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CAPSULAR DEVICES FOR ORAL MODIFIED RELEASE OF DRUGS PREPARED BY INJECTION MOLDING

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BACKGROUND

AND

AIM OF THE WORK

Among modified-release oral dosage forms, increasing interest has currently been addressed to delayed/pulsatile delivery systems [Maroni A. *et al*., 2013b; Maroni A. *et al*., 2005]. They are intended for the liberation of the conveyed active ingredient after a programmed time period (lag phase) that starts with the administration of the dosage form. Such a strategy meets the principles of chronopharmacology, which is based on the knowledge that both the symptomathology of a large number of pathologies and the pharmacokinetics and pharmacodynamics of a variety of drugs are influenced by biological circadian rhythms. The possibility for oral pulsatile delivery systems of finding application in chronotherapy is interesting for those diseases whose symptoms occur mainly during the night or in the early morning, such as bronchial asthma, angina pectoris, rheumatoid arthritis. Indeed, the administration of the drug delivery system (DDS) at bedtime increases patient compliance, since it may circumvent the need for waking up for drug intake; furthermore, the drug liberation in conjunction with the onset of symptoms allows to decrease the dose and/or avoid the useless and potentially dangerous exposition of the patient to the drug in the early hours of sleep, when it wouldn't be necessary [Youan B-B.C., 2004].

Delayed/pulsatile orally-administered systems may also be exploited to achieve site-specific delivery of drugs in the colonic region, based on a time-dependent approach [Maroni A. *et al.*, 2013a; Gazzaniga A. *et al.*, 2006]: a device ensuring a lag time prior to release could target specific regions of the gastrointestinal tract

on the basis of experimental data indicating that the transit time through the small intestine is relatively constant (3 h ± 1 standard error) and independent of the type of dosage form. Indeed, by the application of a gastroresistant (GR) outer film, which allows to overcome gastric emptying variability, the first contact with biological fluids is shifted soon after the dosage form is emptied from the stomach; then, by imparting a lag phase that matches the small intestine transit time (SITT), the drug liberation can occur in the target region. Great interest is focused on colon-specific release for the local treatment of inflammatory bowel disease (IBD), such as Crohn's desease and ulcerative colites, and infective, tumoral as well as neurovegetative colonic pathologies. Moreover, this strategy can also find application in the improvement of the oral bioavailability of peptides and proteins. Although the colon is not considered as an advantageous site for absorption because of its anatomic and physiological characteristics, the long residence time and the lower concentration of local peptidases were proven to partially offset its unfavourable characteristics.

Several strategies for achieving delayed/pulsatile release following oral administration were proposed, generally leading to capsular, osmotic or reservoir systems [Maroni A. *et al.,* 2013b; Gazzaniga A. *et al*., 2008; Bussemer T. *et al*., 2001]. Capsular systems have been designed having an insoluble body filled with a drug preparation. The shell body is sealed with a swellable, erodible or lipophilic matrix plug, which is removed upon swelling and/or erosion, both promoted by the contact with water, or as a consequence of a pressure rise

created inside the capsule by water uptake. Osmotic devices are formed by the drug formulation core including an osmotic and, in some cases, a semi-permeable membrane. Such membrane is provided with a calibrated orifice, from which the drug is pumped out at a constant rate; drug release starts after a silent phase, coinciding with the time required for water penetration into the core of the osmotic pump. Lastly, reservoir systems are also available: they consist of a drug-containing core, which could be either a single- or multiple-unit one, covered by at least one coating layer. On the basis of the composition of such layer, these systems can be distinguished in erodible, rupturable or diffusive. In particular, erodible devices show the ability to impart a lag phase before release, following the dissolution/erosion of a polymeric barrier separating the drug-containing core and the aqueous environment. The lag phase can be modulated by the characteristics of the polymer and the thickness of the coating. Hyroxypropyl methylcellulose (HPMC), hydroxyethylcellulose (HEC) and hydroxypropylcellulose (HPC) are the most commonly employed polymers.

The research group where I carried out my PhD project had already developed an oral DDS for delayed and site-specific release of drugs in the form of an erodible reservoir system, named Chronotopic™ [Cerea M. *et al.*, 2008; Zema L. *et al*., 2007; Sangalli M.E. *et al*., 2004; Sangalli M.E. *et al*., 2001; Gazzaniga A. *et al*., 1995; Gazzaniga A. *et al*., 1994a; Gazzaniga A. *et al*., 1994b ; Maffione G. *et al*., 1993]. It is based on a functional coating composed of a hydrophilic polymer

(generally HPMC) applied by different techniques (press-coating, spray-coating and powder-layering) onto several types of cores (tablets, capsules, pellets). This layer of few hundreds microns of thickness delays the contact of the biological fluids with the drug-containing core through hydration, swelling and dissolution/erosion phenomena, as outlined in Figure 1. The effectiveness of the Chronotopic™ system, its flexibility in terms of duration of the lag phase, both i*n vitro* and *in vivo*, as well as the possibility of scaling up the manufacturing process have been demonstrated.

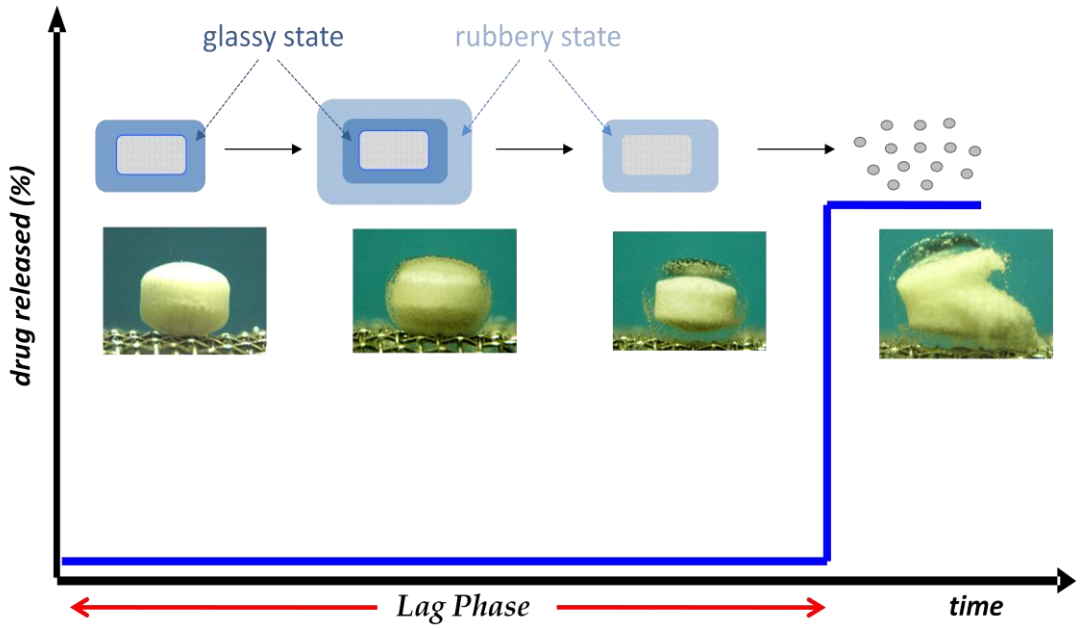


Figure 1: Scheme of the interaction and release performance of a Chronotopic™ system with aqueous fluids.

On the basis of the encouraging results obtained, the possibility of exploiting hydrophilic polymers for the development of a functional container with the same ability of delaying drug release was investigated [Gazzaniga A. *et al.,* 2011a; Gazzaniga A. *et al.,* 2011b]. Such a device was conceived in the form of a capsular shell conveying a variety of drug formulations (solid, semisolid, liquid) filled by means of conventional equipment already available on the market. Notably, such a device would reduce the technical and regulatory burden associated with development and, thus, the relevant costs with respect to coated systems. In an early phase, the use of the hot melt extrusion (HME) technique to prepare the container was explored: it requires that raw materials have a thermoplastic behavior, which is the property of undergoing a transition to a viscous state upon heating [Foppoli A. *et* al., 2009]. HPMC, however, exhibited the desired plastic behavior only when worked at temperatures at which its thermal degradation occurs, even in the presence of plasticizers. Hence, another hydrophilic derivative of cellulose, HPC, was considered for the preparation of functional containers, since it shows thermoplastic properties and the same behavior of HPMC when in contact with aqueous fluids. Moreover, HPC is available on the market in several viscosity grades, suggesting the possibility of achieving a high flexibility in the performance of the delivery platform. The plasticized polymer was extruded into films, which were then press-molded into semi-spherical halves of shells, filled with a drug tracer, paired and sealed to form two-piece spherical capsules. Preliminary data from such devices demonstrated their ability

to delay the release of the contents as a function of the composition. However, the manufacturing technique involved a number of limitations especially with regard to the industrial scalability.

In view of these premises, a different approach was preferred based on the application of the injection molding (IM) technique that gives several advantages, such as the possibility of an easy scale-up, versatility and prospects of patentability. Indeed, although IM has been widely exploited in the plastic field, only few pharmaceutical applications have been reported [Zema L. *et al*., 2012; Vilivalam V.D. *et al.*, 2000; Rothen-Weinhold A. *et al.,* 1999; Stepto R.F.T. and Tomka I., 1987]. IM can be defined as the forced injection of heated thermoplastic polymers into a 3D mold to be shaped while cooling and hardening. The IM equipment employed for the manufacturing of capsular devices was a bench-top press, particularly indicated for the production of small items with high precision (micromolding press). Raw materials, loaded in a hopper, are conveyed by means of a piston into a heated-controlled plastication block, where they undergo softening and mixing; then, the molten mass is forcedly injected through a nozzle into the mold. This consists of two units, one mobile and the other mounted on a fixed platen: once the two halves are combined, the cavity image of the item to be molded is formed. When injected into the mold in the closed configuration, the molten material spreads into the cavity and assumes the desired shape, which can be maintained thanks to the solidification promoted by cooling. Finally, the molded product is ejected. Figure

2 shows an outline of a general IM cycle, which combines two synchronized phases: one comprises filling/opening/closing steps of the mold, while the other refers to the movement of the injection tool (generally a screw) moving forward to the injection position and being pushed back to the pre-injection one. After the automatic ejection of the molded product, a new IM cycle can start, allowing a continuous manufacturing process to take place.

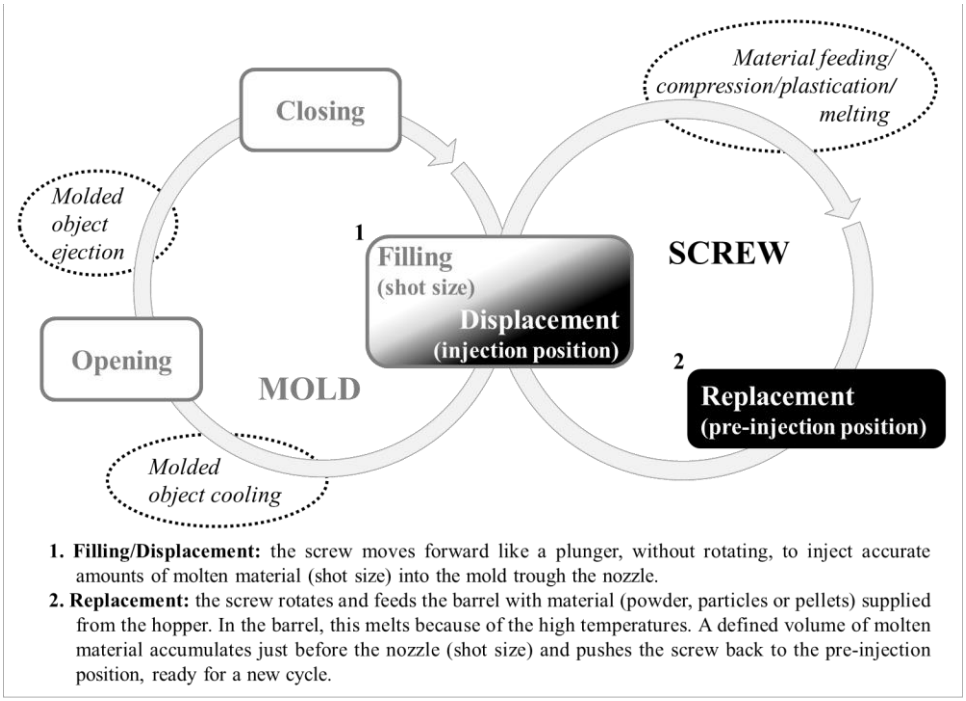


Figure 2: IM cycle in screw-type machine [adapted from Zema L. *et al.*, 2012].

With respect to the mold used for preparing capsular devices, given the need for carrying out early formulation studies, a highly versatile prototype mold that could also be exploited for improving skills in such new technique was designed:

indeed, it could guarantee high flexibility with respect to ongoing adjustments of both the formulation and the thickness of shells. Moreover, the mold was designed able to fulfill a number of requirements for the final device: *i)* the capsular system needed to have a size suitable for oral administration and to consist of matching body and cap items; *ii)* the shape and mechanical characteristics of the device were expected to be suitable for established filling procedures; *iii)* once assembled, the device was expected not to allow for leaks and to preferably have a plane external surface. The prototype mold was provided with a two-cavity set filled simultaneously for the production of cap and body within a single manufacturing cycle; moreover, it bore three couples of cavities to be selected prior to assembling (preselection of the differing thicknesses) (Figure 3).

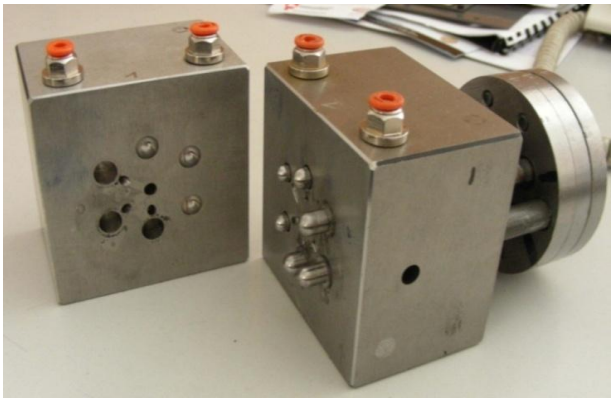
a)  b) 

Figure 3: Prototype mold for the preparation of cap and body items with differing thicknesses (a); detail of the injection orifice and adaptable pouring gate (b) [adapted from Gazzaniga A. *et al.*, 2011a].

A locking mechanism based on the mutual pressure of the contact areas of matching caps and bodies was studied, not entailing any junction gap and less complicated than that of regular gelatin and HPMC capsules (Figure 4). On the basis of previous studies on hydrophilic cellulose ethers used as delaying agents, nominal thicknesses of 300, 600 and 900 µm were hypothesized for capsules, in order to provide lag times of different duration. Because of the shape of the device, such values applied to the thinner wall areas, *i.e.* those where cap and body are not overlapped because of the locking system. Moreover, capsules were designed having the same external dimensions but decreasing inner volume, because of the increase in the wall thickness.

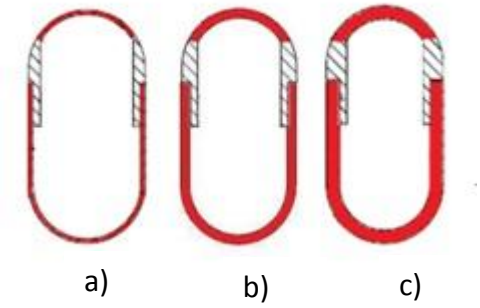


Figure 4: Lengthwise representation of prototype capsules of 300 (a), 600 (b) and 900 (c) µm thickness; areas colored in red are those which nominal thickness refers to.

By the adjustment of process parameters, capsules of the selected shell thicknesses made of Klucel® LF with the addition of 10 w/w of polyethylenglycol (PEG) 1500 as the plasticizer could be obtained (Chronocap™) (Figure 5).

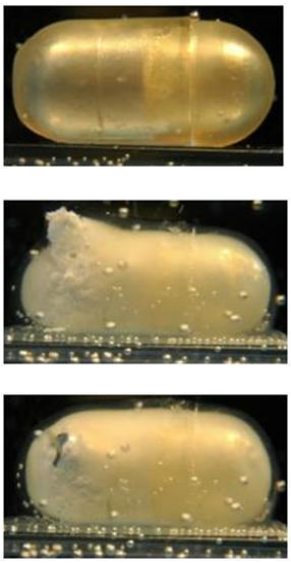
a)  b) 

Figure 5: Assembled capsule (a) and capsule bodies of 300, 600 and 900 µm shell thickness (b).

Capsular devices with shell thickness of 300, 600 and 900 µm based on Klucel® LF exhibited satisfactory technological characteristics as well as release performance (Table 1): reproducible *in vitro* lag phases (calculated as the time to 10% release, $t_{10\%}$), increasing as a function of the wall thickness, were obtained. A prompt and complete release of the drug, promoted by the breakage and rapid dissolution/erosion of capsule shells, was observed both under static and hydrodynamic conditions (Figure 6).

Table 1: Characteristics of Chronocap™ devices with differing shell thicknesses.

nominal thickness µm	**thickness µm (CV)**	**elastic modulus N/mm^2 (CV)**	**$t_{10\%}$ *in vitro* min (CV)**
300	346 (12.30)	2.672 (15.04)	29.3 (22.2)
600	645 (13.20)	5.342 (15.72)	53.5 (10.6)
900	880 (4.64)	8.451 (3.44)	91.7 (3.16)

a) 

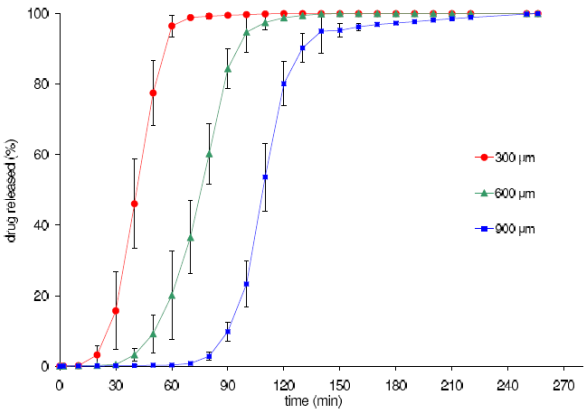
b) 

Figure 6: Photographs at successive time points showing the opening of a 600 µm thick Chronocap™ capsule immersed in deionized water under static conditions (a); mean release profiles of Chronocap™ capsules with differing shell thicknesses (b) [adapted from Gazzaniga A. *et al.*, 2011a].

The *in vivo* release performance of Klucel® LF devices was preliminarily evaluated on healthy volunteers [Zema L. *et al.,* 2013; Gazzaniga A. *et al*., 2011c]. The appearance of drug in saliva samples was delayed with respect to the time of intake as a function of the shell thickness of capsular devices. After the lag phase, the drug concentration showed a fast increase in all cases (Figure 7; Table 2).

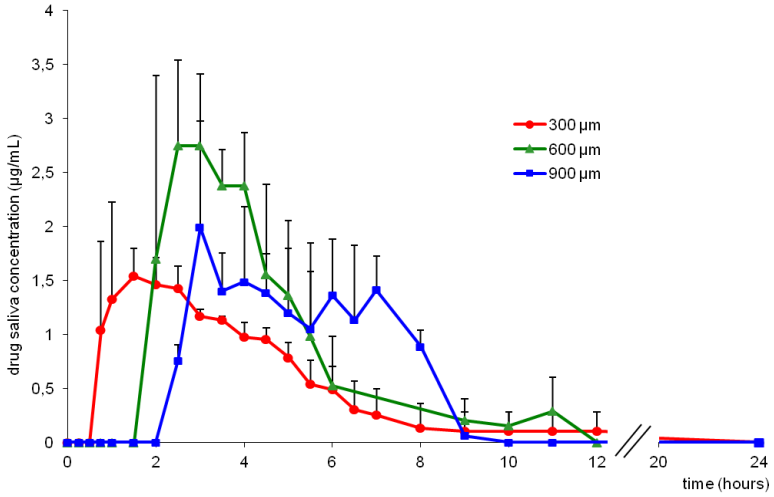


Figure 7: Average acetaminophen saliva concentration *vs* time profiles after oral administration of capsular devices with differing shell thicknesses [adapted from Gazzaniga A. *et al*., 2011c].

Table 2: Pharmacokinetics parameters after oral administration of capsular devices with differing shell thicknesses (standard deviation in brackets) [adapted from Gazzaniga A. *et al*., 2011c].

nominal shell thickness (µm)	**t_{lag} (h)**	**C_{max} (µg/ml)**	**t_{max} (h)**	**AUC_{0-24} (µg*h/ml)**
300	1.25 (0.17)	1.70 (0.46)	1.17 (0.29)	7.43 (3.25)
600	2.34 (0.38)	5.07 (0.97)	2.50 (0.50)	16.06 (4.55)
900	3.53 (0.71)	4.60 (0.97)	3.33 (0.58)	12.62 (0.28)

Based on these premises, the aim of the present work was the further development of the Chronocap™ platform in order to improve its robustness and versatility, as well as to enhance the industrial scalability of the relevant

manufacturing process. In particular, the PhD project focused on: *i)* the development of a dedicated mold for the preparation of HPC-based capsules [Results - Chapter 1]; *ii)* the use of HPC molded capsules as substrates for coating processes in view of the development of a colonic delivery system based on a time-dependent approach [Results - Chapter 2]*; *iii)* the modulation of the lag phase of capsules through formulation changes [Results - Chapter 3].

Most of the results obtained from this research activity have been published/submitted for publication, in the form of experimental articles or poster communications:

E. Macchi, L. Zema, A. Maroni, A. Gazzaniga, L.A. Felton. 2014. Enteric-Coating of Pulsatile-Release Capsules Prepared by Injection Molding, submitted for pubblication.

