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Research paper

A study on maize proteins as a potential new tablet excipient

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Abstract

This investigation has examined the use of zein proteins from maize as the major component in oral controlled-release tablets, such formulations often being required to improve patient compliance. Tablets containing ground zein proteins, calcium hydrogen orthophosphate, polyvinyl pyrrolidone, theophylline and magnesium stearate were produced by wet granulation and compression on a single station tablet press and were compared to directly compressed tablets based on zein proteins, calcium hydrogen orthophosphate and theophylline. Non invasive techniques such as Fourier Transform infrared spectroscopy and Fourier Transform Raman spectroscopy were employed to investigate any changes in the secondary structure of zein proteins during tablet production. Random coils, α helices and β sheets predominated and their relative content remained unaffected during grinding, wet granulation and compression, indicating that formulations based on zeins will be robust, i.e. insensitive to minor changes in the production conditions. Drug release from the tablets was studied using a standard pharmacopoeial dissolution test. Dissolution profiles in water, 0.1 M HCl (pH = 1) and phosphate buffer (pH = 6.8) show that only a limited amount of theophylline was released after 4.5 h, suggesting that zein proteins could act as a potential vehicle for oral controlled drug release. Analysis of the theophylline release profiles using the Peppas and Sahlin model reveals that diffusion and polymer relaxation occurred in acidic (pH = 1) and buffered (pH = 6.8) conditions for wet granulated tablets, whereas diffusion was predominant in directly compressed tablets. In conclusion, the present study has shown that zeins can be successfully used as a pharmaceutical excipient, and in particular as a matrix in monolithic controlled release tablets.

Keywords: Zein; Maize; Tablet; Protein; Secondary structure; FTIR; FT Raman; Dissolution

1. Introduction

Over the last decade, studies on zein proteins and their applications in pharmacy have been steadily increasing, with particular interests in encapsulating and coating [1,2]. Being a food ingredient generally recognized as safe [3], these biopolymers would present no potential harm as a drug delivery system. Furthermore, no evidence of their allergenicity has been reported [4,5]. zeins could also be an alternative for lactase deficient patients [6] for whom solid dosage forms containing lactose are contraindicated.

Zein proteins originate from maize seeds [7]. Despite the considerable annual tonnage of maize production [8], the protein extraction is still costly but as the importance of maize starch as a source of biofuel [9] increases there will be more interest in the use of zeins as co-products [10,11]. Indeed, knowledge has strongly developed in the food area with regard to the use of zeins in packaging [12] and coating [13], but little is known about using these biopolymers as excipients in pharmaceutical products [14]. Commercially available zein protein extracts are soluble in alcohol but insoluble in water, potentially making them a good candidate for a controlled oral drug delivery matrix. These proteins include several categories: α zeins, β zein, δ zein and γ zeins, depending on their molecular weights and modes of extraction [15,7,16,17]. α zeins comprise two polypeptides of estimated molecular weight 22 and 24 kDa and its amino acid composition is similar to that

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of whole zein. β zein is a methionine-rich polymer of 17 kDa and δ zein is a minor fraction of 10 kDa, both having a similar solubility profile to α zeins. γ zeins include two polypeptides of 27 and 18 kDa. A recent model has been proposed for the secondary structure of α zeins [18] and consists of nine helical segments modeled into three sets of three interacting coiled–coil helices with segments positioned end to end.

This paper examines the potential use of zeins as a tablet excipient, in particular the use of zeins as a controlled release matrix in monolithic devices. Standard tests including measurement of the dissolution profile of a model drug from the tablets and analysis of the drug release mechanism have been conducted. Additionally, we present here the results of the first study of the effect of the formulation and tabletting processes on the structure of zein proteins, observed using Fourier Transform Infrared (FT-IR) and Fourier Transform Raman (FT-Raman) spectroscopy.

2. Materials and methods

2.1. Materials

Zein purified material (Acros Organics, Geel, Belgium) was used either as received or after grinding using a glass mortar and pestle, preliminary experiments having demonstrated that commercial zein proteins are coarse with a flaky appearance and required grinding in order to obtain a fine homogenous particle size distribution. Calcium hydrogen orthophosphate $CaHPO_4$ (CHO) (BDH Laboratory Supplies, Poole, UK), polyvinyl pyrrolidone (PVP) (Sigma–Aldrich Company Ltd., Gillingham, UK) and magnesium stearate (Sigma–Aldrich Company Ltd., Gillingham, UK) were used as received. The model drug was anhydrous theophylline (Sigma–Aldrich Company Ltd., Gillingham, UK). All other reagents (e.g. buffer components) used in this study were analytical grade and were used without further purification.

2.2. Particle size analysis by sieving

Unground and ground zein powders were tested. Different particle size fractions were obtained by sieving using a Fritsch Analysette sieve shaker (Fritsch GmbH, Germany) and sieves of the following mesh sizes: 355, 250, 180, 125 and 65 μm (Fisher Scientific UK Ltd., Loughborough, UK).

