Development and analysis of a novel loading technique for FFF 3D printed systems: microwave-assisted impregnation of gastro-retentive PVA modular models

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ABSTRACT

Fused Deposition Modelling (FDM) 3D printing technology was exploited for the rapid prototyping of a modular floating system (caps-in-cap). The optimized models were produced as blank PVA scaffolds, and a morphological analysis on the FDM printed models was conducted to set out an easy re-tune of the desired model with respect to the patients' needs. The printed gastro-retentive systems were then subjected to microwave irradiation in oversaturated solutions of anhydrous caffeine for drug loading, and the research was focused on the impact of the radiations on the chemical and physical properties of the polymer and the drug. Therefore, the drug-loading efficiency, thermal and chemical characteristics of components, the morphology and the stability of the drug and the processed printouts were disclosed. Parameters of this unexplored microwave-assisted post-loading technique were optimized, and the process resulted in the preservation of the polymeric matrix and enhancement of drug adhesion. Hence, the microwave impregnation confirmed its potentialities in supersede the traditional post-loading methods, such as the soaking technique, being faster and more efficient.

1. INTRODUCTION

Stereolithography 3D printing (SLA) offers greater accuracy and reproducibility compared to the more prevalent filament deposition modelling 3D printing (3DP) (M.R. Penny & S. Hilton, 2020). Thus, SLA technology could be exploited for the rapid prototyping of functional tools and preliminary models, in order to obtain the highest resolution during their production.

Fused Deposition Modelling (FDM) manufacturing is one of the most used techniques in the pharmaceutical field for the development of drug delivery systems. In fact, extensive studies have been conducted on the potential use of FDM for the production of medical devices and printed tablets, with bespoke geometries, sizes and release profiles (Pietrzak K., Isreb A & Alhnan M.A., 2015; Kempin W. et al., 2017). However, drug-loaded filaments for FDM-3DP are not available yet, therefore FDM must be coupled with other technologies, i.e. pre-loading *via* hot melt extrusion, in which the APIs undergo thermic processing (Goyanes A. et al., 2015), or soaking post-loading, where the polymeric matrix is loaded through the immersion in a drug-solution, with poor results in terms of loading efficiency (W. Jamróz, et al., 2018).

Microwave radiations are characterized by high and rapid penetration power which has a significant effect particularly on polar compounds. Microwave irradiation of polymers has proved to be an economical, fast and green technique for chemical synthesis, structural cross-linking and drug incorporation (L. J. Waters, S. Bedford & G. M. B. Parkes, 2011).

However, the microwave systems have not been deepened as a post-loading technique of printed structures, thus studies need to be conducted to highlight the potentiality of this method in enhancing the loading of drugs into the polymeric matrix. Therefore, microwave-assisted drug-loading approaches need to focus on the characterization of the impact of the radiations on the chemical and physical properties of polymeric scaffolds and on the stability of the used drug.

In fact, the microwave systems have superior heating features which are based on the quick achievement of high temperatures (up to 300°C) and pressures (up to 30 bar) beyond traditional reflux heating. These characteristics could be applied in the pharmaceutical compounding with the adequate analysis of their effect on the final drug delivery system.

Under the effect of ionizing irradiation, polymers, such as poly(vinyl alcohol) (PVA), may undergo variations in chemical structure and the polymer chains could go through cross-linking or chain scission, depending upon the radiation power, chemical structure, crystallite size and the environment of the analyte (H.F. Afzal et al., 2020).

In this research project, the effect of the impregnation with the model drug caffeine *via* microwave irradiation of PVA floating systems, printed *via* FDM 3DP, was evaluated in terms of drug-loading efficiency, thermal and chemical stability of components, and morphology of the drug and the processed printouts.

Moreover, polyethylene glycol (PEG) (with a melting range of 33-40°C), one of the safest organic co-solvents and pharmaceutical excipients, (IIG limits for oral administration: up to 1.5197 mg) (A.A. D'souza & R. Shegokar, 2016) has been added to improve the post-loading process, exploiting its enhancing properties on the absorbing performances of systems undergoing to microwave processing (L. Liu et al. 2012).

Hence, the aim of this research was to develop and characterize a novel method for the post-loading of FFF printouts, facilitating the transition from traditional methods, such as solvent casting or soaking, to microwave enhanced techniques.

