Lego-inspired capsular devices for the development of personalized dietary supplements: proof of concept with multimodal release of caffeine

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Abstract

Dietary supplement companies have recently started to focus on personalization of products and improvement of the relevant performance. In this respect, a versatile, easy-to-handle capsular delivery platform with customizable content and release kinetics was here proposed and evaluated after filling with caffeine as a model dietary ingredient. In particular, capsular devices comprising 1 to 3 independent inner compartments were attained by Lego-inspired assembly of matching modular units with different wall composition, manufactured by injection molding and fused deposition modeling 3D printing. Accordingly, one-, two- and three-pulse release profiles of the dietary ingredient were obtained from differently assembled devices following breakup of the compartments occurring promptly (immediate release), on pH change (delayed release) or after tunable lag times (pulsatile release). The latter release mode would enable the onset of the stimulating effect of caffeine at different times of the day after a single administration when convenient. The performance of each individual compartment only depended on the composition (*i.e.* promptly soluble, swellable/soluble or enteric soluble polymers) and thickness of its own wall, while it was not affected by the composition and number of joined modular units. Moreover, the delivery platform was extended to include an external gastroresistant shell enclosing previously assembled devices.

1. Introduction

Over the last years, the worldwide demand and consumption of dietary supplements, mainly formulated as tablets, capsules, powders and liquids, has continued to increase, so that the relevant market is expected to exceed $ 200B in 2022.[1] This significant growth may have been promoted by the changes in lifestyle and nutritional habits observed during the last decades as well as by the increasing awareness about preventative healthcare.[2-4] Indeed, most of the users of dietary supplements are healthy subjects who want to take an active role in personal wellness and consider these products as a tool for prevention of possible future diseases. For instance, calcium and omega-3 are taken, respectively, to prevent osteoporosis and reduce blood cholesterol levels as a risk factor for cardiovascular disease.[5] Users are also becoming more and more demanding in terms of perceived quality and performance of dietary supplements.[6] In such a competitive environment, dietary supplement companies are focused on the rapid development of innovative products also containing new food ingredients. In this respect, they have started considering design, formulation and manufacturing approaches borrowed from the pharmaceutical field, drawing inspiration from drug delivery systems (DDSs).[7,8] For example, oral bioavailability of folate and vitamin B12 as well as compliance with fish oil/fatty acids intake could benefit from release of such compounds in the small intestine.[9,10] Melatonin could take advantage of a pulsatile-release strategy, enabling its presence in the bloodstream at specific times when the relevant ability to manage insomnia is mostly needed.[11] Moreover, the possibility of conveying within the same dosage form dietary ingredients that interact with each other (*e.g.* zinc, calcium and vitamin C with iron or magnesium) and releasing them separately would decrease the risk of undesired mutual influence.[12] At the same time, demonstration of close relationships among the human genome, nutrition and health (*i.e.* nutrigenetics and nutrigenomics) has fed the interest towards personalized nutrition, aimed at meeting the unique needs of people with different age, health conditions, lifestyle and eating habits.[13,14]. Examples could be the adjustment of the recommended doses of iron, vitamin D, and omega-3 fatty acids. Also in this field,

the advent of personalization would be eased by the development of versatile dosage forms and by the availability of flexible production models.

Caffeine, the dietary ingredient most widely used as a legal stimulant, would benefit from modulation in both the amount to be administered and release kinetics.[15,16] Being a non-selective antagonist for adenosine receptors located in the brain while favoring release of endogenous adrenaline and noradrenaline, caffeine is employed by consumers to concentrate, memorize, and improve reaction time as well as learning abilities. The amount needed to produce these effects varies from person to person, depending on weight, age, degree of developed tolerance and genetic polymorphism. Currently, in addition to conventional dosage forms that release caffeine immediately after administration, prolonged-release formulations are available. However, the possibility of determining *a priori* the onset of caffeine release after oral intake, *i.e.* by pulsatile release formulations, in the hours of the day when its stimulating action is necessary, is not presently made available by any marketed product.

Based on these considerations, the aim of the present work was the design, fabrication and evaluation of a versatile innovative platform for oral delivery of dietary ingredients, using caffeine as a model molecule. The seminal work for this study was presented in previous publications, focusing on DDSs aimed at pulsatile release of drugs based on swellable/soluble hydrophilic polymers, primarily cellulose derivatives.[17-19] Such systems ranged from reservoir coated dosage forms up to single- and two-compartment capsular devices obtained by injection molding (IM) and fused deposition modeling (FDM) 3D printing.[20-22] The delivery platform here proposed was conceived to enable customizable dietary ingredient content and controlled release performance, while resembling the well-established and easy-to-use shape of hard-gelatin capsules. It entailed from 1 to 3 inner compartments, having diverse capacity and polymeric composition, obtained following assembly of modular units, fabricated by IM and FDM, in a way that is similar to Lego building systems. While standard units could be manufactured on a larger scale *via* IM, customized ones could also be extemporaneously printed. In this respect, a new joint unit was designed enabling the definition of a

third inner compartment. In addition, external enteric-soluble capsule shells were devised to enclose previously assembled capsular devices and evaluated for ability to prevent the relevant opening in the stomach. Starting polymeric formulations with proven suitability for IM and FDM processing were selected based on their different interaction behavior with aqueous fluids, *i.e.* promptly (Kollicoat® IR, KIR; low molecular weight polyvinyl alcohol, PVA03) or more slowly (hydroxypropyl cellulose, HPC; hydroxypropyl methyl cellulose HPMC; high molecular weight polyvinyl alcohol, PVA05) soluble in water and gastroresistant (hydroxypropyl methyl cellulose acetate succinate, HPMCAS).[23] In particular, the swellable/soluble hydrophilic polymers HPC, HPMC and PVA05 employed are known to undergo glass-rubber transition with the formation of a gel structure. The progressive erosion/dissolution of this barrier would lead to deferred breakup of the shell followed by prompt and complete release of its contents after a lag phase. Based on the composition of the modular units, the delivery platform could combine multiple release kinetics for the doses of caffeine loaded into each compartment, such as immediate (KIR and PVA03-based compartments), pulsatile (HPC-, HPMC- and PVA05-based compartments) and delayed (HPMCAS-based compartments) release.