2.3. Powder mixing and wet granulation

A range of formulations were produced via direct compression (DC) and wet granulation (WG) as described in Table 1. Initially, zein powder (ground and unground) was compressed alone and then after dry mixing with CHO, to investigate its compression properties. Subsequently, a DC formulation of theophylline, zein and CHO was prepared by dry mixing using a glass mortar and pestle. A WG formulation of theophylline, zein, CHO, PVP and magnesium stearate was prepared by dry mixing as before and subsequent granulation using 15% w/v PVP aqueous solution. The wet granules were then dried overnight in a fan oven at 45 °C until constant weight was reached. After grinding with mortar and pestle, 1% (w/w) magnesium stearate was added to the dried granules prior to compression. Enough material was produced to fill the feed shoe of the single station press.

2.4. Tablet production and physical tests

A Manesty E-2 single station tablet press (Manesty Machines Ltd., Liverpool, UK) fitted with 12.7 mm round, normal concave, non-engraved punches was used to produce tablets under power. Tablet hardness was measured using an Erweka TBH 28 hardness tester (Erweka GmbH, Germany) and friability was determined using Erweka TAR friabilator (Erweka GmbH, Germany) at 100 rotations. The disintegration time was measured in 0.1 M HCl at 37 ± 0.5 °C in a Copley DTG 2000 apparatus using disks ($n = 3$) (Copley Scientific Ltd., Nottingham, UK).

2.5. Scanning electron microscopy (SEM)

Samples were mounted on an aluminium pin stub using conductive self-adhesive carbon label. The powder specimens were sputter coated with a layer of gold approximately 50 nm thick in a sputter coater S150B (Edwards, UK). Fractured samples from tablets were coated with a layer of carbon approximately 50 nm in a Polaron CC7650 (Quorum Technologies Ltd., UK). All samples were examined in a JEOL 5900 LV scanning electron microscope (JEOL Ltd., UK) at an accelerating voltage of 20 kV.

2.6. Dissolution studies

The tablet dissolution studies were carried out using a British Pharmacopoeia Apparatus II dissolution bath (Copley Scientific Ltd., Nottingham, UK). The media used were

Table 1
Formulations and physical tests data for DC and WG tablets

Process	Formulation (% w/w)					Physical tests		
	Zeins	CHO	Theophylline	PVP	Magnesium stearate	Weight (mg) $n = 10$	Hardness (N) $n = 4$	Friability (%) $n = 6$
DC	33	34	33	–	–	645 (3)	90 (8)	0.2
WG	61	30	7	1	1	740 (3)	40 (4)	0.6

Results are expressed as mean (standard deviation).

distilled water, 0.1 M HCl (pH = 1) and phosphate buffer (pH = 6.8) to simulate conditions in the stomach and colon, respectively; the dissolution medium volume was 900 mL; the stirring speed was 50 rpm; and the temperature was maintained at 37.0 ± 0.5 °C. At appropriate time intervals, aliquots of 10 mL were withdrawn and filtered with a 0.22 μm syringe driven filter unit (Millipore, Cork, Ireland) and measured spectrophotometrically (S-22 Boeco UV/vis spectrophotometer, Boeckel and Co., Hamburg, Germany) at $\lambda_{max} = 270$ nm corresponding to the λ_{max} of theophylline. The experiments were carried out in triplicate. Fitting of the dissolution profiles was performed using the software package GenStat (VSNI, Hemel Hempstead, UK).

2.7. FT-IR spectroscopy

The objective here was to investigate any structural changes in the zeins occurring during the tabletting process. Spectra of powder samples were recorded on a Bio-Rad FTS 165 FTIR spectrometer with a mercury/cadmium/telluride detector (Bio-Rad, USA). Samples were placed on a single-reflection diamond attenuated total reflectance (ATR) accessory (SPECAC, Orpington, UK) and carefully pressed down to ensure a good contact with the ATR crystal. For each sample, 200 spectra at 2 cm^{-1} resolution were averaged. The empty ATR crystal served as a reference. For further analysis, spectra for deionised water and water vapor were measured and subtracted from the sample spectra using Omnic v6.1 A software (Thermo Nicolet Cooperation, Madison, USA). Smoothing was carried out at 11 points at which the resolution was found not to be significantly affected. Each spectrum was zeroed at 1800 cm^{-1} where the baseline was relatively flat. Fourier self deconvolution (FSD) was also carried out with an enhancement factor of 1.3 and bandwidth of 30 [19]. Positions of the absorbance peaks located in the amide I region were determined using the second derivative. Intensities at different wavenumbers were measured and normalized to the glutamine absorbance peak taken at 1596 cm^{-1}. Measurements were triplicated.