2. MATERIALS AND METHODS

2.1 Materials

Poly(vinyl alcohol) (Ultimaker, density 1.23 g/cm3, Netherlands) was used as pristine material for the FDM 3D printing of drug-free models. Anhydrous caffeine (Sigma-Aldrich, aqueous solubility 21.6 mg/mL, 25°C, pH 7.0) (Yalkowsky S.H. et al., 2010) was chosen as model drug. Acetone, ethanol, 2-propanol and ethyl acetate (Sigma Aldrich) were selected as solvent for oversaturated solutions. Moreover, poly(ethylene glycol) PEG 1000 (BioUltra, Sigma) has been added to the acetonic carrier solutions.
For the mobile phase preparation HPLC grade solvents were used: tetrahydrofuran, acetonitrile, sodium acetate trihydrate, and glacial acetic acid.

2.2 Digital modelling of caps-in-cap

CAD models of the caps-in-cap formulations were drawn using Fusion360 (Autodesk, California, USA) and Rhinoceros 6 (Robert McNeel & Associates, McNeel Europe) and then exported as .stl extensions. Graphical designs were then imported to the slicing software:

- PreForm version 3.3.1 (Formlabs, Massachusetts, USA) prior to printing *via* SLA 3D printer Form3 (Formlabs, Massachusetts, USA);
- Cura 4.6.0 (Ultimaker, Netherlands), creating the gcode file used by the FDM 3D printer Ultimaker^3 (Ultimaker, Netherlands).

2.3 3D printing of caps-in-cap

The SLA fast prototyping led to define as final blank geometries the caps-in-cap showed in figure 1.

<Figure 1 near here>

The production of the models was then translated in the FDM manufacturing process with the following printer and slicing parameters (Table 1):

Table 1. Operative specifics and slicing parameters used for FDM 3DP

FDM

3D-Printer and slicing parameters	
Material	PVA
Nozzle diameter	0.4 mm
Printing Temperature	215°C
Layer Heigh	0.10 mm
Line Width	0.19 mm
Print Speed	8-20 mm/s according to the object geometry

2.4 PVA impregnation *via* soaking and *via* microwave-assisted process

Oversaturated solutions of caffeine were prepared with different solvents (i.e. ethanol, acetone, IPA and ethyl acetate) for soaking and microwave processing. Oversaturated solutions with the drug were prepared in relation to the caffeine solubility in these organic solvents (Kulathooran Ramalakshmi & B Raghavan, 1999). Additionally, PEG 2% w/v have been added to acetonic solutions to enhance the propagation of the microwaves.

For the soaking process, blank models of PVA were immersed in 15 mL of acetone oversaturated with caffeine, sealed, and kept under magnetic stirring for 48 hours at room temperature.

For the microwave-assisted impregnation, microwave heating *via* Biotage® Initiator Microwave Synthesizers (Biotage, Uppsala, Sweden) was used. Particularly, the PVA blank model (one cap or one body) was added into 15 mL of the oversaturated solutions of selected solvents and caffeine, sealed, and then processed with the following settings:

- Solvent temperature: from 40°C to 140°C
- Heating rate: 2 - 5 °C/s
- Power range: 1 - 400 W (with defined wattages or gradual ramps)
- Magnetron: 2.45 GHz
- Absorption level: normal, high or very high (according to the polarity and conductivity of the used solvents)
- Stirring range: 600 rpm
- Contact time of the models in the solutions during the process: 10 s - 20 min or Cycles
- Cooling with pressurized air (> 60 L/min, 2.5-4 bar)

Both the soaked and the irradiated models were dried at 40 ± 2 °C until constant weight was obtained. The drug content of soaked and irradiated samples was analysed *via* HPLC as described below.

2.5 Analysis of FDM 3D printed models and processed models

At least three units for each formulation have been weighted using analytical balance (Analytical Balance Sartorius Research R200D, USA):

- after the printing process of blank models;
- after the soaking process and drying;
- after the microwave impregnation and drying.

In particular, the weight acquired after the impregnation or drug loading was calculated and expressed as percentage, following the equation:

$$Weight\ acquired\ (\%) = \frac{mg_f - mg_i}{mg_i} * 100$$

Where mg_f represents the mass of the formulation after loading and drying, while mg_i represents the initial mass of the sample.

All the formulations have been accurately measured with a digital micrometre in terms of height, diameter and width, and the outputs expressed following the equation:

$$Dimensions\ acquired\ (\%) = \frac{d_f - d_i}{d_i} * 100$$

Where d_f represents the dimension of the formulation after loading and drying, while d_i represents the initial dimension of the sample.

Blank models and post-loaded dried formulations were observed in their macro- and micro-architecture by using Scanning Electron Microscopy (Tescan Solaris, Tescan Orsay Holding, Czech Republic), after metallization *via* Leica EM SCD005, Leica-microsystem, Italy.

