

29 **Abstract**

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31 This study evaluates the potential use of zein as an excipient in hot-melt extrusion for
32 controlled delivery of diclofenac sodium (DS). Mixtures of zein, polyethylene glycol and
33 drug were hot melt extruded and cut into 2 mm extrudates. Extrudates were characterised
34 using differential scanning calorimetry, X-ray powder diffraction and scanning electron
35 microscopy. The drug in the extrudates was found to be in the non-crystalline state,
36 independent of the drug loading. Moreover, the drug release from extrudates was
37 investigated. The release was directly dependent on the drug loading: a controlled and nearly
38 zero-order release was obtained at the lowest drug loading (12.5% w/w), whereas almost
39 immediate release was achieved at higher drug loadings, i.e. 25% and 37.5%. The release was
40 inversely dependent on the ionic strength of the medium. The influence of digestive enzymes
41 on drug release was also studied. Pancreatin, but not pepsin, was found to have a significant
42 influence on the drug release as well as on the microstructure of zein extrudates. These data
43 therefore support the potential use of zein as excipient in hot melt extrusion for controlled
44 release purposes.

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46 *Keywords: Zein, Hot melt extrusion, Controlled release, Solid dispersion, Amorphous,*
47 *Diclofenac sodium*

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56 **1. Introduction**

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58 Controlled-release oral dosage forms are designed to achieve drug release characteristics over
59 a specific time course that can offer certain therapeutic objectives over conventional dosage
60 forms. Polymeric excipients have been used in oral formulations to control drug release rate.
61 Although synthetic and semisynthetic polymers have been extensively used for this purpose,
62 natural excipients, being both sustainable and biodegradable, are being increasingly used.
63 Zein, for example, is a plant-derived protein, that has been extensively studied for its
64 potential use as an excipient in the pharmaceutical industry (Berardi et al., 2018).

65

66 Zein is the major storage protein of corn (Shukla and Cheryan, 2001), and constitutes 44-79%
67 of the endospermic proteins (Lawton, 2002). It is composed of a mixture of different
68 peptides that can be classified on the basis of their solubility and molecular weight into α (19
69 and 22 kDa), β (17-18 kDa), γ (16 and 27 kDa) and δ (10 kDa) zeins (Esen, 1986). Zein is
70 particularly rich in hydrophobic amino acids, but deficient in polar or ionisable amino acids
71 (Cabra et al., 2006; Shukla and Cheryan, 2001). Zein is a natural, biocompatible and
72 biodegradable material produced from sustainable sources (Berardi et al., 2018). Due to its
73 amino acid composition, zein is insoluble in water, yet is soluble in aqueous ethanol
74 solutions. Despite its insolubility in water, zein tends to swell in the presence of aqueous
75 medium (Beck et al., 1996). This is because zein has hydrophilic regions in addition to the
76 hydrophobic domains, and thus behaves as an amphiphilic protein (Wang et al., 2008).
77 Indeed, zein matrices were found to swell in a hydrophilic-like fashion, but were non-
78 erodible as hydrophobic matrices (Berardi et al., 2017b). Being swellable, yet insoluble and
79 nonerodable, zein has been also studied for its potential use as an excipient for controlled
80 drug release in nano- and microscale as well as macroscale formats. Zein tablets (Berardi et

81 al., 2017a; Georget et al., 2008; Katayama and Kanke, 1992; Li et al., 2010; O'Donnell et al.,
82 1997; Raza et al., 2020, 2019) and capsules (Berardi et al., 2017b) were successfully
83 developed for controlled release purposes. However, the potential use of zein biopolymer as
84 an excipient for direct compression could be limited by its poor flowability and tableability,
85 compared to the commonly used excipients, as suggested by Berardi et al. (Berardi et al.,
86 2017a). Similarly, Georget et al. (Georget et al., 2008) reported that tablets core were striated
87 in appearance, due to the elastic recovery of zein upon removal of the compression force.