2.8. FT-Raman spectroscopy

Raman spectra were recorded using a Bruker IFS 33 FT-IR spectrometer equipped with a FRA 106 Raman module and a liquid nitrogen-cooled germanium detector (Bruker Optics, Coventry, UK). A diode laser operating at 1064 nm was used as the excitation source, with ~100 mW of laser power at the sample. The spectral resolution was 4 cm^{-1} and each spectrum was an average of 500 or 1000 scans. The sample was carefully deposited onto the capsule.

3. Results and discussion

3.1. Tablet appearance and physical tests

Fig. 1 shows representative samples of tablets produced using the single station tablet press. The tablets produced from unground zein alone had a rough surface and uneven colouration due to the coarse starting material (Fig. 1a). After grinding of the zein, the resultant tablets are more uniform in colour and texture (Fig. 1b). Tablets composed of 100% zein had a very low density and showed a striated appearance when cut vertically, indicating some elastic recovery of the material upon removal of the compression force. The addition of CHO resulted in a denser, less striated tablet, indicating that the plastic nature of the zeins could be balanced by the brittle-fracture nature of the CHO (Fig. 1c). Further improvement of the tablet appearance was achieved by WG after which the tablet surface was smooth and shiny (Fig. 1d).

SEM micrographs are presented in Fig. 2. Particles from ground zein powder are hollowed, fragmented and irregular in shape (Fig. 2a). When directly compressed, the fracture surface of the tablet appears granular with round particles less than 10 μm wide (Fig. 2b). After wet granulation, granules are formed and appear very smooth at their surface with diameter ranging from 200 to 300 μm. After tabletting, the granules disappear to give place to a more homogenous structure, evidence of a more cohesive material. Weight uniformity, hardness and friability values for the tablets are shown in Table 1. The weight uniformity values were acceptable, the hardness values are typical of tablets with these dimensions and the friability is within the limits of the British Pharmacopoeia. Tablets were tested for disintegration times in 0.1 M HCl because, as will be further described, they show the highest degree of dissolution under acidic conditions. All tablets remained intact, even after being exposed to acid for 5 h, although some swelling was evident as shown in Fig. 3. WG tablets swelled more than the DC specimens, which is probably due to the presence of PVP, with the water-soluble binder dissolving in the disintegration medium allowing ingress of fluid into the tablet core.

3.2. FT-IR data

Fig. 4 shows typical FT-IR spectra for zein (unground), CHO, PVP and magnesium stearate. Zein proteins show a typical absorption at around 1650 cm^{-1} (amide I) and 1550 cm^{-1} (amide II) in line with previously published data [19,20]. The secondary structure of zein proteins is dominated by α helices, with the presence of β sheets and β turns. CHO and PVP show absorbance peaks below 1500 cm^{-1} for CHO due to P=O vibration and at 1650 cm^{-1} (amide I), 1420 cm^{-1} and 1280–1270 cm^{-1} (due to aliphatic functional groups [21]) for PVP. Magnesium stearate has peaks assigned to COO^- above 3000 cm^{-1} and around 1700 cm^{-1}, and aliphatic vibrations between 2900 and 2800 cm^{-1} and at 1450 cm^{-1}. In the amide I region PVP and zein proteins have overlapping absorbance peaks, which make the analysis of the spectra of PVP-containing tablets potentially ambiguous. How-

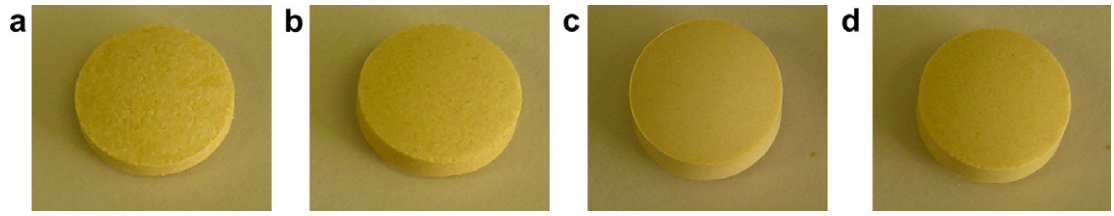

Fig. 1. Representative tablets produced from zein proteins: (a) tablet from unground zein using DC; (b) tablet from mortar and pestle ground zein using DC; (c) tablet from ground zein and CHO using DC; and (d) tablet from ground zein, CHO, PVP and magnesium stearate, using WG.

