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# 1 Tuning pharmaceutically active zein-based formulations for additive manufacturing

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5  
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## 11 Abstract

12 Zein, a biopolymer from corn, was plasticized by glycerol and an Active Pharmaceutical  
13 Ingredient-Ionic Liquid (API-IL) for its melt processing at 130 °C. In order to enhance the  
14 rheological properties of the formulation for 3D printing, the ratio of glycerol to API-IL was  
15 adjusted while maintaining a consistent addition of 20% plasticizer. A 50/50 ratio allowed  
16 obtaining an initial melt viscosity suitable for extrusion-based processes, at approximately  
17 1 kPa.s at a shear rate of 10 s<sup>-1</sup>, with a shear thinning behavior. This viscosity remained stable  
18 during a processing window of about 6 min, before zein proteins start to aggregate, leading to  
19 an apparent gelation phenomenon for long residence times. The processability of this  
20 formulation containing API-IL for the printing of tablets for potential therapeutic applications  
21 was confirmed by tests on a 3D printer. Nonetheless, in comparison to a reference formulation  
22 containing only glycerol, the printing accuracy experienced a decrease. This was ascribed to  
23 slower viscous sintering kinetics in presence of API-IL, evidenced by monitoring fusion-  
24 bonding during dynamic X-ray tomography trials carried out at synchrotron SOLEIL.

## 26 **Keywords**

27 Hot Melt Extrusion; Processing window; Protein; In-situ synchrotron micro-CT; Sintering

28

## 29 **1. Introduction**

30 The advent of Additive Manufacturing processes is a great opportunity for personalized  
31 medicine. In particular, edible 3D printed objects containing active ingredients with customized  
32 porous structures may allow their controlled release *in vivo* [1-3]. Among the numerous existing  
33 technologies, those based on extrusion of thermoplastic formulations, allow obtaining high  
34 quality structures faithful to their initial numerical models, thanks to fast stiffening of deposited  
35 layers upon cooling [4-7]. However, they require optimized formulations combining edible  
36 thermoplastic biopolymer matrices with active ingredients, matching both processing  
37 specifications and therapeutic end-uses, *i.e.* passive diffusion of active molecules [8,9]. The  
38 time and temperature processing window of such formulations must be tuned taking into  
39 consideration the thermo-sensitivity of active ingredients, the rheology of the matrix in the  
40 molten state, and the impact of the hot melt extrusion process on the material, especially when  
41 shaping pharmaceutical tablets [10, 11].

42 Zein, a protein extracted from corn, can be formulated with plasticizers for hot melt extrusion  
43 [12, 13]. Indeed, materials based on zein plasticized by 20w% glycerol show a rheological  
44 behavior at 130 °C that is similar to that of standard polymers used in additive manufacturing  
45 such as PLA and ABS at their own processing conditions for 3D printing (respectively 190 °C  
46 and 230 °C) [14-16]. Furthermore, glycerol-plasticized zein has efficient sintering abilities, also  
47 similar to standard printed materials [17, 18]. These fusion-bonding abilities, resulting from  
48 both surface tension (as a driving force) and viscosity (potentially limiting the sintering  
49 phenomenon) of the plasticized zein are of major interest to control the deposition of 3D printed  
50 structures consisting of juxtaposed and superimposed filaments [19].

51 Furthermore, zein can be plasticized directly by [Lidocainium][Ibuprofenate] ([Lid][Ibu]) [20].  
52 This salt is an Active Pharmaceutical Ingredient - Ionic Liquid (API-IL), a new family of  
53 promising compounds for future drugs, because they can be easily dosed and processed, even  
54 thermo-mechanically, and constitute molecules showing better bioavailability and stability than  
55 solid forms [21].

56 In the case of zein, [Lid][Ibu] both favors processing and provides the final therapeutic  
57 properties after hot melt extrusion: From extruded filaments, a progressive release of this active  
58 ingredient was obtained in simulated physiological conditions, thanks to high affinity between  
59 zein and [Lid][Ibu].

60 Moreover, the zein-based matrix, which undergoes thermomechanical treatment for 3 minutes  
61 at 130 °C, demonstrates its structural integrity as evidenced by thermal gravimetric analysis  
62 (TGA), with the primary decomposition temperature observed above 250 °C. Additionally,  
63 during the same duration and temperature (3 minutes at 130 °C), the matrix retains both the  
64 anesthetic effect of Lidocaine and the analgesic effect of Ibuprofen [22].

65 In the present work, our objective is to combine glycerol and [Lid][Ibu] as co-plasticizers of  
66 zein in order to tune the 3D printing processability. We investigate in detail the rheological  
67 behavior, sintering properties, 3D printing abilities and porous structures of resulting plasticized  
68 zein-based materials. We first characterize the rheological properties of zein plasticized by  
69 various glycerol/API-IL ratios in steady-state flow under extrusion conditions and dynamic  
70 oscillatory testing. Then, the sintering abilities of plasticized zein are finely characterized with  
71 an original experimental set-up, based on the monitoring of the fusion-bonding kinetics between  
72 filaments by real-time X-ray microtomography using synchrotron radiation. Indeed, the  
73 impressive development of X-ray computed microtomography led to application in monitoring  
74 the sintering of powders with high resolution allowed by synchrotron radiation, in particular to  
75 qualify materials for additive manufacturing during powder particle laser sintering [23-25].

76 Nevertheless, it can also be applied to bigger objects such as filaments, benefiting from fast  
77 scans (hundreds of 2D radiographs acquired per second) and high resolution (typically below  
78 10  $\mu\text{m}$  voxel size) during a long period of several minutes for filaments viscous sintering with  
79 surface tension as sole driving force [26, 27]. Such time-resolved tomography, adapted for  
80 dynamic processes, could be of prime interest in testing materials as candidates for 3D printing  
81 by comparing their sintering abilities to standard synthetic polymers, or biopolymer-based  
82 printable materials [28]. These data are of special importance in order to guarantee an efficient  
83 adhesion to control the porosity and the release of active molecules included in the matrix for  
84 pharmaceutical applications.

85 Finally, based on these results, which lead to a more accurate definition of the processing  
86 window, zein-based tablets are 3D printed by hot melt extrusion and their porous structure is  
87 assessed with static X-ray tomography using laboratory equipment. This allows evaluating the  
88 potential of zein plasticized by glycerol and [Lid][Ibu] to be printed as drug delivery systems.

89

## 90 **2. Materials and methods**

### 91 *2.1. Raw materials*

92 All reagents used were of analytical grade. Zein-Z3625 and glycerol-G7893 were purchased  
93 from Sigma-Aldrich. The Active Pharmaceutical - Ionic Liquid (API-IL),  
94 [Lidocainium][Ibuprofenate], was synthesized as in Jouannin et al.'s study [29] and provided  
95 by the UTINAM Institute (UMR CNRS 6213, Université de Bourgogne Franche-Comté,  
96 Besançon, France).

97

### 98 *2.2. Formulation*

99 Physical mixtures of plasticized zein were prepared by adding plasticizers to powdery raw zein  
100 following Table 1. Powder was compressed into small tablets ( $\text{\O}\approx 3$  mm) to feed the single  
101 screw of the 3D printer during subsequent additive manufacturing trials.

102

103 **Table 1.** Formulations of zein-based materials, based on 100 g raw zein (5% water content) from supplier with  
104 added glycerol and/or API-IL [Lid][Ibu].

Formulation ID	Glycerol [g]	[Lid][Ibu] [g]
Z20GLY	20	0
Z15GLY5LI	15	5
Z10GLY10LI	10	10
Z5GLY15LI	5	15
Z20LI	0	20

105

### 106 *2.3. Melt processing and rheological measurements*

107 Rheological assessment was conducted under two distinct conditions: first one was under  
108 extrusion conditions, using either constant rotation speed of the screws or by varying the  
109 rotation speed. The second set of measurements was made under dynamic conditions using a  
110 cell with two parallel plates in oscillatory rotational movements.