2. Materials and Methods

2.1 Materials

Main polymeric components: hydroxypropyl cellulose (HPC, Klucel® LF, Aqualon, US-NJ); hydroxypropyl methyl cellulose (HPMC; Affinisol™ 15cP, Dow, US-CA); hydroxypropyl methyl cellulose acetate succinate (HPMCAS; AQUOT-LG, Shin-Etsu, J); polyvinyl alcohol of different molecular weight (PVA 03 and PVA 05; Gohsenol™ EG 03PW and 05P, Nippon Gohsei, J); polyvinyl alcohol-polyethylene glycol graft copolymer (KIR; Kollicoat® IR, BASF, D). Plasticizers: glycerol (GLY; Pharmagel, I); polyethylene glycol (PEG; PEG 400, 1500 and 8000, Clariant Masterbatches, I). Dietary ingredient tracer: caffeine (CFF; A.C.E.F., I).

2.2 Methods

2.2.1 Design concept of the Lego-inspired delivery platform

The delivery platform consisted in capsular devices having separate inner compartments with different volume and composition of the walls, obtained following assembly of modular units. The compartments are intended to breakup in succession and release their contents, leading to multiple-pulse release profiles. Additional external capsule shells able to enclose single- and two-compartment capsular devices already assembled were also conceived in the form of matching modular units. The opening behavior of compartments and external shells would depend on the composition and thickness of the walls.

Modular units were devised in the form of hollow parts (*i.e.* type A, A_1, A_2, B, B_1 and B_2) and joints (*i.e.* type 1 and 2), schematically represented with dimensional details in Figure 1.

Hollow parts had a closed round end and an open end. The open end was characterized by halved wall thickness to enable overlapping with matching modular units. By progressively increasing the length and dimeter of type A and B hollow parts, while maintaining the same nominal thickness, type A_1 and B_1 as well as type A_2 and B_2 hollow parts were designed. These were intended to enclose capsular devices already filled and assembled.

Type 1 joints were composed of two hollow cylinders with the same diameter, height and thickness, grounded in a common 600 µm thick base. Type 2 joints also had the form of two hollow cylinders resting on opposite sides of the 600 µm thick base that closed both. However, the two cylinders forming type 2 joints had different height and diameter. The open ends of type 1 and type 2 joints had halved thickness enabling overlapping with matching parts.

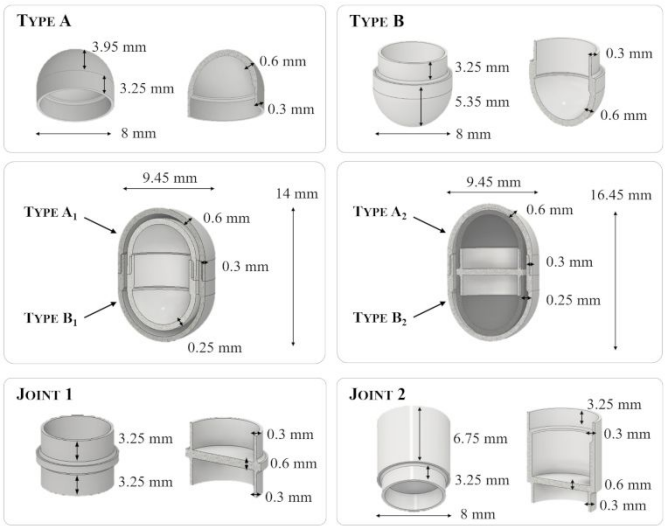

Figure 1: outline of the modular units with dimensional details.

2.2.2 Manufacturing and characterization of modular units

Modular units were manufactured by IM (*i.e.* type A and B hollow parts) and FDM 3D printing (*i.e.* type 1 and 2 joints; type A_1, A_2, B_1 and B_2 hollow parts).

2.2.2.1 Preparation of polymeric formulations

All materials, except for PEGs, GLY and CFF, were kept in an oven at 40 °C for 24 h prior to use. Plasticized polymeric formulations were prepared by mixing or granulating the main polymeric components in a mortar with the selected solid or liquid plasticizer. The amount of plasticizer was expressed as % by weight on the dry polymer (%wt).

2.2.2.2 Injection molding

IM was carried out by a bench-top micro-molding press (BabyPlast 6/10P, Cronoplast S.L., Rambaldi S.r.l., I) equipped with a mold composed of two interchangeable inserts for the manufacturing of type A and B hollow parts having nominal thickness of 600 µm.[24,25] This is a single cavity mold entailing *i)* a hot runner system, *ii)* a length/diameter ratio of 1.5, *iii)* a central injection position, *iv)* halved thickness in the open contact areas between parts and *v)* a duct for injection of compressed air.

Polymeric formulations were loaded into the press through a hopper. An amount of material (charge, C) defined by the final position of the injecting plunger (ø = 10 mm) was forced into a plasticating chamber containing heated spheres and accumulated in the injection chamber. Both the injection and holding phases were performed in pressure control (injection pressure P_1 for 2.5 s and packing pressure P_2 for 1.5 s). The pressure values set were reached by moving the injection piston at selected rates (r_1 and r_2 for injection and holding, respectively) expressed as a percentage of the maximum rate achievable. The diameter of the injection nozzle was 1 mm. Based on the experience previously gained in hot-processing of different polymers of pharmaceutical grade, 4 different temperatures (T_1-T_4) were set throughout the press, where the last value was the hot runner temperature.

Type A and B hollow parts were fabricated with all the polymeric formulations. The relevant IM operating conditions are reported in Table 1.