L. Zema, G. Loreti, E. Macchi, A. Foppoli, A. Maroni, A. Gazzaniga. 2013. Injection-Molded Capsular Device for Oral Pulsatile Release: Development of a Novel Mold, J Pharm Sci 102, 489-499.

L. Zema, G. Loreti, A. Melocchi, F. Casati, E. Macchi, A. Gazzaniga, Oral delivery platforms in the form of “functional containers” prepared by injection molding. Thematic workshop of CRS Italian Chapter on "Design and industrial development of advanced drug delivery systems", Pavia, Italy, November, 2013.

* Part of this work was performed in the Department of Pharmaceutical Science at the University of New Mexico (USA) under the supervision of Prof. Linda A. Felton.

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Results

Chapter 1

Injection-Molded Capsular Device for Oral Pulsatile Release: Development of a Novel Mold

The content of this chapter has already been published in:

L. Zema, G. Loreti, E. Macchi, A. Foppoli, A. Maroni, A. Gazzaniga. 2013. J Pharm Sci 102, 489-499.

Abstract

The development of a purposely-devised mold and a newly set up injection molding (IM) manufacturing process was undertaken in order to prepare swellable/erodible hydroxypropyl cellulose (HPC)-based capsular containers. When orally administered, such devices would be intended to achieve pulsatile and/or colonic time-dependent delivery of drugs. An in-depth evaluation of thermal, rheological and mechanical characteristics of melt formulations/molded items made of the selected polymer (Klucel® LF) with increasing amounts of plasticizer (PEG 1500, 5-15 % by weight) was preliminarily carried out. On the basis of the results obtained, a new mold was designed that allowed, through an automatic manufacturing cycle of 5 s duration, matching cap and body items to be prepared. These were subsequently filled and coupled to give a closed device of constant 600 μm thickness. As compared with previous IM systems having the same composition, such capsules showed improved closure mechanism, technological properties, especially in terms of reproducibility of the shell thickness, and release performance. Moreover, the ability of the capsular container to impart a constant lag phase before the liberation of the contents was demonstrated irrespective of the conveyed formulation.

Keywords: Injection molding (IM); capsular device; microcompounder; pulsatile release; colon delivery; hydroxypropyl cellulose (HPC).

1. Introduction

Injection Molding (IM) was recently explored as a manufacturing technique for the preparation of pharmaceutical dosage forms because of the several advantages it may offer with respect to production costs and technological/biopharmaceutical characteristics of the molded items [Zema L. *et al.*, 2012]. In particular, molded capsular shells with different shape, design and composition were prepared, potentially suitable for a variety of filling formulations (*e.g.* powders, granules/pellets, semi-solid or liquid preparations). IM capsules made of starch or gelatin may represent an alternative to dip-molded commercially-available ones for the formulation of immediate-release oral dosage forms, the administration of pellets or the production of coated modified-release dosage forms [Eith L. *et al.*, 1986; Vilivalam V.D. *et al.*, 2000; Watts P. *et al.*, 2005]. However, innovative devices intended as a viable approach to new Drug Delivery Systems (DDSs) were also proposed. In this respect, the feasibility of capsular containers based on functional polymers (*i.e.* polymers with pH-dependent solubility such as hydroxypropyl methylcellulose acetate succinate, or matrix-forming agents) was demonstrated, and gastroresistant as well as pulsatile delivery systems were so far obtained [Gazzaniga A. *et al.*, 2011a; Gazzaniga A. *et al.*, 2011b; Zema L. *et al.*, 2013].

Swellable hydrophilic cellulose derivatives were successfully employed as release delaying barriers or coating agents in the development of an oral erodible delivery platform (Chronotopic™) intended for pulsatile and/or colonic delivery

[Conte U. *et al.*, 1989; Gazzaniga A. *et al.*, 1994; Gazzaniga A. *et al.*, 2008; Zema L. *et al.*, 2007; Maroni A. *et al.*, 2010]. A lag phase prior to drug release was indeed achieved that could be exploited to obtain a site-selective release to the ileo-colonic region based on a time-dependent strategy [Sangalli M.E. *et al.*, 2001; Del Curto M.D. *et al.*, 2009; Maroni A. *et al.*, 2009]. By the use of hydroxypropyl cellulose (HPC), which offered a good balance between release-controlling performance and IM processability, capsular devices (Chronocap™) having good technological and stability characteristics were subsequently prepared. Such DDSs showed the pursued *in vitro* and *in vivo* release patterns, with lag times increasing as a function of the wall thickness and HPC viscosity grade [Gazzaniga A. *et al*., 2011c]. With respect to coated formulations for pulsatile delivery, the peculiar advantage of Chronocap™ would consist in the possibility of undergoing an independent pharmaceutical development irrespective of its final contents and manufacturing process, which could bring about important repercussions in terms of patentability and industrial scale-up.

The mold prototype employed was highly versatile, as convenient for early formulation studies. Indeed, it allowed the preparation of matching caps and bodies within a single manufacturing cycle as well as the selection of differing nominal shell thicknesses in the 300-900 µm range. However, some practical limitations were entailed by the particular joint mechanism of the capsules, in which the contact areas of bodies and caps overlapped inside the shell cavity thus also leading to a thicker wall region. Moreover, the variability in the shell

thickness and its poor consistency with the nominal value needed to be overcome. In the prospect of an industrial scale-up, the production rate and extent of automation of the manufacturing process also required to be improved.

Based on the dimensional characteristics (*i.e.* length in the order of 10 mm *vs.* thickness of few hundreds of micrometers that is reduced in the proximity of certain details) and tolerances involved, the proposed capsule shell would fall within the definition of micromolded products [Heckele M. and Schomburg W.K., 2004; Giboz J. *et al.*, 2007; Koç M. and Özel T., 2011]. Microinjection molding (µIM) is not only a simple scale-down of classical IM because it entails introducing radical changes into the equipment, the mold construction and the raw materials to be used. In particular, the mold design, formulation development and setup of process parameters (*e.g.* mold temperature, injection speed and pressure, holding time and pressure) should be carried out concomitantly. The aim of the present work was therefore the design of a special mold, dedicated to the production of HPC capsules, and the subsequent development of a manufacturing process able to enhance the industrial scalability of the Chronocap™ pulsatile delivery device.

2. Materials and methods

2.1. Materials

Hydroxypropyl cellulose (HPC, Klucel® LF; Eigenmann & Veronelli, I); polyethylene glycol 1500 and 6000 (Clariant Masterbatches, I); colloidal grade Avicel® CL611 microcrystalline cellulose (IMCD S.p.A, I); acetaminophen (AAP) fine powder (Atabay, TR) and granules (Compap™ Coarse L; Mallinckrodt, US); hard-gelatin capsules (DBcaps®, type B; Capsugel, US).

2.2 Methods

2.2.1. Rheological measurements

The apparent viscosity of polymeric melts was measured using a Haake MiniLab II capillary rheometer (Thermo Scientific Haake, D). The microcompounder was equipped with conical co-rotating twin screws (diameter 5/14, length 109.5 mm) and a backflow channel (capillary: width 10 mm, height 1.5 mm, length 75 mm) with integrated pressure transducers. 5 g samples were manually fed into the extruder. At each extrusion temperature a 10 to 300 rpm screw speed ramp was used. All screw speeds were held for 60 s, and the pressure build-up of the material in the capillary was measured. For each formulation the extrusion temperature was varied from the minimum value that allowed torque ≤ 3.5 N·m to be registered up to 190 °C.

Apparent viscosity data were fitted according to the power equation $\eta=K\cdot\gamma^{n-1}$ where η is the shear viscosity, γ is the shear rate, K and n are the consistency and

power-law index, respectively [Kontopoulou M., 2012]. The latter describes the thinning nature of the melt.

A rotational plate (diameter 25 mm) rheometer (ARES, TA instruments, US) was also used to collect data under isothermal conditions (190 °C); time: from 0 to 1400 s; frequency: 10 rad/s; strain: 0.2 %; gap 1 mm.

2.2.2. Manufacturing of molded items

Mixtures of HPC and polyethylene glycol 1500 were prepared in Turbula® (Type T2C, WAB, CH) and then transferred into a bench-top micromolding machine (BabyPlast 6/10P, Cronoplast S.L.; Rambaldi S.r.l., I). Before use, HPC was dried in a ventilated oven for 24 h at 40 °C.

Molded items were prepared by means of two different molds: *i)* a disk-shaped mold (diameter: 30 mm; height: 1 mm) provided with a central gate and *ii)* a capsular mold with two interchangeable inserts for the manufacturing of matching cap and body items of 600 µm nominal thickness. The IM process conditions are reported in Table R1.1.

Table R1.1: IM process conditions.

		Disk 	capsule 
compression zone temperature; *°C*		120-150	100
metering zone temperature; *°C*		130-160	130
nozzle temperature; *°C*		140-170	140
hot runner temperature; *°C*		-	160
charge; *mm*		15-16	4
1st injection	pressure; *bar*	50	30
	time; *s*	0.6	0.5
	rate; *%*	50	30
2nd injection	pressure; *bar*	40	10
	time; *s*	2	0.3
	rate; *%*	40	10
cooling temperature; *°C*		15	15
cooling time; *s*		2.5	2.5
closing pressure; *bar*		80	120
opening rate; *%*		90	90

2.2.3. Characterization of molded items

Molded items, *i.e.* disks and assembled capsule shells, were checked for weight (analytical balance BP211, Sartorius, D; n = 10) and thickness (digimatic indicator ID-C112X, Mitutoyo, J; n = 10). Moreover, the height and diameter of assembled capsules were determined (analogical micrometer CD15D, Mitutoyo, J; n = 10). Digital photographs (Nikon D70, Nikon, J) of molded items were acquired. The mechanical properties of assembled capsule shells were evaluated by means of a TA.HD.plus Texture Analyzer (Stable Micro Systems, UK; n = 3) as previously described [Gazzaniga A. *et al.*, 2011a]. Elastic modulus (Newton per square millimeter) was calculated from the initial linear portion of stress *vs.* strain curves. Percentage moisture content (MC %) of molded disks was calculated as

[(Ww-Wd)/Ww]·100, where Ww is the wet weight of the sample and Wd is its weight after drying (thermal-scale, Gibertini, I).

The characterization of molded items was generally carried out immediately after ejection and after 1, 3 as well as 7 days storage at ambient conditions (24 ± 2 °C / 55 ± 5 % RH). The mechanical properties and release performance of assembled capsule shells were evaluated after 7 days storage at ambient conditions.

Evaluation of the release performance

Capsule bodies were manually filled with differing formulations each containing 50 mg of acetaminophen (AAP) as a tracer drug and closed with matching caps. For the release test (n = 6), an adapted six-position USP34 disintegration apparatus was used [Gazzaniga A. *et al.*, 1995]. Each unit was inserted in a sinker and positioned in one of the six available tubes of the basket-rack assembly. The basket-rack assemblies moved at 31 cycles/min rate in separate vessels containing 900 mL water at 37 ± 0.5 °C. Fluid samples were withdrawn at fixed time points and assayed spectrophotometrically (248 nm). Lag time ($t_{10\%}$), *i.e.* the time to 10 % release, and pulse time ($t_{90\text{-}10\%}$), *i.e.* the time elapsed between 10 and 90 % release, were calculated from the release curves. Sealing of the assembled capsules was avoided after verifying consistency in the performance of unsealed capsules and capsules sealed as previously reported [Gazzaniga A. *et al.*, 2011a].

Tracer drug formulations and relevant preparation:

i) AAP fine powder (d_{10} = 14 µm; d_{50} = 29 µm; d_{90} = 58 µm) as such;

ii) commercially available AAP granules with 90 % drug content (d_{10} = 230 µm; d_{50} = 480 µm; d_{90} = 900 µm);

iii) pellets in the 710-1000 µm dimensional range prepared by extrusion-speronisation. AAP fine powder (50 % w/w) and microcrystalline cellulose, Avicel® CL611 (50 % w/w), were dry blended and granulated with water (45 % w/w with respect to dry powders) in a planetary mixer (KM 010, Kenwood, UK) fitted with a K-shaped mixing arm. Granulation was performed by slowly pouring water at a constant agitation speed of 250 rpm for 10 min. 350 mg of the wet mass was extruded by a radial basket apparatus (Nica® E140, GEA Pharma Systems, B) equipped with a multi-holed screen (internal hole diameter, 1 mm; screen thickness, 1.25 mm) and the resulting extrudates were spheronised in a Nica® S320 spheroniser with a 64 cm radial-hatched friction plate. Operative conditions were as follows: feeder speed, 90 rpm; extrusion speed, 70 rpm; spheronizer speed, 700 rpm; spheronization time, 1 minute. Pellets were dried in a vacuum oven at 40 °C for 24 h.

iv) AAP solid dispersion in polyethylene glycol 6000 prepared by manually dispersing AAP fine powder (30% w/w) in the molten carrier at 60 °C.

Capsular systems in which approximately 3 mg of a powder dye (methylene blue) was added to the filling formulations were analogously tested. The opening time of capsules was determined by visual inspection. It was defined as the time of

first rupture of the hydrated capsule shells highlighted by the very rapid dissolution of the dye inside the capsule. The morphological changes undergone by the capsular devices when exposed to aqueous fluids and the dissolution behavior of the dye were evaluated on units immersed in unstirred deionized water at the temperature of 37 ± 0.5 °C. Digital photographs were taken at successive time points.

3. Results and discussion

With the aim of developing a special mold, dedicated to the production of hydroxypropyl cellulose (HPC)-based capsules, and a robust injection molding (IM) manufacturing process, it was deemed necessary to focus on a single formulation and evaluate its thermal, rheological and mechanical characteristics. A previous promising composition based on Klucel® LF (KLF), plasticized with polyethylene glycol 1500 (PEG) to improve the mechanical properties of molded capsules, was thus selected as the starting formula [Gazzaniga A. *et al.*, 2011a]. At first, the amount of plasticizer and the method of its incorporation (mixing process) appeared worthy of an in-depth investigation on account of their expected impact on the melt processability and the physical stability of molded items. The rheological properties of KLF melts with increasing amounts of PEG were thus evaluated by a twin-screw extruder integrated with pressure transducers in the backflow channel (capillary rheometer). Because of the relevant mixing ability, the use of co- or counter-rotating screws is well

established in the manufacturing of solid dispersions and of wet- or hot-melt extruded preparations [Repka M.A. *et al.*, 2012; Fukuda M. *et al.*, 2008]. Particularly lab-scale models, which allow to work with few grams of material (microcompounders), were also proposed as tools for rheological measurements since they may provide shear and extensional viscosity parameters at rates experienced during processing, especially when extrusion steps are involved (*e.g.* the plasticating phase of IM) [Cheng B. *et al.*, 2001; Chen Z. *et al.*, 2006; Chen Z. *et al.*, 2008; Ralston B.E. and Osswald T.A., 2008]. Indeed, it is well known that the viscosity characteristics may affect the processability of polymeric melts. Formulations with viscosity values ranging between 103 and 104 Pa·s at operating temperatures are generally used for extrusion processes, whereas lower viscosities (from 101 to 5x102 Pa·s) are needed to face the small cross-sections and/or long flow path entailed by IM [Klemens Kohlgrüber H., 2008]. Polymeric melts are usually characterized by a shear-thinning (or pseudoplastic) behavior that is evident from shear stress *vs.* shear rate profiles. By employing screw-driven capillary rheometers (such as the microcompounder used), shear stress profiles can be obtained as a function of the screw rotation speed thus allowing apparent viscosity values to be calculated [Bialleck S. and Rein H., 2011]. In Figure R1.1, rheograms relevant to KLF as such or in admixture with 5, 10 and 15 % by weight (wt %) of PEG (with respect to the amount of polymer) at different temperatures are reported. Different ranges of processing temperatures were taken into account. The lower limit was that defined by the

resistance of the screw to rotation (torque values ≤ 3.5 N·m). On the other hand, even though the higher limit was set at 190 °C, only data relevant to temperatures ≤ 170 °C were considered because of a browning tendency of the polymer that was highlighted beyond such a value [Gazzaniga A. et al., 2011a].

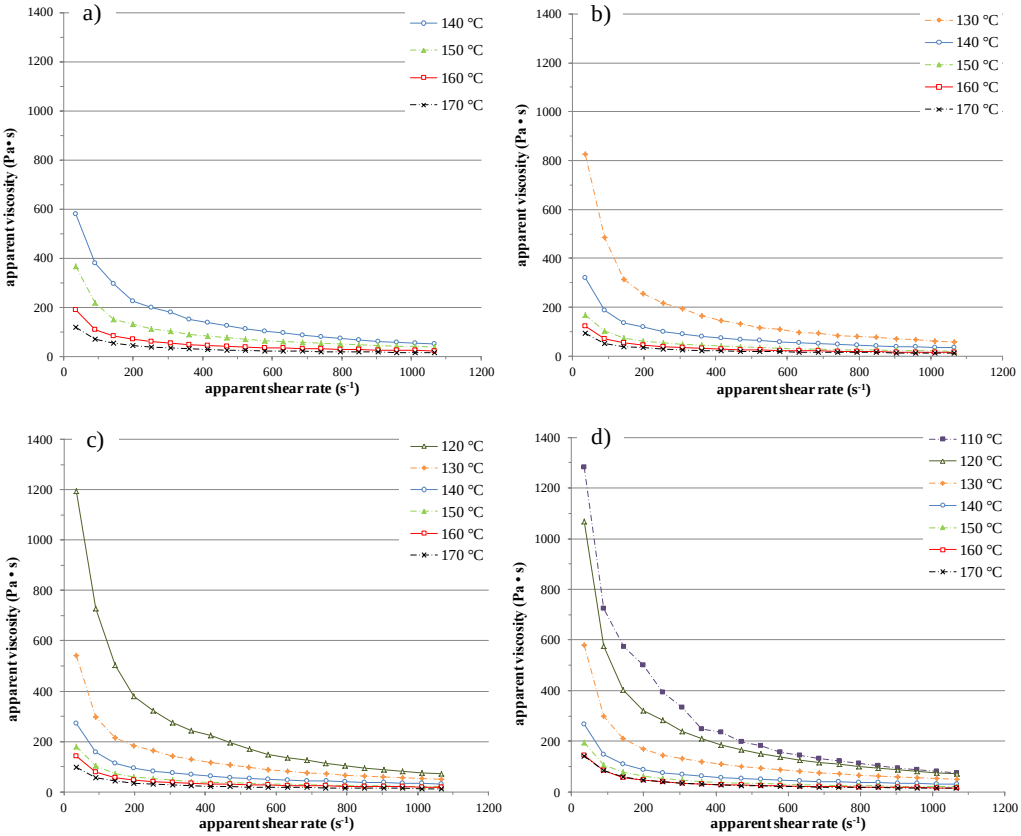


Figure R1.1: Apparent viscosity profiles of Klucel® LF melts at different temperatures: polymer as such (a) and plasticized with 5 (b), 10 (c) or 15 % (d) of PEG.

As expected, the apparent viscosity of KLF-based melts was largely influenced by temperature, especially at the low share rates. Rheograms of the plasticizer-containing mixtures showed an analogous trend as the polymer as such, although apparent viscosity was generally reduced. Moreover, the addition of increasing amounts of PEG was demonstrated to improve the flow behavior of the melts thus allowing the latter to be extruded at progressively lower temperatures (up to 110 °C in the case of 15 % PEG formulation).

In the range of shear rates considered, the shear flow is known to be well described by the power-law equation. Hence, it was thereby possible to calculate the relevant parameters, *n* and *K* [Kontopoulou M. *et al.*, 2012; Paradkara A. *et al.*, 2009]. The power-law index *n* describes the thinning nature of the melt (*i.e.* the smaller the value of *n*, the more shear-thinning the melt), whereas *K* is a consistency index representing the hypothetical shear viscosity extrapolated to zero wall shear rate. The fitting parameters of the above-discussed viscosity profiles are reported in Table R1.2.

Table R1.2: Calculated power-law parameters (n, K and regression coefficient R^2) relevant to Klucel® LF-based melts at different temperatures.

	amount of plasticizer, wt %											
	0			5			10			15		
°C	*n*	*K*	R^2	*n*	*K*	R^2	*n*	*K*	R^2	*n*	*K*	R^2
110	nd	Nd	nd	nd	nd	nd	nd	nd	nd	0.13	41037	0.968
120	nd	Nd	nd	nd	nd	nd	0.14	35406	0.977	0.20	22124	0.989
130	nd	Nd	nd	0.21	16650	0.991	0.30	7332	0.989	0.30	7113	0.996
140	0.26	11026	0.969	0.35	3659	0.992	0.37	2657	0.996	0.37	2510	0.998
150	0.34	4322	0.993	0.38	1644	0.997	0.37	1766	0.999	0.34	2136	0.999
160	0.40	1706	0.998	0.40	1099	0.999	0.43	1043	0.997	0.35	1500	0.999
170	0.41	1026	0.999	0.42	747	0.999	0.40	820	0.999	0.32	1721	0.999

Good correlation coefficients were always obtained, and *n* values were in agreement with those reported in the literature for the same type of polymer [Paradkara A. *et al.*, 2009]. The rheological parameters were determined under temperature conditions analogous to those expected during IM processes though necessarily at lower shear rates. Data should therefore be considered descriptive of the melt behavior from a merely qualitative point of view. In this respect, interesting remarks were drawn from the comparative evaluation of the results obtained. Firstly, the shear-thinning nature of the molten polymer was confirmed to progressively decrease in the temperature range from 140 °C (*n* value 0.26) to 170 °C (*n* value 0.41). Analogously, it was reduced by addition of 5 % of plasticizer at any temperature. However, increasing the amount of this excipient, in particular from the 10 to 15 % PEG-containing formulation, an opposite tendency was observed, especially at the higher temperatures. As far as

the consistency index *K* is concerned, it is known to depend on the degree of polymeric chain mobility and was accordingly supposed to also vary with the temperature and extent of plasticization. *K* values decreasing from the lower to the higher processing temperature were generally obtained for all the formulations except the one containing 15 % of PEG at 170 °C. Moreover, at all the operating temperatures, *K* was higher for the polymer as such than for the PEG 5% formulation. However, by rising the amount of PEG, an opposite and unexpected tendency to increase was noticed for *K* at temperatures > 140 °C, which could be ascribed to a chain rearrangement or a mechanically- and/or thermally-induced degradation of the polymer [Yan D. *et al.*, 1999]. Indeed, the molten KLF and the 5 as well as 10 % PEG formulations also showed a similar behavior, *i.e.* an increase in *K*, though only above 170 °C (data not shown). These results were confirmed by complex viscosity data obtained under isothermal conditions at 190 °C that, for all the formulations, exhibited an increasing trend over time. By way of example, patterns relevant to the 10 % PEG formulation are reported in Figure R1.2.

