AAPS PharmSciTech

Industrial development of a 3D printed nutraceutical delivery platform in the form of a multicompartment HPC capsule

--Manuscript Draft--

Manuscript Number:	AAPSPT-D-17-00748R1
Full Title:	Industrial development of a 3D printed nutraceutical delivery platform in the form of a multicompartment HPC capsule
Article Type:	Research Article
Section/Category:	INVITED ONLY: Printing and Additive Manufacturing (Guest Eds. Sandler and Rantanen)
Keywords:	fused deposition modeling, microextrusion, capsular device, pulsatile release, caffeine.
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Manuscript Region of Origin:	ITALY
Abstract:	Following recent advances in nutrigenomics and nutrigenetics, as well as in view of the increasing use of nutraceuticals in combination with drug treatments, considerable attention is being directed to the composition, bioefficacy and release performance of dietary supplements. Moreover, the interest in the possibility of having such products tailored to meet specific needs is fast growing among costumers. To fulfill these emerging market trends, 3D printed capsular devices originally intended for conveyance and administration of drugs were proposed for delivery of dietary supplements. Being composed of separate inner compartments, such a device could yield customized combinations of substances, relevant doses and release kinetics. In particular, the aim of this work was to face early-stage industrial development of the processes involved in fabrication of nutraceutical capsules for oral pulsatile delivery. A pilot plant for extrusion of filaments based on pharmaceutical grade polymers and intended for 3D printing was set up, and studies aimed at demonstrating feasibility of fused deposition modeling in 3D printing of capsule shells according to Current Good Manufacturing Practices for dietary supplements were undertaken. In this respect, the stability of the starting material after hot-processing and of the resulting items was

	investigated, and compliance of elemental and microbiological contaminants, as well as of by-products, with internal specifications was assessed. Finally, operating charts highlighting critical process variables and parameters that would serve as indices of both intermediate and final product quality were developed.
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Prof. Dr. Niklas Sandler
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Guest Editors for AAPS PharmSciTech

Milan, December 19th 2017

Subject: manuscript submission

Dear Editor,

I am pleased to submit for publication in AAPS PharmSciTech our manuscript entitled "3D printed capsular devices for personalized supplementation" by Alice Melocchi, Federico Parietti, Simone Maccagnan, Marco Ortenzi, Stefano Antenucci, Francesco Briatico-Vangosa, Alessandra Maroni, Andrea Gazzaniga and Lucia Zema.

The manuscript focuses on the potential application of 3D printed two-compartment capsular systems, originally devised for personalization of dosage and delivery of drugs, to the nutraceutical field, in response to a growing market trend that envisages customization of dietary supplements in terms of composition and release performance. In this respect, production-scale manufacturing of filaments intended for 3D printing by fused deposition modeling based on pharmaceutical-grade polymers was set up, and development of an industrially viable printing process for the manufacturing of such capsular devices was started. Prototype filaments and capsule parts meeting pre-set specifications were obtained and the assembled capsular devices showed the desired two-pulse release performance.

Also on behalf of Co-authors, I hereby state that the manuscript comprises new, original unpublished material, which is not under consideration for publication elsewhere. Manuscript publication is approved by all Authors and tacitly by the Dean of the Department.

No ethical issues are involved. All Authors declare no financial or non-financial conflicts of interest.

Sincerely,

Dr. Alessandra Maroni

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Abstract:

Following recent advances in nutrigenomics and nutrigenetics, as well as in view of the increasing use of nutraceuticals in combination with drug treatments, considerable attention is being directed to the composition, bioefficacy and release performance of dietary supplements. Moreover, the interest in the possibility of having such products tailored to meet specific needs is fast growing among costumers. To fulfill these emerging market trends, 3D printed capsular devices originally intended for conveyance and administration of drugs were proposed for delivery of dietary supplements. Being composed of separate inner compartments, such a device could yield customized combinations of substances, relevant doses and release kinetics. In particular, the aim of this work was to face early-stage industrial development of the processes involved in fabrication of nutraceutical capsules for oral pulsatile delivery. A pilot plant for extrusion of filaments based on pharmaceutical grade polymers and intended for 3D printing was set up, and studies aimed at demonstrating feasibility of fused deposition modeling in 3D printing of capsule shells according to Current Good Manufacturing Practices for dietary supplements were undertaken. In this respect, the stability of the starting material after hot-processing and of the resulting items was investigated, and compliance of elemental and microbiological contaminants, as well as of by-products, with internal specifications was assessed. Finally, operating charts highlighting critical process variables and parameters that would serve as indices of both intermediate and final product quality were developed.

Keywords: fused deposition modeling, microextrusion, capsular device, pulsatile release, caffeine.

1. Introduction

Innovative drug delivery systems (DDSs) in the form of functional containers have recently been proposed, wherein polymer coating barriers of traditional reservoir systems, manufactured by film-coating or layering processes, have been replaced by capsule shells ready for filling. Such shells would be able to control the rate, time and/ or site of release based on their design and composition (1-4). As the capsular device can extemporaneously be filled with different formulations, it offers great flexibility and potential for customization, particularly in its recently described configuration including separate compartments (5).

Starting from a variety of pharmaceutical grade polymers, single- and multi-compartment capsular devices with different release (immediate, prolonged and delayed/pulsatile) performance were fabricated by injection molding (IM) and fused deposition modeling (FDM) 3D printing (4-6). While IM could be exploited for large-scale production of a variety of capsule shells with pre-determined release behavior, FDM holds promise as a prototyping tool and would also enable on-demand fabrication of small batches of drug products designed to meet the needs of single patients (7-9).

Given the recent advances in nutrigenomics and nutrigenetics, as well as the growing attention towards the use of dietary supplements in support of drug therapies, personalization is currently drawing interest not only in the area of pharmaceutics but also in the nutraceutical field, *e.g.* for modification of the type or amount of supplement intake over time or use of vegan and kosher ingredients (10-12). Furthermore, there is a fast-increasing awareness about the benefits that may arise from the application of modified release strategies to dietary supplements (13-14). To meet the demand for more and more sophisticated nutraceuticals, young companies or start-ups have lately been founded (*e.g.* Nootropics, Nootrobox, KalibrateV, Elysium, Ritual, Panaceutics).

Based on such premises, the purpose of the present work was to assess the feasibility of the capsular device as a nutraceutical delivery platform starting from prototypes made on a laboratory scale and

accomplish early industrial development of the 3D printing fabrication process, which has never been faced before. Accordingly, stability of the starting materials after hot-processing and of the resulting items needed to be assessed, to rule out formation of any hazardous degradation product. In addition, the potential for industrialization of the first step involved in the fabrication of the capsular devices, consisting in hot melt extrusion (HME) of filaments based on pharmaceutical grade polymers and intended for FDM, was evaluated, and studies aimed at the development of all production stages, including 3D printing of capsule shells to be automatically filled in-process, were undertaken. In this respect, the construction of a pilot plant for capsule printing in agreement with Current Good Manufacturing Practices (CGMPs) for dietary supplements was finally approached by highlighting critical processing stages that may affect the product quality and by assaying the performance of printed prototypes with customizable design.

2. Materials and Methods

2.1. Materials

Hydroxypropyl cellulose (HPC; Klucel™ LF, Ashland, US-MA; two different batches indicated as 1 and 2); caffeine (ACEF, I); polylactic acid (PLA) filament (L-PLA natural, ø 1.75 mm; MakerBot® Industries, LLC, US-NY); blue and yellow dye-containing powder products ready for use (Kollicoat® IR Brilliant Blue and Kollicoat® IR yellow, BASF, D); deuterium oxide (Aldrich; 99.9 % D); ethylene glycol (Aldrich, anhydrous, 99.8%, I); di-sodium hydrogen phosphate dodecahydrate (Merck KGaA, 99%, D); methanol (Sigma-Aldrich, ACS reagent, reg. ISO, reag. Ph. Eur., ≥ 99.8%, I); poly(ethylene glycol)/poly(ethylene oxide) (PSS Polymer standards service GmbH, D); sodium phosphate monobasic monohydrate (Aldrich, puriss. p.a., ACS reagent, ≥ 99.0%, I); water (Aldrich, HPLC grade, I).

2.2. Methods

92 2.2.1. Preparation and characterization of filaments

93 Filaments were prepared by HME starting from HPC powder.

94 - In-house prepared filaments (HMELab) - Filaments were obtained as reported in (6) starting from
95 batch 1 of neat HPC powder (PD1) kept in an oven at 40 °C for 24 h prior to use. A twin-screw
96 extruder (Haake™ MiniLab II, Thermo Scientific, US-WI) equipped with counter-rotating screws
97 and a custom-made aluminum rod-shaped die (ø = 1.80 mm) was employed. The filament diameter
98 was verified every 5 cm in length, and portions having diameter out of the 1.75 ± 0.05 mm range
99 were discarded.

