2 h, the medium was completely replaced with phosphate buffer pH 6.8 to simulate intestinal fluids.

Coated mini-tablets were incubated under the same conditions as free films (2 h in 0.1 M HCl pH 1.2 followed by 6 h in PBS pH 6.8) using a USP III dissolution apparatus, also called reciprocating cylinder apparatus (Bio-Dis, Varian, Paris, France) at 20 dips *per* minute (dpm) and 37 °C. 20 dips *per* minute were considered as harsh conditions in this work [154].

At predetermined times, 3 mL samples were withdrawn and measured using UV-spectrophotometry (UV-1650 PC, Shimadzu, Kyoto, Japan) at λ = 275 nm for theophylline concentration. The range of concentrations of the calibration curve was [1-10 mg/L] and r^2 was 0.99998 and 0.9997 for HCl and PBS, respectively.

ii. Entire GIT - Upon exposure to gastrointestinal and simulated colonic fluids

After incubation in simulated gastric and intestinal fluids, mini-tablets were transferred into 100 mL flasks containing: (1) 100 mL culture medium inoculated with fresh human feces (obtained from IBD patients giving written informal consent), and (2) culture medium without feces for reasons of comparison. Mini-tablets were incubated in triplicates for each aspect tested (ratio or coating level). The samples were gently agitated (50 rpm, Stuart, Cole-Parmer, Villepinte, France) at 37 °C in anaerobic atmosphere (AnaeroGen 2.5 L, Thermo Scientific, Illkirch, France). Culture medium was produced as follows, according to previous publications [154–157]: 1.5 g beef extract, 5 g tryptone, 3 g yeast extract, 2.5 g NaCl and 0.3 g L-cysteine hydrochloride hydrate were dissolved in 1 L distilled water (pH 7.0 ± 0.2). Afterwards, the solution underwent autoclaving. Culture medium containing patients' feces was prepared by diluting fecal samples (approximately 1 g) 1:200 with cysteinated Ringer solution. At pre-determined times, 2 mL of culture medium were withdrawn, centrifuged for 10 min at 15000 rpm (Centrifuge Universal 320, Hettich, Tuttlingen, Germany), filtered (0.22 µm,Millex-HU, Merck Millipore, Tullagreen, Ireland) and quantified by HPLC (Waters E2695 ALLIANCE HPLC) for their drug content using an equipment with a pump, an auto sampler and coupled to UV-Vis detector. For the detection of theophylline as a model drug, the mobile phase was prepared by mixing 90 % phosphate buffer saline 6.8 with 10 % acetonitrile (v/v). Samples were injected into a C18 column (Gemini® 5 µm C18 110 Å, 100 mm x 4.6 mm; Phenomenex, Le Pecq, France) at a 0.6 mL/min flow rate. The drug was detected at λ = 275 nm [158]. The range of concentrations of the calibration curve was [5-150 mg/L] and r^2 was 0.9998.

All experiments were carried out in triplicates.

III. RESULTS AND DISCUSSION

III. Results and discussion

III.1 From polymeric films: upper GIT

III.1.1 Theophylline release from polymeric films

To materialize the coating layer of the upcoming coated dosage forms, polymeric films were produced for the first phase of our study. These polymeric films had the same composition as the coatings of interest and were much simpler to test. Indeed, the coating step is very time consuming and requires using many tablet cores, while our stocks were limited. Polymeric films associated EC, as a thermoplastic polymer, with different types of polysaccharides. Different blend ratios were tested as follows: 90:10, 80:20, 70:30, 60:40 (EC:Polysaccharide). For instance, 90:10 ratio meant that film was based on 90 % of EC and 10 % of polysaccharide. Films were loaded with 1 % anhydrous theophylline (w/w, referring to the total dry mass of the film) and incubated in 0.1 HCl (2 h) followed by PBS 6.8 (6 h), representing the upper GIT. Polymeric films simplified the screening step and yielded numerous and diversified outcomes.

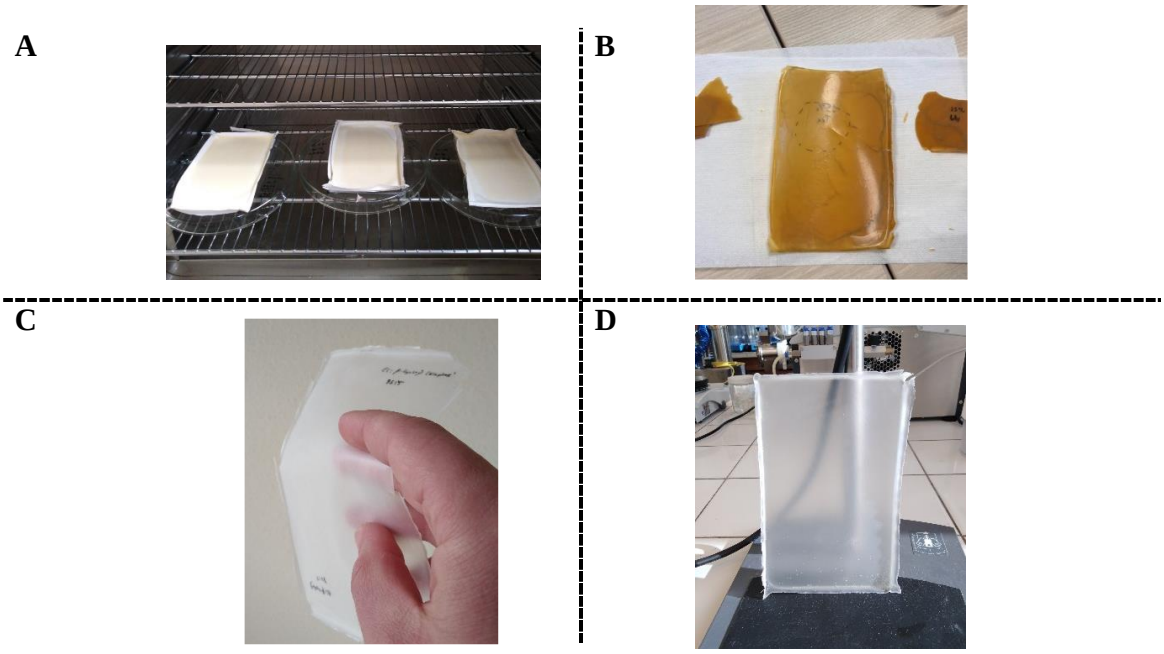
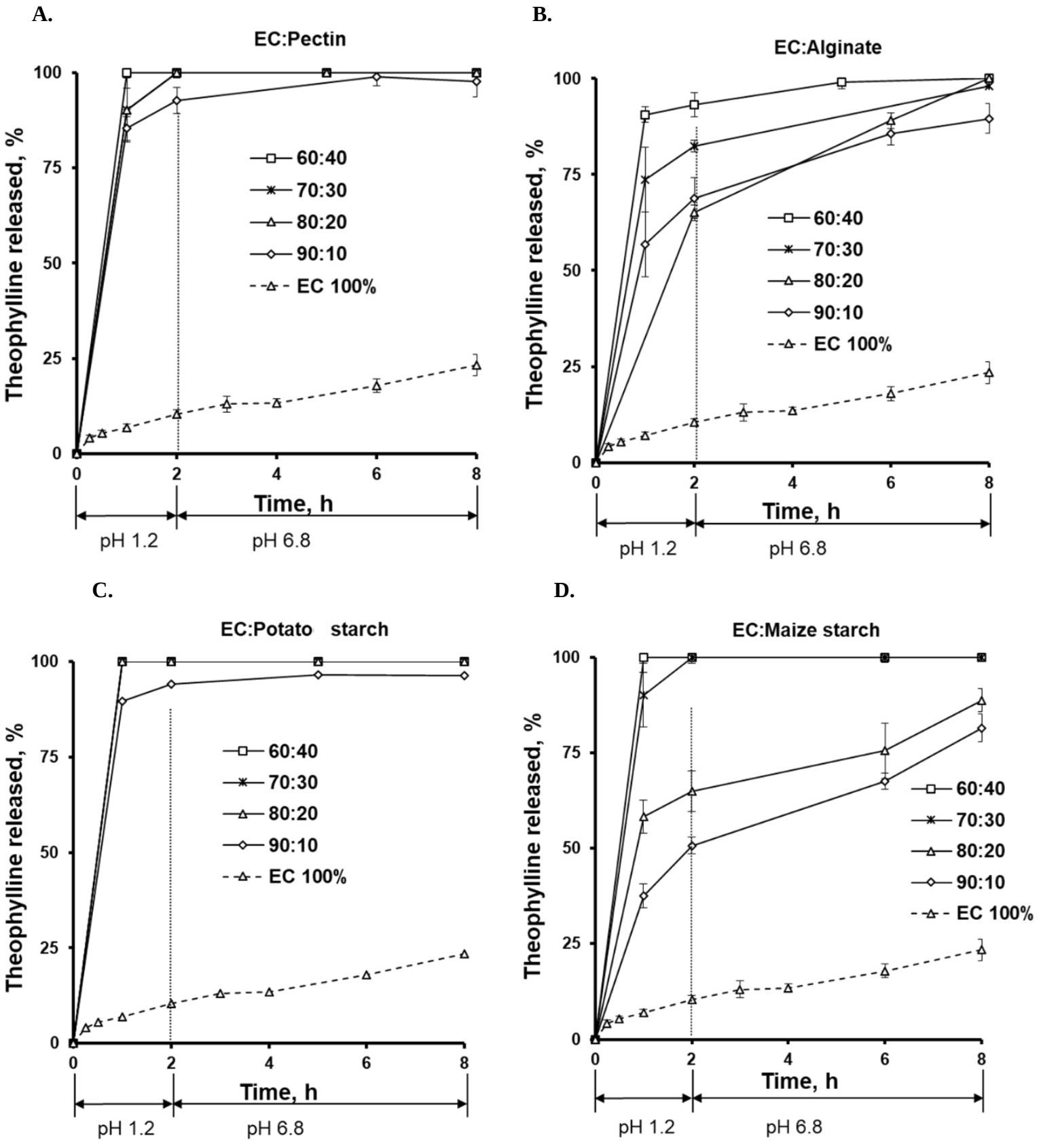


Figure 30: Photographs of polymeric films in molds before (A) and after drying (B-D).

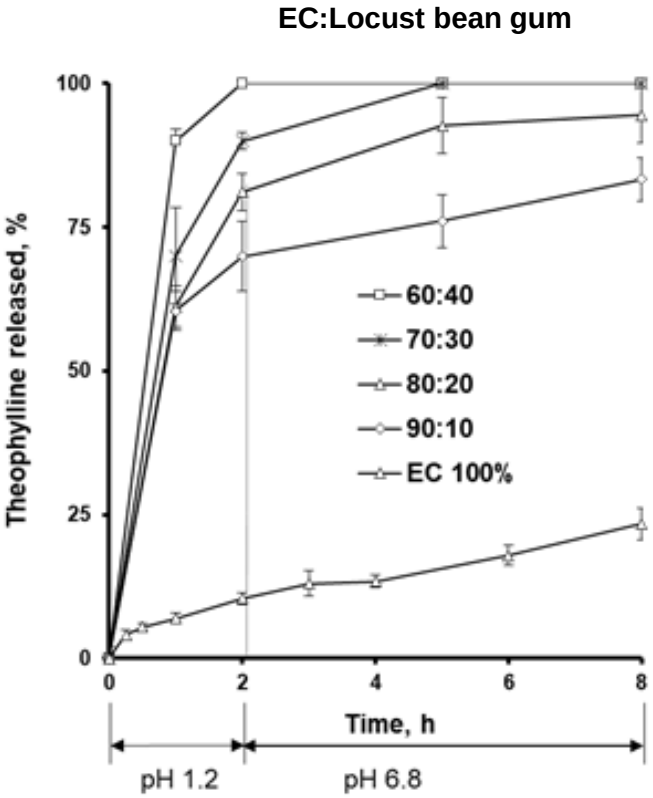
Figure 31 represents the different results obtained. The most interesting kinetic profiles to keep were the ones displaying a sustained release along time. Please note that those graphs were presented in my own Pharm. D. (*Ciblage de la partie distale du tractus gastro-intestinal dans*

le traitement des maladies inflammatoires chroniques de l'intestin, Samuel STRICH, 2023. University of Lille, France).

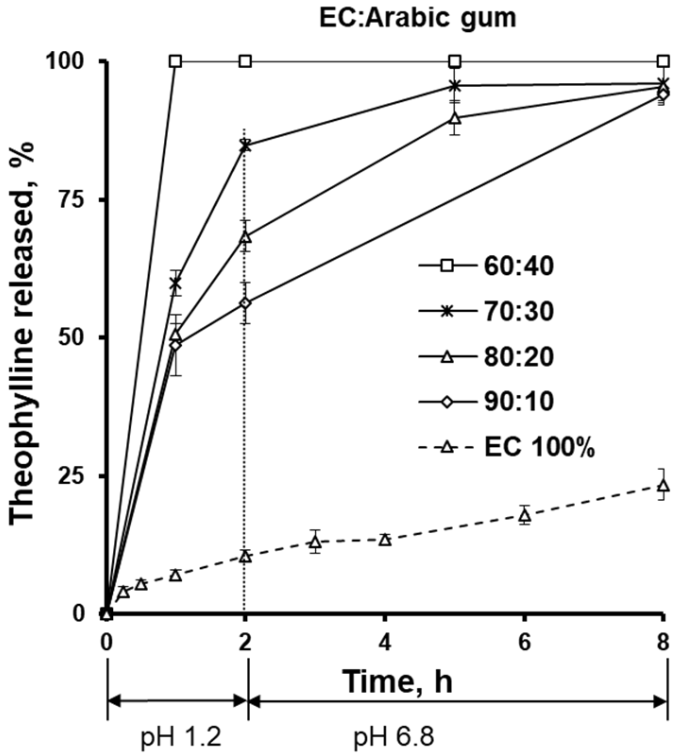
Drug release kinetic will be varying upon the nature and the quantity of polysaccharide.



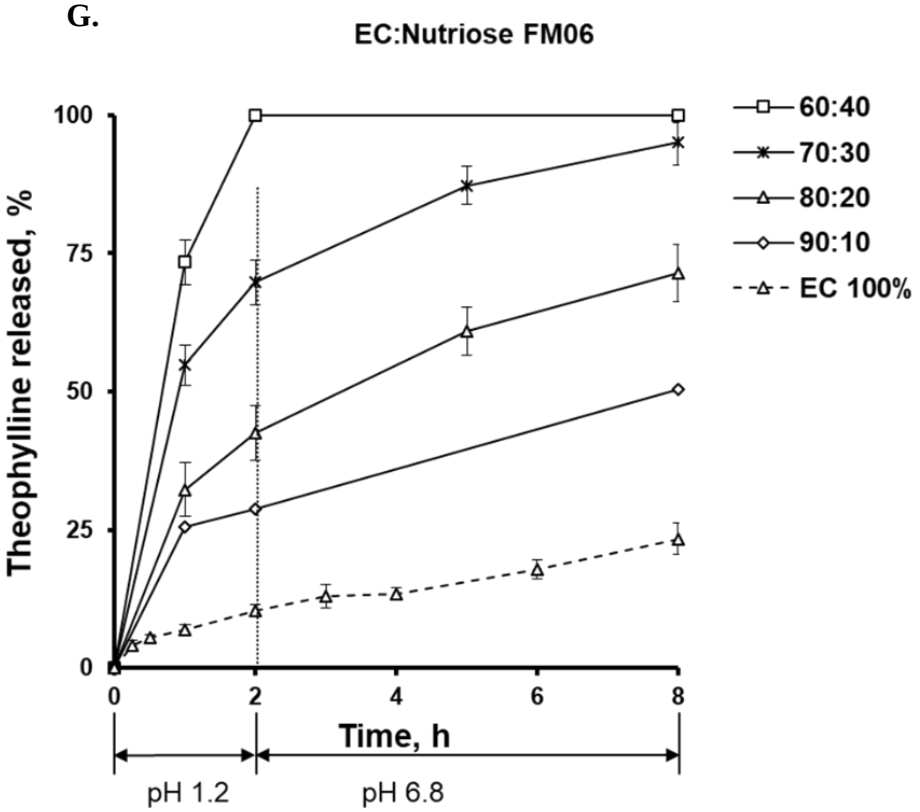
E.


EC:Locust bean gum
Theophylline released, %
100
75
50
25
0
0
2
4
6
8
Time, h
pH 1.2
pH 6.8
60:40
70:30
80:20
90:10
EC 100%

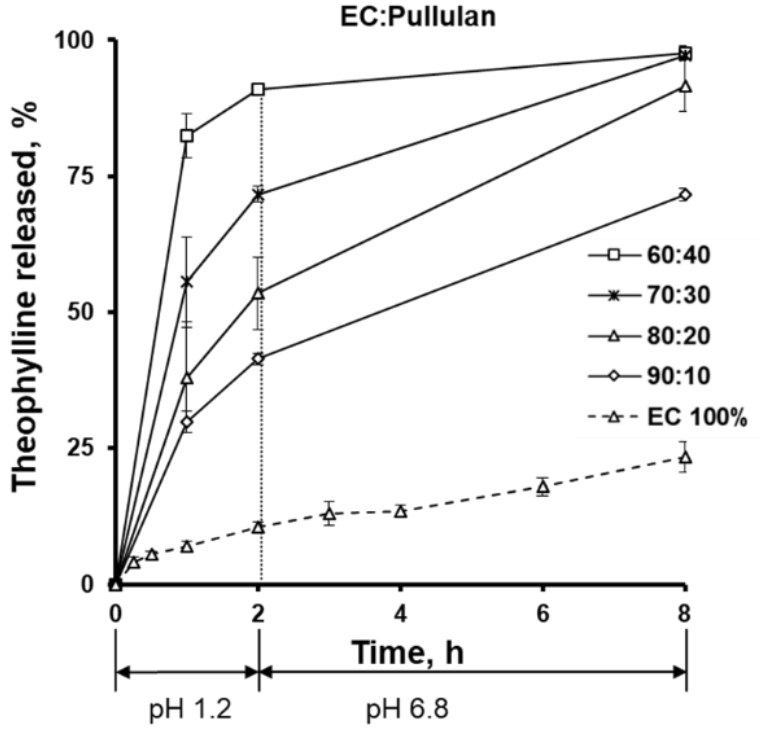
F.


EC:Arabic gum
Theophylline released, %
100
75
50
25
0
0
2
4
6
8
Time, h
pH 1.2
pH 6.8
60:40
70:30
80:20
90:10
EC 100%

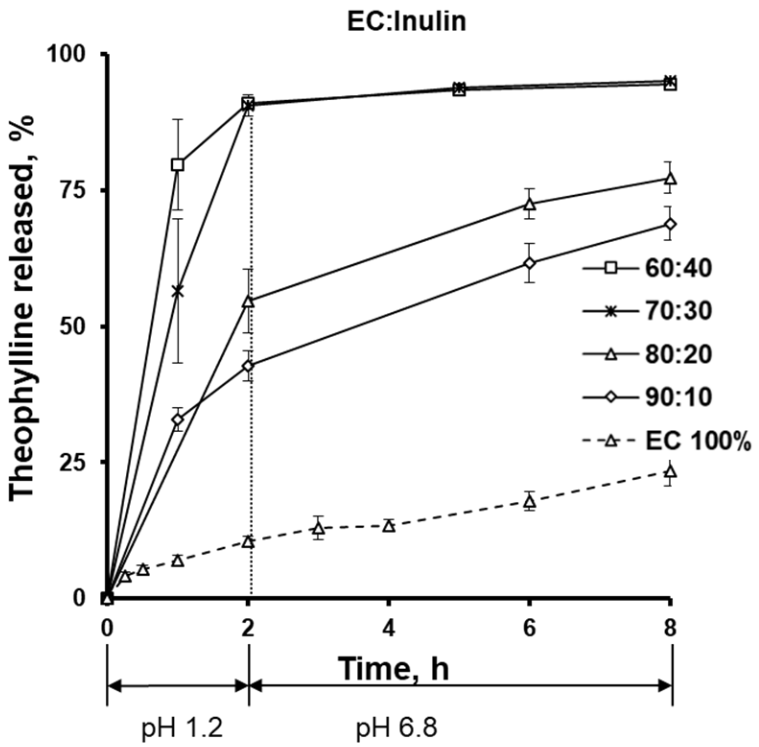
G.


EC:Nutriose FM06
Theophylline released, %
100
75
50
25
0
0
2
4
6
8
Time, h
pH 1.2
pH 6.8
60:40
70:30
80:20
90:10
EC 100%

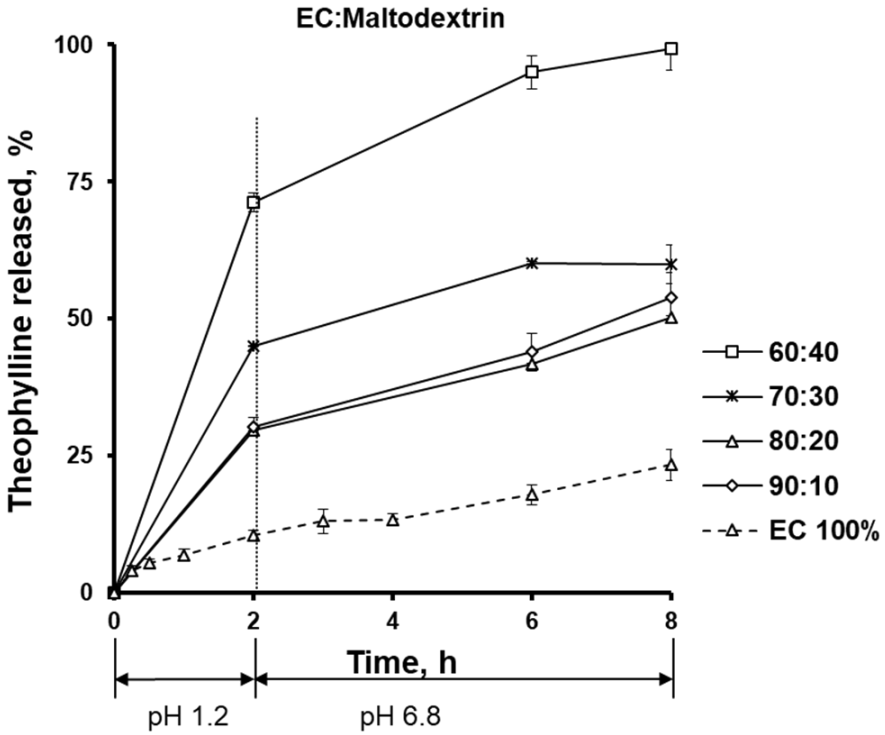
H.


EC:Pullulan
Theophylline released, %
100
75
50
25
0
0
2
4
6
8
Time, h
pH 1.2
pH 6.8
60:40
70:30
80:20
90:10
EC 100%

I.


EC:Inulin
Theophylline released, %
100
75
50
25
0
0
2
4
6
8
Time, h
pH 1.2
pH 6.8
60:40
70:30
80:20
90:10
EC 100%

J.



K.

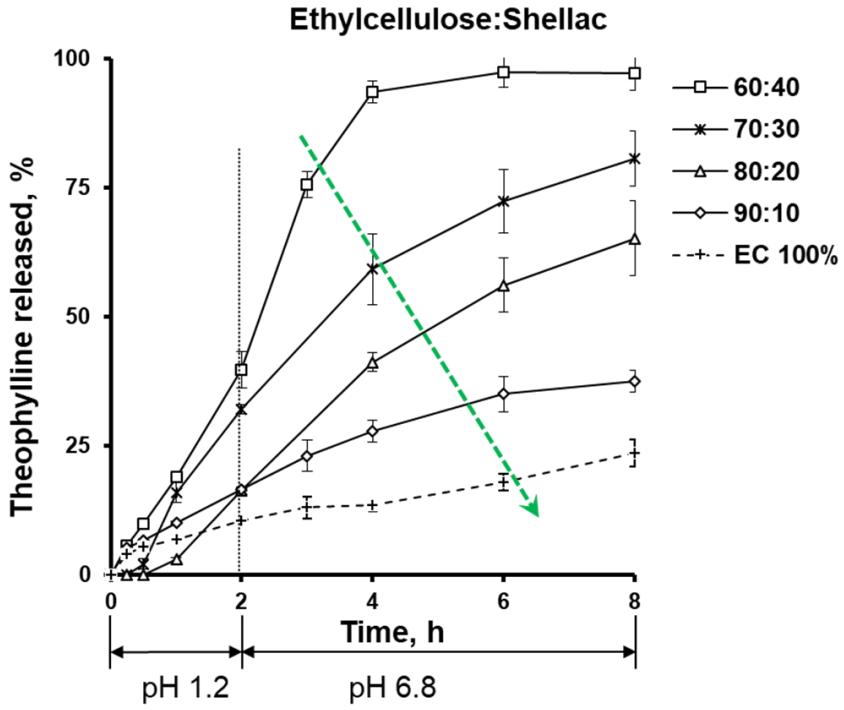


Figure 31: Impact of the polymer:polymer blend ratio (indicated in the diagram) on *in vitro* theophylline release from polymeric films based on ethylcellulose with a natural compound upon exposure to 0.1 M HCl pH 1.2 (2h) followed by phosphate buffer pH 6.8 (6h). All films were loaded with 1 % anhydrous theophylline and plasticized with 25 % TEC. Natural compounds associated with EC were:

A. Pectin; B. Alginate; C. Potato starch; D. Maize starch; E. Locust bean gum; F. Arabic gum; G. Nutriose FM06; H. Pullulan; I. Inulin; J. Maltodextrin; K. Shellac.

In vitro, the drug release pattern from a polymeric film can possibly give an estimation of that from coated solid dosage form. Unlike films (matricial systems), coated mini-tablets are reservoir systems. Drug delivery is slower, due to the polymeric barrier to be crossed around mini-tablets. It is clear that drug release increased when EC fractions lowered. EC possesses about 50 % ethoxy groups and has good filmogenicity, rendering coating layers flexible. Due to its high hydrophobicity, EC controls water uptake and hinders the swelling of the matrix. The more polysaccharide, the more water uptake and the more API released. Polysaccharide provides hygroscopic and hydrophilic characteristics to the film coating. With water absorption, macromolecules have more mobility and, thus, drug diffusion is better [159]. Molecules with low molecular weight such as 5-ASA (131.5 Da) or theophylline (180.2 Da) are likely to diffuse a lot *via* such polymeric films [160]. Beyond the proportions of thermoplastic polymer (EC) and polysaccharide, drug release profiles also depend on the nature of polysaccharide. Figure 31, graph A shows EC:Pectin polymeric films upon incubation in the upper GIT (2 h 0.1 N HCl followed by 6 h PBS pH 6.8). Irrespective of the blend ratio, total and premature release occurred. Pectin is a highly hydrophilic polysaccharide giving brittle and rigid films due to its bad mechanical properties. These particularities were reported in several references [161, 162]. For reasons of comparison, pure EC films were made and tested in the same conditions (dotted lines). The amount of drug release was low. As mentioned previously, EC is a very hydrophobic material and it has low permeability [163, 164]. This is a reason why EC films can be used as waterproofing agents. Graph I stands for EC:Inulin based polymeric films. Drug diffusion is low, as compared to other polymeric blends, and drug release is controlled. Inulin displays numerous advantages: due to its β-osidic bounds, it cannot be degraded by digestive enzymes. Yet, it is fermentable by Bifidobacterium genus located in the colon, which secretes inulinases [165, 166]. Other properties such as good flexibility can be quoted. As a matter of fact, films containing inulin are more flexible and resist to mechanical stress [167, 168]. On another hand, a study of Benzine *et al*. compared drug release profiles from hot melt extrudates based on the same types of blends. Those blends associated EC with different sorts of polysaccharides, as in the present study. The polymeric blend associating EC and inulin showed the best release profile, with only 13 % of Cmax achieved up to 24 h [158]. The incubation implied both the same media: 0.1 N HCl for 2 h, followed by PBS 6.8 for 22 h. This study supported the aforementioned observations. For these different reasons, this polysaccharide was selected as additional excipient for further experiments. Nutriose based films (graph G) also exhibited interesting kinetic properties. At 90:10 (EC:Nutriose FM06) ratio, only 40 % release were observed up to 8 h. Nutriose undergoes about 85 % fermentation in the colon [169]. Its prebiotic

and controlled release properties make it an appropriate candidate for colon targeting. By contrast, films based on potato starch (graph C) were brittle as soon as they reached 80:20 (EC:PA5PH) ratio. This could explain the early drug release profile obtained with potato starch. Even though chitosan is widely used for colon targeting, it was not exploited in our experiments for reasons of too fragile films. Films based on chitosan were highly brittle and unexploitable.
Films based on locust bean gum and arabic gum (graphs E and F) also showed premature and total drug release. Generally, gums (also called hydrocolloids) form very viscous solutions and gels. They are used as thickening agents in food industry. Due to their important swelling, concentrations lower to 1 % are sufficient to get viscous solutions. To get polymeric blends, the amount of water required to dissolve them is huge, as compared to the volume of EC. For this reason, their utilization was quite limited.
Graph K represents drug release from EC:Shellac based films. As mentioned in chapter I.4, shellac is a bio-based hydrophobic polymer with terpenes and sesquiterpenes acids. The Food and Drug Administration (FDA) has approved it as safe, and it provides good film-forming qualities [128]. It is commonly used as an enteric coating material for oral dosage formulations. There have also been reports of its use in formulations aimed at the colon and sustained release [170]. Interestingly, low levels of coating are required to deliver negligible drug amounts in the upper part of the GIT [140].
80:20 blend ratio only displayed 16 % release after 2 hours incubation. 90:10 and 80:20 blend ratios respectively achieved 37 % and 65 % drug release after total transit in the upper GIT. Even from polymeric films, drug release remained quite controlled along time.

Dry shellac starts to swell at pH 6.8 and creates more space between polymeric chains. Nonetheless, a close network is formed at acidic pH. The latter can be relevant for the protection in the upper GIT.
As EC:Shellac blends manifested interesting kinetic properties, this formulation was selected for all the next experiments. Although shellac is not a substrate of microbiota by itself, its enteric properties are public knowledge [170]. The pH-dependency of shellac and its dissolution pH, located around 7.3 [170–172], are interesting features for colon targeted delivery. This film coating composition deserved to be, in a first time, further exploited in the upper GIT. By incorporating a polysaccharide, this innovative approach could lead to a dual stimuli-triggered system: pH-dependent and microbiota-sensitive. This concept is the most reliable in colon targeting.

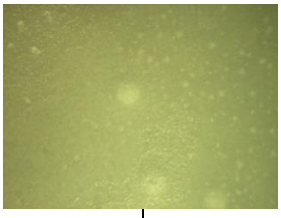
The following pictures represent polymeric films before (T_0) and after incubation in the upper GIT (T_{8h}) for the different blend ratios used. Magnification was ×30 with a stereoscopic microscope.

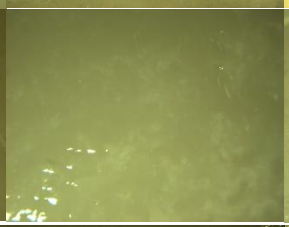
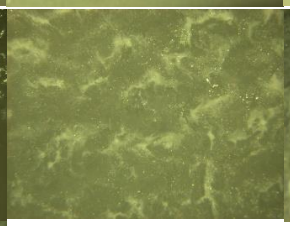
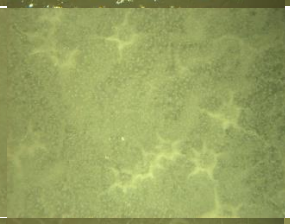
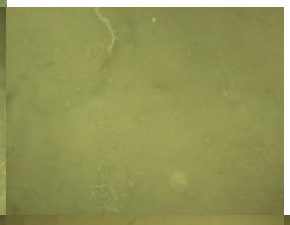
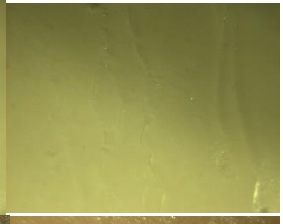
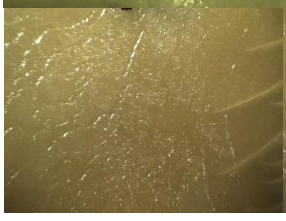
III.1.1.1 Microscopic pictures of polymeric films upon incubation in the upper GIT

i. Before incubation (T0)

T0

EC 100 %

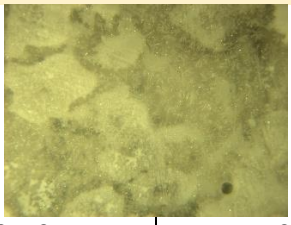


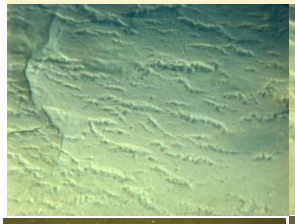
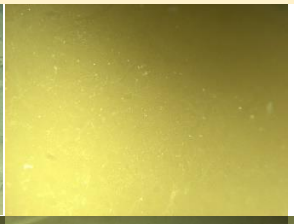
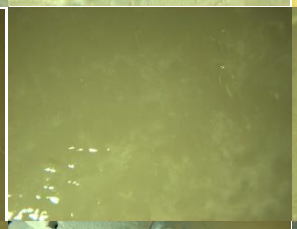
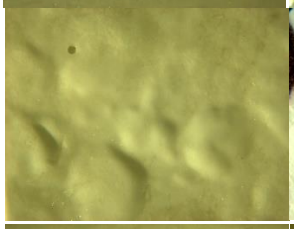
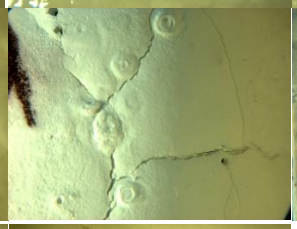
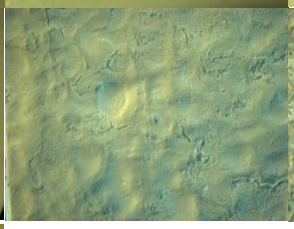
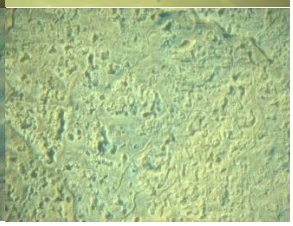
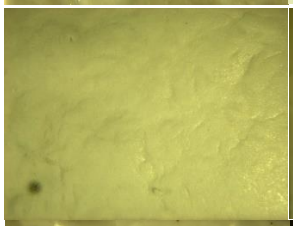
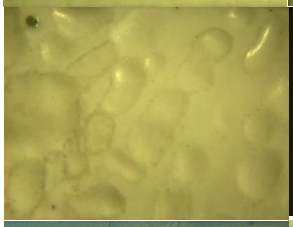
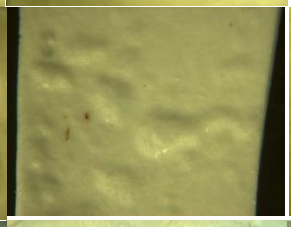
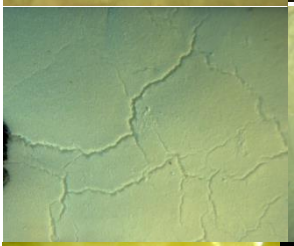
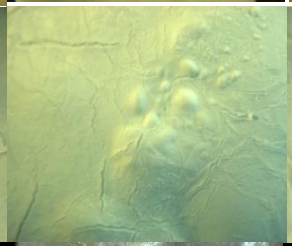
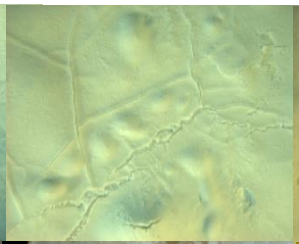
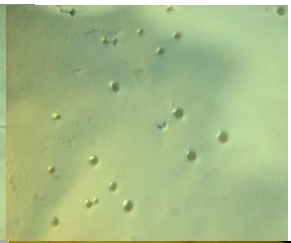
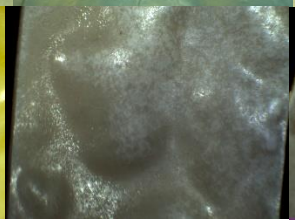
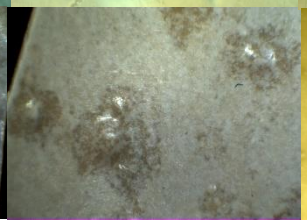
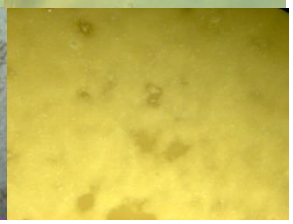
Composition	Blend ratios			
	90-10	80-20	70-30	60-40
EC:Pectin				
EC:Pullulan				
EC:Inulin				
EC:Maize starch			Too brittle	Too brittle
EC:Arabic gum				Too brittle
EC:Alginate				
EC:Shellac				

ii. After incubation (8h)

8h (2h 0.1 N HCl & 6h PBS 6.8)

EC 100 %



Composition	Blend ratios			
	90-10	80-20	70-30	60-40
EC:Pectin				
EC:Pullulan				
EC:Inulin				
EC:Maize starch				
EC:Arabic gum				
EC:Alginate				
EC:Shellac				

III.1.1.2 Selection of EC:Shellac blends and complementary tests

i. Qualitative tests with an additional polysaccharide: maltodextrin

Due to the presence of bacteria in high amounts in the lower GIT (ileocecum and colon), triggered drug release is possible in these very regions. As in the conditions depicted in graph K (figure 31), we investigated polymeric films based on EC:Shellac (from Swanlac® ASL10 solution) including maltodextrin. This one provides hydrophilicity and can be fermented by the microbiota in the lower GIT. Figure 32 stands for theophylline release from thin polymeric films based on EC associated with an equimolar mix of shellac and maltodextrin (50 % shellac blended with 50 % maltodextrin) at different blend ratios: [60:(40)], [20:(80)], [30:(70)] [EC:(Shellac 50% + Maltodextrin 50%)].

