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

Dear Robert O. Williams III, Ph.D.
Editor-in-Chief of AAPS PharmSciTech,

also on behalf of co-authors, I would like to thank the Reviewers for providing helpful comments. Please find below a point-by-point outline of how the manuscript has been modified accordingly.

Reviewer #1:
At the example of pulsatile release capsules with two compartments Melocchi et al. present an overview of how fused deposition modeling based printing techniques could be translated from desk based hobby printers to industrial production. The study is interesting but unfortunately weakened by the lack of detail and rigor which results in a superficial contribution. If the authors cut down on the introduction and add further data and detail regarding the production processes it could be suitable for this journal. In its present form it reads more like a contribution to a glossy magazine.
The introduction is rather detailed with regards to the market opportunities for nutraceuticals and I feel the manuscript would benefit from cutting some of this information as it is not relevant for the topic of study.
We agree with the referee that our manuscript could benefit from reduction of the introduction, especially as regards passages relevant to the nutraceutical market. For this reason, we have cut down related text and also literature references. According to the referee's criticisms about the need for improving the information on the production processes, further details (Figures included) have been added, as highlighted by point-by-point responses.

l. 121 'specially-assembled prototype equipment' provide detail
We have added details on how the extrusion plant was devised to the Results and Discussion section, along with a related schematic (Figure 5).

l. 123 'After production, these filaments were characterized for diameter, using a digital caliper' - what do you mean by that? Did you measure the diameter of the filament at select locations? If so, what was the protocol that was used?
The filament diameter was measured every 30 cm in length, normally to the filament axis in two mutually perpendicular directions.

l. 183 'isothermal scansion' - is that a typo?
"Isothermal scansion" was an oversight and has been changed in the text into "isothermal analysis".

l. 189 'resolution of 4.0 and' - units missing, presumably cm-1
The referee is right. Unfortunately, the unit of measurement (cm^{-1}) was missing and has been added to the text.

l. 201 'the polydispersity index Mw/Mn.' I think this should just be referred to as the 'dispersity' rather than the 'polydispersity index' according to IUPAC recommendations.
'Polydispersity index' has been changed into "dispersity" according to IUPAC recommendations, as suggested by the referee.

l. 297 'A special system was designed to keep the powder inside the hopper at 30 °C and in continuous motion, thereby avoid caking phenomena (i.e. adhesion and cohesion of powder particles without effective feeding of the microextruder). Moreover, the geometry of the screw was purposely conceived to allow efficient movement of the powder within the first part of the barrel as well as proper flow of the melt beyond.' One of the areas I feel this manuscript could improve in is adding actual information to the text. A lot of passages, such as this one highlighted above, are very vague and read like a marketing brochure rather than a scientific paper. A lot of the novelty in the present work is to demonstrate the feasibility of the methods for commercial production and hence these details need to be provided. If the authors feel this would compromise proprietary information that would compromise commercial exploitation they should reconsider publishing the work as it is not sufficient for this journal as it stands in my point of view.
We agree with the referee that our manuscript would benefit from improving information on the production processes. Accordingly, details and schematics (current Figures 5 and 6) about microextrusion and 3D printing have been added to the Results and Discussion section.

'A strict control of extrusion temperature was needed because of the impact of such a parameter on the pressure developed by the melt according to its rheological characteristics. In this respect, the temperature was progressively increased along the barrel and decreased in its last zone as well as in the die.' There is no quantitative information here. What rheological requirements are in place? How was this achieved?

The rheological properties of hydroxypropyl cellulose melts and their relationship with temperature were described in a previous paper. In particular, the viscosity of the polymer showed a tendency to decrease at temperatures above 140 °C. However, stability issues were highlighted at higher temperatures, also depending on the exposure time. For this reason, the sequence of temperatures selected along the barrel was 90, 130, 170, and 130 °C, while the die was maintained at 130 °C, as reported in Table 2.

The above-mentioned previous paper has now been quoted in this respect (reference 2) and a comment has been added to the manuscript.

l. 309 'using an air flow under pressure' - do you mean pressurised air?

The referee is right, it was pressurized air and we have changed the text accordingly.

l. 315 '...and only exerting a mild pulling [force] in order...' - there is no mention of any means of pulling the rod from the extruder; again there is a complete lack of engineering detail. This could be quite critical if the pulling process results in a preferred order of the macromolecular chains and hence a change in crystallinity.

We have added information to the Results and Discussion section about the haul-off system that was used to pull the rods from the extruder. This system was also included in the schematic (current Figure 5) that was drawn to comply with the referee's comment on the need for providing engineering details about production process. In contrast to extrusion plants commonly used for filament production, wherein the diameter of the extrusion head cavity can even be markedly greater than that desired for the filament and the latter has thus to be pulled in order to meet diameter specifications, in the plant we developed the extrusion head was devised and constructed so that the diameter of its cavity would exactly correspond to that of the final product. Therefore, the haul-off system was only needed to hold the outgoing filament and trail it through the double-laser units without causing deformation, deflection or rupture, as well as to ensure that minimal dimensional changes (< 5% of nominal 1.75 mm diameter) would be counteracted. The selected pulling parameters (please see Table 2) and the regulation of the mutual position of the pulling rollers coated with an FDA-approved elastomer allowed these goals to be achieved. All the above concepts have now been specified in the text.