111

#### 112 *2.3.1. Steady-state flow measurements in extrusion conditions*

113 Steady-state flow measurements were carried out on melted materials using a micro-  
114 compounder (Haake Minilab, Thermo Scientific GmbH, Karlsruhe, Germany) to reproduce  
115 extrusion process conditions. The instrument can be used to circulate melt samples through a  
116 conical twin-screw system ( $L_{\text{screw}} = 12$  cm) followed by a backflow channel ( $64 \times 10 \times 1.5$  mm<sup>3</sup>)

117 equipped with two pressure sensors, before their recirculation in a loop, or extrusion through  
118 the exit die.

119 Approximately 7 to 8 g of material were introduced in the system at 130 °C with a screw  
120 rotation speed of  $N = 50$  rpm for steady shear rate measurements. Engine torque ( $T$ ) was  
121 recorded, as well as pressure drop in the channel ( $\Delta P$ ) to calculate the apparent viscosity:

$$122 \quad \eta_a = \frac{\tau}{\dot{\gamma}_a} \quad (1)$$

123 With  $\tau$  (Eq. 2) the shear stress and  $\dot{\gamma}_a$  (Eq. 3) the apparent shear rate,

$$124 \quad \tau = \frac{h \cdot \Delta P}{2 \cdot \Delta L} \quad (2)$$

$$125 \quad \dot{\gamma}_a = \frac{6}{w \cdot h^2} \cdot Q \quad (3)$$

126 where  $h = 1.5$  mm and  $w = 10$  mm are, respectively, the height and width of the channel,  
127  $\Delta L = 64$  mm and  $\Delta P$ , the length and the pressure drop between the sensors.  $Q$  is assumed as  
128 proportional to the screw rate according to

$$129 \quad Q = C \cdot N, \quad (4)$$

130 with  $C = 6 \times 10^{-10} \text{ m}^3 \cdot \text{s}^{-1} \cdot \text{rpm}^{-1}$ , a calibration constant obtained for a commercial polystyrene  
131 (LACQRENE® 6631) of known rheological behavior in co-rotative screws extrusion and  $N$  in  
132 rpm [30].

133 Measurements involving different screw rotation speeds were also conducted during the  
134 recirculation process within the micro-compounder, by decreasing the rotation speed in 10-  
135 second intervals from 200 rpm via the discrete values 150, 100, and 50 rpm down to 10 rpm  
136 during a cycle sweep. This cycle was repeated several times. For a backflow channel of such a  
137 rectangular geometry, values of viscosity ( $\eta$ ) were calculated using corrected shear rate values  
138 in order to consider the shear-thinning properties of the materials and the dimensions of the  
139 channel [30, 31]. The slope of the curve  $\log(\tau)$  vs  $\log(\dot{\gamma}_a)$  provides the pseudo plasticity index  $n$   
140 that allows the Rabinowitsch correction

141  $\dot{\gamma}_w = \dot{\gamma}_a \times \frac{2}{3} \left( 1.047 + \frac{0.452}{n} \right)$  (5)

142

### 143 2.3.2. Shear oscillation rheometry

144 Oscillatory measurements were performed using a rheometer with a plate-plate geometry pre-  
145 heated at 130 °C. Samples were placed between two parallel plates of  $\phi_{\text{plate}} = 20$  mm diameter  
146 oscillating in order to apply a strain of  $\gamma_0 = 0.7\%$  in the linear viscoelastic domain [16].  
147 Approximately 0.5 g of extruded filaments obtained by direct extrusion using the micro-  
148 compounder (see the following paragraph, §2.3.1), were placed onto the basement plate to be  
149 melted for these oscillatory experiments. First series of measurements were done at constant  
150 frequency  $\omega = 1.7$  Hz (10 rad/s), shear viscosity  $\eta$  and elastic and viscous moduli  $G'$  and  $G''$   
151 were recorded by the software device (Eq. 6, 7 and 8).

152  $\eta^* = K \cdot \omega^{n-1}$  (6)

153  $G' = L \cdot \omega^m$  (7)

154  $G'' = P \cdot \omega^r$  (8)

155 The second series of measurements consisted in frequency sweep with oscillation decreasing  
156 from  $\omega \approx 630$  rad/s to 0.630 rad/s. This cycle was repeated over time for about 40 min.

157

### 158 2.4. Filaments sintering characterization

159 In order to fully investigate the thermoplasticization of zein, considering the presence of  
160 glycerol and/or other potential plasticizers, it is necessary to thoroughly characterize the  
161 sintering capabilities between the extruded filaments. To achieve this objective, it is crucial to  
162 go beyond the acquisition and analysis of 2D images to characterize the material while  
163 mitigating inherent limitations, primarily the drop-shadow effect caused by backlighting and  
164 the challenge of accurately locating the focal plane due to the length of filaments and their  
165 continuous movement during hot melt isothermal sintering in the instrumented oven.



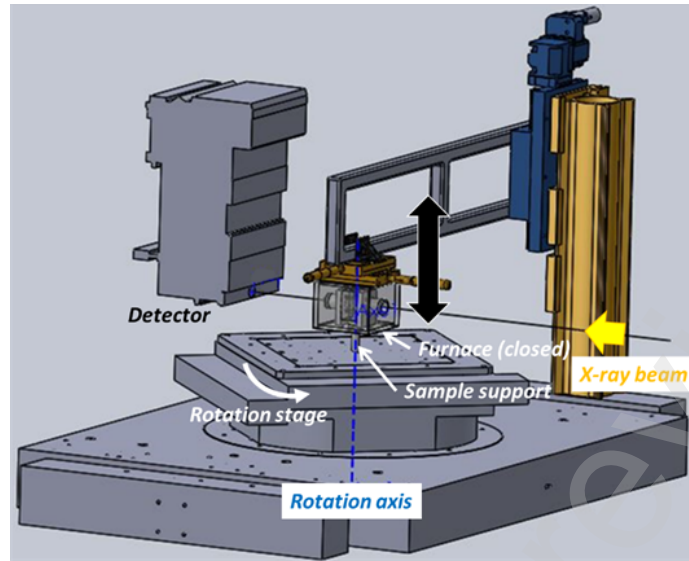
166

167 *2.4.1. Experimental set up for synchrotron dynamic X-ray tomography*

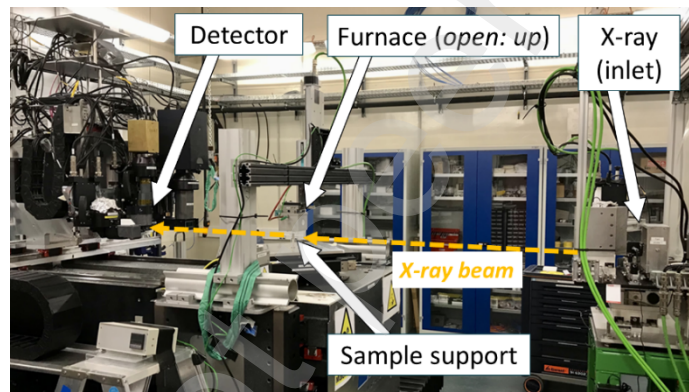
168 The high intensity of the synchrotron X-ray beam is required to record data with sufficient  
169 signal-to-noise ratio in a short time, here of the order of a few seconds per volume scan. The  
170 availability of inline phase contrast is an additional plus over laboratory X-ray tomography. The  
171 synchrotron X-ray tomography measurements were performed using the microtomography  
172 station [32] at the ANATOMIX beamline [33] of Synchrotron SOLEIL. A furnace dedicated to  
173 synchrotron microtomography at high temperatures ( $105 \times 100 \times 80 \text{ mm}^3$ ; heating power 40 W;  
174  $T$  in the range  $[100 \text{ }^\circ\text{C}; 140 \text{ }^\circ\text{C}] \pm 0.1 \text{ }^\circ\text{C}$ ), was designed for this purpose by the BIA-INRAE  
175 group in close exchange with the ANATOMIX beamline team (Fig. 1). The heated volume  
176 inside the furnace is essentially a horizontal cylindrical cavity ( $\varnothing 20 \text{ mm}$ ) lined with aluminum  
177 and insulated with Teflon -polytetrafluoroethylene (PTFE), through which the synchrotron X-  
178 ray beam can pass, with one Kapton window at each end to limit the thermal convection while  
179 ensuring X-ray transmission near 100%. A vertical hole ( $\varnothing 10.5 \text{ mm}$ ) in the bottom of the  
180 chamber, also with aluminum lining, enables insertion of the sample from below. The furnace  
181 was fixed on a vertical motorized translation, suspended above the tomography rotation stage.  
182 Two extruded filaments based on zein ( $\varnothing_{\text{filament}} = 2 \text{ mm}$ ,  $L_{\text{filament}} = 5 \text{ mm}$ ) were placed side-by-  
183 side on the flat top of the sample holder, a tailor-made cylindrical sample support ( $\varnothing 10 \text{ mm}$ )  
184 made of PEEK and topped with an aluminum plate (thickness 1 mm). To carry out sintering  
185 trials under isothermal conditions, the furnace was first heated up for 10 minutes at the desired  
186 set temperature, while kept in a position 20 cm above the samples and the X-ray beam. Then it  
187 was lowered in about 10 s over the samples and into the X-ray beam using the motorized  
188 translation, enabling tomography scanning of the two filaments during their sintering thanks to  
189 the high temperature of the pre-heated furnace.