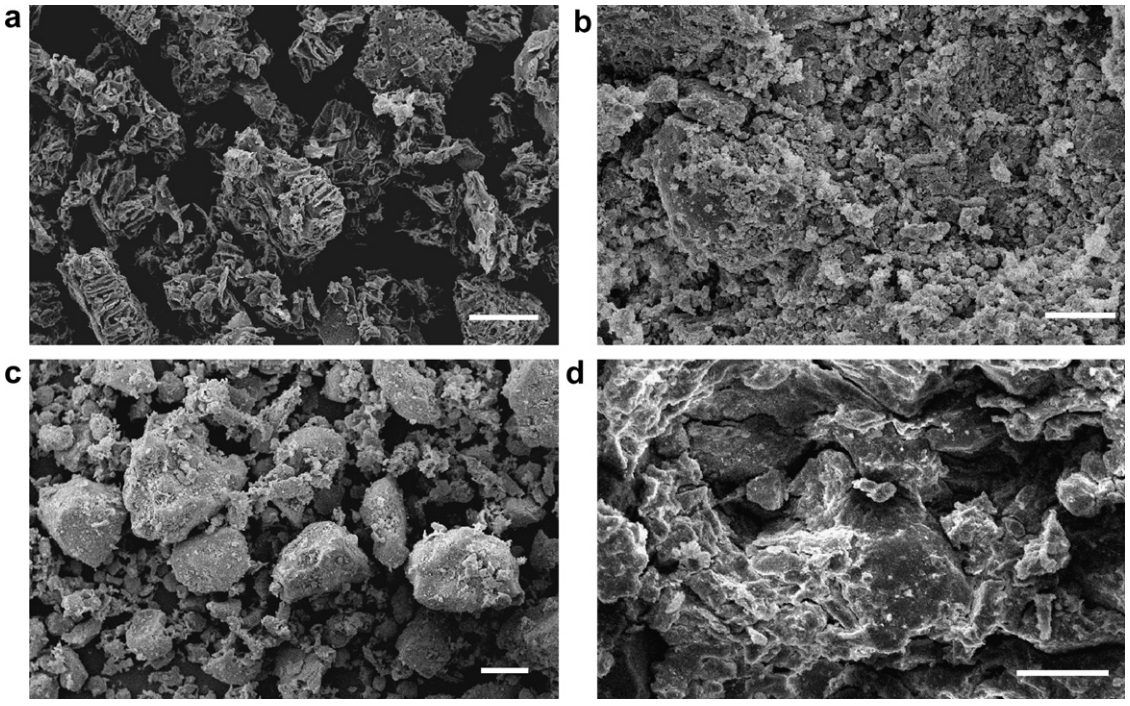

Fig. 2. SEM micrographs of ground zein (a), fracture surface of DC tablets (b), WG powder (c) and fracture surface of WG tablets (d). The scale bars are as follows: 200 μm (a), 50 μm (b), 100 μm (c) and 100 μm (d).

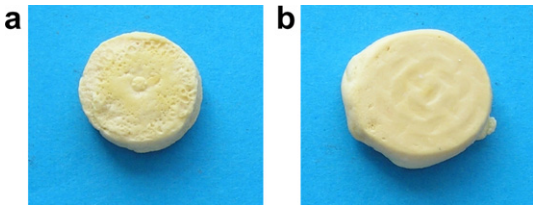

Fig. 3. Appearance of DC (a) and WG (b) tablets after 5 h disintegration testing in 0.1 M HCl.

ever, as the relative contents of zein and PVP within the tablets are so different, it is believed that the zein spectra are essentially unaffected by the presence of PVP.

It has been suggested that the secondary structure of proteins used as a formulation excipient might impinge on the drug release mechanism from such formulations [22]. Therefore, from a development and manufacturing point of view, it is vital to assess the stability of the secondary structure of the zein proteins to typical processing steps, such as granulation, drying and compression. Fig. 5 shows an example of the detailed analysis of the FT-IR spectrum for ground zein in the amide I region. From the Fourier self-deconvoluted spectrum, a second derivative was determined with valleys appearing as separate components. The band assignment shown in Fig. 5 closely follows that reported earlier [19]. The secondary structure of zein can be fully characterized by four major bands in the amide I region: β turns (1662 cm^{-1}), random coils and α helices (1645 cm^{-1}), intramolecular β sheets (1631 cm^{-1}) and intermolecular β sheets (1614 cm^{-1}). Their relative content in the secondary structure of zein before and after grinding and compression are summarised in Fig. 6. Firstly, considering the effect of grinding, it was found that the particle size distribution was bimodal for both unground and ground materials. For unground zeins, maxima were between 63 and 125 μm (17%) and at size greater than 355 μm (63%). For the ground material, maxima were between 125 and 180 μm (27%) and between 250 and 355 μm (27%) (Fig. 2). Hence, the FT-IR results would suggest that despite the change in particle size upon grind-