Table 1: IM operating conditions

Polymeric formulation	**T_1 (°C)**	**T_2 (°C)**	**T_3 (°C)**	**T_4 (°C)**	**C (mm)**	**P_1 (bar)**	**r_1 (%)**	**P_2 (bar)**	**r_2 (%)**
HPC	100	130	145	165	5	50	40	45	30
HPC + 5% PEG 1500	100	130	140	165	5	40	30	20	20
HPC + 10% PEG 1500	100	130	140	160	4	30	30	10	10
HPMC + 15% PEG 400	120	150	165	175	6	40	45	30	35
KIR + 12% GLY	120	145	155	165	4.5	30	30	20	15
PVA03 + 15% GLY	130	150	155	160	4	40	45	30	25
PVA05 + 15% GLY	140	160	165	170	4	45	50	40	25
HPMCAS + 35% PEG 8000	130	135	160	170	6	30	40	20	30

2.2.2.3 3D printing

Extrusion of filaments - Filaments were prepared by hot melt extrusion (HME), starting from HPC-, HPMC-, PVA05-, HPMCAS-based formulations, employing a twin-screw extruder (Haake™ MiniLab II, Thermo Scientific, US-WI) equipped with counter-rotating screws and a custom-made aluminum rod-shaped die (ø = 1.80 mm).[26-28] After production, filament diameter was verified every 5 cm in length and the portions having diameter outside the acceptable range (1.75 ± 0.05 mm) were discarded.

FDM - Starting from the filaments produced, FDM was performed with a Kloner3D 240® Twin (Kloner3D, I) equipped with 0.4 mm tip and using specifically developed computer-aided design (CAD) files for the fabrication of joints (*i.e.* type 1 and type 2) and type A_1, A_2, B_1, B_2 hollow parts. The design step was performed using Autodesk® Autocad® 2016 software version 14.0 (Autodesk, Inc., US-CA). The files were then saved in .stl format and imported to the 3D printer software (Simplify 3D, I). 3D printing in some cases required the use of supports to avoid the collapse of the item during the additive manufacturing process. HPMCAS-based filaments were used for printing type A_1, A_2, B_1, B_2 hollow parts, while type 1 and 2 joints were fabricated starting from HPC-, HPMC- as well as PVA05-based ones. The FDM process parameters employed for each polymeric formulation are reported in Table 2.

Table 2: FDM operating conditions

Polymeric formulation	T (°C)		flow rate (%)	layer height (mm)	printing speed (mm/s)	use of supports
	Nozzle	build plate				
HPC	175	50	100	0.125	47	yes
HPMC + 15% PEG 400	200	50	100	0.100	30	yes
PVA05 + 15% GLY	195	60	105	0.125	47	no
HPMCAS + 35% PEG 8000	190	90	110	0.200	7	no

2.2.2.4 Characterization of modular units

Modular units were checked for weight (analytical balance BP211, Sartorius, D; n = 10) and thickness (MiniTest FH7200 equipped with FH4 probe, ø sphere = 1.5 mm, ElektroPhysik, D; n = 10). Digital photographs (Nikon D70, Nikon, J) were also taken. Examples are reported in Figure 2.

2.2.3 Assembly of modular units

Depending on the number of inner compartments the final system should be provided with, capsular devices were manually filled and assembled as schematically shown in Figure 2:

- single-compartment capsular device (Figure 2a): a type B hollow part was filled and closed with a type A hollow part. Type A and B hollow parts had the same composition;
- two-compartment capsular device (Figure 2b): a 1st type A hollow part was filled and closed with a type 1 joint. The latter assembly was used to close a 2nd type A hollow part already filled. The 1st type A hollow part and the type 1 joint had the same composition, different from the 2nd type A hollow part;
- three-compartment capsular device (Figure 2c): a 1st type A hollow part was filled and closed with a type 1 joint. A 2nd type A hollow part was filled and closed with a type 2 joint. Subsequently, the longer and wider cylinder of the type 2 joint was also filled and closed with the assembly composed of the type 1 joint and 1st type A hollow part. The type 1 joint and 1st type A hollow part had the same composition. The 2nd type A hollow part and type 2 joint differed in composition from each other and from the type 1 joint and 1st type A hollow part.

Modular units were manually filled on the analytical balance with 30 mg of CFF (cv ≤ 2.5) using a micro spatula. To hold the unit to be filled with the open end facing up, avoid the relevant tilting and allow its correct placement on the balance, a purposely developed support was used.

Single- and two-compartment capsular devices already assembled were inserted into type B_1 and B_2 hollow parts, respectively. The latter were then closed with a type A_1 or a type A_2 hollow part of the same composition.

All the resulting capsular devices were weighted and visually checked for integrity, especially in the matching area. Indeed, the seal closure of the systems was ensured by appropriate overlapping of the halved-thickness area of the different modular units employed for assembly. The effectiveness of such a locking mechanism, already demonstrated with HPC-based capsular devices in stressful conditions (*i.e.* during pan coating), relied on the mutual pressure exerted by the contact areas of matching units leading to thigh adherence without any junction gap.[29]