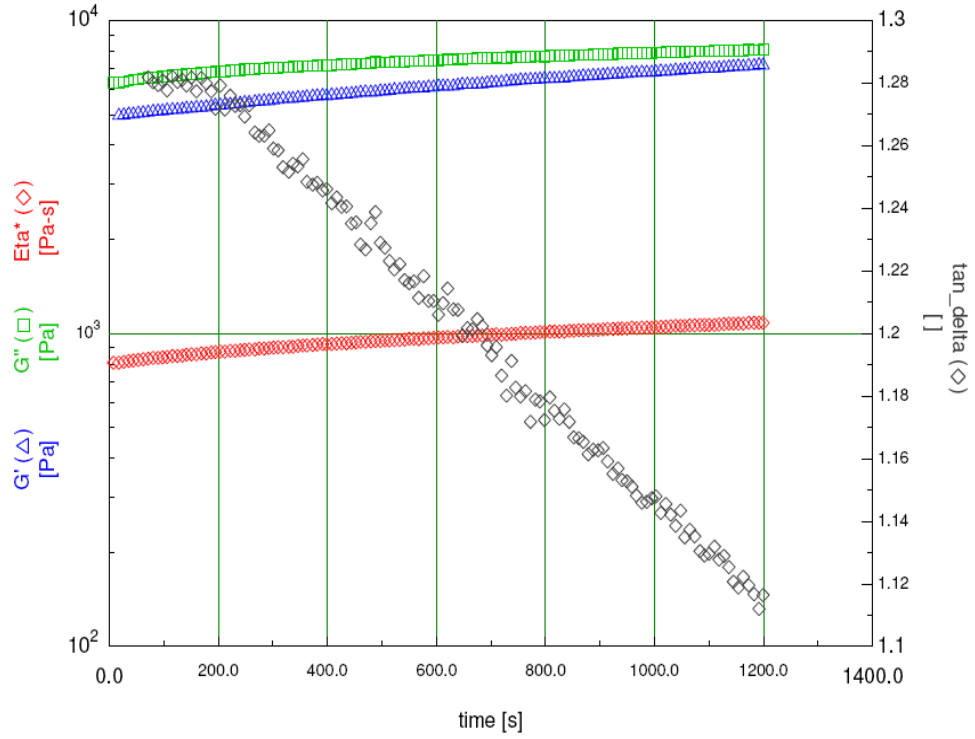


Figure R1.2: Absolute viscosity (red diamonds), G' storage modulus (blue triangles), G'' loss modulus (green squares) and G''/G' tan-delta (black diamonds) profiles of KLF melt with 10% of PEG.

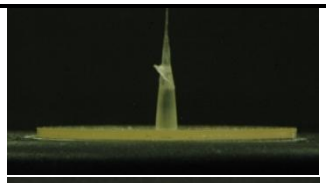
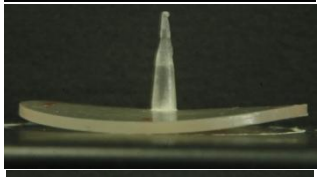
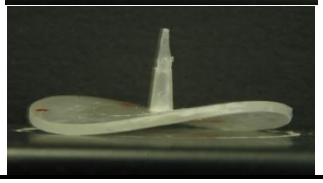
The overall results of rheological studies demonstrated the need for dealing with a plasticized KLF formula in order to improve not only the mechanical properties of molded items but also the processability of the melt. Moreover, they indicated that not only operating temperatures would be a critical issue, but also the time over which the material is maintained at a relatively high temperature that is related to the IM process cycle time. In order to avoid overheating, special changes were introduced into the press configuration, such as a reduction in the

diameter of the piston and nozzle so that the amount of material heated at each molding cycle, *i.e.* the mean residence time of the material under heating, was minimized.

The influence of the operating temperatures and amount of plasticizer on the actual IM processability and on the characteristics of molded products was then investigated. In particular, the dimensional changes (contraction or expansion) undergone by the item inside the mold, immediately after demolding or in the successive hours/days, needed to be considered because they may impair the relevant quality and performance, and might also have an impact on the design of the mold cavity [Fischer J.M., 2003]. Disk-shaped items (1 mm thickness and 30 mm diameter) especially suitable for the evaluation of dimensional changes (shrinkage test) were therefore prepared [Zema L. *et al.*, 2013], providing the modified press with a centrally-gated circular mold. A 120-170 °C range of temperatures was investigated setting a 10 °C-based ramp. Different combinations of compression, metering and nozzle temperatures were thus considered, namely from 120/130/140 °C to 150/160/170 °C. The change in the operating temperatures only involved a minimal adjustment of all the other process parameters. Moreover, the effect of the amount of plasticizer on processability was limited to a slight sticking tendency observed with the 15 % PEG formulation. With regard to the physico-mechanical characteristics of molded items, while no significant influence of the manufacturing temperature was noticed, the degree of plasticization was confirmed as a critical aspect. In

particular, the molded items containing only 5 % of PEG turned out glassy and brittle but maintained their shape after demolding, whereas in the presence of higher amounts of plasticizer deformation of the disks occurred. By way of example, data relevant to molded disks obtained under a mid-range combination of temperatures (*i.e.* 140/150/160 °C) are shown in Table R1.3.

Table R1.3: Characteristics of molded disks (standard deviation in brackets); scale bar of photographs = ⟼ 5 mm.

% of PEG	weight *mg*	thickness *µm*			moisture content *%*		
	t = 0	t = 0	t = 72 h	t = 72 h	t = 0	t = 72 h	t = 7 days
5	937.57 (1.24)	1080 (5)	1121 (12)		1.7 (0.12)	4.5 (0.42)	5.5 (0.48)
10	931.86 (2.57)	1078 (8)	1118 (10)		1.5 (0.21)	4.2 (0.45)	4.5 (0.53)
15	927.88 (3.61)	1087 (13)	1117 (15)		1.1 (0.15)	2.9 (0.32)	3.7 (0.58)

KLF-based molded disks generally showed an increment of thickness, with respect to the mold nominal dimension, of about 8 %, increasing up to 12 % over 72 hours. The degree of plasticization was demonstrated not to have a significant influence on the final disk thickness ($p < 0.1$). On the contrary, the molded disks

exhibited a warping tendency that could be related to the amount of plasticizer in the formulation. Indeed, only with the items containing PEG at 5 %, bending was observed neither after demolding nor storage. According to previous findings relevant to HPC-based capsular devices prepared by IM [Gazzaniga A. *et al.*, 2011a], the molded disks reached a re-equilibrium in terms of moisture content within 7 days of storage at ambient conditions from preparation, whereas no dimensional changes were highlighted after 72 h.

Taking the overall results obtained into account, a novel mold for the production of capsular devices with 600 µm thickness was specially designed for use with the 10 % PEG formulation, improved in that: *i)* a centered position of the injection orifice would enable a consistent flow length in all directions, *ii)* a hot runner keeping the melt heated up to the mold cavity would prevent overheating of the material thanks to the possibility of maintaining the desired temperature while this is moving throughout the mold, *iii)* a halved thickness in the body/cap contact areas would lead to a constant thickness of the closed device also in the overlap region, *iv)* a length/diameter ratio lowered to 1.5 would reduce the flow path of the melt inside the unheated mold cavity, and *v)* a duct for injection of compressed air into the mold would facilitate the ejection of the molded item thus allowing the use of lubricants to be avoided. The amount of plasticizer selected was the minimum able to improve the KLF melt processability and the molded item brittlness. The process parameters required to be defined by means of preliminary trials. By the selected operating

conditions, a fully automated process was finally achieved with a cycle time per cap or body item reduced to about 5 s and no need for adding either internal or external lubricants (Table R1.1 in the Material and methods section). The highest temperature of 160 °C was set in the final step only, *i.e.* in the hot runner, whereas the plasticating/injecting phases of the process could be carried out at lower temperatures. An outline and some pictures of the capsule shells obtained are shown in Figure R1.3.

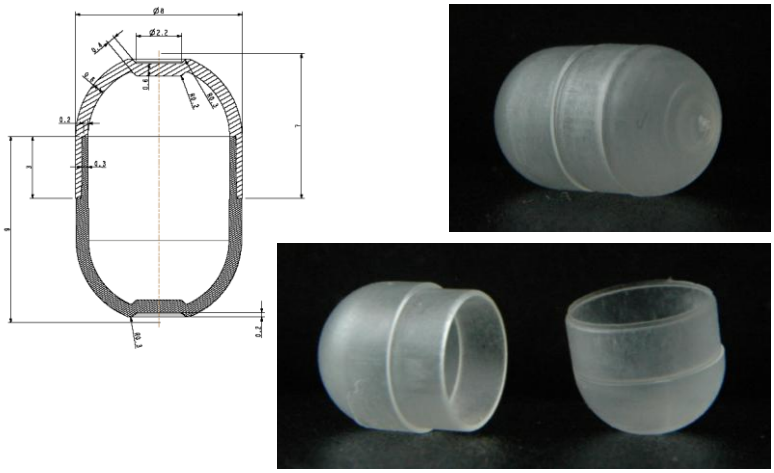


Figure R1.3: Technical drawing and photographs of the capsular device.

The capsules manufactured by using the novel mold were evaluated in terms of technological properties and release performance. When comparing such units with those prepared by the former mold, the average shell thickness turned out closer to the nominal 600 μm value and, importantly, the extent of thickness variability was considerably restrained (Table R1.4). This result was also reflected

in a higher reproducibility of the mechanical properties of the shells as expressed by elastic modulus (EM). Probably because of their modified shape, the novel capsules exhibited a lower EM. However, this was still comparable with that of immediate-release gelatin capsules thus supporting the possible utilization of the filling equipment employed for the latter [Gazzaniga A. *et al.*, 2011a].

Table R1.4: Physico-technological characteristics of capsule shells prepared by the novel and the former mold (CV in brackets); data for the former devices are adapted from [Gazzaniga A. *et al.*, 2011a].

	Capsule shells prepared by the	
	novel mold	**former mold**
Weight, mg	228 (0.51)	336 (0.70)
Height, mm	13.11 (0.13)	16.08 (0.12)
Diameter, mm	8.05 (0.02)	8.19 (0.37)
Thickness, µm	610 (3.30)	645 (13.20)
Elastic modulus, N/mm^2	3.509 (2.01)	5.342 (15.72)

Furthermore, the ability of the HPC capsule-based devices to impart a lag phase to the release of their content was investigated, and the relevant release profiles were comparatively evaluated *vs.* the dissolution curves of gelatin capsule dosage forms with an analogous filling. In particular, acetaminophen (AAP) was selected as a tracer drug, and formulations with a possibly different dissolution behavior were considered, namely fine powder, commercially available granules,

microcrystalline cellulose-based pellets prepared by extrusion-spheronization and a solid dispersion of the fine powder in polyethylene glycol 6000. Moreover, particular HPC capsule devices that also contained a dye were tested under analogous hydrodynamic conditions and in an unstirred medium in order to assess the rupture mode (time and position) of the shell.

Important information was drawn from the evaluation of the morphological changes undergone by the HPC capsule-based devices when exposed to aqueous unstirred fluids and of the dissolution behavior highlighted by the dye-containing fillings. The capsule shells remained intact during the hydration and dissolution/erosion of the external polymeric layers, independent of the contents. The first rupture of the device took place in the cap item at the cap/body overlap region, where the body wall ends. A very rapid dissolution of the dye inside the capsule, before its contents were quantitatively delivered, demonstrated that aqueous fluids initially diffuse into the shell cavity through this cleft. An opening time was thus defined. By way of example, photographs of the device containing the fine powder formulation are reported in Figure R1.4. Such a behavior of HPC-based systems was confirmed under the conditions used for the release test and opening times were determined.

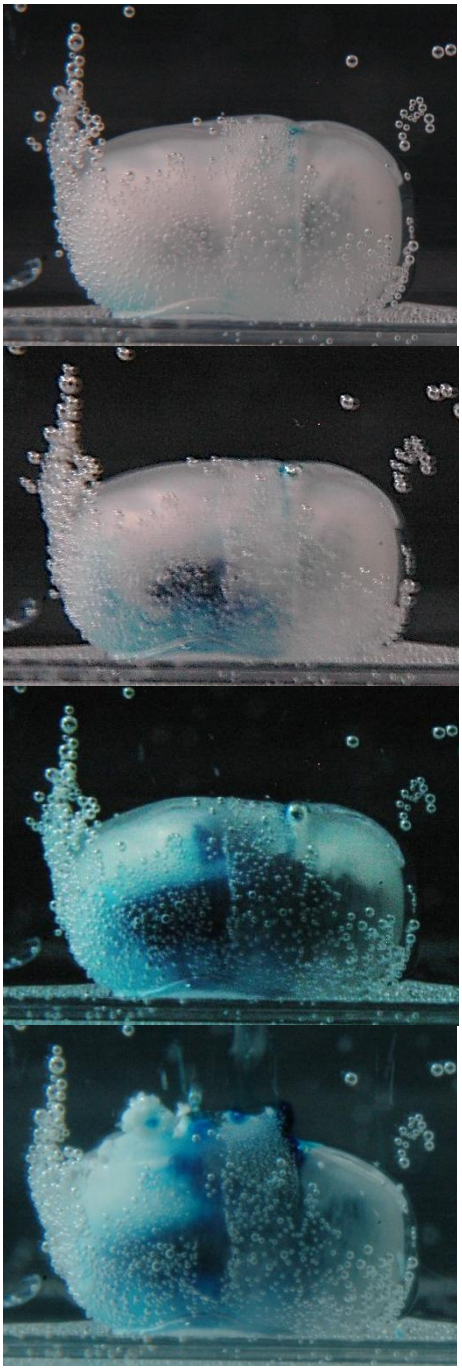
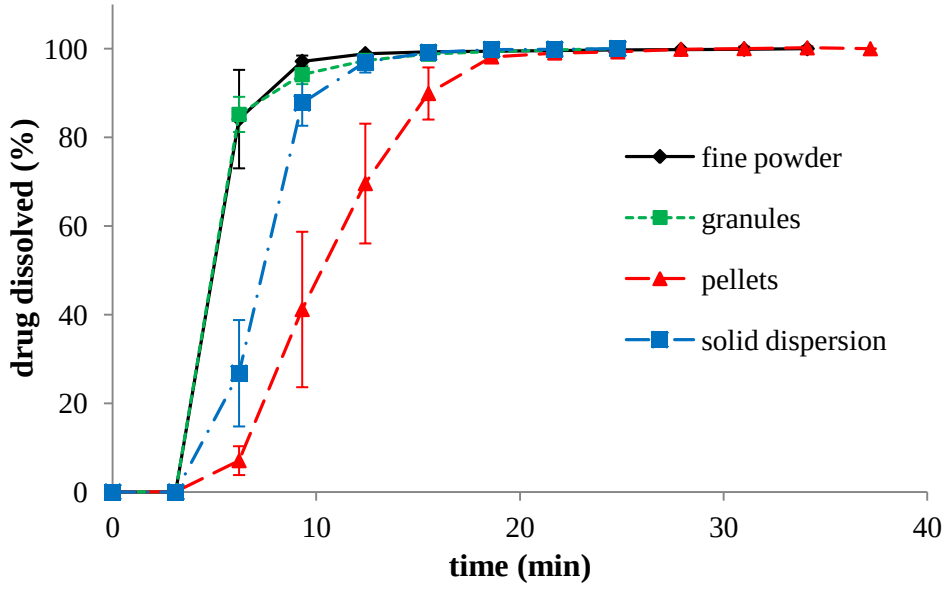
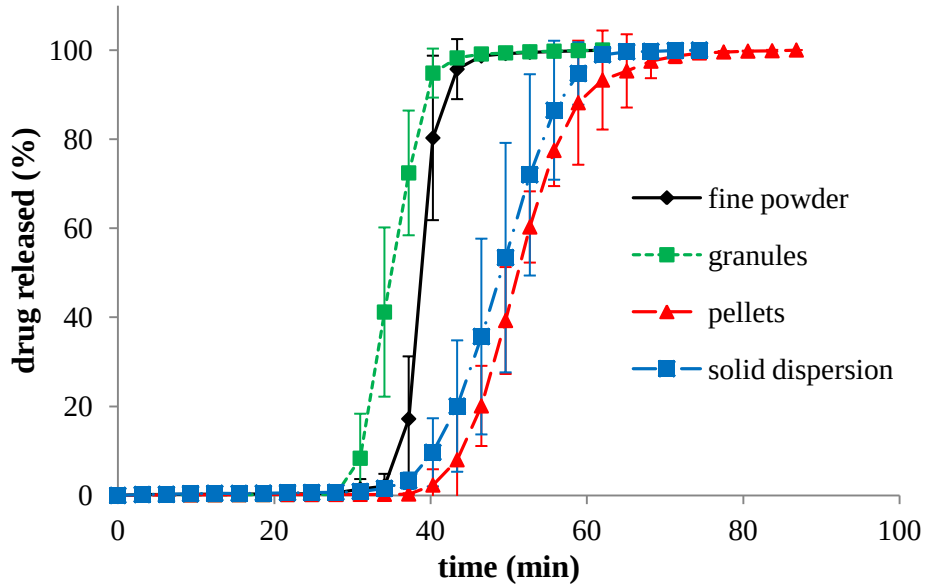


Figure R1.4: Photographs of a capsular device, containing AAP fine powder along with methylene blue, taken at successive time points (< 1 min intervals) during immersion in unstirred deionized water.

The dissolution and release profiles from gelatin and KLF capsule-based systems, respectively, are reported in Figure R1.5. The obtained data of opening time, along with those of lag time before drug release (expressed as $t_{10\%}$, time for 10 % of drug to be released) and pulse time, *i.e.* the time elapsed between onset and completion of the release process (expressed as $t_{90\text{-}10\%}$), relevant to the profiles of Figure R1.5 are collected in Table R1.5. For comparison purposes, $t_{10\%}$ and $t_{90\text{-}10\%}$ values were analogously calculated from the dissolution profiles of gelatin capsule-based dosage forms.



a)



b)

Figure R1.5: Dissolution/release profiles of gelatin (a) or HPC (b) capsule-based systems containing different AAP formulations.

Table R1.5: Release parameters of gelatin and HPC capsule-based systems containing different AAP formulations (standard deviation in brackets).

	gelatin capsule-based systems		**HPC capsule-based systems**		
filling formulation	lag time min	pulse time min	opening time* min	lag time min	pulse time min
fine powder	3.5 (0.1)	3.8 (1.1)	33 (1)	36.0 (1.3)	5.0 (1.0)
granules	3.5 (0.1)	4.3 (0.9)	35 (2)	32.6 (3.1)	5.1 (0.5)
Pellets	6.6 (0.5)	8.7 (1.7)	35 (1)	45.0 (2.9)	13.9 (5.6)
solid dispersion	4.9 (1.2)	5.1 (1.1)	34 (2)	41.7 (3.1)	13.9 (0.9)

* determined by visual inspection from HPC capsule-based devices that contained a dye added to the filling

The HPC capsular containers developed proved able to achieve a lag phase prior to drug release. Moreover, the opening time was independent of the filling. On the other hand, some minor differences were found in the release parameters, such as in particular extended lag and pulse times of capsules filled with pellets or with the solid dispersion, which could be attributed to the dissolution behavior of these particular formulations. Indeed, while from the fine powder and the granules, containing disintegrant and wetting agents, the drug tracer was promptly available for dissolution, its contact with the aqueous fluid was somewhat delayed because of the reduced surface area exposed by highly dense matrix pellets made of microcrystalline cellulose and coarse fragments of the solid dispersion [Zema L. *et al.*, 2008]. An analogous trend was observed with gelatin-based dosage forms with the same contents. With respect to the capsular devices prepared by the former mold, the duration of release (pulse time) was reduced probably due to the relatively lower amount of polymer that has to erode/dissolve to enable the breakup of the capsule.

In order to explore whether the method of preparation of KLF blends with 10 % of plasticizer would impact on the IM process, capsule shells were prepared starting from pre-extruded KLF/PEG blends obtained under different temperatures; no influence on the manufacturing (process conditions, cycle time) nor on the physico-technological characteristics of the capsules was thereby highlighted (data not shown).