We also agree with the referee on the possibility of a preferred orientation of the macromolecular chains of HPC as a consequence of pulling. In this respect, however, the potential impact of the very mild pulling exerted was disregarded because of the filament quality proving acceptable with respect to Current Good Manufacturing Practices (CGMPs) for dietary supplements and suitable for printing of the nutraceutical delivery system.

l. 353 'and moving mechanical parts were insulated in order to prevent the build plate from contamination'. What do you refer to here? In the absence of any technical drawings or schematics this is impossible to interpret.
We understand the difficulties encountered in interpreting this passage. Therefore, we have added a schematic that may hopefully help, and a more detailed explanation has been introduced into the text.

l. 385 '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.' That makes sense intuitively but I wonder whether there would be significant impact on the porosity of the capsule wall if one were to print the wall in more layers. There has been quite some interest in how the microstructure of a printed dosage form needs to be considered when one determines the performance of a printed dosage form. See for example Pharm. Res., 2017, 34, 1037-1052 and J. 3D Print. Med., 2018, 2, 27-33. You refer to 'thickness consistency' (l. 392) but the point raised by Markl et al. in the two papers cited above is equally, or even more, critical i.e. the pore structure and connectivity that is a direct result of the printing process that is inherent to all fused deposition modeling technologies.
We agree with the referee about the possible impact of multiple layer deposition on the microstructure of the printed items and are aware of the literature studies on this topic. It would be very interesting to investigate this aspect, and we will be committed to go into depth in the specific field in the future. Currently, we are experiencing the issue of porosity changes associated with attempts at increasing printing resolution when dealing with insoluble/diffusive barriers (e.g. based on ethylcellulose), and promptly soluble layers (e.g. based on polyvinyl alcohol/polyethylene glycol graft copolymer). However, since the goal of our work was to accomplish early industrial development of 3D printing for a nutraceutical product, we actually focused on the attainment of a proper balance between production time and resolution of the printed capsule on the one hand, and of the desired release performance on the other, which was fully met by the obtained prototypes. By the way, the extent of variability in lag time ($t_{10\%}$) before release from capsules was fairly limited in

spite of the greater variability exhibited by their shell thickness. It is indeed known that, when hydrophilic polymers, such as hydroxypropyl cellulose, are employed as main components of swellable-erodible barriers, porosity poorly affects the permeability of such barriers following swelling (A. de Leyva et al., Critical points and phase transition in polymeric matrices for controlled drug release. In: V. K. Thakur, M. K. Thakur Eds., Handbook of Polymers for Pharmaceutical Technologies, Structure and Chemistry, volume 1, pp. 101-135, John Wiley and Sons, Hoboken, US-NJ, 2015). This would be due to the glassy-rubbery transition of the polymers in contact with aqueous fluids and formation of a gel layer, which may offset initial differences in the matrix structure.

Figure 2: There is no y-axis provided for the DSC traces. The resolution is too poor to read the x-axis.

Because in Figure 2 a series of DSC traces is shown, wherein the various curves would overlap when using a graduated y-axis, this has been left out to have the thermograms spaced and make them visible. However, in order to comply with the comment of the referee, we have added the concerned parameter (heat flow), the relevant interval axis and its value in brackets, as found in analogous works.

In both axes, we have now used a larger font size to improve readability. As regards resolution, all the images loaded met the journal requirements (1200 dpi).

Figure 3: Y-axis is missing in all subfigures. X-axis in subfigures b and c is too small and impossible to read.

We have added y axis in all subfigures, along with the concerned parameter and its unit of measurement. In both axes, we have now used a larger font size to improve readability. Moreover, we have enlarged all the subfigures. As regards resolution, all the images loaded met the journal requirements (1200 dpi).

Figure 7: y-axis missing, same font size problems as Figure 3

For Figure 7 (current Figure 9) please see responses to the above comment relevant to Figure 3.

Figures 8 and 9: these are simplified illustrations but it is not clear to me that the outlined parameters are exhaustive and/or authoritative. There is no systematic experimental or theoretical underpinning presented in the present study and these figures suggest a much better level of process understanding than is actually demonstrated in this study.

Figure 8 and 9 (gathered in current Figure 10) report schematics that were drawn for clarity and integration purposes, summarizing the work done in order to face the early industrial development of a nutraceutical product in compliance with CGMPs for dietary supplements. The latter require that any step in the manufacturing process where control is necessary be identified and relevant specifications be set, to ensure the quality of the product (for instance by setting specifications for components, for the in-process production and for the finished batch). All the actions taken, and depicted in current Figure 10, ultimately allowed to fulfill such goals, as testified by the successful outcome of inspection of the Multiply Labs production site in San Francisco and review of the inherent quality assurance system by California Public Health Department, Food and Drug Branch (Processed Food Registration n. 97587, expiration date 10/12/2018).

Reviewer #2:

The manuscript titled "3D printed capsular devices for personalized supplementation" deals with the physico-chemical characterisation of hydoxypropyl cellulose (HPC) filaments intended for fused deposition modelling, and with the analysis of possible degradation products after the extrusion of HPC under different conditions.

The "device" mentioned in the manuscript title is in fact just a two-compartment capsule with two different wall thicknesses, which has already been published several times by the same authors. There is no new idea or scientifically relevant finding in the current manuscript. In fact, the manuscript resembles more a technical report for GMC process validation rather than a scientific publication.

Actually, the concept of a two-compartment capsular device was first and only proposed in Maroni A, Melocchi A, Parietti F, Foppoli A, Zema L, Gazzaniga A. J Control Release. 2017;268:10-8 (reference 5 in the manuscript), which reported the original design of the system, now modified and improved, along with preliminary data about the relevant fused deposition modeling (FDM) feasibility starting from a range of materials. By the way, such materials did not include hydroxypropyl cellulose (HPC) that was studied and used in the current work.

The underlying idea and the relevance of the scientific findings presented in this work reside in the particular subject dealt with, i.e. the early industrial development of 3D printing for the manufacturing of a nutraceutical product, which bring about peculiar technical and regulatory implications and has never been faced before to our knowledge. Notably, this involves that process compliance with regulatory requirements and quality attributes consistent with safe product intake

have not been addressed in any previous pharmaceutical/nutraceutical literature articles on FDM. Useless to say, all FDM equipment used so far for printing of dosage forms could never be acceptable from a regulatory point of view due to their construction materials (e.g. aluminum, brass), cleaning limitations and possible contamination issues. Moreover, safety requisites for the resulting printed items have never been taken into proper account.
In the end, we deem that the particular topic we focused on well matched the subject area outlined by the invitation to contribute we received from the Guest Editors of the Journal Issue on Additive Manufacturing, specifically mentioning "still missing broadly accepted processing principles, key equipment, optimized excipients, computational methods, as well as quality control tools for additive manufacturing of innovative pharmaceuticals".
We do regret that the referee did not catch any of these hints from our manuscript.