2.6 HPLC quantitative analysis of Caffeine-loaded printouts

Caffeine from drug-loaded samples was quantified *via* HPLC (Agilent Technologies 1200 series, 1260 Infinity II) with Infinity Lab Poroshell 120 EC-C18 column (Agilent Technologies, Cheadle, UK) at a wavelength of 275 nm. The calibration curve was performed in a range of 12 – 450 μg/mL (regression coefficient value R^2 0.99998).

The mobile phase was prepared as recommended by European Pharmacopoeia 8th Edition, and consisted of 20 volumes of tetrahydrofuran, 25 volumes of acetonitrile and 955 volumes of a

solution containing 0.82 g/L of anhydrous sodium acetate previously adjusted to pH 4.5 with glacial acetic acid.

The drug content (DC) was expressed as percentage w/w considering the mass of the caffeine ($mg\ Caffeine$) obtained for the single formulation *via* HPLC analysis and the mass of the post-loaded formulation after drying (mg_f), according to the equation:

$$DC\ (\%) = \frac{mg\ Caffeine}{mg_f} * 100$$

2.7 Buoyancy tests

Buoyancy tests on drug-loaded models (body2 and cap2 enclosed in body1 and cap1) have been carried out in acidic medium (dissolution buffer 0.1 M HCl solution) at 37°C under constant magnetic stirring (50 rpm). The lag time and the floating time have been visually detected and reported in hours.

2.8 FT-IR analysis

Solid State Fourier Transform Infrared Spectroscopy was performed via FT-IR/NIR spectrophotometer (Perkin Elmer Inc., Spotlight 400N FT-NIR Imaging System, USA) equipped with the Universal ATR accessory crystal plate and Spectrum Software version 10.5.2. All the raw materials, dried caffeine powders pre and post microwave, and blank/loaded printed formulations were analysed in a spectral range of 600-4000 cm^{-1}; moreover, resolution of 1 cm^{-1}, scan number of 128 and scan speed of 0.2 cm/s were used in specular reflectance sampling configuration.

2.9 Thermal Analysis via TGA/DSC

Pristine chemicals and dried caffeine powders obtained from the acetonic solutions (pre and post microwave processing) have been analysed *via* Differential Scanning Calorimetry (DSC822e Differential Scanning Calorimeter, Mettler Toledo, Germany) to highlight thermal events. Each sample was accurately weighted in a 40 µL aluminum pan and sealed by a predrilled lid. The thermal cycle set was: a dynamic segment from 25 °C to 400 °C, with a heating rate of 10 °C/min and 70 mL/min of nitrogen as inert gas. The analysis of the data was conducted by STARe Evaluation software v16.20.

Thermogravimetric Analysis (Discovery TGA, TA instruments) was conducted on raw materials, air-dried samples and heated-dried samples with an average mass of 7 mg in TA pans, heating from ambient temperature to 400°C, with a rate of 10°C/min, using N_2 as inert sample purge gas to control the sample environment (20 mL/min). The analysis of the data was conducted by TA Instruments Trios V5.1.1.46572.

2.10 Dissolution tests

Drug release profiles were obtained placing the loaded formulations (caps and bodies 2 enclosed in 1) in 750 mL of acidic dissolution medium (0.1 M HCl) and using USP Dissolution Tester TDT-06L (Electrolab), configuration 1 (baskets) at 75 rpm and 37 °C, coupled with 850-DS Dissolution Sampling Station (Agilent Technologies). For all drug loaded formulations, the withdrawal times were 5', every 15' minutes until the first hour and then every 30 minutes up to 6 hours. The samples were quantized undiluted *via* HPLC technique. For all batches analysed, mean values and standard deviations were reported.

3. RESULTS AND DISCUSSION

3.1 Model geometries development and mass/morphological analysis

The modelling of caps-in-cap geometries was defined for the possibility of enclosing models in decreasing size consecutively one inside another, as a matryoshka of modular capsules. The prototyping for the FDM 3D printing of caps-in cap was carried out with the aim of obtaining, using a 0.4 mm nozzle, the thinnest wall width for the smallest models (model 3), gradually increased for the largest ones (models 2 and 1) (Figure 2).

<Figure 2 near here>

The total exposed area to the solvents, calculated *via* software on the digital models, was about 3000 mm^2, remarkably higher compared to floating models with the same dimensions and low infill percentage for the floating necessity (Giri el al., 2020; Vo et al., 2020). The average total mass of the whole formulation (figure 3) was about 1133 mg. Weighted units underlined the high printing reproducibility as confirmed by the low standard deviations of masses (table 2).