88

89 Hot melt extrusion (HME) technique involves forcing raw materials using a rotating screw(s)
90 through a die of a defined geometry under elevated temperature (Crowley et al., 2007). HME
91 is a continuous and solvent free process, reducing the number of steps and eliminating the
92 drying step (Crowley et al., 2007). Recently, Zein has proved to be a promising new excipient
93 for HME for the development of controlled drug delivery systems (Bouman et al., 2016,
94 2015). Despite being a protein, it has been shown that zein is relatively heat stable and can be
95 extruded at high temperatures (up to 160 °C) without significant changes in its molecular or
96 physical properties (Selling, 2010). Zein and paracetamol physical mixtures were hot-melt
97 extruded injection moulded into single-matrix phase, homogenous caplets by Bouman et al.
98 (Bouman et al., 2015). Controlled drug release was obtained and was found to be strongly
99 dependent on device dimensions but nearly independent of drug loading. In another work,
100 Bouman et al. studied the influence of drug hydrophobicity as well as the influence of
101 electrostatic interactions between drug and polymer, which is governed by pH medium, on
102 drug release (Bouman et al., 2016). They found out that the release kinetics were not mainly
103 determined by water solubility of the drug, yet the electrostatic attractions between zein
104 matrix and drug can significantly slow down the release.

105

106 In this work, we developed controlled release zein matrices of diclofenac sodium (DS) using
107 hot melt extrusion. DS is a BCS class II drug that is insoluble at acidic pH, yet soluble at
108 more basic intestinal pH (Chuasuwana et al., 2009), justifying its widespread use in controlled
109 release dosage forms (Jantratid et al., 2009; Rani and Mishra, 2004). The physical state of the
110 model drug was characterised by differential scanning calorimetry (DSC) and X-ray powder
111 diffraction (XRPD). Dissolution studies were performed to investigate the effect of drug
112 loading and composition of dissolution medium on drug release. The influence of the medium
113 ionic strength on drug release from zein extrudates was evaluated here for the first time. We
114 also studied the influence of pH switch, i.e. pH 1.2 to pH 7.5, and that of the presence of
115 digestive enzymes on the drug release and on the microstructure of zein extrudates. The
116 objective of this article is therefore to gain an understanding of what are the key factors that
117 might influence the performance of zein extrudates in conditions simulating the
118 heterogeneous environment of the upper gastrointestinal tract, and could ultimately dictate
119 zein's applications.

120

121 **2. Materials and Methods**

122 **2.1 Materials**

123

124 Zein from maize (Z3625) was obtained from Sigma-Aldrich (Germany) and used as received.

125 This product was plasticized with polyethylene glycol 400, PEG 400, obtained from Merck-

126 Schuchardt (Germany). The model drug, diclofenac sodium was obtained from Aarti drugs

127 Ltd. (India) and was used as received. Diclofenac sodium was chosen on the basis that an

128 extended release formulation of this, some are already available in market, can prove useful

129 in management of signs and symptoms of patients with osteoarthritis and rheumatoid

130 arthritis. Pepsin (p7125) and pancreatin (p8096) enzymes were purchased from Sigma-

131 Aldrich (USA). Other materials used for the preparation of the dissolution media were
132 reagents of standard grades and were supplied by AZ chem and Alpha Chemika (India).

133

134 **2.2 Methods**

135

136 **2.2.1 Hot melt extrusion**

137 Zein powder was first granulated in a wet granulation step in order to improve its flow
138 properties. PEG 400, added as an aqueous solution during granulation, was used to lower the
139 glass transition temperature (T_g) of zein. Zein and PEG 400 (10 or 20% of the total weight)
140 were mixed using a mortar and pestle until a suitable granule was formed. At concentrations
141 greater than 20% PEG, the granulation process was not possible. The wet granulation was
142 sieved through a 1 mm sieve, and then dried in oven at 50 °C overnight. The dried granules
143 were sieved again through the same sieve before being stored in a plastic bottle. The residual
144 moisture content was measured by the "loss on drying" (LOD) in triplicate using a moisture
145 balance (Mettler PE 160, USA). The LOD of granules containing 10% and 20% PEG 400
146 was 3.75% (± 0.78) and 3.98% (± 0.32), respectively.