The title of the manuscript is misleading. There is absolutely nothing about "personalized supplementation" in the actual manuscript. Figure 6 shows two different colorants, but that does not warrant the title (similar figures have been published in previous papers about this two-compartment capsule). The manuscript just repeats a previously published (several times) concept of a two-compartment capsule, without providing any credible data as to why should delayed pulsatile release of different supplements provide any pharmacokinetic advantage over taking all of them at once with food. The actual technical focus of the manuscript is thermal stability of HPC during filament extrusion in different types of extruders, so this should be in the title. I am aware of the sensationalist tone that many publications nowadays follow in an effort to increase citation rate (it is an unfortunate reality that many people cite papers because of their title without actually reading them) but I hope AAPS will not go down this way.
We agree with the referee that the focus of the manuscript is not exactly on supplement personalization. The manuscript actually deals with industrial development of HPC-based capsular devices intended for personalized nutraceutical delivery. This also involves assessment of HPC stability after hot-processing, which thus plays a pivotal role in the experimental plan. According to the referee's comment, we have now changed the title of the manuscript to better reflect its contents, highlighting the central role of HPC and avoiding any emphasis on the potential for personalization. As the development of a fabrication process compliant with Current Good Manufacturing Practices (CGMPs) for dietary supplements was the final objective of the experimental activity undertaken, and the thermal stability of HPC was not the only focus of the manuscript, the new title we propose is "Industrial development of a 3D printed nutraceutical delivery platform in the form of a multicompartment HPC capsule".

In addition, we would like to point out that dye tracers were only employed to monitor the macroscopic behavior of the capsules when immersed in aqueous fluid. The release performance of the system was then studied with prototypes containing caffeine, a dietary supplement among the most widely used.
The possible advantages of having supplement release modified according to the consumers' needs are extensively described in the literature (please see current references 11, 13, 14 in the manuscript), and the goal of our work was not actually to provide credible data in this respect. However, in the specific case of dietary supplements such as caffeine and melatonin, largely used to ameliorate adaptation to daily sleep and awakening rhythms, special benefits would arise from formulation as pulsatile-release systems. Indeed, their effect may thereby be exerted when especially needed without modifying the administration schedule with possible negative repercussion on the consumer compliance.

Finally, I find the manuscript rather poorly structured -- the Results section is just one long monolithic bloc of text without any division or sub-sections, very difficult to read.
We agree that shorter paragraphs relevant to each specific study stage may help reading and understanding of the Results and Discussion section. Following this suggestion, we have now subdivided the text into five briefer sections.

Reviewer #3:

1. On the reference side in the introduction section it may be suggested to add a reference to:
   Sun, Y. and Soh, S. (2015), Printing Tablets with Fully Customizable Release Profiles for Personalized Medicine. Adv. Mater., 27: 7847-7853. doi:10.1002/adma.201504122
   As this paper also describes how to make a 3D printed capsular devices with highly customizable release profiles.
   The indicated reference has been added to the introduction section (current reference 9).

2. In line 373 "Table IV" is cited. Perhaps change it to "Table 4" in order to be consistent.
   Table IV has been changed into Table 4. Thank you for pointing out the oversight.

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

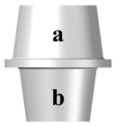
Table 3: Internal quality specifications set for the filament.