190

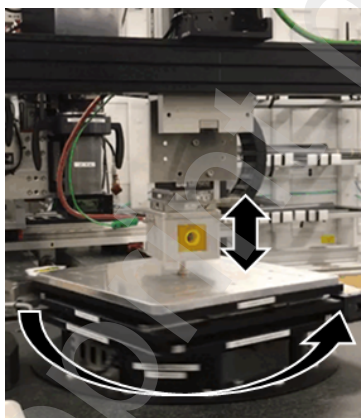
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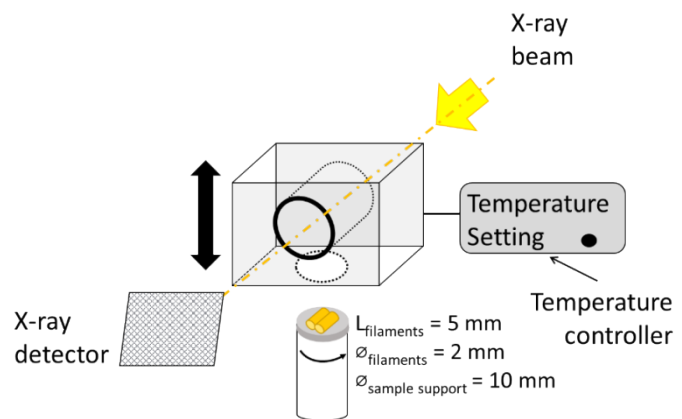
b-



c-



d-



**Fig. 1.** Experimental set up for dynamic X-ray tomography at the ANATOMIX beamline to assess filaments sintering kinetics: (a) 3D drawing and (b) photo. (c) Front view of the furnace with Kapton window and (d) scheme of the devices and samples (*i.e.*, two juxtaposed filaments) placed on the holder.

192 Each tomography volume scan was performed by acquiring 1000 projection radiographs over  
193 an angular range of  $180^\circ$  while the sample stage (model RT500, LAB Motion Systems,  
194 Bekkevoort, Belgium) was rotated at a speed of  $150^\circ \cdot \text{s}^{-1}$ . The overall acquisition time for the  
195 projections of each scan was thus 1.2 s, where the camera acquisition time for each projection  
196 was set to 1 ms. Scans were acquired every 6 s (*i.e.*  $10 \text{ scans} \cdot \text{min}^{-1}$ ) during 2.5 min.

197 The detector was an indirect lens-coupled system consisting of a single-crystal lutetium  
198 aluminum garnet scintillator ( $\text{Lu}_3\text{Al}_5\text{O}_{12}$ , thickness 300  $\mu\text{m}$ , supplier: Crytur, Turnov, Czech  
199 Republic), a pair of Hasselblad photo objectives with focal lengths of, respectively, 100 mm  
200 and 210 mm mounted in tandem geometry [34], resulting in an optical magnification of 2.1,  
201 and a scientific-grade fast CMOS detector (model pco.dimax HS4; supplier: PCO, Kelheim,  
202 Germany;  $2000 \times 2000$  pixels, physical pixel size 11  $\mu\text{m}$ ). The effective pixel size was 5.2  $\mu\text{m}$ .

203 The beamline was operated with an electron current of 450 mA in the SOLEIL storage ring,  
204 using a filtered polychromatic (“white”) X-ray beam from the U18 cryogenic in-vacuum  
205 undulator of the beamline set to a magnetic gap of 7.5 mm and X-ray attenuation filters of gold  
206 with a total Au thickness of 20  $\mu\text{m}$ , resulting in a central X-ray photon energy around 25 keV.

207 At the camera exposure time of 1 ms, this gave a signal of 26,000 ADU (analog-digital units)  
208 in the flat beam from the 16-bit analog-digital converter (ADC) of the pco.dimax.

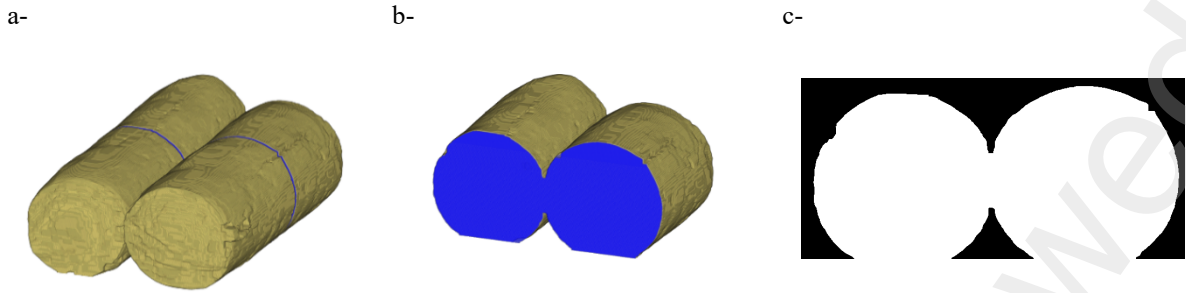
209 The camera was operated with a hardware region of interest (ROI) set to  $1600 \times 560$  pixels, *i.e.*  
210 a field of view of 8.3 mm width and 2.9 mm height. The distance between the sample and the  
211 detector was set to 760 mm, to allow for X-ray phase contrast to form, in order to be sensitive  
212 to potential defects in the filaments, such as pores. Tomographic reconstruction was performed  
213 using the standard data processing pipeline at ANATOMIX, in which reconstruction parameters  
214 are set with an iPython script [35] and the reconstruction itself was performed by the computer  
215 program PyHST2 [36] using the filtered back-projection method.

216

217 *2.4.2. Image analysis to characterize sintering kinetics*

218 Image analysis and adjustments are semi-automated and carried out with Matlab® software  
219 (The MathWorksInc, Natick, MA, US). For image analysis, the 3D reconstructed volumes  
220 stored in 32-bit floating-point format obtained from X-ray tomography were first converted into  
221 8-bit files to reduce the computational time. For each 3D image, an adapted median filter was  
222 applied to remove noise, and a grey level threshold was determined to segment the sample from  
223 the background. This threshold was set to the median value of the first pixels analyzed on the  
224 image. Subsequent image processing consisted of morphological filters to reduce the artefacts  
225 at the edges of the filaments in order to obtain binary images of a ROI corresponding to the  
226 filaments, faithful to the experimental phenomenon. From these binary images, virtual sections  
227 with a total thickness of 57 µm (stack of 11 slices of the image x 5.2 µm thick each) were  
228 selected in the center part of the two juxtaposed filaments to characterize the sintering  
229 phenomenon in detail (Fig. 2). Those 11 segmented slices were combined with a logical “OR”  
230 operation into a single 2D image. The length of the fusion bonding neck between the two  
231 filaments and the corresponding bonding angle was calculated following Hopper’s model [17,  
232 37].

233



**Fig. 2.** Illustration of the characterization of sintering between two extruded zein-based filaments (formulation Z20GLY, after 100 s at 130 °C) from dynamic X-ray tomography. Three steps: (a) 3D rendering of the whole volume of the juxtaposed filaments; (b) 3D rendering cut in half at the location to monitor fusion-bonding between filaments; (c) 2D segmented image of the section shown in blue in (a) and (b) with the zein-based material in white pixels and surrounding air in black.