Table 2. Mass analysis of FDM printouts

Model (n=6)	**Average mass (mg) ± SD**
Cap 1	370 ± 16
Body 1	375 ± 15
Cap 2	126 ± 10
Body 2	151± 14
Body and Cap 3	114± 3

The total height of the closed model was 23.00 ± 0.10 mm (digital output: 22.875 mm), and the obtained dimensions of the biggest model 1 (that enclosed the tiniest models 2 and 3) were comparable to the 00 capsules size. Moreover, the general shape of the formulations is related

to the well-known geometry of capsules, thus this should suggest high patient compliance and treatment adherence for these 3D printed novel products.
Analysing the dimensional data set of 12 caps-in-cap from 2 batches (6 each) produced with slightly different widths, predictive linear relationships between the digital and the real models has been found (figure 3). This result paves the way for an easily re-tuning of the heights, the diameters and the widths of the models, for the purpose of personalization of the shape, dimension and release profiles, according to the patients' dosage needs and swallowing capabilities.

<Figure 3 near here>

Particularly, considering the PVA expansion along the three axes XY and Z, the digital-real correlation resulted in an accurate prediction for obtaining the desired dimensions of the printouts. For the wall width expansion, the linear relationship was less accurate (R^2: 0.9925), but still realistic, probably due to the bigger nozzle diameter compared to the tiny dimensions of the prints. The accuracy of the prediction could be easily enhanced using nozzles with reduced diameters, such as 0.2 mm, in order to improve the resolution of small models and reduce the variability of widths during printing. Finally, the predictive relationship has been considered fundamental for the thinnest models, to whom the absolute value of the difference between digital and real dimensions was higher than for the biggest models.

3.2 PVA matrix microwave-assisted impregnation

PVA has been chosen for its absorbing properties in the microwave system (Salimbeygi G. et al., 2013) thanks to its good polarity; while caffeine, belonging to the class I of the Biopharmaceutics Classification System (BCS), was chosen as model drug due to its compatibility with hydrophilic matrix.
All the four selected solvents and two mixtures thereof (2-propanol:acetone 50:50 or ethyl acetate:acetone 80:20) were used to load caffeine into the blank printouts, collecting data about drug loading and the morphology of the processed models (data not shown).
All the used solvents led to a higher loading than the traditional soaking method (greater than 0.95% w/w for all the microwave processes *versus* 0.55% w/w for soaking). In this case, acetone has been selected as appropriate solvent for caffeine loading thanks to the higher drug

loading of the drug in this matrix and the good shape preservation of the processed models, compared to the other solvents.

The best process parameters were optimized for the subsequent impregnations considering that:

• the increase in temperature always led to increased loading, but to frequent delamination;

• the increase in the impulse exposure time (i.e. 2', 10' or 20') did not affect the loading;

• the increase in wattage has often caused the physical breakdown of the polymer matrix.

Particularly, focusing on short cycling pulses, increasing the numbers of cycles (i.e. 5 or 10 pulses with 10 seconds of stay at the maximum temperature), no quantitative enhance was found. After the screening of different wattage sets (0-375 W), 5 short pulses, with gradual power, ending with 10 seconds of stay at the final temperature 80°C have been selected as the most promising process to load samples preserving the shape of caps and bodies after the microwave irradiation (namely 5 x 10" g.p., batch 510gp48).

A drying step on a glass plate of loaded samples at 40-42°C was used to reduce the amount of acetonic residual present in the formulation and speed up the drying process without affecting the shape of the formulations.

Table 3. MW cycles on samples placed in acetone and caffeine 2% w/v solutions (in italic: broken/delaminated samples; in bold: the best loading cycle using acetone)