100 - Industrially-manufactured filaments (HMEInd) - Filaments were also manufactured at Gimac's
101 facilities in Castronno (VA), Italy, starting from batch 2 of neat HPC powder (PD2) by means of a
102 specially-assembled microextrusion system; 3 different batches were produced (HMEInd1,
103 HMEInd2 and HMEInd3). Details on the equipment and process are reported and discussed within
104 the Results and Discussion section. The obtained filaments were cut into pieces of 1 m in length and
105 packed into moisture-protective bags in common use for nutraceuticals. After production, the
106 filament diameter was checked every 30 cm in length using a digital caliper (Mitutoyo, J).
107 Measurements were performed normally to the filament axis in two mutually perpendicular
108 directions, and roundness index was calculated as the ratio between the diameters measured in the x
109 and y axis. Mechanical properties of filaments were evaluated using an Instron 1185-5800R
110 dynamometer (Instron, US-IL) equipped with a 10kN load cell. Commercially-available PLA
111 filament was taken as a reference. Tensile tests were carried out on 200 mm long cylindrical
112 samples (n = 3) with nominal diameter of 1.75 mm. The initial clamping distance, L_0, was about
113 100 mm and the crosshead speed was 1.25 mm/min. Applied displacement, ΔL, and the relevant
114 load, F, were measured, from which strain, $\varepsilon = \Delta L/L_0$, and stress, $\sigma = F/(\pi R^2)$, were estimated.
115 From the resulting stress-strain curve, the Young modulus, E, *i.e.* the slope of the first linear part of
116 the curve, was determined. Moreover, the peak stress to initial failure, *i.e.* the value of stress
117 obtained at the first strain beyond which such a parameter started to decrease, was selected as an

index of the material strength (σ*). As an example, Figure 1 reports a typical curve for the PLA filament. Moreover, elemental (*i.e.* lead, arsenic, cadmium, mercury) and microbiological contamination was evaluated according to the relevant USP monographs [USP <2232> and <2023>] in a certified laboratory (Micro Quality Labs, Inc., US-CA) specialized in pharmaceutical, nutraceutical and cosmetic testing.

2.2.2 Printing of capsular devices

Capsular devices were 3D printed by FDM, starting from HPC filaments.

- Laboratory-scale printed capsular devices (FDMLab) - 3D printing was performed by a MakerBot Replicator 2 (MakerBot® Industries, US-NY) starting from HMELab filaments as described in (6).

- Industrially-fabricated capsular devices (FDMInd) - 3D printing was performed at Multiply Labs production site in San Francisco, US-CA, by a commercial 3D printer (Series 1 Pro, Type A Machines, US-CA) purposely modified, starting from HMEInd filaments. Computer-aided design (CAD) files were designed using an engineering 3D modeling software, saved in .stl format, imported to the 3D printer software and then converted in GCode instructions for the 3D printer. The printer trajectory and extrusion rates were improved to ensure the correct wall thickness, while minimizing printing defects such as gaps and over- or under-extruded sections. Details on the equipment and process are reported and discussed within the Results and Discussion section.

2.2.3. Characterization of capsular devices

Capsule parts 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) of samples were also acquired.

The morphological changes undergone by capsular devices when exposed to aqueous fluids were evaluated by immersing two-compartment units, filled with approximately 25 mg of a blue and a yellow dye-containing formulation, respectively, in unstirred deionized water at the temperature of

144 37 ± 0.5 °C. Digital photographs were taken every 30 sec using a digital camera (GoPro Hero
145 Session, US-CA). The opening time of each compartment was determined by visual inspection and
146 defined as the time of first rupture of the hydrated shells, as highlighted by rapid dissolution of the
147 dye inside the capsule compartment and its outward diffusion.
148 The release performance of capsular devices (n = 6) having each compartment filled with 25 mg (cv
149 ≤ 2) of caffeine was evaluated according to a method previously developed (5). The assembled
150 capsules were inserted into sinkers and tested using a modified USP38 three-position disintegration
151 apparatus (Sotax, CH), wherein each basket-rack assembly moved at a 31 cycles/min rate in a
152 separate vessel filled with 800 mL of distilled water at 37 ± 0.5 °C (15). Fluid samples were
153 withdrawn at fixed time points and assayed spectrophotometrically (λ = 205 nm). By linear
154 interpolation of release data immediately before and after the time point of interest, time to 10%
155 release ($t_{10\%}$) was calculated.
156
157 2.2.4 Thermal studies
158 2.2.4.1 Differential Scanning Calorimetry (DSC)
159 DSC analyses were performed by a Mettler Toledo DSC1 (Mettler Toledo, CH), weighing 5-10 mg
160 of each sample in a standard 40 μL aluminum pan. An equal empty pan was used as the reference.
161 In order to overcome any effect of their thermal history, specimens were heated from 25 °C to 250
162 °C at 10 °C min^{-1}, kept for 5 min at 250 °C, cooled to 25 °C at -10 °C min^{-1}, left for 2 min at 25 °C
163 and finally heated again to 250 °C at 10 °C min^{-1}, under nitrogen flux.
164
165 2.2.4.2 Thermal Gravimetric Analysis (TGA)
166 TGA analyses were performed on 6 mg of neat HPC powder using Perkin-Elemer TGA 4000
167 (Perkin Elmer, US-MA) under 20 mL/min nitrogen purge. A temperature ramp from 30 °C to 160
168 °C at 20 °C/min was set followed by an isothermal analysis at 160 °C for 20 min.
169

2.2.5 Analyses of by-products

2.2.5.1 Fourier-transform infrared spectroscopy (FT-IR)

FT-IR spectra of powder, extruded filaments and 3D printed samples were acquired by a 100 spectrophotometer (Perkin Elmer, US-MA) in the attenuated total reflection (ATR) mode, at a resolution of 4.0 cm^{-1} and 256 scans and in a 4000 and 400 cm^{-1} wavenumber range. A single-bounce diamond crystal was used with an incidence angle of 45°.

2.2.5.2 Proton Nuclear Magnetic Resonance (^{1}H-NMR)

^{1}H-NMR spectra were collected at 25 °C using a Bruker 400 MHz spectrometer (Bruker, D) from samples prepared by dissolving 10 mg of powder, extruded filaments or 3D printed samples in 1 cm^3 of D_2O. In order to detect by-products soluble in organic solvents, powder, extruded and printed samples were kept for 24 h at room temperature in methanol, and ^{1}H-NMR analyses were performed on methanolic fractions dried under nitrogen flux and then solubilized in D_2O.

2.2.5.2 Gel Permeation Chromatography (GPC)

GPC analysis was performed using a size exclusion chromatography (SEC) system consisting of a Waters 1515 isocratic HPLC pump (Waters, US-MA), a PlySep three-column set [(20-300)Da; (100-10000)Da; (3000-400000)Da] (Phenomenex, US-CA) and a refractive index (RI) detector (Knauer RI Detector 2300, Knauer, D). The flow rate and the injection volume were set at 0.35 mL/min and 50 µL, respectively. 30 mg samples were dissolved in 1 mL of phosphate buffer pH 6.3 (100 mM), and solutions were filtered (0.45 µm) before injection. Ethylene glycol was used as the internal reference in each analysis and data collected were normalized with respect to the main peak. Molecular weight data, *i.e.* number average molecular weight (M_n) and weight average molecular weight (M_w), were obtained using linear poly(ethylene glycol)/poly(ethylene oxide) as the calibration standard in the 62-490000 Da range. Molecular weight distribution (D) was also estimated from dispersity (M_w/M_n).

196

197 2.2.6 Rheological studies

198 Viscosity of 5% w/w HPC solutions in distilled water obtained from powder and filament was
199 measured using a HAAKE Viscotester C (Thermo Fisher Scientific, US-MA). The method
200 employed was that described by the producer of Klucel™ LF, except for R4 spindle and 60 rpm
201 rotational speed in that they enhanced the reproducibility of data (16).

202

203 ## 3. Results and discussion

204 Based on the extensive experience gained on hot-processing of HPC and on its availability in
205 different viscosity grades, which makes it a versatile main component for capsule shells intended
206 for different release patterns (*e.g.* prompt release or pulsatile release after lag phases of programmed
207 duration), such a polymer was identified as an advantageous raw material to start with for setting up
208 a fabrication process compliant with Current Good Manufacturing Practices (CGMPs) for dietary
209 supplements (17-19).

210 In order to commercialize a dietary supplement based on the new delivery platform, the quality of
211 the raw materials and intermediates, such as the polymeric filament undergoing 3D printing, has to
212 be demonstrated, and fulfillment of all requirements of the final product needs to be ensured (20). In
213 this respect, besides confirming the identity of raw materials, a number of other specifications are to
214 be met, which should be assessed by relying on the certificate of analysis provided by a qualified
215 supplier and also performing appropriate tests. Because neither extruded filaments based on HPC
216 nor FDM-printed products based on such a polymer are currently available on the market, it was
217 necessary to assess stability of the starting material to hot-processing.