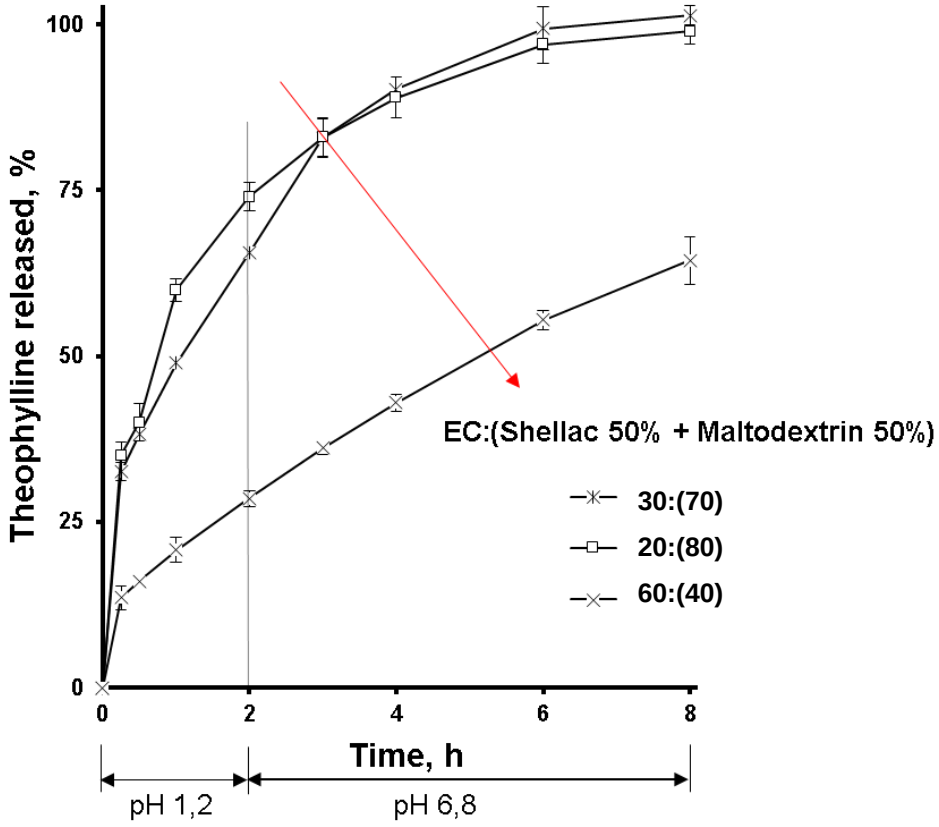


Figure 32: Theophylline release from polymeric films based on [EC:(Shellac 50% + Maltodextrin 50%)] in the upper GIT. Blend ratios are indicated in the figure.

In such a formulation, each ratio is read as follows: the first part is about EC fraction, the second one is about the equimolar mix of both shellac and maltodextrin. For example, 60:(40) means 60 % EC and 40 % of equimolar blend of shellac and maltodextrin (50 % shellac + 50 % maltodextrin).

Figure 32 showed an interesting trend: 60:40 blend ratio still unveiled controlled release profile. Less than 30 % theophylline were delivered within 2 h, and 64 % after 8 h. It is noteworthy that 60:(40) ratio led to better resistance with this formulation, as compared to 60:40 (EC:Shellac formulation) ratio (figure 31). The two other ratios ([30:(70)] and [20:(80)]) showed a total and rapid drug release. Below a certain ratio threshold, the formulation lost its retaining potential. This experiment provided a range of ratios exploitable with this formulation: while decreasing EC proportions until 60:(40) ratio, the drug could be protected even in a matricial system comprising a hydrophilic excipient.

Involving a hydrophilic polysaccharide into the formulation did not loudly impact drug release. As we knew, we could obtain resistance in the upper GIT. We also knew that incorporating a substrate of microflora was still possible, without harming enteric properties up to 8 h. This is a very good path for further experiments, as well using films as using mini-tablets. The next experiment (Figure 33) was carried out with the same formulation and two different blend ratios: 70:(30) and 50:(50). Each ratio was configured in films with different thicknesses.

Please note that the main part of the upcoming results and data can be consulted in my first-author article (AAPS PharmSciTech 24(7). DOI: 10.1208/s12249-023-02652-2).

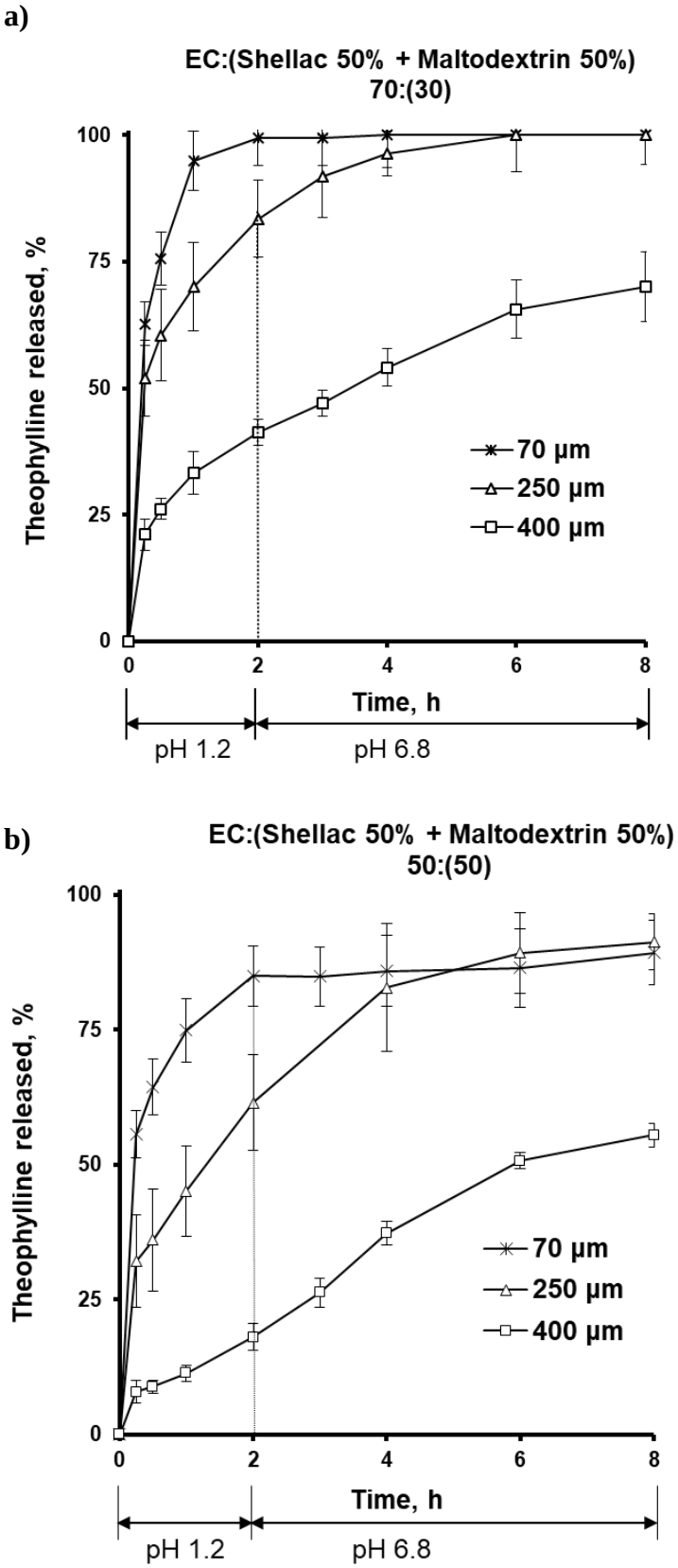


Figure 33: Impact of the thickness on *in vitro* theophylline release from polymeric films based on [Ethylcellulose:(Shellac + Maltodextrin)] and plasticized with 25 % TEC. Thicknesses are indicated in the diagram. Blend ratios were: a) 70:(30), and b) 50:(50). Polymeric films containing 1 % theophylline were incubated in 0.1 M HCl pH 1.2 (2h) followed by phosphate buffer pH 6.8 (6h).

The aim of this experiment was to assess the impact of thickness from thin films upon drug release with different blend ratios with this formulation.

Thin polymeric films were characterized by different thicknesses: 70 μm, 250 μm and 400 μm, using different blend ratios: [70:(30)] and [50:(50)] [EC:(Shellac 50 % + maltodextrin 50 %)], as indicated in the diagrams above.

The interest was a better understanding of the behavior and properties from these polymeric film coatings, in view of: (*) polymer:polymer blend ratio, (**) film thickness, and (***) the influence of maltodextrin upon drug release.

As the mean thickness increased, we could see that theophylline release evolved negatively. This could be explained by the tighter polymeric network which renders the diffusion pathway longer to cross [173]. Evidently, 400 μm thicknesses were associated with slowest kinetics.

At 70 μm thickness, immediate release could be noticed for both [70:(30)] and [50:(50)] polymeric blend ratios. A lesser polymeric network density and a reduced diffusion pathway explain this.

This partial release highlighted that, as a single thin film coating, this formulation was enough impermeable to protect the drug under these conditions. Surprisingly, reducing EC in favor of the second fraction (shellac 50% + maltodextrin 50%) from 70:(30) to 50:(50) had negligible impact over drug release. This could be due to the hydrophobic backbone of shellac, which interacted and significantly increased the hydrophobicity of these new systems. This association would deserve to be tested until the colon as a dual stimuli-triggered system (pH and microbially activated). Importantly, shellac provides in this case a pH-dependent aspect, while maltodextrin, or other natural polysaccharides, could be obviously substrates of the microbiota.

Promising films were identified with optimal drug protection upon exposure to digestive fluids.

These features augured good estimations as for drug delivery from oral dosage forms. In fact, from an inner tablet core, a longer pathway must be crossed: first, the drug must solvate into the tablet core. Then, it must go across the polymeric barrier to diffuse into the surrounding medium.

In the reservoir system, drug should get across the film coating, or through hydrophilic pores, to diffuse in bulk medium.

Figure 34 illustrates photographs with a 30-fold magnification of thin films based on [EC(:Shellac 50% + maltodextrin 50%)], before and after incubation in the upper GIT.

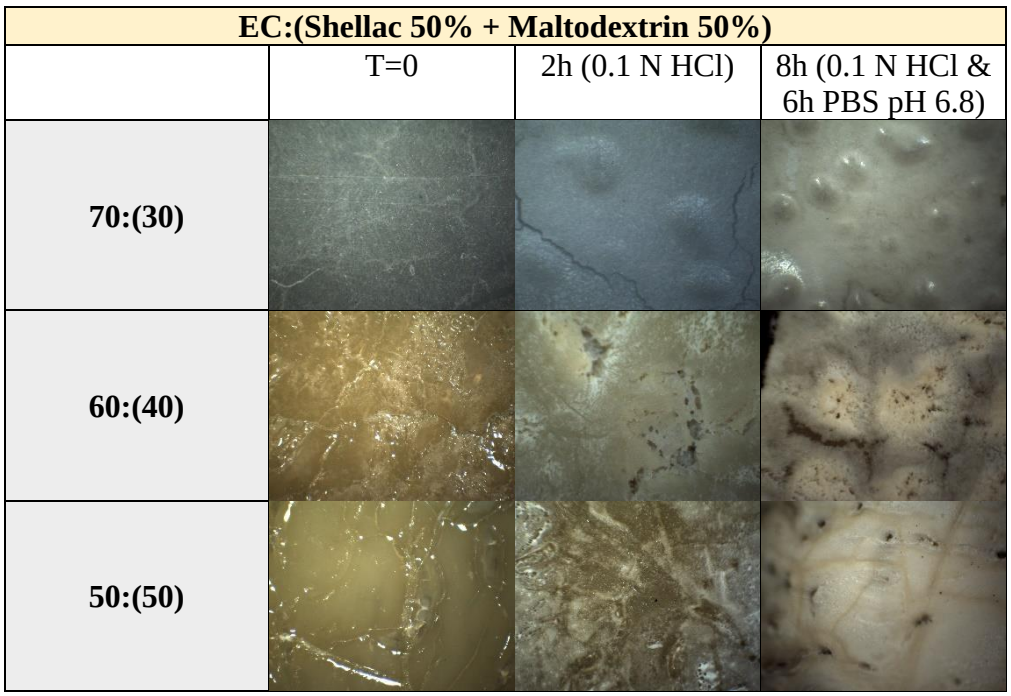


Figure 34: Surface morphology of polymeric films based on [EC:(shellac+maltodextrin)] blends before and after incubation in the upper GIT (2h HCl 0.1 N followed by 6h PBS 6.8) using ×30 magnification. Blends ratios were: 70:(30), 60:(40), 50:(50).

In a same way, figure 35a (below, on the left) was conducted with 90:10 (EC:Shellac) blend ratio and the adjunction of 5 and 10 mL of a xanthan gum solution (0.3 % mass concentration) inside.

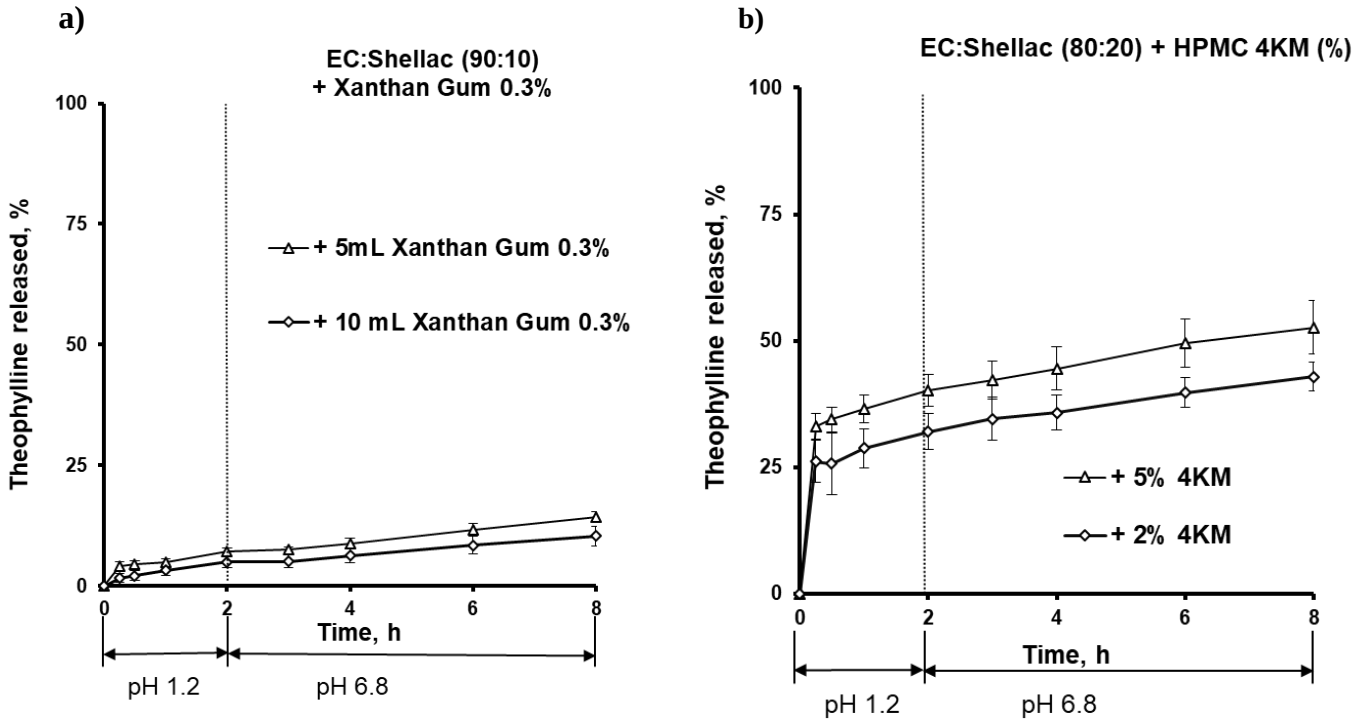


Figure 35: Impact of the addition of a natural polysaccharide upon drug release from polymeric films based on EC:Shellac blend ratios. Polysaccharides added were: a) xanthan gum 0.3 % (mass concentration), and b) HPMC K4M. Films were incubated in the upper GIT (2h HCl 0.1 N followed by 6h PBS 6.8).

As a reminder, xanthan gum is a hydrocolloid and generates highly viscous solutions. This is why its concentration was below 1 %.

Even if the variation in volume was negligible, we could see the slight influence on drug release due to the increasing amount of hydrocolloid. Hydrophobicity rather diminishes in favor of hydrophilicity, but still in a negligible manner. Same conclusion could be stated for figure 35b, involving HPMC K4M. Please note that the percentage of K4M added was based on the total mass of films (including EC, shellac and plasticizer TEC masses). The current formulation was adjustable for microbially triggered delivery without compromising its robustness in the upper GIT. We could consider a ternary mixture comprising EC, shellac, and polysaccharide, for a microbiota-sensitive delivery in the lower GIT. This ternary composition will be further used.

The next experiments will aim to determine a range of polysaccharide concentrations for a microbiota sensitive formulation, still ensuring protection in the upper GIT.

ii. Quantitative tests with an additional polysaccharide: inulin

We previously worked on a basis formulation (EC:Shellac) which manifested enteric protection for 8 h, even involving hydrophilic excipients as maltodextrin, xanthan, or HPMC. The previous experiments were qualitative. They aimed to determine the impact of a hydrophilic polysaccharide upon drug release profile, whatever compound was used.

This basis formulation could be optimized in various ways. Predetermined and rising amounts of inulin were integrated to see the impact upon global hydrophobicity. As inulin is required as a substrate of microbiota for colon targeting, the challenge was to get little changes in hydrophilicity, but still enough to be degraded by the microflora. The interest of this quantitative study was to better define the precise threshold from where addition of inulin should be stopped, to avoid premature delivery within 8 h. Please note that inulin was selected for its properties such as fermentability and mechanical resistance, as mentioned in chapter III.1.1.

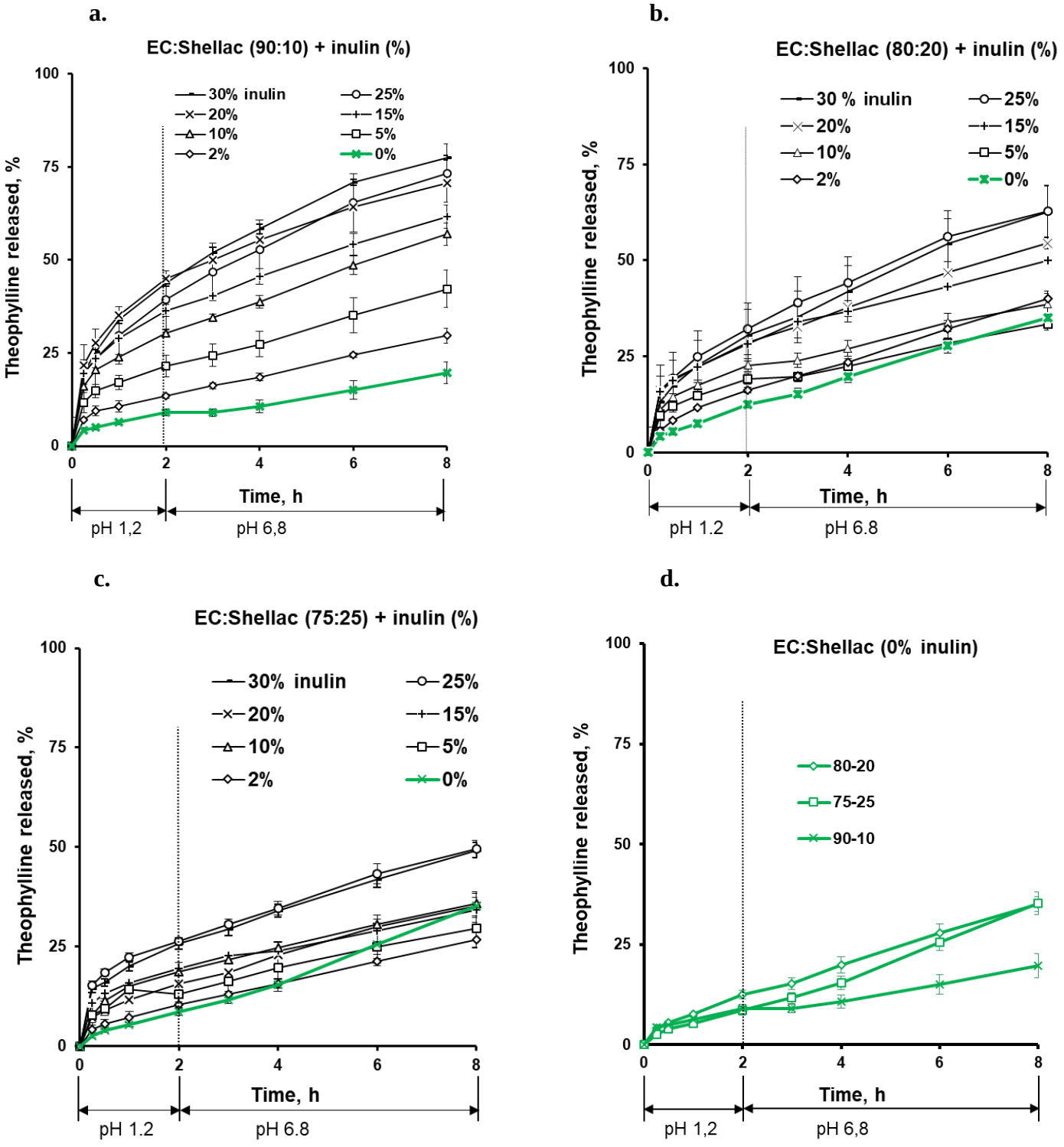


Figure 36: Impact of inulin amount (0 to 30 %; w/w referring to the total mass of the film) on *in vitro* theophylline release from polymeric films based on Ethylcellulose:Shellac blend ratios: (a) 90:10, (b) 80:20, and (c) 75:25. For reasons of comparison, films were represented without inulin on graph (d). Polymeric films were incubated in 0.1 M HCl (2h) followed by phosphate buffer pH 6.8 (6h).

Figure 36 exposed theophylline release as a function of additional inulin (in percentage) in (EC:Shellac) films. The 3 following ratios were tested: 90:10, 80:20, 75:25. The rate of supplemental inulin varied from 0 % to 30 % (w/w, referring to the total dry mass of the film, including EC, shellac, and TEC masses).

For comparison, all films were standardized with an average thickness by 600 µm. As graph b (Figure 36b) showed, 80:20 ratio with 30 % of added inulin (w/w, referring to the total dry mass of the film) led to 63 % drug release (mean value) after 8 h.

Similarly, graph c showed the negligible influence of inulin over drug release from films based on 75:25 ratio (EC:Shellac). Less than 50 % release were achieved with the two highest amounts of inulin (25 % and 30 % inulin), while lower concentrations led to 20 to 30 % release up to 8 h. The influence of this polysaccharide over the system was low. Indeed, 75:25 ratio showed a same trend in theophylline release between 0 and 20 % of inulin content. For example, 10 %, 15 % and 20 % additional inulin led to 35 % drug release, as did the reference graph, without inulin (green line). The positive variation in terms of hydrophilicity was clearly visible but progressive.

Quite similarly to 75:25 ratio 80:20 ratio exhibited a progressive evolution: from 0 % to 10 % additional inulin, the kinetics of curves could be easily discriminated between 0 and 2 h. Up to 8 h, they gathered around a same order of magnitude, in the range [27-35 %] (theophylline release) for 75:25 ratio, and [35-40 %] for 80:20 ratio. The following rates of inulin (15 % to 30 %) presented a better curves separation for 75-25 ratio.

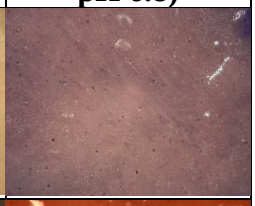
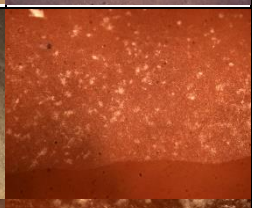
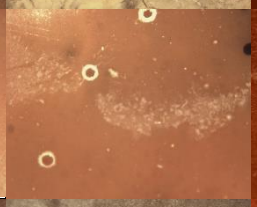
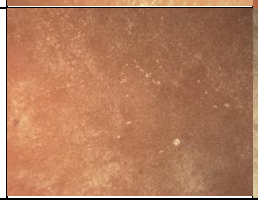
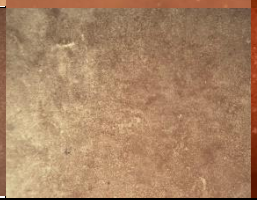
Unlike 75:25 and 80:20 blend ratios, 90:10 (figure 36a) ratio showed another type of behavior: all graphs could be discriminated as long as inulin amount rose, and the maximal drug release rate was 77 %. Even the lowest adjunctions of inulin (2 %, 5 %, 10 %) could display separated curves.

Interestingly, inulin had a louder impact over drug release when film coatings were rather hydrophobic. This could be noticed with 90:10 film coating and its evolution if inulin was added. The improved hydrophilicity would be more visible from a more hydrophobic film. Graph d represents drug release kinetic from each blend ratio without added inulin. As expected, 90:10 ratio was the more hydrophobic film coating, owing to the presence of 90 % EC in the composition. Yet, this film coating was the easiest to modify as for its permeability. Considering that polymeric films are expected to show a burst effect when placed into an aqueous medium, we can state that the influence of inulin does not sharply harm the controlled release properties of these polymeric films. Drug release will take more time in case of mini-tablets.

These films properties provided a good estimation as for their use onto mini-tablets, as the drug undergoes a longer pathway to cross from the inner core to the outer bulk medium. These polymeric films can withstand their transit through the upper GIT and may allow for *in situ* microbial degradation. Regarding these observations, same compositions and blend ratios were tested as coating layers onto mini-tablets.

This optimization step will correspond to the second part of tablets experiments, in chapter III.2.4. The following pictures represent polymeric films before (T_0) and after incubation in the upper GIT (T_{8h}) for different rates of inulin added. Magnification was ×30 with a stereoscopic microscope.

a)

80:20 + inulin (%)			
% of inulin	**T=0**	**2h (0.1 N HCl)**	**8h (2h 0.1 N HCl & 6h PBS pH 6.8)**
0			
5			
10			
30			

b)

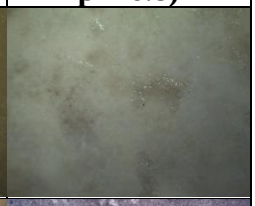
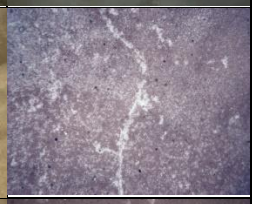
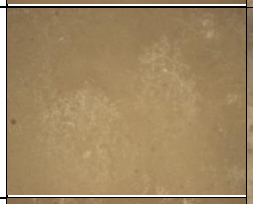
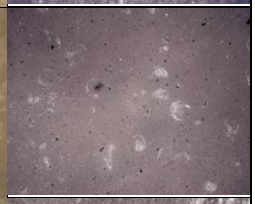
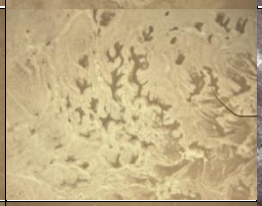
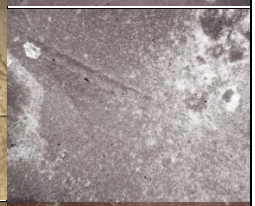
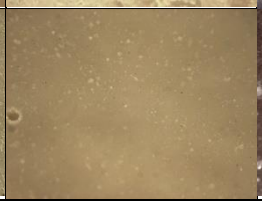
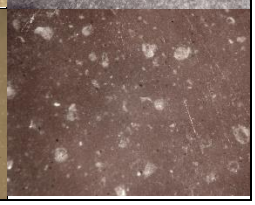
75:25 + inulin (%)			
% of inulin	**T=0**	**2h (0.1 N HCl)**	**8h (2h 0.1 N HCl & 6h PBS pH 6.8)**
0			
5			
10			
25			
30			

Figure 37: Surface morphology of polymeric films based on (EC:shellac) blends with additional inulin before (T_0) and after incubation in the upper GIT (2h HCl 0.1 N followed by 6h PBS 6.8) using ×30 magnification. Blends ratios were a) 80:20 and b) 75:25. The quantities of inulin added are indicated in the left-hand side.

III.1.1.3 Selection of plasticizer

Plasticizers are low molecular weight compounds that intercalate between polymers, break hydrogen bonds, lessen the forces between molecules, reduce the glass transition temperature, increase the mobility of the polymer network, the flexibility, and facilitate their processing [174, 175]. The significant influence of the plasticizer content upon the mechanical properties of polymeric films are known. Generally, the plasticizer increases mechanical stabilities (tensile strength, elongation at break) by increasing the energy required to break thin films in the dry state, at room temperature.
Herein, 25 % plasticizer was chosen because TEC contents below 25 % w/w would compromise the fusion of ethylcellulose nanoparticles and film formation, the mobility of the polymer chains being pivotal at this stage. A sticking propensity during coating and curing (= post-thermal treatment) can appear when TEC contents are higher than 30 % w/w, and should, thus, be avoided [160]. 25 % TEC and glycerol (GLY) were incorporated into films, and 10 % glycerol was also assessed for reasons of comparison.

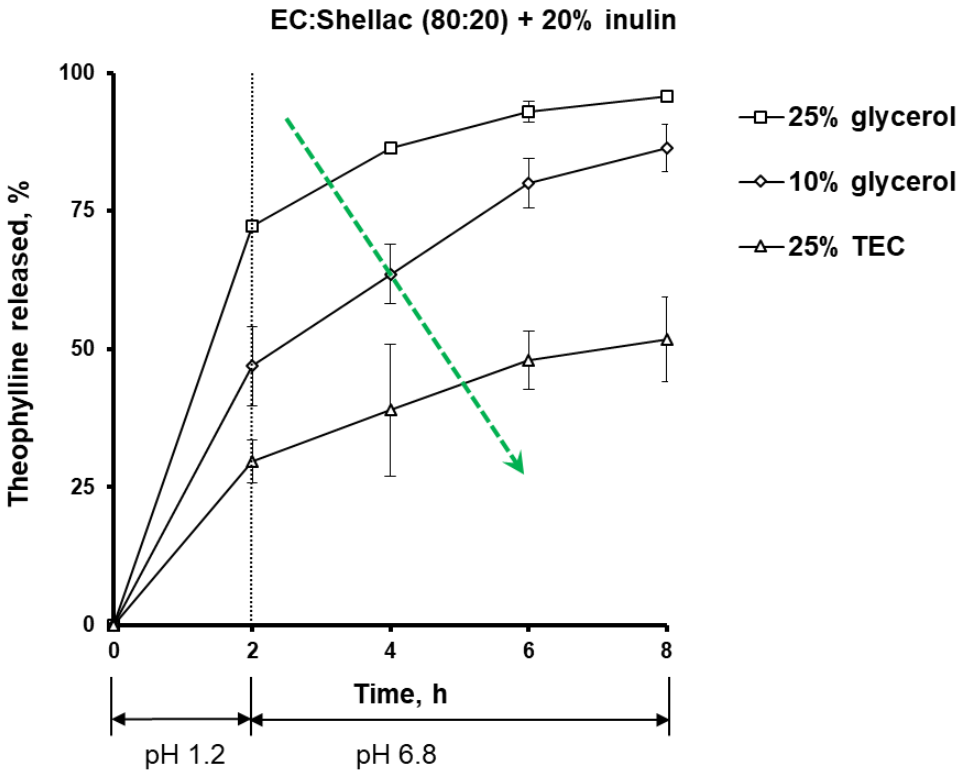


Figure 38: *In vitro* drug release from polymeric films based on EC:Shellac blends + 20 % inulin (w/w, referring to the total mass of films). Different plasticizers were assessed: triethyl citrate (TEC) and glycerol (GLY). Films were incubated in the upper GIT (2h HCl 0.1 N followed by 6h PBS 6.8).

Up to 2 h, we could already notice a sharp difference between 25 % plasticizers. TEC displayed 30 % release *versus* (*vs)* 72 % for glycerol. Glycerol released 3 times more API at this rate, whilst 100 % release were achieved after 8 h.

Unlike glycerol, only partial release could be noticed for 25 % TEC with 52 % (± 7.6 %) of Cmax achieved after complete incubation time. For sake of comparison, 10 % glycerol led to 40 % release after 2 h, and 80 % release after 8 h.

Increasing the plasticizer level favors drug release by increasing the rates and extents of water uptake and dry mass loss, after exposure to simulated digestive fluids. This may lead to a more important drug permeability in the polymeric film and premature drug release [160]. An increment in glycerol concentration is known to increase the solubility in water, moisture content, and film thickness.

Similar findings were observed by Razavi *et al*. and Muscat *et al*., who described that glycerol produced more significant tensile strength reduction than other polyols [176, 177]. Thus, the lower molar mass of glycerol chains may be the cause of this tendency.

In another study, some cellulose acetate films underwent GLY addition in the polymeric matrices and displayed the following increased properties: water vapor permeability, thickness, and opacity. Films employing TEC as a plasticizer led to opposite behavior to those containing GLY [178]. For obvious reasons of controlled release, TEC was kept as the plasticizer to be used for dosage forms.