147

148 Blends of pre-plasticized zein granules and different amounts of diclofenac sodium were
149 prepared to obtain formulations with drug loading ranging from 12.5% to 37.5% (w/w). The
150 mixtures were hot-melt extruded using a single-screw extruder (Randcastle microtruder,
151 USA) (Andrews et al., 2008; Young et al., 2002), fitted with a cylindrical die with an inner
152 diameter of 5 mm. The extrusions were performed at a temperature of 140 °C for all zones,
153 i.e. zone 1, 2 ,3 and 4, and a screw speed of 20 rpm. Extrudates were allowed to cool down
154 and then stored in sealed containers at room conditions.

155

156 The composition of zein extrudates is reported in Table 1. A special notation (e.g.,
157 E_12.5_20) was used to describe the preparation conditions: the letter E stands for extrudate,
158 the first number is the concentration of diclofenac sodium (w/w%) and the second number is
159 the plasticizer level (w/w%) with respect to the total weight of zein and PEG 400.

160

161 2.2.2 Drug Content

162 Extrudates were ground using mortar and pestle. An amount equivalent to 25 mg of
163 diclofenac sodium was weighed and transferred to a 100 ml volumetric flask and phosphate
164 buffer pH 7.5 was added. The flask was stirred for 1 hour using a magnetic bar and was then
165 sonicated in a bath sonicator for another 1 hour with intermittent shaking. The volume was
166 made to 100 mL and a 10 mL sample was taken and filtered using 0.45 μ m PTFE filters. The
167 samples were analysed for diclofenac sodium by high-performance liquid chromatography
168 (HPLC; Knauer, Leeds, UK). The mobile phase consisted of water- methanol (30:70 v/v)
169 adjusted to pH 3.5 with glacial acetic acid and pumped at 1 mL.min⁻¹ through a C18, 5 μ m,
170 250 x 4.6 mm column (Thermo Scientific, USA). UV detection was carried out at 276 nm.
171 The measurements were performed in triplicate and the averages and standard deviations
172 were calculated. The drug content of zein extrudates is reported in Table 1.

173

174 2.2.3 Differential Scanning Calorimetry (DSC)

175 DSC analysis of diclofenac sodium (DS) raw powder, zein raw powder, physical mixtures of
176 DS and zein powder or granulated zein as well as DS loaded zein extrudates was carried out
177 using DSC 250 (TA Instruments, USA). Approximately 5 mg samples were sealed in
178 standard aluminium DSC pans. Extrudates were crushed using mortar and pestle before
179 measurements. The samples were heated from 25°C to 350°C at a heating rate of 10°C/min,
180 under nitrogen purge (50 ml/min).

181

182 **2.2.4 X-Ray Powder Diffraction (XRPD)**

183 X-Ray Powder Diffraction analysis of diclofenac sodium (DS) raw powder, zein raw powder,
184 physical mixtures of DS and zein powder or granulated zein as well as DS loaded zein
185 extrudates was carried out using XRD-7000 X-Ray Diffractometer (Shimadzu, Japan). The
186 diffraction pattern was recorded using monochromatic Cu radiation ($\lambda = 1.54 \text{ \AA}$) as anode
187 material and operated at a voltage of 40 kV and a current of 30 mA. The samples were
188 mounted on Al sample holder and XRD patterns were recorded in the range of $2\text{--}60^\circ$ at the
189 speed of $2^\circ/\text{min}$ and a sampling pitch of 0.02° .