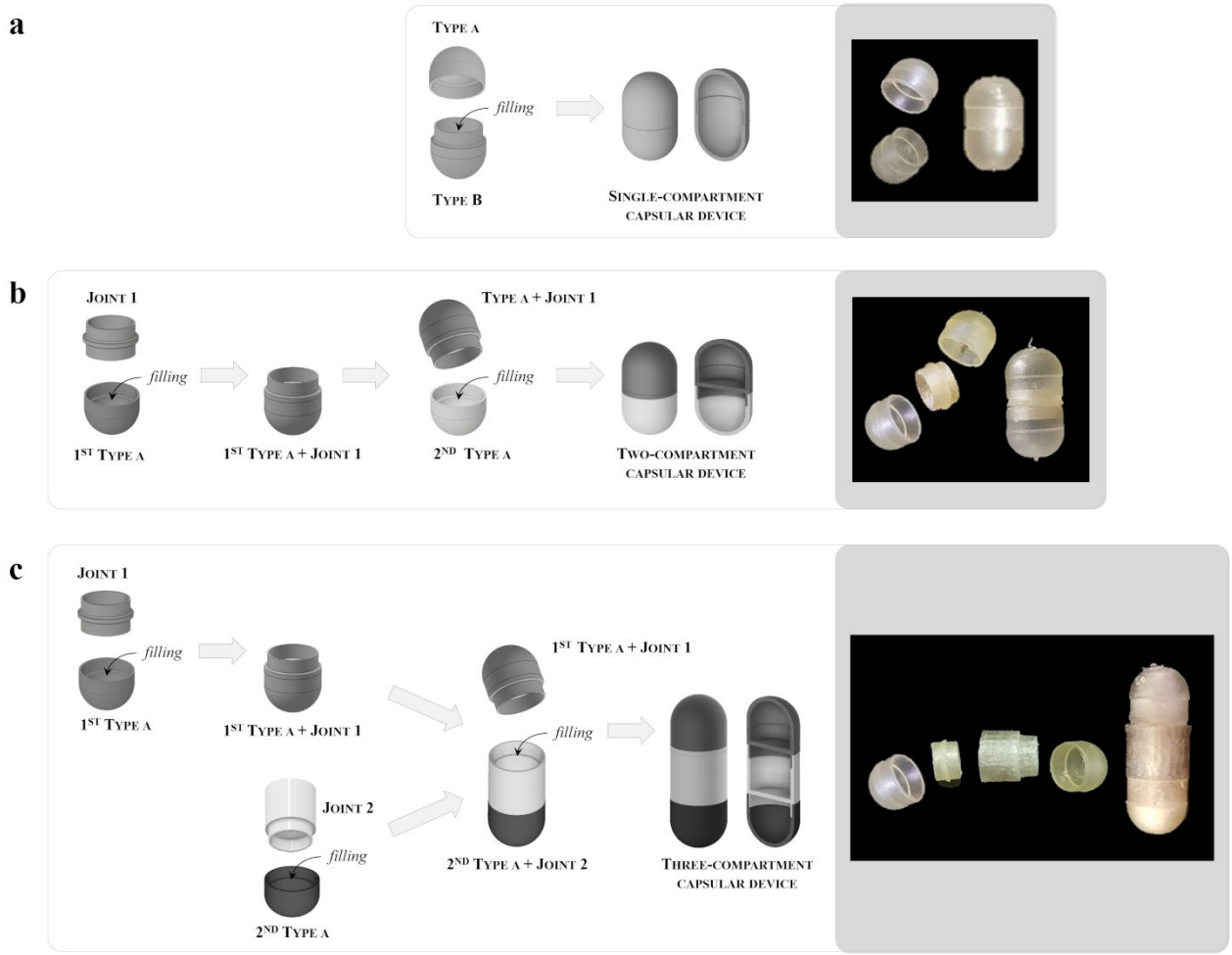

Figure 2: outline and photographs of (a) single-, (b) two- and (c) three-compartment capsular devices and details of the relevant assembly.

2.2.4 Evaluation of the release performance of capsular devices

The release performance of capsular devices was studied by an adapted three-position USP38 disintegration *apparatus* (Sotax, CH) to avoid sticking phenomena previously observed when testing

units based on swellable/soluble hydrophilic polymers by dissolution *apparatus* (Figure 3)[20,23]. Each capsule, inserted into a sinker, was positioned in one of the 6 available tubes of a basket-rack assembly. During the test, all the assemblies moved at 31 cycles/min in separate vessels containing 800 mL of distilled water (pH = 6.8) at 37 ± 0.5 °C. When HPMCAS-based modular units were tested, release was evaluated according to "Dissolution Test for Delayed-Release Dosage Forms" (Method B, USP38) using the same disintegration equipment above described.

Fluid samples were withdrawn at fixed time points and assayed spectrophotometrically (λ = 248 nm). Time to 10% ($t_{10\%}$), 80% ($t_{80\%}$) and 90% release ($t_{90\%}$) were calculated by linear interpolation of the release data immediately before and after the time point of interest. In the case of HPMCAS-based-modular units, $t_{10\%}$ and $t_{90\%}$ referred to the phosphate buffer stage only, thus subtracting 120 min of testing in the acidic medium. Release parameters were reported with relevant standard deviation (sd).

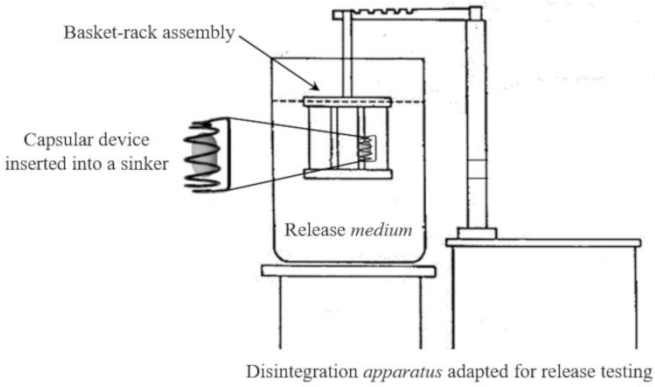

Figure 3: outline of the *apparatus* set up for release testing.

3. Results and discussion

3.1 Results

Type A and B hollow parts were manufactured by IM starting from polymeric formulations based on HPC, HPMC, KIR, PVA03, PVA05 and HPMCAS. By setting suitable operating parameters, molded

units with satisfactory and reproducible physico-technological characteristics were obtained (Table 3).

Table 3: weight and thickness of molded hollow parts.