218

219 ### 3.1. Assessment of HPC stability following laboratory-scale HME and FDM

220 In the beginning, it was investigated whether any degradation phenomena would occur following
221 the laboratory-scale processes. In particular, the thermal and rheological behavior of extruded

intermediates (*i.e.* filaments, HMELab) and printed capsules (FDMLab), as well as possible formation of by-products, were evaluated in comparison with the pharmaceutical-grade HPC powder selected (*i.e.* Klucel® LF powder batch 1, PD1), by a range of suitable techniques. DSC and TGA were exploited to study the thermal properties of samples, particularly in the temperature ranges to be used in HME and FDM processes. Moreover, micro- and macro-molecular changes possibly brought about by hot-processing, *i.e.* changes of molecular weights and molecular weight distribution of the polymer and formation of low-molecular weight compounds, were investigated by FT-IR, ^{1}H-NMR and GPC.

HPC powder was shown to lose approximately 2% of its initial mass when kept at 160 °C for 20 min (TGA data not shown), which was ascribed to removal of adsorbed water. As expected, no water signal was observed in DSC curves relevant to the extruded filament, most likely due to the high temperature it was subjected to throughout manufacturing (Figure 2). With FDM products, fabricated at ambient conditions, water loss was still evident probably due to moisture uptake within processing (printing time ≈ 5 min). No other differences in DSC data of processed *versus* unprocessed specimens were highlighted, thus indicating that neither for HME nor for FDM the processing of the material affected the thermal properties of the samples. Major differences relevant to extruded and printed products with respect to the starting powder were not even found in either FT-IR and ^{1}H-NMR spectra or ^{1}H-NMR spectra from the methanolic fractions, in which less polar low molecular weight by-products might have partitioned (Figure 3).

GPC curves and molecular weight data relevant to HPC powder, filaments and printed samples are reported in Figure 4 and Table 1, respectively. Two peaks relevant to HPC-based materials (*i.e.* peak 1 and 2) were highlighted, while the peak at about 4800 s refers to the internal reference used (*i.e.* ethylene glycol). The wide peak in the 2500-3500 s retention time range (peak 1) was observed both in the HPC powder and in the processed samples, which could be attributed to a broad molecular weight distribution of the polymer, also confirmed by relevant D values. However, the shape of such a peak may have resulted from aggregation phenomena. Peak 2, indicating the

presence of lower molecular weight components, was also detected in all specimens, thus ruling out the formation of by-products during the process. This GPC pattern (*i.e.* a wide peak at lower retention times and a small peak at higher times) was already described for HPC and attributed to the synthetic nature of the polymer, characterized by the presence of different macromolecular species with relatively higher or lower degree of hydroxylation (21). The presence of aggregates resulting from the same retention time region of higher molecular weight components was also investigated, and the relevant formation was demonstrated to be promoted by the experimental conditions. Peak 1 was the widest for PD1 among all samples, which may be associated with the highest extent of aggregation.

The overall results support the possibility of using HPC for the manufacturing of filaments and printing of capsular devices under the experimental conditions used without causing major degradation phenomena.

3.2 Development of a pilot plant for microextrusion of HPC filaments

The issue of the lack of filaments ready for printing and consequent need for their large-scale production was subsequently addressed. An industrial partner specialized in microextrusion was involved in the development and construction of an extrusion plant for one-step production of filaments that could be compliant with regulatory requirements in force in the nutraceutical field. Microextrusion does not represent a simple scale-down of traditional HME being characterized by the ability *i)* to produce items having very complex geometry and narrow tolerances, *ii)* preserve the chemical properties of the starting materials and *iii)* process polymeric blends expected to perform according to their physical and chemical characteristics. Indeed, such a technique was already exploited for fabrication of devices intended for biomedical applications. Particularly, the feasibility of microextrusion in the production of tubes, filaments or porous wires to be composed into patches or scaffolds was evaluated, and critical aspects, such as their mechanical properties, the tolerances achieved and the potential degradation of the processed formulation, were dealt with (22-26).

The main constraints in the production of the HPC-based filaments were related to the utilization, for the equipment to be devised, of construction materials that may fulfill the requirements involved by the intended application. For instance, all components of the extruder should be inert and easily cleanable. Moreover, the strict dimensional tolerances of produced filaments should be met, while taking the need for setting up simple and cost-effective processes into proper account. The use of machineries and tools for pilot-scale trials, engineered and supplied by the industrial partner, was explored with the aim of preliminarily assessing the process feasibility, and any necessary modifications were step-by-step introduced.

While HME is traditionally carried out starting from free-flowing pellets in order to ensure a constant output of material and, consequently, dimensional consistency of the resulting filament, the challenge in this specific instance was to deal with HPC in powder form, thus skipping the compounding phase that commonly precedes such an operation. The powder (Klucel® LF powder batch 2, PD2) was dried at 70 °C for 8 h to remove residual water before hot-processing. HME was performed horizontally by a microextrusion system (Figure 5) equipped with a thermoregulated hopper (internal volume 0.1 L), wherein HPC was kept at 30 °C and in continuous motion by an angled-palette stirrer (1 rpm) in a dried and nitrogen-protected environment. Under such conditions, caking phenomena (*i.e.* adhesion and cohesion of powder particles without effective feeding of the microextruder) were avoided, and starve-feeding of the barrel through a conical opening was carried out. Along the barrel, four distinct thermoregulation zones were defined and independently controlled by means of fluid-thermoregulated bushings, each covering in length approximately four flights of the inner screw. The geometry of the latter was purposely conceived to allow efficient conveying of the powder within the first part of the barrel and proper flow of the melt beyond. Particularly, the screw had 30 L/D length, and its first four flights were characterized by palette shape on the ridge and reduced diameter of the core in order to break powder aggregates while rotating.

At the end of the barrel an extrusion head was mounted. It terminated with a spinning opening having land length of 10 mm and diameter of 1.75 mm, corresponding to the nominal filament diameter. The extrusion head was provided with a heating element sunk into brass and enclosed in a heat-exchange jacket cooled by a circulating fluid (water). Moreover, it was designed to be especially compact in the longitudinal direction, *i.e.* the extrusion axis, as this made it easier to limit undesired temperature instabilities, which would involve changes in viscosity of the melt and, therefore, in the relevant flow as well as in the filament diameter. Continuous contact of the head with the heat-exchange jacket allowed thermal energy to be distributed in such a way as to yield a practically constant temperature profile at the inner surface of the cavity where the filament diameter was defined, thus avoiding flow alterations and uncontrolled surface texture. The possibility of strictly controlling the extrusion temperature was of utmost importance also considering the impact of such a parameter on the pressure developed by the melt according to its rheological characteristics (2). In particular, HPC viscosity was shown to decrease by increasing the temperature above 140 °C, but critical issues related to overheating were also highlighted. Accordingly, the operating temperature was studied by progressively raising it from 85 to 175 °C along the barrel, and decreasing it under 140 °C in its last zone as well as in the extrusion head. Indeed, by reducing the temperature at the end of the barrel, the viscosity of the melt was settled, and a constant pressure of about 300 bar was exerted against the 40 µm filter here positioned. Such a pressure turned out sufficient to maintain a constant rate of material output, thus helping attain consistency in the filament diameter.

As traditional water cooling devices could not be employed because of water solubility of the polymer, the outgoing filament was initially cooled using pressurized air and then at room temperature only. While cooling, the extruded product was pulled by a purposely-built haul-off system having servocontrolled series of rollers coated with Food and Drug Administration (FDA)-approved elastomer. In order to avoid deformation of the hauled filament, the mutual position of the pulling rollers was defined by means of mechanical parts having highly-sensitive pneumatic

regulation. The filament was trailed through two subsequent double-laser units (Zumbach Electronic S.r.l., I) measuring the diameter in two perpendicular directions (measurement frequency 0.5 s), thus allowing possible ovalization to be highlighted, *i.e.* lack of correspondence between diameters measured in the x and y axis, respectively. The former laser unit was positioned close to the extrusion head, and the latter was located at the end of the cooling path to check the dimensional parameters of the final filament.

By progressively setting up the operating conditions, filaments consistently meeting the established dimensional specifications, *i.e.* diameter of 1.75 ± 0.05 mm and roundness index in the 0.989-1.011 range, could be attained (Table 2). Only the lowest pulling speed and force enabled by the haul-off system were employed, which turned out suitable for holding the outgoing filament, driving it through the laser units and counteracting possible dimensional changes, without causing its deformation or rupture. In particular, pulling speed was automatically adjusted in the 1.5 - 1.7 m/min range to deal with possible over- and under-sizing (< 5% of nominal 1.75 mm diameter), based on the measurements performed by the former laser unit.

Combined setup of extrusion and pulling parameters was meant to be automated on the basis of the filament dimensions in the final production-scale equipment, in which a winding machine could also be included.