190

191 **2.2.5 Dissolution Studies**

192 Dissolution studies were carried out at $37 \pm 0.5^\circ\text{C}$ in a BP dissolution apparatus I (ERWEKA
193 DT 600, ERWEKA GmbH, Germany), with a rotation speed of 100 rpm. Extrudates were
194 manually cut into pieces of 2 mm long using a cutting blade. An appropriate amount of the
195 previously cut extrudates (2 mm) of each formulation (Table 1), equivalent to 25 mg drug,
196 was weighed and placed in the basket. The tests were performed in 50 mM phosphate buffer
197 (pH 7.5), as recommended by USP monograph for diclofenac sodium extended release
198 tablets, or otherwise in 900 mL of water; 100 mM phosphate buffer (pH 7.5); 200 mM
199 phosphate buffer (pH 7.5); 50 mM phosphate buffer (pH 7.5) + 0.1 M NaCl; 50 mM
200 phosphate buffer (pH 7.5) + 0.2 M NaCl. Ten mL samples were withdrawn at predetermined
201 times using a syringe, filtered through a $0.45 \mu\text{m}$ PTFE membrane filter (Macherey-Nagel
202 GmbH & Co. KG, Germany) and replaced with an equivalent volume of fresh medium. The
203 diclofenac sodium concentration was determined using a UV-VIS spectrophotometer
204 (SpectroScan 80D, Cyprus) set at a wavelength of 276 nm and with reference to an

205 appropriate standard curve. The dissolution studies were carried out in triplicate and the
206 average drug release (calculated based on actual drug content) \pm SD was calculated.

207

208 Dissolution studies under conditions that more closely resemble those in the upper
209 gastrointestinal tract were also carried out. In this case, freshly prepared simulated gastric
210 fluid (SGF) (2 g L⁻¹ NaCl and 3.2 g L⁻¹ pepsin) pH 1.2 was used as the dissolution medium
211 for the first 2 hours. This medium was then replaced by freshly prepared simulated intestinal
212 fluid (SIF) (6.8 g L⁻¹ KH₂HPO₄, 10 g L⁻¹ pancreatin) pH 7.5 for an additional 22 hours.
213 Control experiments using SGF without pepsin for 2 hours followed by 22 hours in SIF
214 without pancreatin were run in parallel. Diclofenac is a weak acid (pK_a=4) and thus
215 practically insoluble in acid. In order to measure the amount of the drug released in acidic
216 media, the media were neutralized at the end of the 2-hour test in SGF with or without pepsin
217 by adding 20 mL of 5M NaOH and stirring using paddles for 5 minutes. Then a sample was
218 withdrawn and filtered through 0.45 μ m filters prior to analysis for diclofenac sodium by
219 high-performance liquid chromatography (HPLC; Knauer, Leeds, UK). In SIF with
220 pancreatin, ten-mL samples were removed at predetermined times over a 22-hour period,
221 centrifuged at 13,000 rpm for 30 minutes, and filtered through 0.45 μ m filters prior to
222 analysis for diclofenac sodium by high-performance liquid chromatography (HPLC; Knauer,
223 Leeds, UK), using the same method described in Section 2.2.2.

224

225 **2.2.6 Scanning Electron Microscopy (SEM)**

226 The surface morphology of zein extrudates prior and after testing in media was studied using
227 SEM. Samples were mounted onto stubs using double-sided tape and were platinum coated
228 by a Emitech K550 X sputter coater manufactured by Quorum Technologies (England). The

229 imaging process was performed in a high vacuum environment. Imaging process was
230 performed with a FEI Inspect F50 SEM (Netherlands), mounted with a tungsten filament with
231 an acceleration voltage of 1-30 kV.

232

233 **3. Results**

234

235 **3.1 Characterization of Drug-Loaded Zein Extrudates**

236

237 DSC and XRPD studies were engaged for determination of the physical state of the model
238 drug, diclofenac sodium, in the prepared zein extrudates. Physical mixtures of DS and zein or
239 granulated zein as well as DS and zein raw powders were used as controls.