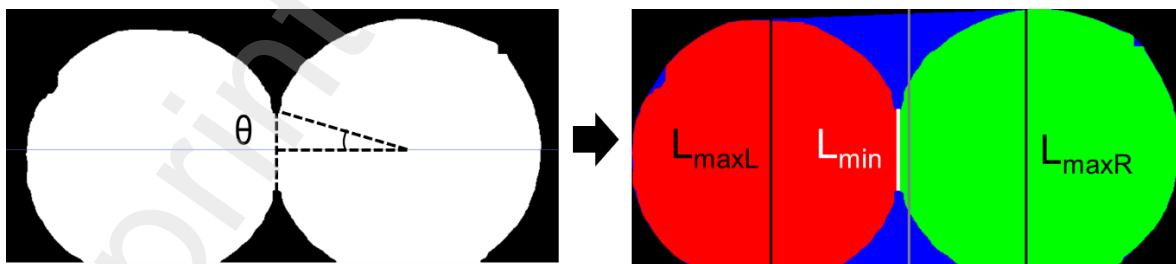
234

235 Hopper's model was then used to assess the evolution of the geometry of the sintered filaments  
 236 with time at high temperature in the furnace: the length of the fusion bonding neck,  $L_{min}$ , the  
 237 average maximum diameters of the right (R) and left (L) filament seen as side view,  $L_{max}$ , and  
 238 the bonding angle  $\theta$  (Fig. 3).

$$239 \quad L_{max} = \frac{L_{maxR} + L_{maxL}}{2} \quad (9)$$

$$240 \quad \theta = \sin^{-1}\left(\frac{L_{min}}{L_{max}}\right) \quad (10)$$

241



**Fig. 3.** Left: Binary segmented image of a filament cross-section during sintering (stack of 11 tomographic slices in the middle part of the filaments during their sintering; Z20GLY sample at 130 °C after ~100 s in the furnace). Right: illustration of the quantities retrieved by image analysis.

242

## 243 2.5. Additive manufacturing

244 Model cylindrical tablets were 3D printed with formulations based on zein in collaboration with  
245 West Waste Print (Nantes, France), Octoplus-3D (Barjouville, France) and 3D printer  
246 manufacturer Tumaker® (Irun, Spain). A 3D printer by hot melt deposition with a single screw  
247 ( $\text{Ø}_{\text{screw}} = 8 \text{ mm}$ ) was equipped with a 0.8-mm-diameter nozzle. Layers of 0.4 mm were  
248 produced with an extruding rate of 25 mm/s (setting at 750 step.mm<sup>-1</sup> for the step-by-step  
249 engine). The extrusion temperature (barrel of the single-screw extruder) was set at 115 °C and  
250 the nozzle temperature at 140 °C (to obtain a temperature of the flowing material measured at  
251 about 130 °C, because of heat loss, the printer environment being open). The plate was  
252 regulated at 100 °C, to obtain temperature on the surface above 80 °C (the temperature for  
253 material flowing [16]). Six successive layers were superimposed for 3D printed tablets, to reach  
254 a total thickness of about 2.4 mm. Two types were processed based on zein-based materials:  
255  $\text{Ø}_{\text{tablets}} = 35 \text{ mm}$ , for three complex porous structures presenting the same density at 0.5 but  
256 different printed porous patterns and  $\text{Ø}_{\text{tablets}} = 20 \text{ mm}$  for dense -100% infill- model, needing  
257 less place to obtain a simple rectilinear non-patterned porous structure).

258

## 259 2.6. Static X-ray tomography scans at laboratory scale

260 Static X-ray tomography images were obtained using a Skyscan 1174 apparatus (Bruker France  
261 SAS, Champs-sur-Marne, France) located at Oniris (Nantes, France). Samples were placed in  
262 a foam support and scanned in rotation steps of 0.6°, for a total exposure time of about 20 min.  
263 The reconstructed slices obtained were TIFF files of 16-bit depth and a pixel size of 29.7 µm.

264

## 265 3. Results and discussion

### 266 3.1. Steady-state flow rheology under extrusion conditions

267 Measurements carried out with the twin-screw micro-compounder allow screening  
268 formulations of zein plasticized by various amounts of glycerol and/or [Lid][Ibu], by comparing  
269 their rheological behavior as a function of residence time (Fig. 4).  
270 Apparent viscosity values measured after 1 min of recirculation are given in Table 2.  
271 Plasticization with the same amount of pure glycerol (Z20GLY) leads to a much lower melt  
272 viscosity compared to pure [Lid][Ibu] (Z20LI). This may be attributed to a different  
273 plasticization mechanism of the amphiphilic zein macromolecules, which have a prismatic  
274 structure combining hydrophilic top and bottom surfaces and hydrophobic lateral surfaces [38].  
275 Glycerol being hydrophilic and [Lid][Ibu] hydrophobic, the two substances can only interact  
276 with the respective matching surfaces of zein prisms. Viscosity values thus suggest that the  
277 overall reduction of zein intermolecular interactions is higher for glycerol which reduces the  
278 hydrophilic interactions between zein prisms. When glycerol is partially substituted by 5% of  
279 [Lid][Ibu] (Z15GLY5LI), the initial viscosity slightly decreases suggesting a synergy between  
280 the two plasticization mechanisms. However, for higher [Lid][Ibu] content, the viscosity  
281 increases almost linearly.  
282

283 **Table 2.** Apparent initial viscosity of zein-based materials after 1 min recirculating in twin-screw micro-  
284 compounder at 130 °C and duration of the processing window *i.e.* time to protein aggregation.

Formulation ID	Apparent initial viscosity at t=1 min [Pa.s]	Duration of the processing window [min]	Gelation time [min]
Z20GLY	770	6	12
Z15GLY5LI	750	9	26
Z10GLY10LI	970	10	92
Z5GLY15LI	1020	-	286
Z20LI	1330	-	

285  
286 During the recirculation inside the micro-compounder at 130 °C, the viscosity increases with  
287 time for all formulations. However, the shape of viscosity vs time curves strongly differs. For  
288 pure [Lid][Ibu] (Z20LI), a sharp, almost linear increase is observed, reaching  $5.4 \times 10^3$  Pa.s after  
289 approximately 180 sec (Fig. 4). This suggests a fast aggregation of zein macromolecules by  
290 stacking of their hydrophilic top and bottom surfaces [38]. Such a rheological behavior is  
291 incompatible with a melt 3D printing process. Concurrently, for pure glycerol plasticization,  
292 the slope of the viscosity curve remains low for approximately 360 sec (6 min) before  
293 increasing to above  $2 \times 10^3$  Pa.s. This leaves a time window for 3D printing, before zein  
294 macromolecules aggregation by side-by-side association through hydrophobic residues, and/or  
295 sulfide bond formation (the cysteine residues being hydrophobic).