Polymeric formulation	Weight mg (cv)		Thickness µm (cv)			
	Type A	Type B	Type A		Type B	
			overlapping area*	round area**	overlapping area*	round area**
HPC	109.93 (0.30)	120.96 (1.28)	333 (6)	658 (7)	335 (5)	620 (4)
HPC + 5% PEG 1500	112.23 (1.89)	121.03 (0.43)	322 (6)	647 (5)	317 (4)	617 (5)
HPC + 10% PEG 1500	115.06 (1.07)	122.09 (0.37)	331 (3)	610 (3)	319 (2)	615 (2)
HPMC + 15% PEG 400	111.51 (1.95)	123.19 (0.29)	355 (4)	740 (6)	351 (4)	657 (2)
KIR + 12% GLY	119.05 (1.98)	122.41 (0.23)	350 (4)	645 (3)	353 (5)	616 (2)
PVA 03 + 15% GLY	126.74 (0.72)	138.54 (0.57)	339 (1)	743 (3)	353 (3)	632 (4)
PVA 05 + 15% GLY	126.55 (0.58)	138.70 (0.28)	355 (6)	755 (4)	348 (3)	615 (4)
HPMCAS + 35% PEG 8000	115.30 (0.33)	128.64 (0.53)	323 (3)	778 (1)	334 (2)	632 (2)

*nominal 300 µm; **nominal 600 µm

Type 1 and 2 joints were fabricated by FDM 3D printing starting from in-house made filaments based on HPC, PVA05 and HPMC formulations. Moreover, type A_1, A_2, B_1 and B_2 hollow parts were also fabricated by FDM starting from in-house made filaments based on the HPMCAS formulation. FDM was performed by a trial-and-error approach, which consisted in introducing successive changes into the virtual models depending on the evaluation of the characteristics of the printed prototypes (*e.g.* weight and thickness, matching ability of the modular units and closing efficiency). The weight of type 1 and 2 joins fabricated starting from different polymeric formulations was in the 102 -125 mg (cv < 8) and in the 220 - 245 mg (cv < 7) range, respectively. On the other hand, the thickness of walls nominally set at 300 µm was for both joints in the 410-500 µm range (cv < 10) and, for walls

nominally set at 600 µm, was always in the 630-690 µm range (cv < 10). Data relevant to the printed enteric-soluble hollow parts are reported in Table 4.

Table 4: weight and thickness of printed hollow parts based on the HPMCAS formulation.

		Weight mg (cv)	**Thickness µm (cv)**	
			overlapping area*	round area**
	Type A_1	170.89 (8.98)	441 (8)	623 (7)
	Type B_1	215.51 (8.12)	436 (9)	668 (8)
	Type A_2	191.22 (7.86)	430 (9)	633 (8)
	Type B_2	234.11 (8.66)	428 (9)	674 (9)

*nominal 300 µm; **nominal 600 µm

By assembling *i)* molded type A and type B hollow parts, *ii)* molded type A hollow parts and printed type 1 joints or *iii)* molded type A hollow parts and printed type 1 and 2 joints, single-, two- and three-compartment capsular devices were obtained. The performance of prototypes in which the different compartments were filled with CFF as a model dietary ingredient was investigated and the release parameters relevant to capsular devices resulting from different combinations of modular units are reported in Table 5. Particularly, $t_{80\%}$ was calculated from the release profiles of promptly-soluble compartments (*i.e.* composed of KIR- and PVA03-based modular units), while $t_{10\%}$ and $t_{90\%-10\%}$ from the curves of swellable/soluble and gastroresistant ones (*i.e.* composed of HPC-, HPMC- or PVA05- and HPMCAS-based modular units, respectively). While $t_{10\%}$ was used to define the lag time , $t_{90\%-10\%}$ indicated the pulse time, *i.e.* the time required to complete release after breakup. By way of example, in Figure 4 release profiles of selected single-, two- and three-compartment capsular devices are reported.

Table 5: release parameters (sd in brackets) of (a) single-, (b) two-, and (c) three-compartment capsular devices.

A

Polymeric formulation		Compartment 1		
Type A	**Type B**	$t_{80\%}$	$t_{10\%}$	$t_{90\%}$-$t_{10\%}$
KIR + 12% GLY		14.74 (0.51)		
PVA03 + 15% GLY		23.51 (1.01)		
HPC			59.06 (3.03)	9.48 (2.62)
HPC + 5% PEG 1500			50.52 (0.27)	8.70 (0.95)
HPC + 10% PEG 1500			40.68 (0.22)	7.28 (3.52)
HPMC + 15% PEG 400			51.03 (4.72)	8.14 (0.86)
PVA05 + 15% GLY			91.87 (0.78)	16.88 (9.50)
HPMCAS + 35% PEG 8000			64.20* (8.90)	6.33* (2.82)

*calculated in phosphate buffer pH 6.8.

B

Polymeric formulation			Compartment 1 (Type A + Joint 1)			Compartment 2 (Joint 1 + Type A)		
Type A	**Joint 1**	**Type A**	**$t_{80\%}$**	**$t_{10\%}$**	**$t_{90\%}$-$t_{10\%}$**	**$t_{80\%}$**	**$t_{10\%}$**	**$t_{90\%}$-$t_{10\%}$**
KIR + 12% GLY	HPC		16.10 (0.61)				50.32 (8.50)	8.07 (3.36)
	HPC + 5% PEG 1500		13.23 (1.91)				45.62 (5.07)	6.57 (1.70)
	HPC + 10% PEG 1500		12.11 (2.03)				40.42 (2.94)	7.77 (2.41)
	HPMC + 15% PEG 400		9.96 (0.75)				47.70 (3.03)	12.03 (2.08)
	PVA05 + 15% GLY		12.58 (2.00)				95.23 (1.07)	15.12 (6.55)
PVA03 + 15% GLY	HPC + 10% PEG 1500		21.11 (1.23)				41.65 (3.01)	7.49 (1.98)
HPC + 10% PEG 1500	PVA05 + 15% GLY			41.33 (4.12)	8.42 (1.58)		91.23 (1.56)	16.63 (7.05)

C

Polymeric formulation				Compartment 1 (Type A + Joint 2)			Compartment 2 (Joint 2 + Joint 1)			Compartment 3 (Joint 1+ Type A)		
Type A	**Joint 2**	**Joint 1**	**Type A**	**$t_{80\%}$**	**$t_{10\%}$**	**$t_{90\%}$-$t_{10\%}$**	**$t_{80\%}$**	**$t_{10\%}$**	**$t_{90\%}$-$t_{10\%}$**	**$t_{80\%}$**	**$t_{10\%}$**	**$t_{90\%}$-$t_{10\%}$**
KIR + 12% GLY	HPC + 10% PEG 1500	PVA05 + 15% GLY		15.84 (0.83)				36.02 (3.6)	15.32 (1.67)		85.68 (4.42)	14.55 (2.13)