240

241 DSC thermograms of DS and zein raw powders, physical mixtures of DS and zein powder or
242 granulated zein and zein extrudates of two different drug loadings are demonstrated in Figure
243 1. Zein powder showed a very broad endotherm with onset at 50 °C and peak temperature
244 around 90 °C. This can be associated with water loss (Tillekeratne and Easteal, 2000). The
245 glass transition temperature of zein was observed at 168.4 °C which is in agreement with
246 previous reports (Doğan Atik et al., 2008; Tillekeratne and Easteal, 2000). After 255 °C, the
247 degradation of zein started with an exothermic peak coupled with an endothermic peak at 300
248 °C that has been attributed to thermal denaturation of zein through the breakdown of
249 hydrogen bonds, electrostatic interactions, and dipole-dipole interactions and subsequent loss
250 of tertiary protein structure (Gaona-Sánchez et al., 2015; Tillekeratne and Easteal, 2000).
251 Beyond 330 °C, the exothermic degradation prevailed.

252

253 The glass transition temperature of zein was still evident in the physical mixture of zein and
254 DS (PM_Zein-DS 12.5%), however, it disappeared upon the incorporation of PEG used in the

255 granulation of zein in PM_Granulated zein-DS 12.5%. PEG acts as a plasticizer reducing the
256 glass transition temperature of zein and making it difficult to be determined as it is shifted
257 towards a lower temperature and is possibly overshadowed by the evaporative endotherm
258 (Tillekeratne and Easteal, 2000). The onset of the thermal degradation peak of PM_Zein-DS
259 12.5% started at the same temperature as that of the zein alone sample (≈ 255 °C). However,
260 the degradation of samples containing PEG 400 was seen at a lower temperature. This is
261 possibly due to the thermal degradation of PEG 400 which starts at around 200 °C, as shown
262 in previous thermogravimetric analysis data (Phaechamud and Chitrattha, 2016).

263

264 The DSC thermogram of DS showed two endothermal events with a sharp peak at 53.44 °C
265 and a broad one at 80.49 °C which correspond to the dehydration of DS tetrahydrate. A sharp
266 endothermic melting peak at 287.55 °C was observed and followed by decomposition (Bettini
267 et al., 2004, 2000; Pasquali et al., 2007). The endothermic melting peak however disappeared
268 in the DSC thermogram of PM_Zein-DS 12.5%. This is because the thermal degradation of
269 zein overshadowed the melting transition of the drug. Similarly, the drug peak was absent in
270 all other samples containing drug and zein. Therefore, the overlap of zein and drug transitions
271 precluded the determination of the physical state of the drug using DSC.

272

273 X-ray diffraction was used to analyze the degree of drug crystallinity in the studied samples.
274 As indicated by X-ray examinations (Figure 2), DS raw powder manifested the distinct peaks
275 of highly crystalline DS tetrahydrate, while there were no clear peaks in the X-ray
276 diffractogram for the amorphous zein. The X-ray spectra of physical mixtures of zein and DS
277 showed that the intensities of typical peaks for raw drug were lowered by the dilution effect
278 of zein, particularly at low DS content (i.e. PM_Zein-DS 12.5%).

279

280 PM_Granulated zein-DS 12.5%, unlike PM_Zein-DS 12.5%, did not show any discernible
281 peaks and the diffraction pattern corresponded closely to that of amorphous zein. However,
282 the diffraction peaks of DS were seen in PM_Granulated zein-DS 37.5% sample, albeit at
283 lower intensity compared to those observed with PM_Zein-DS 37.5%. The reduction in the
284 crystallinity of the granulated samples containing PEG 400 could be explained by the
285 solubilisation of DS by PEG 400 (Khalil et al., 2000). These results suggest that the 12.5% of
286 DS was completely solubilized by PEG 400, whereas PEG 400 was saturated with DS at 37%
287 drug loading and the remaining fraction of insolubilized drug contributed to the peaks
288 observed in the diffractogram of that sample.