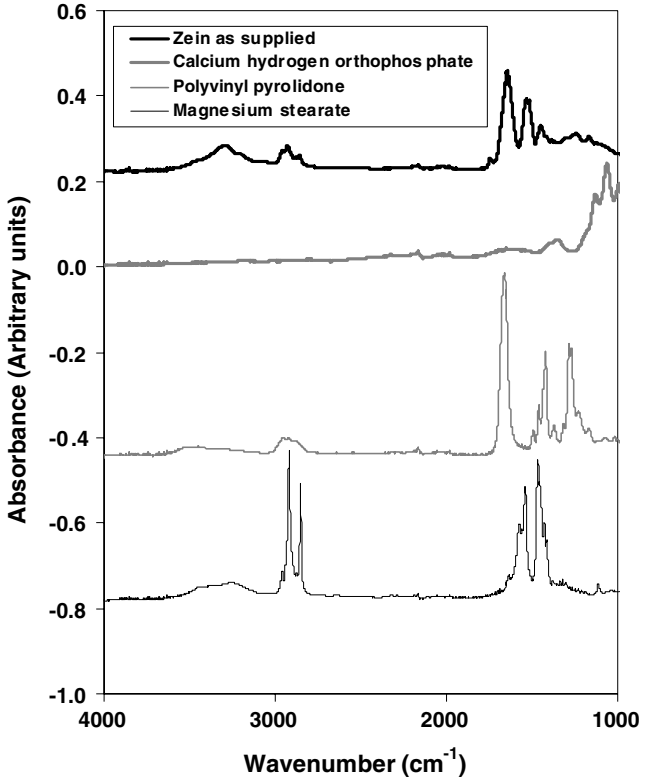

Fig. 4. Typical FT-IR spectra of zein, calcium hydrogen orthophosphate, polyvinyl pyrrolidone and magnesium stearate.

ing, the secondary structure of the zein proteins is substantially unaltered. Furthermore, when the ground materials was compressed using a single station press, only a small increase in the relative content of intermolecular β sheets was detected, which might be attributed to the alignment of proteins chains through hydrogen bonds. Overall, these data indicate that the grinding and compression has no deleterious effect on the secondary structure of the zein proteins, suggesting that a tablet formulation made from this material would be robust, i.e. insensitive to minor changes in the production conditions.

3.3. FT-Raman data

Fig. 7 shows the FT-Raman spectra of zein upon grinding and compression. Several regions are characteristic of proteins and absorbance peaks were assigned according to previously published work [23]. The amide function has absorbances centered at 1650 cm^{-1} (amide I) and 1340 cm^{-1} (amide III). Single amino acids also show absorbance, i.e. phenylalanine at 1000 cm^{-1} and tyrosine at 850 and 830 cm^{-1}. A closer examination of the amide I and amide III regions confirms the data from FT-IR; that is, the secondary structure of zein proteins is not affected by grinding and tabletting. The peaks due to tyrosine vibrations can be used as an indicator for change of structure but in the case of zein proteins used here, no such alteration was observed.

3.4. Dissolution profiles

Figs. 8 and 9 show the dissolution of theophylline from tablets containing zein proteins over a period of 4.5 h in different media (pH = 1, pH = 6.8 or water). The overall extent of theophylline dissolution is relatively low, with the WG tablets showing a slower drug release than the

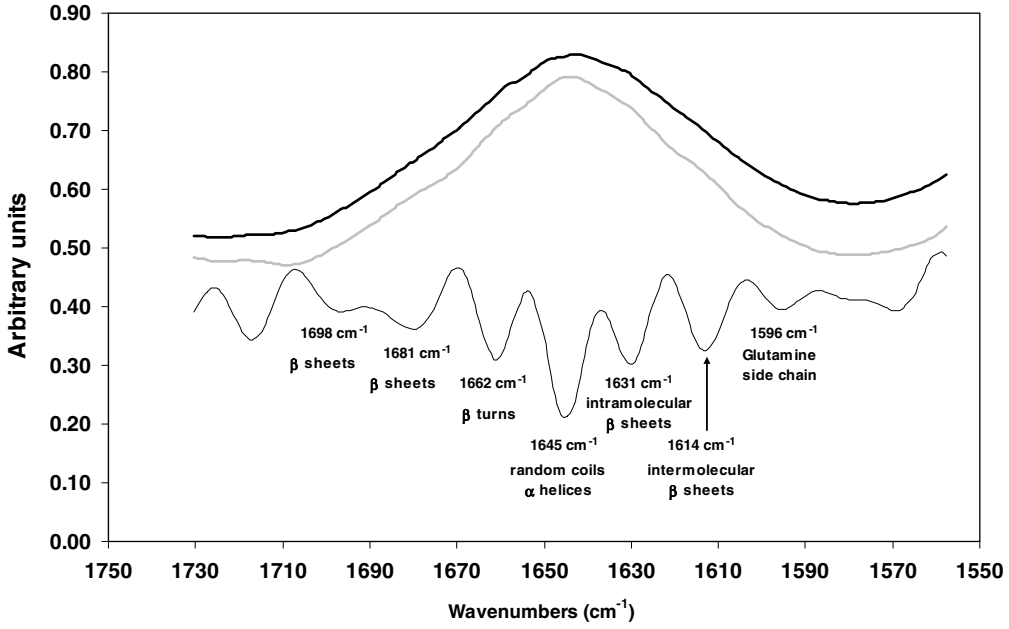

Fig. 5. Detailed analysis of the FT-IR spectrum for ground zein in the amide I region: top line, water vapor subtracted and smoothed spectrum; middle line, Fourier self-deconvoluted spectrum; bottom line, second derivative showing the valleys associated with the secondary structure of the proteins. The assignment of the valleys is also included.