4. Conclusions

An oral capsular device (Chronocap™) intended for pulsatile and/or time-based colonic delivery was recently prepared by injection molding (IM). The prototype capsule shells obtained from a swellable/erodible thermoplastic polymer (HPC) were able to delay, both *in vitro* and *in vivo*, the release of a model drug. The lag phase duration was found dependent on the shell composition and wall thickness. In the prospect of a scale-up of this technology, the development of a novel mold and manufacturing process was pursued in order to improve the technological characteristics of capsules on the one hand (*e.g.* shell thickness variable and poorly consistent with the nominal value), and the production rate as well as extent of automation on the other. The capsular devices prepared by this mold through an automatic manufacturing cycle (duration 5 s) were demonstrated to possess good technological properties and, as compared with the previous systems, higher reproducibility of shell thickness, mechanical properties, opening mechanism and release performance. In particular, although filled with different drug formulations, they showed a reproducible time to breakup (opening time) that was followed by release patterns dependent on the dissolution behavior of their contents.

Acknowledgments

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Chapter 2

Enteric-Coating of Pulsatile-Release Capsules Prepared by Injection Molding

The content of this chapter has already been submitted for publication:

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Abstract

Erodible capsular devices based on hydroxypropyl cellulose (Klucel® LF) intended for pulsatile release were prepared by injection molding (IM). In the present work, the possibility of exploiting such capsules for the development of colonic delivery systems based on a time-dependent approach was evaluated. For this purpose, it was necessary to demonstrate on the one hand the ability of molded cores to undergo a coating process and, on the other, that of coated systems to yield the desired performance (gastric resistance). Although no information was available on the coating of IM substrates, some issues relevant to that of commercially-available capsules are known. Thus, preliminary studies were conducted on molded disks for screening purposes prior to facing the spray-coating of HPC capsular cores with Eudragit® L 30 D 55. The ability of the polymeric suspension to wet the molded substrate, spread above it, start penetrating and bring about hydration/swelling phenomena, as well as to provide a gastroresistant barrier was demonstrated. The coating of prototype HPC capsules was carried out successfully, leading to coated systems with good technological properties and able to withstand the acidic medium with no need for a sealing. Such systems maintained the original pulsatile release performance after failure of the enteric film in pH 6.8 fluid. Therefore, they appeared potentially suitable for the development of a colon delivery platform based on a time dependent approach.

Keywords: injection molding; capsular device; enteric coating; oral pulsatile release; oral colon delivery; swellable/erodible polymers.

1. Introduction

In the field of oral delivery, besides a widespread use of prolonged-release dosage forms [Grassi et al., 2004; Sangalli et al., 2001b], increasing interest has been focused on the development of formulations able to release active pharmaceutical ingredients after programmed lag times [Conte et al., 1989; Maroni et al., 2013b, 2010] or to specific regions of the gastrointestinal (GI) tract [Pinto, 2010]. In particular, the possibility of exploiting the colon as a site for the release of drugs for the treatment of both local and systemic pathologies has been investigated: while several applications for the management of local symptoms/pathologies (*e.g.* inflammatory bowel disease, IBD) can be already found in the literature, colon delivery is currently considered an interesting approach for increasing the bioavailability of peptides and proteins [Maroni et al., 2013a, 2012]. The colon has long been considered an unsuitable site for the absorption of molecules because of the reduced surface area; moreover, the lack of free fluids, the viscous contents and the presence of gas produced by bacterial fermentation processes may impair the disintegration of oral dosage forms and dissolution of drug particles. However, the unfavorable anatomical and physiological characteristics of the colon are offset by its low concentration of local peptidases and the long residence time of drug delivery systems.

Over the years, several strategies have been attempted for targeting the colonic region. The exploitation of enzymatic reactions by unique bacteria at the site is one (*chemical/microbiological* approach): among substrates undergoing enzymatic activation reactions (*i.e.* reduction and hydrolysis of azo and glycoside bonds, respectively), both prodrugs and polymeric materials have been investigated [Patel and Amin, 2011]. The high variability in the composition of the microflora, influenced by everyday events (*e.g.* stress, modification of the diet and concurrent therapies), regulatory burden involved by the use of prodrugs and synthetic materials as well as poorly reliable performance of natural polysaccharide carriers represent the main critical aspects of this delivery strategy. The *technological/physiological* approach, on the other side, makes use of the difference in some parameters along the GI tract, namely pressure, pH or transit time. With the pressure-controlled delivery strategy, the disintegration of the drug delivery system is enhanced by the combination between intense muscle contractions and the higher viscosity of luminal contents [Takaya et al., 1995]. The pH-based approach relies on the hypothesis of a progressive increase in the pH values along the GI tract. Relevant systems are composed of a drug-containing core coated with polymers which dissolve in media with neutral to weakly alkaline pH. However, the possibility that this pH threshold is exceeded in the small intestine or a weakly acidic pH is found in the caecum and in the right colon segments may lead to an inconsistent performance of devices [Gazzaniga et al., 2006]. Finally, the time-dependent approach to colon delivery is based on

the relatively constant small intestinal transit time (SITT; 3 h ± 1 standard error) of dosage forms [Davis, 1985]. On the contrary, the duration of gastric residence of solid dosage forms, which depends on their size and density as well as fasted or fed conditions of subjects, is unpredictable; hence, by the application of an outer gastroresistant layer, which dissolves after the dosage form is emptied from the stomach, the variability in gastric emptying can be overcome. Subsequently, a lag phase imparted to the drug-containing core allows the system to reach a delay duration comparable to SITT, thus targeting the colonic region of the GI tract [Gazzaniga et al., 2006].

Among time-based devices for colonic release, a platform for delayed and site-specific release of drugs in the form of a *reservoir* system, named *Chronotopic™*, has already been developed [Del Curto et al., 2011, 2009; Sangalli et al., 2004, 2001a; Zema et al., 2007]. Such device is based on single- or multiple-unit drug cores (tablets, capsules, *pellets*) coated with a functional layer composed of swellable/erodible polymers (namely, hydroxypropyl methylcellulose, HPMC) of few hundreds microns of thickness applied by different techniques (*press-coating*, *spray-coating* and *powder layering*). When intended for time-based colon delivery, an outer enteric film is subsequently applied. Once the device is emptied from the stomach, the enteric coating dissolves and the HPMC-based layer delays the contact of the biological fluids with the core, allowing the release of the drug only after a programmed period of time. The effectiveness of the *Chronotopic™* system, its flexibility in terms of duration of the lag phase,

both i*n vitro* and *in vivo*, as well as the possibility of scaling up the manufacturing process has been widely demonstrated. A step forward in the development of the *Chronotopic™* system was represented by a capsular device (*Chronocap™*) that combined the release functionality of the polymeric coating with the ability to convey a variety of drug preparations (solid, semi-solid, liquid), thus bringing about both technical and regulatory advantages. The feasibility of the injection molding (IM) technique to prepare capsules from a thermoplastic swellable ether of cellulose, hydroxypropylcellulose (HPC), as an alternative to the HPMC-based coating of the *Chronotopic™* delivery platform, has previously been demonstrated [Gazzaniga et al., 2011; Zema et al., 2012].

Preliminarily, shells of different thickness (300, 600, and 900 μm) were prepared and showed good technological characteristics. Moreover, pulsatile release patterns were obtained from drug-containing capsules, both *in vitro* and *in vivo*, with a lag time that was dependent on the shell composition and thickness [Zema et al., 2013a]. In the prospect of an industrial scale up, a purposely devised mold with a nominal thickness of 600 μm was designed on the basis of thermal, rheological and mechanical characteristics of the polymeric formulation selected [Zema et al., 2013b]. The new mold allowed a fully automated manufacturing to be developed with cycle times of few seconds. However, no information is available on the application of pharma-grade polymer coatings onto the molded HPC capsule shell devices.

Based on these premises, the aim of the present work was to evaluate *Chronocap™* capsules as cores for the application of a gastroresistant film, in order to make them possibly suitable for the development of a time-dependent colonic release system. The feasibility of coating IM capsules with an aqueous-based enteric dispersion (Eudragit® L 30 D-55) was preliminarily studied using a prototype shell of 600 µm thickness composed of Klucel® LF plasticized with polyethylene glycol (PEG 1500, 10 % by weight) and filled with a tracer drug.

2. Materials and methods

2.1. Materials

Hydroxypropyl cellulose (HPC): Klucel® LF, Eigenmann & Veronelli, Italy; hydroxypropyl methylcellulose (HPMC): Methocel™ E50 Premium LV, Dow, Italy; polyethylene glycol (PEG) 1500, Clariant Masterbatches, Italy; low-density polyethylene (LDPE), Polimeri Europa, Italy; acetaminophen (AAP) fine powder, Atabay, Turkey; Eudragit® L 30 D-55, Evonik, Germany; triethyl citrate (TEC), Vertellus Specialties Inc., IN, USA; fluorescein sodium salt, Sigma-Aldrich Corp., MO, USA; size 2 hard-gelatin and HPMC capsules, Capsugel, SC, USA.

2.2. Methods

2.2.1. IM processes

A mixture of HPC, previously dried in a ventilated oven for 24 h at 40 °C, and PEG 1500 (90 % and 10 % w/w, respectively) was prepared in Turbula® (Type T2C;

WAB, Switzerland) and then transferred into a bench-top micromolding machine (BabyPlast 6/10P; Cronoplast S.L., Spain; Rambaldi S.r.l., Italy). Molded items were prepared by means of two different molds: (1) a disk-shaped mold (diameter: 30 mm; height: 1 mm) provided with a central gate and (2) a capsular mold with two interchangeable inserts for the manufacturing of matching caps and bodies of 600 µm nominal thickness. Process conditions were applied as reported in [Zema et al., 2013b]. Disks made of LDPE were also prepared.

The physical characteristics, mechanical properties and release performance of capsule shells were evaluated after 7 days of storage at ambient conditions (24 ± 2 °C / 55 ± 5 % relative humidity).

2.2.2. Capsules filling

Gelatin, HPMC and HPC-based capsule shells were manually filled with 80 mg of AAP. Banded samples of each type of system were prepared: gelatin capsules were manually sealed with a 20 % w/v gelatin aqueous solution, HPMC with a 20% w/v Methocel™ E50 aqueous solution and HPC-based capsules with a 3 % w/v Klucel® LF aqueous solution.

2.2.3. Coating processes

2.2.3.1. Preparation of the coating suspension and fluorescein solution

The coating suspension was prepared by dissolving TEC (20 % w/w, based on the dry polymer weight) into the commercially available Eudragit® L 30 D-55

dispersion, previously diluted to decrease the solid contents to 20 %; when required, fluorescein (0.01 % w/w, based on the dry polymer weight) was added. The suspension was stirred for at least 30 min, filtered through a 0.3 mm sieve and maintained under stirring during the coating process.

A solution of fluorescein alone for comparison purposes was prepared by dissolving the fluorescent marker in pure water at the same concentration used in the coating suspension.

2.2.3.2. Coating of molded disks and glass slides

The coating suspension containing fluorescein was sprayed by a two-fluid nozzle (Series 970, Düsen-Schlick, Germany) with 1.2 mm port size onto a rotating drum, carrying disks and glass slides, covered by a siliconized liner under a constant heating air flow in order to maintain the film surface at approximately 30 °C [Felton, 2007]. Each sample was sprayed with the amount of suspension needed to reach theoretical 10 mg/cm^2 of polymer applied. Molded HPC-based disks were sprayed under the same conditions with the fluorescein solution for 5 s.

2.2.3.3. Coating of capsules

Batches of 500 capsules, both banded and unbanded, were coated in a Hi-coater (Vector corporation LDCS-3, Vector Corp., IA, USA) equipped with a 1.3 L perforated pan. Process conditions were: inlet air temperature, 30 °C; air

pressure, 12 psi; pan speed, 15 rpm; spraying rate, 1 g/min. For HPC capsules only, the spraying rate was also increased up to 2 g/min. During the process, samples with different coating levels were withdrawn and replaced with placebo capsules to maintain the batch size.

2.2.3.4.Curing

After the coating process, all samples (glass slides, disks and capsules) were cured in a ventilated oven at 40 °C for 2 h. In order to allow re-equilibrium with ambient humidity, all measurements were performed after 7 days of storage at ambient conditions (24 ± 2 °C/55 ± 5 % RH) from preparation.

2.2.4. Characterization of substrates and coated systems

2.2.4.1. Contact angle

A Tantac CAM-Micro Contact Angle Meter (Chemsultants International, Inc., OH, USA) was used to determine contact angles between the Eudragit® L 30 D-55 suspension and different substrates: HPC-based molded disks, LDPE molded disks and glass slides. A 10 µl droplet of the polymeric suspension was delivered onto the surface of each substrate and contact angles were measured after 1 s and 15 s (n = 3).

2.2.4.2. Gastroresistance and release performance

Uncoated substrates (molded disks and capsules) were tested in phosphate buffer pH 6.8 according to the *Dissolution Test for Immediate-Release Dosage Forms* (USP 34); coated systems (glass slides, molded disks and capsules) were tested according to the *Dissolution Test for Delayed-Release Dosage Forms, Method A* for HPC-based capsules and *Method B* in all other cases; n = 3.

Molded disks were fixed to an inert support in order to avoid floating during the test: pre-weighed samples were dried in a ventilated oven at 40 °C for 2 h and then transferred into a USP 34 Apparatus 2; test conditions: 900 mL of dissolution medium (HCl 0.1 N, pH 1.2 or phosphate buffer, pH 6.8), 37 ± 0.5 °C, 50 rpm. At fixed time intervals, samples were withdrawn from the medium and dried in a ventilated oven at 40 °C for 24 h. The residual dry mass was calculated from the dried (m_{dried}) to starting (m_0) mass ratio according to Eq. (1):

$$\text{Residual dry mass \%} = 100 - [(m_{dried}/m_0) * 100] \qquad \text{Eq. (1)}$$

Conventional capsules (gelatin and HPMC) were tested in Apparatus 2, at 50 rpm, in 900 ml of medium at 37 °C with wire sinkers. IM capsules were tested in a six-position USP 34 disintegration apparatus in order to avoid sticking of the swollen shells to the vessels [Gazzaniga et al., 2011, 1995]: each unit was inserted in one of the six available tubes of the basket-rack assembly, moving at 31 cycles/min rate in separate vessels that contained 675 mL of dissolution

medium (HCl 0.1 N, pH 1.2 or 800 mL phosphate buffer, pH 6.8), 37 ± 0.5 °C (n = 3). Fluid samples were spectrophotometrically assayed at 248 nm. 10 % drug dissolved/released ($t_{10\%}$) was calculated from the relevant curves, that was defined as the lag time in the case of HPC capsules. Moreover, the time elapsed between 10 % and 90 % release was calculated from the profiles of HPC-based systems that was defined as pulse time ($t_{90\%-10\%}$).

2.2.4.3. Dimensions

The diameter of uncoated capsules and coated systems was determined by micrometer (Craftsman Micrometer, Mechanical Digital, 0-1 in. range, n = 10). The coating layer thickness was calculated from the difference between diameters of coated (d_c) *vs*. uncoated (d_{uc}) capsules according to Eq. (2):

$$\text{Coating thickness} = (d_c - d_{uc})/2 \qquad \text{Eq. (2)}$$

2.2.4.4. Mechanical resistance

The mechanical properties of uncoated and coated HPC-based capsules were evaluated (n = 3) using a Chatillon universal tension/compression tester (model TCD-200 MS, Wagner Instruments, CT, USA) equipped with a DFGS digital force gauge and a flat-ended probe with 13 mm diameter. Capsular samples were placed on the lower stationary platform, centrally positioned under the probe [Missaghi et al., 2006]; the upper platen was then lowered and compressed the

capsule at a rate of 10 mm/min. Capsule failure under crushing was determined and the inherent load strength (N) was measured.

2.2.4.5. Moisture sorption

Uncoated disks and disks coated with up to 10 mg/cm^2 of dry polymer were analyzed by means of a dynamic vapor sorption apparatus (DVS Advantage-1, Surface Measurement Systems Ltd., UK). Pre-weighed samples of about 50 mg cut from disks were added to 10 mm video quartz pans, which were placed in the sample chamber. Experiments were conducted isothermically (either 25 or 40 °C) under N_2 purge of 200 sccm. Samples, initially dried at 0 % RH until a dm/dt of 0.002 %/min was reached, were then exposed to a ramping experiment from 0 % to 98 % RH in 600 min. Finally, each sample was equilibrated at 98 % RH, determined by a dm/dt of 0.002 %/min.

2.2.4.6. Photographs

Digital photographs of molded disks uncoated and coated up to 10 mg/cm^2 of dry polymer were acquired by a Nikon D70 camera (Nikon, Japan).

Samples of glass slides and molded disks treated with the fluorescein-containing solution or suspension and tested for gastroresistance were analyzed by confocal laser scanning microscopy (CLSM). Images were taken with a Zeiss LSM 510 camera (ZEISS, Germany) coupled with a microscope (AxioPlan 2 MOT, ZEISS, Germany); a ×20 objective lens (Numerical Aperture 0.5) was used and laser

excitation wavelength was set to 488 nm. Single plane images of the surface of samples and optical cross sections were acquired.

Photomicrographs of gelatin, HPMC and HPC-based capsules, uncoated and coated up to 10 mg/cm^2 of dry polymer were taken by a Jeol Model 35 scanning electron microscope (Jeol, MA, USA); details of the surface and of the cap and body closing area were acquired.

Photomicrographs of HPC-based capsules were taken by an optical microscope (Inverted Olympus 1x70, Olympus, PA, USA) coupled with a camera (Olympus DP2-BSW, Olympus, PA, USA); details of the cross section of capsule cleaved by means of a scalpel were acquired.

3. Results

With the aim of demonstrating the feasibility of a colonic delivery system based on the *Chronocap™* platform, the coating of a molded prototype with a gastro-resistant polymer (Eudragit® L 30 D-55) was investigated. Data relevant to the characterization of HPC-based capsules as a substrate for coating processes, the development of the coating process itself and the evaluation of coated capsules compared with conventional gelatin or HPMC ones are reported.

3.1. Characteristics of core units

In order to develop a coating process for molded HPC capsules, some critical issues related to other types of capsule cores needed to be considered. For

example, the smooth surface of the shell of hard gelatin capsules may lead to poor adhesion of the coating film [Nagata, 2002]; moreover, during the initial phase of wetting, partial solubilization of gelatin may result in sticky shells [Thoma and Bechtold, 1992], whereas shell brittleness was noticed after exposure to low humidity conditions [Osterwald, 1982]. In addition, the irregularity of the closing area could prevent the formation of a continuous layer [Felton et al., 2002; Plaizier-Vercammen et al., 1992]. Some of these drawbacks were partly overcome by using a subcoat that, however, involves a further process step, potentially problematic and time consuming. More recently, HPMC-based capsules were launched on the market as an alternative to gelatin-made ones for meeting vegetarian needs and incompatibility problems [Ogura et al., 1998]. As these capsules are composed of a non-ionic polymer, they demonstrated less reactivity toward many chemical entities (*e.g.* aldehydes, reducing sugars and metal ions) and a higher compatibility with some filling vehicles (including liquids) in comparison with hard gelatin capsules. Furthermore, the mechanical strength of the HPMC shells was shown to be less influenced by storage humidity conditions [Sherry Ku et al., 2010]. Finally, such capsules showed a rougher surface with respect to gelatin shells, suggesting a potential for better film adhesion, although the need for a sealing step was demonstrated [Cole et al., 2002; Felton et al., 2002; Sherry Ku et al., 2010].

As mentioned, little information is available about the impact of the IM process - *i.e.* of high temperatures and pressures applied to the polymeric formulation in

order to have it melt and flow through a relatively long narrow mold section- on the properties of the material (*e.g.* surface characteristics, porosity, hydration rate and swelling ability). Therefore, the ability of a Eudragit® L 30 D-55 coating suspension to adhere to molded substrates and the possible influence of the enteric film on the physical properties as well as dissolution/erosion behavior of molded HPC needed to be verified. For this purpose, 1 mm thick disk-shaped specimens, also prepared by IM and with the same composition as capsules, were used as a simple model for physico-mechanical and release characterization studies [Zema et al., 2013b, 2013c]. Preliminary data relevant to the wettability of HPC disks were acquired. Glass-slides and molded disks made of low-density polyethylene (LDPE) were used for comparison, as these inert materials were expected to display little/no interaction when in contact with aqueous fluids. The wettability of the different surfaces (*i.e.* molded HPC, molded LDPE and glass) by the coating suspension was investigated by means of contact angle analysis. During the test, droplets showed a certain tendency to spread on the contact surface over 30 s approximately. Therefore, contact angles were determined after 1 and 15 s and the difference between values at the two time points was used to estimate the spreading ability of the coating suspension (Table 1).

Table 1: Contact angles of Eudragit® L 30 D-55 coating suspension with different substrates after 1 and 15 s contact-time (s.d. in brackets) and their percentage difference (Δ %).

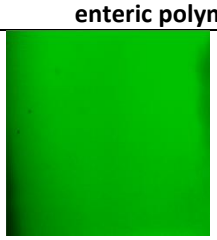
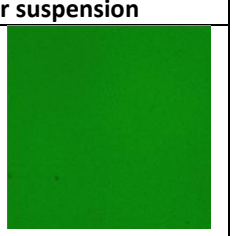
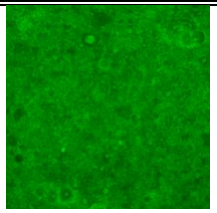
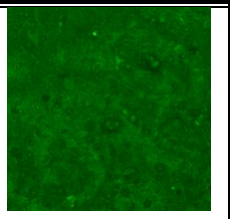
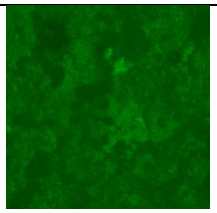
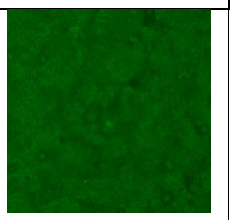
	Contact time, s	Contact angle, ° (s.d.)	Δ %
HPC-based molded disk	1 15	33.3 (1.26) 24.5 (4.43)	26.4
LDPE-based molded disk	1 15	57.5 (3.70) 41.8 (1.26)	27.3
glass slide	1 15	23.7 (1.53) 17.7 (0.58)	25.3

Similar contact angles were observed for HPC-based molded disks and glass slides; much higher contact-angle values were obtained for LDPE disks, likely due to the hydrophobicity of the material. The spreading ability of the aqueous polymeric dispersion, as indicated by the Δ % values, seemed to be less influenced by the characteristics of the surfaces. Based on these results, glass slides were selected as reference inert controls for subsequent experiments.