289

290 Finally, the extruded samples (E_12.5_20; E_37.5_20) were characterized by the absence of
291 the diffraction peaks in XRPD, signifying a drug amorphousization. The fact that amorphous
292 pattern was obtained even at the highest DS content, i.e. 37.5% indicates that the extrusion
293 process has higher capacity to amorphousize the drug compared to the granulation process.

294

295

296

297 **3.2 *In Vitro* Drug Release Studies**

298

299 **3.2.1 Effect of Drug Loading**

300

301 The effect of drug loading on drug release was first evaluated. Figure 3 compares the
302 dissolution profiles of zein extrudates of four different drug loadings: 12.5%, 18.75%, 25%
303 and 37.5% w/w in phosphate buffer pH 7.5. The higher the drug loading the faster was the
304 drug release. Samples with the higher drug loadings, i.e. 25% and 37.5% showed almost
305 immediate release profiles with circa 75% and 80% of drug being released in 1 hour,

306 respectively. The drug release was the slowest from 12.5% DS loaded extrudates and
307 approached zero order release kinetics over the test duration, without initial burst release. The
308 release profile from 18.75% DS loaded extrudates was biphasic with a rapid release of the
309 drug easily accessible at the surface of the extrudates and a slower second phase where
310 diffusion occurs. Bouman et al. (Bouman et al., 2015) have previously found that the drug
311 release from zein hot melt extruded injection moulded caplets was dependent on drug loading
312 in the case of ranitidine, but not in the case of paracetamol and indomethacin.

313

314 Zein is a swellable, nonerodable polymer, and drug release is thus expected to occur via
315 diffusion through pores between the polymeric chains or those created upon the dissolution of
316 the soluble components. In other words, increasing the drug % increases the soluble
317 component, and simultaneously decreases the insoluble zein component, in the formulation,
318 which upon dissolution creates more pores through which the drug can be released. In
319 addition, the higher the concentration of the hydrophilic drug salt within the formulation, the
320 greater is the overall hydrophilicity of the whole system. Higher hydrophilicity might
321 translate into more rapid hydration (Bouman et al., 2016) and drug release. However, the
322 differences in drug release could not be attributed to the physical state of the drug in the
323 extrudates, as DS was present in the non-crystalline state at the lowest and highest drug
324 loadings, as shown by the solid-state characterization discussed earlier.

325

326 **3.2.2 Effect of Buffer Concentration and Ionic Strength of Medium on Drug** 327 **Release**

328

329 In addition to the standard compendial medium, i.e. 50 mM phosphate buffer of pH 7.5,
330 diclofenac sodium release from zein extrudates was tested in media with different ionic
331 strengths, i.e. in phosphate buffers of different buffer concentrations and in 50 mM phosphate

332 buffer with added amounts of NaCl. These tests have been done because zein swelling is
333 known to be dependent on pH and ionic strength of the medium. Formulation E_12.5_20 was
334 selected for the studies described in this section. This is because this formulation exhibited a
335 controlled release behaviour, and it is therefore more likely to be discriminative, compared to
336 the other formulations.