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

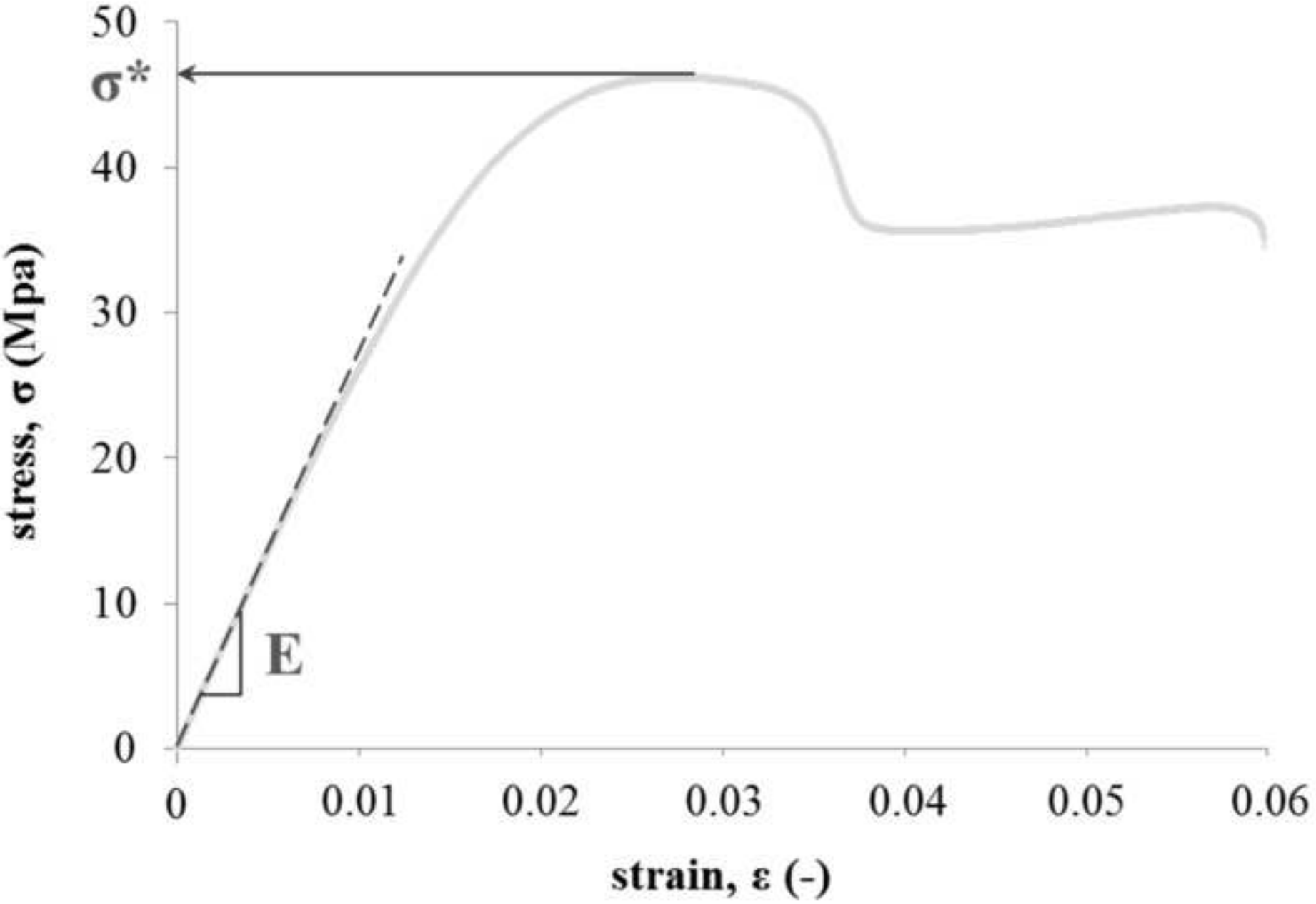


Figure 2 revised

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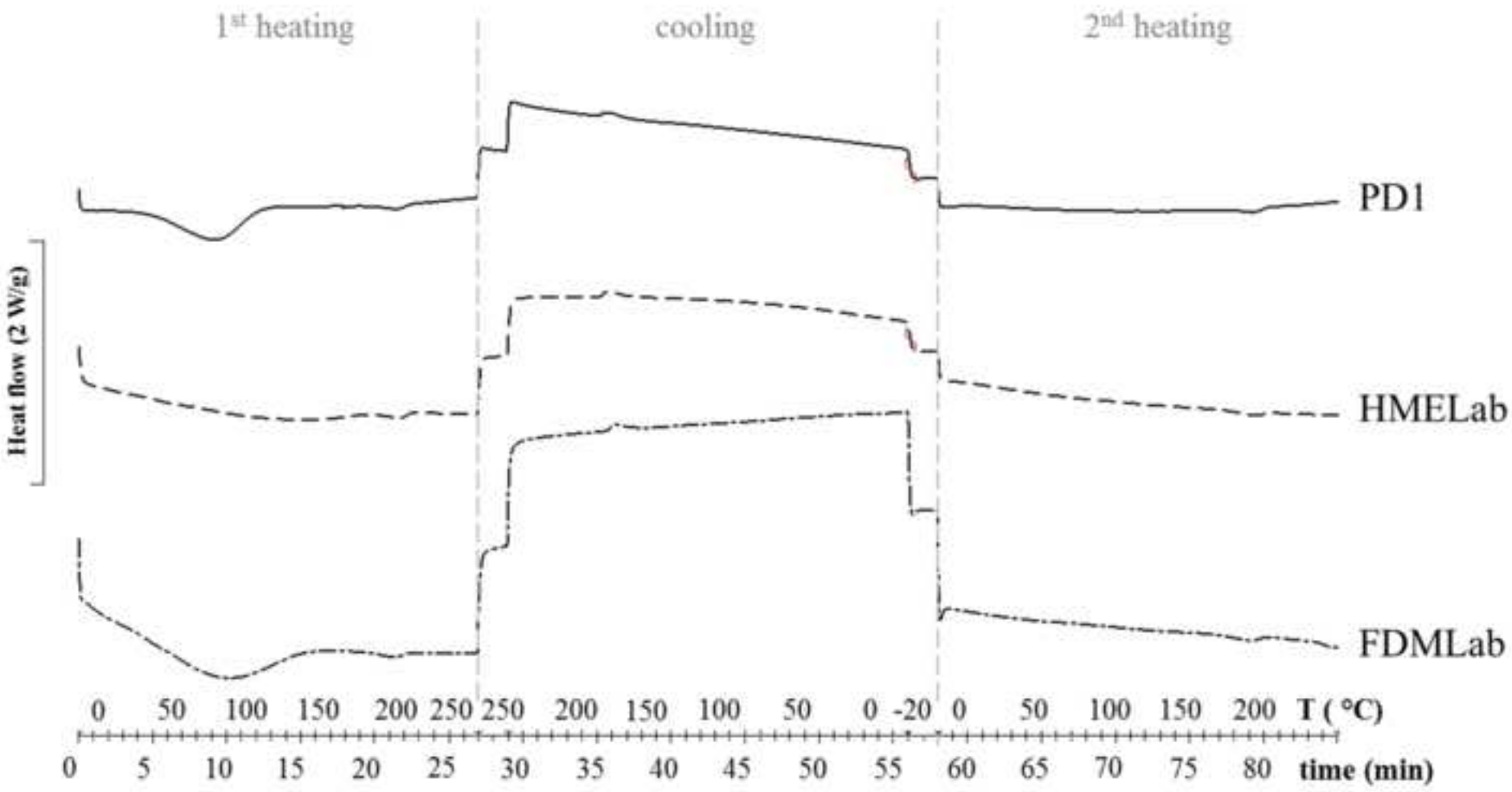