III.1.1.4 Water uptake and dry mass

i. From EC:Shellac based films

An ideal film coating permitting colon targeting should only absorb small amounts of water at a low rate in both simulated gastric and intestinal fluids, to prevent drug release in the upper GIT. In addition to the water uptake kinetic, dry mass loss behavior of polymeric films is a witness as for the permeability to the drug molecules [163, 164]. Indeed, if a film loses a lot of its dry mass upon exposure to the release media, it may be permeable for many drugs. The permeability of a polymeric film varies with its water content [179]. Increasing water content favors the mobility of macromolecules, the free volumes available for diffusion and, thus, also the mobility of incorporated drug molecules [180]. The following figure shows the gravimetrically measured water uptake of polymeric films based on EC:Shellac blends after incubation in 0.1 N HCl followed by phosphate buffer pH 6.8 at 37 °C.

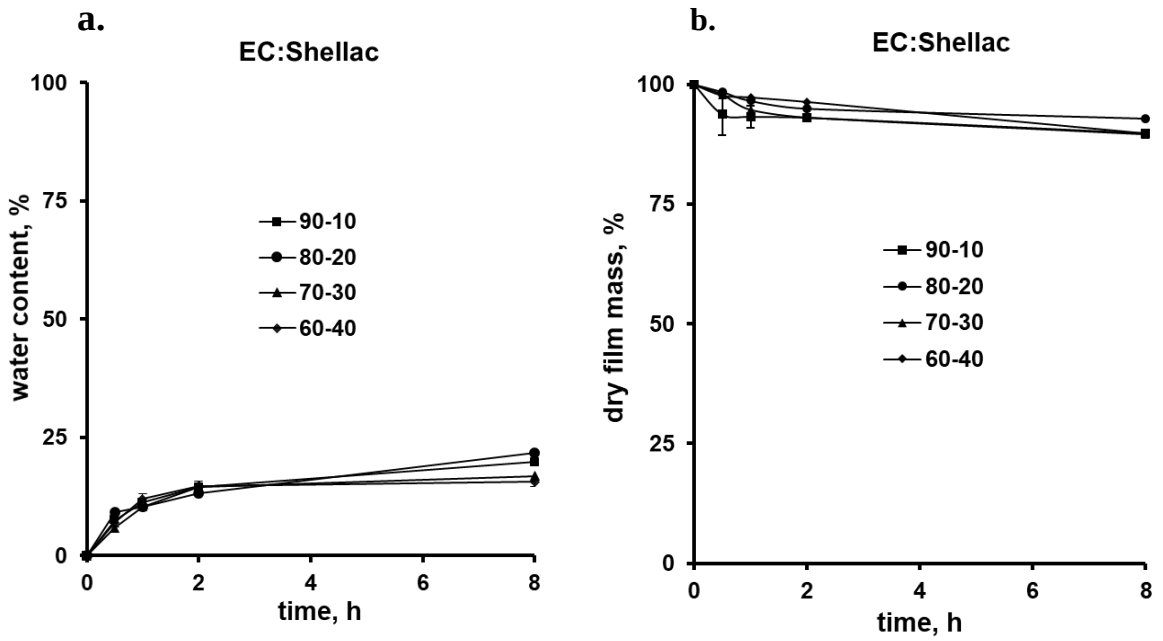


Figure 39: Water uptake (a) and dry mass loss (b) of polymeric films based on EC:Shellac blends after incubation in the upper GIT (2h HCl 0.1 N followed by 6h PBS 6.8). Ratios were: 90-10, 80-20,70-30, 60-40.

Please note that also the diffusion of the water-soluble plasticizer TEC into the aqueous media can contribute to the observed results. Indeed, increasing water contents into the films fosters the mobility of polymeric chains and, thus, the mobility of the low molecular weight plasticizer. The water uptake rates and extents are limited in all case thanks to the presence of the water-

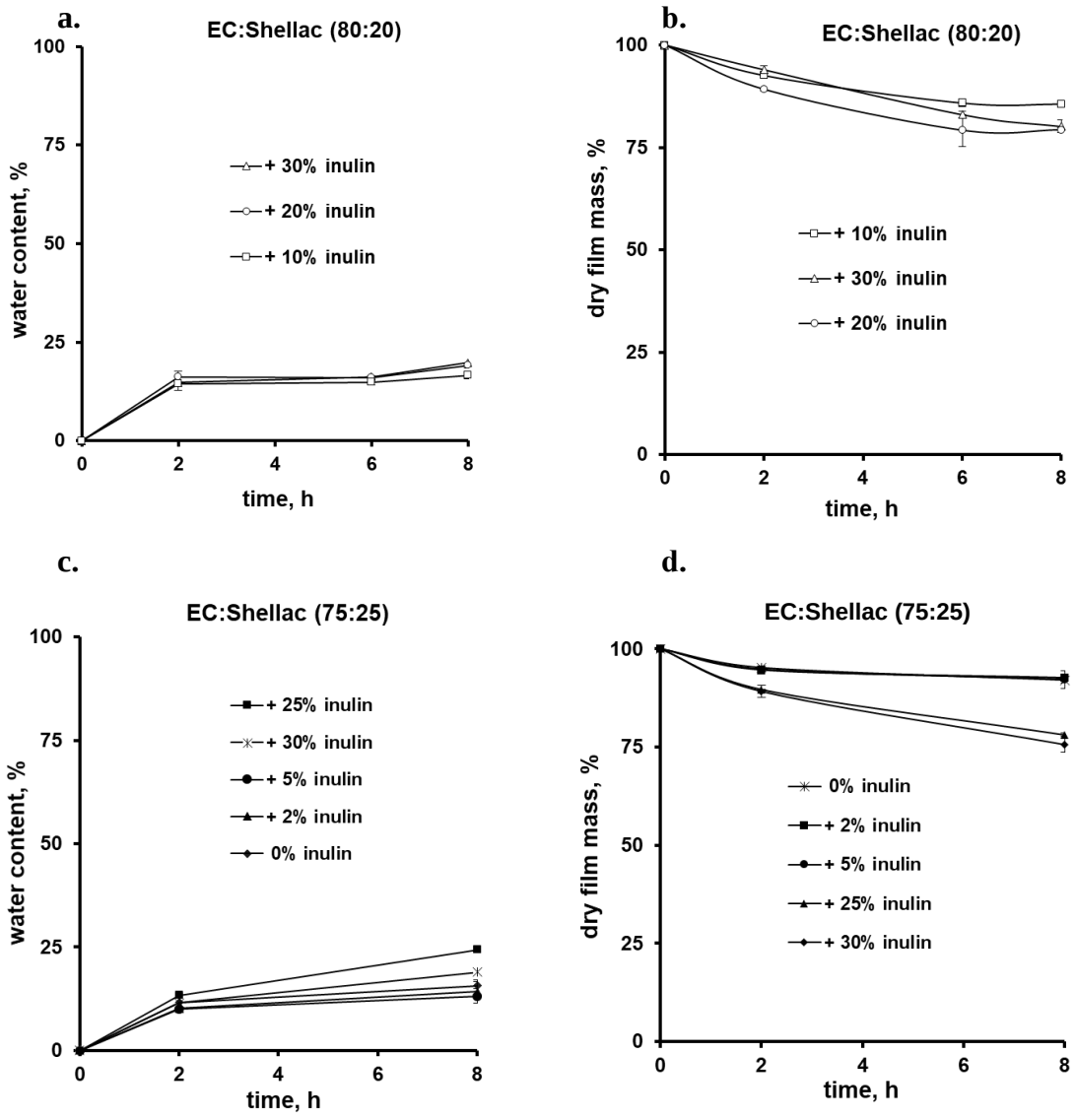
insoluble EC and acid-insoluble shellac. Globally, EC limits premature dissolution of film in the upper GIT. The ideal film coatings should only lose negligible amounts of dry mass (or no mass at all), assuring impermeable and dense polymeric networks with respect to the incorporated drug, in these conditions.

In some systems, polymers can undergo a glassy to rubbery phase transition from a certain water content threshold. This leads to a progressive increase in polymer and drug mobility [160].

ii. From optimized films (*cf.* **films with inulin**)

The investigated polysaccharide (inulin) is water-soluble and provides the partial sensitivity of the coating and its permeability to drug.

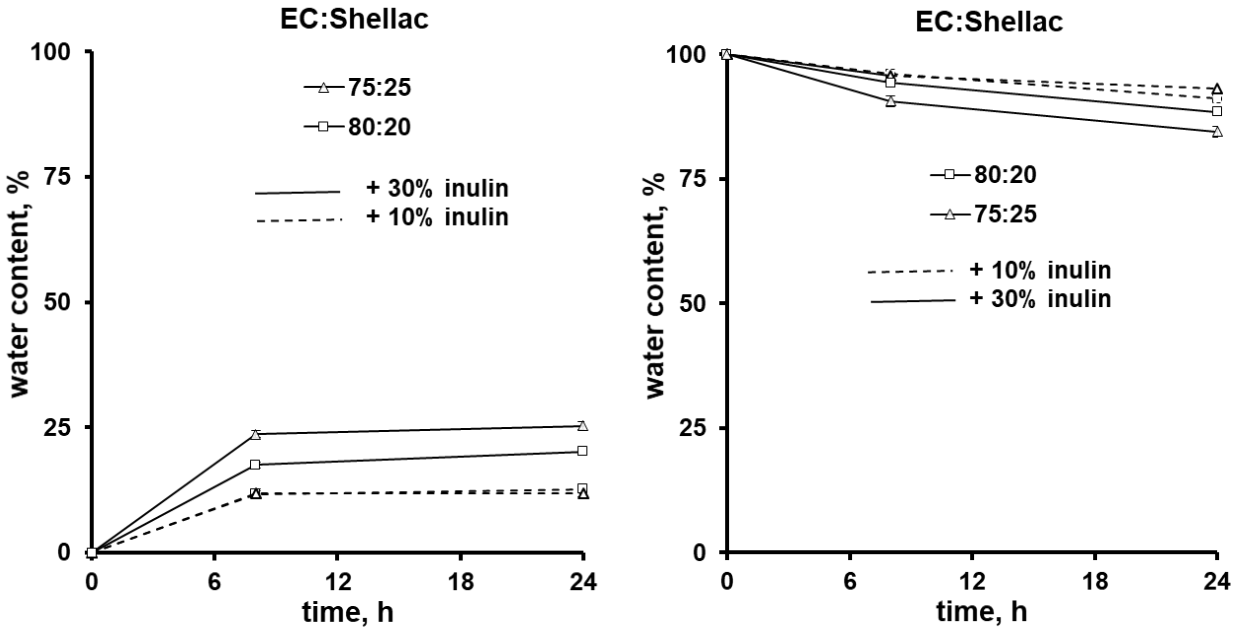
It may be permeable for many drugs, namely those with a low molecular weight such as theophylline (180,17 Da).



The polymeric blend ratios accelerated the water uptake rates and extents. The amount of water increased with the augmentation of inulin. This can be due to the hydrophilic and hygroscopic nature of inulin, facilitating the absorption of water within the matricial film. The mobility of drug molecule within this type of polymeric films was clearly promoted by the rising addition of inulin. As for dry mass loss, this can be due to the leaching of this water-soluble polysaccharide in bulk fluids. Please note that even the most important water uptake rates and extents of the different films are quite low. Drug release in the upper GIT can be objectively expected to be limited with such films, used as coating layers onto tablets. The loss of TEC into the bulk fluids can be expected to be more important in films containing 30 % (w/w) water-soluble inulin as compared to pure EC:Shellac films, because of the expanded water uptake and extents of the blend, leading to higher polymer chains mobility.

It is important to note that these results were obtained in the absence of any enzymes. The degradability of certain polysaccharides by pancreatic enzymes (amylase, protease, lipase) is known and should induce higher water uptake and dry mass loss *in vivo*.

The following figures represent the same experiments along 24h-release (2 h HCl 0.1 N followed by 22 h PBS 6.8) without bacteria.



The outcome was more important with 75:25 blend ratio, above all with 30 % added inulin, rendering the system more hydrophilic. Water uptake study showed 25 % water content for 75:25 blend ratio *vs* 20 % for 80:20 ratio. At this stage, the mean thickness of film might be a limit for 24h-release study, and it should be more relevant to test the same experiment, but with oral dosage forms.

III.2 From coated mini-tablets: upper and entire GIT

III.2.1 Reminder

Mini-tablets designate solid dosage forms with a diameter inferior or equal to 3-4 mm and divided into smaller fractions of conventional tablets. The use of numerous punches is the only difference from typical tablets production processes. They present an advantage in patients with swallowing disabilities and receiving various drug treatment. They reduce the variation in drug release profile and ensure a more effective treatment [181].

III.2.2 Quality control of mini-tablet cores

Quality control of mini-tablet cores was achieved in accordance with Ph. Eur. 11.1. The following ensemble of figures (gathered as figure 40) displays the different quality tests performed and also highlights the conformity of mini-tablets. The latter were conform to Ph. Eur. 11.1 and could be used for coating process.

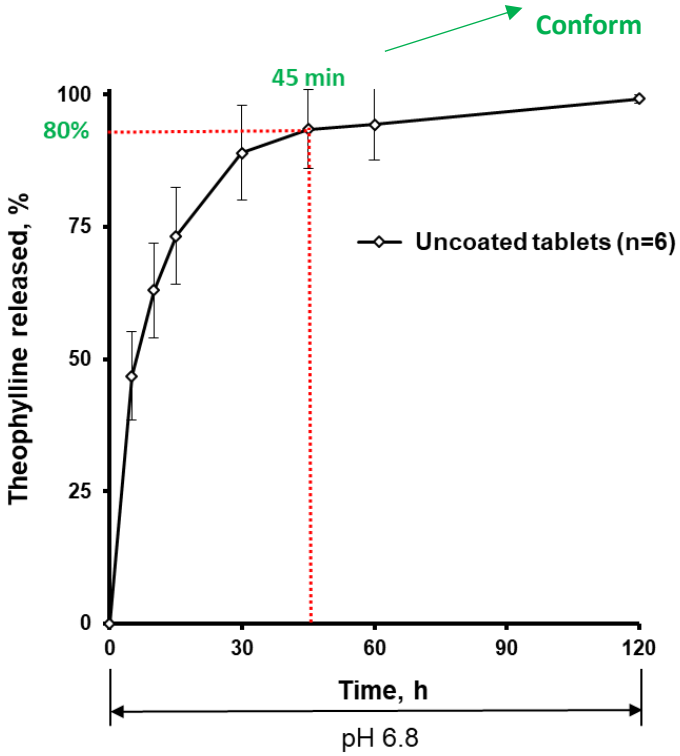
a) Uniformity of mass of uncoated tablets

tablet n°	mass, mg
# 1	61.2
# 2	60.9
# 3	52.1
# 4	54.7
# 5	51.8
# 6	52.3
# 7	54.2
# 8	55.4
# 9	55.2
# 10	55.2
# 11	56.9
# 12	55.4
# 13	52.7
# 14	55.7
# 15	52.2
# 16	61.7
# 17	60.5
# 18	50.8
# 19	57.9
# 20	52
mean **sd** **min** **max**	55.4 3.3 **< 10 %** 51.8 61.7
mean +10% **mean -10%** **nb of tablets out of specification**	61 49.9 ⇩ **2 Conform**

b) Uniformity of drug content of uncoated tablets

tablet n°	drug content, mg
# 1	3.81
# 2	3.28
# 3	3.23
# 4	3.54
# 5	3.71
# 6	3.39
# 7	4.02
# 8	3.30
# 9	3.71
# 10	3.41
mean **sd** **min** **max**	3.54 0.25 3.23 4.02
85% mean **115% mean**	3.01 4.07 ⇩ **Conform**

c) Dissolution test of uncoated tablets



Quality control of uncoated mini-tablets according to Ph. Eur. 11.1: a) mass uniformity, b) drug content uniformity, c) dissolution test.

d) Hardness of uncoated tablets

tablet n°	hardness, N
# 1	35
# 2	25
# 3	25
# 4	36
# 5	40
# 6	34
# 7	35
# 8	34
# 9	29
# 10	34
# 11	29
# 12	35
# 13	31
# 14	39
# 15	34
# 16	23
# 17	26
# 18	30
# 19	25
# 20	37
mean	31.8
sd	4.92 **< 5 %**
min	23
max	40

e) Dimensions of uncoated tablets

tablet n°	diameter, mm	height, mm
# 1	5.069	3.157
# 2	5.089	3.709
# 3	5.078	3.048
# 4	5.037	2.975
# 5	5.041	2.901
# 6	5.023	3.016
# 7	5.069	3.424
# 8	5.066	3.225
# 9	5.069	3.210
# 10	5.068	3.145
# 11	5.076	3.210
# 12	5.039	2.990
# 13	5.078	3.161
# 14	5.045	2.942
# 15	5.076	3.143
# 16	5.076	3.654
# 17	5.065	3.510
# 18	5.071	3.150
# 19	5.077	3.267
# 20	5.047	2.922
mean	5.06	3.19
sd	0.017 **< 5 %**	0.22
min	5.023	2.901
max	5.089	3.709

Quality control of uncoated mini-tablets according to Ph. Eur. 11.1: d) hardness, e) dimensions of uncoated tablets.

f) Friability of uncoated tablets

initial tablets weight, mg	m_0=6.5145g
tablets weight after test, mg	m_t=6.5103g
friability, % according to (1)	0.06 % **< 1 % ⟹ Conform**

(1) Friability = $\frac{m0-mt}{m0} * 100$ = 0.06 %

g) Disintegration of uncoated tablets

number of tablets	n=6
time of disintegration	1 min ⟹ **Conform**

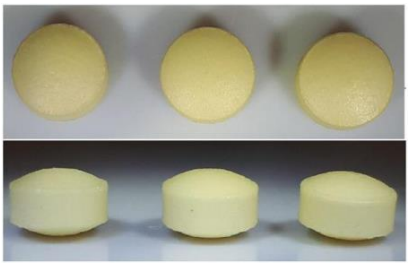
Figure 40: Quality control of uncoated mini-tablets according to Ph. Eur. 11.1. Tests were:

a) Uniformity of mass;
b) Uniformity of drug content;
c) Dissolution;
d) Hardness;
e) Dimensions;
f) Friability;
g) Disintegration.

III.2.3 Mini-tablets coated with EC-Shellac blends

coating process:

- 4 g/min spray rate
- 25 rpm



Before any test with mini-tablets, their quality control was carried out in accordance with the latest version of the European Pharmacopeia (Ph. Eur. 11.1). Uncoated mini-tablets complied with Ph. Eur. 11.1 and could therefore be used for film coating process.

Ratios and coating levels were compared to better appreciate the impact of each of the latter upon theophylline release, in these conditions. Also, to select the best candidate for colon targeting. Please note that coating level, also called weight gain, corresponds to the tablet weight after the coating, respective to uncoated tablet weight. The increase in coating level leads to a thicker and denser coating material around the tablet core, which increases the diffusion pathway. 40 g of mini-tablets were withdrawn at predetermined time points during coating process, for each coating level indicated in the diagrams. The following EC:Shellac ratios were chosen: (80:20); (75:25); (60:40); and the coating levels were: 10, 12, 13.5, 15, 18, 20, 25, 30, 35 %, depending on the polymer:polymer ratio.

Afterwards, mini-tablets were immersed in 0.1 M HCl for 2 h, and then in phosphate buffer pH 6.8 for 6 h, thus simulating the upper GIT. Drug release was quantified spectrophotometrically (UV-Visible) in both these media.

As a first point of view, Figure 41 below displays theophylline release as a function of blend ratios for different coating levels (15 %; 20 %; 25 %; 30 %) in the upper GIT. This compilation permitted to assess drug release evolution with increasing EC fraction, and to pick the best candidate for colon targeting. We could also evaluate the influence of weight gain (= coating level), irrespective of the blend ratios. We can easily appreciate the variation in drug release with increasing coating level. 30 % coating level (figure 41d) revealed slightly reduced drug release amounts, notably with (60:40) et (75:25) ratios, exhibiting 14 % and 4 % of Cmax, respectively.

The drug release rate is inversely proportional to the amount of coating around tablet cores. The thicker the film coating, the slower the release rate [182]. The mechanism obeys the theory of diffusion applicable to reservoir-type systems. Several release rates may be obtained with the same formulation by adjusting the level of coating and hence altering the diffusional path length. A bigger coating level would lead to thicker and denser film coating, hence inhibiting the easy and quickly formation of pores, and subsequently the diffusion of drug in the outer medium. This phenomenon can be seen as advantageous as for the protection of drug in the upper GIT, but disadvantageous for drug delivery into the colon.

One can state that gastro-resistance was achieved. According to Pharmacopeial criteria for delayed release, gastro-resistance corresponds to less than < 10 % release after 2 h in pH 1.2

[139]. The variation in kinetic profiles according to blend ratios (80:20, 75:25, 60:40) will be discussed afterwards.

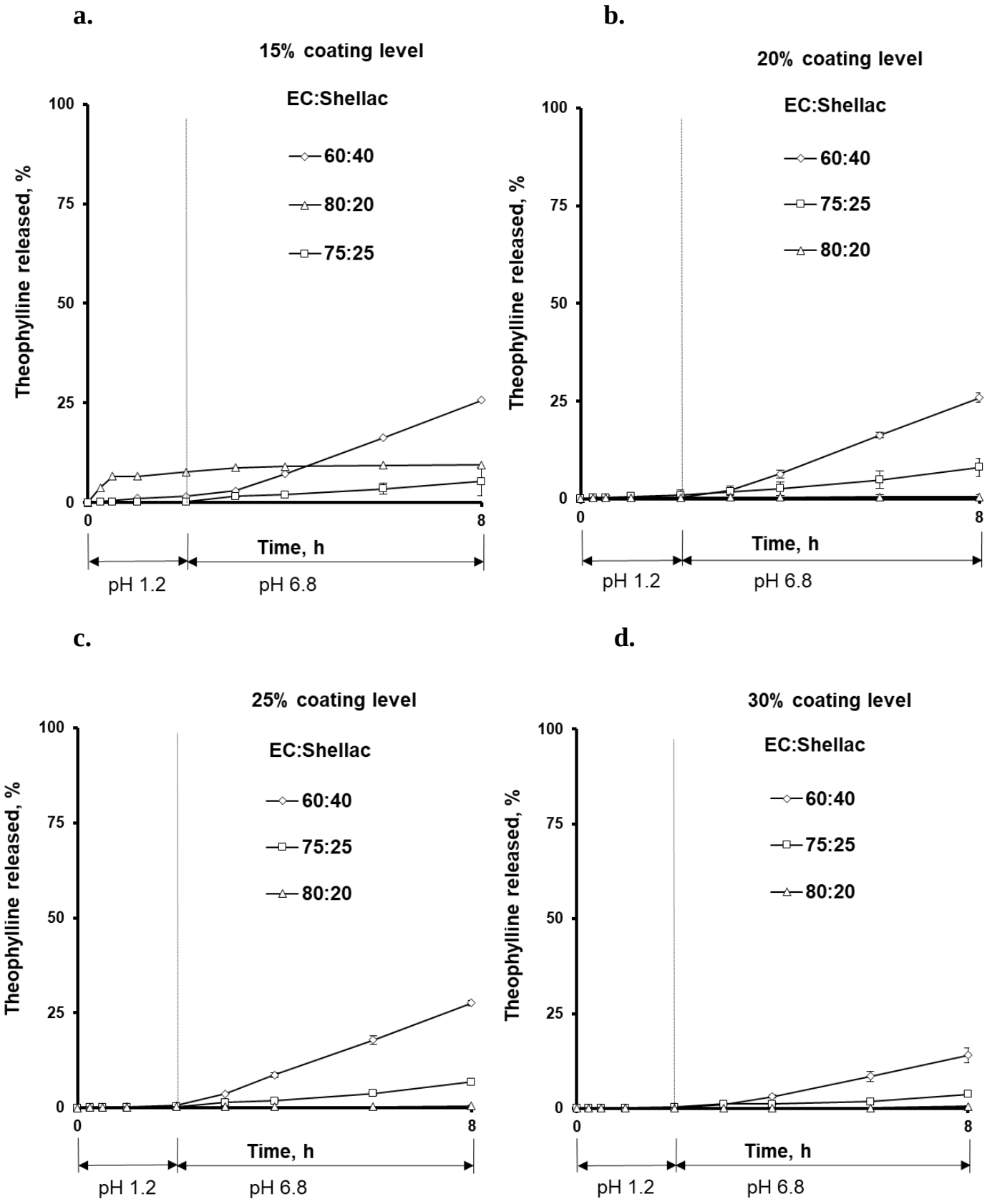


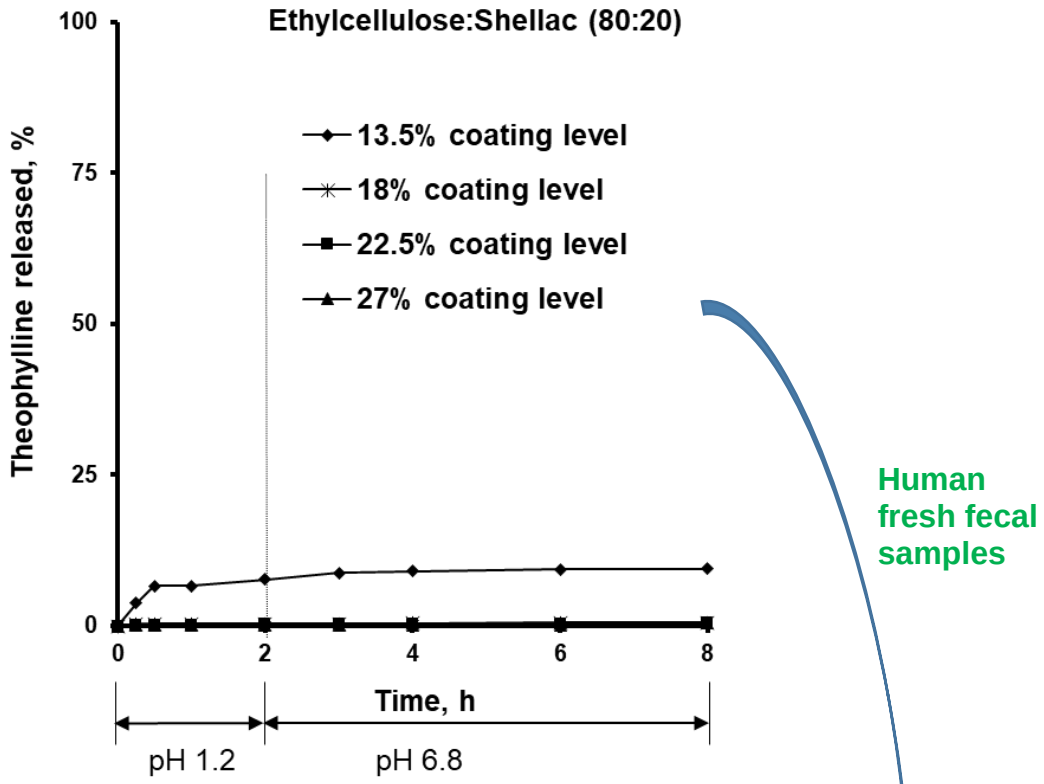
Figure 41: Impact of the blend ratio (EC:Shellac) on *in vitro* theophylline release from coated mini-tablets incubated in 0.1 M HCl (2h) followed by phosphate buffer pH 6.8 (6h) using different coating levels: a) 15 %, b) 20 %, c) 25 %, and d) 30 %.

Figures 42, 44 and 46 illustrate the *in vitro* drug release kinetic of theophylline from mini-tablets coated with EC:Shellac blends at different ratios (80:20; 75:25; 60:40, respectively) and coating levels. As mentioned previously, mini-tablets were first immersed for 2 h in 0.1 M HCl, then in phosphate buffer pH 6.8 for 6 h, to simulate the upper GIT.

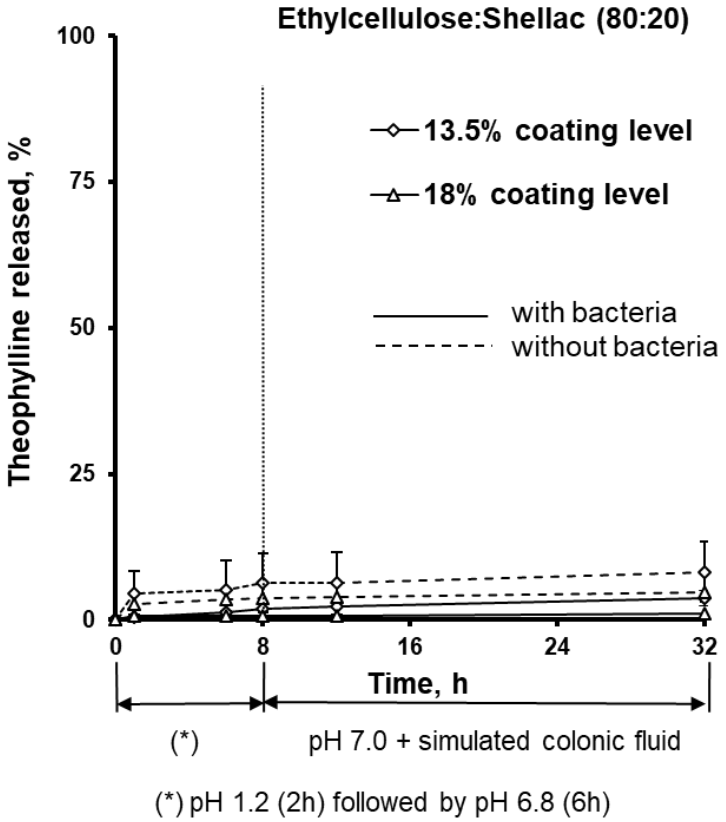
To reproduce the entire GIT, same solid dosage forms were incubated in the upper GIT (as quoted above) before subsequent incubation in culture medium with and without fresh feces samples, for reasons of comparison. Culture medium containing feces constitutes simulated colonic fluid (SCF).

An ideal microbially-triggered system for prolonged release should protect the drug in the upper GIT and release it in the distal part of the GIT (*e.g.*, ileocecal, and colonic region) due to the presence of the microbiota.

a) **Upper GIT**



b) **Entire GIT**



(*) pH 1.2 (2h) followed by pH 6.8 (6h)

Figure 42: Impact of the coating level on *in vitro* theophylline release from mini-tablets coated with Ethylcellulose:Shellac blend (80:20) upon exposure to (a) the upper GIT: 0.1 M HCl (2h) followed by phosphate buffer pH 6.8 (6h), and (b) the entire GIT: simulated gastric and intestinal fluids followed by culture medium inoculated with or without fresh fecal samples (24h), for reasons of comparison.

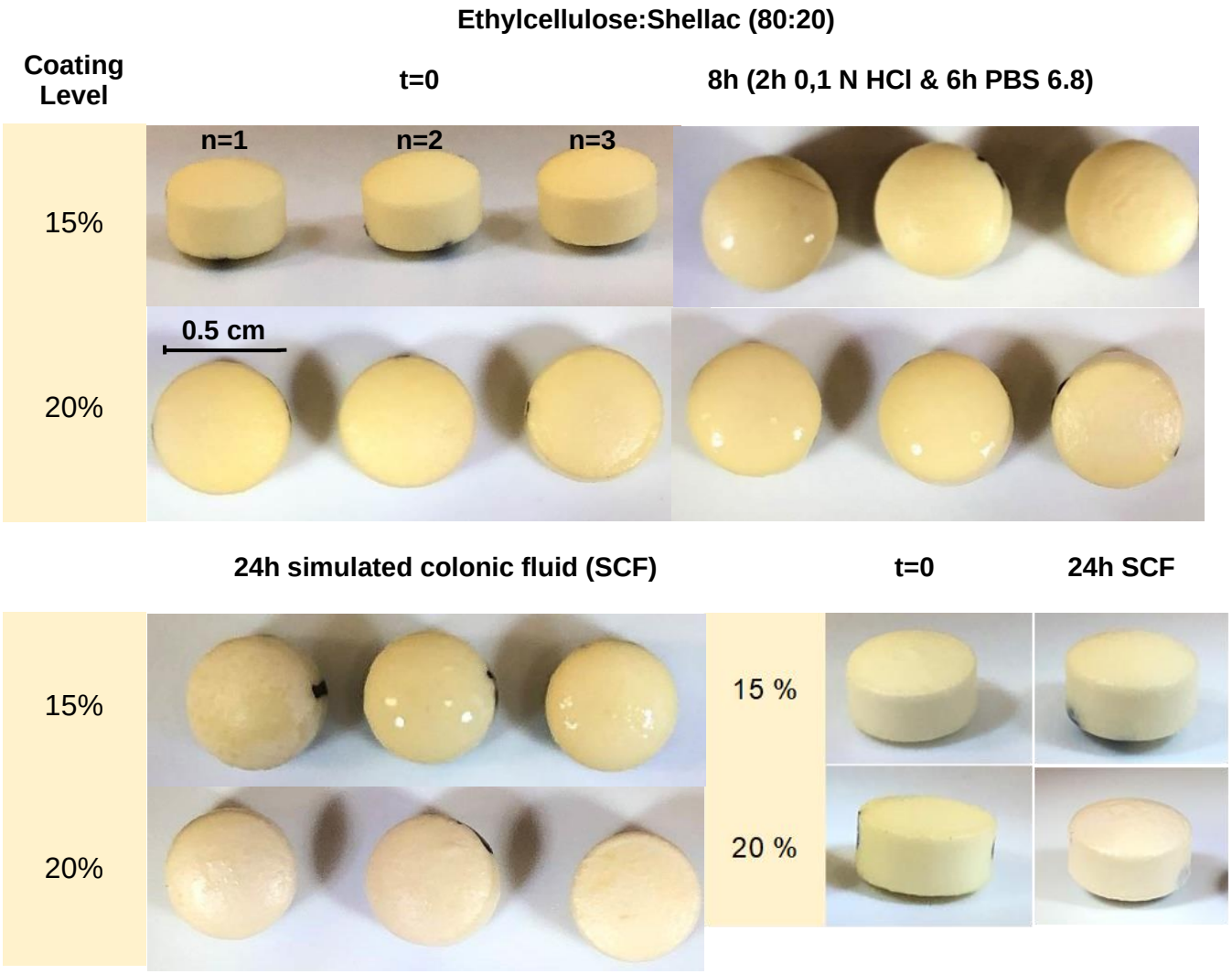
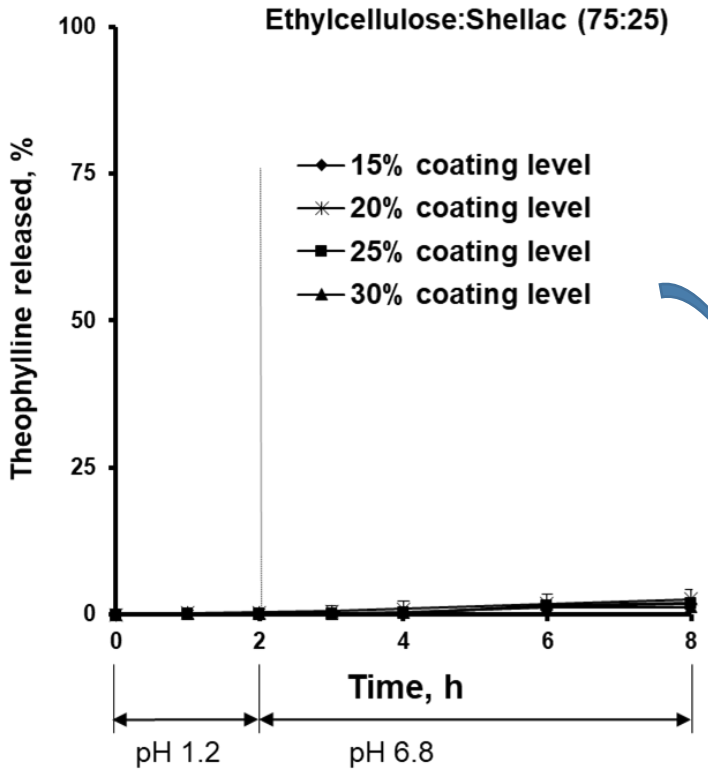


Figure 43: Macroscopic pictures of mini-tablets coated with Ethylcellulose:Shellac blend (80:20). The coating levels are indicated on the left-hand side. The potential exposure to the release media is indicated at the top.