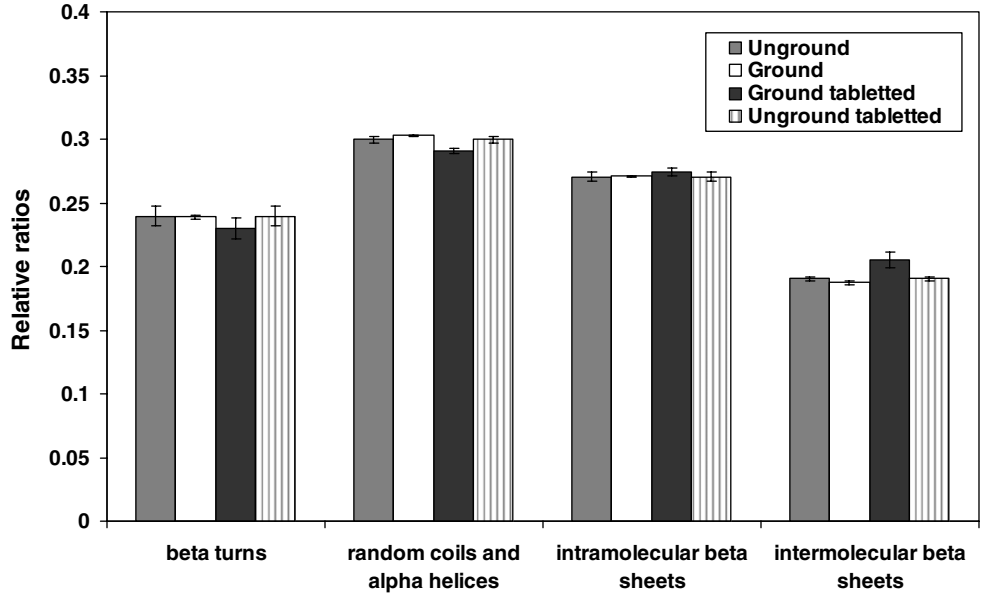

Fig. 6. Relative content of random coils and α helices, β sheets and β turns in the secondary structure of zein before and after grinding and compression.

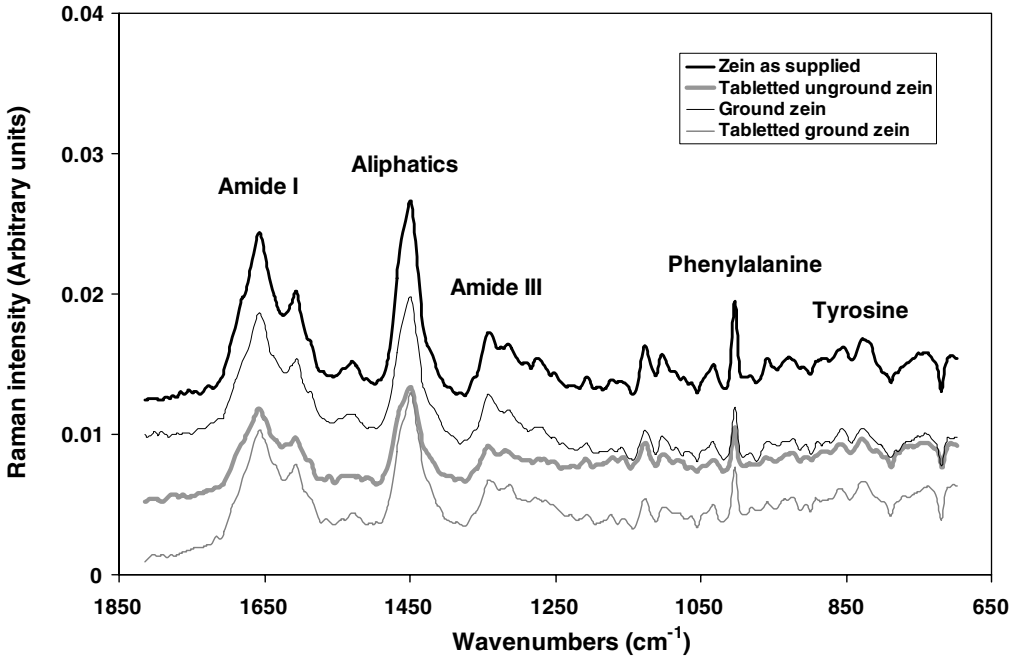

Fig. 7. Typical FT-Raman spectra for unground zein, compressed unground zein, ground zein and compression ground zein.