3.3 Assessment of quality specifications for industrially-manufactured HPC filaments

Three different batches of filaments were prepared for validation purposes (*i.e.* HMEInd1, HMEInd2, HMEInd3) by the developed microextrusion plant. Because filaments as supplied would represent the starting material to be employed for fabrication of printed products, selected quality specifications needed to be established based on parameters that were identified as critical, and routine tests for ascertaining the relevant fulfillment had to be developed. The latter should be performed not only by the manufacturer of the filament in order to control its quality, but also by the dietary supplement producer in order to verify compliance with the certificate of analysis and

accept the incoming batch. Particularly, diameter, roundness, microbiological attributes and presence of by-products as well as elemental contaminants were identified as quality indices, and proper internal specifications thereof were defined (Table 3). The three validation batches turned out compliant. With respect to by-products, FT-IR, ^{1}H-NMR, ^{1}H-NMR from the methanolic extract and GPC analyses were initially performed on both the filaments and the starting powder. As the rheological behavior of a polymeric solution is known to depend on the molecular weight of the solute, aqueous solutions of HPC prepared from polymer powder and filaments were compared with each other in terms of viscosity in order to highlight major changes in length of the macromolecular chains. Viscosity of 5% w/w solutions, as measured according to HPC producer, turned out to be in the 75 - 115 cps range (producer specifications 75-150 cps) for samples prepared from both powder batch PD2 and filament, thus indicating that such a parameter would not be affected by any changes in the polymer molecular weight and ruling out major degradation phenomena taking place during extrusion. On account of the obtained results, compliance with internal specifications for by-products was ascertain on the basis of FT-IR, ^{1}H-NMR and viscosity measurements.

3.4 Development of a scalable FDM plant for printing of HPC capsule shells

The manufacturing of capsule shells was subsequently faced, and the setup of a FDM 3D printing process that could be compliant with current regulatory requirements was the focus of the further part of the work. First of all, the facility where the process was intended to be run was brought up to CGMPs for dietary supplements standards. A prototype printer was then designed, which could easily be disassembled and cleaned (Figure 6). Particularly, commercially available and purposely fabricated parts were combined for the printer construction, having all components that would come in contact with the filament/product, *i.e.* the build plate, the extruder assembly and the filament feeding system, made of 316L stainless steel or of FDA-compliant materials. The equipment was also conceived to be operator-safe by adding an outermost enclosure, so that it could not unintentionally be accessed while working. Issues of potential contamination from moving

mechanical parts were also faced. Indeed, the extruder assembly of the 3D printer was suspended above the build plate and attached to carriages sliding along linear guides (Figure 6, detail a). The carriages were actuated by stepper motors, which controlled the motion of a timing belt transmission. In such a system, the main source of particle generation was represented by frictions between the carriages and the linear guides, and between the timing belt and the pulleys. Moreover, because lubrication of the linear guides was needed, the motion of the carriages may have generated oil droplets. Therefore, having the moving mechanical parts located over the build plate, particles and oil droplets may have fallen onto the latter, thereby contaminating products in fabrication. This risk was circumvented by enclosing the sources of potential contamination in a shielding system that consisted in a rigid barrier, sealed on all sides except above the moving parts to allow access for maintenance. Particles could not escape from the open side, because the 3D printer was operating inside a clean room with downward laminar airflow, dragging contaminants towards the bottom of the containment barrier.

The extruder assembly (Figure 6, detail b), intended to melt the filament and deposit it onto the part being formed, was also enclosed into an analogous particle containment system. With respect to the original Type A printer, it was extensively modified in order to ensure compliance with CGMP for dietary supplements. Indeed, not only were parts in contact with the filament constructed in 316L stainless steel instead of aluminum, but also the overall assembly was devised to easily be opened, as opposed to most commercially-available printers. Furthermore, the heating chamber and the nozzle of the extruder assembly were modified according to the polymer employed, *e.g.* in terms of nozzle length and position of the heating elements and of the thermocouple. Such modifications were aimed at avoiding both overheating and rapid changes in temperature, which had already been demonstrated critical in that they impact on HPC stability and cause undesired alterations in the melt viscosity (2). During laboratory-scale printing trials, in fact, nozzle clogging, material browning and lack of reproducibility in the deposited amounts were observed. The overall shielding

system and the extruder assembly were subjected to periodical inspection and cleaning as a part of the equipment standard maintenance.

The printer feeding mechanism was adjusted based on the mechanical properties of the filament in use in order to exert lower stress onto the latter, thus leading to an efficient and constant loading of the material into the equipment as deformation was thereby prevented. When compared with the standard PLA filament commonly supplied along with commercially available printers, the HPC filament was indeed characterized by lower strength and stiffness (HPC: E = 1.19 GPa, cv 4.43; σ^* = 9.94 MPa, cv 21.56; PLA: E = 2.64 GPa, cv 4.13; σ^* = 43.41 MPa, cv 6.47).

As regards the design and dimensions of the capsule shell, as well as the mode and accuracy of printing, helpful hints were derived from the experience already acquired (4,5). The dimensions of size 00 gelatin capsules were chosen as a reference to be mimicked because they were deemed to yield an acceptable balance between inner capacity and swallowing compliance (see sketches in Table 4). The capsule shell was composed of two hollow parts having same length and width, with nominal thickness of 400 and 800 µm, respectively, and of a middle joint part. The latter was composed of two hollow truncated cones connected at their larger bases through a disk. When assembled by partial overlapping of the hollow parts with the joint, the capsular device comprised two separated internal compartments intended to break up at successive time points (two-pulse release performance) as a function of their wall thickness. The design characteristics for the joint were specially conceived to impart mechanical resistance to the printed piece and ease insertion into the hollow parts so that tight closure of the system would be possible. The nominal wall thickness of the truncated cones was of 800 µm, which was achieved through deposition of two adjacent layers of material, by means of a 0.4 mm nozzle. In addition, the printing software was adjusted so that changes in the deposition direction during fabrication, which may lead to structural weaknesses, could be avoided. Prototypes of hollow and middle parts were thus printed in order to assess the feasibility of the capsular device (Figure 7 and Table 4). Although it would have been possible to attain higher resolution and thinner multilayered wall thicknesses by the use of nozzles

with smaller orifice diameter (*e.g.* 0.2 mm), this would have increased the overall process time, which would be critical in the prospect of large-scale production and commercialization.

3.5 Evaluation of HPC capsular devices and assessment of quality specifications

To check the effectiveness of the locking system and the opening mechanism of the capsular device, release tests of samples filled with either different dyes or caffeine were carried out under unstirred and stirred conditions.

As expected, thickness consistency turned out to be a critical goal especially in the case of thinner hollow parts. Although printing accuracy could still be improved, changes undergone over time in unstirred water by a device containing different dye colors in each compartment showed the desired pattern: *i)* swellable/erodible behavior consistent with the nature of the starting material, with evidence of formation of a gel layer followed by dissolution of the polymer, and *ii)* opening based on occurrence of a first tear at the least thick area of each compartment (Figure 8). Air bubbles associated with leakage of the dye were seen where hollow parts were not superimposed on the joint, and the opening time was dependent on their wall thickness. Accordingly, capsular devices having both compartments filled with caffeine as a tracer supplement, tested under stirred conditions, exhibited a two-pulse release profile due to successive opening of the 400 and 800 µm thick compartments ($t_{10\%}$ = 27.15 min ± 4.72 SD and 89.12 min ± 15.63 SD, for the thinner and thicker compartments, respectively).

In addition to assessing the performance of the printed device, quality specifications also needed to be established. In particular, it was important to assess process temperature ranges that would not only allow consistent printed objects with tightly adhering layers to be achieved but also prevent any negative impact on HPC stability during the latter heating step. During the FDM process, HPC filaments (HMEInd) were progressively pulled out from a dryer, set at 40 °C, connected with the printer in order to avoid moisture adsorption. Batches consisting of 15 printed items, either joints or hollow parts, were fabricated requiring a maximum processing time of 12 min *per* capsule.

Preliminary printing trials were performed keeping the HPC filament within the heating chamber for 30 min at the temperatures of 175 °C or 200 °C, in order to study the effect of harsh conditions. FT-IR and ^{1}H-NMR spectra of the fabricated parts showed degradation of the material maintained at the higher temperature only. After verifying that the temperature of 175 °C could be used, stability of units printed at 175 and 180 °C (*i.e.* FDMInd175 and FDMInd180) was investigated in view of 5 °C fluctuations that might occur within the heating chamber of the equipment. Major differences were neither observed when comparing the two sets of samples with each other, nor with the starting batch PD2 of HPC powder (Figure 9).

Following the printing trials carried out to define design features of the capsular device and appropriate FDM process conditions, specifications needed to be established with respect to critical parameters that impact on product quality. As regards microbiological attributes, by-products and elemental contaminants, the same specifications as for the starting filament were applied to the printed items that turned out compliant, thus ruling out any impact of the process on those variables. Further internal specifications relevant to weight of capsule parts and wall thickness of hollow halves are to be set on the basis of opening and release performance, which would be derived from fabrication and evaluation of a sound number of batches of suitable size. Validation of all specifications should then be accomplished.

4. Conclusions

3D printed multi-compartment capsular systems, originally devised for personalization of dosage and delivery of drugs, were proposed for application to the nutraceutical field in response to a growing market trend that envisages more and more complex needs in terms of composition and release performance of dietary supplements. In this respect, caffeine was the first ingredient to be considered during early development of the nutraceutical delivery platform because of the highly inter-individually variable response it may elicit in terms of awakeness and attention based on body weight, age, tolerance and genetic polymorphism of users. Moreover, caffeine would greatly benefit

from a pulsatile release mode so that the onset of its effects may be scheduled throughout the day when mostly needed.