Concerning 80:20 (EC:Shellac) blend ratio, 13.5 to 27 % coating levels were investigated. Please note that the following coating levels were initially desired: 15 %, 20 %, 25 %, 30 %. A mistake occurred with the calculation of volumes to spray and explains these values. As we can see on figure 42, negligible amounts of API were released in simulated gastric and intestinal fluids up to 8 h (figure 42a). Clearly, 3 out of 4 of the investigated coating levels did not deliver drug molecules at all. The curves were confounded with the abscissa. Only the lowest coating level (13.5 %) revealed 9,5 % release, and this might be rather due to the single aberrant value registered. The other tablets belonging to this coating level cracked prematurely and did not permit to get a mean value and a standard deviation. The protection of drug was total under these conditions, and both gastric and enteric resistance were achieved. Same outcomes could be observed despite 24 further hours into colonic medium. There was no significant difference in drug release profile whether in the presence or in the absence of fecal samples up to 32 h (figure 42b). A good estimation from films to mini-tablets could be affirmed in terms of protection and permeability.

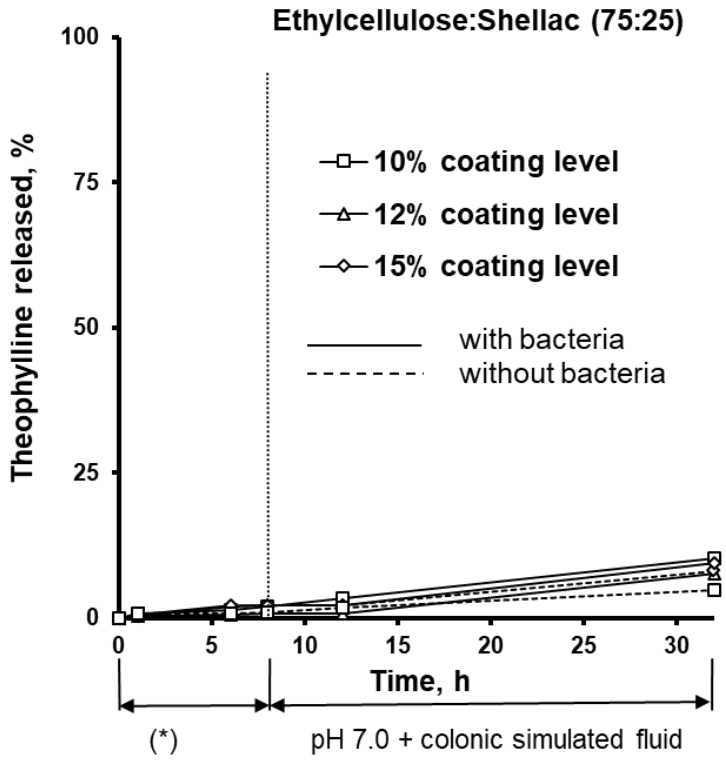
The next blend ratio (75:25) shew the same trend, with a total protection of the API up to 8 h (figure 44a) as well as 32 h (figure 44b).

a) Upper GIT



Human fresh fecal samples

b) Entire GIT



(*) pH 1.2 (2h) followed by pH 6.8 (6h)

Figure 44: Impact of the coating level on *in vitro* theophylline release from mini-tablets coated with Ethylcellulose:Shellac blend (75:25) upon exposure to (a) the upper GIT: 0.1 M HCl (2h) followed by phosphate buffer pH 6.8 (6h), and (b) the entire GIT: simulated gastric and intestinal fluids followed by culture medium inoculated with or without fresh fecal samples (24h), for reasons of comparison.

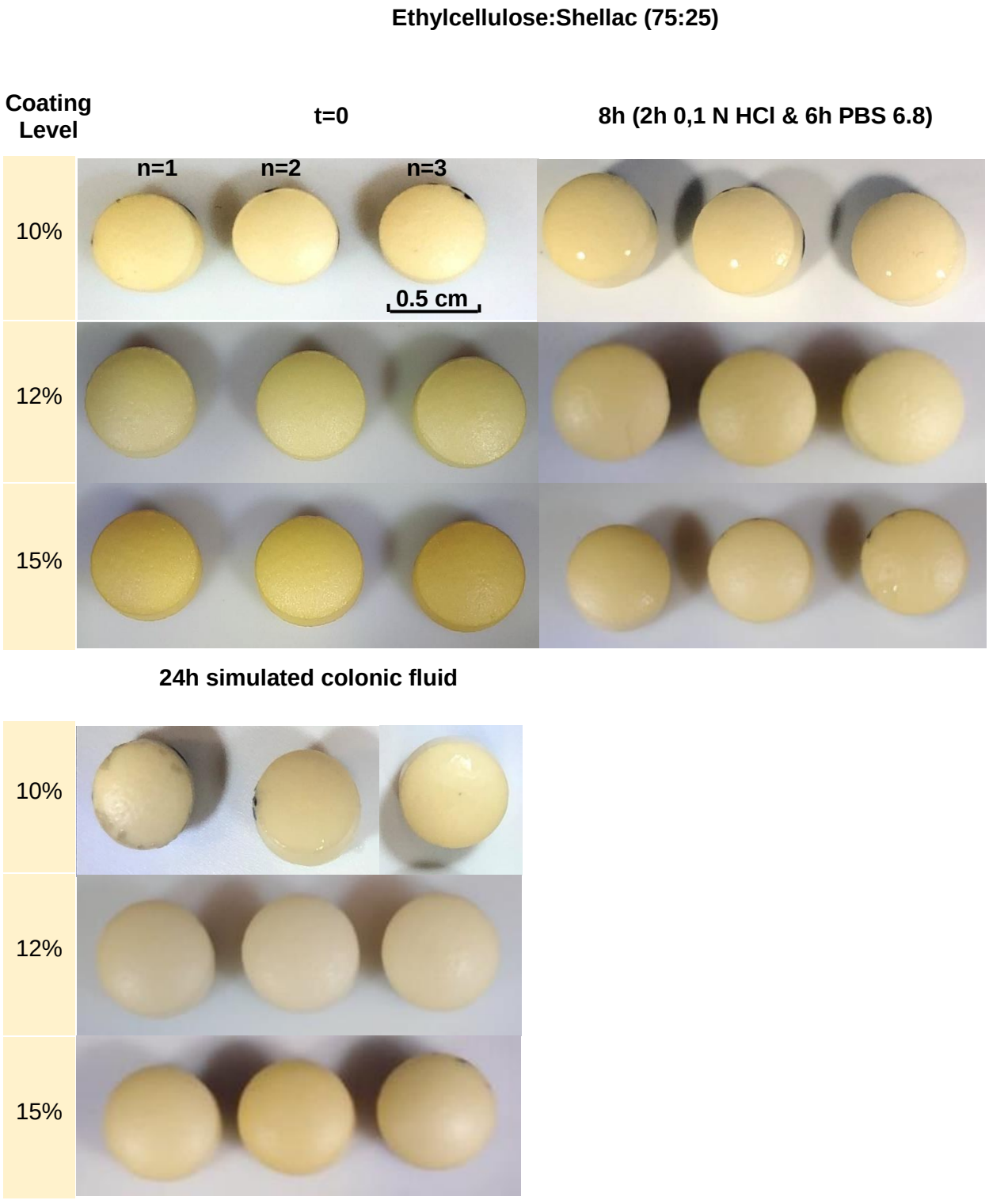


Figure 45: Macroscopic pictures of mini-tablets coated with Ethylcellulose:Shellac blend (75:25). The coating levels are indicated on the left-hand side. The potential exposure to the release media is indicated at the top.

Only a slight onset of diffusion was observed after 24 h of incubation in simulated colonic medium, with 9,5 % theophylline release. It can be argued that both (80:20) and (75:25) ratios were fully reliable as enteric coatings for colon targeted delivery, under these conditions.

It has to be pointed out that 24 h as colonic incubation time was decided for the following reasons : (i) to let sufficient time for bacterial cell proliferation (long process), ensuring a better exploitation of microbiota *in vitro*, (ii) to be closer to colonic transit time, ranging from 18 h to 34 h, as well as varying from one to another individual (depending on sex, age, healthy or sick state, etc.) [183].

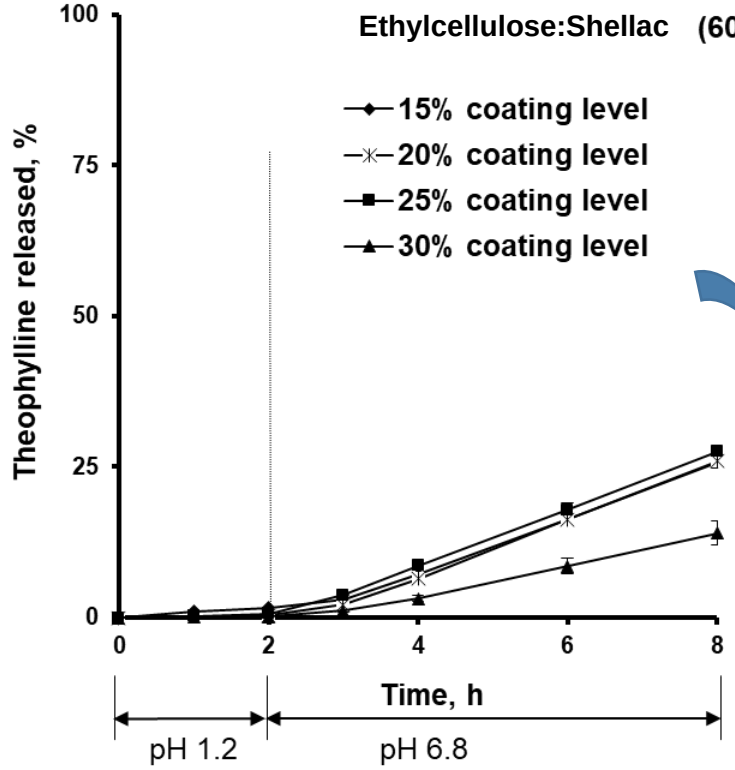
Though these systems provided a perfect protection within the upper GIT, no triggering action occurred in the lower GIT, even in the presence of the microbiota. Drug diffused by a slow time-controlled manner. These formulations afforded these very advantages: (1) suitable for lower-dose therapies, (2) easy to administer.

These outcomes agreed with literature: shellac-based coatings resist in the stomach as long as their pH threshold (> 7.0) is not reached. Shellac layers enable drugs to be delivered into the colonic area for a topical action [184–187]

As a comparison, Karrout *et al*. [188] studied *in vitro* 5-ASA release from commercial dosage forms. They brought out premature 5-ASA release from Pentasa® pellets and Asacol® capsules in the upper GIT. Numerous reports in the literature confirmed this peculiarity [189, 190].

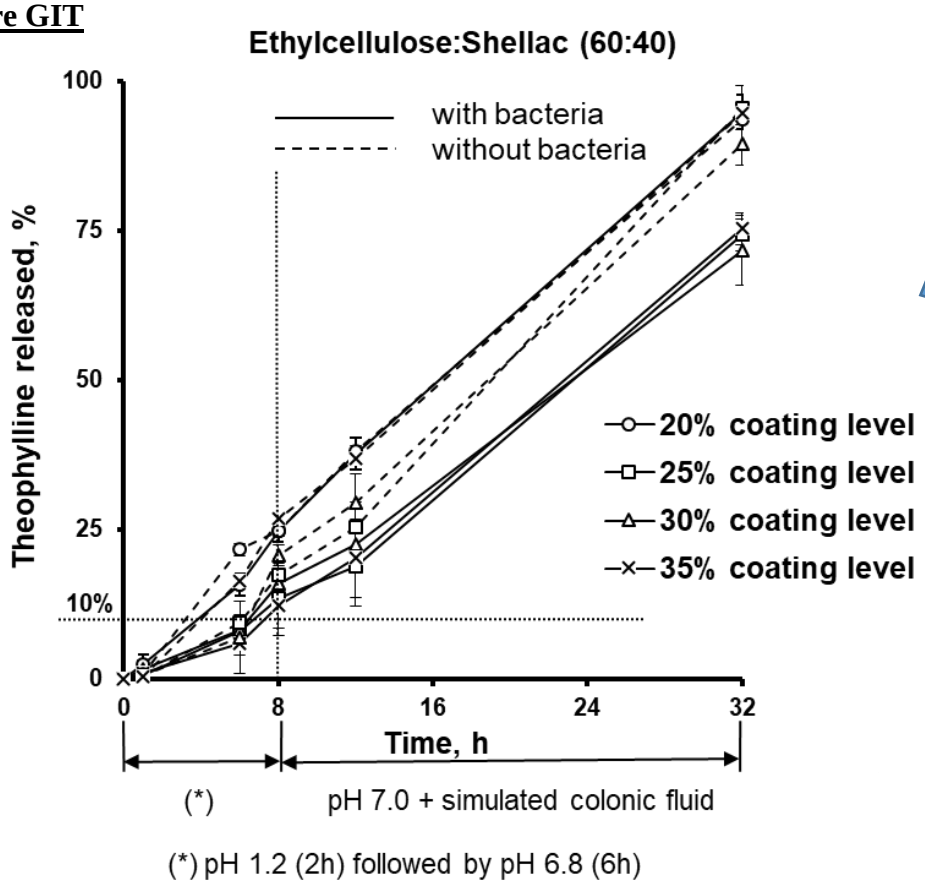
Figure 46 below presents *in vitro* theophylline release from mini-tablets coated with (60:40) blend ratio.

a) Upper GIT



Human fresh fecal samples

b) Entire GIT



(*) pH 1.2 (2h) followed by pH 6.8 (6h)

Figure 46: Impact of the coating level on *in vitro* theophylline release from mini-tablets coated with Ethylcellulose:Shellac blend (60:40) upon exposure to (a) the upper GIT: 0.1 M HCl (2h) followed by phosphate buffer pH 6.8 (6h), and (b) the entire GIT: simulated gastric and intestinal fluids followed by culture medium inoculated with or without fresh fecal samples (24h), for reasons of comparison.

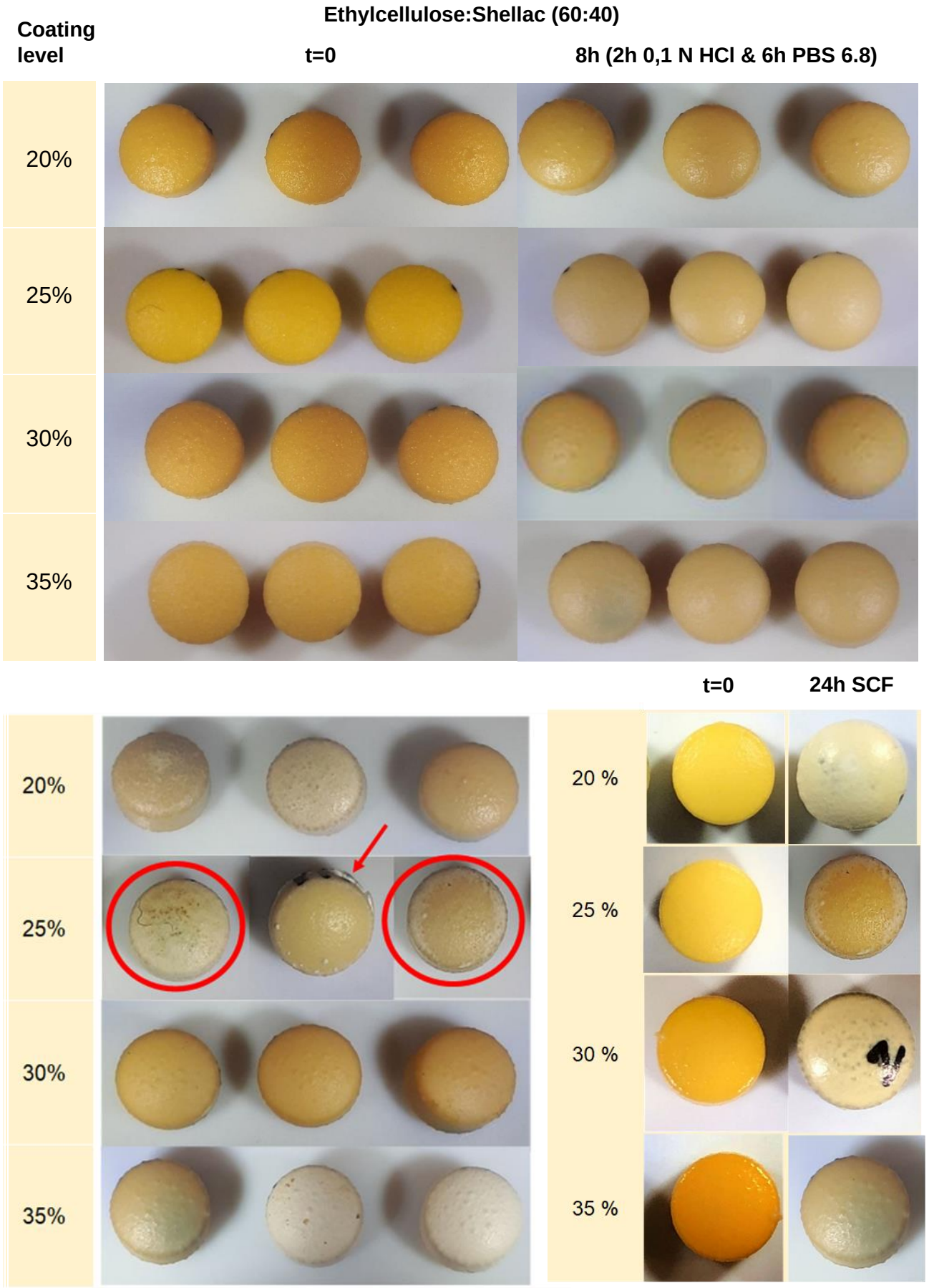


Figure 47: Macroscopic pictures of mini-tablets coated with Ethylcellulose:Shellac blend (60:40). The coating levels are indicated on the left-hand side. The potential exposure to the release media is indicated at the top.

After incubation in the upper GIT (Figure 46a), drug release evolved as a function of coating level. Please note that mini-tablets were selected for tests in colonic medium only when they released very low amounts of drug in the upper GIT, for each exploited formulation. For example, each of (75:25) and (60:40) blend ratios were tested with different coating levels from each other throughout the entire GIT. The aim was to adjust the appropriate coating level permitting prolonged drug delivery until degradation in the distal GIT.

Shellac is not dissolved at pH 1.2 and has good barrier functions, allowing for gastric resistance. Mini-tablets coated with 30 % weight gain most resisted, with only 14 % drug released after 8 h incubation.

All other coating levels (15 %, 20 % and 25 %) achieved around 30 % of Cmax. The ethylcellulose fraction dominated water uptake and dry mass loss, as we observed with polymeric films in the first part (chapter III.1.1). The influence of EC upon drug release is noteworthy. In such conditions, lowering EC fraction in aid of shellac reduced the overall hydrophobicity of the coating, as we could observe with 60:40 (EC:Shellac) polymeric film (figure 31, graph K).

In figure 46b, the same experience was followed by incubation in simulated colonic fluid for 24 more hours. As in figure 46a, 20 % coating level (full line) released 27 % of Cmax after 8 h (corresponding to the upper GIT). This was more important than the other coating levels. Almost the whole amount of API diffused after 32 h, unlike the others, attaining by 72 % drug release (coating levels and ratios are designated in the diagram). In all conditions, no significant difference in drug release was visible as well with bacteria as without bacteria. Shellac is not degraded by bacterial enzymes and cannot serve as a substrate of microbiota. Only passive diffusion of drug occurred through the coating layer, rising as long as shellac fraction increased in the blend ratio. A different color of mini-tablets along time could be noticed in figure 47. After 24 h in simulated colonic fluids, mini-tablets turned white, namely 35 % coating level (bottom line of figure 47). This could be due to the reduced fraction of ethylcellulose and, thus, to less hydrophobicity. The most important shellac content for this ratio provided the darker orange tint. Shellac has good film-forming properties and is exploited as enteric coating, making this polymer reliable for colon targeting [170, 191].

Concerning its molecular structure, shellac is made of cyclic terpene acids with carboxyl groups on, providing weak acid properties. Depending on the type and grade, the pKa can vary between 5.6 and 7.0 [192, 193].

When pH < pKa, carboxyl groups are protonated and produce strong intermolecular hydrogen bonding. This makes the shellac polymer tightly organized and leads to a film coating with high modulus of rigidity [170, 171]. As the pH of the surrounding medium increases until it reaches 6.8, carboxylic groups start to dissociate and shellac swells. The total dissolution of shellac occurs around pH 7.3 (6.8 to 7.4) due to its ionization above this pH value.

The different shellac mechanisms reactions are indicated below:

$R - COOH + NH_4^+ + OH^- \longrightarrow R - COO^- + NH_4^+ + H_2O$

(Shellac ammonium salt solution)

After coating and drying

$R - COO^- NH_4+$ (Ammonium shellac salt film)

After ingestion and contact within 0.1 M HCl (pH 1.2)

$R - COOH + NH_4Cl$

The swelling of shellac reduces its barrier functions and allows for water to penetrate the tablet core from the coating layer. In the case of Swanlac® ASL10 solution, two more parameters are interesting: mechanical properties and brittleness.

Combining these features with the global low hydrophobicity of the system helps to understand the kinetic profiles of this composition. The dissolution of shellac is governed by the pKa and the number of carboxyl groups [134]**.** As the proportion of shellac increases, its pH properties predominate upon the overall system and earlier release occurs (Figure 46).

By the high dissolution pH threshold of shellac, the previous different outcomes constitute interesting data. These characteristics permit prolonged and controlled release as well in the upper as in the whole GIT. As a reminder, (80:20) and (75:25) blend ratios illustrated total protection up to 32 h. Moreover, the distal part of the GIT has lower pH in IBD patients, upstream the dissolution pH threshold of shellac.

This phenomenon can be advantageous as for drug protection in the upper part of the GIT, but disadvantageous for drug delivery into the colon.

After incubation till pH 6.8, drug release is related to shellac swelling, and followed by drug diffusion through the coating [187]**.** Though shellac coating resists to acidic pH, little water can penetrate inside of it with aqueous products based on ammonium salts, which were used in this study [171].

Despite the pronounced hydrophobicity of shellac, its proportions must be considered within these drug delivery systems. Indeed, (60:40) ratio seemed to be adapted for colon targeting, with a zero-order kinetic profile, irrespective of bacterial triggering action (Figure 46b). Considering these observations, an optimization step was chosen for the toughest ratios, (80:20) and (75:25). Inulin was added to induce a microbially triggered release. (60:40) ratio was not kept because of its fragility. Drug diffusion towards the surrounding medium is tributary of water uptake and dry mass loss of dosage form, both connected with the quantity and the nature of the polysaccharide added. Drug release from coated mini-tablets with bacteria and pH-dependent polymeric films implies swelling of the coating, erosion, and dissolution of the polysaccharide, leading to pores formation into the coating layer. Numerous drug release patterns could be noticed in this study. Some of them could suppress drug release until 8 h in the upper part of the GIT. Others manifested prolonged drug delivery, whether in the presence or in the absence of microbiota. This characteristic seems appealing for prolonged therapies.

An appropriate equilibrium must be found between too resistant and proper microbially triggered form. Shellac is still a promising material, as it has demonstrated superior performance, as a single-layer coating, over other enteric polymers (Eudragit S100 and cellulose acetate phthalate) [46].

To achieve an enteric release with dissolution from pH 6 with shellac, the use of a water-soluble polymer as a pore-former is usually recommended, *e.g.,* hydroxypropyl methylcellulose (HPMC) [185]. Herein, a natural polysaccharide will be used as a substrate of the microflora for a colonic drug delivery. Polysaccharides are of utmost importance due to their prebiotic and other physiological activities, which can be beneficial for IBD patients [186]. As with polymeric films, inulin was chosen for this stage.

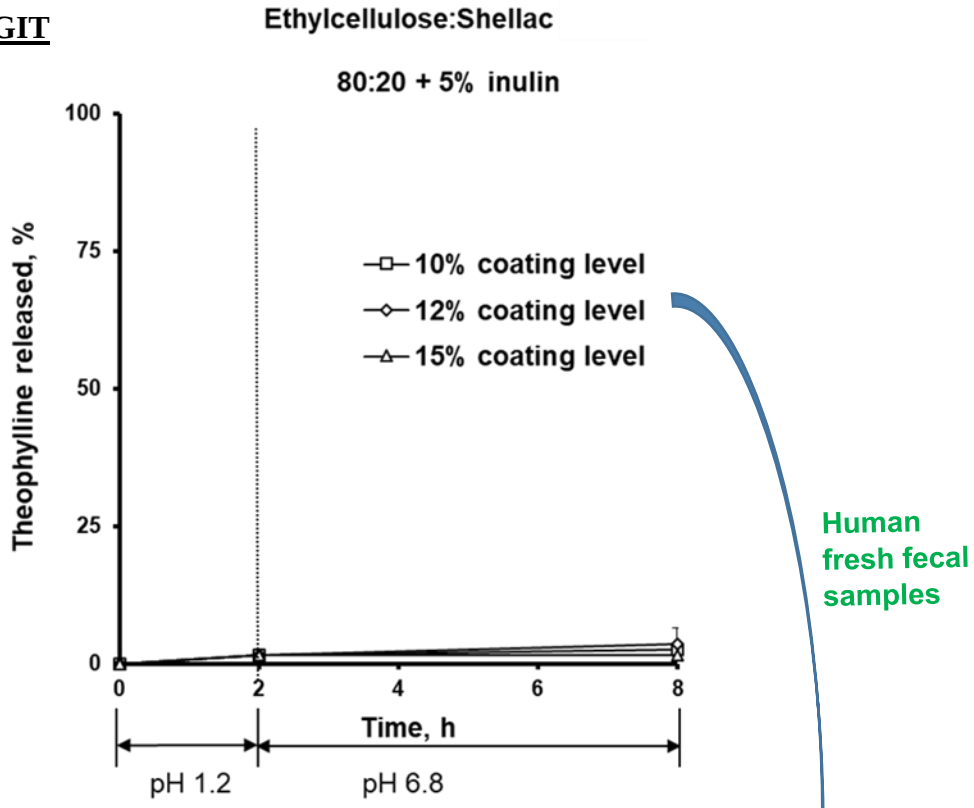
III.2.4 Optimized coated mini-tablets

As studied in the quantitative test, inulin was selected and integrated into polymeric films based on EC:Shellac at different percentages (figure 36). The goal of this experiment was to determine the impact of this biodegradable polysaccharide, as well as the minimal quantity susceptible to be degraded in the distal part of the GIT, without harming its robustness in the upper GIT. This study allowed for a wide range of supplemental amounts that could be included. We concluded that these polymeric films could protect drug in the upper part of GIT, enabling *in situ* degradation, in view of gut microbiota metabolism. Based on these observations, same parameters (compositions and blend ratios) were tested onto mini-tablets. We designed a ternary formulation involving EC, shellac, and inulin, as a coating monolayer. Besides acting as a substrate of microbiota, the presence of inulin would increase hydration level of coating and favors enzymes accessibility to film *via* pores and fissures.

The blend ratios of EC:Shellac used were the same as for the optimized films (described above), namely 80:20 and 75:25.

Estimation from polymeric films to tablets was quite good and led to the following results:

a. Upper GIT



b. Entire GIT

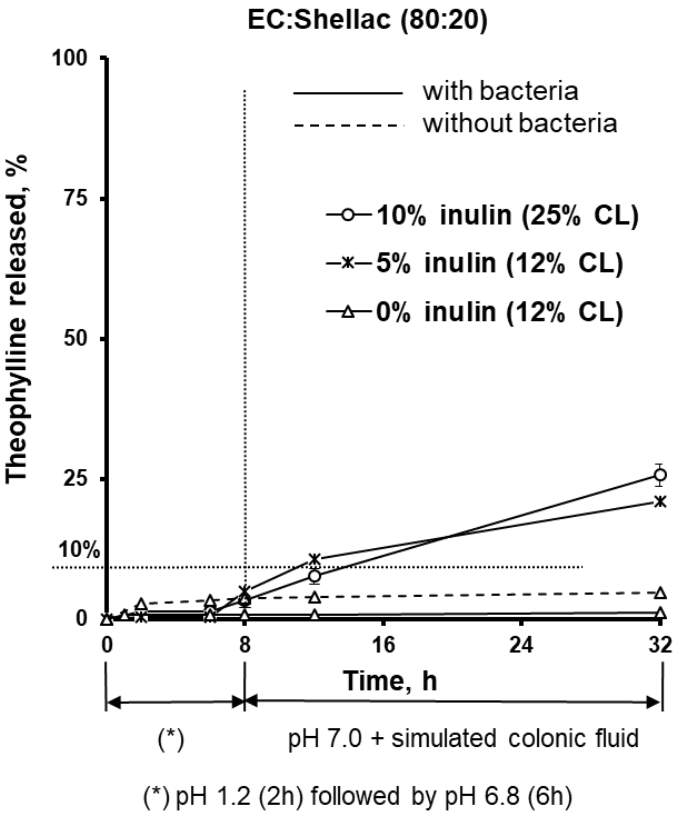


Figure 48: Impact of (a) the coating level on *in vitro* theophylline release from mini-tablets coated with Ethylcellulose:Shellac blend (80:20) containing 5 % inulin added, and (b) the amount of inulin (0, 5, and 10 %) into the film coating. Coated mini-tablets were exposed to (a) 0.1 M HCl (2h) followed by phosphate buffer pH 6.8 (6h), and (b) simulated gastric and intestinal fluids as well as culture medium with and without fresh fecal samples.

Figure 48 depicts *in vitro* theophylline release from mini-tablets in the GIT, coated with EC:Shellac (80:20) blends comprising different rates of inulin, as a function of time.

Analogously to polymeric films (Figure 36), additional inulin did not impact drug release in the upper GIT. The formulation was resistant, irrespective of coating levels, revealing less than 5 % theophylline release up to 8 h (figure 48a). This formulation was subsequently incubated in culture medium with and without fresh fecal samples to assess its reliability for colon targeting. Noticeably, the same drug release pattern was achieved as for 80:20 (EC:Shellac) blend without inulin up to 8h. This system suited for the upper GIT.

Figure 48b, involving 32 h of total incubation (entire GIT), indicated negligible differences for both 5 % and 10 % inulin. An onset of drug release started from 8 h and reached 21 % and 26 % of Cmax, for 5 and 10 % inulin added, respectively. Herein, higher amounts of polysaccharide would be required for colon-targeted drug delivery.

As a reminder, this ratio did not show any differences either with polymeric films: only 35 % (5 % added inulin) and 40 % (10 % added inulin) drug release were achieved. The following figure illustrates photographs of these tablets along the incubation time.

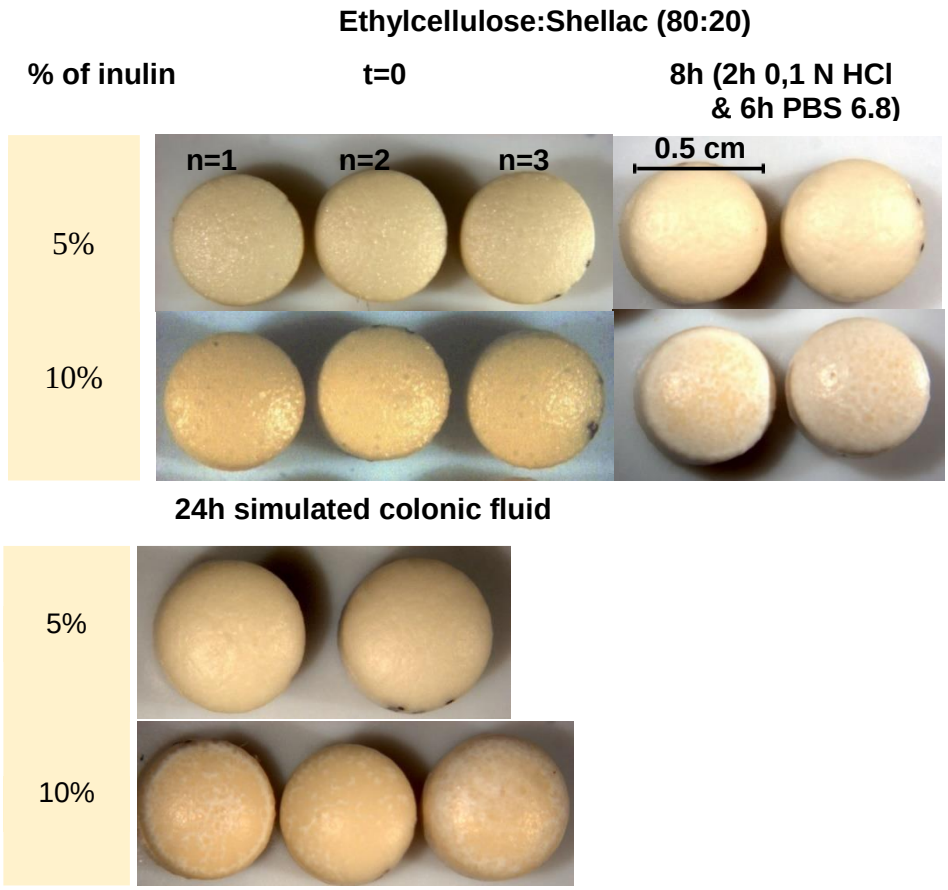
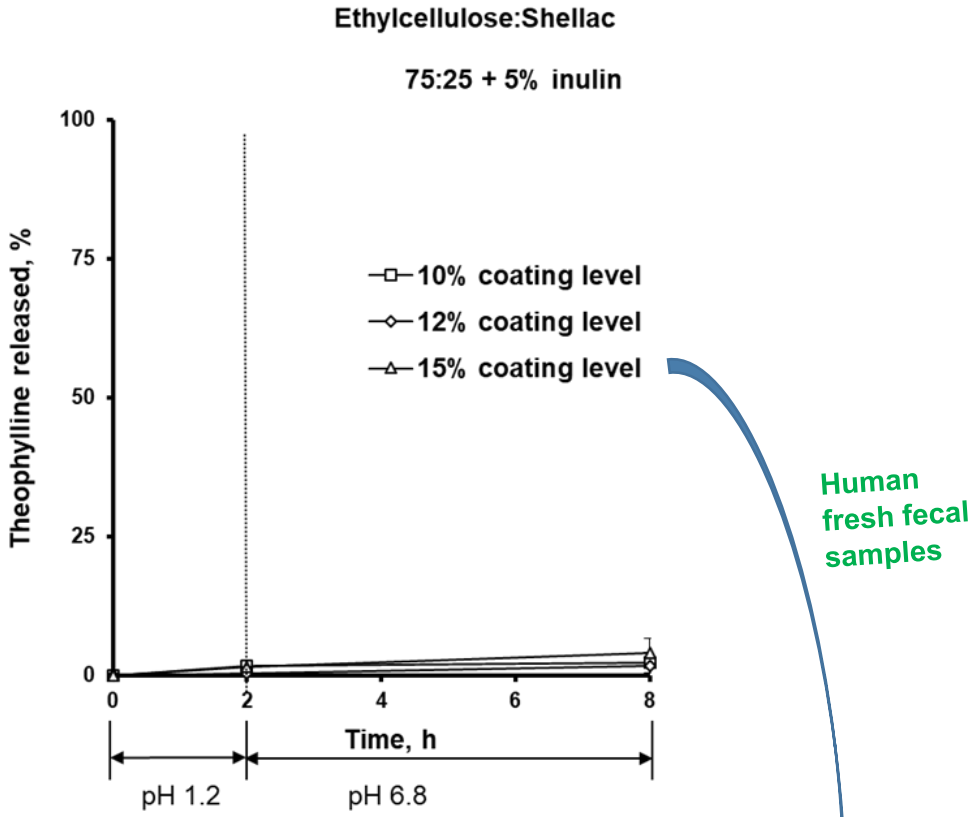


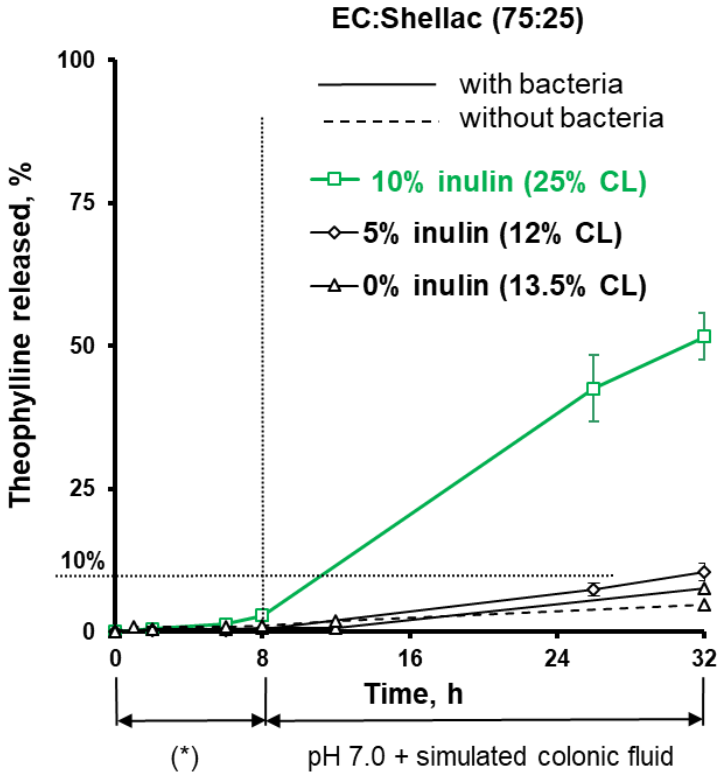
Figure 49: Macroscopic pictures of mini-tablets coated with Ethylcellulose:Shellac blend (80:20) containing 5 % to 10 % inulin added. The percentages of inulin added are indicated on the left-hand side. The potential exposure to the release media is indicated at the top.

