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Hot melt extruded zein for controlled delivery of diclofenac sodium: effect of drug loading and medium composition

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Abstract

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

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

1. Introduction

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

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

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

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

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

2. Materials and Methods

2.1 Materials

Zein from maize (Z3625) was obtained from Sigma-Aldrich (Germany) and used as received. This product was plasticized with polyethylene glycol 400, PEG 400, obtained from Merck-Schuchardt (Germany). The model drug, diclofenac sodium was obtained from Aarti drugs Ltd. (India) and was used as received. Diclofenac sodium was chosen on the basis that an extended release formulation of this, some are already available in market, can prove useful in management of signs and symptoms of patients with osteoarthritis and rheumatoid arthritis. Pepsin (p7125) and pancreatin (p8096) enzymes were purchased from Sigma-

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

2.2 Methods

2.2.1 Hot melt extrusion

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

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

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

2.2.2 Drug Content

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

2.2.3 Differential Scanning Calorimetry (DSC)

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

2.2.4 X-Ray Powder Diffraction (XRPD)

X-Ray Powder Diffraction analysis of diclofenac sodium (DS) raw powder, zein raw powder, physical mixtures of DS and zein powder or granulated zein as well as DS loaded zein extrudates was carried out using XRD-7000 X-Ray Diffractometer (Shimadzu, Japan). The diffraction pattern was recorded using monochromatic Cu radiation (λ= 1.54 A°) as anode material and operated at a voltage of 40 kV and a current of 30 mA. The samples were mounted on Al sample holder and XRD patterns were recorded in the range of 2–60° at the speed of 2°/min and a sampling pitch of 0.02°.

2.2.5 Dissolution Studies

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

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

Dissolution studies under conditions that more closely resemble those in the upper gastrointestinal tract were also carried out. In this case, freshly prepared simulated gastric fluid (SGF) (2 g L^{-1} NaCl and 3.2 g L^{-1} pepsin) pH 1.2 was used as the dissolution medium for the first 2 hours. This medium was then replaced by freshly prepared simulated intestinal fluid (SIF) (6.8 g L^{-1} KH_2HPO_4, 10 g L^{-1} pancreatin) pH 7.5 for an additional 22 hours. Control experiments using SGF without pepsin for 2 hours followed by 22 hours in SIF without pancreatin were run in parallel. Diclofenac is a weak acid (pKa=4) and thus practically insoluble in acid. In order to measure the amount of the drug released in acidic media, the media were neutralized at the end of the 2-hour test in SGF with or without pepsin by adding 20 mL of 5M NaOH and stirring using paddles for 5 minutes. Then a sample was withdrawn and filtered through 0.45 µm filters prior to analysis for diclofenac sodium by high-performance liquid chromatography (HPLC; Knauer, Leeds, UK). In SIF with pancreatin, ten-mL samples were removed at predetermined times over a 22-hour period, centrifuged at 13,000 rpm for 30 minutes, and filtered through 0.45 µm filters prior to analysis for diclofenac sodium by high-performance liquid chromatography (HPLC; Knauer, Leeds, UK), using the same method described in Section 2.2.2.

2.2.6 Scanning Electron Microscopy (SEM)

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

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

3. Results

3.1 Characterization of Drug-Loaded Zein Extrudates

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

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

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

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

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

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

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

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

3.2 *In Vitro* Drug Release Studies

3.2.1 Effect of Drug Loading

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

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

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

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

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

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

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

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

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

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

3.2.3 Effect of pH Medium and Enzymes on Drug Release

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

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

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

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

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

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

4. Conclusions

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

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