Batch	***Time***	***Power***	***Temperature***	***Acquired Mass***	***Acquired Height***	***Acquired Diameter***	***Acquired Width***	***Drug loading***
n	***Cycle n, s***	***W***	***°C***	***%***	***%***	***%***	***%***	***% w/w***
510gp48	**5 x 10''**	**g.p.**	**40-80**	**4.6 ± 0.8**	**4.0 ± 1.1**	**-1.2 ± 0.8**	**1.9 ± 0.8**	**2.83 ± 0.30**
510p148	5 x 10"	100	40-80	2.1 ± 0.1	2.0 ± 0.5	-0.6 ± 0.9	-1.1 ± 3.1	2.20 ± 0.32
310gp48	3 x 10"	g.p.	40-80	6.3 ± 0.4	4.2 ± 0.0	0.2 ± 0.9	1.2 ± 2.1	2.54 ± 0.11
310p348	3 x 10"	375	40-80	3.1 ± 0.1	4.1 ± 0.3	0.7 ± 0.5	-1.2 ± 2.1	2.47 ± 0.09
510gp41	*5 x 10''*	g.p.	*40-100*	*3.5*	*14.1*	*-4.9*	*8.6*	*3.05*
510p141	5 x 10"	100	40-100	3.7 ± 0.0	3.0 ± 0.7	-0.9 ± 0.7	-1.1 ± 1.1	2.36 ± 0.44
310gp41	*3 x 10''*	g.p.	*40-100*	*4.1 ± 0.5*	*11.6 ± 1.6*	*-2.9 ± 1.3*	*8.4 ± 6.2*	*2.64 ± 0.16*
310p341	*3 x 10''*	*375*	*40-100*	*4.5*	-	-	-	*2.40*
510gp56	5 x 10"	g.p.	50-60	3.9 ± 0.3	2.1 ± 0.6	-0.1 ± 0.5	1.1 ± 7.4	2.20 ± 0.19
510p268	5 x 10"	200	60-80	-	-	-	-	2.23
510gp68	5 x 10"	g.p.	60-80	5.5 ± 1.9	2.9 ± 1.3	-0.8 ± 1.2	-2.2 ± 1.9	2.73 ± 0.81
510gp71	*5 x 10''*	g.p.	*70-100*	*7.7 ± 0.7*	*15.6 ± 4.4*	*2.6 ± 2.0*	*-2.4 ± 5.5*	*3.78 ± 0.53*

g.p.: gradual power (ramp from 0 to 375 W)

Moreover, PEG 1000 was added into the acetonic solution (table 4), exploiting its capability in enhancing the electromagnetic absorbing properties of the whole system and in reducing the impedance of the medium and, then, the dissipation of microwaves during the irradiation (Li Y. et al., 2018). Furthermore, PEG has been added as a suspending agent in order to enhance the homogeneity of caffeine physical dispersion, ensuring de-aggregation of caffeine and thus uniform drug distribution into the oversaturated solution.

Table 4. MW cycles on samples placed in acetone, PEG 2% w/v and caffeine solutions (in bold: the best loading cycle using Acetone-PEG)

Batch	***Time***	***Power***	***Temperature***	***Acquired Mass***	***Acquired Height***	***Acquired Diameter***	***Acquired Width***	***Drug loading***
n	***Cycles n, s***	***W***	***°C***	***%***	***%***	***%***	***%***	***% w/w***
P510gp	5 x 10"	g.p	40-80	2.5 ± 0.4	4.7 ± 1.9	-0.2 ± 2.5	2.5 ± 4.4	2.41 ± 0.14
P510p1	**5 x 10''**	**100**	**40-80**	**3.3 ± 0.3**	**2.1 ± 0.4**	**-0.6 ± 0.5**	**1.2 ± 4.1**	**3.04 ± 0.40**
P510p2	5 x 10"	200	40-80	2.8*	2.9*	-0.8*	1.2*	2.49
P510p3	5 x 10"	300	40-80	3.0*	3.1*	-1.4*	3.7*	2.45
P510gp	5 x 10"	g.p.	40-100	3.1*	12.1*	-2.3*	4.8*	3.12

g.p.: gradual power (ramp from 0 to 375 W)
** Standard deviations have not been recorded due to the deformation of samples*

Particularly, the addition of PEG allowed to reach 100°C of maximum temperature with gradual increase of power without breaking the models (batch P510gp). But in this case, the height acquired was excessive preventing the enclosing of the small models (models 2 and 3) into the biggest one (model 1).

Accordingly, the best process in terms of loading and dimensions maintenance with PEG was: a cycle at 100 Watts from 40 to 80°C, then 10 seconds at 80°C, and at last a fall from 80°C to 40°C speeded up *via* air cooling, all repeated five times (batch P510p1) (figure 4).

<Figure 4 near here>

In conclusion, the addition of PEG in the oversaturated acetonic solution led to the production of the formulations with the highest drug loading at 100 W, corroborating the enhancing properties of PEG in microwave absorbing and in avoiding the attenuation of the waves as it propagates into the absorbing medium. Particularly, the effect was related to the set wattage and then to the released energy for molecules vibrations.

A comparison of the amount of drug (μg/mm^2) adsorbed onto the PVA matrix for some models is shown in table 5.