Otherwise, figure 50 was conducted in the same conditions as figure 48 with (75:25) blend ratio (EC:Shellac).

a. Entire GIT



b. Upper GIT



(*) pH 1.2 (2h) followed by pH 6.8 (6h)

Figure 50: Impact of (a) the coating level on *in vitro* theophylline release from mini-tablets coated with Ethylcellulose:Shellac blend (75:25) containing 5 % inulin added, and (b) the amount of inulin (0, 5, and 10 %) into the film coating. Coated mini-tablets were exposed to (a) 0.1 M HCl (2h) followed by phosphate buffer pH 6.8 (6h), and (b) simulated gastric and intestinal fluids as well as culture medium with and without fresh fecal samples.

The same trend was noticed in the upper GIT. These dosage forms could withstand 8h-incubation (upper GIT), despite the incorporation of a hydrophilic excipient. Nonetheless, figure 50b highlighted interesting data within the lower GIT. Different drug release patterns could be described upon exposure to the whole gastrointestinal tract, encompassing 24 h in simulated colonic fluid. Interestingly, 10 % additional inulin showed 52 % release after 32 h exposure to digestive media, unlike 5 % additional inulin, which only displayed 10.5 % drug release. The latter was not sufficient to maximize drug delivery in the colon. As a negative control, *in vitro* drug release from the same formulation (10 % inulin) was carried out in the same conditions without fecal samples. Especially, drug release was not relevant in culture medium without bacteria. Of course, inulin is a substrate of microbiota and is of great importance. Drug release may be faster and better by increasing the quantity of polysaccharide (15, 20, 25, 30 % inulin) in these conditions. Please note that we selected higher coating levels as the proportion of inulin increased, for a better protection in the upper GIT, since the system became more hydrophilic. In this way, 12 % CL was exploited with 5 % inulin and 25 % CL with 10 % inulin. As long as hydrophilicity rises, the drug needs to be more protected, by thickening the coating. Please note that the enzymatic activity and secretions are saturated within the used closed test dissolution equipment. The viability of this microflora is limited *in vitro*. *In vivo,* bacteria secrete their enzymes continuously. *In vivo,* this phenomenon would not get limited due to peristalsis and intestinal motility, ensuring continual degradation of the formulation *via* an opened ecosystem. Thus, the drug release rate, which was below 100 % in our case, might be superior *in vivo* [160]. The following figure illustrates macroscopic pictures of these mini-tablets coated with Ethylcellulose:Shellac (75:25) and containing 5 or 10 % inulin.

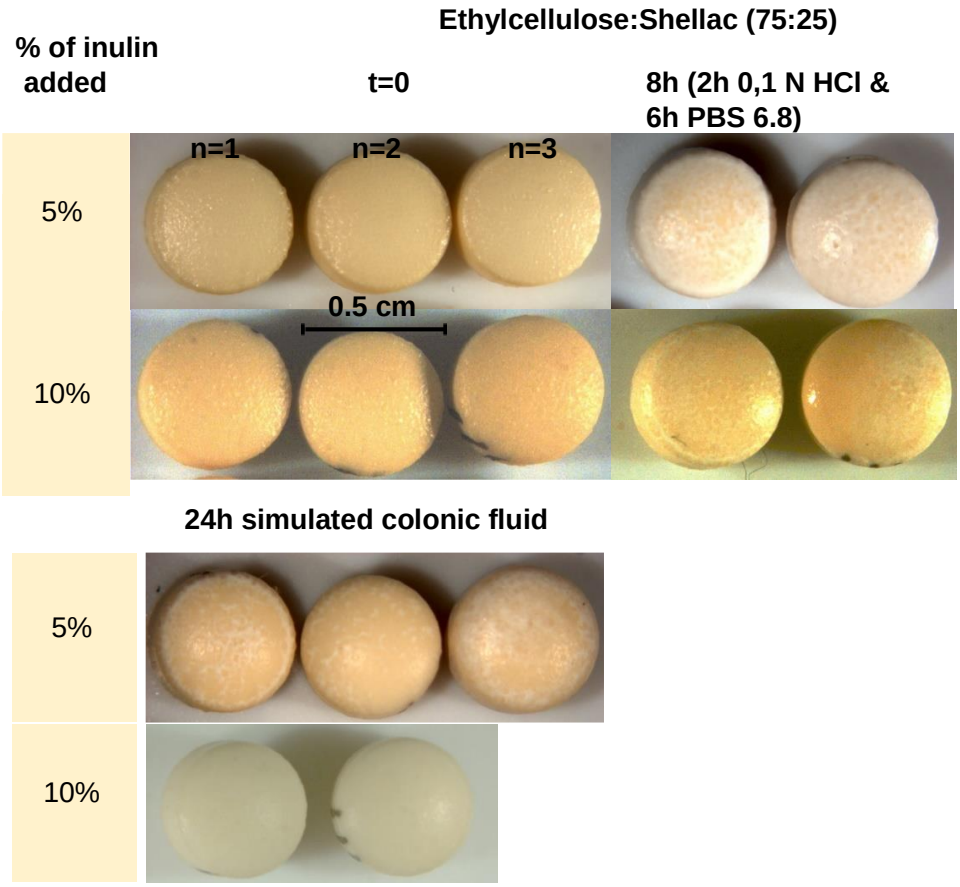
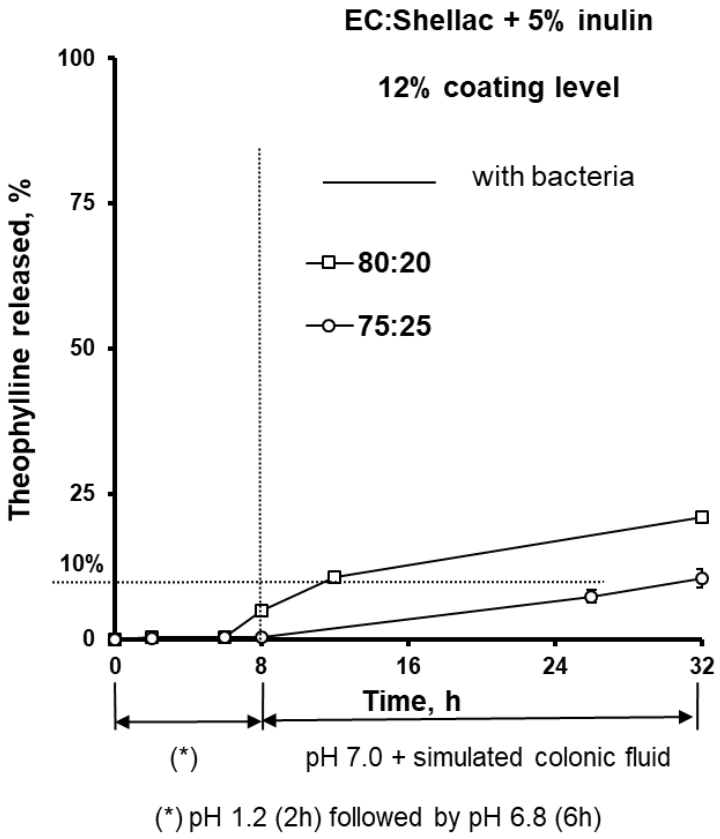


Figure 51: Macroscopic pictures of mini-tablets coated with Ethylcellulose:Shellac blend (75:25). The percentages of inulin are indicated on the left-hand side. The potential exposure to the release media is indicated at the top.

In another perspective, figure 52 represents theophylline release as a function of blend ratios (80:20 and 75:25) and inulin amounts in the film coating.

a. Entire GIT



b. Entire GIT

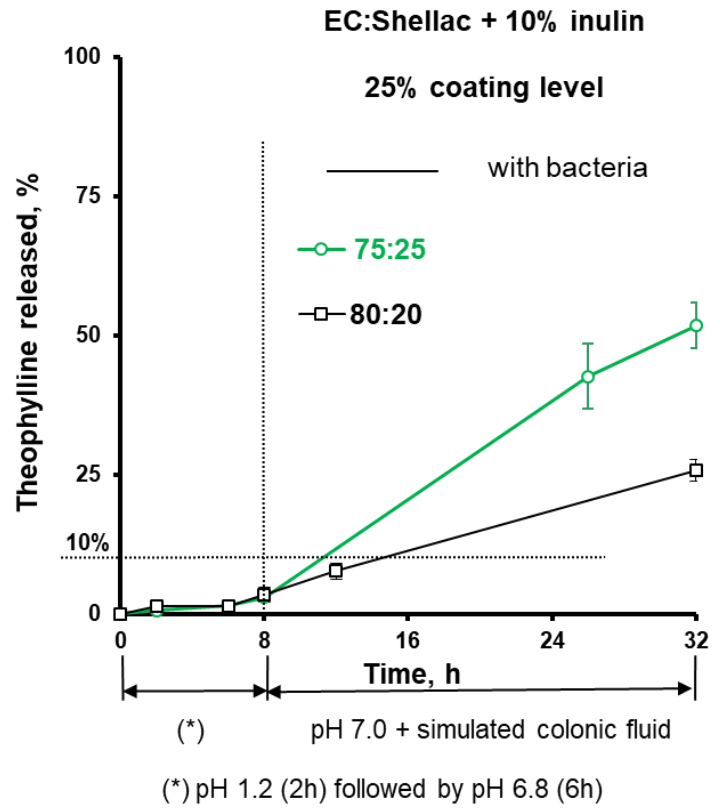


Figure 52: Impact of the blend ratio on *in vitro* theophylline release from mini-tablets coated with Ethylcellulose:Shellac blends containing (a) 5 % and (b) 10 % inulin added upon exposure to 0.1 M HCl (2h) followed by phosphate buffer pH 6.8 (6h) and simulated colonic fluid with fresh fecal samples.

Interestingly, 80:20 blend ratio containing 5 % inulin displayed a double percentage of theophylline release compared to (75:25) ratio (21 % *vs* 10.5 % respectively, figure 52a).

Even from 8 h of incubation, drug started to diffuse in the aqueous medium whilst the other ratio (75:25) still totally resisted.

This could be paralleled with the analogous observations met with optimized film coatings (figure 36b *vs* figure 36c). A louder impact of inulin over hydrophilicity was noticed when the system was more hydrophobic.

The opposite behavior could be observed in case of 10 % added inulin (Figure 52b). In the lower GIT, 75:25 ratio pointed double release rate compared to 80:20 ratio (52 % *vs* 26 %).

For reasons of comparison, EC:Inulin coatings with same blend ratios (80:20 and 75:25) were made in order to see the contribution of shellac upon drug release, when coatings comprised this polymer. Unfortunately, these tablets batches could not withstand their incubation in simulated digestive media. EC based film coatings all peeled-off from tablet cores, as well EC:Inulin as EC:Shellac based coating layers.

This batch issue will be further detailed in part III.3 (Problems with batches production).

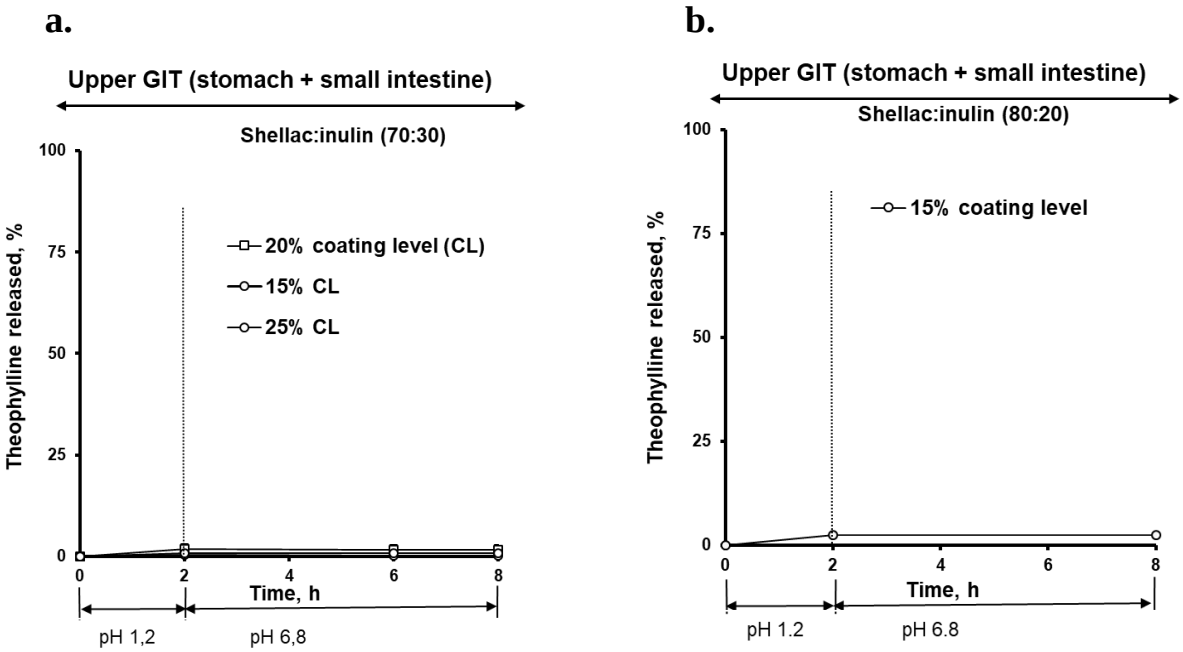
Based on the previous observations, 10 % inulin in such basis formulations revealed relevant clues for colonic drug delivery. It has to be emphasized that 10 % inulin-based coatings was the minimum amount for a colon targeted system under these conditions. The amount of inulin is all the more important that colonic fluid volumes are low [14, 44, 194] and, in a situation where a fast transit time could limit optimal exposure of mucosa to the delivered API, a fast onset of coating dissolution is needed. Here, inulin as an additional excipient showed interesting and significant variations upon theophylline release along time, providing a potential dual (pH and bacteria sensitive) stimuli-triggered system. This new formulation, containing 10 % inulin as a biodegradable polysaccharide, proved its *in vitro* efficacy, and would deserve to be further exploited in preclinical studies. Progressive and increasing proportions of inulin were to be assessed in these oral forms until 30 %. They could not be tested because of batches issues.

Shellac provides a pH-dependency with enteric properties up to pH 7.3.
Inulin is a hydrophilic pore-former excipient, but also acts as a substrate of microflora.
EC hinders water uptake and limits the swelling of the system.

This ternary mixture, as an innovative film coating formulation, still needs to be optimized and exploited. First, by rising the amount of inulin, then by modulating the coating level to get a reproducible and reliable drug release profile *in vitro*. Numerous criteria and steps remain to be engaged, but those outcomes seemed to pave the way for a promising monolayer technology.

For reasons of comparison, mini-tablets were coated with blends of shellac and inulin (without ethylcellulose) in order to determine drug release profile and, thus, the contribution of EC upon the global behavior *in vitro*.

Figure 53: Tablets coated with Shellac:Inulin blends without ethylcellulose.



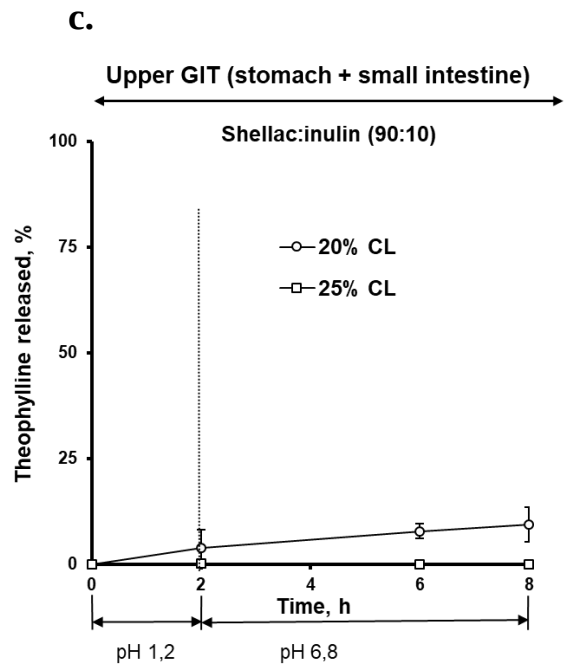
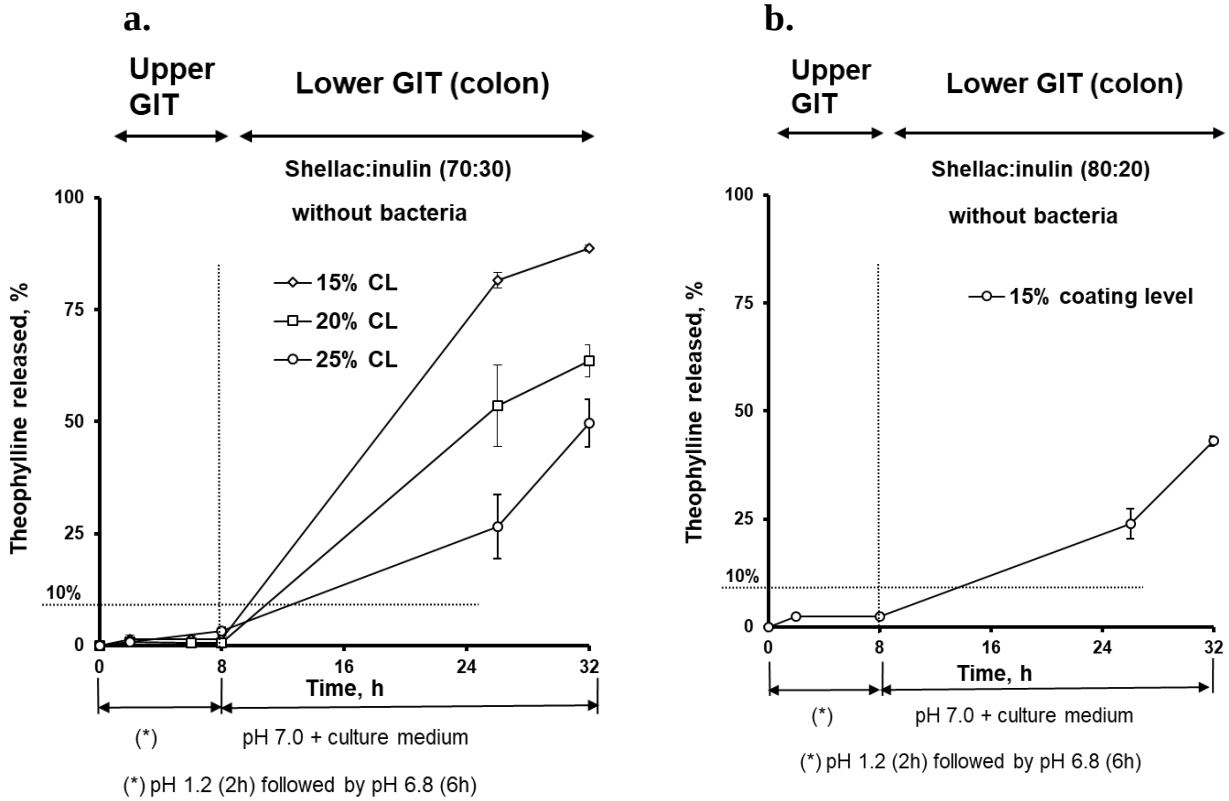


Figure 54: Impact of blend ratio and coating levels on *in vitro* theophylline release from mini-tablets coated with Shellac:Inulin blends upon exposure to 0.1 M HCl (2h) followed by phosphate buffer pH 6.8 (6h). Blend ratios were (a) 70:30, (b) 80:20, and (c) 90:10.



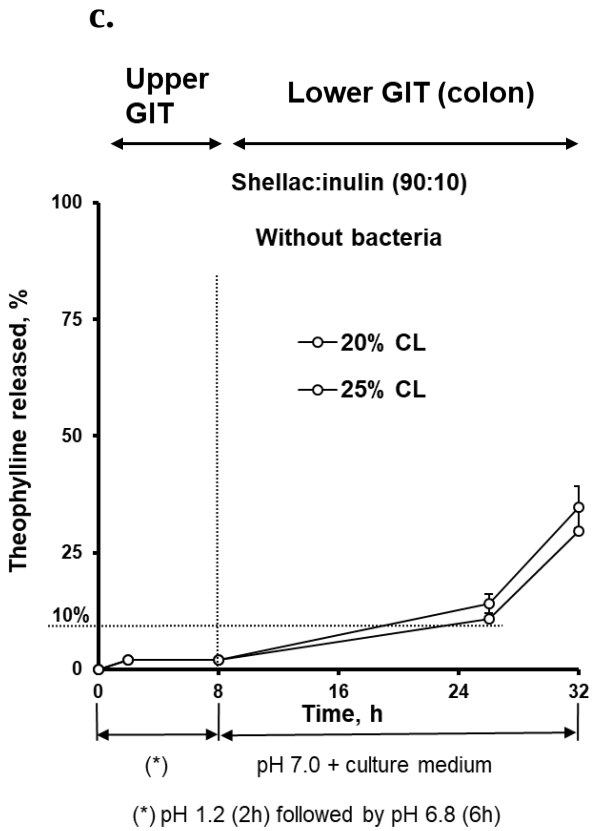


Figure 55: Impact of blend ratio and coating levels on *in vitro* theophylline release from mini-tablets coated with Shellac:Inulin blends upon exposure to 0.1 M HCl (2h) followed by phosphate buffer pH 6.8 (6h) and culture medium without fresh fecal samples (negative controls). Blend ratios were (a) 70:30, (b) 80:20, and (c) 90:10.

We previously noticed in figure 46 (60:40 ratio of EC:Shellac blend) that reducing EC fraction in favor of shellac was likely to accelerate theophylline release. We could assume that pure shellac would lead to total and rapid release. As a reminder, shellac has a dissolution pH around 7.3 and tends to dissociate at this value.

Figures 54 and 55 (above) present theophylline release in the upper GIT from mini-tablets coated with the following blend ratios of shellac and inulin: 90:10, 80:20, 70:30 (Shellac:Inulin). Different coating levels were exploited: 15 %, 20 %, 25 %.

Interestingly, no drug release occurred, and the protection was optimal up to 8 h. These observations are in conformity with a publication from Habashy *et al*. [144], who got similar trends with tablets containing theophylline and coated with a blend of shellac (from Swanlac® ASL10) and alginate at 70:30 ratio. This publication showed that with 12 % coating level, they could achieve less than 20 % release in 6 h (purple line on the figure below). Drug release was very low, and no API at all diffused up to 4 h. The following figure illustrates the kinetic profiles obtained from this publication.

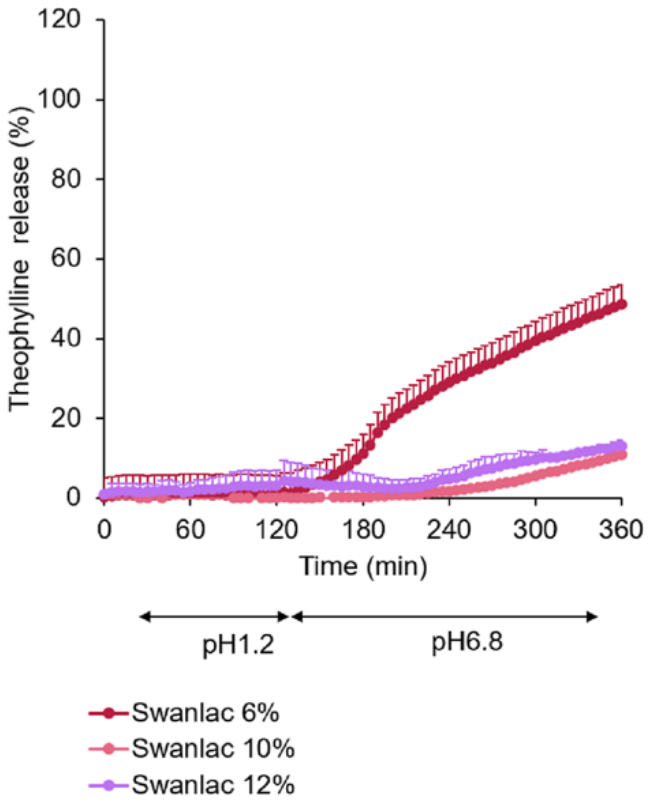
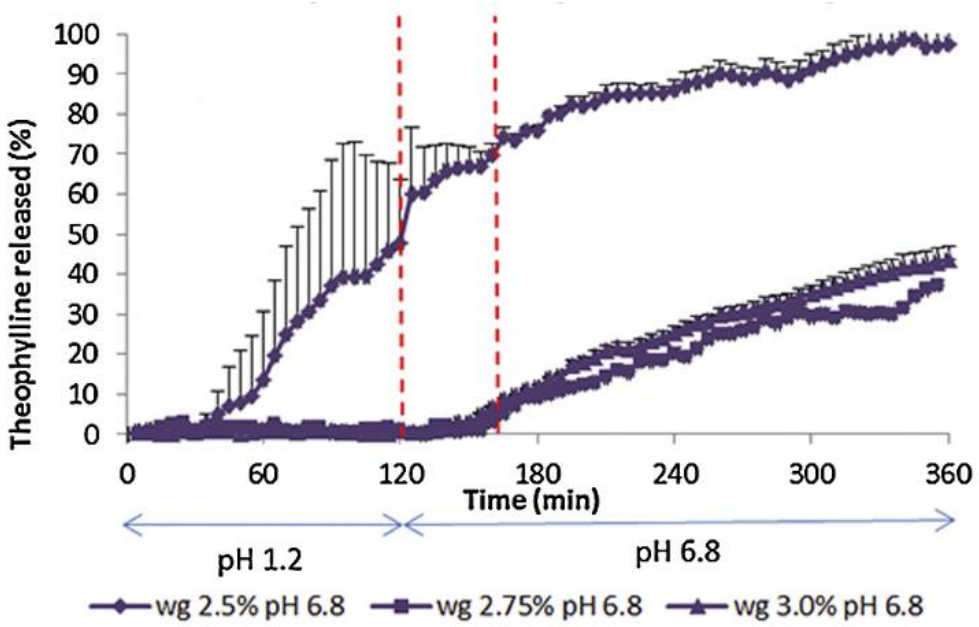


Figure 56: *In vitro* dissolution profile of theophylline tablet coated with commercial Swanlac® ASL 10 at pH 6.8. Figure taken to Habashy et al. (2020, [144]).

All coating levels, ranging from 6 % to 12 %, shew 100 % resistance in gastric medium. As compared to 70:30 (Shellac:Inulin) blend ratio, we can see common features such as total resistance in pH 1.2 and controlled release up to 6 h, mainly for both 10 % and 12 % coating levels (figure 56). Since 70:30 blend ratio was investigated with 15 % to 25 % coating levels in our case, this could explain the absence of drug release in the upper GIT.

Shellac has good barrier properties and is very hermetic. It is used as a coating system known as Protect® by Sensiet Pharmaceutical [195]. Even if shellac has a dissolution threshold located about 7.3, Habashy *et al*. did a dissolution test at pH 7.4 and yet still reported slow theophylline release at this pH [144].

Same trends were observed in a previous article from Czarnocka *et al*. (2015), who assessed Shellac-Alginate coating with Protect™ formulation:

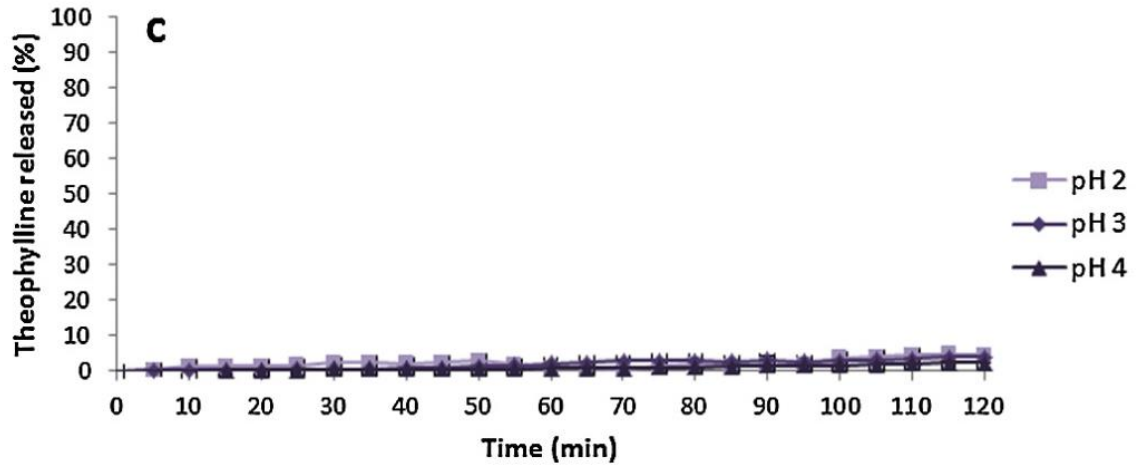


***In vitro* drug release of theophylline from tablets coated at different coating levels with Shellac-Alginate blends using USP II pH change dissolution. Figure taken to Czarnocha et al. (2015, https://doi.org/10.1016/j.ijpharm.2015.03.039).**

The authors stated that 2.75 % coating level was the minimal thickness able to provide gastric resistance from Shellac-Alginate coated tablets [139].

They also obtained little changes in drug release after changing their tablets from pH 1.2 to pH 6.8, despite using 2.75 % and 3 % coating levels [139]. As compared to EC based coatings, shellac-based coating provided a total protection in elevated pH gastric environment.

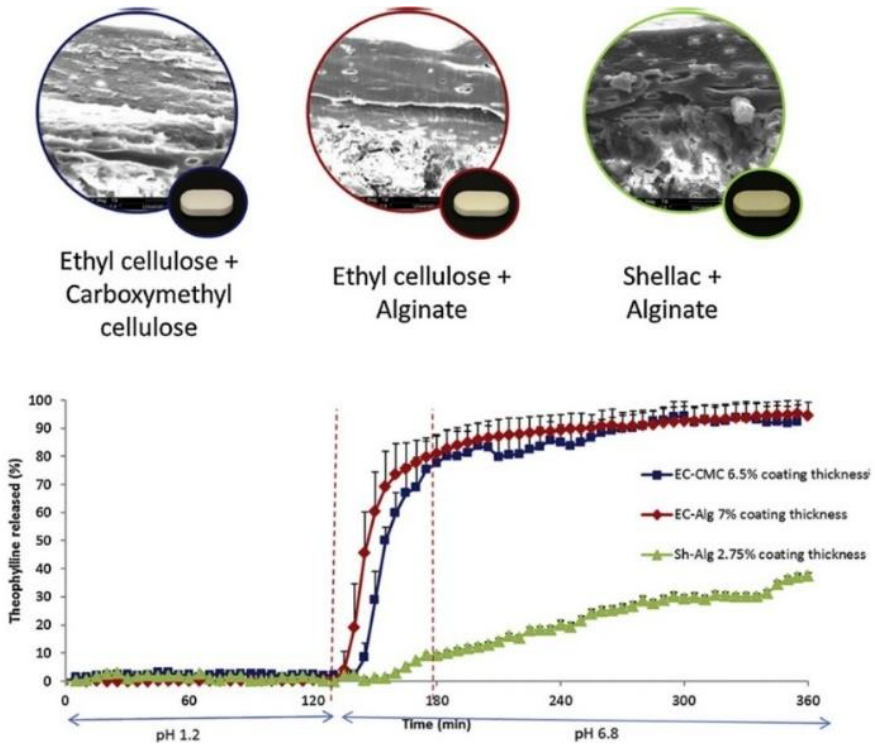
In a same way, their results in the upper GIT were comparable with our profiles. The following figure displays theophylline release in SGF (2 h pH 1.2):



***In vitro* theophylline release in different pH media for 2 h, 37 ± 0.5 °C, 50 rpm (n = 3), Shellac-Alginate. Figure taken to Czarnocha et al. (2015).**

This level of protection with shellac coating was qualified as similar to the level of drug release suppression in elevated pH gastric medium that could be achieved using coated synthetic polymers (Eudragit L100-55) [196].

This publication suggested that only shellac-based coating provided a reliable protection in elevated pH gastric environment. In a more global point of view, the following figure, extracted from the same works, exposed the superiority of shellac based coating over other polymer blends:



Graphical abstract of Czarnocha et al. (2015).

In addition to its enteric applications, shellac has a high potential as a coating material for moisture protection and taste-masking. In contrast to commonly used cellulose derivatives, much lower coating levels are required to achieve similar effects while keeping drug release unaltered [140].

It is important to note that inlet air temperature during coating process clearly influences drug release from the coated tablets. Indeed, a minimum inlet air temperature has to be exceeded to obtain a continuous shellac film coating. Under such a temperature, cracks in the coating can appear and impact drug release profiles, namely about gastric resistance.