In order to gain qualitative information about the adhesion of the acrylic film to the molded substrates and the surface phenomena arising from HPC interaction with aqueous fluids, molded disks and controls were coated with a polymeric suspension (equivalent to 10 mg/cm^2 of polymer) containing a fluorescent marker, namely fluorescein, that could be detected by confocal laser scanning microscopy (CLSM). Some disks were also sprayed for a few seconds with an aqueous solution of fluorescein alone at the same concentration as in the

coating suspension to gain insight into the interaction between HPC and the polymeric suspension early in the coating process. The ability of the coated disks to withstand acidic media and dissolve in buffer pH 6.8, *i.e.* their gastroresistance performance, was thus investigated. In Table 2, CLSM images of the surface of disks and glass slides coated with the marker-containing polymeric suspension or maintained 2 h in acidic medium and then transferred in phosphate buffer pH 6.8 are reported. CLSM images of the coated disks withdrawn from the pH 6.8 medium after 15-40 min were also compared with those of molded disks sprayed with the fluorescein solution and immersed into pH 6.8 buffer for an equal time period. In this case, images of the optical cross section of the disks are reported (Table 3) where the coating layer thickness or the position of the solvent penetration front through the thickness of uncoated disks could be appreciated.

Table 2: CLSM images of the surface of molded HPC-based disks and glass slides coated with a fluorescein-containing Eudragit® L 30 D-55 suspension following immersion in either HCl 0.1N or phosphate buffer pH 6.8 for differing time periods.

Medium	Exposure time (min)	Molded disks	Glass slides
		sprayed with fluorescein-containing enteric polymer suspension	
-	-		
HCl 0.1N	10		
HCl 0.1N	120		

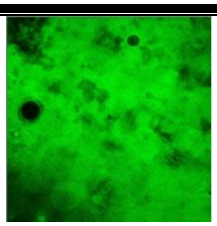
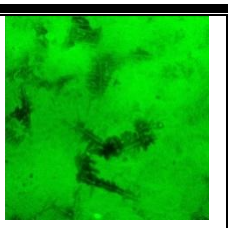
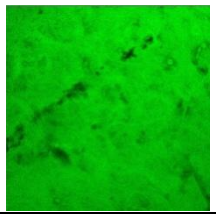
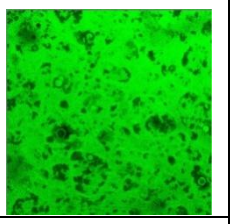
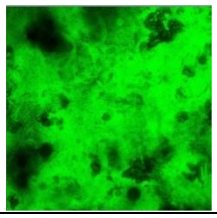
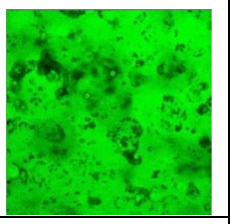
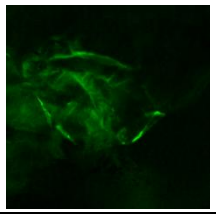
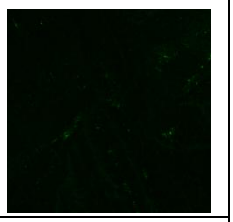
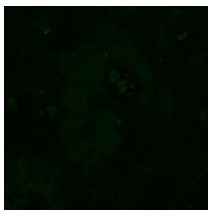
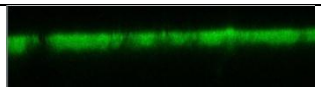
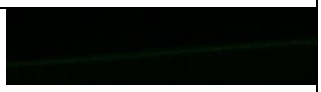
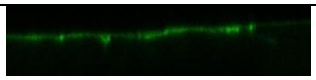
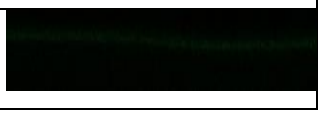
Phosphate buffer pH 6.8 (after 2h in HCl 0.1N)	5		
	10		
	20		
	25		
	30		

Table 3: CLSM images of the optical cross section of molded disks coated with a fluorescein-containing Eudragit® L 30 D-55 suspension or sprayed with a fluorescein solution following immersion in phosphate buffer pH 6.8 for differing time periods.

Medium	Exposure time (min)	Molded disks	
		sprayed with fluorescein-containing enteric polymer suspension	sprayed with fluorescein solution
Phosphate buffer pH 6.8	15		
	25		
	40		

In CLSM images the fluorescent marker appears green and helps identify the polymeric coating layer. Gradual shading or discontinuous coloring of the green surface (Table 2), as well as decrease of the thickness of the green layer in the cross section images (Table 3), were related to the dissolution of the coating. Both coated disks and glass slides showed a homogeneously colored surface before and after being exposed to the acidic medium up to 120 min. On the other hand, 25-30 min were necessary for the green color to almost disappear from samples once they were transferred into the buffer solution at pH 6.8 (Table 2). In the optical cross section images of coated disks, a green layer that becomes thinner after 25-30 min is evident, which was confirmed to be consistent with the time required for the dissolution of the polymeric coating

(Table 3). Notably, the coloring of coated disks was generally very similar to that of glass controls, which might have suggested an analogous dissolution time course for the coating. However, a faded green coloration could still be detected after 40 min in pH 6.8 buffer on the molded HPC disks only. Similarly, a green shadow was also present at each time point (15-40 min) on disks that were sprayed with an aqueous solution of the fluorescent marker. It was thus hypothesized that the fluorescein solution could rapidly penetrate through the pore network of the glassy matrix, leading to the appearance of the green coloration. On the contrary, the diffusion of the marker through the swollen HPC molecular chains, both inward and outward, might be relatively slower thus making the color leaching out negligible. The faded coloration could therefore disappear following dissolution/erosion of the gel layer only. In order to verify this hypothesis, the HPC mass loss profile of disks in buffer medium pH 6.8 was evaluated (Figure 1): data from coated disks were collected after the dissolution of the Eudragit® L film only.

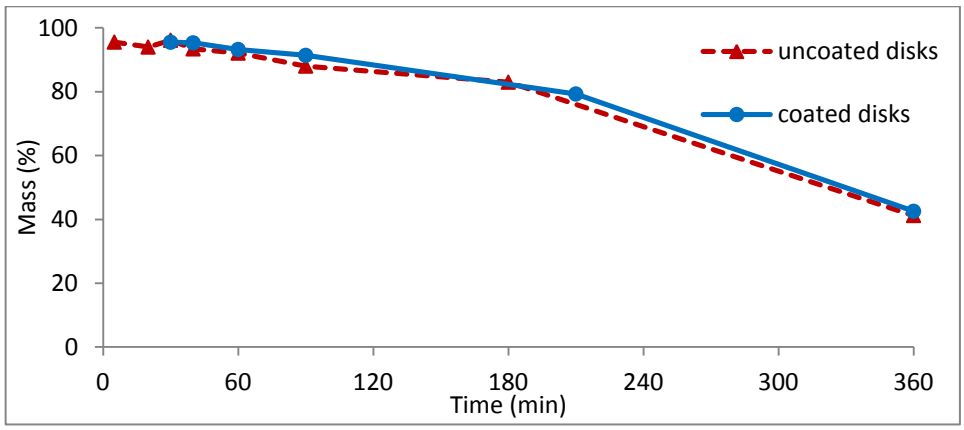


Figure 1: HPC mass loss profiles in phosphate buffer pH 6.8 of molded disks.

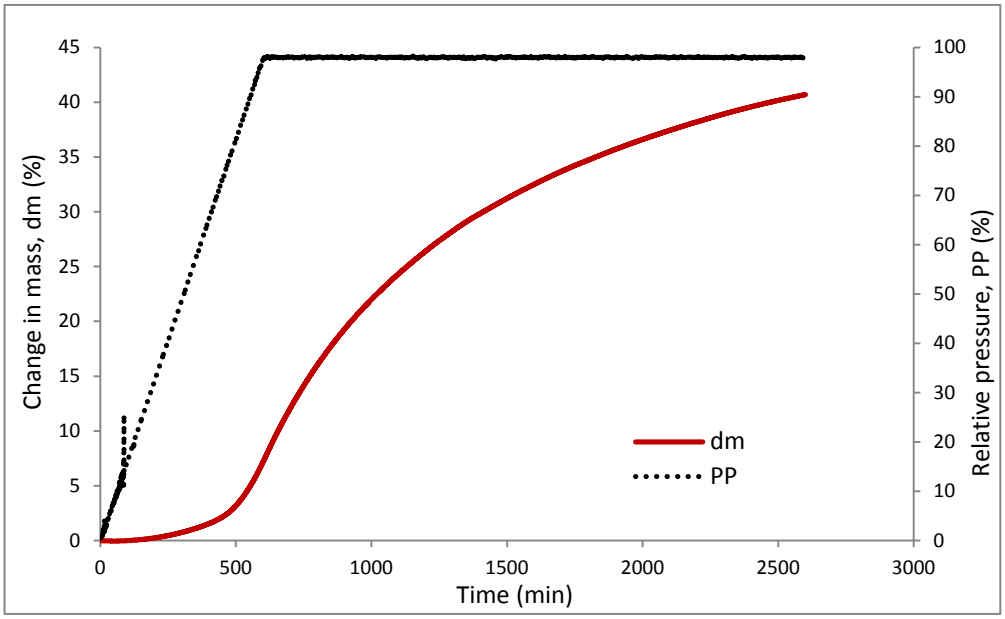
The mass loss of uncoated disks under the conditions employed was very slow (about 45 % within 6 h); in particular, a < 5% decrease in weight was noticed during the first hour. The profile of HPC mass loss relevant to coated disks after the dissolution of the Eudragit® L film was almost superimposed. Assuming that a gel layer could form on the surface of disks on wetting after a few seconds (with both the fluorescein solution and the coating suspension) and as no dissolution/erosion phenomena had occurred to change the disk surface, the faded coloration observed on the disks was confirmed to coincide with the front of the initial solvent penetration through the glassy matrix pore network. Mass loss profiles also pointed out that the dissolution behavior of molded HPC disks was not affected either by the application of an enteric coating (coating process) or by the test conditions (2 h in pH 1.2). This was considered a promising result in the prospect of achieving coated HPC capsules able to ensure the same pulsatile-release performance after the dissolution of the enteric coating.

Considering HPMC capsules, it is known that the glassy-rubbery transition of the polymer promoted by the first contact with the coating suspension can bring about a sticking tendency of cores, and thus potential issues related to the process yield that were also encountered for gelatin cores [McGinity and Felton, 2008; Thoma and Bechtold, 1992]. As HPC involved the same issue, when finalizing the coating process of molded capsules, it will be necessary to identify conditions to prevent sticking (*i.e.* problems related to the tumbling of cores within the rotating coating pan or to an incomplete exposure of the shell surface

to the sprayed polymeric suspension) and enabling a balance between wetting and drying efficiency.

Finally, as a certain tendency of HPC to water uptake, which could impact the mechanical characteristics of shells and stability profile of the filled capsules, was already highlighted [Gazzaniga et al., 2011], the potential of the coating layer as a barrier for gas diffusion was evaluated by means of Dynamic Vapor Sorption (DVS), both at 25 and 40 °C. Evidences that water can act as a plasticizer lowering the glass transition temperature of polymers, which leads to a decrease in mechanical resistance and adhesive strength, is widely reported in the literature [Aulton et al., 1984; Felton and McGinity, 1997; Hancock and Zografi, 1997; Okhamafe and York, 1985; Stanley et al., 1981]. Uncoated and coated disks maintained under the same storage conditions (24 ± 2 °C/55 ± 5 % RH) showed differences in water content (higher for the uncoated) that were also reflected in a different duration of the desorption phase (longer for uncoated disks). Accordingly, the moisture sorption profiles of uncoated and coated disks turned out to be different in terms of both the actual amount of water sorbed and rate of sorption. By way of example, results obtained at 25 °C are shown in Figure 2.

a)



b)

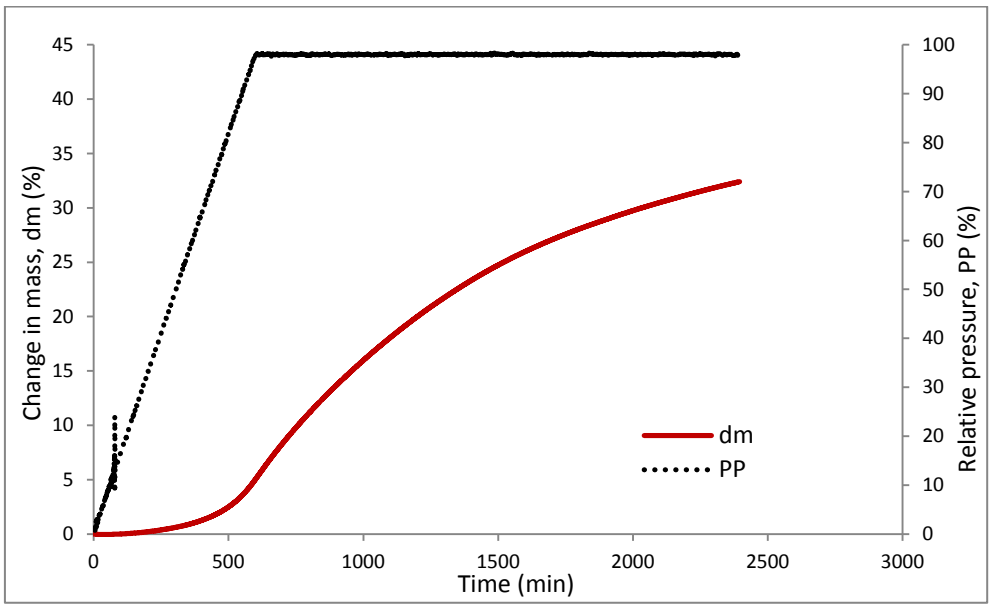


Figure 2: Moisture sorption profiles of uncoated (a) and coated (b) disks at 25 °C.

Coated samples reached a final water content of 32.41 % vs. 40.70 % of uncoated ones. Analogous results were obtained under 40 °C, but in this case a higher rate

of water sorption was observed with both types of samples. Lower equilibrium moisture contents (≤ 20 %) are reported in the literature for HPC, both untreated and processed by hot melt extrusion [Alvarez-Lorenzo et al., 2000; Harwood, 2003; Prodduturi et al., 2004]. However, the ability of PEG 1500 to promote the moisture sorption of plasticized HPC-based blends was already demonstrated for IM capsules [Gazzaniga et al., 2011]. The overall results demonstrated the potential of the enteric film for limiting water exchange of the molded substrate. In the case of coated HPC capsules, this could be advantageous in terms of product stability.

Proof of the ability of the Eudragit® L film to protect molded disks from the environment could also be appreciated in the reduction of the warping tendency. The coating layer prevented molded items from undergoing a previously described deformation, occurring within 72 h from demolding and attributed to the presence of the plasticizer in the shell composition [Zema et al, 2013b]. In fact, coated disks, stored at the same conditions as uncoated ones, maintained their morphology over time (Figure 3).

a) 

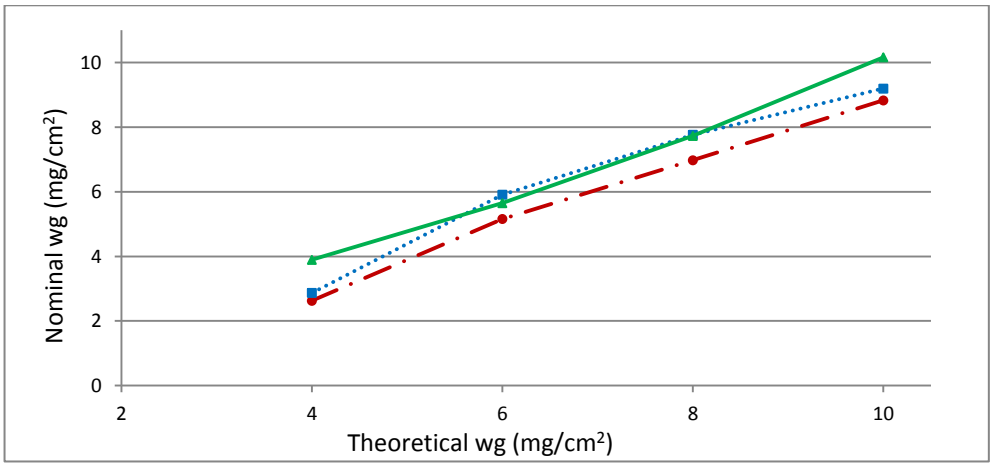
b) 

Figure 3: Photographs of uncoated (a) and coated (b) disks stored at ambient conditions after 15 days.

3.2. Coating process and characteristics of coated capsules

As the closing system of a capsule may become a critical issue during the coating process, both banded and unbanded units of HPC on the one hand, and gelatin or HPMC on the other, were considered. During the Eudragit® L 30 D-55 coating process, samples with increasing weight gains, *i.e.* 4, 6, 8, and 10 mg/cm^2, were withdrawn. The process yield can be calculated from the actual vs. theoretical amount of dry polymer applied; in the case of optimal polymer deposition, a linear relationship with slope approaching 1 is expected. The rate of polymer deposition under the same process conditions was found analogous for the three types of cores, and showed the desired slope (Figure 4). Moreover, with the aim of reducing the duration of the HPC capsule coating process, the spraying rate was doubled still avoiding the sticking of cores and without impacting the physico-technological characteristics of the coated units.

a)



b)

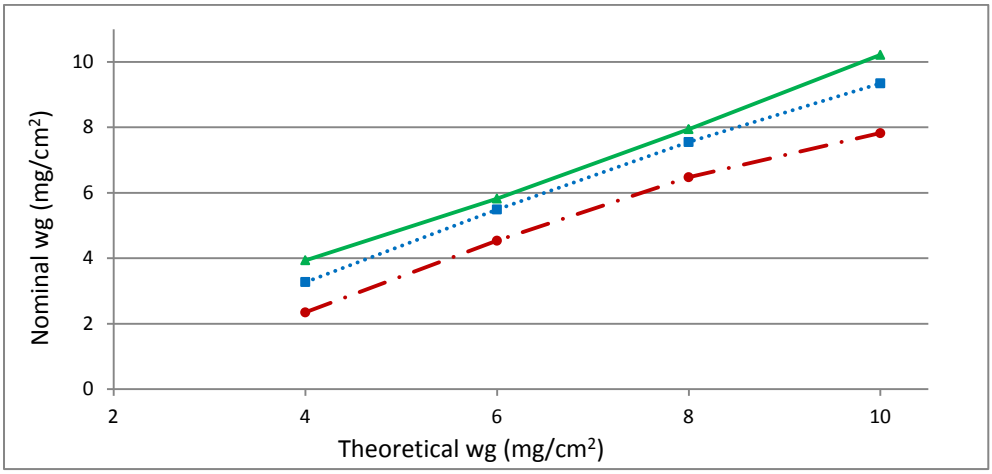


Figure 4: Profiles of nominal vs actual weight gain (wg) of gelatin (●), HPMC (■) and HPC (▲)capsules, unbanded (a) and banded (b).

In Table 4 the physico-technological characteristics of coated systems are compared with those of uncoated IM capsules: by way of example, data relevant to unbanded cores as such or coated with increasing amounts of Eudragit® L are reported.

Table 4: Characteristics of HPC-based capsules uncoated and coated with increasing amounts of Eudragit® L (CV in brackets).

	Uncoated capsules	**Capsules coated with increasing nominal amounts of polymer (mg/cm^2):**			
		4	**6**	**8**	**10**
Weight, mg	229.2 (0.26)	323.6 (0.64)	329.4 (0.47)	336.2 (0.63)	344.3 (0.47)
Actual amount of polymer, mg/cm^2	-	3.58	5.65	7.73	10.16
Coating thickness, µm	-	63.40	88.25	107.20	132.15
Mechanical resistance, N	71.02 (5.02)	84.56 (2.99)	92.23 (1.09)	95.54 (3.06)	97.01 (4.62)

The actual amount of pure polymer applied onto IM capsules turned out close to the theoretical, confirming the ability to maintain an almost constant deposition rate even under the more stressful process condition of increased spraying rate. Moreover, the increase in the coating thickness appeared nearly linear within the range of weight gain considered (data not shown). When applied onto IM capsules, the enteric coating demonstrated to improve the mechanical resistance of the system. In the presence of a coating film, the maximum force borne by capsules before breaking was higher with respect to uncoated ones, but the increase in the mechanical resistance was not directly proportional to the amount of Eudragit® L applied. The coating film would not only impact on the hardness of capsules directly, but also by affecting the relevant humidity content [Bley et al., 2009; Cerea et al., 2004]. Indeed, the potential ability of the Eudragit® L film to reduce the final water content of HPC-based substrates was shown earlier with the molded disks.

The quality of the enteric coating applied to molded capsules was finally investigated by means of optical microscope and SEM analysis. When considering capsular cores, the application of the coating film onto the closing system, *i.e.* the overlapping area between the cap and body, may be a critical issue. In Figure 5 technical drawings of capsules and SEM images of uncoated and coated (10 mg/cm^2) systems are reported: in particular, details of closing systems of both banded and unbanded cores are shown.