Table 5. Comparison between soaking technique and microwave-enhanced impregnation of the absorbed drug related to the exposed surface area of the models, using the best selected cycles of irradiation

Model	**Digitally obtained exposed area**	***MW Impregnation in Acetone***		***MW Impregnation in Acetone-PEG***		***Soaking in Acetone***	
		μg/mm^2		*μg/mm^2*		*μg/mm^2*	
	mm^2	*Average*	*St Dev*	*Average*	*St Dev*	*Average*	*St Dev*
Cap2	**387.0**	**8.13**	**0.98**	**8.85**	**1.04**	**1.65**	**0.14**
Body2	**452.5**	**7.05**	**1.59**	**-**	**-**	**-**	**-**
BodyCap3	**349.8**	**10.27**	**2.40**	**-**	**-**	**-**	**-**

3.3 Morphology of processed materials and FFF 3D printed models

Analysing the micrographs of the processed samples (figure 5), the PVA models resulted in slightly distorted layers adhesion due to the processes carried out on them. Furthermore, it can be evidenced that the drug adhesion onto the PVA model was more homogeneous for samples processed *via* microwave, compared to the soaked ones.

<Figure 5 near here>

Moreover, both after soaking and microwaving, the growth behaviour of caffeine crystallites onto the PVA surface followed preferentially a directional growth, favouring a flat-on orientation with respect to the substrate, particularly considering the bundles of packed acicular crystals of caffeine β polymorphs (Park & Yeo, 2008; Röthel et al., 2017). Thus, the orientation of adsorption after nucleation during crystal growth mostly coincided with the long needle axis, regardless of the roughness of the surface.

On samples processed in acetone *via* microwave, tubular fashions of the metastable α polymorphs were highlighted with the loss of polyhedral stability. These were organized as slightly disturbed hexagonal or rectangular motifs with a central cavity within the crystals, which favoured instead an edge-on orientation perpendicular to the PVA surface (M.D. Eddleston & W. Jones, 2010a; C. N. Nanev & A. N. Penkova, 2002).

With regards to samples loaded in acetone-PEG *via* microwave, high organized hexagonal structures of caffeine crystallites were identified, characteristic of the metastable polymorph α, as further highlighted also by DSC and IR data. This high organized crystal habit occurs when the growth rate of the hexagonal face is greater at the centre than at the edges. Thus, PEG retarded the drop of acetone solution in evaporating and supersaturation levels increase in the dome of the hexagonal face. This event leads to the diffusion of caffeine molecules in the centre of the cavity in the slowly growing hexagonal crystals, while the growth rate is reduced to the apexes of the structure (M.D. Eddleston & W. Jones, 2010b).

Finally, even not penetrating deeply into the polymeric matrix, caffeine accumulated not only on the surface of PVA, but also in the hollow cavities inter and intra layers, existing as thick and short needles grown along random directions, slightly rotated to adjacent needles.

Hence, both after soaking and microwave process, caffeine crystallites tended to rearrange in adsorption geometries more energetically advantageous to adapt for the polymeric surface constraints and evaporation conditions.

<Figure 6 near here>

An interesting aspect of the drug adhesion onto the PVA surface was evidenced by the acquisition of magnifications of the inner part of the caps and bodies (figure 6). Here caffeine adsorbed particularly on the initial few layers of the concave area of the models, while on the internal part the drug distribution was not homogeneous and comparable to the outer surface. This occurrence may be explained by the development of vortices in the boundary layer flows on curved walls, leading to a convective instability of flow and turbulent motions of the liquid into cavity of caps and bodies (Kim et al., 2010). The turbulences prevented the drug to interact and adsorb homogeneously onto the polymeric concave inner surface.

3.4 FT-IR analysis

<Figure 7 near here>

All the acquired spectra are shown in figure 7.

Considering the air-dried Caffeine obtained from Acetone and Acetone-PEG solutions (pre and post microwave), all the characteristic bands of the drug were found in the spectra.