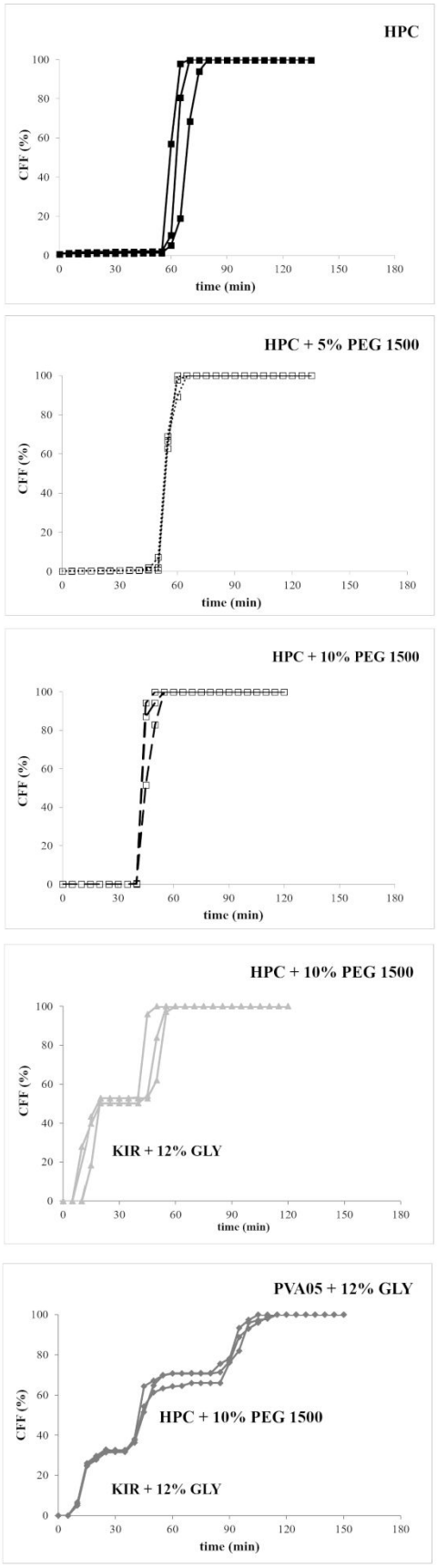

Figure 4: release profiles of single-, two- and three-compartment capsular devices.

Printed HPMCAS-based type A_1, A_2, B_1, B_2 hollow parts were used to enclose prototypes of single- and two-compartment capsular devices previously assembled. The release profiles of HPC-based single-compartment capsular devices and of KIR/HPC-based two-compartment capsules inserted into HPMCAS-based printed shells are reported in Figure 5 by way of example. Both systems showed the ability to withstand testing in the acidic medium and subsequently gave rise to CFF release consistent with the type of capsular device contained inside the gastroresistant shell. Indeed, by taking account of the time necessary for the dissolution of the gastroresistant shell (*i.e.* approximately 60 min), the $t_{10\%}$ value calculated for the HPC-based single-compartment capsular devices (*i.e.* 107.93 min, sd = 6.1) and $t_{80\%}$ as well as $t_{10\%}$ relevant to the KIR- (*i.e.* 82.14 min, sd = 4.45) and the HPC-based compartments (*i.e.* 115 min, sd = 0.1) of the two-compartment capsular devices turned out consistent with those obtained from capsular devices of the same composition and number of compartments when tested as such.

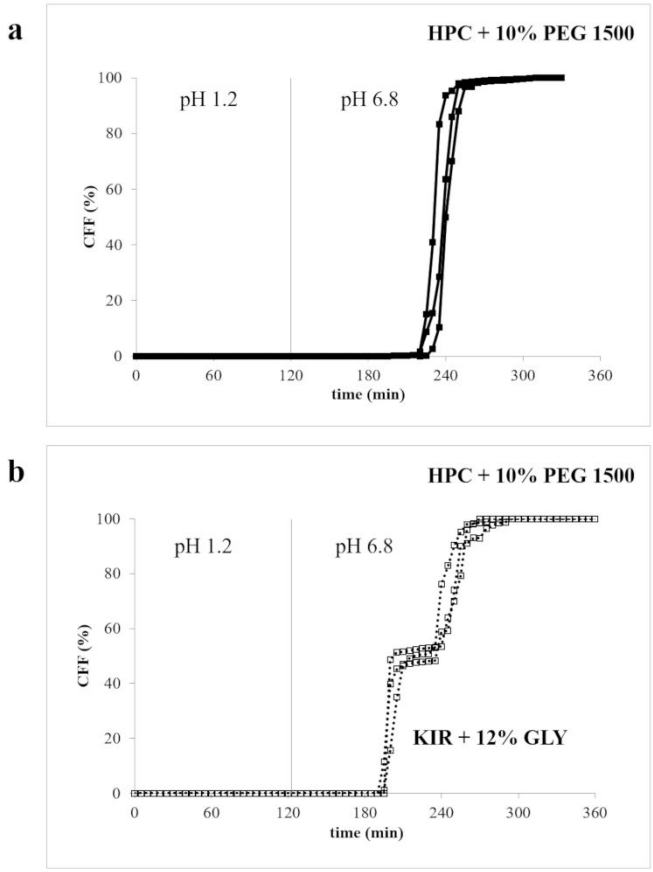

Figure 5: release profiles of (a) single- and (b) two-compartment capsular devices inserted in HMCAS-based shells