HPC capsules were produced with a locking mechanism based on the mutual pressure exerted by the contact areas of matching items so that the body and cap components, once assembled, would not entail any junction gap [Zema et al., 2013b]. This was confirmed by SEM images of the overlapping areas: in particular, the very tight adherence of HPC cap and body items with respect to gelatin and HPMC ones was pointed out. The plane external surface of the closing area that was devised for HPC capsules in order to ensure a constant shell thickness also turned out advantageous to the application of coatings. In fact, no discontinuities of the film applied to the overlapping area were observed, independent of whether the cores were banded or unbanded, thus suggesting that a sealing step could be avoided. Conversely, a continuous coating layer covering the closure of gelatin and HPMC capsules was only obtained with band-sealed cores. These findings were then confirmed by the release performance of the coated systems, as described in the subsequent section of the paper. Finally, the effective adhesion of the coating to HPC capsules, as demonstrated by a smooth and homogeneous surface with a lack of observable cracks or pores, could be attributed not only to the improved locking system but also to the rough surface of the molded devices, similar to that previously reported with HPMC capsule shells [Felton et al., 2002].

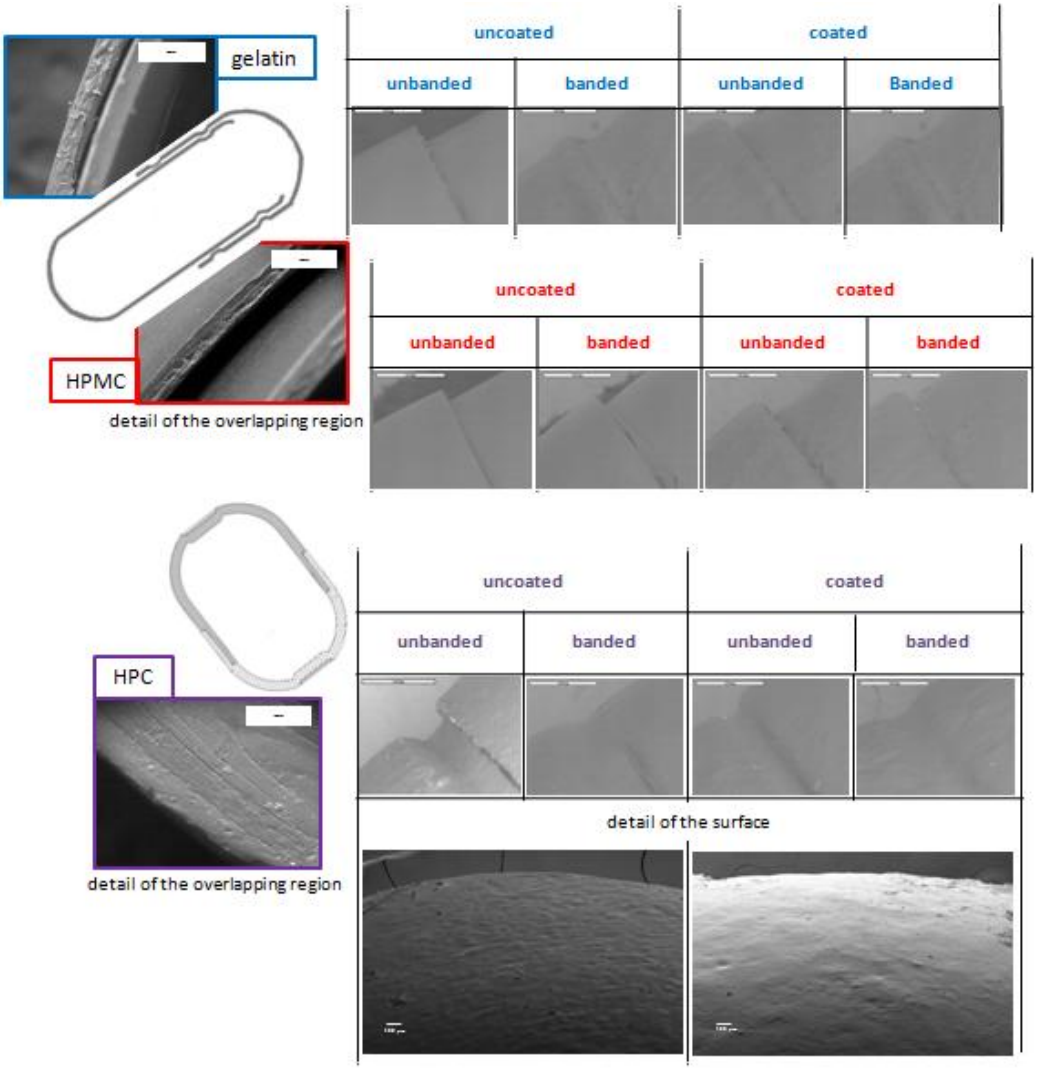


Figure 5: Technical drawings and SEM images of gelatin, HPMC and HPC capsules, unbanded and banded, uncoated and coated (10 mg/cm^2).

Optical microscope images of the cross section of HPC capsules, uncoated, withdrawn from the coating pan after 1 min of processing or coated up to 10 mg/cm^2 are reported in Figure 6.

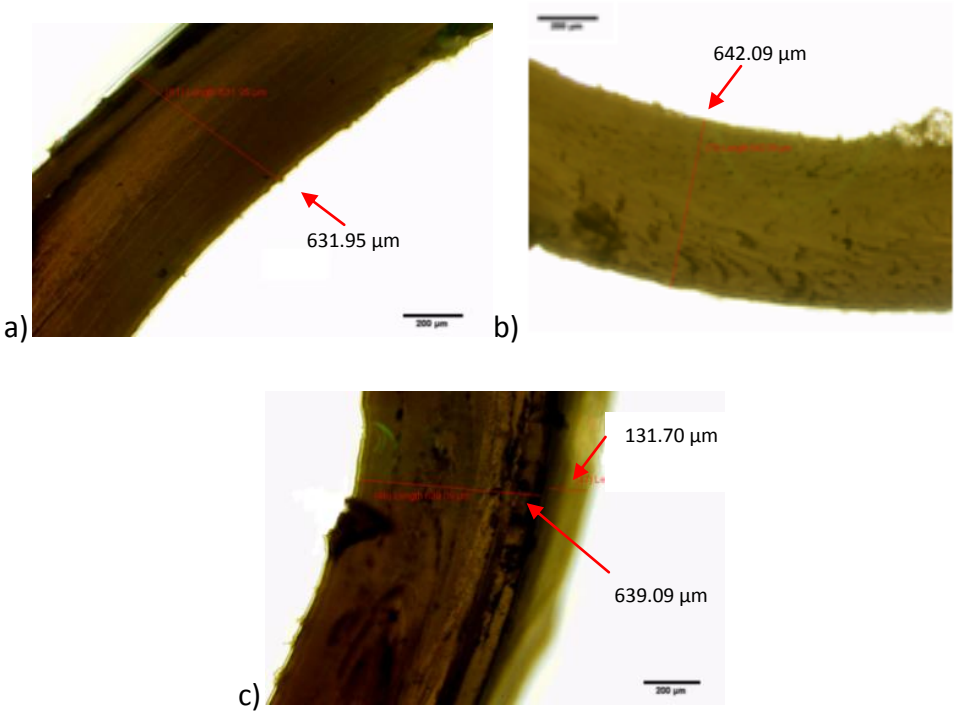


Figure 6: Optical microscope images of the cross section of cleaved capsules uncoated (a), withdrawn from the coating pan after 1 min (b) and coated with 10 mg/cm^2 of polymer (c).

The wall thickness measured from the uncoated capsule image (around 630 µm) was in agreement with data previously reported [Zema et al., 2013b]. On the other hand, the wall thickness of the sample withdrawn after 1 min of coating was increased slightly. This was probably due to a slight swelling of the HPC capsule surface promoted by contact with the aqueous Eudragit® suspension, as previously observed with the molded disks. Looking at the coated capsules, a ~130 µm thick homogeneous layer, relevant to the enteric film, can be seen on

the external side. Such a layer perfectly matches the curved surface of the capsular core. Also in this image, the shell thickness is of ~640 µm as after 1 min of coating. Indeed, the measured thickness of the enteric coating is in agreement with the calculated mean value that is reported in Table 4.

Finally, the release performance of the coated devices was studied: banded and unbanded capsules, coated with increasing amounts of polymer, were evaluated and compared with conventional gelatin and HPMC ones. In table 5, $t_{10\%}$, *i.e.* the time to 10 % dissolution/release, and $t_{90\text{-}10\%}$, *i.e.* the time elapsed between 10 % and 90 % release, are listed; in the case of HPC capsules, these parameters were used as an index of the duration of the lag phase and of the release process, respectively.

Table 5: Dissolution/release data of uncoated and coated systems: (a) $t_{10\%}$ of gelatin and HPMC capsules; (b) $t_{10\%}$ and $t_{90\text{-}10\%}$ of HPC capsules.

a)

		Gelatin capsules		HPMC capsules	
	wg (mg/cm^2)	$t_{10\%}$, min (CV)		$t_{10\%}$, min (CV)	
		Banded	Unbanded	Banded	Unbanded
Uncoated	-	0.50 (32.90)	0.32 (2.93)	8.01 (17.47)	6.09 (34.20)
Coated	4	121.46 (0.01)	48.37 (97.83)	121.32 (0.51)	100.42 (21.62)
	6	120.98 (0.54)	89.96 (60.30)	122.05 (0.79)	102.70 (31.45)
	8	121.50 (0.00)	121.35 (0.11)	122.91 (7.07)	121.66 (0.07)
	10	123.25 (0.45)	121.60 (0.57)	129.51 (5.62)	121.91 (0.40)

	wg (mg/cm^2)	HPC capsules			
		$t_{10\%}$, min (CV)		$t_{90\text{-}10\%}$, min (CV)	
		Banded	Unbanded	Banded	Unbanded
Uncoated	-	44.98 (11.88)	36.31 (2.37)	8.54 (12.56)	10.08 (8.47)
Coated	4	146.99 (5.22)	144.66 (4.67)	11.60 (1.29)	12.61 (3.83)
	6	149.51 (2.47)	150.88 (0.31)	10.34 (4.59)	11.89 (5.46)
	8	159.52 (1.67)	159.51 (1.90)	9.88 (4.38)	9.51 (7.65)
	10	171.98 (2.95)	168.64 (5.82)	11.57 (1.99)	10.14 (0.35)

b)

The good quality of the enteric-coated HPC capsules was confirmed by the relevant release behavior. Uncoated IM capsules provided a pulsatile release performance characterized by a lag time ($t_{10\%}$), due to the dissolution/erosion of the shell, and a pulse time, *i.e.* the time elapsed between onset and completion of release ($t_{90\text{-}10\%}$): the obtained $t_{10\%}$ and $t_{90\text{-}10\%}$ values from this study are in agreement with previously reported values [Zema et al., 2013b]. The lag time of enteric coated capsules was longer with respect to that of uncoated ones, as expected, and increased as a function of the amount of polymer applied. In the case of the coated systems, in fact, both the ability of the film to withstand acidic media and the time needed for the opening of pulsatile delivery capsules would contribute to the overall lag time. Although it was not possible to discriminate, the lag phase seemed to be consistent with effective gastric resistance (2 h in acidic media) and opening time of the uncoated shells, at least for systems with 10 mg/cm^2 coating level. Moreover, no impact of the coating layer on the release performance after capsule opening was highlighted: indeed, pulse times of

coated systems were analogous to those of uncoated capsules. The tightness of the shell closing system was further supported as no differences in drug release were observed between banded and unbanded capsules, thus confirming that the sealing of IM cores could be avoided. In contrast, in the case of gelatin and HPMC capsules, the minimum amount of polymer applied (4 mg/cm^2) was sufficient to ensure gastric resistance of banded cores only, whereas this amount needed to be increased to 8 mg/cm^2 with unbanded units.

4. Conclusions

The feasibility of coating HPC-based molded capsules intended for pulsatile release of drugs with Eudragit® L 30 D-55 was evaluated. When enteric coated, such capsules would come into contact with aqueous GI fluids only after stomach emptying thus being able to target the colonic region through lag phases matching the small intestinal transit time of dosage forms (time-dependent approach).

Prototype capsules of 600 µm thickness that were already demonstrated to provide, both in vitro and in vivo, a lag phase prior to the onset of release were employed. However, in view of the peculiar substrate involved, the application of the Eudragit® L film and characterization of coated products were preliminarily carried out on molded disks intended for screening purposes. The polymeric films generally exhibited consistent behavior independent of whether they were applied to the molded disks or to reference glass slides: in both cases, they were

able to withstand the acidic medium and dissolve in 25-30 min after switching to phosphate buffer.

Interestingly, the enteric coat showed a potential for improving the physical stability of molded products by reducing their moisture sorption and warpage tendency as well as increasing the overall mechanical resistance. IM capsules were successfully coated with increasing amounts of Eudragit® L leading to systems with good technological properties and the desired release performance, *i.e.* a lag time compatible with the maintenance of gastric resistance and same pulsatile profile in the pH 6.8 medium as the uncoated devices. The capsule closing system turned out effective with no need for a sealing to be applied before the coating process and suitable for the achievement of a homogeneously layered coating of constant thickness.

The obtained results highlighted the potential of Chronocap™ capsules for being exploited as core units in the development of colon delivery systems based on a time-dependent approach.

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Chapter 3

Formulation Development of an Injection-Molded Capsular Device for Oral Pulsatile and/or Time-Based Colon Delivery

1. Objective

Within a general research project aimed at the development of a capsule-shaped drug delivery system (DDS) prepared by injection molding (IM) and intended for pulsatile release and/or time-dependent colonic delivery of drugs (Chronocap™ platform), the modulation of the typical lag phase of such a device was in-depth investigated. The possibility of preparing containers for drug formulations, purposely designed for achieving pre-defined lag times prior to release, would be advantageous in chronotherapy. Moreover, the time-dependent approach to target the colonic region is based on the administration of gastroresistant pulsatile delivery systems able to impart a lag phase, starting after stomach emptying, of ~~a~~ defined duration, *i.e.* 3 h ± 1 standard error, consistent with the small intestinal transit time of dosage forms.

A correlation between the duration of the lag phase and the thickness of IM capsule shells was previously demonstrated by means of a prototype mold that allowed capsules with 300, 600 and 900 µm thick walls [Gazzaniga A. *et al.,* 2011] to be manufactured. In the present chapter, formulation strategies possibly suitable for the modulation of the opening time of the Chronocap™ shells were therefore evaluated. In particular, an extension of the lag phase was pursued. A first approach was based on slowing down the hydration/swelling phase of HPC, which is the basic component of the capsule shells. As a consequence, the break-up of systems after the dissolution/erosion of the shell is expected to occur later and the liberation of capsule contents to be further delayed. For this purpose

the use of alternative swellable polymers and/or swelling adjuvants (HPC grades with higher molecular weight and disintegrants), as well as the addition of insoluble and inert adjuvants (ethylcellulose and talc) were evaluated.

2. Materials and methods

2.1. Materials

Hydroxypropyl cellulose (HPC): Klucel® LF and GF, Eigenmann & Veronelli, I; sodium starch glycolate, Explotab CLV®, JRS Pharma, US; ethyl cellulose: Ethocel® 100std, Colorcon, US; talc, Carlo Erba Reagenti, I; polyethylene glycol (PEG) 1500, Clariant Masterbatches, I; acetaminophen (AAP) fine powder, Atabay, TR.

2.2. Methods

2.2.1. IM process

A mixture of formulation components, previously dried in a ventilated oven for 24 h at 40 °C, and 10 % by weight of PEG 1500 was prepared in Turbula® (Type T2C; WAB, CH) and then transferred into a bench-top micromolding machine (BabyPlast 6/10P; Cronoplast S.L., ES; Rambaldi S.r.l., I). In Table R3.1 the composition (%, by weight) of the polymeric formulations processed by IM is reported.

Table R3.1: Codes and composition of molded formulations.

Code	Formulation composition (% by weight)
KL	Klucel® LF (90%) + PEG 1500 (10%)
KLKG 7/3	(Klucel® LF 70% + Klucel® GF 30%) (90%) + PEG 1500 (10%)
KLKG 5/5	(Klucel® LF 50% + Klucel® GF 50%) (90%) + PEG 1500 (10%)
KG	Klucel® GF (90%) + PEG 1500 (10%)
KLEX5	(Klucel® LF + PEG 1500 (10%)) (95%) + Explotab CLV® (5%)
KLEX10	(Klucel® LF + PEG 1500 (10%)) (90%) + Explotab CLV® (10%)
KLEX20	(Klucel® LF + PEG 1500 (10%)) (80%) + Explotab CLV® (20%)
KLEX30	(Klucel® LF + PEG 1500 (10%)) (70%) + Ethocel® 100std (30%)
KLEC20	(Klucel® LF + PEG 1500 (10%)) (80%) + Ethocel® 100std (20%)
KLTA20	(Klucel® LF + PEG 1500 (10%)) (80%) + Talc (20%)

The behavior of polymeric formulations upon heating or IM was evaluated as follows:

Hot-plate experiment: samples of 2-3 g of the Klucel® LF as such or in admixture with the selected excipients, were placed in an aluminum pan on a hot plate. Samples, under continuous manually stirring, were gradually heated from 30 to 200 °C in few minutes and the temperature was checked with a laser thermometer. Changes of color, aspect and mechanical characteristics of the materials upon heating were observed.

Air shot test: 50 g of Klucel® LF as such or in admixture with the selected excipients, loaded into the molding press through the hopper, were expelled from the injecting unit as operating a purge under different operating temperatures [Rosato D.V. *et al.*, 2000]. Expelled samples were checked for overall aspect, color and mechanical characteristics immediately after ejection and when solidified.

Some of the polymeric formulation were processed by hot-melt extrusion in a microcompounder (Minilab II, HAAKE Rheomex CTW5, Thermo Scientific, D) equipped with conical twin screws (diameter 5/14, length 109.5 mm) which can either co- or counter-rotate. Operating temperatures were selected in the range of 130-160 °C; the rotation speed of the screws was set at 15 rpm. The extruded product was milled before being loaded in the IM press.

IM items were prepared by means of either a capsular mold with two interchangeable inserts for the manufacturing of matching caps and bodies of 600 µm nominal thickness, or a disk-shaped mold (diameter: 30 mm; height: 1 mm) provided with a central gate. Process conditions were varied within different ranges of values depending on the item produced (disk or capsule) (Table R3.2).

Table R3.2 : IM process parameters.

		disk	**capsule**
compression zone temperature; *°C*		140-160	100-130
metering zone temperature; *°C*		150-160	130-140
nozzle temperature; *°C*		160-170	140-150
hot runner temperature; *°C*		-	160-170
charge; *mm*		15-16	4
1st injection	pressure; *bar*	50-60	30-60
	time; *s*	0.6	0.4
	rate; *%*	50-60	30-60
2nd injection	pressure; *bar*	10-50	10-50
	time; *s*	2	0.3
	rate; *%*	10-40	10-50
cooling temperature; *°C*		15	15
cooling time; *s*		2.5	2.5-3
closing pressure; *bar*		80	120
opening rate; *%*		90	90

2.2.2. Characterization of molded items

IM disks and capsules were checked for weight by an analytical balance (Sartorius BP211D, Sartorius AG, D; n=10) and for thickness by a digital micrometer (Mitutoyo IT-012U, Mitutoyo, J; n=10) immediately after ejection and after 24, 48, 72 h storage at ambient conditions (24 ± 2 °C/55 ± 5% relative humidity, RH).

Digital photographs of molded capsules were acquired by a Nikon D70 camera (Nikon, J).

Dissolution/erosion properties of molded disks upon contact with aqueous fluids were assessed by a mass loss test performed in the pharmacopoeial apparatus 2 (Dissolution System 2100B, Distek, US) at 100 rpm. Each sample, weighed (p_i) and inserted in a closed system prepared from a polyethylene net (2 mm mesh), was then weighed again in order to determine the tare (t) and placed in a vessel containing 600 mL water kept a 37 °C. Samples (n=3) were withdrawn every 30 min, then placed in a ventilated oven at 40 °C for 24 h and weighed (p_f). Percentage of residual mass was calculated according to Eq. (1):

$$\text{residual mass } \% = \frac{[(p_i - t) \times 100]}{p_f} \qquad \text{Eq. (1)}$$

Both the release performance and the opening behavior under unstirred conditions of capsular systems were evaluated.

The *in vitro* release test of capsules (n=6) containing 150 mg of AAP powder was performed in a six-position USP 34 disintegration apparatus [Gazzaniga A. *et al.*, 1995]. Each capsule was inserted in one of the six available tubes of the basket-rack assembly, which moved at 31 cycles/min rate in separate vessels; each of these contained 800 mL deionized water kept at 37 ± 0.5 °C. Fluid samples were withdrawn at fixed time points and assayed spectrophotometrically at 248 nm. 10 and 90 % drug released were calculated for each sample from the relevant release pattern (n = 6); 10 % value ($t_{10\%}$) was defined as lag time (*i.e.* the time required for the opening of the system), while the time elapsed between 10 % and 90 % release ($t_{90\%–10\%}$) was defined as pulse time (*i.e.* the time required for completion of the release process).

The morphological changes of filled capsules exposed to deionized water at ambient temperature under unstirred conditions were evaluated over 6 h. Digital photographs were taken every 5 min.

3. Results and discussion

HPC is commercially available in several types (e.g. Klucel® products) based on the molecular weight (average 50000-1250000 Da) and the grade of substitution of hydroxyl groups. Such characteristics may influence the behavior of polymeric chains when in contact with aqueous fluids, *i.e.* the rate and extent of hydration/swelling, and the viscosity of the polymer aqueous solutions.