Farag *et al*. demonstrated that 60°C inlet air temperature was the best temperature setting for controlled release with shellac coated dosage forms [197], which was used in our experiments. Likewise, 24 h curing at 60°C provides good mechanical properties and ensures the film integrity, which was consistent with the profiles obtained above.

The following figure accounts for macroscopic pictures of Shellac:Inulin coated tablets before incubation (T=0) in simulated digestive media.

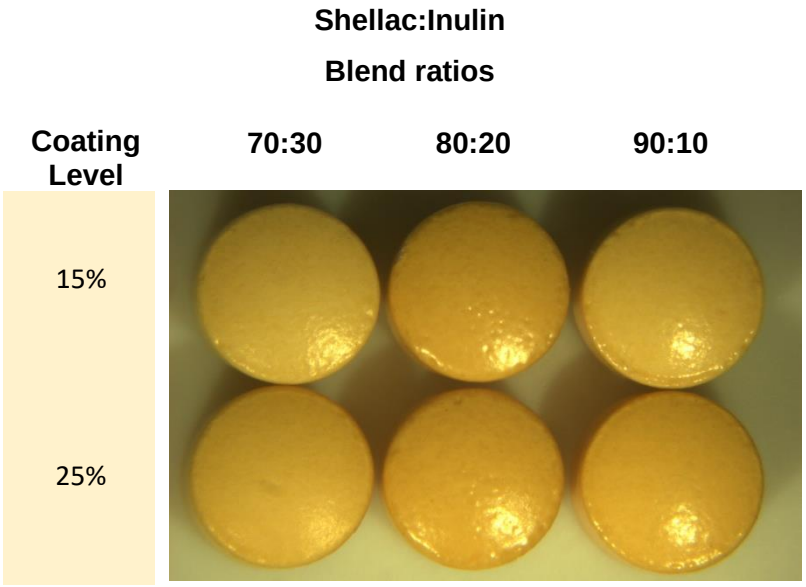


Figure 57: Macroscopic pictures of mini-tablets coated with Shellac:Inulin blends before incubation (T=0). Coating levels are indicated on the left-hand side. Blend ratios were: 70:30, 80:20, 90:10.

Limits of drug release in the lower GIT [161]

Numerous methodologies are employed for the study of *in vitro* dissolution with colon targeted drug delivery systems, since the USP dissolution methods do not ensure appropriate provisions for systems depending on colonic bacteria. No standardized criteria for fermentation (*e.g.*, media composition, volume and pH, enzymes, agitation intensity, time…) are available for *in vitro* dissolution testing of colonic drug delivery. Thus, the comparison of *in vitro* release from different formulations is hard to consider. Biorelevant dissolution testing is in developmental stages and has been extensively reviewed [44]. In sequential testing, the duration of analysis is generally 2 hours in SGF, 3 hours in SIF, and up to 19 hours in SCF. The volumes of dissolution media range from 100 mL to 900 mL, with no defined limitations on the nature and quantity of additives used to make the medium biorelevant. For example, simulated colonic fluid, rat cecal contents, human fecal slurries, and polysaccharide-metabolizing enzymes can be used without distinction.

Furthermore, *in vitro* dissolution studies regularly include phosphate buffer to represent small intestinal environment and fluid composition. But in fact, bicarbonate is the main buffer type in the GI fluid, not phosphate [198–201]. Thus, bicarbonate buffer is more relevant to simulate physiological conditions, and it represents a promising alternative to phosphate buffer, with its ability to accurately discriminate the drug release patterns of dosage forms [201, 202]. The reliability of bicarbonate buffer was further demonstrated by a study of Goyane *et al.* [203], where drug release profiles of different mesalazine products were compared. Notably, drug release pattern for Lialda® (Mezavant XL®) displayed a good correlation with gamma-scintigraphy outcomes in humans [204]. Several sources mention that Hanks buffer closely resembles the luminal composition of small intestinal fluid [61, 205]. Additionally, colonic drug delivery is still a challenge, owing to the low colonic fluid volume and more viscous luminal content, compromising drug absorption through mucosa [44, 194]. Indeed, colonic free fluid volumes were reported to be close to 13 mL [206], and the total colonic fluid volume (including free volume and volume stock in discrete fluid pockets) was estimated as 372 mL. Considering this average value, it has to be noted that results displayed considerable deviation around the means [207]. This is due to the fact that 90 % of water entered is absorbed in the colon [194].

Limits of bacteria and pH-sensitive polysaccharide-polymer films

On the other hand, while bacteria and pH-sensitive films can be exploited with most small organic molecules, it may show limitations in the delivery of biomolecules to the colon. The stability of proteins and peptides may be harmed due to the heat involved during the coating

process. Even if Leong *et al*. [208] achieved film formation thanks to a solvent-casting process below 37 °C, this method is not possible with the coating of tablets or pellets. Of course, inlet air temperature and product bed temperature in coating processes are 60 °C and 38 °C, respectively. Proteins can hardly retain activity at these processing conditions [209, 210]. The capability to adapt the formulation processes to the new approaches in IBD therapy, such as biological therapy and gene therapy [211], are critical.

III.3 Problems with batches production

III.3.1 Capping phenomenon

Unfortunately, the entire last year of the project faced a serious production issue, leading to unusable tablets. The main part of optimized coated tablets was sacrificed, and numerous further experimentations were aborted (notably from inulin rates > 10 % in film coatings). For this reason, only 5 and 10 % inulin based coated tablets were previously tried out in bacterial media.

A coating defect was observed, reported, and impeded all the next experiments. As soon as tablets were immersed into aqueous medium, tablets swelled and separated in two parts. This is called capping phenomenon (figure C below). It is defined by the partial or complete separation of the top or bottom of tablet. Another one encountered was lamination (figure B below), which is the separation of tablet into 2 or more distinct horizontal layers. Lamination is very similar to capping, but occurs in the main body of the tablet, not at the top [212]. Figure A shows the uncoating of tablets.

A) **B)**

Lamination

C)

Capping

III.3.2 Troubleshooting for issues in tablets coating

Among the different alternatives to solve this problem, the main principal ones relied on the atomization aspect of coating.
Indeed, microdrops are sprayed on the tablet cores, and the latter need to land at a dry state. If they keep wet, the coating will be harmed by the flow of tablets on each other.
Then, it is recommended to rather spray dry the polymer dispersion during the coating process. This can be corrected *via* the distance between nozzle and tablets bed, and the spray rate also.

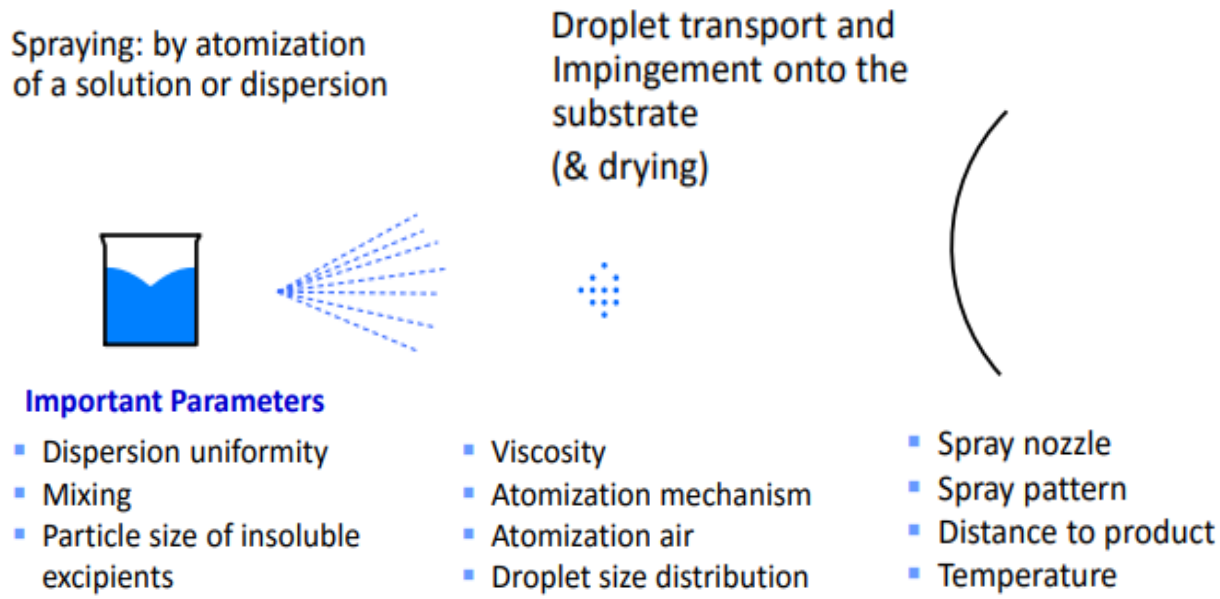


Figure 58: Film coating process with atomic layer deposition of polymer particles onto the substrate (tablet cores herein). Distance between nozzle and tablets bed as well as spray rate play a role for the film formation. Figure taken from Nyamweya et al. (2019, DOI: 10.13140/RG.2.2.34800.28164).

The following coating protocols could never be achieved because of this issue:

- EC:Shellac (80:20) + **15 %** inulin
- EC:Shellac (80:20) + **20 %** inulin
- EC:Shellac (80:20) + **30 %** inulin

EC:Shellac (75:25) + **15 %** inulin
EC:Shellac (75:25) + **20 %** inulin
EC:Shellac (75:25) + **30 %** inulin
EC:Shellac (75:25) + **10 %** **Pullulan** / **Maltodextrin** / **Alginate**

- EC:Inulin (80:20)
- EC 100 %

EC:Inulin (75:25)

- (EC+TEC) +Shellac *vs* (EC+TEC)+(Shellac+TEC)
- (EC+TEC) + (Shellac + PEG or Glycerol)

By changing several parameters, various combinations of operating conditions were attempted:

- Distance between nozzle and tablets bed was shortened
- 0.5 mm nozzle was switched to 0.8 mm nozzle for a wider spray
- Adjunction of plasticizer for shellac such as glycerol 5 % (m/m, referring to the dry mass of shellac)
- Substitution of EC with Eudragit RS30D
- Spray rate was augmented from 4 to 10 g/min: increasing the rate allows for a uniform spray print. The following figure illustrates this:

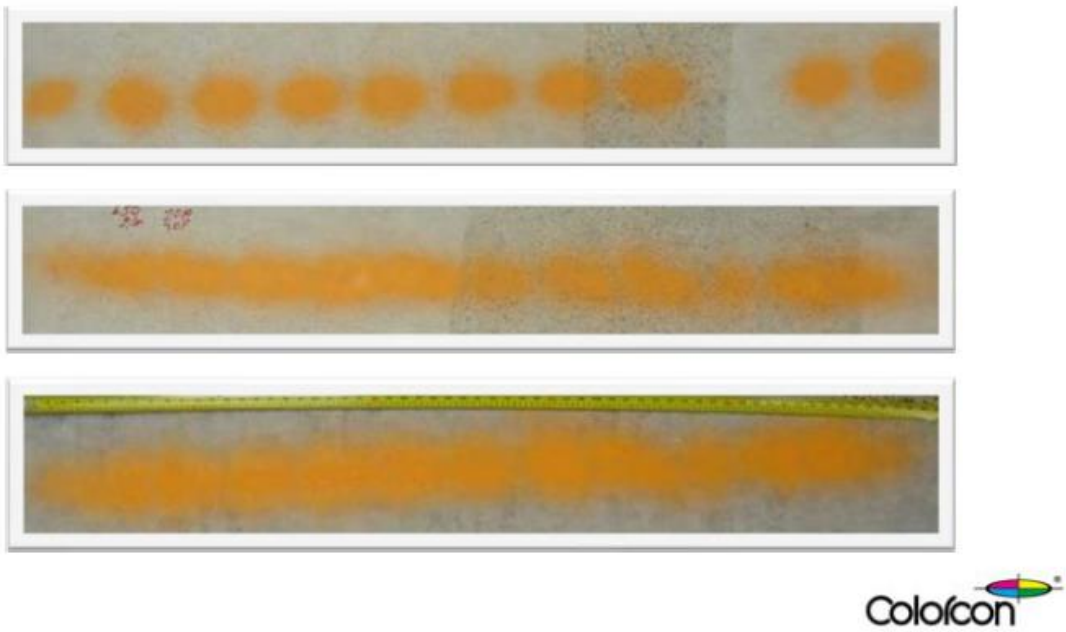


Figure 59: Spray print depending on the spray rate value. Figure reused from Colorcon.

- Shape air pressure was increased to 1 bar. See explanations below:

Explanation

Shape air system allows for a more homogeneous distribution of the sprayed dispersion. In the following figure, we can see the lateral air flows (“Air”, A and B zones) on both sides of the central air flow (“water”). More pressure leads to a wider stream. Shape air creates an ellipsis to spray the same quantity of polymer on all the tablets.

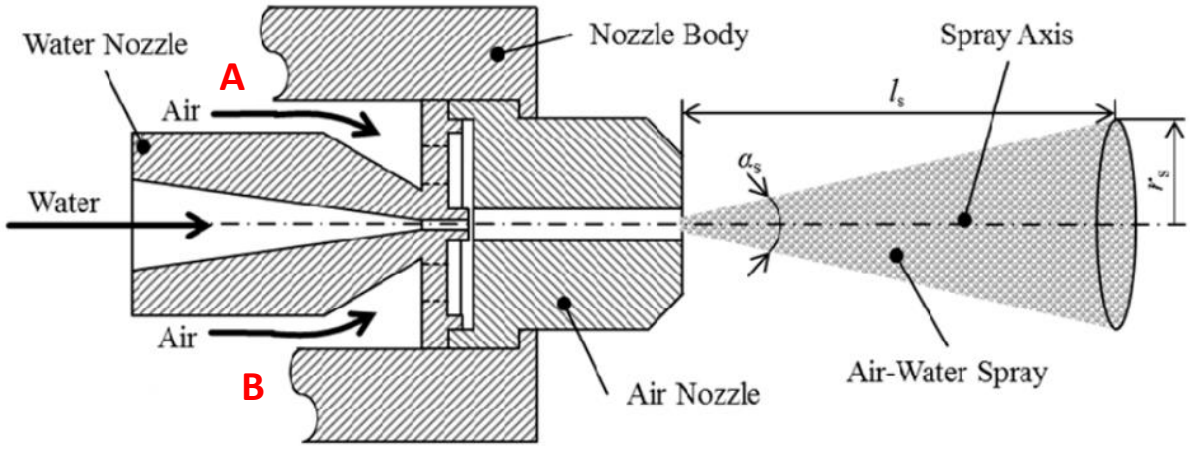


Figure 60: Schematic representation of the nozzle and its air flows. Shape air system can be seen on A and B zones. Figure reused from Herbst et al. (2016, https://doi.org/10.1504/IJMMP.2016.079149).

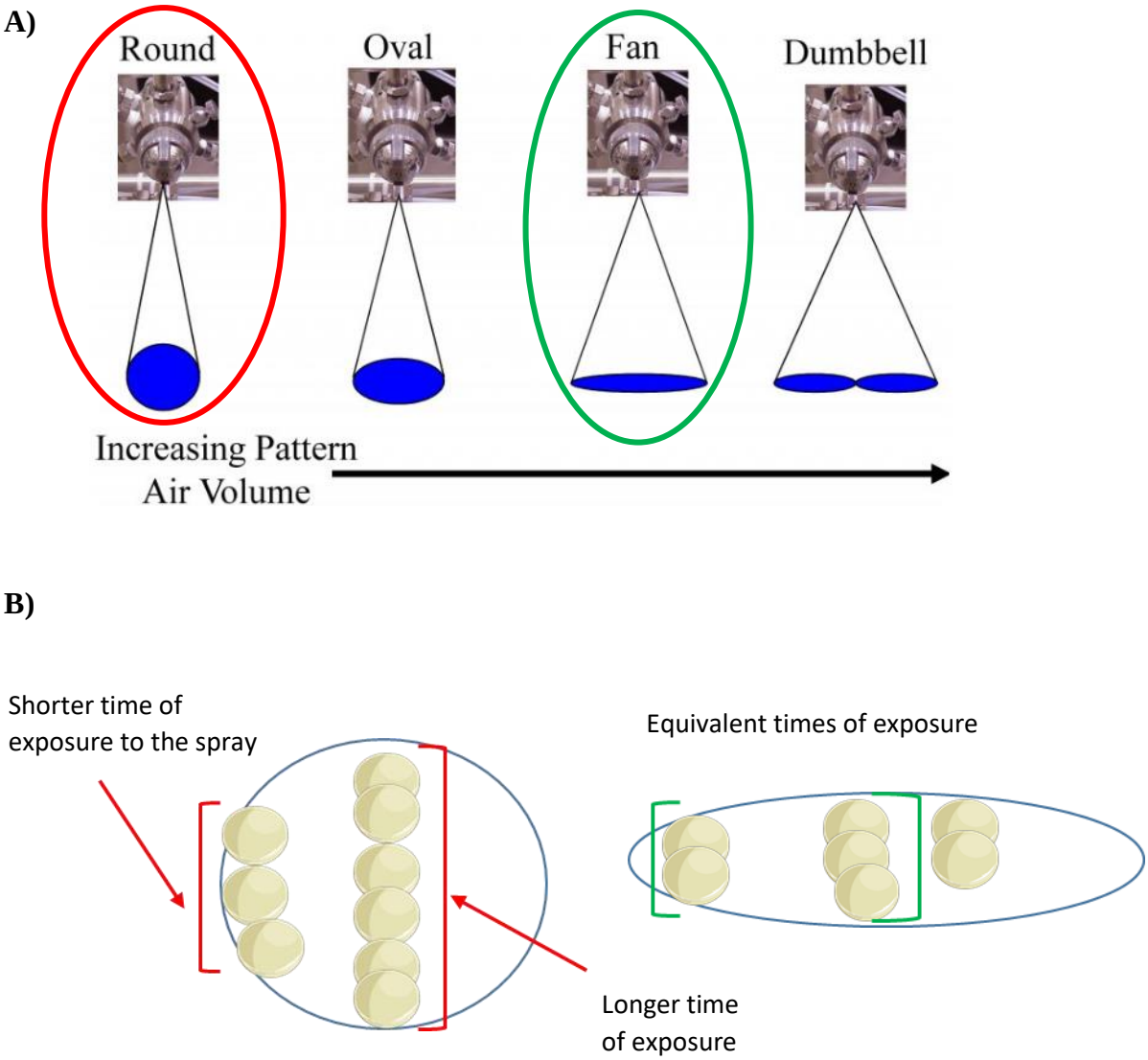


Figure 61: Uniform « curtain » of spray. Figures A) and C) reused from Freund Vector [36].

- Outlet air temperature was set to 70°C
- Atomizing air pressure was risen to make smallest spray drops and favors their drying onto tablet cores
- For pre-heating step, the rotation speed of pan was reduced from 25 rpm to 5 rpm, to avoid tablet cores stress

After these modifications, disintegration time was lagged from 1 min to 4-6 h. This was a start of improvement, but not sufficient yet. Figure 62 hereafter displays the slight onset of improvement in tablets stability along the year.

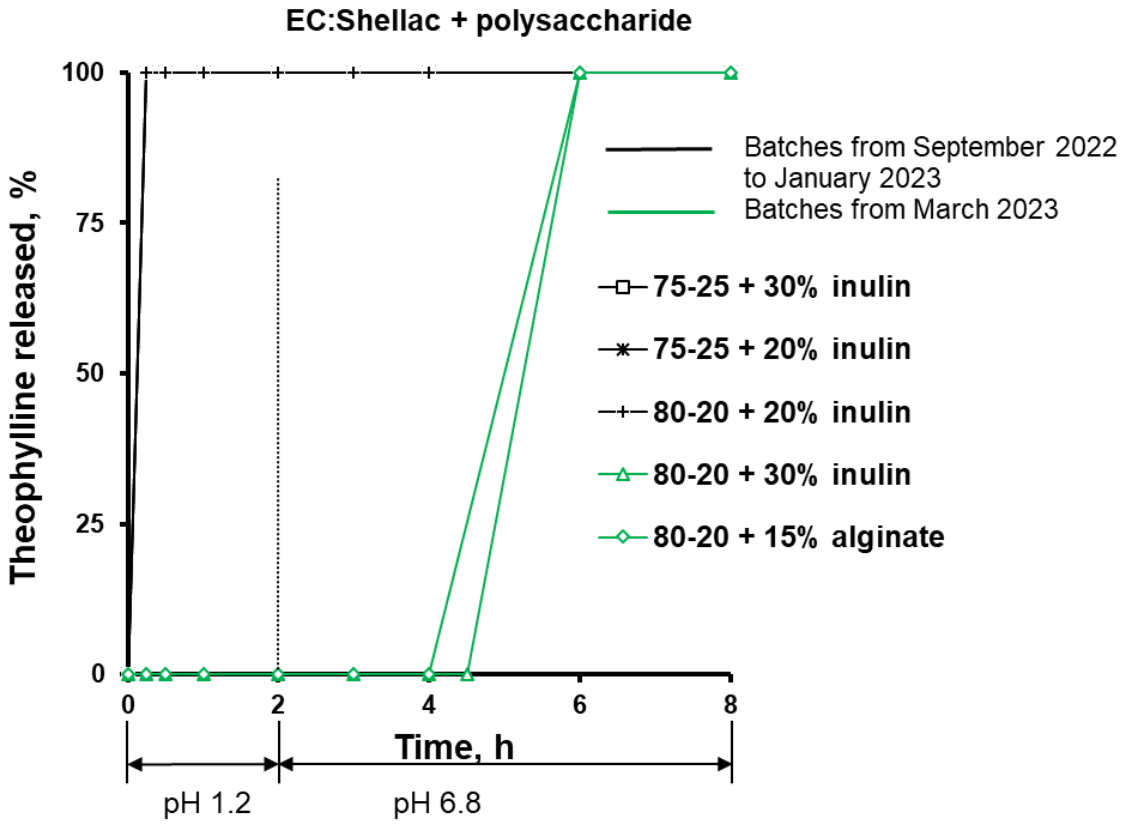


Figure 62: Changes in tablets stability in aqueous media during 2022-2023 academic year. Batches from March 2023 (green lines) were made after attending a training planned by Colorcon about tablets coating.

Please note that tablets coated with pure shellac (without EC) did not expose this issue. Shellac coated tablets could withstand their incubation in aqueous fluids for one week without any issue. It is possible that EC was the source of this difficulty. Indeed, tablets coated with EC without shellac still showed capping phenomenon, but also EC:Inulin manufactured coated tablets (80:20 and 75:25 blend ratios). It has to be noted that many coating process considerations can suggest a range of air flow such as [374-442] (m^3/hr) for Aquacoat ECD coating [152], depending on the machine used. As for Hüttlin Solidlab1, air flow value

recommended is 70 m^3/hr [213]. The fact that 30m^3/hr air volume was exploited might partially explain this coating instability. The situation was even more complex that composite film coatings were made, including both shellac and EC, each one having different physicochemical properties. Nonetheless, Swanlac® ASL 10 is reputed to be highly compatible with other natural or synthetic resins and polymers [214].

Among other alternatives, the following ones should also be undertaken:

- Lowest rotative speed of pan for a fluid and continuous tablets bed
- Pre-coating for the application of a sub-layer with HPMC (*e.g.*) to better protect the edges with 1 % weight gain. Indeed, the coating layer is thinner by the edges, and they represent the most delicate parts. See explanation below.

Explanation

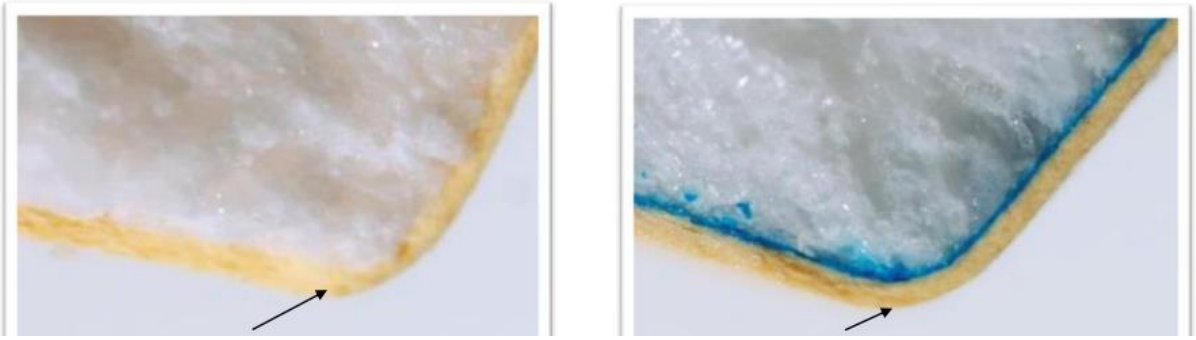
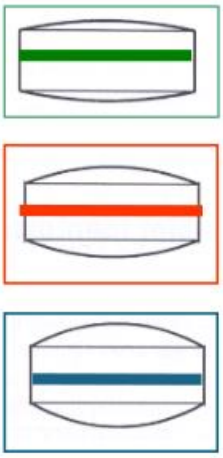


Figure 63: A sub-coat protects the edges of tablets. Figure reused from Colorcon.

- Change the radius of curvature: increasing the latter lowers sharp edges
- To make tablets cores with 2 fillers in equal proportions (50 %-50 %), instead of 1: MCC + sorbitol, lactose, or mannitol
- Magnesium stearate < 0.5 % (rather 0.25 %)
- Correct hardness to avoid lamination / cleavage during coating processes



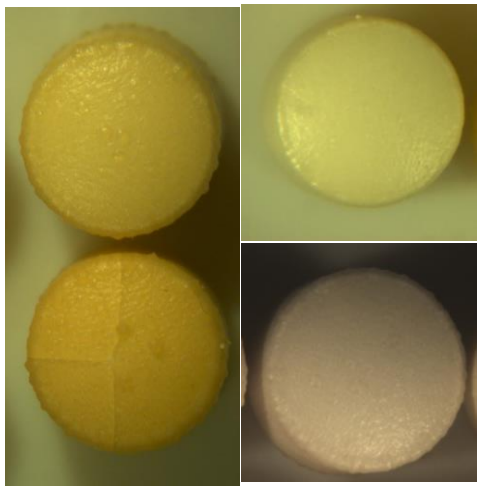
Please note that this imponderable constrained us to increase tenfold the quantities of tablets to be used for *in vitro* drug release tests.

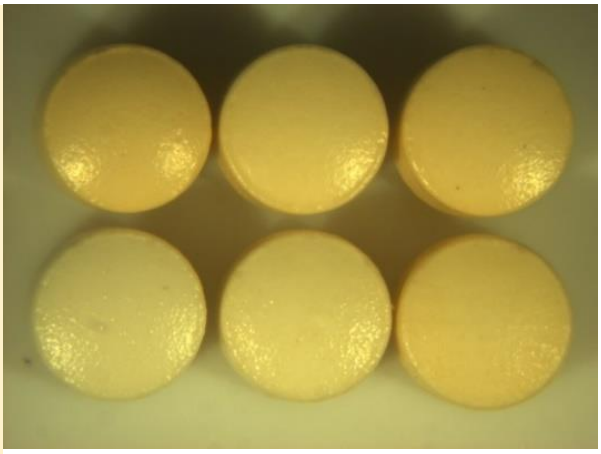
Since tablets could not withstand aqueous medium, we could not work with triplicates anymore. They all resulted in capped dosage forms. Instead, we ended up using 56-plicates (instead of triplicates) to be sure that at least one tablet would be remaining. This considerably disturbed the way to work, the material to use, and time management.

The following pictures represent the different formulations that were made to be tested. Due to capping phenomenon in aqueous fluids, only T0 were shot.

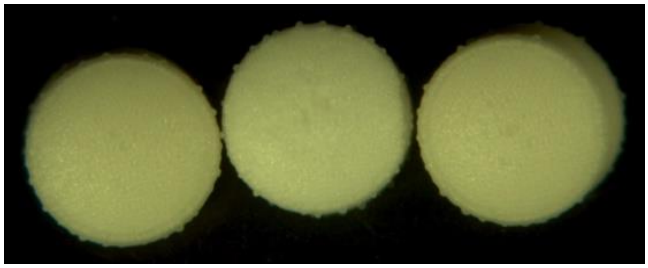
EC:Shellac + Inulin

Blend ratios

Inulin amount (%)	75:25	80:20
20		
30		

Nature of polysaccharide	15% CL	20% CL	25% CL
10 % Alginate			
10 % Maltodextrin			

EC:Inulin (75:25)



A.

Issue[5]	Major causes	Recommendation
Formulation related	Large amount of fines and low bulk density of tableting blend	Increase bulk density and remove excessive amount of fines in formulation
	Low moisture content	Increase the moisture content (loss on drying in between 1 % and 3 %)
Machine related	High rotative speed of pan resulting in low dwell time	Lower pan speed (increase in dwell time)
	Lower precompression load and higher main compression load	Increase precompression load and decrease main compression load
	Poorly finished dies	Use tapered, defect free, properly polish dies

B.

Figure 64: Capping phenomenon and its solutions.

Figure B reused from Biogrund [216]

[5] Recommandations obtained from Shin-Etsu Chemical [215]

Issue[6]	Major causes	Recommendation
Formulation related	Low moisture content	Increase the moisture content (loss on drying in between 1 % and 3 %)
	Too much of hydrophobic lubricant	Decrease amount of lubricant or change the type of lubricant
Machine related	Rapid decompression / elastic recovery	Use pre-compression step
	High main precompression pressure and less precompression	Reduce pan speed and reduce the final compression pressure
	Rapid relaxation of the peripheral regions of a tablet, on ejection from a die	Use tapered dies

Figure 65: Lamination phenomenon and its solutions.

[6] Recommandations obtained from Shin-Etsu Chemical [215]

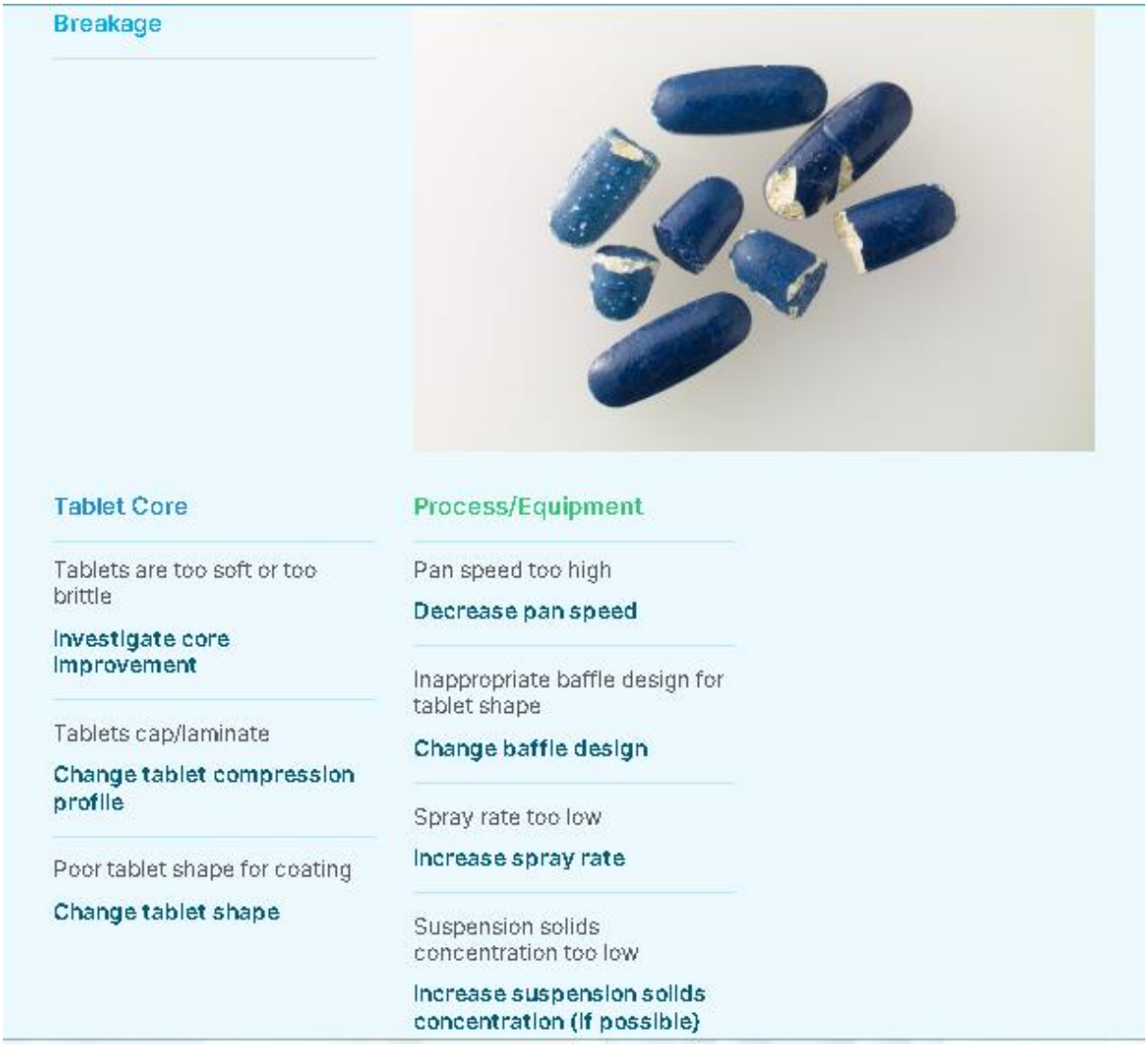


Figure 66: Recap tablet breakage, encompassing capping and lamination. Figure reused from Colorcon Troubleshooting.

Figure 67: Peeling phenomenon and its remedies. Figure reused from Colorcon troubleshooting [217].

*** During pre-warming step, the air expands and can inflate the tablet cores**

IV. CONCLUSION AND PERSPECTIVES

IV. Conclusion and perspectives

A novel polymeric film coating based on ethylcellulose and shellac was designed. Despite being a GRAS product and enteric polymer, the use of shellac has got rare. An aging-related instability was reported due to self-esterification of shellac, occurring *via* cross-linking between free hydroxyl and carboxylic groups. Swanlac® ASL 10, an ammonium-based aqueous solution of shellac, avoids this issue and ensures better stability and reliability. The properties of shellac make it a suitable polymer for colon targeting, as its pH dissolution threshold is close to colonic standardized pH values.

Apart from pH-sensitivity, a supplemental stimulus was integrated in order to favor colonic drug delivery. Polysaccharide-based systems remain the most reliable and efficient ones. We could obtain a formulation combining both pH- and microbially triggered approaches.

This concept catches up the recent and world's best technologies patented, such as Phloral® or Opticore®, all relying upon dual-responsive stimulation. Mini-tablets were manufactured and coated with this basis formulation and led to promising outcomes. A significant amount of drug was delivered within the colon in the presence of bacteria. Of course, the presence of shellac contributed to partial release, owing to its pH-sensitivity. That being said, from little amount of polysaccharide added, this primary composition demonstrated its colon-targeted potential.

10 % additional inulin in this polymeric film coating clearly showed an onset of microbial degradation and drug release catalysis. As for ethylcellulose, it protected drug and hindered premature film dissolution in the upper GIT, thus remaining of overriding importance.

It is clear that this invariant formulation deserves to be further optimized in order to provide appropriate drug release pattern in the very colon, in both physiological and pathological conditions. First, by rising the quantities of inulin. Then, by attempting to determine an optimal equilibrium between both shellac and ethylcellulose polymers. Finally, by screening various polysaccharides to see whether a better biodegradation occurs or not. It has to be pointed out that this new formulation founded on Ethylcellulose:Shellac blend, whether including or not a polysaccharide, is reliable for prolonged drug delivery in the lower GIT. This is advantageous for polypeptide and protein-based drugs, which get destroyed in the upper GIT.