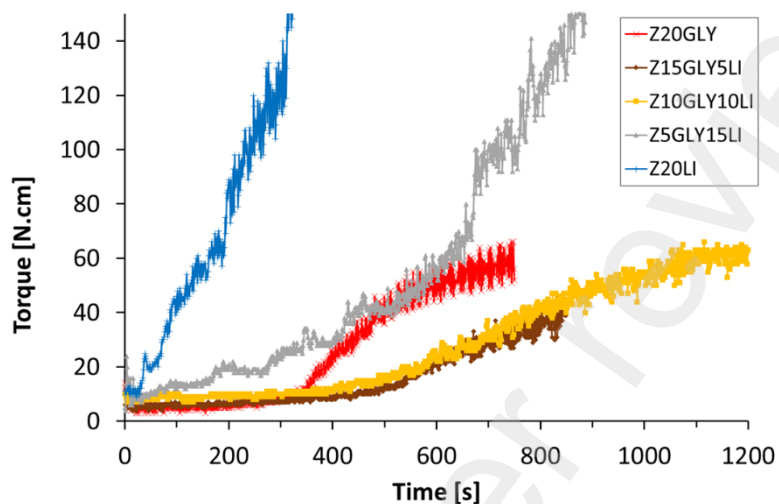
296 It is noteworthy that the partial substitution of glycerol by up to 50% of [Lid][Ibu]  
297 (Z10GLY10LI) increases the processing window before the change of slope, suggesting a  
298 synergy between the plasticizers in terms of delaying protein aggregation, both through their  
299 hydrophobic and hydrophilic residues. The formulation Z10GLY10LI thus combines a



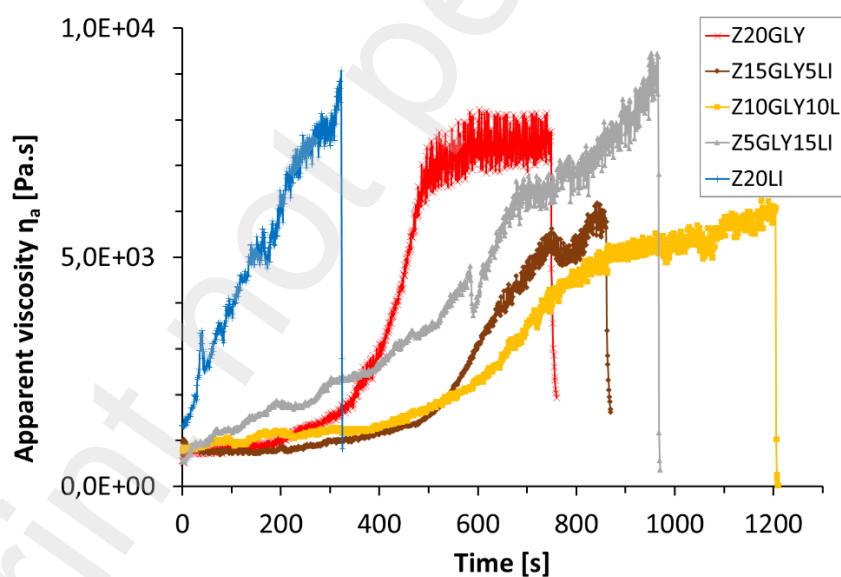
300 relatively low initial viscosity, and a large processing window. It was therefore selected for  
301 further investigation for 3D printing of zein material based with pharmaceutical ingredient.

302

a-



b-



**Fig. 4.** (a) Engine torque and (b) apparent viscosity,  $\eta_a$ , of molten zein-based materials recirculating in the micro-compounder at 130 °C.

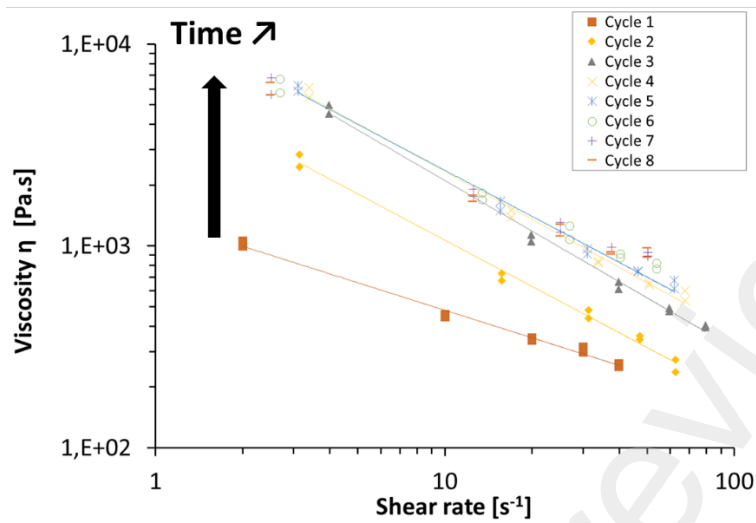
303

304 For Z20GLY and Z10GLY10LI, the flow curves obtained from measurements at various  
305 rotation rates are shown in Figure 5. Melt viscosity decreases with the increase of shear rate,

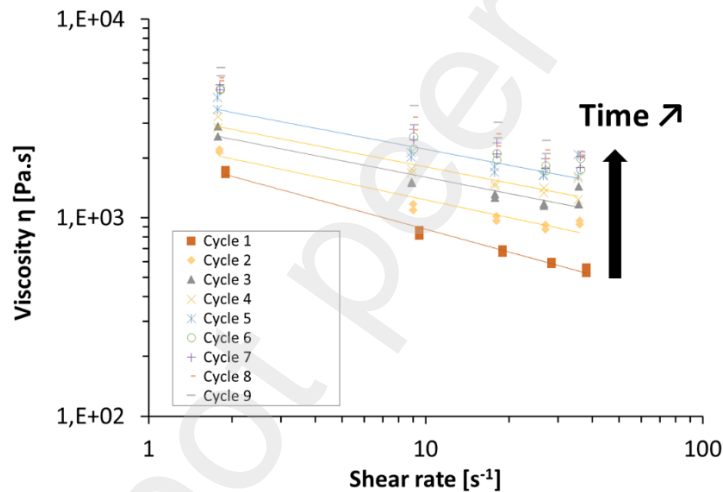
306 showing the shear thinning behavior of the materials, and increases with residence time at  
307 130 °C as flow curves are shifting upward. This corresponds to protein aggregation, as  
308 mentioned above in paragraph §3.1.1. (Fig. 5). Cycle 1 (residence time  $\approx$  1 min) highlights the  
309 lower initial viscosity of formulation Z20GLY compared to Z10GLY10LI with an apparent  
310 viscosity between 260 and 1000 Pa·s, against 560-1700 Pa·s for Z10GLY10LI. Measurements  
311 of the following cycles show greater shear-thinning properties for Z20GLY, as viscosity gap  
312 between the highest and the lowest shear rate is around  $5 \times 10^3$  Pa·s against  $2 \times 10^3$  Pa·s for  
313 Z10GLY10LI. However, rheological properties of Z20GLY seem to reach a plateau from  
314 cycle 3 ( $\approx$  8 min), as curves of the following cycles are superimposed, whereas curves of  
315 Z10GLY10LI continue to shift to higher viscosity values. This behavior demonstrates the  
316 protein aggregation as being delayed when formulation includes 10% of [Lid][Ibu]  
317 (Z10GLY10LI).

318

a-



b-



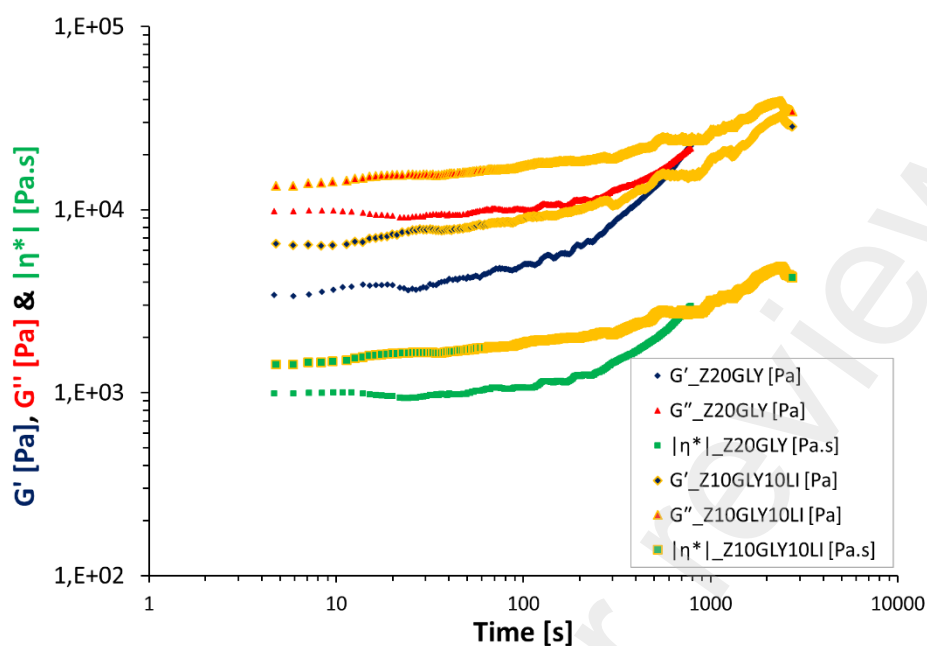
**Fig. 5.** Evolution of the viscosity,  $\eta$ , determined during recirculation in micro-compounder at 130 °C and presented as a function of the applied shear rate for zein-based materials: (a) Z20GLY, (b) Z10GLY10LI. Each cycle corresponds to 5 rotation rates of the screws repeated over time (approximately at cycle 1: 1 min, cycle 4: 10 min, cycle 8: 25 min).