In the prospect of commercializing capsular devices for customizable supplement delivery, production-scale manufacturing of HPC filaments intended for 3D printing was set up, and development of an industrially viable 3D printing process for two-compartment capsule shells was started. It was thereby possible to demonstrate the suitability of the produced filaments and fabricate prototype capsule parts meeting pre-set elemental, microbiological and by-product specifications. Manually filled and assembled capsular devices showed the desired two-pulse release of caffeine, in agreement with the swellable/erodible nature of the starting polymer.

Overall, the research activity performed led to outline an HME operating chart highlighting critical process variables and parameters that would serve as indices of filament quality (Figure 10a). Moreover, the results from the present work supported feasibility of FDM in fabrication of capsule shells within an industrial environment according to CGMPs for dietary supplements. Particularly, a compliant FDM printer was designed and built up, and related operating as well as product quality parameters were established (Figure 10b). The development of a filling station to be coupled with the 3D printer is ongoing, which would enable on-demand production of small batches of capsules having customized composition characteristics (*i.e.* type, amount and release mode of dietary ingredients) in a single step, with the additional advantage to have some of the quality parameters monitored in real-time (*e.g.* weight and thickness).

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Table 1: Molecular weight data obtained by GPC from PD1, HMELab and FDMLab.

Table 2: Process parameters for the production of HPC filaments.

Table 3: Internal quality specifications set for the filament.

Table 4: Weight and thickness of hollow and middle parts of a two-compartment capsular device.

Table 1: Molecular weight data obtained by GPC from PD1, HMELab and FDMLab.

		M_n	M_w	D
PD1	**peak 1**	12000	63500	5.3
	peak 2	628	632	1.0
HMELab	**peak 1**	27500	111000	4.0
	peak 2	627	629	1.0
FDMLab	**peak 1**	27100	113500	4.2
	peak 2	662	675	1.0

Table 2 revised

Table 2: Process parameters for the production of HPC filaments.

Barrel temperature, °C	T_1, 90, T_2 130, T_3 170, T_4 130
Extrusion head temperature, °C	130
Screw speed, rpm	26
Cooling temperature, °C	10
Pulling speed, m/min	1.6
Pulling force, kgf	0.08

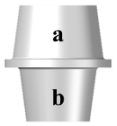
Table 3: Internal quality specifications set for the filament.

Quality parameters		Specifications
Diameter		1.75 ± 0.05 mm
Roundness index		0.989-1.011
Microbiological attributes	Total aerobic microbial count	$< 10^3$ cfu/g
	total combined yeast and mold count	$< 10^2$ cfu/g
	E. Coli in 10 g	absent
	Pseudomonas in 10 g	absent
	S. Aureus in 10 g	absent
	Salmonella/Shigella in 10 g	absent
Heavy metals	Lead	< 1 ppm
	Arsenic	< 0.5 ppm
	Cadmium	<0.3 ppm
	Mercury	< 1 ppm
By-products		absent*

*no significant differences in FT-IR and ^{1}H-NMR spectra as well as in rheological data compared with the starting powder

Table 4

Table 4: Weight and thickness of hollow and middle parts of a two-compartment capsular device.

		Joint	Hollow parts	
				
			Thinner compartment	Thicker compartment
Weight, mg (cv)		163.40 (5.83)	130.98 (4.82)	208.72 (4.85)
Wall thickness, µm (cv)	a, 800*	802 (12)		
	b, 800*	791 (14)		
	a', 400*		405 (18)	
	b', 800*			814 (10)

*nominal thickness, µm

Figure 1: Stress *versus* strain curve for a PLA filament. The linear range for Young Modulus (E) determination and the value of σ* are highlighted.

Figure 2: DSC curves from PD1, HMELab and FDMLab.

Figure 3: (a) FT-IR, (b) ^{1}H-NMR and (c) ^{1}H-NMR on the methanolic extract spectra from PD1, HMELab and FDMLab.

Figure 4: GPC curves from PD1, HMELab and FDMLab.

Figure 5: Schematic of the microextrusion system employed: comprehensive view and details relevant to (a) stirrer, (b) first four flights of the screw and (c) extrusion head.

Figure 6: Schematic of the 3D printer employed: (left) comprehensive view and (right) details relevant to (a) gantry and (b) extruder assembly.

Figure 7: Printed hollow and middle parts of a two-compartment capsular device.

Figure 8: Photographs of a capsular device including two compartments of 400 and 800 μm wall thickness, filled with yellow and blue dye, respectively, taken at successive time points during immersion in unstirred water.

Figure 9: (a) FT-IR and (b) ^{1}H-NMR spectra from PD2, FDMInd175 and FDMInd180.

Figure 10: Operating charts reporting critical process variables and product quality parameters relevant to (a) HME and (b) FDM (potential application to automated in-process capsule filling enclosed in the dotted frame).