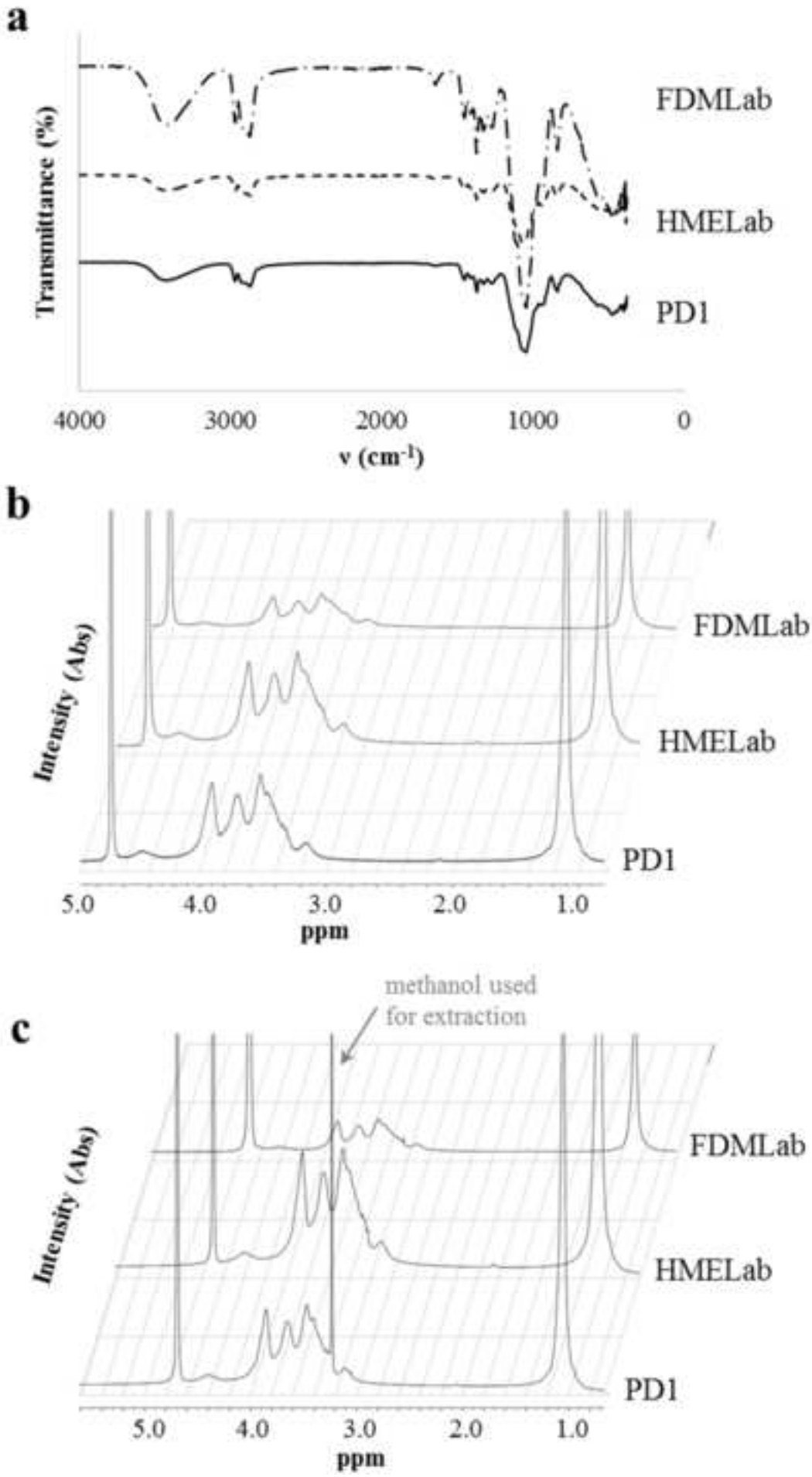
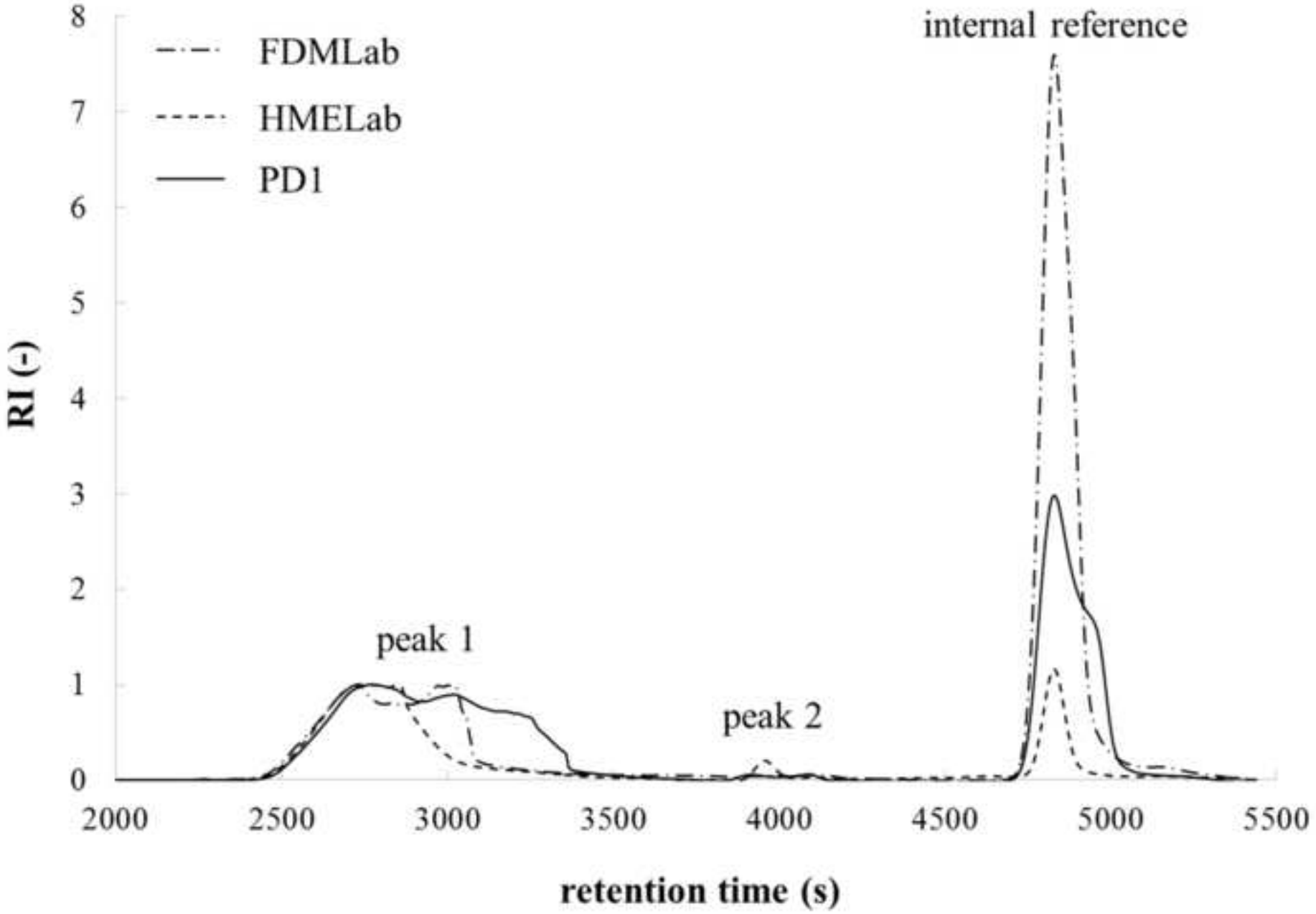