319

### 320 3.2. Dynamic shear rheology

321 Formulation Z10GLY10LI was characterized in plate-plate geometry rheometer in order to  
 322 assess its viscoelastic and rheological properties over a frequency range of several decades,  
 323 allowing to complete the comparison to the reference formulation Z20GLY, including glycerol  
 324 alone as standard plasticizer. Extruded filaments were melted in a parallel-plate rheometer at

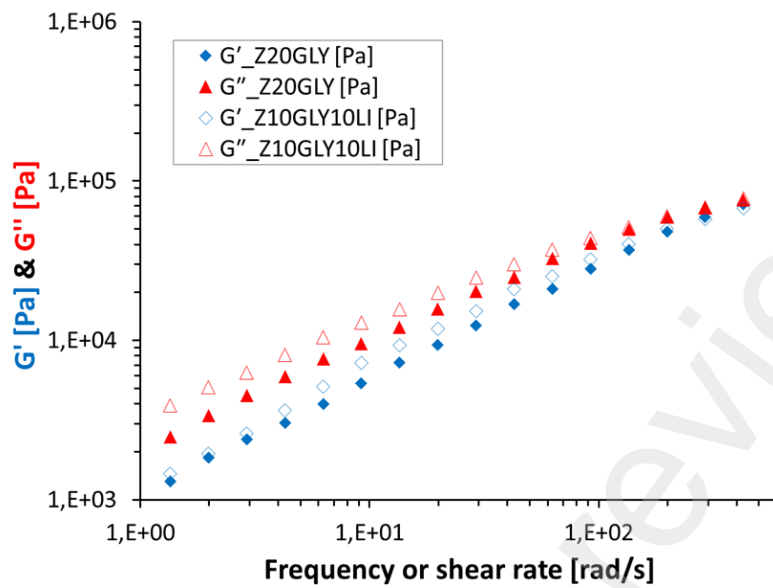
325 130 °C to measure viscoelastic moduli along oscillations. Heating started with an elastic  
326 modulus  $G'$  inferior to viscous modulus  $G''$ , as can be seen in Figure 6, confirming the melted  
327 state of the material. After several minutes at high temperature, moduli eventually increased  
328 and the  $G'$  and  $G''$  values converged up to crossover in the case of Z20GLY indicating a gelation  
329 of the material at  $t = 6$  min. In presence of [Lid][Ibu] (Z10GLY10LI), such a crossover of the  
330 modulus curves was not observed during experiments, which can be ascribed either to an  
331 insufficient residence time at 130 °C to reach gelation, or to an absence of gelation. Indeed,  
332 gelation of plasticized zein is known to involve the formation of sulfide bonds by reaction  
333 between cysteine residues [16], which are hydrophobic. The presence of the hydrophobic  
334 [Lid][Ibu] thus may prevent such crosslinking reactions (and so gelation) or at least delay them.  
335 Assuming a simple delay of gelation, the time of crossover between  $G'$  and  $G''$  can be evaluated  
336 by asymptotic extrapolation of the curves, leading to the gelation time values listed in Table 2.  
337



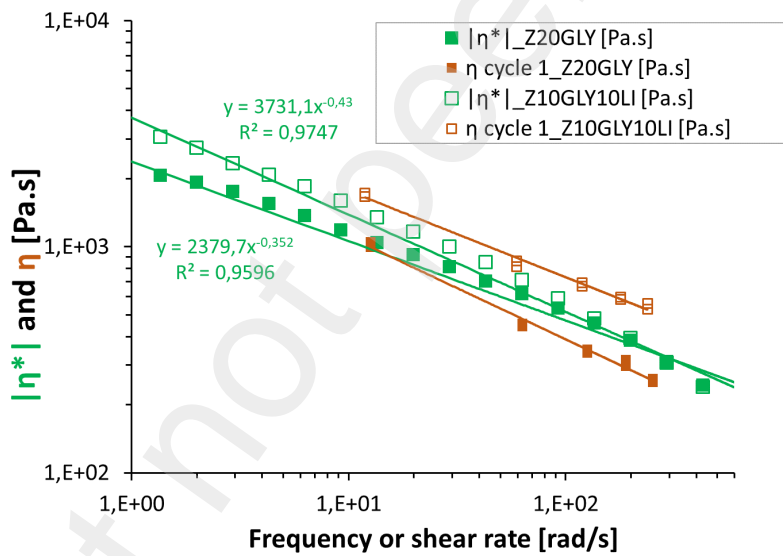
**Fig. 6.** Evolution of the viscoelastic behavior (*i.e.* conservative modulus  $G'$  and dissipative one  $G''$ ) and the modulus of the complex viscosity  $\eta^*$ , obtained at 130 °C in an oscillation rheometer at constant frequency (1.7 Hz), for molten zein-based materials Z20GLY and Z10GLY10LI.

339  
 340 Measurements of viscoelastic modulus were also carried out in oscillatory frequency sweep  
 341 with the same experiment. Figure 7 shows the first cycle of measurements at time  $\approx$  1 min.  
 342 Viscosity decreases with the increase of the frequency showing the shear thinning properties of  
 343 formulations. Z20GLY shows a lower viscosity. than Z10GLY10LI. This viscosity curve is  
 344 shifting to higher values when cycles are repeated at high temperature and during these cycles,  
 345 it is also interesting to note that the viscous modulus  $G''$  is superior to  $G'$  that tends to approach  
 346  $G''$  when frequency oscillation increases and with viscosity increase (please see Appendix A1,  
 347 Fig. S1).  
 348 The viscosity values obtained can be compared to the viscosity measured in cycle 1 with micro-  
 349 compounder – recirculation – rotation speed sweep, and almost follow the application of the  
 350 Cox-Merz empirical rule. For a minimum shear rate, at 1 rad/s and short heating (cycle 1),  
 351 Z20GLY exhibits a lower viscosity ( $\approx$ 2 kPa.s) than Z10GLY10LI ( $\approx$ 3 kPa.s).

a-



b-



**Fig. 7.** Viscoelastic behavior assessed during the first frequency sweep at 130 °C for materials Z20GLY (filled squares) and Z10GLY10LI (hollow squares). (a) Values of the conservative modulus,  $G'$ , and the dissipative modulus,  $G''$ . (b) Modulus of the complex viscosity  $\eta^*$  and superimposed values of the initial flow viscosity  $\eta$  (cycle 1) obtained under extrusion conditions with the micro-compounder set at 130 °C.

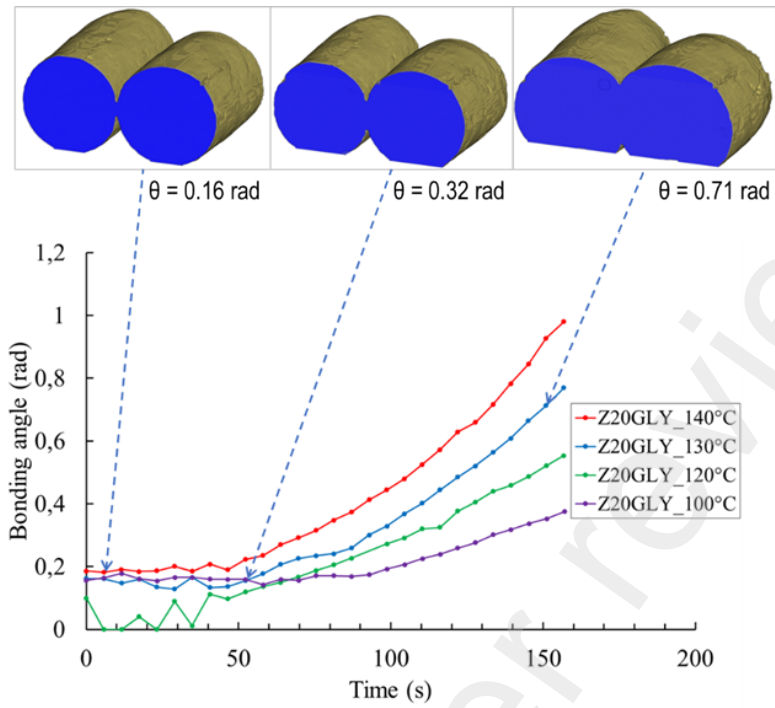
353 *3.3. Filament sintering abilities in relation to melt rheological behavior*

354 Figure 8 shows the evolution of the bonding angles obtained by image analysis of X-ray  
355 tomography data for formulations Z20GLY and Z10GLY10LI at four temperatures ranging  
356 from 100 °C to 140 °C.