Figure 1

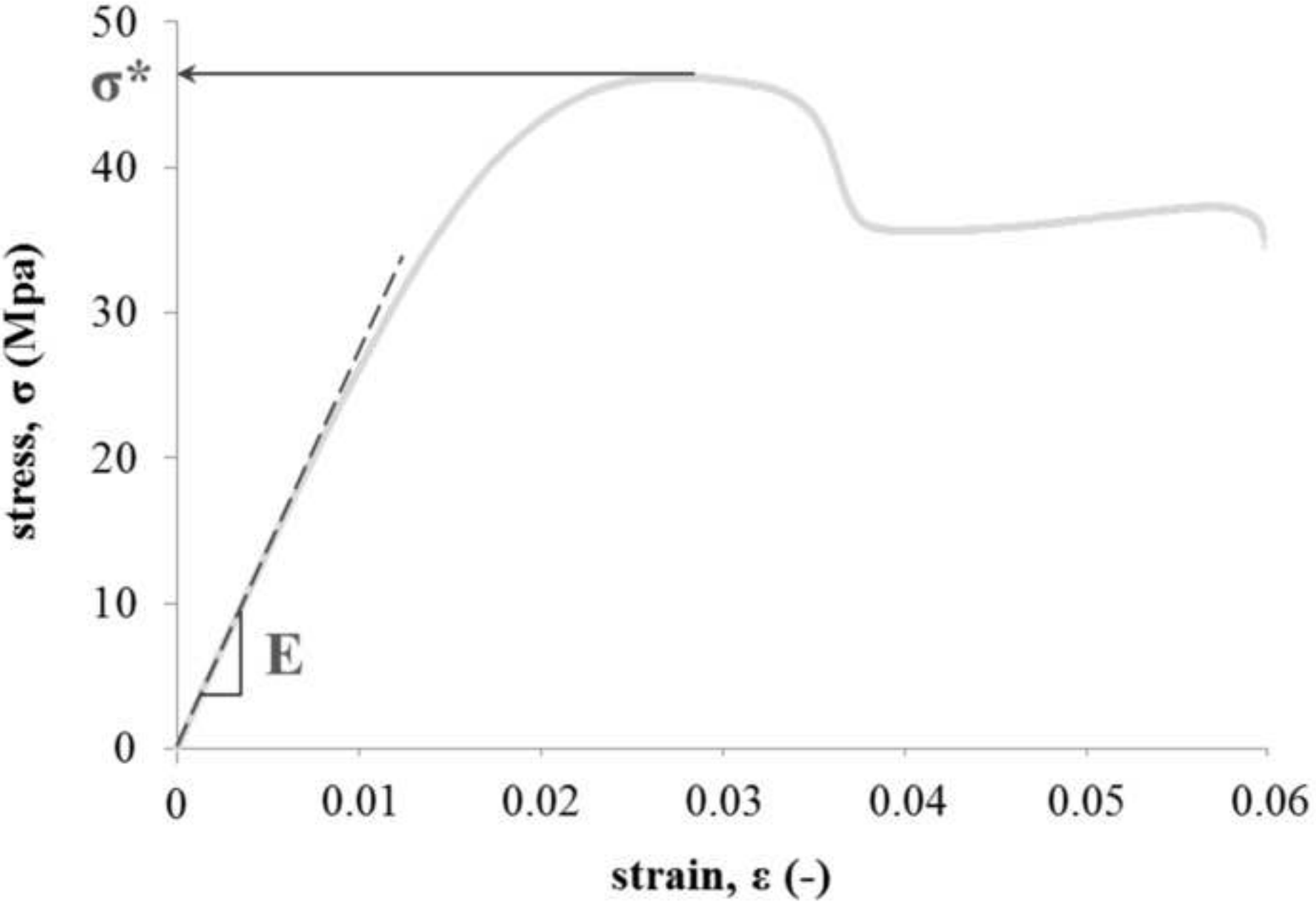


Figure 2 revised

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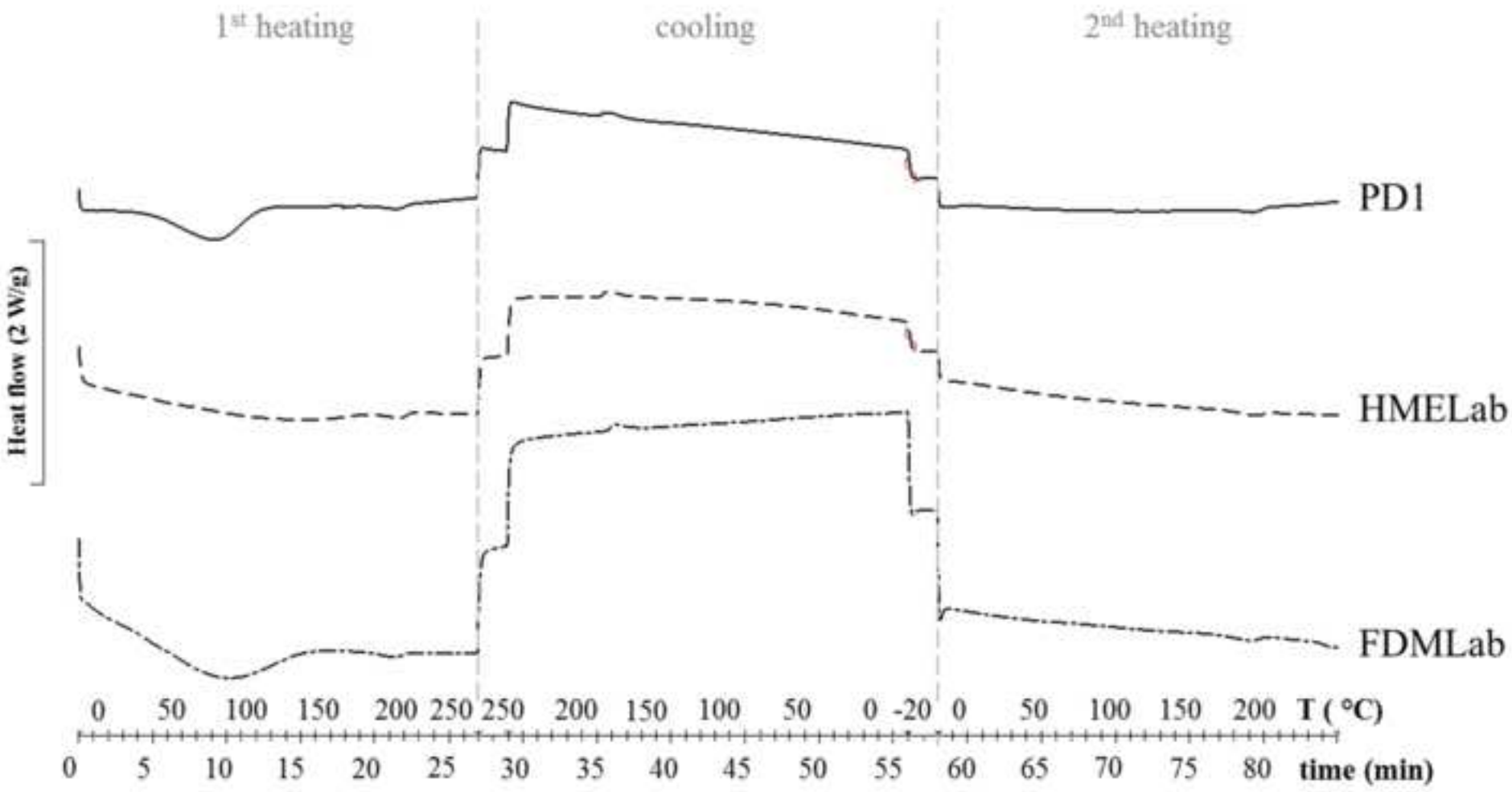