DC tablets (between 5% and 20% from WG tablets and between 20% and 35% from DC tablets, at 4.5 h, respectively). It is not unusual for WG tablets to show a slower dissolution profile as they are generally harder than DC tablets, although this effect is more often related to fully disintegrating tablets. In the case of WG zein based tablets, the proteins content is significant making the tablets less hard than the DC tablets. Dissolution of theophylline from the tablets appears to be essentially constant after an initial slight burst, which is more apparent with the DC tablets than the WG tablets. This slow but constant release pattern suggests that zein proteins may indeed be used as a controlled release tablet matrix. When comparing dissolution in different pH conditions, there is a significant increase in drug dissolution under acidic conditions. This might be explained by the deamidation of glutamine and asparagine present in the zein proteins which can occur under acidic conditions [24,25]. Consequently, the secondary structure of the zein proteins might be altered. To test for this, a small aliquot of mortar and pestle ground zein mate-

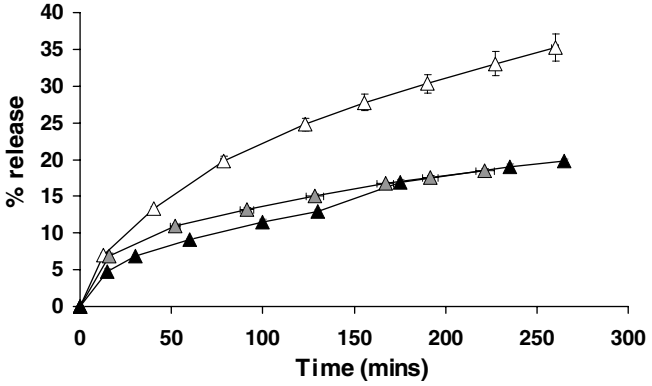

Fig. 8. Dissolution profiles in different media for DC tablets containing theophylline and zein proteins: distilled water (solid triangle), pH = 1 (open triangle) and pH = 6.8 (grey triangle).

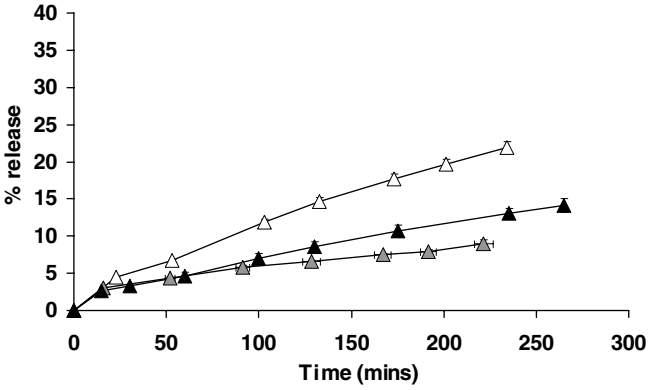

Fig. 9. Dissolution profiles in different media for WG tablets containing theophylline and zein proteins: distilled water (solid triangle), pH = 1 (open triangle) and pH = 6.8 (grey triangle).

rial was mixed with 0.1 M HCl for 4 h to mimic the dissolution conditions and then centrifuged at 3000 rpm. The residue was collected and washed with distilled water. This extraction was repeated 3 times. The washed residue was then dried overnight at 40 °C and then analysed by FT-IR. Fig. 10 represents the second derivatives of the FT-IR spectra of the untreated and 0.1 M HCl treated zeins. An increase in intramolecular β sheets at the expense of random coils and α helices was observed in the acid-treated samples, demonstrating that exposure to low pH conditions may result in a change in the secondary structure of the zein proteins, possibly due to deamidation as discussed above. However, the residence time of a solid monolithic dosage form in the unfed stomach is in the order of 30 min [26] and over this time period, the dissolution of theophylline into the three media was essentially constant. Hence it is considered that exposure to acid conditions for relatively short periods will not result in any deleterious changes in the performance of the zein tablet matrix. In the pH 6.8 buffer, the extent of the theophylline release from the WG tablets was greater than that observed in purified water. It is possible that the solubility of zein pro-

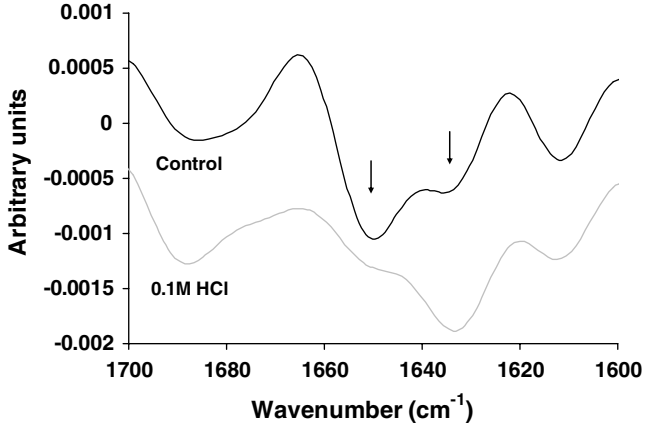

Fig. 10. FT-IR second derivative spectra of zein proteins treated with 0.1 M HCl. Arrows indicate changes in random coils and α helices at 1650 cm^{-1} and β sheets at 1630 cm^{-1}.

teins might increase due to the 'salting in' effect of ions (in this case, phosphates present in the buffer). Binding of phosphate anions would increase the net charge of the zein proteins and hence would increase their solubility [27]. This may then result in an increase in drug release due to erosion of the surface of the tablets.