3.2 Discussion

The delivery platform was initially conceived in the form of capsular devices with separated inner compartments, ranging from 1 to 3 in number. The external dimensions (8 mm in diameter and length in a 12.5 to 21.8 mm range) were selected to be consistent with convenient oral administration, in agreement with the capsule sizes commonly used for dietary supplements (*i.e.* size ≤ commercially-available 00el hard-gelatin capsules). Like in Lego building systems, the compartments were obtained by assembling matching modular units having different geometry and polymeric composition. This way, multiple compartments having different volume and composition could be combined in a single system, which would overall increase the versatility of the delivery platform in terms of *i)* type and amount of dietary ingredients that could be contained in each capsule and *ii)* release profiles that could be achieved. Moreover, the availability of compartments with different composition but having

similar performance would make the platform compatible with a wide range of active substances. An optional feature that may increase the versatility of the delivery platform would be the capability of preventing the release of active ingredients into the stomach, while enabling it soon after gastric emptying or following a further lag phase of programmable duration. Several dietary ingredients would benefit from this release mode (*e.g.* fish oil, probiotics, lactase, vitamin B12 and B6, sodium, potassium, magnesium, calcium and iron) because of being poorly tolerated in the stomach, potential irritants for the gastric mucosa, unstable in acidic media, preferentially absorbed in the upper small bowel or intended for local activity in the intestinal tract.[30] In this respect, various formulation strategies have been proposed, involving the exploitation of coatings based on GRAS materials that are in principle compliant with the quality and safety requirements of dietary supplements. Notable examples include modified starch, shellac resins, water-insoluble polymers mixed with pore formers having pH-dependent solubility and swellable/soluble hydrophilic polymers. The operating mechanisms of such barriers are based on solubility at different pH values, slow dissolution/erosion in aqueous fluids or progressive increase in permeability. However, their potential is often limited due to various reasons, such as *i)* stability issues and erratic dissolution behavior, possibly leading to failure in both withstanding the acidic pH and releasing the active ingredient in the intestinal tract; *ii)* early onset of slow release. In this respect, despite daily intake limits and restrictions in the use as food additives, semisynthetic polymers with pH-dependent solubility already approved for pharmaceutical applications (*i.e.* HPMC derivatives and polymethacrylates) were also evaluated for the preparation of enteric-coated dietary supplements, resulting in an increase in their effectiveness.[10,31,32] Based on these considerations, gastroresistant modular units to be directly filled with dietary ingredients or used to house assembled capsular devices was deemed worth investigating. IM and FDM, due to their well-known versatility in the fabrication of a wide range of part geometries, were employed for manufacturing of modular units.[33,34] Thermoplastic polymers that had successfully been subjected to hot-processing were thus employed. Such polymers were KIR and PVA03,

promptly soluble in water, enteric soluble HPMCAS, and HPC, HPMC as well as PVA05, characterized by swelling and slower dissolution properties.

In a first attempt to manufacture 600 µm thick single-compartment capsular devices, feasibility of matching type A and B hollow parts by IM was evaluated using a single mold that had previously been used.[25] In a subsequent step, it would be possible to develop specific molds, dedicated to capsules with different wall thickness or composed of different materials. Following the first molding trials, the need for including adjuvants in the polymeric formulations was assessed (Table 3). When using HPC, hollow parts were successfully fabricated by employing the polymer as such and relevant plasticized formulations containing different amounts of PEG 1500. As the concentration of the plasticizer increased, due to its expected effect on melt viscosity and flow properties, it was possible to reduce pressures and rates of injection and improve the rate and uniformity of filling of the mold cavity, especially in the thinnest areas (Table 1).[25] Accordingly, the frequency of interruption of the manufacturing cycle to allow for mold lubrication was also reduced. For these reasons, the presence of a plasticizer turned out essential with all the other polymers. The minimum amount of plasticizer enabling automatic ejection of molded hollow parts with appropriate physico-technological characteristics was selected in each case. In particular, when using PEGs, the least necessary concentration was identified based on the recently released guidelines on safety-related risk assessment of the European Food Safety Authority Panel on Food Additives and Nutrient Sources added to Food.[35] The weight of molded units was generally reproducible (cv < 2), confirming that robust formulation parameters and operating conditions appropriate for melt flow were set up. The wall thickness of the molded parts, both in the overlapping areas and in the round ones, was quite reproducible. However, average data diverged from the nominal values to a different extent for each formulation. In this respect, the tendency of hot-processed materials to expand after ejection had already been described.[25,36] This behavior should be taken into account for the development of dedicated molds, enabling hollow parts of defined thickness to be attained with each formulation.

For the assembly of two- and three-compartment capsular devices, joints to be matched with hollow parts were used. The two-compartment devices were composed of two type A hollow parts of different composition and of a type 1 joint. The joint base and walls helped delimit the two adjacent compartments. As the joint should remain intact until the opening of the longer-lasting compartment to ensure two-pulse release profiles, its composition had to be analogous to that of the more persistent type A hollow part it was connected to, based on HPC-, PVA05-, HPMC- and HPMCAS formulations. Assembling of three-compartment capsular devices involved the use of two type A hollow parts of different composition, one connected to a type 1 joint and the other matching a type 2 joint. For the achievement of a three-pulse release pattern, the composition of type 1 joint should be analogous to that of the longer-lasting modular unit it was matched with (*i.e.* either a type 2 joint or type A hollow part). On the other hand, the composition of type 2 joint could be analogous to that of type 1 joint, thus being responsible for the third release pulse, or be different from that of both the modular units it was connected to, thus giving rise to the second pulse. In addition, matching hollow parts (*i.e.* type A_1, A_2, B_1, B_2) to be assembled into shells able to enclose the final multi-compartment capsular devices were designed and fabricated. For the manufacturing of the joints and hollow parts *via* IM, appropriate molds should have been developed, which would turn out time-consuming and challenging at this early-development stage.[25] Therefore, they were fabricated by 3D printing, on the basis of the already demonstrated prototyping ability of FDM with respect to IM technique.[21,23] The results obtained with these prototypes could indeed ease the design of dedicated molds subsequently. The printed modular units showed lower reproducibility in terms of weight with respect to molded ones and higher differences in thickness values with respect to the nominal ones, especially in the geometric features with lower wall thickness. These results were attributed to the performance limits of the 3D printer in execution of micrometric details and to the characteristics of the in-house made polymeric filaments used to feed it. Their diameter variability with respect to commercially available filaments, which are not approved as food components even when made of the same polymer (*e.g.* PVA), is already known to affect the printing outcome.[26] Moreover, the cylindrical sections of the

units revealed a tendency to expand after deposition, making assembly of the modular units more difficult. This also happened when they were fabricated through the deposition of a single layer with a nominal thickness equal to the nozzle diameter (0.4 mm). Such an issue was addressed by modifying the CAD file of the joint, *i.e.* progressively reducing the external diameter of the cylindrical portions and thereby introducing a virtual gap in the overlapping region.[21,23] The same approach was followed to ensure appropriate matching of type A_1, A_2, B_1, B_2 hollow parts. Moreover, to allow for proper housing of single- and two-compartment capsular devices in HPMCAS-based shells, a 0.25 mm gap was envisaged between the outer surface of the conveyed systems and the inner surface of the external shell (Figure 1).