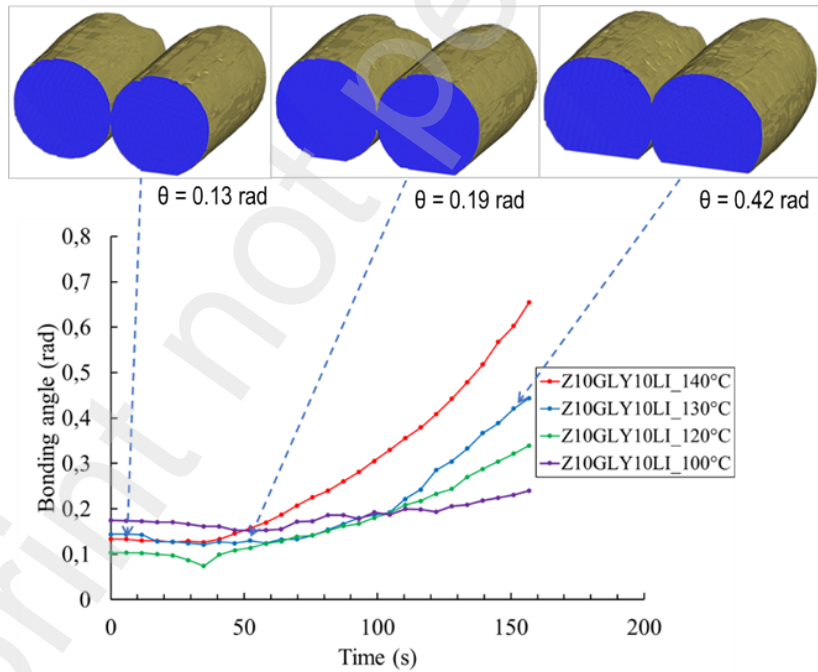
357 The slope of the curves, initially close to zero, starts increasing around 50 s after the start of the  
358 sintering process, except for the measurements at 100 °C, for which sintering remains very  
359 slow, due to the fact that the molten state is only reached at temperatures significantly above  
360 80 °C [16].

361

a-



b-



**Fig. 8.** Sintering kinetics monitored via the evolution of the bonding angles assessed by dynamic X-ray tomography for Z20GLY (a) and Z10GLY10LI (b).



363 Sintering rates  $V$  [ $\text{rad}\cdot\text{s}^{-1}$ ], defined as the slope of the linear part of the curves (in the range  
 364 between 100 and 150 s) are given in Table 3: An increase with temperature is observed, as  
 365 previously found for such plasticized biopolymer [17].

366 Lower rates are observed when [Lid][Ibu] is present, which is consistent with the higher melt  
 367 viscosity compared to pure glycerol. It was also checked that no dilation force was involved in  
 368 the fusion-bonding phenomenon, indeed, measurements carried out on one filament in the  
 369 furnace showed no volume increase (please see Appendix A2, Fig. S2).

370  
 371 At temperatures above 100 °C, the ratio between the sintering rates observed for the two  
 372 formulations is almost constant ( $V_{\text{Z10GLY10LI}} / V_{\text{Z20GLY}} \approx 60\pm 5\%$ ). This ratio is close to that of  
 373 melt viscosities at 130 °C measured after 150 s at 130 °C (typical sintering time) with micro-  
 374 compounder during rotation rate sweeps measurements ( $\eta_{\text{Z20GLY}} / \eta_{\text{Z10GLY10LI}} \approx 61\%$  at  
 375 10  $\text{rad}\cdot\text{s}^{-1}$ , Fig. 7-b) and in dynamic rheometry ( $|\eta^*|_{\text{Z20GLY}} / |\eta^*|_{\text{Z10GLY10LI}} \approx 68\%$  at 1  $\text{rad}\cdot\text{s}^{-1}$  and  
 376  $|\eta^*|_{\text{Z20GLY}} / |\eta^*|_{\text{Z10GLY10LI}} \approx 61\%$  at 10  $\text{rad}\cdot\text{s}^{-1}$ , Fig. 7-b and Fig. 6, respectively). This suggests  
 377 that the surface tension, the other parameter affecting sintering rate theoretically, is probably  
 378 not significantly modified by the presence of [Lid] [Ibu].

379  
 380 **Table 3.** Sintering rate of Z20GLY and Z10GLY10LI and their ratio at different temperatures (*\*beginning of the*  
 381 *sintering phenomenon, slightly starting at 100 °C).*

V [ $\text{rad}\cdot\text{s}^{-1}$ ]			
Temperature [°C]	Z20GLY	Z10GLY10LI	Ratio: $V_{\text{Z10GLY10LI}} / V_{\text{Z20GLY}}$
140	0.0093	0.0060	65%
130	0.0075	0.0047	63%
120	0.0050	0.0028	56%
100*	0.0032	0.0008	25%

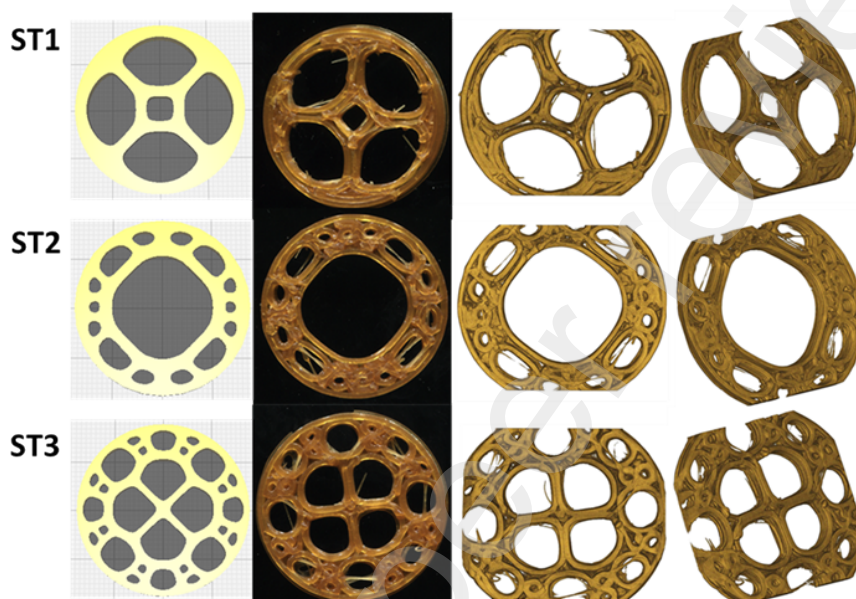
382

383

### 384 3.4. 3D printed tablets based on zein and sintering abilities of the deposited melts

385 Three virtual structures were generated by multi-physics modelling with identical 50% infill  
386 density, leading to different simulated release kinetics (ST1, ST2 and ST3 from [12], Fig. 9).

387

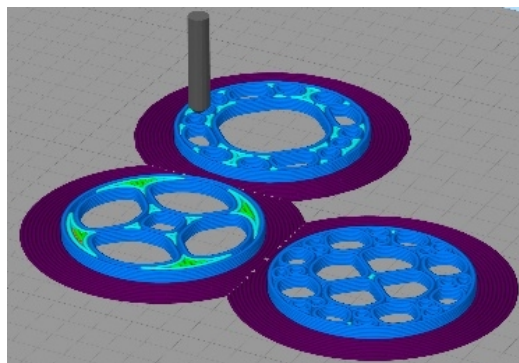


**Fig. 9.** 3D digital models of tablets with different geometries for the same density set at 0.5 (left); images of Z20GLY-printed tablets (left center) and 3D renderings of volumes obtained by static X-ray tomography with a laboratory instrument (right center and right).