Before the microwave process, the absorption in the 3500–3150 cm^{-1} region may come from the OH stretch of residual minute quantities of water in pure acetone (> 0.5%) that was trapped in Caffeine crystals (J.J. Max & C. Chapados, 2003), and/or from the formation of organic solvates Caffeine-Acetone in a cocrystal related system (solvent evaporation may give rise to a solvent occlusion into the crystal faces as multicomponent systems) (A.M. Healy et al., 2017). Both phenomena are confirmed by the slight variation of CO stretching vibrations (from 1695 to 1700 cm^{-1}) and of the band characteristic of ring =C-H stretching (from 3110 to 3120 cm^{-1}). Instead, regarding the air-dried Caffeine obtained from Acetone-PEG solutions, the spectrum obtained before the microwave process, displayed: a slight shift (from 1105 to 1110 cm^{-1}) for the C-O-C asymmetric stretching of PEG, a variation of the band shape in the range 1480-1430 cm^{-1}, and broadening of the band at 3400 cm^{-1}. All confirming the presence of intramolecular non-covalent interactions (such as hydrogen bonding and Van der Waals forces) between Caffeine ring and the PEG trunk and OH terminals. Moreover, in the spectrum of the dried caffeine after the microwave process, a further variation of the C-O-C band was observed, shifting from 1105 to 1095 cm^{-1}. Furthermore, in the range 1340-1360 cm^{-1}, the two bands characteristic of the PEG branching became a single band at 1358 cm^{-1} and a reduction in the relative intensity of PEG bands at 2885 cm^{-1} due to the CH stretching and deformations vibrations was highlighted. From these variations, a re-arrangement of the PEG branching pattern caused by the microwave process may be supposed and the further broadening of the band at 3450 cm^{-1} may suggest a more intimate interaction amid the Caffeine structure and the PEG shaft with reduced segments.

Finally, regarding the samples processed in Acetone (Gradual Power) and Acetone-PEG (100 W), both the spectra displayed the typical bands of purines (from 610 to 745 cm^{-1} and 1240 cm^{-1}); besides the CO alcoholic stretching region (1020-1090 cm^{-1}) and CH deformation region (1300-1440 cm^{-1}) of PVA were unchanged. For both processed samples, similar variations in band shifts were underlined, particularly:

- from 1645 to 1655 cm^{-1} of the caffeine ring stretching,
- from 1695 to 1705 cm^{-1} and from 1730 to 1735 cm^{-1} of the carbonyl groups of caffeine and PVA respectively,
- from 2950 to 2940 cm^{-1} of the methyl groups of Caffeine.

Moreover, a broad band at 3330 cm^{-1} increased in relative intensity. All these data highlighted the presence of intramolecular non-covalent interactions between Caffeine structure and PVA, particularly with hydroxylic terminals, enhanced by the microwave processing. Moreover,

these shifts highlighted the presence of the α polymorphs of caffeine crystals adsorbed onto the polymeric matrix. The polymorphic transition was therefore mediated by the supersaturated acetonic solution during evaporation and crystals nucleation and growth, as evidenced also by SEM micrographs and DSC analyses.

3.5 Thermal Analysis via DSC and TGA

<Figure 8 near here>

Starting from the thermogram of pure Caffeine (figure 8), the drug, as irregular crystalline particles at room temperature, experienced a partial sublimation and recrystallization in the range of temperatures 140-170°C. This endothermic peak of a solid-solid transition (Dong et al. 2007) led to the growth of long needles and rod-shaped crystals. The melting of the β form of the drug was evident at 240°C, finally the melted product underwent evaporation process from 260 to 310ºC (R. Ruiz-Carol & M.D. Veiga-Ochoa, 2009). Furthermore, the caffeine did not show thermal events until 140°C, confirming its anhydrous state.

The acquisition of raw PEG 1000 displayed the melting process at 30-45°C and decomposing starting after 380°C.

The thermograms of air-dried caffeine obtained from acetone solutions (both pre and post microwaving) showed the evaporation of the residual solvent in the range 30-70°C, with a peak at 60°C, and a slight decrease of the melting peak (237 and 235°C respectively). Moreover, the range of evaporation was highlighted at 260-330°C. Minor peaks of recrystallization during solid-solid transition were slightly visible at 155°C, with transition heat in the range -8/-27 mJ. These data confirm the change in the crystalline structure, from irregular particles to needle-like crystals, and the processed crystals are thermally different compared to the unprocessed caffeine. Furthermore, the hypothesis derived from FT-IR about the composition of organic solvates Caffeine-Acetone in a cocrystal related system may be confirmed.

Finally, the thermograms of air-dried caffeine obtained from acetone-PEG solutions corroborated the deduction of the PEG chain scission due to the decrease melting range of PEG at 25-32°C found after the microwave process. Furthermore, for both pre and post microwaving caffeine, the solvent of crystallization desorbed and evaporated from 45 to 100°C, evidence of the stronger non-covalent interactions with the interstitial acetone trapped into the crystals. The melting range of the Caffeine turned out in a broader peak starting from 170 up to 230°C, as

well as the evaporation process from 270 to 370°C. All these data underlined the probable effect of PEG in the reduction of the Gibb's free energy of the surface of the crystals with the construction of small sharp crystals with different thermal stability.