When employed as hydrophilic swellable barriers in the form of capsule shells, different HPC grades may lead to a diverse opening of the device and, thus, to a range of modified release profiles of the conveyed drug. With respect to the processability of HPC by IM, neither the glassy-rubbery transition (T_g) of the polymer nor the relevant hot-melt extrusion temperatures seem to be dependent on the molecular weight [Trey S.M., *et al*., 2007]. Hence, in order to improve the versatility of the Chronocap™ platform by increasing the lag phase prior to release, the feasibility of capsular containers prepared from different Klucel® grades or blends was considered. For this study, the previously established formulation based on Klucel® LF (KL: Klucel® LF + 10% by weight PEG 1500) and the molded capsules obtained were considered as the reference for the evaluation of both the processability of the new materials and the release performance of resulting devices. The alternative to Klucel® LF (MW_{LF} 95000) taken into account was Klucel® GF, which has a higher molecular weight (MW_{GF} 370000). The possibility of exploiting the same plasticizer was preliminarily explored: hot-plate experiments and air shot tests were performed on polymeric blends with increasing amount of PEG 1500. By heating the material under continuous manual mixing, while gradually increasing the temperature up to 200°C, not only the range of working temperatures can be defined, but also possible issues of the heating process may be highlighted, such as a rapid transition of the molten mass to a liquid state, a lack of homogeneity or the occurrence of degradation phenomena at each stage. Moreover, the aspect and

mechanical characteristics of products after cooling can be checked. On the other hand, by expelling the material loaded in the IM press from the injection unit, the characteristics of the product can be related to the working parameters, which may be helpful for gathering information about the suitability of the material for IM processes. Also in this case, the material can be evaluated not only as soon as ejected from the nozzle, but also after its cooling/hardening. Such preliminary studies confirmed the processability of Klucel® grade GF by employing the same type and amount of plasticizer already used for the LF one (KG: Klucel® GF + 10 % w/w PEG 1500).

Capsules could be prepared from formulations KL and KG and their blends (KLKG 7/3: 70% KL + 30% KG and KLKG 5/5: 50% of both KL and KG). However, failures in the automatic ejection of molded half shells and minor imperfections of the surface were noticed in some cases.

When dealing with polymeric blends, the poor mixing ability of the IM press used could turn out critical. In this respect, some attempts were made to improve the homogeneity of distribution by pre-extruding blends of raw materials. For this purpose, a lab-scale twin-screw extruder (microcompounder) equipped with conical co-rotating and counter-rotating screws was used. The extrusion temperature was defined within the range of temperatures already involved in the IM process: 160 °C was identified as the temperature leading to the complete glassy/rubbery transition of raw materials (no evidence of particles in the extrudate) and to the lowest value of torque (*i.e.* the strain/stress of the

rotating screw measured in N·m) registered. For the manufacturing of capsules based on pre-extruded formulations, minor changes of process parameters were needed with respect to those not treated. However, molded units were obtained with no imperfections and major issues related to the automatic ejection of molded products could be overcome.

The characteristics of weight and thickness of capsules based on Klucel® LF, GF and their blends are reported in Table R3.1: product codes followed by "-e" refers to pre-extruded formulations.

Table R3.1: Weight and thickness of capsules based on Klucel® LF, GF and their blends.

formulation	weight (mg)		thickness (µm)	
	mean	CV	mean	CV
KL	221	0.83	609	1.35
KL co-e	228	0.05	613	1.02
KL counter-e	228	0.04	616	2.38
KLKG 7/3	226	1.59	614	1.78
KLKG 5/5	225	0.68	614	1.86
KLKG 5/5 co-e	226	0.23	611	1.59
KLKG 5/5 counter-e	227	0.27	616	2.60
KG	219	0.81	614	0.81

The characteristics of capsules made of blends of Klucel® GF turned out not to be significantly different ($p < 0.05$) from those based on the LF formulation. Moreover, even the pre-extrusion treatment of raw materials demonstrated to impact on the weight and thickness of molded shells.

The *in vitro* release performance of capsules filled with a powder drug tracer was then assessed. Two parameters were used to describe release profiles (Table R3.2): the lag time (calculated as the time to 10% release, $t_{10\%}$) and the pulse time ($t_{90\text{-}10\%}$), *i.e.* the time elapsed between onset ($t_{10\%}$) and completion ($t_{90\%}$) of the release.

Table R3.2: Release parameters of capsules based on Klucel® LF, GF and their blends.

Formulation	lag time (min)		pulse time (min)	
	mean	CV	Mean	CV
KL	25.22	10.47	6.24	16.05
KL co-e	27.00	5.62	5.13	6.93
KL counter-e	28.37	14.36	7.36	9.57
KLKG 7/3	34.13	6.54	5.86	14.50
KLKG 5/5	38.27	3.46	6.93	35.93
KLKG 5/5 co-e	38.08	8.82	5.62	14.21
KLKG 5/5 counter-e	42.25	5.28	8.54	14.29
KG	n.d.	n.d.	n.d.	n.d.

The lag time of capsules made of blends of formulations KL and KG turned out 10-15 minutes longer ($p < 0.05$) with respect to the KL reference. The application of an extrusion pre-treatment generally demonstrated not to affect the performance of devices, neither in the case of the KL formulation as such nor in that of its blends with the KG. Only with the KLKG 5/5 formulation pre-treated by the counter-rotating twin-screw extruder, the device obtained showed a lag time few minutes longer ($p < 0.05$) than that of the same formulation not previously

extruded or pre-treated by co-rotating screws. The presence of different amounts of Klucel® GF in the shell formulation, 30 or 50 %, seemed to not affect the lag time of release. With respect to the time for the complete opening of the systems (pulse time) containing Klucel® LF, it turned out independent of the composition and manufacturing procedure of capsule shells and always < 9 min. On the contrary, the release performance of KG systems was definitely different from that of all the other and was characterized by a high variability (Figure R3.1). Lag times of hours and slow release profiles were observed, which could indicate a problematic opening mechanism: the drug tracer, in fact, had generally started to diffuse out through the gelled structure of the shell wall before it broke. Moreover, few capsules didn't even start to release their contents by 6 hours (duration of the test). Release parameters for capsules made of the GL formulation could not be determined.

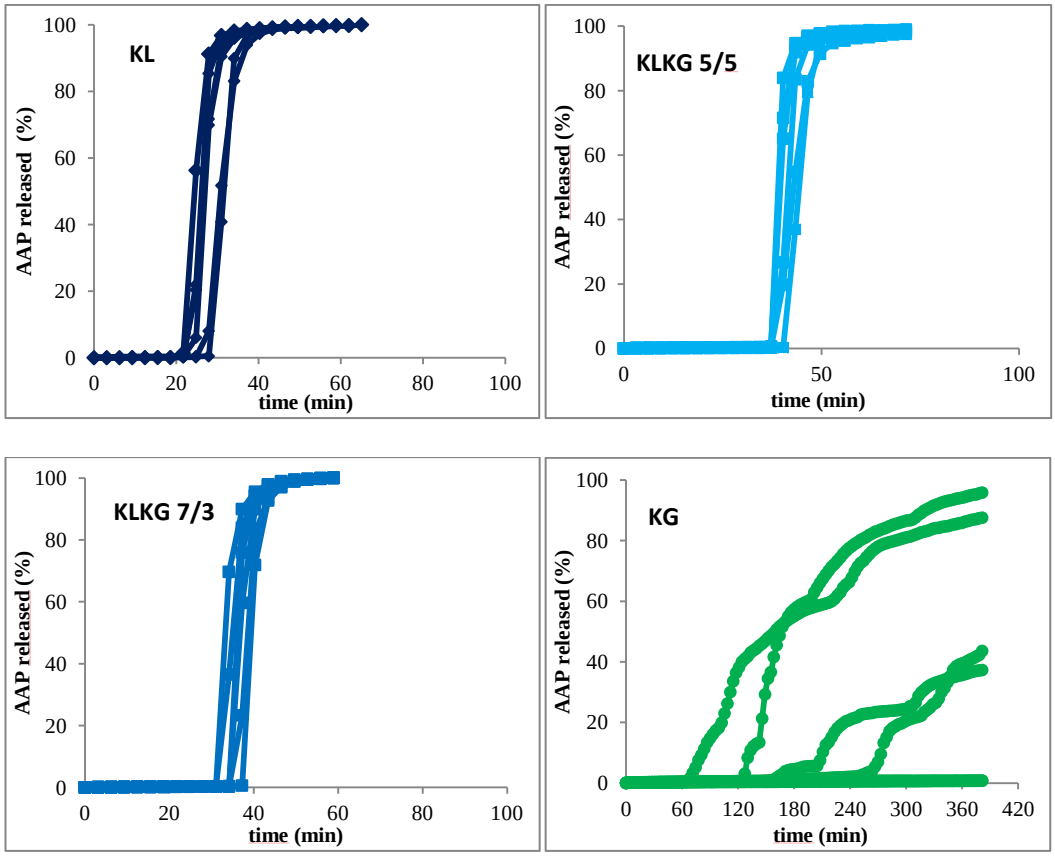

Figure R3.1: *In vitro* release profiles of capsules based on Klucel® LF, GF and their blends.

Based on the results obtained, it was hypothesized that the contribution of Klucel® GF to the lag time duration of capsules made of blends of formulations KL and KG was that of slowing down the rate of hydration and dissolution/erosion of the external polymeric layers that leads to the formation of first aperture in the shell. Afterwards, the overall resistance of the remaining gel barrier seems not to be affected as similar pulse time were calculated for all the devices based on polymeric blends. However, such a result could be attributed to the

hydrodynamic conditions of the test promoting the dissolution/erosion rate of the swollen barrier.

In order to confirm these hypothesis and evaluate the influence of the hydrodynamic conditions, release tests were carried out under unstirred conditions at ambient temperature. Photographs of capsules of the formulation KL, KG and their 1/1 mixture representing crucial steps of the release performance are reported in Figure R3.2: in particular, the first bubble coming out from the initial crack of the capsule shell (image framed in red) and the complete opening of the device that leads to the liberation of the powder tracer contained (image framed in blue) are depicted.

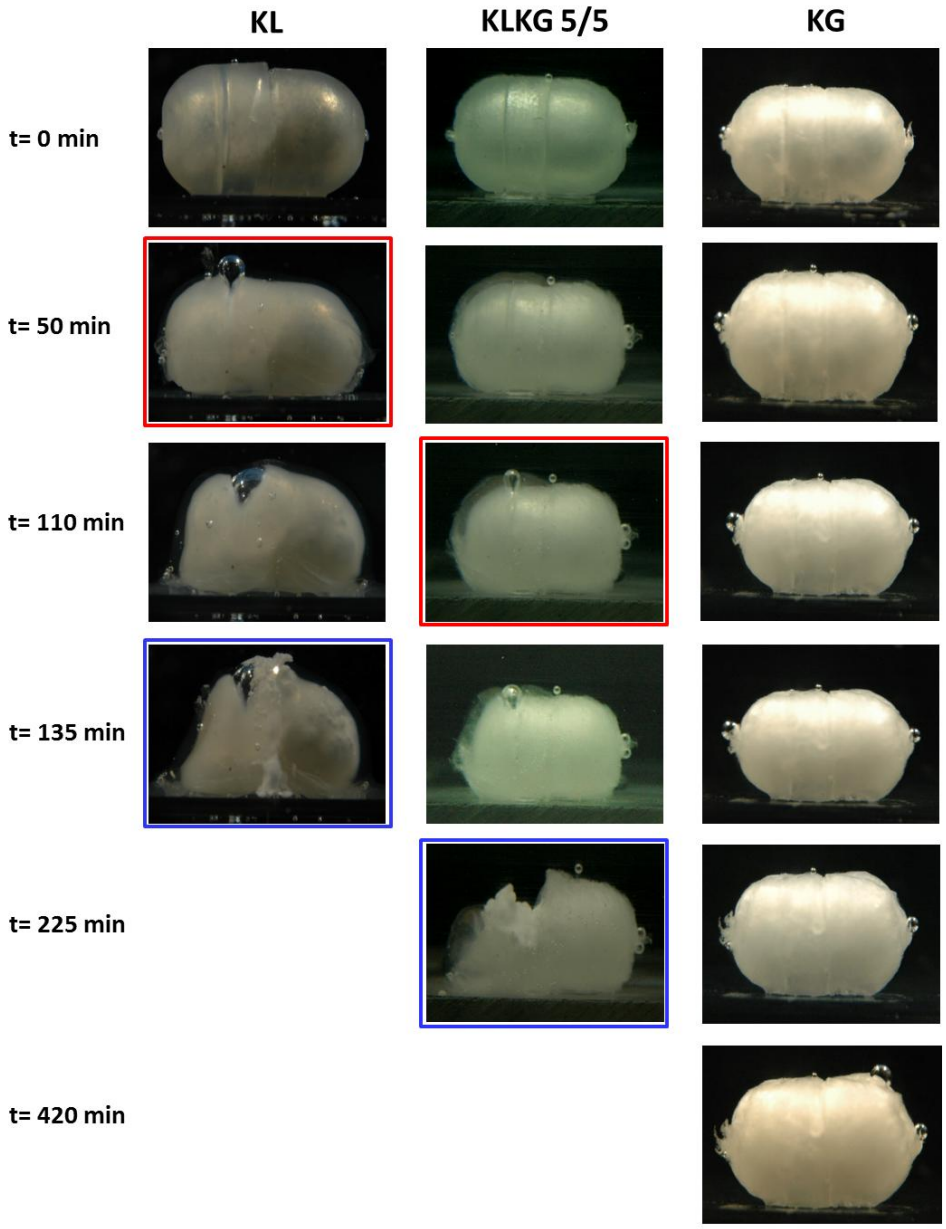

Figure R3.2: Photographs of capsules of Klucel® LF, GF and their 1/1 blend immersed in water under unstirred conditions at different time points.

The LF reference capsule properly underwent hydration with the formation of a gel layer that gradually dissolved/eroded until the formation of a first tear; the presence of the rupture was evidenced by the leakage of an air bubble that occurred after about 50 min of contact with the aqueous fluid. The complete loss of integrity of the capsule shell required 85 min more. As expected, the timing of capsule opening under unstirred conditions turned out to be noticeably longer with respect to both the lag phase and pulse time obtained under the release test conditions. Accordingly, the KG shell remained intact over 6 hours; at the end of this period, only a thin gel layer was present around the device. The capsule composed of the KL and KG formulation blend showed a behavior more similar to that of the KL device, but with longer latency before breakage. However, even the time to complete the opening of the device turned out longer, thus confirming that the resistance of the gel barrier after the formation of the first crack is largely affected by the unstirred conditions.

The objective of improving the duration of the Chronocap™ lag phase by introducing a higher viscosity grade HPC in the reference formulation based on Klucel® LF was not completely fulfilled. This could be partially attributed to difficulties in promoting the intimate contact or the interpenetration of polymeric chains during the hot processing. In fact, capsules prepared from polymeric blends showed a behavior much more similar to that of one of the components (Klucel® LF), even if a ~40% increase of lag time was achieved.

However, a further moderate increase of capsule lag time was only obtained when the formulation was counter-extruded before being processed by IM.

Other polymeric materials that could be introduced in the LF formulation of capsule shells in order to increase the lag phase were identified. Based on the previous experience with Klucel® GF, the most likely mechanism to be exploited should be that of hindering the hydration of HPC and its early transition to the rubbery state. Accordingly, two different types of excipients, having opposite properties upon contact with water, but expected to lead to the same outcome in extending the lag time of capsules, were considered: swellable polymers, which are intended to partially remove water by absorbing it (competitors), and insoluble/inert adjuvants, simply unable to draw water to the system. From the former category, L-HPC and sodium starch glycolate (Explotab® CLV) were selected. L-HPC is a low-substituted HPC, having about 15 times less hydroxypropyl groups than Klucel® LF (molar substitution L-HPC = 0.2 vs molar substitution Klucel® LF = 3). Moreover, while HPC is soluble in water, L-HPC is insoluble, although it strongly retains water molecules and swells [L-HPC ShinEtsu, technical data. 2011; Kibbe A.H, 2000]; on the basis of this characteristic, it is recommended as a disintegrant for the preparation of solid oral dosage forms. Also the second material considered, Explotab® CLV, is a widely employed and one of the most efficient disintegrants. On the other side, with respect to insoluble/inert excipients, some preliminary results were obtained with talc and ethylcellulose.

As far as the processability of swellable polymers is concerned, since little information about their characteristics upon heating is reported in the literature, both L-HPC and Explotab® CLV were tested on hot-plate: they didn't undergo melting phenomena typical of thermoplastic materials upon glassy-rubbery transition, but showed signs of degradation around 200-250 °C, temperatures remarkably higher than those involved in the molding of Klucel® LF-based capsules. As no modifications could be observed at the operating temperatures, the selected disintegrants were expected to remain suspended in the HPC molten mass. For this reason, the processability by the IM press of the KL formulation containing L-HPC or Explotab® CLV was evaluated and disk-shaped screening items were prepared. In particular, formulations containing 10 and 30 % by weight of the disintegrant (KLLH10 and KLLH30 with L-HPC; KLEX10 and KLEX30 with Explotab® CLV, respectively) were considered. While KL disks turned out clear after demolding, those based on the polymeric blends with powder disintegrants were opaque with white particles visible, more frequent in disks containing 30 % by weight of the excipient.

All the disks were characterized in terms of thickness immediately after demolding and over time (up to 1 month) (Figure R3.3).

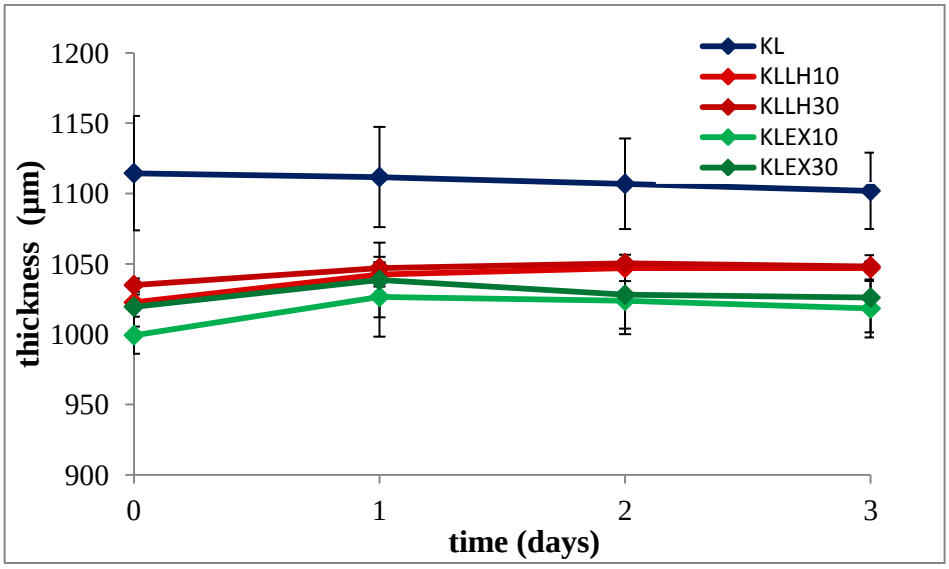

Figure R3.3: Variation over time of the thickness of molded disks based on Klucel® LF as such and blended with 10 and 30 % by weight of Explotab® CLV or L-HPC.

As already observed while working with HPC [Zema L. *et al*., 2013], the thickness of KL disks turned out higher (around 10 %) with respect to nominal 1000 µm. This tendency, mainly related to inner tensions formed during the rearrangement of the polymeric chains inside the mold [Fisher J.M., 2003], seemed to be limited by the presence of suspended particles of the disintegrants: by way of example, the thickness of KLEX30 disks after demolding was around 2.5 % greater than the nominal. Moreover, thickness values tended to increase over time, at least for 48 hours.

In order to gain preliminary information about the dissolution/erosion performance of the molded items based on polymeric blends containing disintegrants, the mass loss trend over time was studied; tests were carried out on disks stored for a week (Figure R3.4).