Mathematical analysis to elucidate the mechanism of drug release was carried out by fitting the experimental data to the power law equation using Eq. (1) discussed by Ritger and Peppas [28]:

$$\frac{M_\infty}{M_o} = kt^n \tag{1}$$

where M_∞/M_o is the cumulative drug release ratio, k is the kinetic constant and n is the release exponent, indicative of the mechanism of drug release. Table 2 gives the values for n and k and shows that the exponent values lie between 0.37 and 0.74. With the exception of DC tablets, these n values signify a non-Fickian or anomalous mechanism of drug release. In the latter case, Fickian diffusion through the tablet matrix and polymer relaxation/erosion govern drug dissolution. Peppas and Sahlin [29] proposed an alternative, empirical model, which is described by Eq. (2) below:

$$\frac{M_\infty}{M_o} = k_1 t^m + k_2 t^{2m} \tag{2}$$

where M_∞/M_o was defined earlier, k_1 and k_2 are the kinetic constants associated with diffusional and relaxational release, respectively, and m is the purely Fickian diffusion exponent. Given the geometry of our tablets [29] a value of 0.45 for m was approximated. In Table 2, values for k_1 and k_2 are presented. The ratio k_1/k_2 was calculated and shows that the relaxational process of the zein matrix was significant during theophylline release for WG tablets in pH = 1 and pH = 6.8 media. The contribution from the relaxation/erosion part of the drug dissolution might be explained by the plasticizing effect of water, lowering the glass transition temperature (T_g) of zein proteins [30].

Table 2
Diffusional exponent (n), kinetic constant (k), diffusional (k_1) and relaxational (k_2) kinetic constants for the dissolution of DC and WG tablets in water, HCl and buffer

Process	Dissolution medium	Ritger and Peppas			Peppas and Sahlin			
		n	k (h^{-n})	R^2	k_1 ($h^{-0.45}$)	k_2 ($h^{-0.90}$)	k_1/k_2	R^2
DC	H_2O	0.37 (0.024)	1.514 (0.182)	0.9994	1.242 (0.069)	−0.024 (0.007)	−51	0.9998
	pH = 1	0.51 (0.019)	1.231 (0.081)	0.9996	1.432 (0.054)	0.027 (0.010)	54	0.9956
	pH = 6.8	0.52 (0.020)	0.654 (0.059)	0.9984	0.755 (0.040)	0.018 (0.004)	41	0.9925
WG	H_2O	0.48 (0.023)	0.390 (0.039)	0.9990	0.408 (0.025)	0.004 (0.003)	99	0.9897
	pH = 1	0.74 (0.011)	0.239 (0.003)	0.9994	0.325 (0.010)	0.075 (0.007)	4	0.9961
	pH = 6.8	0.74 (0.011)	0.186 (0.020)	0.9989	0.265 (0.027)	0.035 (0.002)	8	0.9963

Results are expressed as mean (standard deviation).

Indeed, they reported that the T_g of zein proteins decreases from 150 °C when dry to 25 °C at 27% water content. Despite the low content of PVP, its T_g might also be depressed as reported earlier [31], potentially enhancing this effect.

4. Conclusions

This study has shown that zeins, storage proteins from maize, can be successfully used as a matrix in monolithic controlled release tablets. The rate of release of the model drug theophylline was essentially constant after an initial short burst, more evident with DC than WG formulations. Release of the drug was partly by Fickian diffusion and partly governed by relaxation/erosion of the zeins and could be fitted using the Peppas–Sahlin model. Drug release was greatest in acidic conditions mimicking those in the stomach, although the expected residence time of such a tablet within the stomach is short, circa 30 min compared to the 4.5 h exposure here, and at short time intervals the release was essentially the same in all three media studied. FT-IR and FT-Raman spectroscopic data indicated that the secondary structure of zeins is mainly governed by α helices, in agreement with published literature. This secondary structure was not altered by standard pharmaceutical processing steps, such as grinding and compression, suggesting that zein will be a robust excipient, resistant to changes during processing. In conclusion, this study has shown that zeins, and potentially other biopolymers from renewable sources, may successfully be used as pharmaceutical excipients.

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