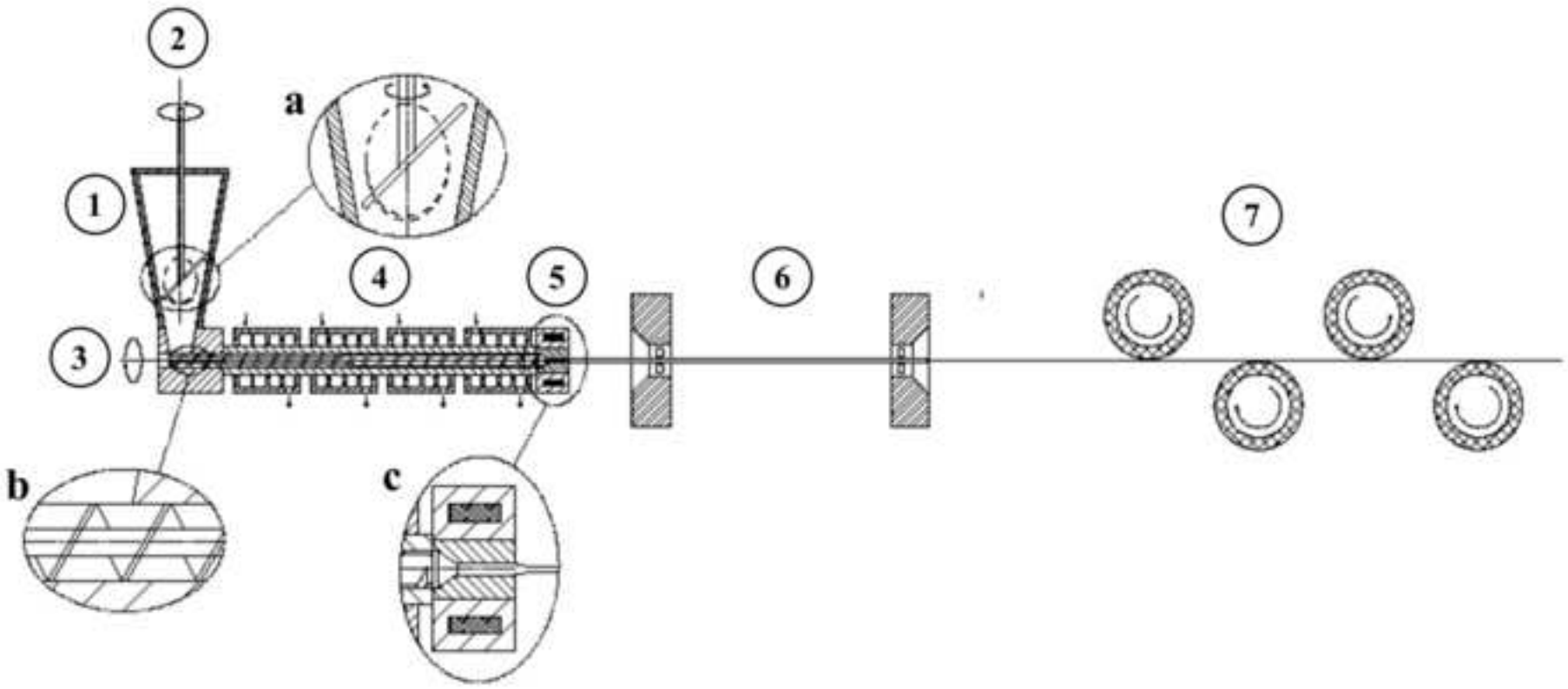
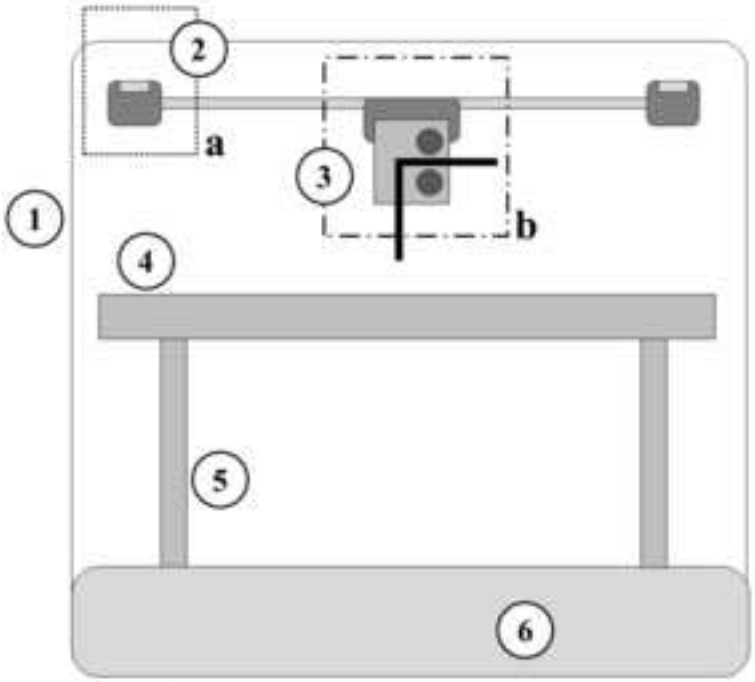