<Figure 9 near here>

The thermogravimetric analysis (figure 9) of neat caffeine indicated that the compound was stable up to 200ºC. Then the onset when the weight loss began of the melting event took place at 245-283°C and the total mass loss occurred in a single step. The peak of the first derivative indicated at 278°C the point of greatest rate of change on the weight loss curve, thus the inflection point with 100% weight loss confirmed the total evaporation of the drug.

Regarding the unprocessed PEG 1000, the onset of the pyrolysis was found at 391°C, and from 85 to 173°C a 4.6% of weight loss was evidenced (DTGA), due to the evaporation of residual water and formation of volatile products.

For the blank printed PVA models, the weight loss peak was found at 325°C, beginning at 306°C and ending at 348°C (with 30.3% of residual weight at 400°C corresponding to carbonised products). Furthermore, up to 206°C only 5.0% of weight loss occurred due to residual adsorbed water, underlining the high thermal stability of this thermoplastic polymer used for 3D printing.

The air-dried samples from acetone displayed a peak at 91°C, starting from 50°C, with a weight percent loss of 6.7% of residual adsorbed moisture and/or acetone. While the heated-dried samples from acetone experienced at 85°C, starting from 44°C, just 2.5% of weight loss, highlighting the reduction of residual organic solvent and water after the drying step at 40-42°C. For both samples the step transition occurred at 328°C (onset 302°C, endset 349°C) with a change in weight of 71.8%.

Regarding the samples processed in acetone-PEG solution, the air-dried samples showed a peak at 97°C with a variation in weight of -6.3%; while the heated-dried sample displayed a peak at 84°C and underwent 3.1% of weight loss. These differences in variations underlined again the enhancement of the evaporation of residual solvent of the heated drying process, reducing the potential toxicity of these floating drug delivery systems when administered during long therapeutic regimen.

The weight change was 72.4 and 71.1% for the air and heated dried samples, with step transition at 328 or 318°C respectively (onset 304°C and endset 350°C).

Peaks at 135°C (weight percentage loss of 3.9%) and at 131°C (weight percentage loss of 2.4%) were found in the thermograms of heated-dried samples deriving from Acetone and Acetone-PEG solutions respectively, due to the partial sublimation of the Caffeine α crystals. Thus, these events further confirmed the presence of the α polymorph derived from the microwave-assisted drug-loading and solvent evaporation at 40°C.

3.6 Buoyancy tests

Blank formulations and drug loaded formulations showed the same buoyancy behaviour with no lag time during tests in beakers, both in distilled water and acidic medium. The floating time of blank and drug loaded formulations lasted until complete erosion of the PVA matrix (6.0 ± 0.5 hours). This was due to an optimal fraction of the volume of voids over the total volume, even when models are grouped altogether. Regarding MW post-loaded formulations, after 20 hours a minimal sticky residual mass was slightly visible adhered onto the glass surface of beakers or into the metallic mesh of the dissolution baskets. These data were indeed confirmed visually also during dissolution tests of post-loaded formulations (models 1 and 2) placed in USP dissolution apparatus.

3.7 Dissolution tests

Analysing the release kinetics of the samples processed in acetone or acetone-PEG (figure 10 respectively AC and APC), the caffeine dissolution profiles can be considered over imposable. Hence, the impact of small pulses using two different parameters of power set (gradual power or 100W) did not affect particularly the PVA matrix in terms of erosion, even if this assumption should be confirmed for higher wattages (i.e. 200 or 300W). Furthermore, caffeine adsorbed onto the Acetone and Acetone-PEG processed samples displayed the same dissolution behaviour, determining a similar *in vitro* drug release rate.

<Figure 10 near here>

4. CONCLUSIONS

In this research, an innovative and unexplored methodology for post-loading of FDM 3D printed pharmaceutical formulations, applying microwave irradiation, was analysed, fully deepened in terms of parameters (time of irradiation, pulses effect on polymeric models, temperature, pressure and energy released from the system) and optimized. Moreover, the intensify of drug-loading was found when a biocompatible pharmaceutical excipient with polar characteristics, i.e. PEG, was added to the selected oversaturated acetonic solutions, thanks to the reduction of wave dissipation during irradiation and to the interaction with the selected drug and polymeric matrix.

It is therefore confirmed the potentiality of this process in improving the adhesion of the drug on printed drug delivery models, compared to the traditional soaking technique (5.53 times more for the most promising carrier solution).

In conclusion, the novel developed technique for post-loading and impregnation of FFF 3D printed blank polymeric models, enhancing drug adsorption *via* microwave irradiation, could be implemented in clinical settings and manufacturing scale-up for the production of drug delivery systems, taking into account all the variables and parameters highlighted in this scientific research.

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