Technical issues with tablet coating did not allow for these steps, but these preliminary data seem to be promising, all the more given that mini-tablets offer advantages, such as good swallowability for elder patients.

Coated mini-tablets were already exploited for oral administration of antibody. Vorabody®, a

TNF-α neutralizing antibody that stepped into phase II in 2017, was formulated into enteric-coated mini-tablets.

Otherwise, inulin, among other polysaccharides, exhibits prebiotic properties. By normalizing the microflora and enzyme patterns in the colon of patients, this could be particularly beneficial for patients suffering from inflammatory bowel diseases.

Although interrupted, this study may lay the first stone for concrete perspectives.

V. RESUME IN DETAIL (FRENCH)

V. Resume in detail (french)

Dix millions dans le monde, trois millions en Europe et deux cent mille en France. Ces chiffres sont ceux des personnes atteintes de maladies inflammatoires chroniques de l'intestin (MICI) dans chaque région mentionnée.

Les MICI, qui comprennent la maladie de Crohn et la rectocolite hémorragique, se caractérisent par une inflammation de la paroi du tube digestif, qui évolue par poussées, et sont surtout diagnostiquées entre 15 et 35 ans. Ces pathologies intéressent notamment les pays occidentalisés.

Dans les populations d'Amérique du Nord, d'Australie et d'Europe, la prévalence de ces pathologies dépasse désormais 0,3% [4]. En France, 273100 personnes étaient concernées par les MICI en 2019 [218].

Décrite pour la première fois par Burril Bernard Crohn en 1932, la maladie de Crohn (MC) peut toucher n'importe quelle partie du tractus gastro-intestinal (TGI), de la bouche à l'anus. L'iléon et le gros intestin (côlon) restent les principales régions concernées. Les lésions de la MC sont transmurales : muqueuse, sous-muqueuse et séreuse sont impactées.

Reconnue officiellement en 1875, la rectocolite hémorragique (RCH) se cantonne au gros intestin et au rectum. L'intestin grêle n'est jamais touché. La plupart des RCH sont distales (60 %) et touchent le rectum, ainsi que l'ensemble rectum et côlon sigmoïde.

Les autres formes de RCH peuvent être pancoliques (attaquant tout le côlon et le rectum, formant 15% des RCH) ou de formes intermédiaires (entre distales et pancoliques, comptant pour 25% des RCH). L'inflammation ne touche que la partie superficielle de la muqueuse. Les symptômes sont généralement des douleurs abdominales et des diarrhées, mais ils peuvent mener à de graves complications : sténose, perforation intestinale ou cancer.

Au-delà de l'impact de ces maladies sur la qualité de vie des patients, il n'existe à ce jour aucun traitement curatif. La prise en charge actuelle est d'abord symptomatique, et peut s'avérer conséquente (résection chirurgicale, corticoïdes au long cours).

L'escalade thérapeutique implique la prise d'aminosalicylés par voie orale (5-ASA), d'antibiotiques (métronidazole), d'immunosuppresseurs par voie parentérale (azathioprine) ou encore de biothérapies. Les spécialités orales actuelles ont tendance à se dégrader et à libérer prématurément la substance active dans le haut TGI. Plus de 90% des patients sont traités par aminosalicylés durant les premières années post-diagnostic, et 60% à 90% d'entre eux poursuivent ces traitements encore 15 ans après [5]. 1 patient sur 2 atteint de MC sera opéré dans les 10 ans suivant le diagnostic. Concernant la RCH, c'est 1 malade sur 3, dans les 20 années suivant le diagnostic [219].

Le ciblage de la partie distale du TGI, ou côlon, permet d'optimiser la libération de substance active au niveau des zones lésées en réduisant les effets indésirables des traitements [7]. En sus d'intéresser les MICI, le ciblage du côlon semble prometteur dans d'autres cas tels que le syndrome de l'intestin irritable, la diverticulite ou encore certaines parasitoses [8].

Au-delà d'améliorer l'efficacité thérapeutique *in situ*, cibler le côlon permettrait, dans certains cas, l'administration orale de molécules qui seraient normalement dégradées dans le haut TGI (en raison de l'environnement gastrique) [9–12]. La dégradation des médicaments à base de protéines et de peptides, notamment, pourrait être évitée en raison de la plus faible activité protéolytique dans la partie distale du TGI [13, 14].

Ajoutons que la voie orale est la voie d'administration la plus fréquente. Du fait de l'absence de douleur et de sa commodité, cette voie implique une bonne observance chez les patients.

Différentes stratégies existent pour cibler le côlon par voie orale : *) Les prodrogues, **) Les systèmes sensibles au pH, ***) Les systèmes temps-dépendants, et ****) les systèmes sensibles à la flore bactérienne intestinale.

Les systèmes temps- et pH-dépendants ont montré de fortes variations en termes de résultats et ne sont pas les plus fiables. En effet, malgré leur succès commercial, les polymères pH-dépendants peuvent présenter un manque de spécificité quant à leur site de dissolution. A titre d'exemple, de nombreuses études ont rapporté le manque de performance de l'Eudragit S (pelliculage se dégradant à pH > 7.0) [28, 220]. Cela a pu s'observer par des formes galéniques intactes dans les selles de patients [28]. Les raisons sont nombreuses et concernent des caractéristiques physiologiques comme l'état nourri ou à jeun, le volume de fluide colique, la motilité intestinale, le pouvoir tampon…etc. De toutes, l'approche basée sur l'activité du microbiote reste la plus fiable [1–3]. Les quelques 10^{12} bactéries par gramme de selle dans le côlon peuvent, *via* leurs sécrétions enzymatiques (hydrolases, azoréductases, estérases, nitroréductases, glucuronidases, glycosidases, amidases…), métaboliser les polysaccharides.

Les systèmes pH- et temps-dépendants ayant leurs limites, les approches à plusieurs stimuli leur sont préférées [59, 221, 14].

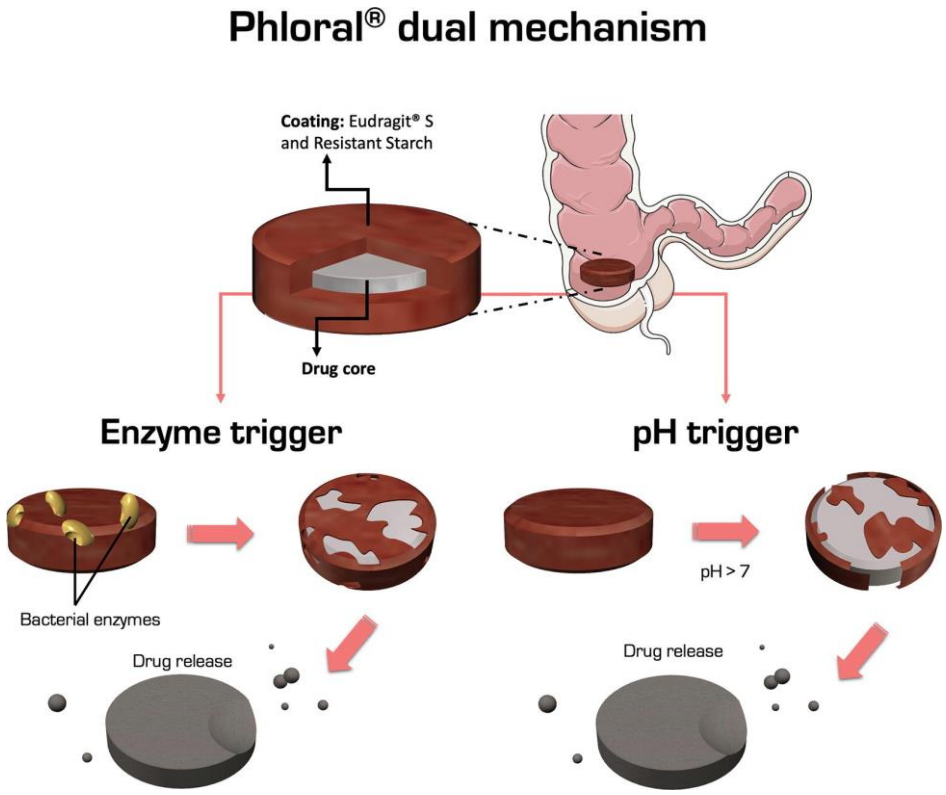
Quelques brevets et technologies pour le ciblage du côlon

La section suivante décrira certaines technologies brevetées et commercialisées pour le traitement des MICI.

Le système multi-matrice (ou MMX®) associe des approches pH- et temps-dépendantes [46]. Des substances actives comme la mésalazine (Lialda™ aux États-Unis, Mezavant® en France) ou le budésonide (Uceris® aux Etats-Unis,

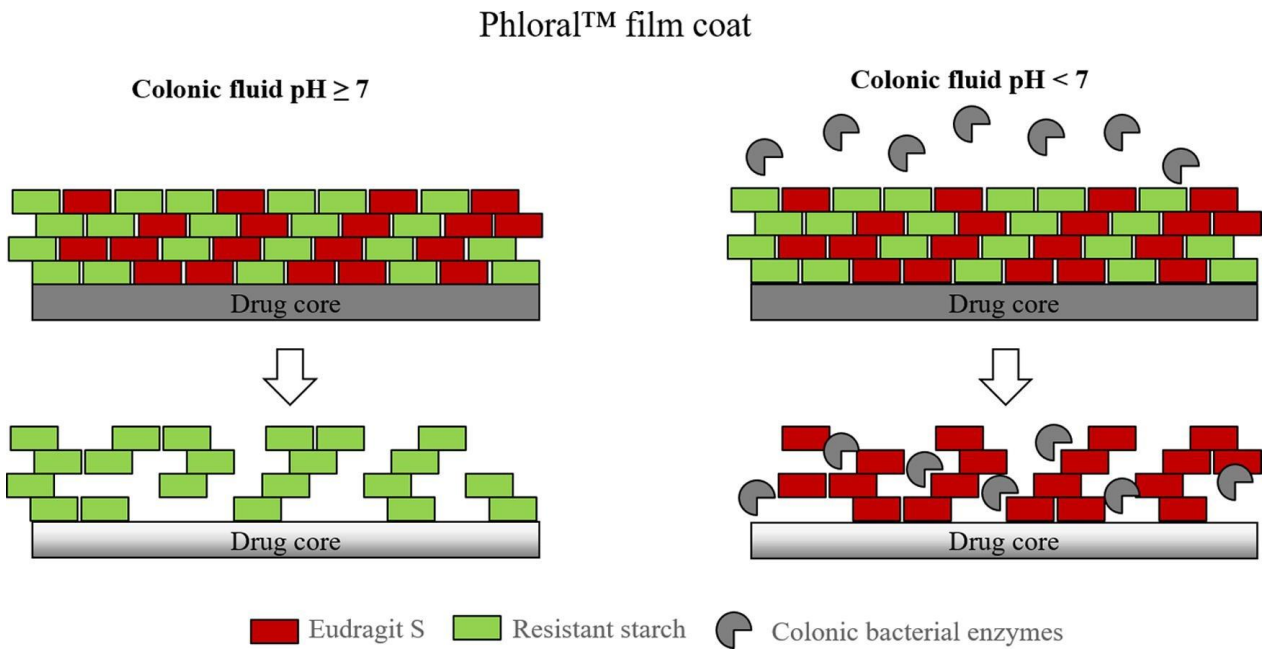
Cortiment® en France) sont incorporées dans de petites matrices lipophiles, au sein d'un ensemble hydrophile. Ce système de matrice « double » est pelliculé par un film à libération entérique (*e.g.* à base d'Eudragit L et S). Après dissolution du pelliculage à pH neutre, la double matrice ralentit la libération du médicament.

Exemple de système combinant des caractéristiques dépendantes du pH et du microbiote intestinal



Mécanisme d'action du système de libération pH- et microbiote-dépendant Phloral®. Figure issue de Awad et al. (2022).

Première technologie à double stimulus commercialisée, Phloral® repose sur un pelliculage associant un polysaccharide (amidon), sensible au microbiote, et un polymère pH-dépendant (Eudragit® S). Phloral est la propriété d'Intract Pharma. L'amidon n'est pas digéré par les amylases digestives, mais par le microbiote intestinal. Phloral® assure une libération précise dans le côlon, autant chez les individus sains que malades. Les stimuli, indépendants mais complémentaires, permettent la délivrance de la substance active par des mécanismes pH- et microbiote-dépendants. Conçu à l'University College London, Phloral® a démontré son potentiel dans le traitement de pathologies touchant le côlon (*e.g.* MICI) dans tous les contextes d'alimentation (états à jeun, pré-alimentés et nourris) [59].



Mécanisme d'action détaillé du système de libération pH- et microbiote-dépendant Phloral®. Figure issue de Varum et al. (2020).

Au terme d'une étape de criblage permettant d'identifier le mélange polymérique le plus résistant dans le haut tractus gastro-intestinal, le mélange Ethylcellulose:Shellac a été retenu. Cette composition de base a présenté une libération contrôlée dans le temps et une faible prise en eau, suscitant notre intérêt.

La shellac est un polymère naturel biosourcé non toxique, hydrophobe, dont l'utilisation pour le ciblage du côlon a été plusieurs fois documentée. Son pH de dissociation, situé autour de 7.0, permet d'utiliser celui-ci comme pelliculage entérique.

Différents tests de prise en eau / perte en masse et de libération *in vitro* ont prouvé la fiabilité de cette formulation de base. En effet, de faibles valeurs de perte en masse ont été relevées et ont démontré que ce mélange polymérique pouvait servir de pelliculage entérique.

La seconde phase, dite d'optimisation, a montré que l'ajout d'excipients hydrophiles, tels que la maltodextrine ou l'inuline, n'avait pas d'impact significativement défavorable sur la perméabilité et la robustesse de ces films dans le haut TGI.

L'ajout de polysaccharide reste indispensable pour assurer la biodégradation du film par le microbiote intestinal. Ces expériences préliminaires ont permis de voir un aspect prometteur à ce pelliculage.

Par la suite, des mini-comprimés de 5 mm de diamètre ont été fabriqués par compression directe. Ces derniers ont subi une étape de pelliculage au moyen d'une enrobeuse à tambour, ou « pan coater ». Différents lots ont été réalisés, tous à base de mélange EC:Shellac, avec différents ratios de mélange et niveaux d'enrobage (autrement appelés gains de masse). Ces

comprimés sont des systèmes réservoirs : ils sont composés d'un noyau central où est logée la substance active, lui-même enrobé du pelliculage.

Les tests de libération *in vitro* de ces formes galéniques ont impliqué différents milieux digestifs :

- 0.1 N HCl ou milieu gastrique reconstitué (2h)
- PBS 6.8 ou milieu intestinal reconstitué (6h)
- Milieu colique reconstitué sous atmosphère anaérobie (24h), contenant ou non des selles de patients.

Les selles de patients MICI ont permis d'être fidèle aux conditions physiopathologiques. La substance active a été totalement protégée dans le haut TGI. Aucune libération, sinon négligeable, n'a été observée après 8h d'incubation en milieu aqueux. Aussi, indépendamment de la présence ou de l'absence de bactéries, le pelliculage a pu retenir la substance active jusqu'à 32h. Cette formulation innovante semble avantageuse en termes de libération contrôlée dans le haut TGI, mais aussi dans le côlon.

En outre, ces mini-comprimés pelliculés présentent les avantages suivants : (1) faciles à administrer, (2) adaptés pour des doses réduites.

La shellac n'étant pas dégradée par le microbiote intestinal, l'ajout d'un polysaccharide s'est avéré nécessaire : d'abord pour améliorer l'hydrophilie globale du système et favoriser sa prise en eau (souvent recommandé avec ce polymère, *via* l'ajout de « pore formers », qui catalysent la création de pores dans le film). Mais surtout, pour permettre la libération provoquée de la substance active dans le côlon grâce à un mécanisme actif. Exploiter l'activité enzymatique de la microflore intestinale semble d'autant plus important que le volume moyen de liquide dans le côlon est faible (environ 13 mL) [206], ne permettant pas la bonne dissolution de la matrice.

La formulation finale, composée d'EC, de shellac et d'inuline, permet :

- Le contrôle de sa prise en eau et de sa dissolution grâce à l'EC
- Une pH-dépendance grâce à la shellac
- D'être substrat du microbiote intestinal grâce à la présence du polysaccharide

Comme dit plus haut, les systèmes à double déclenchement (sensibles au pH et au microbiote *e.g.*) intéressent de plus en plus les chercheurs. Plusieurs brevets existent et s'appuient sur ce type de composition (Phloral®, Colal®, Opticore®...).
Ce pelliculage innovant a fourni des résultats intéressants. A partir d'un taux d'inuline de 10%, la dégradation du film a été catalysée dans le bas TGI, avec 52% de théophylline libérée. La protection de la substance active est restée optimale dans le haut TGI, dans ces mêmes conditions. La formulation exploitée ici semble prometteuse, et de nombreux tests mériteraient d'être menés, d'abord *in vitro,* puis *in vivo,* chez l'animal. Ce mélange présente, entre autres avantages, celui d'être un pelliculage monocouche, facile à fabriquer.
Un souci de fabrication des lots de comprimés a néanmoins freiné les tests de libération *in vitro*. Tout au long de la dernière année de thèse (2022-2023), les comprimés enrobés présentaient une forte instabilité en milieu aqueux et se désagrégeaient en quelques minutes. De nombreux paramètres du procédé de pelliculage ont été corrigés, mais sans réel succès. Suite à cela, les comprimés pelliculés ont prolongé leur stabilité dans l'eau jusqu'à tenir 4 à 5h, mais pas davantage. Les expériences qui suivaient, impliquant des tests quantitatifs (20% et 30% d'inuline ajoutée) et qualitatifs (inuline remplacée par alginate, maltodextrine, pullulan) n'ont pu être menées à bien. De la même manière, d'autres tests *in vitro,* de comprimés enrobés avec d'autres mélanges, n'ont pu être entrepris. Notamment, il était prévu d'évaluer les mélanges EC:Inuline (sans shellac) et Shellac:Inuline (sans EC), afin de voir l'impact de l'un et l'autre de ces polymères sur les profils de libération obtenus. Les seuls résultats obtenus avec 10% d'inuline ont été très favorables et nous encouragent à œuvrer dans ce sens. Notamment, l'inuline a des propriétés prébiotiques, capable de favoriser le développement de la microflore intestinale. Cette propriété n'est pas négligeable dans le cadre des MICI, et mérite d'être d'autant plus exploitée.

VI. REFERENCES

VI. References

[1] Burisch J, Jess T, Martinato M, et al. The burden of inflammatory bowel disease in Europe. *J Crohns Colitis* 2013; 7: 322–337.

[2] Kaplan GG. The global burden of IBD: from 2015 to 2025. *Nat Rev Gastroenterol Hepatol* 2015; 12: 720–727.

[3] Ungaro R, Mehandru S, Allen PB, et al. Ulcerative colitis. *Lancet Lond Engl* 2017; 389: 1756–1770.

[4] Ng SC, Shi HY, Hamidi N, et al. Worldwide incidence and prevalence of inflammatory bowel disease in the 21st century: a systematic review of population-based studies. *Lancet Lond Engl* 2017; 390: 2769–2778.

[5] Barberio B, Segal JP, Quraishi MN, et al. Efficacy of Oral, Topical, or Combined Oral and Topical 5-Aminosalicylates, in Ulcerative Colitis: Systematic Review and Network Meta-analysis. *J Crohns Colitis* 2021; 15: 1184–1196.

[6] Crohn's & colitis foundation, Online leaflet (consulted on November 4, 2023).

[7] Patel MM. Getting into the colon: approaches to target colorectal cancer. *Expert Opin Drug Deliv* 2014; 11: 1343-1350.

[8] Prakash S, Urbanska AM. Colon-targeted delivery of live bacterial cell biotherapeutics including microencapsulated live bacterial cells. *Biol Targets Ther* 2008; 2: 355–378.

[9] Tran PHL, Tran TTD. Current film coating designs for colon-targeted oral delivery. *Curr Med Chem* 2021; 28: 1957–1969.

[10] Jones RGA, Martino A. Targeted localized use of therapeutic antibodies: a review of non-systemic, topical and oral applications. *Crit Rev Biotechnol* 2016; 36: 506–520.

[11] Vass P, Démuth B, Hirsch E, et al. Drying technology strategies for colon-targeted oral delivery of biopharmaceuticals. *J Control Release Off J Control Release Soc* 2019; 296: 162–178.

[12] Truong-Le V, Lovalenti PM, Abdul-Fattah AM. Stabilization challenges and formulation strategies associated with oral biologic drug delivery systems. *Adv Drug Deliv Rev* 2015; 93: 95–108.

[13] Philip AK, Philip B. Colon targeted drug delivery systems: a review on primary and novel approaches. *Oman Med J* 2010; 25: 79–87.

[14] Awad A, Madla CM, McCoubrey LE, et al. Clinical translation of advanced colonic drug delivery technologies. *Adv Drug Deliv Rev* 2022; 181: 114076.

[15] Alqahtani MS, Kazi M, Alsenaidy MA, et al. Advances in Oral Drug Delivery. *Front Pharmacol* 2021; 12: 618411.

[16] Zizzari AT, Pliatsika D, Gall FM, et al. New perspectives in oral peptide delivery. *Drug Discov Today* 2021; 26: 1097–1105.

[17] Granados-Romero JJ, Valderrama-Treviño AI, Contreras-Flores EH, et al. Colorectal cancer: a review. *Int J Res Med Sci* 2017; 5: 4667.

[18] Gala UH, Miller DA, Williams RO. Harnessing the therapeutic potential of anticancer drugs through amorphous solid dispersions. *Biochim Biophys Acta BBA - Rev Cancer* 2020; 1873: 188319.

[19] Gupta SC, Sung B, Prasad S, et al. Cancer drug discovery by repurposing: teaching new tricks to old dogs. *Trends Pharmacol Sci* 2013; 34: 508–517.

[20] Patel MM. Colon: a gateway for chronotherapeutic drug delivery systems. *Expert Opin Drug Deliv* 2015; 12: 1389–1395.

[21] Mehra R. Understanding nocturnal asthma. The plot thickens. *Am J Respir Crit Care Med* 2014; 190: 243–244.

[22] Maqbool I, Akhtar M, Ahmad R, et al. Novel multiparticulate pH triggered delayed release chronotherapeutic drug delivery of celecoxib-β-cyclodextrin inclusion complexes by using Box-Behnken design. *Eur J Pharm Sci* 2020; 146: 105254.

[23] Ozturk N, Ozturk D, Kavakli IH, et al. Molecular Aspects of Circadian Pharmacology and Relevance for Cancer Chronotherapy. *Int J Mol Sci* 2017; 18: 2168.

[24] Sajan J, Cinu T, Chacko A, et al. Chronotherapeutics and Chronotherapeutic Drug Delivery Systems. *Trop J Pharm Res* 2009; 8: 467-475. DOI: 10.4314/tjpr.v8i5.48091.

[25] Mahar KM, Portelli S, Coatney R, et al. Gastric pH and Gastric Residence Time in Fasted and Fed Conscious Beagle Dogs using the Bravo® pH System. *J Pharm Sci* 2012; 101: 2439–2448.

[26] Kararli TT. Comparison of the gastrointestinal anatomy, physiology, and biochemistry of humans and commonly used laboratory animals. *Biopharm Drug Dispos* 1995; 16: 351–380.

[27] Evans DF, Pye G, Bramley R, et al. Measurement of gastrointestinal pH profiles in normal ambulant human subjects. *Gut* 1988; 29: 1035–1041.

[28] Ibekwe VC, Fadda HM, McConnell EL, et al. Interplay between intestinal pH, transit time and feed status on the in vivo performance of pH responsive ileo-colonic release systems. *Pharm Res* 2008; 25: 1828–1835.

[29] Wang YT, Mohammed SD, Farmer AD, et al. Regional gastrointestinal transit and pH studied in 215 healthy volunteers using the wireless motility capsule: influence of age, gender, study country and testing protocol. *Aliment Pharmacol Ther* 2015; 42: 761–772.

[30] Madla CM, Gavins FKH, Merchant HA, et al. Let's talk about sex: Differences in drug therapy in males and females. *Adv Drug Deliv Rev* 2021; 175: 113804.

[31] Fischer M, Siva S, Wo JM, et al. Assessment of Small Intestinal Transit Times in Ulcerative Colitis and Crohn's Disease Patients with Different Disease Activity Using Video Capsule Endoscopy. *AAPS PharmSciTech* 2017; 18: 404–409.

[32] Dieterich W, Schink M, Zopf Y. Microbiota in the Gastrointestinal Tract. *Med Sci* 2018; 6: 116.

[33] Basit AW. Advances in colonic drug delivery. *Drugs* 2005; 65: 1991–2007.

[34] Macfarlane GT, Macfarlane S. Bacteria, colonic fermentation, and gastrointestinal health. *J AOAC Int* 2012; 95: 50–60.

[35] Macleod GS, Fell JT, Collett JH, et al. Selective drug delivery to the colon using pectin:chitosan:hydroxypropyl methylcellulose film coated tablets. *Int J Pharm* 1999; 187: 251–257.

[36] Moens F, Van Den Abbeele P, Basit AW, et al. A four-strain probiotic exerts positive immunomodulatory effects by enhancing colonic butyrate production in vitro. *Int J Pharm* 2019; 555: 1–10.

[37] Tian L, Wang X-W, Wu A-K, et al. Deciphering functional redundancy in the human microbiome. *Nat Commun* 2020; 11: 6217.

[38] Lautenschläger C, Schmidt C, Fischer D, et al. Drug delivery strategies in the therapy of inflammatory bowel disease. *Adv Drug Deliv Rev* 2014; 71: 58–76.

[39] Kotla NG, Rana S, Sivaraman G, et al. Bioresponsive drug delivery systems in intestinal inflammation: State-of-the-art and future perspectives. *Adv Drug Deliv Rev* 2019; 146: 248–266.

[40] Van Den Mooter G, Maris B, Samyn C, et al. Use of Azo Polymers for Colon-Specific Drug Delivery. *J Pharm Sci* 1997; 86: 1321–1327.

[41] Maroni A, Del Curto MD, Zema L, et al. Film coatings for oral colon delivery. *Int J Pharm* 2013; 457: 372–394.

[42] Hanauer SB, Sparrow M. Therapy of ulcerative colitis. *Curr Opin Gastroenterol* 2004; 20: 345–350.

[43] Clive G Wilson, Patrick J Crowley. *Controlled Release in Oral Drug Delivery*. Springer-Verlag New York Inc., 2011, 414 p. Online book (last consulted on November 2, 2023). DOI: 10.1007/978-1-4614-1004-1; ISBN: 978-1-4614-1003-4.

[44] Yang L. Biorelevant dissolution testing of colon-specific delivery systems activated by colonic microflora. *J Control Release Off J Control Release Soc* 2008; 125: 77–86.

[45] FDA NEWS. Alizyme Updates Phase III Product Development, October 10, 2007. https://www.fdanews.com/articles/99592-alizyme-updates-phase-iii-product-development (accessed on November 2, 2023).

[46] Kamm MA, Sandborn WJ, Gassull M, et al. Once-daily, high-concentration MMX mesalamine in active ulcerative colitis. *Gastroenterology* 2007; 132: 66–75; quiz 432–433.

[47] Hoy SM. Budesonide MMX(®): a review of its use in patients with mild to moderate ulcerative colitis. *Drugs* 2015; 75: 879–886.

[48] Cosmo Pharmaceuticals Company. LIALDA® / MEZAVANT® / MESAVANCOL®, https://www.cosmopharma.com/products/lialda-mezavant-mesavancol (accessed on August 25, 2022).

[49] Center for drug evaluation and research. LIALDA (Mesalamine), Approval Package for *NDA 022000S-005*; 370 p. Online consultation (accessed on September 10, 2023).

[50] Development timeline for Lialda. *Drugs.com*, https://www.drugs.com/history/lialda.html (2023).

[51] HAS - Direction de l'Evaluation Médicale, Economique et de Santé Publique. Commission de la transparence - avis du 20 octobre 2010, https://www.has-sante.fr/upload/docs/application/pdf/2010-11/mezavant_-_ct_8220.pdf.

[52] Santarus Receives FDA Approval of UCERIS (Budesonide) for the Induction of Remission in Patients with Active, Mild to Moderate Ulcerative Colitis. *fiercebiotech.com*, https://www.fiercebiotech.com/biotech/santarus-receives-fda-approval-of-uceris-budesonide-for-induction-of-remission-patients.

[53] HAS - Direction de l'Evaluation Médicale, Economique et de Santé Publique. Commission de la transparence - avis du 23 octobre 2019, https://www.has-sante.fr/upload/docs/evamed/CT-17793_CORTIMENT_PIC_REEV_Avis2_CT17793.pdf.

[54] Nardelli S, Pisani LF, Tontini GE, et al. MMX® technology and its applications in gastrointestinal diseases. *Ther Adv Gastroenterol* 2017; 10: 545–552.

[55] Edsbäcker S, Andersson T. Pharmacokinetics of budesonide (Entocort EC) capsules for Crohn's disease. *Clin Pharmacokinet* 2004; 43: 803–821.

[56] Patel M, Shah T, Amin A. Therapeutic opportunities in colon-specific drug-delivery systems. *Crit Rev Ther Drug Carrier Syst* 2007; 24: 147–202.

[57] Katsuma M, Watanabe S, Takemura S, et al. Scintigraphic evaluation of a novel colon-targeted delivery system (CODES) in healthy volunteers. *J Pharm Sci* 2004; 93: 1287–1299.

[58] Yang L. Biorelevant dissolution testing of colon-specific delivery systems activated by colonic microflora. *J Control Release* 2008; 125: 77–86.

[59] Varum F, Freire AC, Fadda HM, et al. A dual pH and microbiota-triggered coating (Phloral™) for fail-safe colonic drug release. *Int J Pharm* 2020; 583: 119379.

[60] Allegretti JR, Fischer M, Sagi SV, et al. Fecal Microbiota Transplantation Capsules with Targeted Colonic Versus Gastric Delivery in Recurrent Clostridium difficile Infection: A Comparative Cohort Analysis of High and Lose Dose. *Dig Dis Sci* 2019; 64: 1672–1678.

[61] Varum F, Freire AC, Bravo R, et al. OPTICORE™, an innovative and accurate colonic targeting technology. *Int J Pharm* 2020; 583: 119372.

[62] Basit A, Varum F, Bravo F. OPTICORE™: A first-in-class colonic targeting technology, https://ondrugdelivery.com/wp-content/uploads/2020/07/109_Jul_2020_Tillotts.pdf (accessed on August 28, 2022).

[63] D'Haens GR, Sandborn WJ, Zou G, et al. Randomised non-inferiority trial: 1600 mg versus 400 mg tablets of mesalazine for the treatment of mild-to-moderate ulcerative colitis. *Aliment Pharmacol Ther* 2017; 46: 292–302.

[64] Soteria® Transforming Injectable Biologics into Oral Pills. *Intract Pharma*, https://www.intractpharma.com/fomulation-technologies/soteria/.

[65] Bardoliwala D, Baradia D, Amrutiya J, et al. Applications of Polymers in Colon Drug Delivery. In: *Applications of Polymers in Drug Delivery*. Elsevier, pp. 187–220.

[66] FDA history of mesalamine products approval, https://www.fda.gov/media/100068/download.

[67] Mesalamine tablets approval - French National Authority for Health. *https://www.has-sante.fr/*, https://www.has-sante.fr/upload/docs/application/pdf/ct-2153_pentasa_.pdf.

[68] Sulfasalzine FDA approval. *https://www.fda.gov/*, https://www.drugs.com/sulfasalazine.html.

[69] Balsalazide FDA approval. *Drugs.com*, https://www.drugs.com/mtm/balsalazide.html (accessed on August 7, 2023).

[70] Olsalazine FDA approval. *Drugs.com*, https://www.drugs.com/mtm/olsalazine.html (accessed on August 7, 2023).

[71] Sulfasalazine - French National Authority for Health. https://www.has-sante.fr/, https://www.has-sante.fr/upload/docs/evamed/CT-16651_SALAZOPYRINE_PIC_RI_Avis2_CT16651.pdf.

[72] Olsalazine - French National Authority for Health. *https://www.has-sante.fr/*, https://www.has-sante.fr/upload/docs/evamed/CT-15611_DIPENTUM_PIS_RI_Avis2_CT15611.pdf.

[73] Entocort EC FDA approval. *Drugs.com*, https://www.drugs.com/mtm/entocort-ec.html (accessed on August 7, 2023).

[74] Budenoside MMX FDA approval. *http://www.fiercebiotech.com*, https://www.fiercebiotech.com/biotech/santarus-receives-fda-approval-of-uceris-budesonide-for-induction-of-remission-patients (accessed on August 7, 2023).

[75] Rayos FDA approval. *Drugs.com*, https://www.drugs.com/pro/rayos.html#s-34067-9 (accessed on August 7, 2023).

[76] Entocort - French National Authority for Health. *https://www.has-sante.fr/*, https://www.has-sante.fr/upload/docs/application/pdf/ct032583.pdf.

[77] Cortiment - French National Authority for Health. *https://www.has-sante.fr/*, https://www.has-sante.fr/upload/docs/evamed/CT-17793_CORTIMENT_PIC_REEV_Avis2_CT17793.pdf.

[78] Zhang J, Zhan P, Tian H. Recent updates in the polysaccharides-based Nano-biocarriers for drugs delivery and its application in diseases treatment: A review. *Int J Biol Macromol* 2021; 182: 115–128.

[79] Eric Sagonowsky. The top 20 drugs by worldwide sales in 2020. *FIERCE Pharma*, https://www.fiercepharma.com/special-report/top-20-drugs-by-2020-sales#5db07ce0-93eb-469d-aa5b-3b71fda03734 (accessed on August 28, 2022).

[80] Global Biopharmaceuticals Market Size, Share, Trends, Growth, COVID-19 Impact Analysis Report - Segmented By Product Type, Therapeutic Type and Region – Industry Forecast (2022 to 2027). *Market Data Forecast*, https://www.marketdataforecast.com/market-reports/bio-pharmaceuticals-market (2022, accessed on September 9, 2022).

[81] Anselmo AC, Gokarn Y, Mitragotri S. Non-invasive delivery strategies for biologics. *Nat Rev Drug Discov* 2019; 18: 19–40.

[82] Fosgerau K, Hoffmann T. Peptide therapeutics: current status and future directions. *Drug Discov Today* 2015; 20: 122–128.

[83] Teruel AH, Gonzalez-Alvarez I, Bermejo M, et al. New Insights of Oral Colonic Drug Delivery Systems for Inflammatory Bowel Disease Therapy. *Int J Mol Sci* 2020; 21: 6502.

[84] Richard J. Challenges in oral peptide delivery: lessons learnt from the clinic and future prospects. *Ther Deliv* 2017; 8: 663–684.

[85] Bak A, Ashford M, Brayden DJ. Local delivery of macromolecules to treat diseases associated with the colon. *Adv Drug Deliv Rev* 2018; 136–137: 2–27.

[86] Intract Pharma. Intract Pharma Limited and Celltrion Group announce collaboration for development of oral Infliximab., https://www.intractpharma.com/2020/08/20/intract-pharma-limited-and-celltrion-group-announce-collaboration-for-development-of-oral-infliximab/ (2020, accessed August 28, 2022).

[87] Zhu Q, Chen Z, Paul PK, et al. Oral delivery of proteins and peptides: Challenges, status quo and future perspectives. *Acta Pharm Sin B* 2021; 11: 2416–2448.