Figure 4

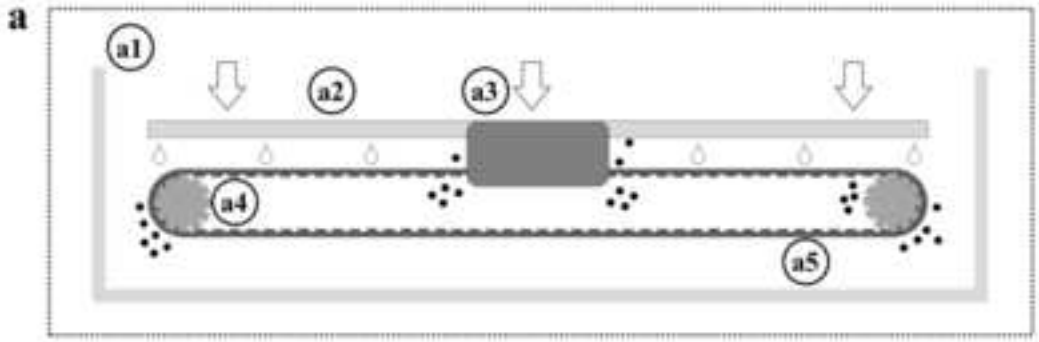




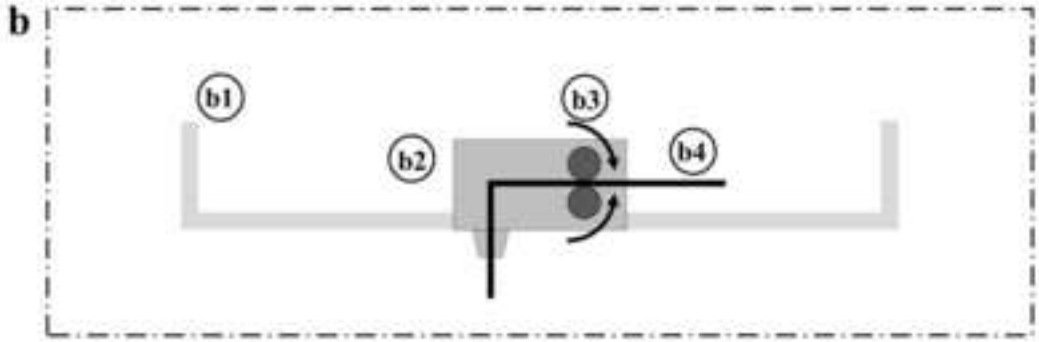
1. thermoregulated hopper
2. angled-palette stirrer (see detail a)
3. screw (see detail b)
4. four-zone barrel
5. extrusion head (see detail c)
6. double-laser units
7. haul-off system with coated rollers



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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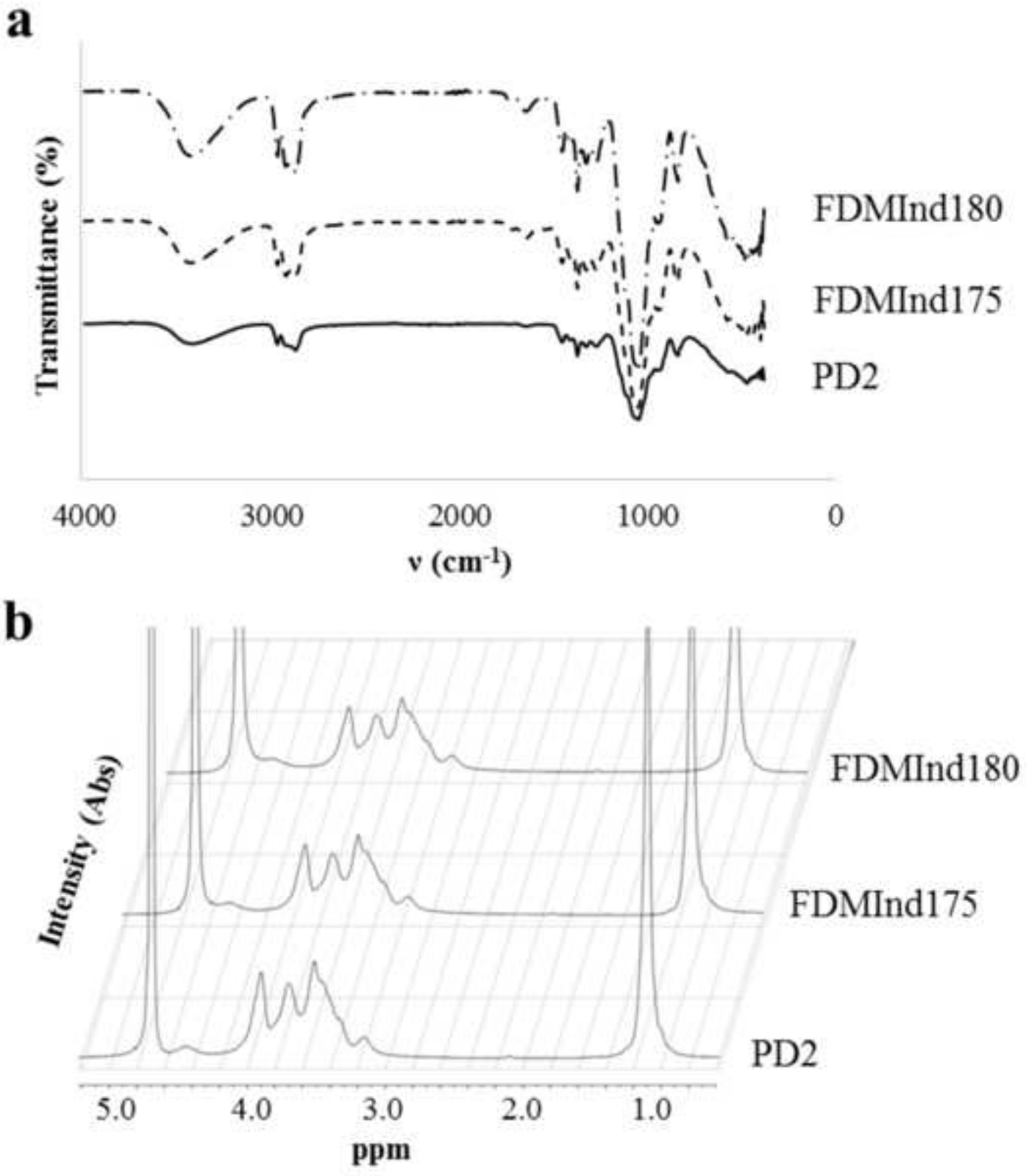