By assembling different modular units filled with CFF as the model dietary ingredient, single-, two- and three-compartment capsular devices were attained. Notably, type A and B hollow parts based on HPC as such were characterized by greater stiffness if compared to those fabricated with plasticized formulations. However, this did not hinder capsule filling and closure. Each compartment of the capsular devices was expected to enable complete release of CFF after breakup in aqueous fluids. Depending on the shell composition, the breakup occurred promptly after contact with the suitable fluids (*i.e.* immediate-release and enteric-soluble compartments) or was deferred for a tunable period of time (*i.e.* pulsatile-release compartments) (Table 5, Figure 4). As regards single-compartment capsular devices, the whole amount of tracer contained in KIR- and PVA 03-based shells was detected in the medium within 25 min of testing, and $t_{80\%}$ varied from 15 min to 23 min depending on the composition of the shell. The opening time could be further shortened by reducing the wall thickness. HPMCAS-based capsules withstood the acidic medium and released CFF at pH 6.8 only. However, this occurred after a lag phase of about 1h. The observed performance was consistent with that of traditional enteric-coated drug products having film coatings of about 100 µm in thickness.[37,38] All the other capsules pointed out a pulsatile release performance, based on the swellable/soluble nature of the main polymeric components of the shells. As expected, the rates of hydration and dissolution/erosion of the polymeric gel barrier were shown to depend on the type and molecular

weight of the polymer. Such phenomena were also influenced by the presence of the plasticizer as shown with HPC-based devices. In those cases, a reduction in the duration of the lag phase was observed, which shifted from about 60 min for the devices made of HPC as such to about 40 min for those containing 10% of plasticizer. At the same time, a decrease in the pulse time values and an improvement in the overall reproducibility of the capsule performance were noticed. This was associated with the presence of PEG itself in the molded formulation, as it is a hydrophilic promptly soluble polymer. Its addition, it might have favored water uptake by the polymeric barrier, thus increasing the relevant rate of dissolution/erosion.

As desired, the multi-compartment capsular devices exhibited two- or three-pulse release patterns. Notably, the performance of each compartment was independent of the composition of the associated modular units. Moreover, it was found in agreement with the release behavior of single-compartment molded capsules with analogous composition. The wall thickness of the joints was shown not to affect CFF release parameters even when it was higher than the nominal value. This could be explained by the fact that the first tear from which the breakup of the capsule wall started was always located on the round end of hollow parts or in the central part of the cylindrical type 2 joint, far from the overlapping area.[22] Indeed, during the release test, no disassembly of the modular units was observed, thus proving the effectiveness of the locking mechanism. Overall, since no interference was observed between aliquots of CFF released from different compartments, the capsular devices were proven suitable for co-administration of dietary ingredients to be kept separated during manufacturing or be released at differing programmed time points. Finally, in the case of enteric-soluble shells designed to house multi-compartment capsular devices, no adherence phenomena between the inner system and the external enteric-soluble one were highlighted, and the release performance in phosphate buffer, after the opening of the outer shell, was that of capsular device contained.

4. Conclusions

In the present work, an innovative and easy-to-handle delivery platform for dietary ingredients in the form of a capsule shell entailing Lego-inspired assembly of modular units was developed. The feasibility of such a delivery platform designed for the achievement of a variety of release kinetics was first proved with CFF. Capsular devices comprising from 1 to 3 independent inner compartments were attained following combination of matching modular units having diverse geometries and composition (*i.e.* promptly soluble, swellable/soluble and enteric soluble polymers). These units were manufactured by IM and FDM and showed satisfactory physico-technological properties (*e.g.* weight, thickness, effective assembly). Notably, fabrication *via* IM would be advantageous for mass-production of pre-formed modular units that could be assembled in different configurations, even extemporaneously, thus enabling customization of combination and filling. On the other hand, the use of FDM 3D printing would enable on-demand manufacturing of small batches of personalized modular units, simplify the supply chain and speed up R&D stages of IM by virtue of its prototyping capability. After filling modular units with the model dietary ingredient and assembling them in capsular devices, these pointed out single- and multi-pulse release patterns, consistent with the configuration of the system (*e.g.* number of compartments, polymeric composition and wall thickness of each compartment). Finally, the delivery platform was extended to include an external gastroresistant capsule shell enclosing previously-assembled devices. This was demonstrated to yield resistance to the acidic release environment of single- and multi-compartment capsular devices.

The present work gives preliminary insight into the possibility of exploiting novel manufacturing techniques for the development of versatile and customizable dietary ingredient formulations provided with innovative features in terms of composition and performance. The delivery platform configuration enables conveyance, in a single dosage form, of dietary ingredients that need to be physically separated for stability reasons or compounds that would benefit from release at different times because of known mutual interaction in the gastrointestinal tract. Indeed, single and multiple release profiles, including immediate and modified (pulsatile and delayed) ones, would be achieved, and also possible improvement of the bioavailability of specific ingredients. Currently, these goals

may be of great interest for dietary supplement users, opening up new business opportunities for companies that may be able to fulfill them by devising novel products. In this respect, like traditional hard-gelatin capsules, the delivery platform would only involve filling and assembly of ready-to-use modular units. Moreover, the availability of a variety of such units could favorably impact on the development of new dietary supplements in terms of versatility, patentability and time-to-market as well as related costs.

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