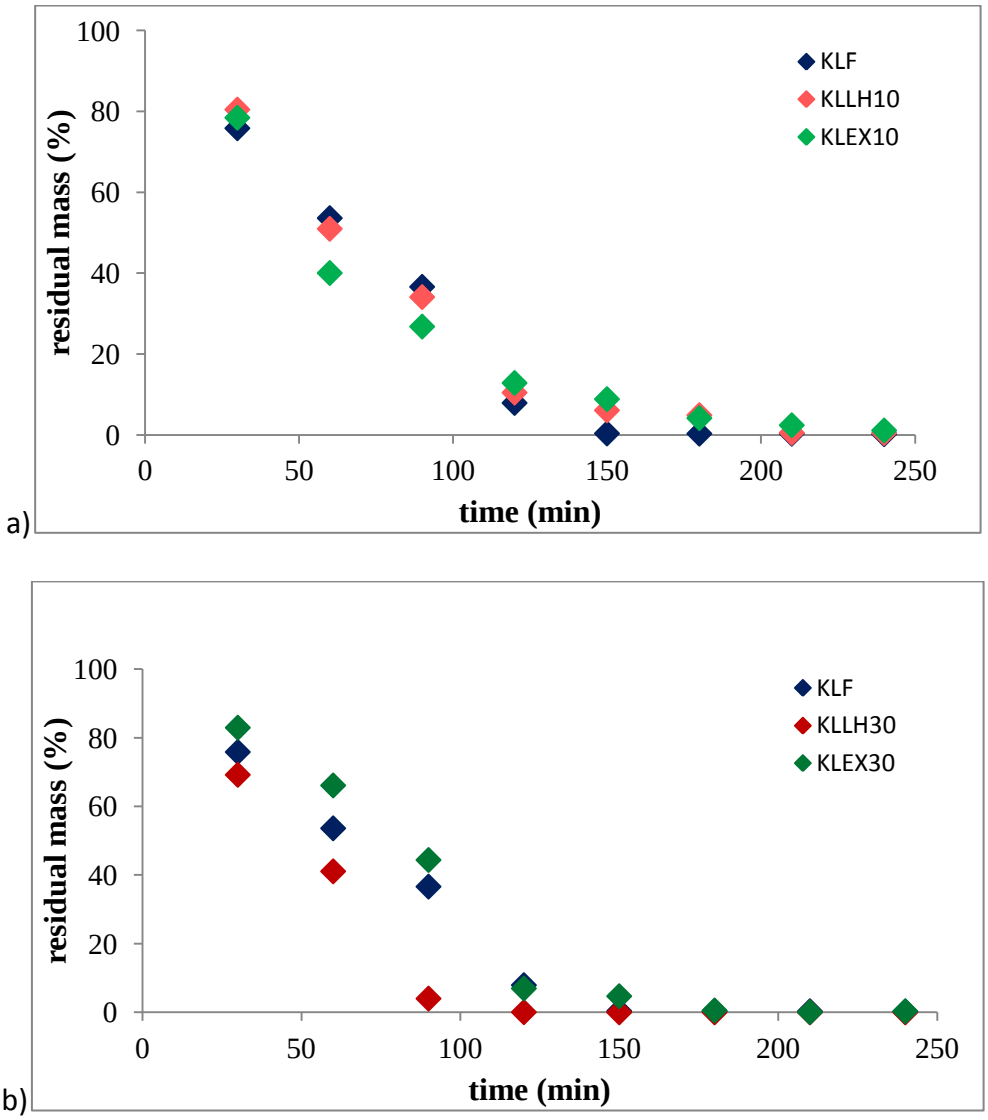

Figure R3.4: Dissolution/erosion profiles of molded disks containing 10 % (a) or 30 % by weight (b) of disintegrants.

The 10% of an additional swelling agent seemed not to influence the erosion rate of molded disks except for Explotab® CLV that showed a slight tendency to slow it down during the first h of the test (Figure R3.4). The same trend was noticed with disks containing the highest amount (30% by weight) of L-HPC. This behavior was attributed to the different ability of disintegrants to compete against Klucel® LF for water. Accordingly, when increasing the amount of Explotab® CLV in the formulation, the effect of inhibiting the hydration rate of HPC was counterbalanced by the swelling of the disintegrant polymeric chains, and thus the result was that of promoting to some extent the erosion of molded disks.

Capsular containers were prepared starting from formulations containing from 5 to 30 % by weight of Explotab® CLV (KLEX5, KLEX10, KLEX20 and KLEX30) or 10 and 30 % by weight of L-HPC (Table R3.4).

Table R3.4: Weight and thickness of capsules based on Klucel® LF and its blends with Explotab® CLV or L-HPC.

formulation	weight (mg)		thickness (µm)	
	mean	CV	mean	CV
KL	211.00	0.83	609	1.35
KLEX5	233.08	0.13	621	1.61
KLEX10	233.55	0.14	626	2.70
KLEX20	239.29	0.09	633	1.89
KLEX30	248.55	0.71	629	2.33
KLLH10	243.08	0.85	631	1.45
KLLH30	245.45	0.47	636	1.72

When preparing capsules with blend formulations, IM parameters needed to be modified with respect to reference KL capsules, especially in terms of injection pressure and rate. This was probably the reason why the weight and thickness of molded shells turned out generally higher.

As far as the release performance of capsules is concerned (Table R3.5 and Figure R3.5), the results obtained were in good agreement with premises: capsules with the lower amounts of disintegrants in the shell formulation showed an increase up to 20 min (~80%) of the lag time with respect to the reference. This increase was proportional to the disintegrant content. Only in the case of devices containing 20 and 30 % of Explotab® CLV, an opposite tendency towards a reduction of the lag time was observed, that was attributed to the early erosion of shell walls promoted by the disintegrant swelling. With respect to devices containing 30 % of L-HPC in the shell formulation, although their mean

lag phase was the longest achieved from the devices tested, it was however characterized by the higher variability clearly evidenced in Figure R3.5. As this could be attributed to a not homogeneous distribution of the disintegrant in the injected molten mass, a preliminary evaluation of the effect of pre-extruding the polymeric formulation according to procedures already tested with Klucel® LF/Klucel® GF blends was carried out. Unfortunately, the release behavior of capsules prepared from co-extruded or counter-extruded formulations turned out not significantly different from the untreated ones.

All capsules, irrespective of the presence of a disintegrant in the formulation composition, demonstrated the desired pulsatile-release profile with a pulse time not different from that of the reference.

Table R3.5: Release parameters of capsules based on Klucel® LF and its blends with Explotab® CLV or L-HPC.

formulation	lag time (min)		pulse time (min)	
	mean	CV	mean	CV
KL	25.22	10.47	6.24	16.05
KLEX5	37.50	6.46	6.15	18.74
KLEX10	46.70	8.46	6.34	10.05
KLEX20	36.96	6.04	6.02	13.09
KLEX30	36.49	6.04	5.79	7.60
KLLH10	35.81	6.78	8.24	10.76
KLLH30	49.50	15.99	6.10	11.07

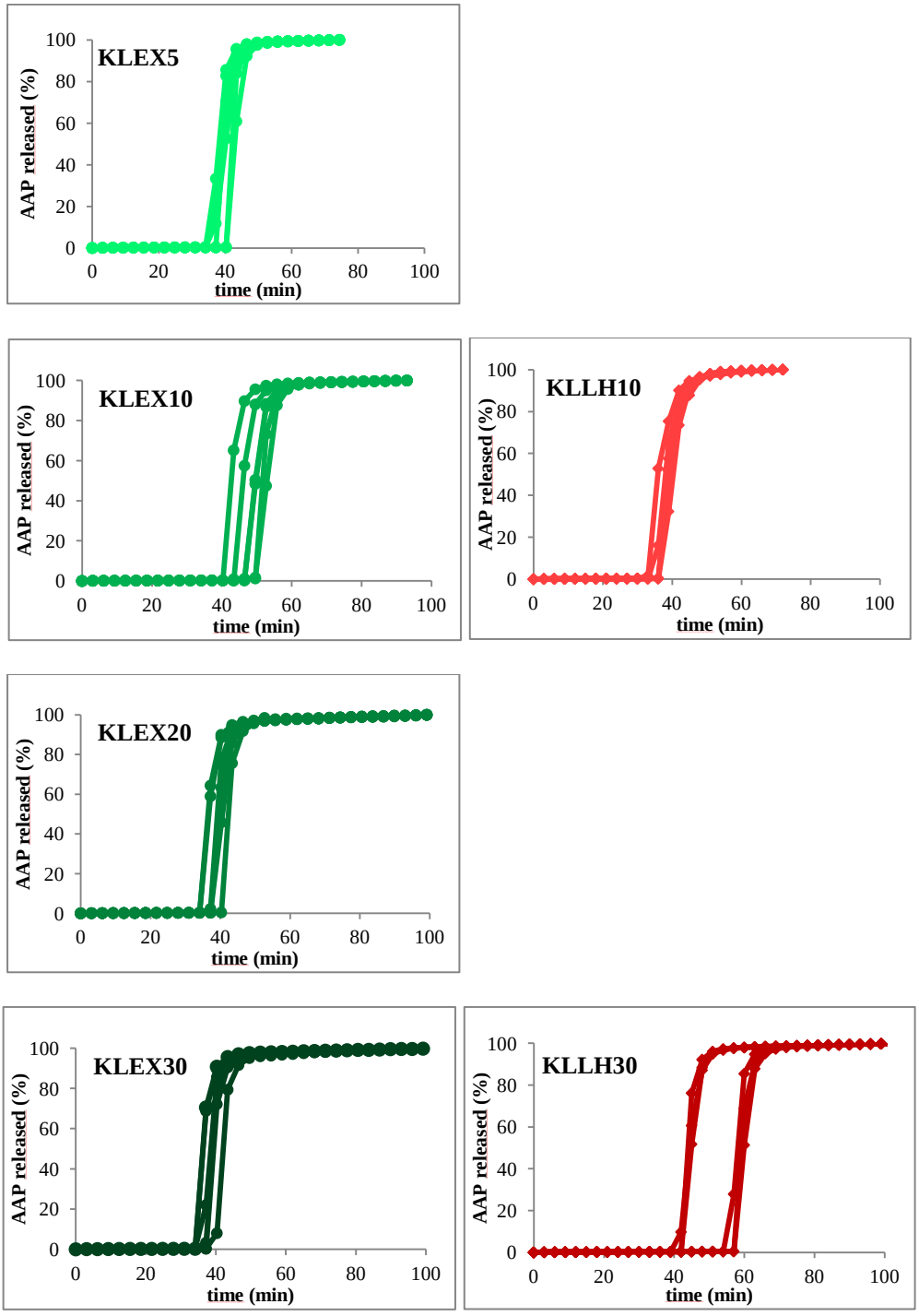

Figure R3.5: *In vitro* release profiles of capsules based on Klucel® LF and its blends with Explotab® CLV (5, 10, 20 and 30 %) or L-HPC (10 and 30%).

Afterwards, the research work went through a further investigation of the opening behavior of HPC capsules containing disintegrants in the shell walls by testing them under unstirred conditions. Devices with 10 or 30 % of Explotab® CLV, the release performance of which had suggested different hypotheses in regard to the opening mechanism, were considered and compared with reference capsules (Figure R3.6).

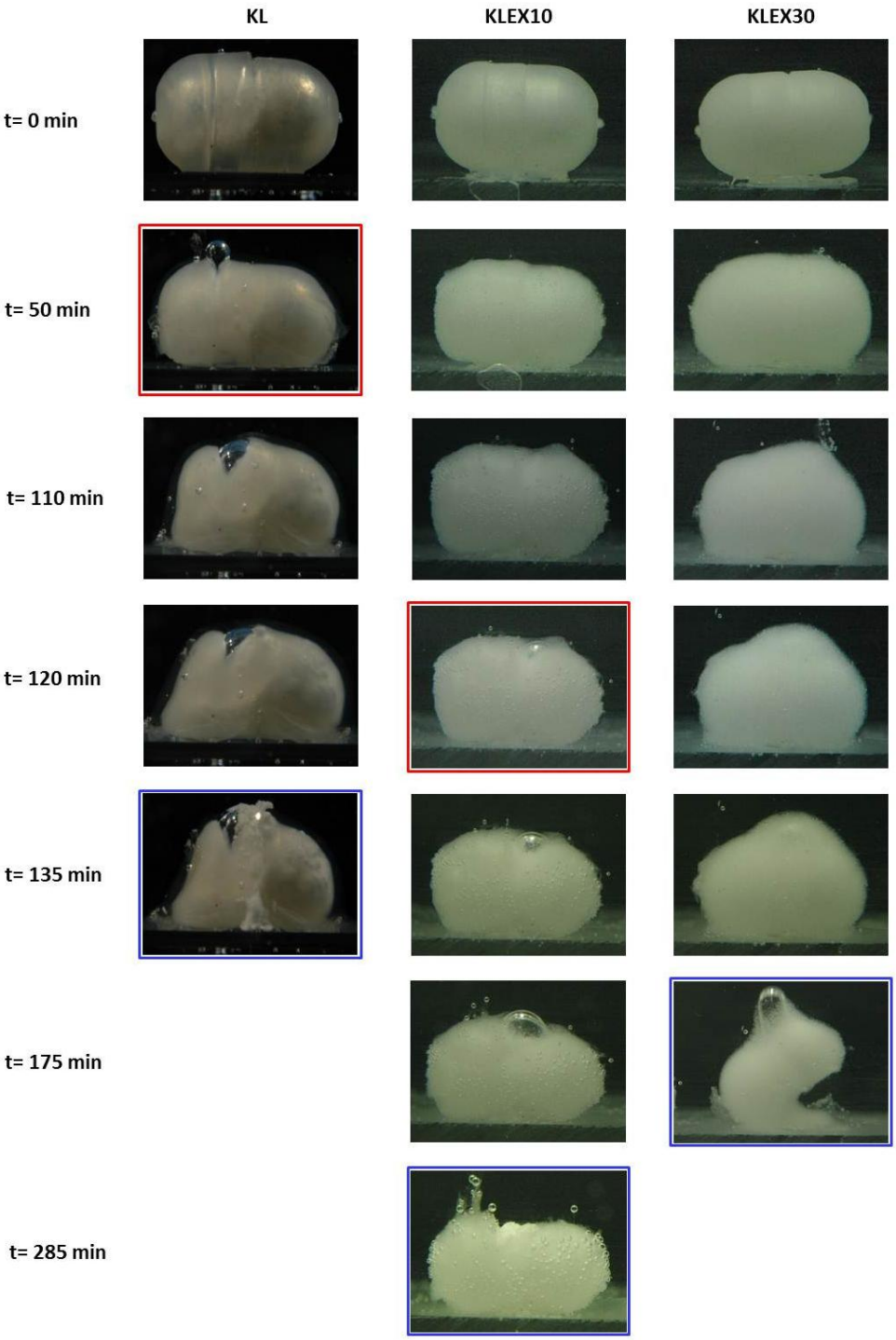

Figure R3.6: Photographs of capsules based on Klucel® LF and its blends with 5 or 10 % of Explotab® CLV immersed in water under unstirred conditions at different time points.

The overall results obtained were useful to confirm the hypothesized working mechanism of disintegrants when present in the formulation of HPC capsules. The ability of the disintegrant to delay the appearance of the first break in the shell wall was evidenced: over 1 hour in the case of the capsule containing 10 % of Explotab® CLV. Indeed, the opening position of such a tear was different with respect to the reference product. Moreover, the highest amount of Explotab® CLV was confirmed to be sufficient to bring about a disintegrating force contributing to the complete break-up of the device. In fact, the KLEX30 capsule broke up leading to the complete release of its content about 2 hours before the one with only 10 % of disintegrant (KLEX10).

With the same purpose of slowing down the hydration rate of the HPC barrier of capsules, the addition to the formulation of insoluble materials such as talc and ethyl cellulose was preliminarily evaluated; blends of Klucel® LF and 20 % by weight of the excipient (KLTA20 and KLEC20, respectively) were therefore considered. Talc was expected to remain dispersed in the molten polymeric mass and carried through the IM phases in the same way as previously used insoluble materials (disintegrants). Accordingly, opaque molded items were obtained with some particles visible on the surface. As far as the EC is concerned, it is a thermoplastic polymer but with a transition temperature around 130 °C, higher with respect to the operating temperatures needed for HPC capsules. However, when dispersed in the KL formulation, in the presence of a plasticizer, it was probably able to melt at lower temperatures giving rise to clear and

homogeneous molded items. With both the formulations the IM process was carried out with no need for changes of parameters, except for a 10 °C increase of the operating temperature.

Weight and thickness of capsules are reported in Table R3.6 while release parameters and profiles are shown in Table R3.7 and Figure R3.7, respectively.

Table R3.6: Weight and thickness of capsules based on Klucel® LF and its blends with 20 % of talc or EC.

formulation	weight (mg)		thickness (µm)	
	Mean	CV	mean	CV
KL	211.00	0.83	609	1.35
KLTA20	256.69	0.54	630	3.78
KLEC20	225.97	0.44	626	1.40

Table R3.7: Release parameters of capsules based on Klucel® LF and its blends with 20 % of talc or EC.

formulation	lag time (min)		pulse time (min)	
	Mean	CV	mean	CV
KL	25.22	10.47	6.24	16.05
KLTA20	33.05	10.40	5.13	9.70
KLEC20	46.81	3.15	6.54	16.67

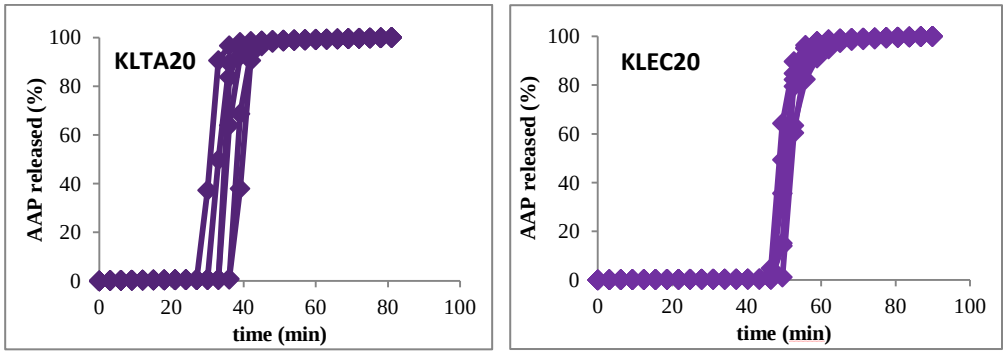

Figure R3.7: *In vitro* release profiles of capsules based on Klucel® LF and its blends with 20 % of talc (KLTA20) or EC (KLEC20).

Once again, the presence of insoluble excipients in the LF formulation resulted in a variation of the thickness of molded items that turned out higher with respect to the nominal value. This behavior will need consideration for the development of purposely devised molds.

Dealing with the release performance, the ability of inert materials to delay the lag phase prior to the break-up of the HPC shell barrier in which they had been incorporated was confirmed. In particular, the lag time of capsules containing EC in the shell turned out to be the longest and about 20 min greater than that of reference ones. The pulse time of both the devices was close to that of the reference, possibly indicating that no important changes in the mechanism of capsule opening had occurred. The effect of talc and EC in the formulation of HPC-based capsule shells needs to be more in-depth investigated to assess

whether modifications in the qualitative or quantitative composition may lead to different lag phase durations or release mechanisms.

The overall results obtained from the addition of both swellable/disintegrant and insoluble adjuvants to the formulation of the HPC-molded shell indicated that the decrease of the hydration rate of its main component, Klucel® LF, could be a promising strategy for improving the duration of the lag time of the device. However, some limitations, likely related to the poor ability of the IM press employed to apply mixing shear forces in the plasticating unit, were highlighted. The possibility of overcoming the problem by hot-compounding of polymeric formulations in a twin-screw extruder before their IM processing was evaluated: it was demonstrated a valuable approach to improve the distribution of solid particles within a molten polymeric mass and gave proof of being a pursuable strategy for promoting a more intimate contact/interaction between different types of polymeric chains. The possibility of achieving different performances with the new polymeric compounds eventually obtained still needs to be confirmed.

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Conclusions

The research project was focused on the development of a pulsatile capsular-shaped drug delivery system (DDS) named Chronocap™. It consists of a capsule shell, made of an hydrophylic swellable/erodible polymer (hydroxypropyl cellulose, HPC) and prepared by injection molding (IM), intended to be filled with different types of drug preparations and to delay their liberation after oral administration. The feasibility by IM of capsules based on Klucel® LF was already assessed by means of a prototype mold allowing shells of different thickness (300, 600 and 900 μm) to be obtained. Such devices were demonstrated able to convey drug preparations giving rise to the expected pulsatile release performance, confirmed by *in vivo* data. The lag phase was attributed to the slow interaction of HPC with aqueous fluids, leading to the formation of a gel-barrier the break-up of which takes place as a consequence of dissolution/erosion phenomena; accordingly, such lag phase turned out to increase as a function of the shell wall thickness. The PhD project was undertaken aiming at improving the robustness and versatility of the Chronocap™ device as well as enhancing the industrial scalability of its manufacturing process. For this purpose, two different tasks were pointed out: the improvement of the manufacturing process and of the technological characteristics of capsules on the one side, and the upgrade of applications of the device on the other. In this respect, the modulation of the lag phase of the capsular device and its possible exploitation in the design of a colon DDS were approached.

A new mold was designed based on the thermal, rheological and mechanical characteristics of the Klucel® LF formulation. It enabled the manufacturing of capsules improved in terms of reproducibility of the wall thickness and relevant consistency with the nominal value, efficiency of the body-cap joint mechanism and reliability of *in vitro* performance. Moreover, the production rate and extent of automation of the process were increased [Results – Chapter 1].

The possibility of exploiting these capsules for the development of colon delivery systems based on a time-dependent approach was initially faced by evaluating the Chronocap™ device as the substrate for film-coating with Eudragit® L. Screening disks were preliminary prepared by IM and employed for studying the interaction of the polymeric coating with the molded surface and the gastroresistant performance of the obtained product. Chronocap™ capsules could be successfully coated with increasing amounts of the enteric soluble polymer. Coated systems showed good technological properties and, at least those with the thickest Eudragit® layer, maintained their integrity for 2 h in acidic media and released their contents in pH 6.8 buffer after a lag time comparable with that of uncoated devices [Results - Chapter 2].

Finally, the lengthening of the lag phase of molded capsules through formulation changes of the composition of the walls was successfully approached. The hydration rate of Klucel® LF was reduced by adding swellable and/or insoluble materials (*e.g.* Klucel® GF, L-HPC, Explotab® CLV EC and talc) to the formulation of the shell walls. As a consequence, the formation of the first break in capsule

walls by dissolution/erosion of the gel barrier was delayed. Pulsatile release profiles with longer lag times were obtained from the new systems based on polymeric blends. On the contrary, the time required for the complete opening of capsules remained < 8 min as for the reference Klucel® LF system. Results obtained by pre-extruding some of the polymeric blends prior to IM processing showed promising perspectives towards the achievement of longer lag phases [Results - Chapter 3].

The overall results obtained may lead to the manufacturing of pulsatile and colon specific drug delivery prototypes for *in vivo* testing.