Figure 10 revised

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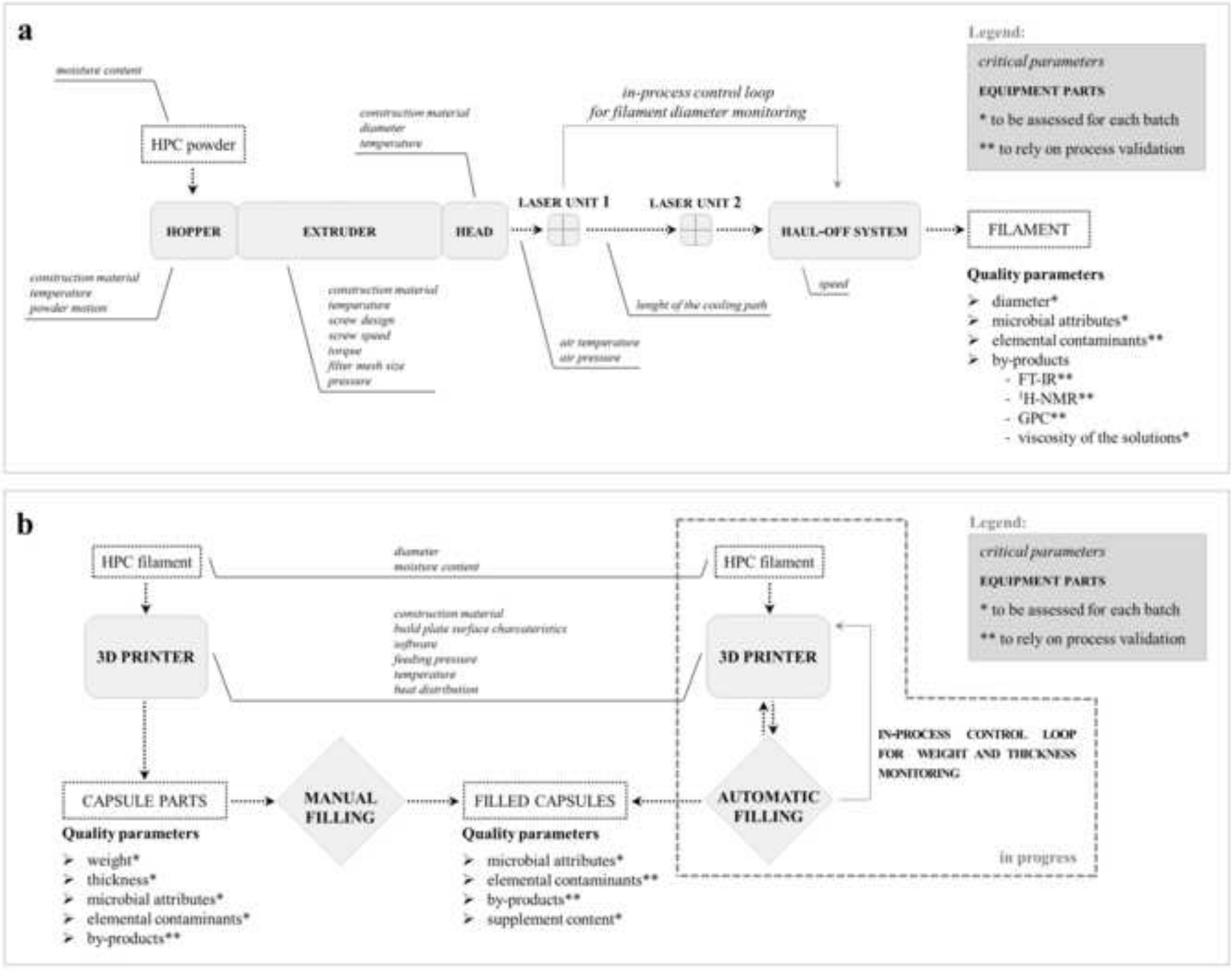

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Journal: ☐ *The AAPS Journal* or ☒ *AAPS PharmSciTech*
Title: 3D printed capsular devices for personalized supplementation

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