[88] Mahmood A, Bernkop-Schnürch A. SEDDS: A game changing approach for the oral administration of hydrophilic macromolecular drugs. *Adv Drug Deliv Rev* 2019; 142: 91–101.

[89] Choonara BF, Choonara YE, Kumar P, et al. A review of advanced oral drug delivery technologies facilitating the protection and absorption of protein and peptide molecules. *Biotechnol Adv* 2014; 32: 1269–1282.

[90] Karsdal MA, Riis BJ, Mehta N, et al. Lessons learned from the clinical development of oral peptides. *Br J Clin Pharmacol* 2015; 79: 720–732.

[91] Maher S, Ryan B, Duffy A, et al. Formulation strategies to improve oral peptide delivery. *Pharm Pat Anal* 2014; 3: 313–336.

[92] Chen G, Kang W, Li W, et al. Oral delivery of protein and peptide drugs: from non-specific formulation approaches to intestinal cell targeting strategies. *Theranostics* 2022; 12: 1419–1439.

[93] Sandimmune capsule FDA approval. *Drugs.com*, https://www.drugs.com/mtm/sandimmune.html (accessed August 7, 2023).

[94] Lopes M, Simões S, Veiga F, et al. Why most oral insulin formulations do not reach clinical trials. *Ther Deliv* 2015; 6: 973–987.

[95] Rubinstein A, Tirosh B, Baluom M, et al. The rationale for peptide drug delivery to the colon and the potential of polymeric carriers as effective tools. *J Controlled Release* 1997; 46: 59–73.

[96] Gibson SA, McFarlan C, Hay S, et al. Significance of microflora in proteolysis in the colon. *Appl Environ Microbiol* 1989; 55: 679–683.

[97] Aguirre TAS, Teijeiro-Osorio D, Rosa M, et al. Current status of selected oral peptide technologies in advanced preclinical development and in clinical trials. *Adv Drug Deliv Rev* 2016; 106: 223–241.

[98] Wen J-Y, Ledger R, McLeod BJ, et al. Protein and peptide degradation in the intestine of the common brushtail possum (Trichosurus vulpecula). *J Comp Physiol B* 2002; 172: 553–559.

[99] Tozaki H, Odoriba T, Iseki T, et al. Use of protease inhibitors to improve calcitonin absorption from the small and large intestine in rats. *J Pharm Pharmacol* 1998; 50: 913–920.

[100] Smart AL, Gaisford S, Basit AW. Oral peptide and protein delivery: intestinal obstacles and commercial prospects. *Expert Opin Drug Deliv* 2014; 11: 1323–1335.

[101] Wen J-Y, Ledger R, Butt AG, et al. Inhibition of proteolysis in luminal extracts from the intestine of the brushtail possum. *J Pharm Pharmacol* 2002; 54: 1365–1372.

[102] Wen JY, Ledger R, McLeod BJ, et al. Enzymatic degradation of luteinizing hormone releasing hormone (LHRH) by mucosal homogenates from the intestine of the common brushtail possum (Trichosurus vulpecula). *Life Sci* 2002; 71: 3019–3030.

[103] Haupt S, Gil E, Tirosh R, et al. Regional peptide uptake study in the rat intestinal mucosa: glatiramer acetate as a model drug. *Pharm Res* 2002; 19: 832–837.

[104] Ungell AL, Andreasson A, Lundin K, et al. Effects of enzymatic inhibition and increased paracellular shunting on transport of vasopressin analogues in the rat. *J Pharm Sci* 1992; 81: 640–645.

[105] Maroni A, Zema L, Del Curto MD, et al. Oral colon delivery of insulin with the aid of functional adjuvants. *Adv Drug Deliv Rev* 2012; 64: 540–556.

[106] Lu RH, Kopecková P, Kopecek J. Degradation and aggregation of human calcitonin in vitro. *Pharm Res* 1999; 16: 359–367.

[107] Hanefeld M, Monnier L, Schnell O, et al. Early Treatment with Basal Insulin Glargine in People with Type 2 Diabetes: Lessons from ORIGIN and Other Cardiovascular Trials. *Diabetes Ther Res Treat Educ Diabetes Relat Disord* 2016; 7: 187–201.

[108] Petersen SB, Nolan G, Maher S, et al. Evaluation of alkylmaltosides as intestinal permeation enhancers: comparison between rat intestinal mucosal sheets and Caco-2 monolayers. *Eur J Pharm Sci Off J Eur Fed Pharm Sci* 2012; 47: 701–712.

[109] Brayden DJ. Localised Delivery of Macromolecules to the Large Intestine: Translation to Clinical Trials. *BioDrugs* 2022; 36: 687–700.

[110] Durán-Lobato M, Niu Z, Alonso MJ. Oral Delivery of Biologics for Precision Medicine. *Adv Mater* 2020; 32: 1901935.

[111] Vesga O, Agudelo M, Salazar BE, et al. Generic vancomycin products fail in vivo despite being pharmaceutical equivalents of the innovator. *Antimicrob Agents Chemother* 2010; 54: 3271–3279.

[112] Vancocin capsules, product label, http://www.accessdata.fda.gov/drugsatfda_docs/label/2011/050606s028lbl.pdf (accessed on August 11, 2023).

[113] Anderson KE, Eliot LA, Stevenson BR, et al. Formulation and evaluation of a folic acid receptor-targeted oral vancomycin liposomal dosage form. *Pharm Res* 2001; 18: 316–322.

[114] Catry B, Cavaleri M, Baptiste K, et al. Use of colistin-containing products within the European Union and European Economic Area (EU/EEA): development of resistance in animals and possible impact on human and animal health. *Int J Antimicrob Agents* 2015; 46: 297–306.

[115] Zhang W, Michalowski CB, Beloqui A. Oral Delivery of Biologics in Inflammatory Bowel Disease Treatment. *Front Bioeng Biotechnol* 2021; 9: 675194.

[116] Crowe JS, Roberts KJ, Carlton TM, et al. Preclinical Development of a Novel, Orally-Administered Anti-Tumour Necrosis Factor Domain Antibody for the Treatment of Inflammatory Bowel Disease. *Sci Rep* 2018; 8: 4941.

[117] Scott Crowe, Mike West, Kevin Roberts, et al. *United States Patent Crowe et al .* US 10,633,438 B2, Cambridge, https://patentimages.storage.googleapis.com/2e/b3/59/4960ef6e6a7ef6/US10633438.pdf.

[118] Nurbhai S, Roberts KJ, Carlton TM, et al. Oral Anti-Tumour Necrosis Factor Domain Antibody V565 Provides High Intestinal Concentrations, and Reduces Markers of Inflammation in Ulcerative Colitis Patients. *Sci Rep* 2019; 9: 14042.

[119] Wahlich J. Oral delivery of a novel domain antibody (Vorabody™) for the treatment of Crohn's Disease, https://www.pharmaexcipients.com/wp-content/uploads/attachments/VHsquared-PDDS-2017-V565-oral-delivery.pdf?t=1502685580 (accessed 8 August 2023).

[120] Sunanda V. Kane. Mongersen Is Not Effective for Crohn Disease. *New England Journal of Medicine*, https://www.jwatch.org/na50620/2020/01/07/mongersen-not-effective-crohn-disease, 2020 (accessed on August 9, 2023).

[121] Revolutionizing oral biologics delivery. *Intract Pharma*, https://www.intractpharma.com/ (accessed on August 9, 2023).

[122] Protagonist Therapeutics. Protagonist Therapeutics Announces Topline Data from Phase 2 IDEAL Study of PN-943 in Ulcerative Colitis, https://www.prnewswire.com/news-releases/protagonist-therapeutics-announces-topline-data-from-phase-2-ideal-study-of-pn-943-in-ulcerative-colitis-301532339.html, 2022 (accessed on September 10, 2023).

[123] Mattheakis L, Tang T, Venkataraman S, et al. 416 – The Oral A4B7 Integrin Specific Antagonist Pn-10943 is More Effective Than Ptg-100 in Multiple Preclinical Studies. *Gastroenterology* 2019; 156: S-80-S-81.

[124] Sandborn WJ, Mattheakis LC, Modi NB, et al. PTG-100, an Oral α4β7 Antagonist Peptide: Preclinical Development and Phase 1 and 2a Studies in Ulcerative Colitis. *Gastroenterology* 2021; 161: 1853-1864.e10.

[125] ISID Meeting; May 10-13, 2023; Tokyo, Japan, https://isid2023.org/ (accessed on September 26, 2023).

[126] Paul B, Kumar S, Das A. Lac cultivation & their host trees found in Bastar Forest Division. *Plant sciences feed* 2013; 3: 8–12.

[127] Thombare N, Kumar S, Kumari U, et al. Shellac as a multifunctional biopolymer: A review on properties, applications and future potential. *Int J Biol Macromol* 2022; 215: 203–223.

[128] Srivastava S, Thombare N. Safety Assessment of Shellac as Food Additive through Long Term Toxicity Study. *Trends Biosci* 2017; 10: 733–740.

[129] Wang L, Ishida Y, Ohtani H, et al. Characterization of natural resin shellac by reactive pyrolysis-gas chromatography in the presence of organic alkali. *Anal Chem* 1999; 71: 1316–1322.

[130] Ben Messaoud G, Sánchez-González L, Probst L, et al. Physico-chemical properties of alginate/shellac aqueous-core capsules: Influence of membrane architecture on riboflavin release. *Carbohydr Polym* 2016; 144: 428–437.

[131] Sharma SK, Shukla SK, Vaid DN. Shellac-Structure, Characteristics & Modification. *Def Sci J* 1983; 33: 261–271.

[132] Phan The D, Debeaufort F, Luu D, et al. Moisture barrier, wetting and mechanical properties of shellac/agar or shellac/cassava starch bilayer bio-membrane for food applications. *J Membr Sci* 2008; 325: 277–283.

[133] Stummer S, Salar-Behzadi S, Unger FM, et al. Application of shellac for the development of probiotic formulations. *Food Res Int* 2010; 43: 1312–1320.

[134] Limmatvapirat S, Limmatvapirat C, Luangtana-Anan M, et al. Modification of physicochemical and mechanical properties of shellac by partial hydrolysis. *Int J Pharm* 2004; 278: 41–49.

[135] Pearnchob N, Dashevsky A, Bodmeier R. Improvement in the disintegration of shellac-coated soft gelatin capsules in simulated intestinal fluid. *J Control Release Off J Control Release Soc* 2004; 94: 313–321.

[136] Farag Y, Leopold CS. Development of shellac-coated sustained release pellet formulations. *Eur J Pharm Sci Off J Eur Fed Pharm Sci* 2011; 42: 400–405.

[137] Oehme A, Valotis A, Krammer G, et al. Preparation and characterization of shellac-coated anthocyanin pectin beads as dietary colonic delivery system. *Mol Nutr Food Res* 2011; 55 Suppl 1: S75-85.

[138] Gately NM, Kennedy JE. The Development of a Melt-Extruded Shellac Carrier for the Targeted Delivery of Probiotics to the Colon. *Pharmaceutics* 2017; 9: 38.

[139] Czarnocka JK, Alhnan MA. Gastro-resistant characteristics of GRAS-grade enteric coatings for pharmaceutical and nutraceutical products. *Int J Pharm* 2015; 486: 167–174.

[140] Pearnchob N, Siepmann J, Bodmeier R. Pharmaceutical applications of shellac: moisture-protective and taste-masking coatings and extended-release matrix tablets. *Drug Dev Ind Pharm* 2003; 29: 925–938.

[141] Sinha VR, Kumria R. Coating polymers for colon specific drug delivery: a comparative in vitro evaluation. *Acta Pharm Zagreb Croat* 2003; 53: 41–47.

[142] Wang A, Lin J, Zhong Q. Enteric rice protein-shellac composite coating to enhance the viability of probiotic Lactobacillus salivarius NRRL B-30514. *Food Hydrocoll* 2021; 113: 106469.

[143] Yan F, Polk DB. Probiotics and Probiotic-Derived Functional Factors—Mechanistic Insights Into Applications for Intestinal Homeostasis. *Front Immunol* 2020; 11: 1428.

[144] Habashy R, Khoder M, Zhang S, et al. An innovative wax-based enteric coating for pharmaceutical and nutraceutical oral products. *Int J Pharm* 2020; 591: 119935.

[145] Bar H, Bianco-Peled H. The unique nanostructure of shellac films. *Prog Org Coat* 2021; 157: 106328.

[146] Somani AA, Thelen K, Zheng S, et al. Evaluation of changes in oral drug absorption in preterm and term neonates for Biopharmaceutics Classification System (BCS) class I and II compounds. *Br J Clin Pharmacol* 2016; 81: 137–147.

[147] Theophylline water solubility at 25°C. *www.drugbank.com*, https://go.drugbank.com/drugs/DB00277 (accessed on October 13, 2022).

[148] Venkat Tumuluri. Pharmaceutical mini-tablets: a revived trend. *Drug Delivery Trends* 2020; 3: 123–139.

[149] Mitra B, Chang J, Wu S-J, et al. Feasibility of mini-tablets as a flexible drug delivery tool. *Int J Pharm* 2017; 525: 149–159.

[150] Tablet coating. *Spraying Systems Co.®*, https://www.spray.com/-/media/dam/industrial/usa/sales-material/catalog/c12b_pharm_tablet-coating.pdf (accessed on July 29, 2023).

[151] Film coating - Mastering formulation and the coating process. *SEPPIC*, https://www.seppic.com/en/technologies/film-coating (accessed on July 29, 2023).

[152] DuPont. AQUACOAT® ECD: Coating process considerations - Technical Memorandum.

[153] Abbas N, Irfan M, Zahoor AF, et al. Polymer blend: a new approach for eliminating curing effect of aqueous dispersion coatings. *Braz J Pharm Sci* 2020; 56: e18562.

[154] Karrout Y, Neut C, Wils D, et al. Novel polymeric film coatings for colon targeting: Drug release from coated pellets. *Eur J Pharm* 2009; 37: 427-433.

[155] Karrout Y, Neut C, Wils D, et al. Peas starch-based film coatings for site-specific drug delivery to the colon. *J Appl Polym Sci* 2011; 119: 1176–1184.

[156] Karrout Y, Neut C, Siepmann F, et al. Enzymatically degraded Eurylon 6 HP-PG: ethylcellulose film coatings for colon targeting in inflammatory bowel disease patients. *J Pharm Pharmacol* 2010; 62: 1676–1684.

[157] Karrout Y, Neut C, Wils D, et al. Colon targeting with bacteria-sensitive films adapted to the disease state. *Eur J Pharm Biopharm* 2009; 73: 74–81.

[158] Benzine Y, Siepmann F, Neut C, et al. Hot melt extruded polysaccharide blends for controlled drug delivery. *J Drug Deliv Sci Technol* 2019; 54: 101317.

[159] Fan LT., Singh SK. Chapter *Diffusion-Controlled Release*. In: *Controlled Release: a quantitative treatment*. Springer Berlin, Heidelberg, 1989. *Polym*; 13: 9-88. https://doi.org/10.1007/978-3-642-74507-2_2

[160] Karrout Y. *Innovative drug delivery systems for colon targeting*. PhD, Freien Universität Berlin (Germany), 2008. https://refubium.fu-

berlin.de/bitstream/handle/fub188/232/Karrout.pdf?sequence=1&isAllowed=y (accessed on June 20, 2023).

[161] Esseku F, Adeyeye MC. Bacteria and pH-sensitive polysaccharide-polymer films for colon targeted delivery. *Crit Rev Ther Drug Carrier Syst* 2011; 28: 395–445.

[162] Macleod GS, Fell JT, Collett JH. Studies on the physical properties of mixed pectin/ethylcellulose films intended for colonic drug delivery. *Int J Pharm* 1997; 157: 53–60.

[163] Lecomte F, Siepmann J, Walther M, et al. Blends of enteric and GIT-insoluble polymers used for film coating: physicochemical characterization and drug release patterns. *J Control Release Off J Control Release Soc* 2003; 89: 457–471.

[164] Lecomte F, Siepmann J, Walther M, et al. pH-Sensitive polymer blends used as coating materials to control drug release from spherical beads: elucidation of the underlying mass transport mechanisms. *Pharm Res* 2005; 22: 1129–1141.

[165] Afinjuomo F, Abdella S, Youssef SH, et al. Inulin and Its Application in Drug Delivery. *Pharm Basel Switz* 2021; 14: 855.

[166] López-Molina D, Navarro-Martínez MD, Rojas-Melgarejo F, et al. Molecular properties and prebiotic effect of inulin obtained from artichoke (Cynara scolymus L.). *Phytochemistry* 2005; 66: 1476–1484.

[167] Shahdadi Sardou H, Akhgari A, Afrasiabi Garekani H, et al. Screening of different polysaccharides in a composite film based on Eudragit RS for subsequent use as a coating for delivery of 5-ASA to colon. *Int J Pharm* 2019; 568: 118527.

[168] Mensink MA, Frijlink HW, van der Voort Maarschalk K, et al. Inulin, a flexible oligosaccharide. II: Review of its pharmaceutical applications. *Carbohydr Polym* 2015; 134: 418–428.

[169] van den Heuvel EGHM, Wils D, Pasman WJ, et al. Short-term digestive tolerance of different doses of NUTRIOSE FB, a food dextrin, in adult men. *Eur J Clin Nutr* 2004; 58: 1046–1055.

[170] Yuan Y, He N, Dong L, et al. Multiscale Shellac-Based Delivery Systems: From Macro- to Nanoscale. *ACS Nano* 2021; 15: 18794–18821.

[171] Theismann E-M, Keppler JK, Knipp J-R, et al. Adjustment of triple shellac coating for precise release of bioactive substances with different physico-chemical properties in the ileocolonic region. *Int J Pharm* 2019; 564: 472–484.

[172] Patel A, Heussen P, Hazekamp J, et al. Stabilisation and controlled release of silibinin from pH responsive shellac colloidal particles. *Soft Matter* 2011; 7: 8549.

[173] Haaser M, Karrout Y, Velghe C, et al. Application of terahertz pulsed imaging to analyse film coating characteristics of sustained-release coated pellets. *Int J Pharm* 2013; 457: 521–526.

[174] Jha P. Effect of plasticizer and antimicrobial agents on functional properties of bionanocomposite films based on corn starch-chitosan for food packaging applications. *Int J Biol Macromol* 2020; 160: 571–582.

[175] Sanyang M, Sapuan S, Jawaid M, et al. Effect of Plasticizer Type and Concentration on Tensile, Thermal and Barrier Properties of Biodegradable Films Based on Sugar Palm (Arenga pinnata) Starch. *Polymers* 2015; 7: 1106–1124.

[176] Razavi SMA, Mohammad Amini A, Zahedi Y. Characterisation of a new biodegradable edible film based on sage seed gum: Influence of plasticiser type and concentration. *Food Hydrocoll* 2015; 43: 290–298.

[177] Muscat D, Adhikari B, Adhikari R, et al. Comparative study of film forming behaviour of low and high amylose starches using glycerol and xylitol as plasticizers. *J Food Eng* 2012; 109: 189–201.

[178] Teixeira SC, Silva RRA, De Oliveira TV, et al. Glycerol and triethyl citrate plasticizer effects on molecular, thermal, mechanical, and barrier properties of cellulose acetate films. *Food Biosci* 2021; 42: 101202.

[179] Siepmann J, Peppas NA. Hydrophilic matrices for controlled drug delivery: an improved mathematical model to predict the resulting drug release kinetics (the 'sequential layer' model). *Pharm Res* 2000; 17: 1290–1298.

[180] Fan L-T, Singh SK. Diffusion-Controlled Release. 13, https://link.springer.com/chapter/10.1007/978-3-642-74507-2_2 (1989, accessed on October 3, 2023).

[181] Timucin U. Mini Tablets: A Short Review-Revision. *Open J Chem* 2017; 012–022.

[182] Qussi B, Suess WG. Investigation of the effect of various shellac coating compositions containing different water-soluble polymers on in vitro drug release. *Drug Dev Ind Pharm* 2005; 31: 99–108.

[183] Stillhart C, Vučićević K, Augustijns P, et al. Impact of gastrointestinal physiology on drug absorption in special populations--An UNGAP review. *Eur J Pharm Sci Off J Eur Fed Pharm Sci* 2020; 147: 105280.

[184] Roda A, Simoni P, Magliulo M, et al. A new oral formulation for the release of sodium butyrate in the ileo-cecal region and colon. *World J Gastroenterol* 2007; 13: 1079–1084.

[185] Maghrabia AE, Boughdady MF, Meshali MM. New Perspective Enteric-Coated Tablet Dosage Form for Oral Administration of Ceftriaxone: In Vitro and In Vivo Assessments. *AAPS PharmSciTech* 2019; 20: 306.

[186] Ravi V, Siddaramaiah null, Pramod Kumar TM. Influence of natural polymer coating on novel colon targeting drug delivery system. *J Mater Sci Mater Med* 2008; 19: 2131–2136.

[187] Farag Y, Leopold CS. Investigation of drug release from pellets coated with different shellac types. *Drug Dev Ind Pharm* 2011; 37: 193–200.

[188] Karrout Y, Neut C, Wils D, et al. Novel polymeric film coatings for colon targeting: Drug release from coated pellets. *Eur J Pharm Sci Off J Eur Fed Pharm Sci* 2009; 37: 427–433.

[189] Schellekens RCA, Stuurman FE, van der Weert FHJ, et al. A novel dissolution method relevant to intestinal release behaviour and its application in the evaluation of modified release mesalazine products. *Eur J Pharm Sci Off J Eur Fed Pharm Sci* 2007; 30: 15–20.

[190] Wilding IR, Kenyon CJ, Hooper G. Gastrointestinal spread of oral prolonged-release mesalazine microgranules (Pentasa) dosed as either tablets or sachet. *Aliment Pharmacol Ther* 2000; 14: 163–169.

[191] SSB® Pharma - Shellac for pharmaceutical applications. https://www.stroever.de/en/. Online leaflet (accessed on November 2, 2023).

[192] Limmatvapirat S, Limmatvapirat C, Puttipipatkhachorn S, et al. Enhanced enteric properties and stability of shellac films through composite salts formation. *Eur J Pharm Biopharm Off J Arbeitsgemeinschaft Pharm Verfahrenstechnik EV* 2007; 67: 690–698.

[193] Buch K, Penning M, Wächtersbach E, et al. Investigation of various shellac grades: additional analysis for identity. *Drug Dev Ind Pharm* 2009; 35: 694–703.

[194] Amidon S, Brown JE, Dave VS. Colon-targeted oral drug delivery systems: design trends and approaches. *AAPS PharmSciTech* 2015; 16: 731–741.

[195] Protect® enteric production information. *Sensient Pharmaceutical*, https://www.sensientpharma.com/product/enterics/protect/ (accessed on July 14, 2023).

[196] Liu F. *A novel approach to accelerate the dissolution of enteric polymer coatings*. PhD, University College London (United Kingdom), 2008. https://www.proquest.com/openview/1db43e46fb34663252350110af67212b/1?pq-origsite=gscholar&cbl=2026366 (accessed on November 2, 2023).

[197] Farag Y, Leopold CS. Influence of the inlet air temperature in a fluid bed coating process on drug release from shellac-coated pellets. *Drug Dev Ind Pharm* 2011; 37: 320–328.

[198] Fadda HM, Merchant HA, Arafat BT, et al. Physiological bicarbonate buffers: stabilisation and use as dissolution media for modified release systems. *Int J Pharm* 2009; 382: 56–60.

[199] Garbacz G, Kołodziej B, Koziolek M, et al. An automated system for monitoring and regulating the pH of bicarbonate buffers. *AAPS PharmSciTech* 2013; 14: 517–522.

[200] Krieg BJ, Taghavi SM, Amidon GL, et al. In vivo predictive dissolution: transport analysis of the CO2 , bicarbonate in vivo buffer system. *J Pharm Sci* 2014; 103: 3473–3490.

[201] Liu F, Merchant HA, Kulkarni RP, et al. Evolution of a physiological pH 6.8 bicarbonate buffer system: application to the dissolution testing of enteric coated products. *Eur J Pharm Biopharm Off J Arbeitsgemeinschaft Pharm Verfahrenstechnik EV* 2011; 78: 151–157.

[202] Merchant HA, Goyanes A, Parashar N, et al. Predicting the gastrointestinal behaviour of modified-release products: utility of a novel dynamic dissolution test apparatus involving the use of bicarbonate buffers. *Int J Pharm* 2014; 475: 585–591.

[203] Goyanes A, Hatton GB, Merchant HA, et al. Gastrointestinal release behaviour of modified-release drug products: Dynamic dissolution testing of mesalazine formulations. *Int J Pharm* 2015; 484: 103–108.

[204] Wray H, Joseph R, Palmen M, et al. Combined pharmacokinetic and scintigraphic analyses for the comparison of 5-ASA release profiles from MMX™ mesalamine and another delayed-release mesalamine formulation: P-0030. *Inflamm Bowel Dis* 2008; 14: S19–S20.

[205] Al-Gousous J, Ruan H, Blechar JA, et al. Mechanistic analysis and experimental verification of bicarbonate-controlled enteric coat dissolution: Potential in vivo implications. *Eur J Pharm Biopharm* 2019; 139: 47–58.

[206] Schiller C, Fröhlich C-P, Giessmann T, et al. Intestinal fluid volumes and transit of dosage forms as assessed by magnetic resonance imaging. *Aliment Pharmacol Ther* 2005; 22: 971–979.

[207] Major G, Murray K, Singh G, et al. Demonstration of differences in colonic volumes, transit, chyme consistency, and response to psyllium between healthy and constipated subjects using magnetic resonance imaging. *Neurogastroenterol Motil* 2018; 30: e13400.

[208] Leong CW, Newton JM, Basit AW, et al. The formation of colonic digestible films of amylose and ethylcellulose from aqueous dispersions at temperatures below 37 degrees C. *Eur J Pharm Biopharm Off J Arbeitsgemeinschaft Pharm Verfahrenstechnik EV* 2002; 54: 291–297.

[209] Katsuma M, Watanabe S, Kawai H, et al. Effects of absorption promoters on insulin absorption through colon-targeted delivery. *Int J Pharm* 2006; 307: 156–162.

[210] Maroni A, Del Curto MD, Serratoni M, et al. Feasibility, stability and release performance of a time-dependent insulin delivery system intended for oral colon release. *Eur J Pharm Biopharm Off J Arbeitsgemeinschaft Pharm Verfahrenstechnik EV* 2009; 72: 246–251.

[211] Dahan A, Amidon GL, Zimmermann EM. Drug targeting strategies for the treatment of inflammatory bowel disease: a mechanistic update. *Expert Rev Clin Immunol* 2010; 6: 543–550.

[212] Tablet Capping and Lamination. *Merlin Powder characterisation*, https://www.merlin-pc.com/services/tablet-capping-and-lamination (accessed on July 31, 2023).

[213] Syntegon Pharma Technology, LLC. Syntegon processing and packaging, Online leaflet (accessed on August 10, 2023).

[214] Shellac – Aqueous Solution – SWANLAC ASL 10. *A.F. Suter and Co. Ltd Shellac, Waxes, Gums and Resins*, https://www.afsuter.com/product/aqueous-shellac-solution/ (accessed August 10, 2023).

[215] Shin-Etsu Chemical. Shin-Etsu excipients in tablet troubleshooting, https://www.shinetsu.co.jp/en/.

[216] Troubleshooting Guide – TOP 10 Film Coating Errors. *Biogrund*, https://www.biogrund.com/top10-troubleshooting/?lang=en#toggle-id-6 (2023, accessed on July 31, 2023).

[217] Troubleshooting Guide. *Colorcon*, https://www.colorcon.com/resource-center/trouble-shooting-chart (accessed on July 31, 2023).

[218] L'Assurance Maladie. Personnes prises en charge pour maladies inflammatoires chroniques intestinales en 2019. *Observatoire national des MICI*, http://www.observatoire-crohn-rch.fr/wp-content/uploads/2021/12/2019_fiche_maladies-inflammatoires-chroniques-intestinales.pdf (accessed on September 21, 2023).

[219] AFA - Crohn, RCH France. Section 'Comprendre la maladie', https://www.afa.asso.fr/maladie-inflammatoire-chronique-de-linstestin/comprendre-la-maladie/mici-maladie-inflammatoire-chronique-intestin/ (accessed on September 21, 2023).

[220] Fiorino G, Sturniolo GC, Bossa F, et al. A Phase 2a, Multicenter, Randomized, Double-Blind, Parallel-Group, Placebo-Controlled Trial of IBD98-M Delayed-Release Capsules to Induce Remission in Patients with Active and Mild to Moderate Ulcerative Colitis. *Cells* 2019; 8: 523.

[221] McCoubrey LE, Favaron A, Awad A, et al. Colonic drug delivery: Formulating the next generation of colon-targeted therapeutics. *J Controlled Release* 2023; 353: 1107–1126.

VII. RESEARCH PEDIGREE

VII. Research pedigree

STRICH Samuel	Former intern of Lille University Hospital in research specialty Full-time Ph.D. funded by the resident salary Doctor of Pharmacy (Pharm.D.)
	Ph.D. from November 2020 to April 2023 at INSERM U1008 – Advanced Drug Delivery Systems Thesis director: Dr Youness KARROUT. PharmD, PhD, HDR. Associate Professor in Pharmaceutics, Biopharmaceutics, and Pharmaceutical technology.
Location:	Laboratoire de Pharmacotechnie industrielle INSERM U1008, 3 rue du Professeur LAGUESSE Faculté de Pharmacie de Lille 59000 Lille, France.

Tutoring

Conferences of residency: preparation of master's degree students in pharmacy for the national competition of hospital residency in the following topics:

- Toxicology: poisons of haemoglobin, toxicomania, psychotropic drugs, cardiotoxicity of antiarrhythmic drugs, hematologic – renal – cardiac toxic mechanism of action.

- Nephrology: renal physiology, hydroelectrolytic and acid-base disorders, renal insufficiencies, nephrotic syndrome.

Lectures, resolution of clinical cases and methodology teaching.

Training of students

Clémence G.	Pharmacy student in 2nd year Université de Lille (1 week)
Céline P.	Pharmacy student in 2nd year Université de Lille (1 week)
Hanene K.	Master's student Université de Lille (6 months)
Clara A.	Pharmacy student in 3rd year Université de Lille (1 week)
Nour H.	Pharmacy student in 3rd year Université de Lille (7 weeks)
Redwan A.	Pharmacy student in 3rd year Université de Lille (7 week)

Nova M.	Bachelor's student in therapeutic innovation and biotechnologies Université de Lille (3 months)
Lamya S.	Pharmacy student in 5th year Université de Lille (1 week)
Faïza B.	Bachelor's student Université de Lille (2 months)
Luka S.	Pharmacy student in 2nd year Université de Lille (1 week)
Yael L.	Master's student Université de Paris (3 months)
Florent M.	Bachelor's student Université de Lille (3 months)
Florian P.	Bachelor's student Université de Lille (3 months)

Participations in conferences and congresses

- 12th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology: 11-14th May 2021 | Virtual conference and exhibition.
  Vienna (Austria, Europe)

- 13th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology: 28-31st March 2022 | Presential conference and exhibition
  Poster presentation: **Strich S**, Lahiani-Skiba M, Amraoui R, Skiba M, Karrout Y. Oral controlled release from thin polymeric films based on polysaccharides.
  Rotterdam (Netherlands, Europe)

- Hot topic day (APGI): Local Controlled Drug Delivery
  29th November 2022 | Presential conference
  Lille (France, Europe)

- Presential coating school by Colorcon: 14-16th March 2023 | Presential courses and practicum about tablets coating
  Certification issued
  Massy (Paris area, France, Europe)

Other courses

Mandatory hospital education with 2 majors to attend for the whole residency:
- Pharmacovigilance and drug safety
- Clinical pharmacy and medication reconciliation

60 European Credits Transfer System (ECTS) acquired from seminaries and conferences attended. Mandatory to validate doctoral school syllabus.

Personal

University degree (2020): Immunotherapies and vaccinology.
Paris Université, Cochin Hospital (Paris, France, Europe)

VIII. APPENDIX

VIII. Appendix

Technology	Polymers and Drug Delivery Details	Applications
Eudracol (Skalsky et al., 2003)	It is pH- and time-controlled drug delivery system. Pellets layered with Eudragit RL/RS and Eudragit FS 30 D.	Technology offers a new aspect for colon drug targeting through oral delivery due to its specific coating structure. Dose frequency reduction.
CODES technologies (Li et al., 2002)	System is based on the two mechanisms pH-dependent release along with microbial-triggered CDDS. Lactulose containing tablet core is coated with Eudragit E, and then it is overall coated with Eudragit L.	Lactulose acts as a trigger for targeted drug release in the colonic region.
MTCT-OP (Liu et al., 2007)	System is combination of osmotic drug delivery technology and microbial-triggered mechanism. Chitosan incorporated drug core was used to produce osmotic systems along with in situ delivery pores for colon-specific drug release. Tablet was coated by semipermeable membrane made by cellulose acetate and lastly coated with Eudragit L-100-55 that could inhibit semipermeable membrane from forming pore or rupture before it reaches to the colon.	System developed for controlled drug delivery system based on chitosan.
MMX (Multimatrix technology) (Nardelli et al., 2017)	Acrylic copolymers are used to prepare pH-resistant drug delivery systems. This system delays the drug release in stomach acid and starts dissolving in intestinal location.	System permits the protection of the drug from harsh stomach pH conditions and enzymes present in the upper GIT. Can be utilized as a platform technology for applications outside the colonic region where a delayed release is necessary.
Peltab (pelletized tablet) (Dey et al., 2008)	Polymer-coated pellets of drug are compressed into tablets for colon-specific drug delivery.	Technology can be used as controlled drug delivery.
PHLORAL (Trivedi and Puranik, 2017)	pH-dependent coating also incorporates a resistant starch component, which is broken down particularly by the microbes in the colon. Dosage units with the combination coating all disintegrated in the colonic area as desired.	Technology provides "fail—safe" delivery of drug to the target site by employing two complimentary mechanisms to trigger the release of drug and hence significant enhancement in colonic delivery.
Colal-Pred system (Hanauer and Sparrow, 2004)	System contains small ethyl cellulose- and amylose-coated pellets containing prednisolone sodium metasulfobenzoate that is broken down in the colon only by enzymes.	System consists of the combination of colonic drug delivery system, COLAL, and prednisolone sodium metasulfobenzoate. Possibly the best treatment of ulcerative colitis without the usual debilitating steroidal adverse effects.

CDDS, *Colon drug delivery system;* GIT, *gastrointestinal tract;* MTCT-OP, *microbial-triggered colon-targeted osmotic pump.*

A_1: Marketed technologies containing various polymer for the colon targeted drug delivery. Table obtained from Bardoliwala et al. (2021, https://doi.org/10.1016/B978-0-12-819659-5.00007-0).

IX. PUBLICATIONS

IX. Publications

- **2023**: S. Strich, H. Azehaf, C. Neut, Y. Lellouche-Jacob, N. Medkour, M. Penning, Y. Karrout.
  Film Coatings Based on Aqueous Shellac Ammonium Salt "Swanlac®ASL 10" and Inulin for Colon Targeting. AAPS PharmSciTech, 2023. 24: 205. Published on October 4th of 2023.
  https://doi.org/10.1208/s12249-023-02652-2

- **2022**: Poster presentation.
  Strich S, Lahiani-Skiba M, Amraoui R, Skiba M, Karrout Y. Oral controlled release from thin polymeric films based on polysaccharides.
  13th World Meeting on Pharmaceutics, Biopharmaceutics and Pharmaceutical Technology. 28-31st March, 2022, Rotterdam (Netherlands, Europe).