337

338 Figure 4 compares the dissolution profiles of DS from the extrudates in phosphate buffers
339 (pH 7.5) of different buffer concentrations (50, 100 and 200 mM). Water (used as a
340 reference), 50, 100, and 200 mM PB have approximately ionic strength values of 0, 90, 180,
341 360 mM, respectively. This range of ionic strength of the medium has been used to simulate
342 the effect of gastro-intestinal fed and fasted states on drug release from extended release
343 matrices (Asare-Addo et al., 2013a, 2013b). An increase in the buffer concentration from 0 to
344 200 mM resulted in slower drug release. This result is in agreement with Berardi et al.
345 (Berardi et al., 2017b) where drug release from zein filled into hard gelatin capsules was
346 inversely dependent on PB concentration. The authors related the differences in drug release
347 in phosphate buffers of different ionic strengths to hydration/ swelling of zein protein.
348 Swelling of zein was found to be inversely proportional to the ionic strength of the medium.
349 Thus, the greater swelling at lower ionic strengths increased the porosity of the matrix, i.e.
350 the number and/or size of the channels, and ultimately increased the drug release. However,
351 in this study it can be observed that the release in water became slower than that in 50 mM
352 PB after 8 hours. This is most likely due to the lack of buffer capacity of water and the
353 subsequent decrease in DS solubility in water as more acid drug dissolves into the medium.

354

355 Human intestinal fluids contain bicarbonate buffer rather than phosphate buffer (Sheng et al.,
356 2009). Moreover, the buffering capacity *in vivo* has been found to be much inferior to that of

357 50 mM phosphate buffers (Hens et al., 2017; Tsume et al., 2012). This means that drugs,
358 especially BCS class II weak acid drugs, could exhibit faster dissolution *in vitro* than *in vivo*,
359 due to the higher pH and buffer capacity *in vitro*. Indeed, it has been calculated that the buffer
360 capacity of bicarbonate buffers *in vivo* is equivalent to that of 8 – 45 mM phosphate buffers
361 (Tsume et al., 2012). Further studies have shown that even lower phosphate buffer
362 concentrations (1– 25 mM) are usually more biorelevant in simulating the influence of
363 bicarbonate buffers on the dissolution of weak acid drugs (Krieg et al., 2015). We can thus
364 speculate that the slower release of diclofenac sodium in water compared to 50 mM PB might
365 also occur, although to a lower extent, *in vivo*, where the buffer capacity is expected to be
366 intermediate between the two media (i.e. water and 50 mM PB) investigated here.

367

368 Next, we tested the effect of addition of NaCl into phosphate buffer on drug release. Figure 5
369 shows the drug release of DS from the extrudates in media containing increasing
370 concentrations of salt. It can be observed that the addition of 0.1 M NaCl (ionic strength 190
371 mM) in the buffer slowed down the drug release. However, no further decrease in drug
372 release was obtained as the amount of NaCl was increased to 0.2 M. The obtained results are
373 qualitatively and quantitatively similar to those of Figure 4. Again here, the decrease in drug
374 release at increasing ionic strengths could be attributed to reduced swelling of zein matrices
375 (Berardi et al., 2017b).

376

377 **3.2.3 Effect of pH Medium and Enzymes on Drug Release**

378

379 Performing a dissolution test with a two hours pre-incubation in SGF (without enzymes),
380 followed by a switch to SIF (without enzymes) for the remaining time enables to simulate the
381 effect of the gastric acid on the performance of the drug delivery system (Berardi et al.,
382 2017a; Corti et al., 2008). This is particularly important for zein-based dosage forms which

383 are known to swell more in acidic compared to more basic environments (Berardi et al.,
384 2017a; Bouman et al., 2016, 2015). DS (pKa = 4) is practically insoluble at pH \approx 1 of the
385 simulated gastric fluids (SGF) (Kincl et al., 2004). The low solubility precludes the drug
386 diffusion from the dosage form. Indeed, the drug release in SGF was lower than in PB during
387 the first two hours (Figure S1). Thereafter, the profiles in SIF and PB became rapidly
388 superimposable, indicating that the pre-treatment in acid did not compromise the ability of
389 the zein matrix to control the drug release. This is probably because, the preferential swelling
390 of zein in acid is thought to be reversible (Berardi et al., 2018, 2017a).