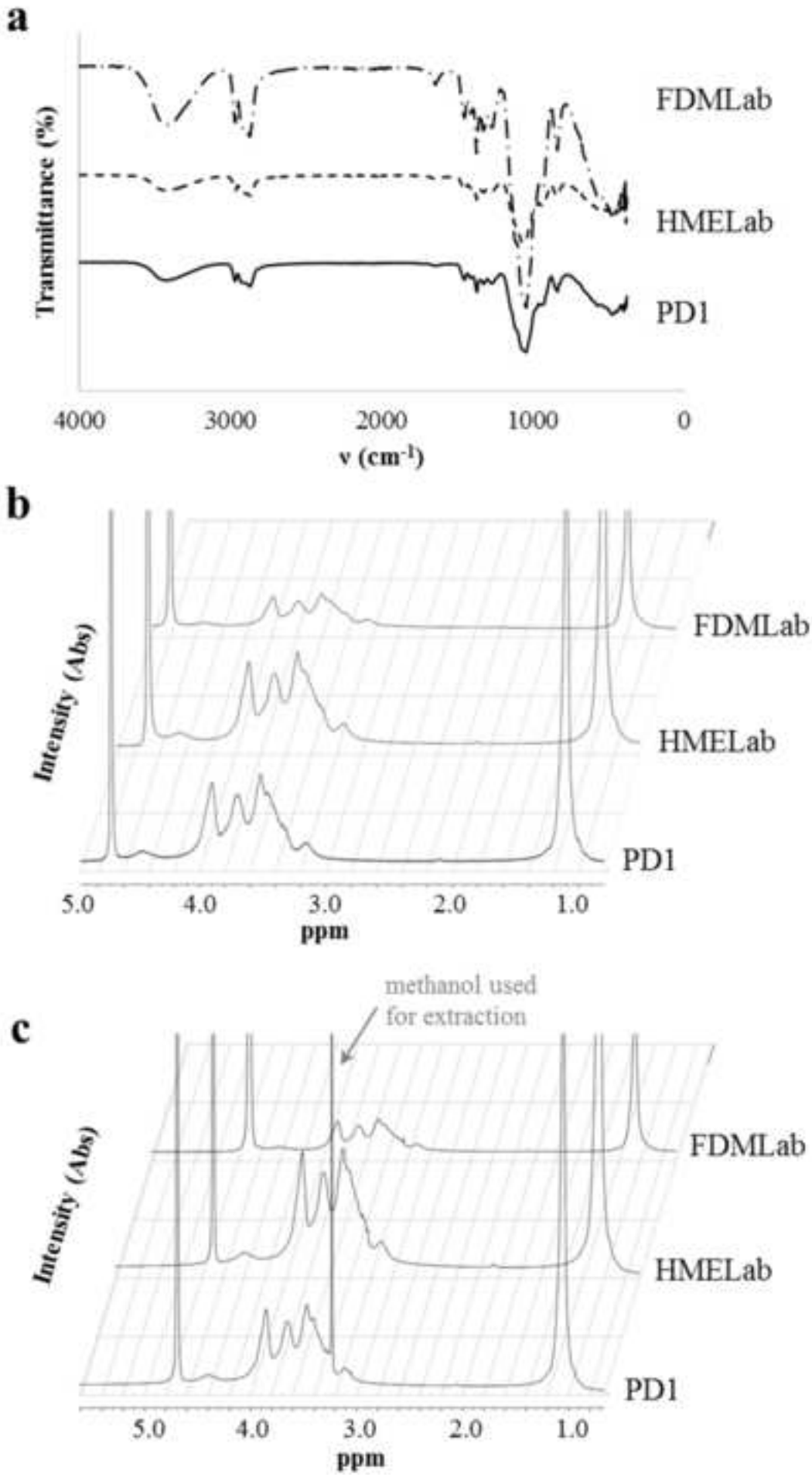
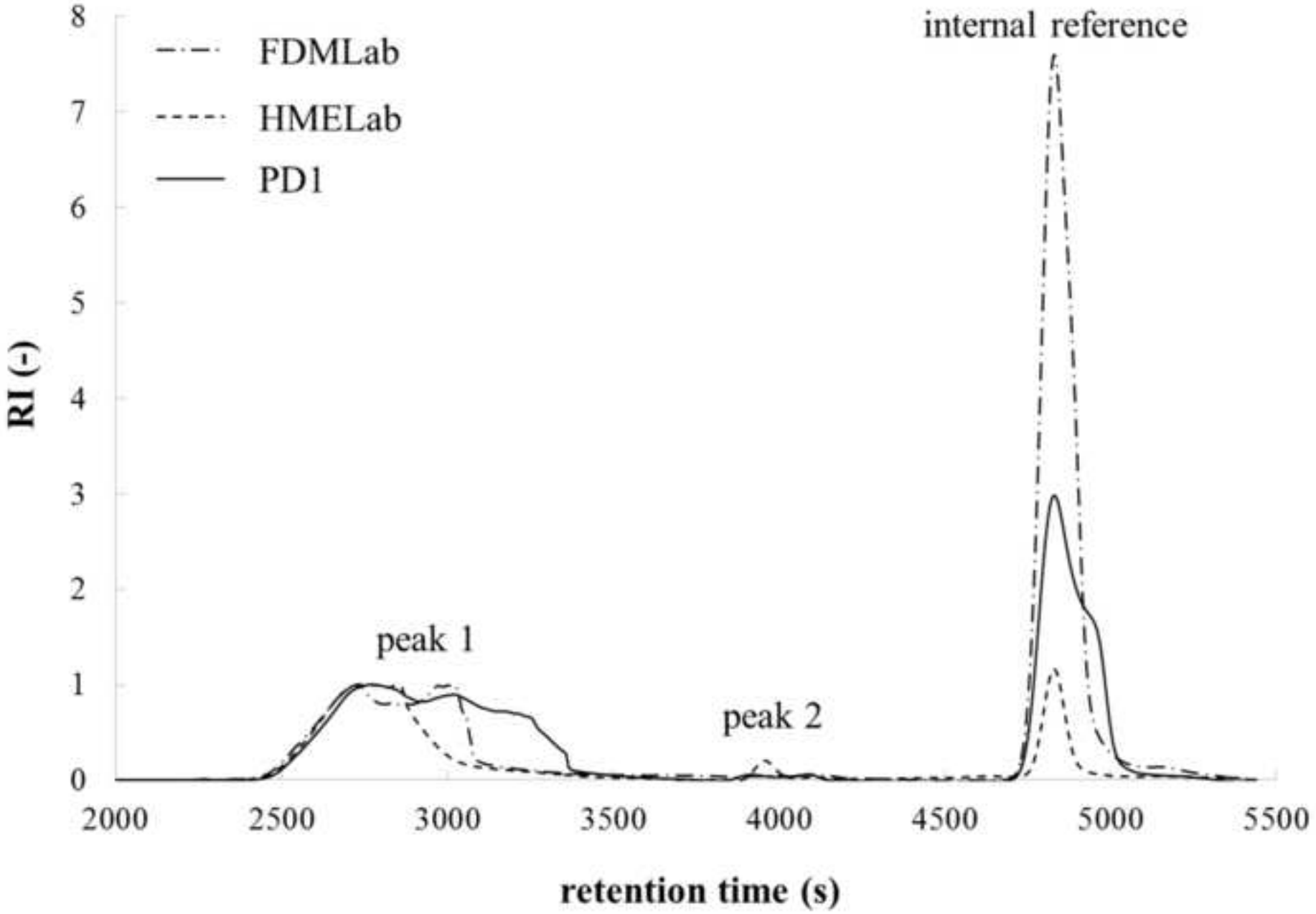
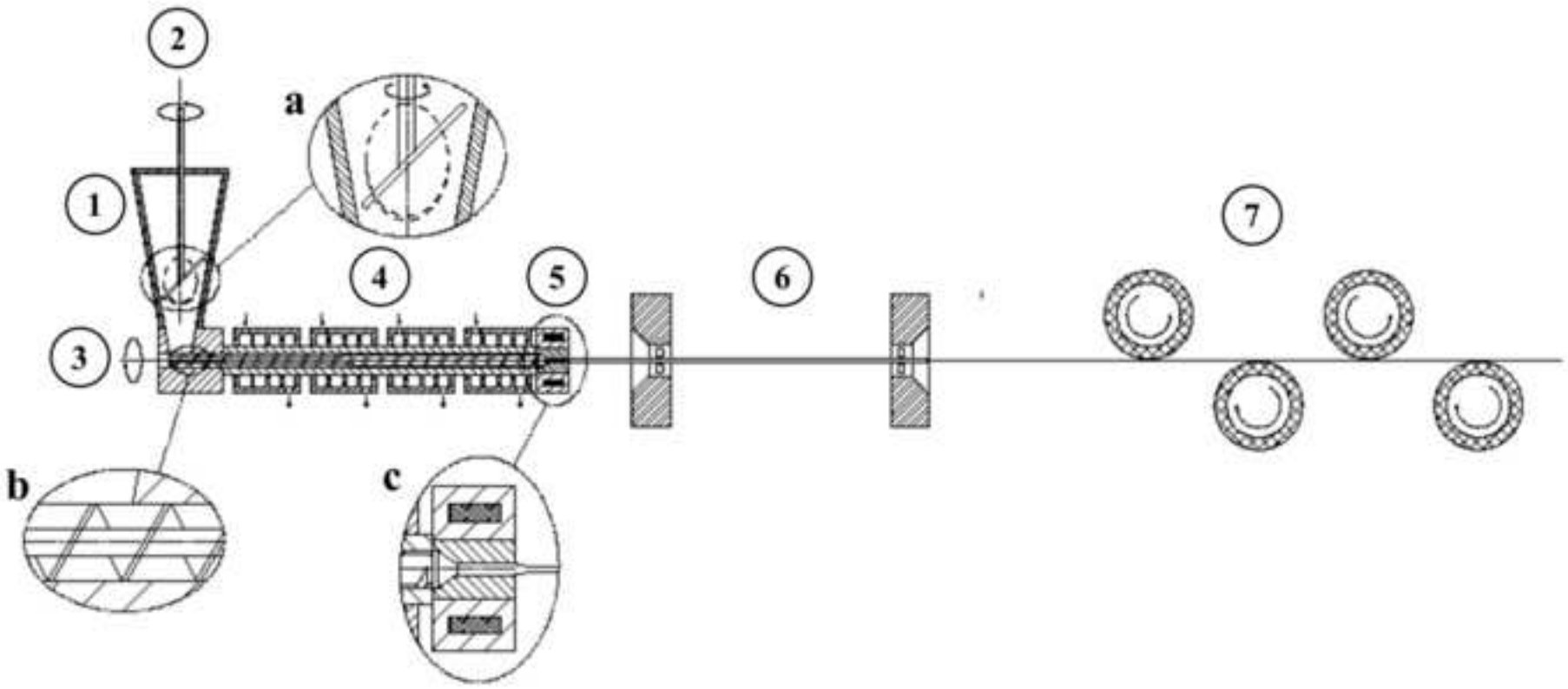
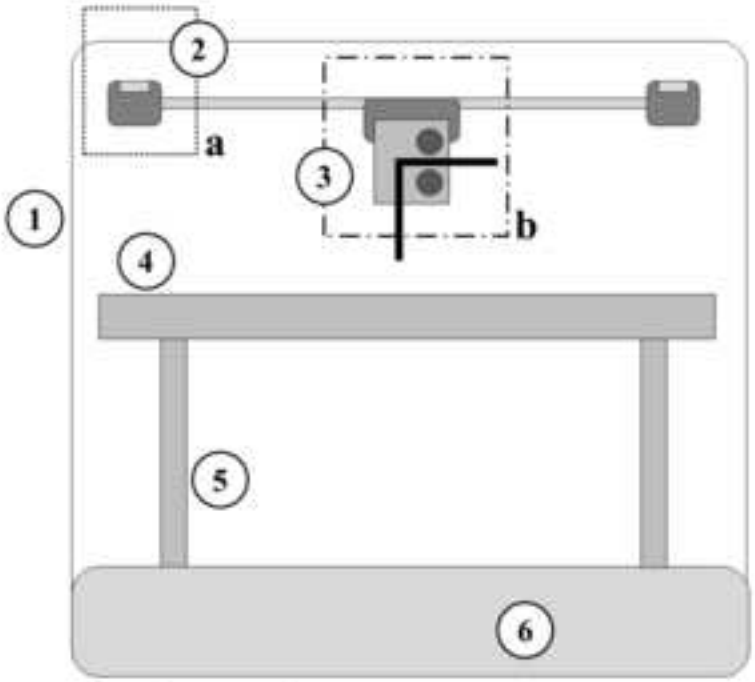


Figure 4

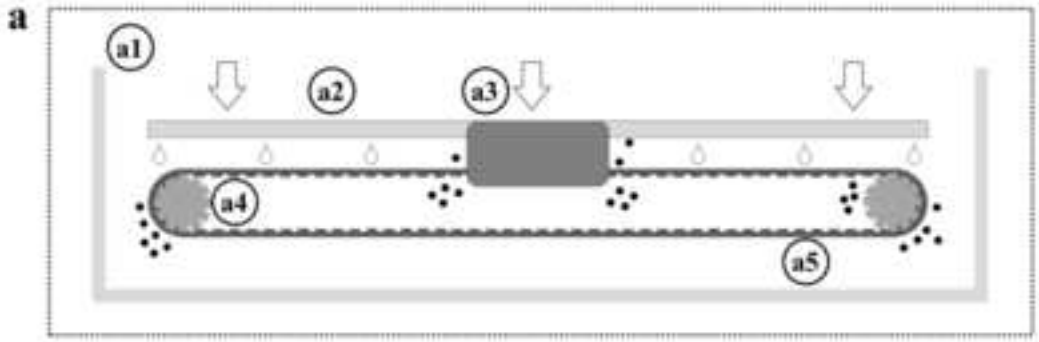




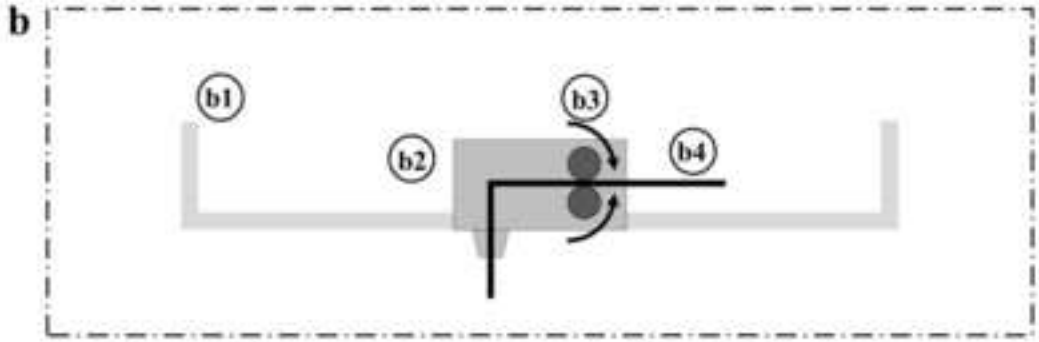
1. thermoregulated hopper
2. angled-palette stirrer (see detail a)
3. screw (see detail b)
4. four-zone barrel
5. extrusion head (see detail c)
6. double-laser units
7. haul-off system with coated rollers