388

389 These tablets were successfully printed with the Z20GLY formulation under conditions  
390 described in paragraph §2.5, with a final porosity of the 3D printed tablets confirmed by X-ray  
391 tomography to be 0.5 on average, with minimum and maximum values of, respectively, 0.47  
392 and 0.55, confirming the suitability of zein plasticized with glycerol for 3D printing (Fig. 10).  
393 However, experiments using the Z10GLY10LI formulation were inconclusive and revealed  
394 poor adhesion of successive layers to one another. (Fig. 10-c). It is nonetheless noticeable that  
395 the adhesion to platform support is very efficient for all tested compositions in 3D printing  
396 trials.

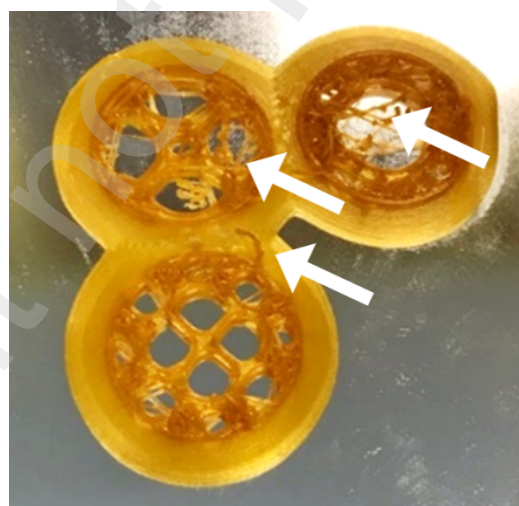
a-



b-



c-



**Fig. 10.** Preview of melt deposition paths for 3D printing with different geometries (a), after 3D printing of Z20GLY (b) and Z10GLY10LI (c). Arrows indicate adhesion defects.

398

399 To enhance the printing of co-plasticized formulation Z10GLY10LI, we tried to optimize the  
 400 process conditions by working on full filled tablets (100% density in printing parameters, with

401 rectilinear filling). Z20GLY was correctly printed (Fig. 11-a) and, to obtain satisfying tablets  
402 with Z10GLY10LI (Fig. 11-b), the nozzle temperature set in the software was increased by  
403 5 °C, as well as the extruded material flow rate that was increased by 15% (860 steps·mm<sup>-1</sup> for  
404 the step-by-step engine). These conditions allowed us to overcome the higher viscosity of  
405 Z10GLY10LI, which leads to degraded sintering abilities as shown in the previous paragraph  
406 (§3.3).

407 The porous structure of the final printed tablets was tested by static X-ray tomography at the  
408 laboratory scale and, as expected, these tablets showed a very low porosity ranging from 0.5 to  
409 1%. Furthermore, to help in adhesion, the flow rate was set significantly higher for  
410 Z10GLY10LI. Therefore, the tablet obtained with this formulation has the smallest porosity  
411 (0.5% in volume of the total printed part, Fig. 11). However, it is noteworthy that in the  
412 reconstructed volumes obtained through tomography, the pores within the Z20GLY tablet  
413 exhibit a higher level of connectivity compared to the Z10GLY10LI tablet. This observation  
414 confirms the greater density of the latter formulation. Moreover, porosity distribution is more  
415 in the small pores for Z10GLY10LI, with 80% pores smaller than 90 µm, whereas it is 55% in  
416 the case of Z20GLY (Fig. 11-c).

417

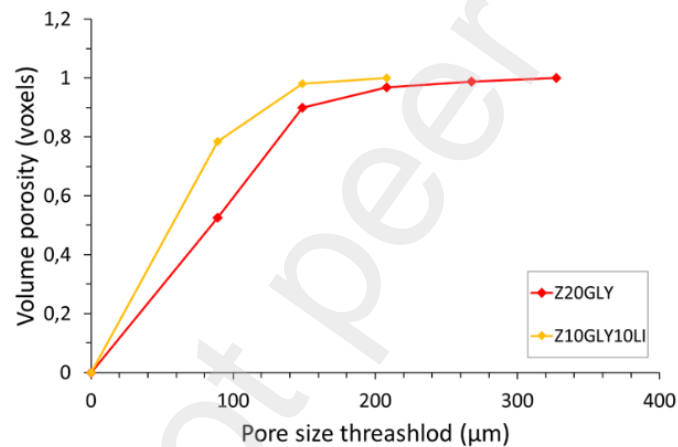
a-



b-



c-



**Fig. 11.** 3D printed full-filled tablets ( $\text{Ø}_{\text{tablets}} = 20 \text{ mm}$ ; 100% filling in melt deposition settings as rectilinear pattern) based on formulation: (a) Z20GLY and (b) Z10GLY10LI. Photos on the left hand and in-line visualization to the right of the reconstructed 3D volumes (static X-ray tomography at laboratory scale): dense material (superposition of 6 layers visible on the edge of the slightly rotated volume) and internal porosity linked to the "defect" during melt deposition. (c) Cumulative size distribution of pores (total porosity of the final printed tablets measured at 1% for Z20GLY and 0.5% for Z10GLY10LI).

418

#### 419 4. Conclusions

420 This work has demonstrated that glycerol can be combined with a hydrophobic ionic liquid in  
421 order to tune the melt rheology of plasticized zein as a feedstock material for additive  
422 manufacturing by hot melt deposition. This technique enables the production of edible materials

423 including an Active Pharmaceutical Ingredient in the form of an Ionic Liquid (API-IL) for the  
424 formulation of 3D printed tablets intended for therapeutic purposes.

425 Flow rheology results obtained on a micro-compounder simulating extrusion at 130 °C showed  
426 that while zein-based melts show a shear thinning behavior, apparent viscosity of plasticized  
427 zein increases with API-IL/glycerol ratio and with residence time. However, adding the  
428 [Lidocainum][Ibuprofenate] API-IL to glycerol delays protein aggregation at 130 °C and  
429 broadens the time window for processing, from 6 minutes when 20w% of glycerol was used as  
430 sole plasticizer to 10 minutes in the case of co-plasticization by 10w% of glycerol and 10w%  
431 of API-IL. This was attributed to [Lid][Ibu] being hydrophobic while glycerol is hydrophilic.  
432 The two substances therefore interact with the amphiphilic zein macromolecules by different  
433 plasticization mechanisms. Nonetheless, this synergy between the plasticizers was not observed  
434 for higher amounts of [Lid][Ibu]. Indeed, the protein aggregation cannot be completely avoided  
435 and the evolution of zein-based melts' viscoelastic behavior studied with oscillatory testing  
436 suggested that a crossover of  $G'$  and  $G''$ , *i.e.* a gelation phenomenon, takes place after longer  
437 durations with increasing amount of API-IL.

438 The partial substitution of glycerol by API-IL also affects the melt sintering kinetics, which  
439 were assessed via a purpose-built dedicated motorized furnace for real-time synchrotron X-ray  
440 microtomography 3D imaging. It was used to monitor the fusion-bonding kinetics of extruded  
441 filaments in high resolution, during isothermal trials. Viscous sintering kinetics were faster  
442 when the temperature was increased in the range from 100 °C to 140 °C, but slowed down when  
443 increasing the API-IL content, presumably due to the induced viscosity increase.

444 Subsequent 3D printing tests at 130 °C showed that such slowed sintering kinetics limits the  
445 printing accuracy of the glycerol/API-IL co-plasticized formulation, despite well bonded  
446 juxtaposed and superimposed layers, while the formulation including glycerol as sole

447 plasticizer allows printing tablets with complex porous structures characterized in detail by  
448 static X-ray tomography.

449 These results open the way for future work on 3D geometries based on zein and Active  
450 Pharmaceutical Ingredients as Ionic Liquid, targeting applications in the biomedical or the  
451 pharmaceutical field by taking advantage of both their plasticizing and therapeutic properties  
452 to set edible and functional printed systems.

453

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