391

392 Next, we studied the influence of the digestive enzymes on the drug release. Understanding
393 the effect of proteases on the delivery system is of foremost important, given that zein is a
394 protein. For this purpose, we compared the drug dissolution in SGF, followed by SIF either in
395 presence or in absence of digestive enzymes, i.e. pepsin and pancreatin, respectively. Results
396 are shown in Figure 6: digestive enzymes had a significant influence on the drug release. In
397 the first two hours in SGF the drug release was only minimally affected by the presence of
398 pepsin. However, upon switching to SIF, the drug release was much faster in the presence
399 rather than in the absence of pancreatin, with circa 86% and 33% of the drug being released
400 after a 12 hour-test duration, respectively.

401

402 To investigate this further, we took optical and SEM images of the extrudates before
403 incubation (Figure 7A) and after incubation in SGF (Figure 7B), SIF (Figure 7C) and SGF
404 followed by SIF (Figure 7D) both in presence and absence of enzymes. A comparison of the
405 images in Figure 7A and B reveals that the extrudates remained smooth both in the dry state
406 and upon incubation in SGF without pepsin. In the presence of pepsin, the surface appeared
407 slightly more striated and some superficial pores showed in the structure. However, the

408 structural changes were microscopic and just on the surface. In contrast, the presence of
409 pancreatin in SIF resulted in major morphological changes (Figure 7C): the structure of
410 extrudates became an open network of pores visible both at the macroscopic and microscopic
411 levels, while it was solid in the absence of pancreatin. This indicates that pancreatin eroded
412 the zein-matrix by digestion. Figure 7D shows that the combined effect of pepsin and
413 pancreatin led to the formation of large and small pores in the structure. However, the erosion
414 was less than that observed with pancreatin alone (Figure 7C). We can hypothesise that the
415 lesser digestion of zein matrix in SGF+SIF compared to SIF is due to an initial swelling and
416 coalescence of the matrix in SGF, which partially impeded the following digestive action of
417 the pancreatin in SIF.

418

419 Overall, these findings indicate that zein extrudates were much more affected by the
420 enzymatic digestion by the intestinal proteases than by the gastric pepsin. In agreement with
421 our study, several other authors, although testing different dosage forms, have shown that
422 zein is more sensitive to pancreatin than pepsin (Alqahtani et al., 2017; Fu et al., 2002; Hu et
423 al., 2016; Hurtado-López and Murdan, 2006; Kanig and Goodman, 1962). Despite these
424 studies, there still is a question mark on whether zein-based dosage forms are affected by the
425 digestive enzymes or not. It has been suggested that a univocal answer cannot be given, as
426 this depends on the type of dosage form and manufacturing process and it should be
427 evaluated on a case by case (Berardi et al., 2018). Our extrudates were sensitive to digestion,
428 yet the digestion only sped up the drug release without causing a sudden and unwanted drug
429 release burst. In other words, the enzymes partially digested zein, causing a modulation of the
430 drug release, but did not abolish the ability of zein matrix to control the drug release.

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434

435 **4. Conclusions**

436

437 In this study, diclofenac sodium loaded zein based hot- melt extrudates were successfully
438 produced. The drug was present in the non-crystalline state in zein extrudates at all drug
439 loadings investigated. Drug loading as well as media parameters including ionic strength and
440 presence of digestive enzymes influence the drug release rate were investigated. The results
441 indicate that the extent of the controlled release is drug loading dependent. A nearly zero-
442 order release with no burst release was obtained at the lowest drug loading (12.5% w/w).
443 Drug release rate can be further reduced by increasing ionic strength of medium, highlighting
444 the potential influence of ionic strength in the gastro-intestinal tract on drug release from
445 zein-based formulations. The presence of the digestive enzyme, namely pancreatin in
446 simulated intestinal fluid significantly increased the drug release from zein extrudates by
447 creating more pores through which the drug can diffuse. These results confirm the possibility
448 of using zein as excipient in hot-melt extrusion for producing controlled release dosage
449 forms.

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465 **6. References**

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