1. printer enclosure
2. x-y axis gantry assembly (see detail a)
3. extruder assembly (see detail b)
4. printing plate
5. z-axis linear guide for printing plate
6. base containing control electronics



a1. particle containment system
a2. linear guide
a3. carriage
a4. pulley
a5. timing belt



b1. particle containment system
b2. extruder assembly
b3. feeding system
b4. HPC filament

Figure 7 revised

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Figure 8 revised

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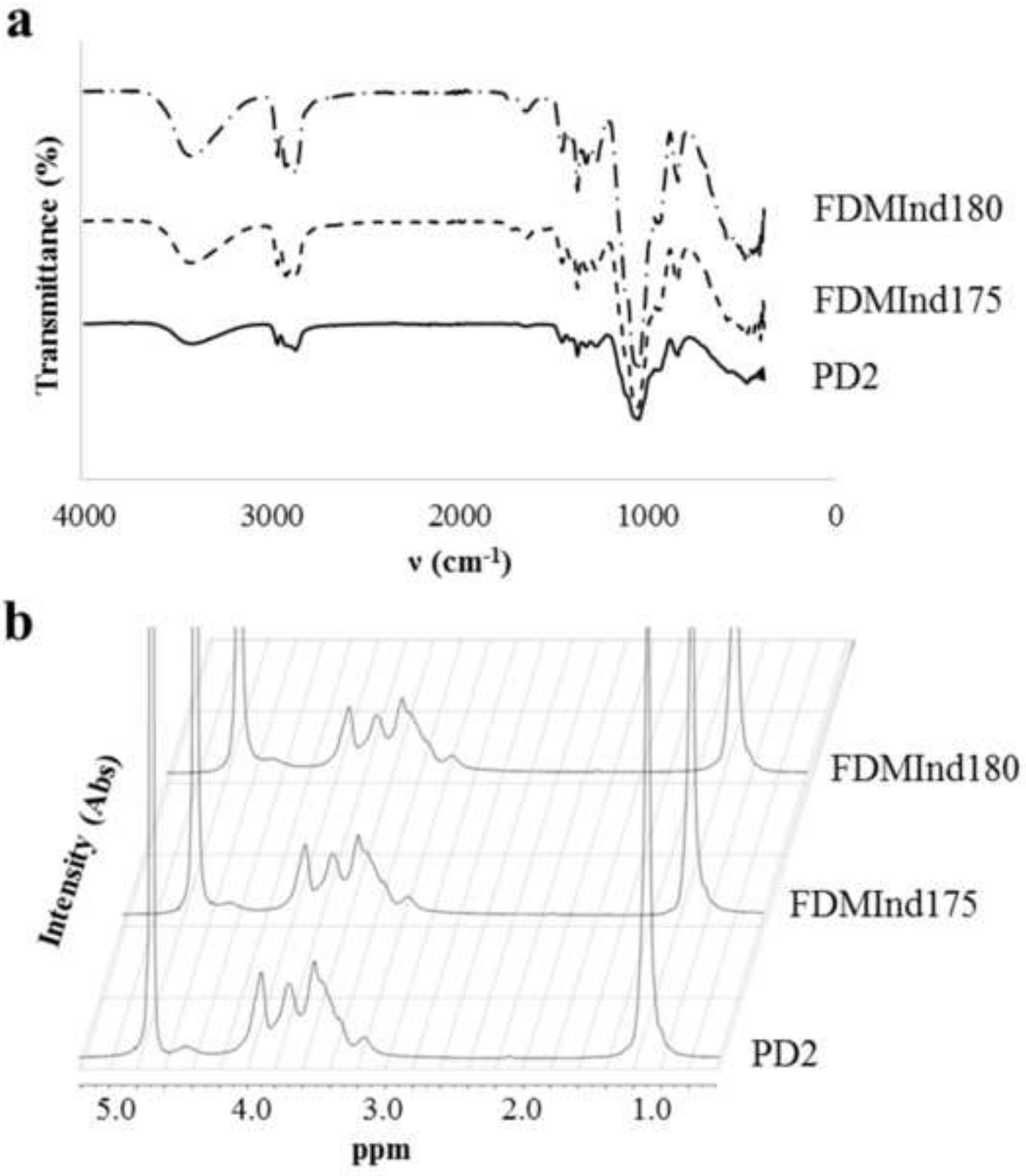

Figure 10 revised

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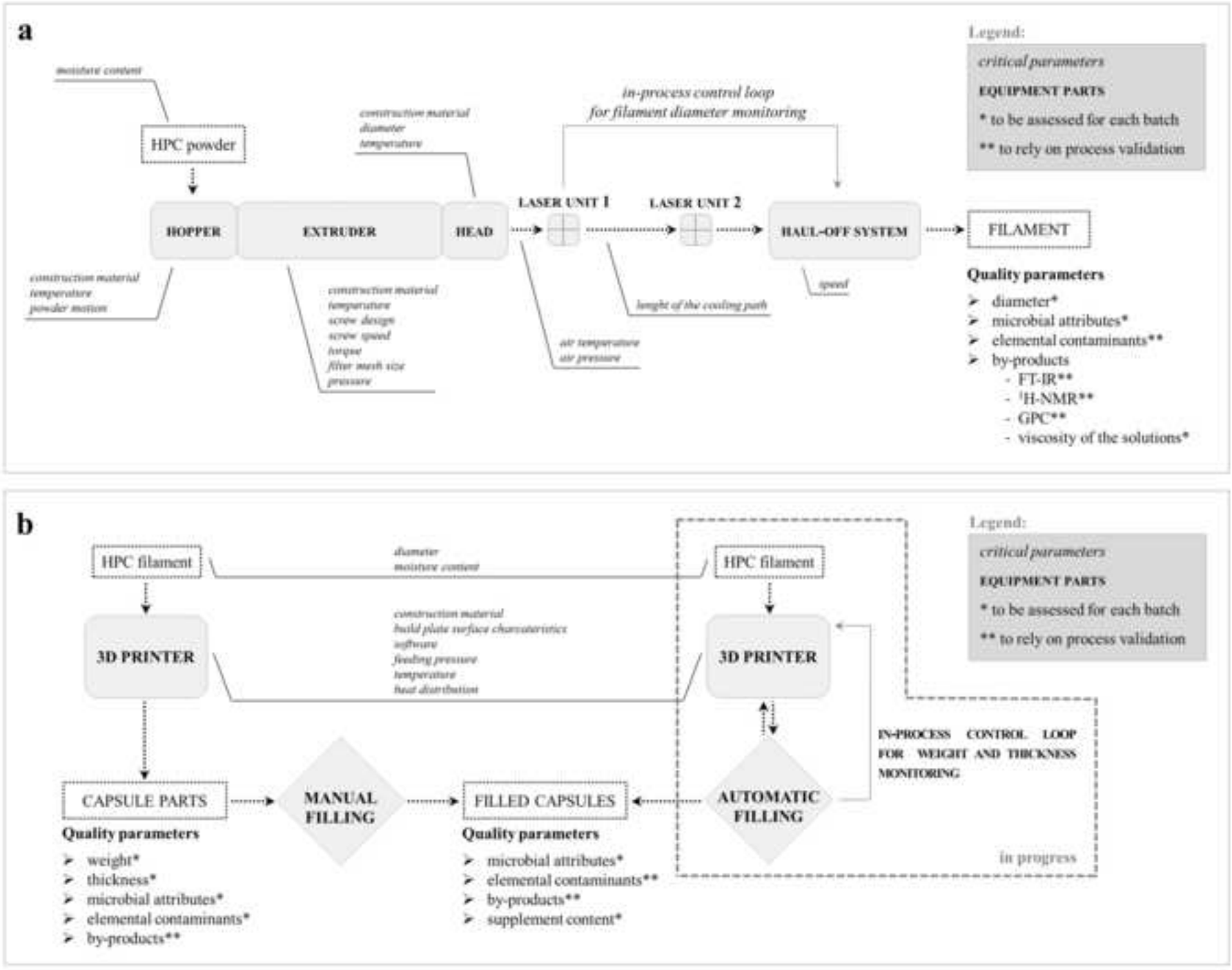

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