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Extraction and Characterization of Zein Protein from Corn for Controlled Drug Release

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Abstract

The aim of the present study was to analyze the effect of β-zein in a coating film and on the controlled drug release properties in pharmaceutical tablets. The zein protein was obtained by solvent extraction using acetic acid, ethanol and isopropanol with different concentrations from 70 to 90% v/v in each solvent. The structure of the extracted zein protein was analyzed using SDS-PAGE and FT-IR. Only the zein extracted using acetic acid had a low molecular weight (17 kDa), which was represented as β-zein. The extracted zein from 70% isopropanol and 70% acetic acid was then used to prepare coating solutions for coating tablets and compared with commercial zein. The film was coated on the tablets containing a model drug, namely theophylline or chlorpheniramine maleate, with the samples prepared using the wet granulation method. Investigation of the film elasticity indicated that the extracted zein from acetic acid had greater flexibility than the commercial zein. The dissolution profiles of the coated tablets from the extracted zeins were slower than that of the commercial zein as zero-order release profiles. The better elongation and flexibility could prevent penetration of the solvent into the core of the tablet and help retain the film function.

Keywords: zein, tablet coating, control release, solvent extraction, physicochemical property

1. Introduction

In recent years, there has been much attention in pharmaceutical development focused on controlled drug release for a better therapeutic outcome. To achieve this, various controlled-release-dosage materials have been developed for coating tablets [1, 2]. The main goals in designing a drug coating are drug protection in the stomach or from moisture during storage, controlled drug release, masking the taste, and reinforcing the tablet [3, 4]. Controlled drug release in a delivery system should also maintain a relatively constant therapeutic blood level of the drug during the desired release period

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[1,5]. Typically, coating materials involve the application of a sugar or polymer onto the tablet. Pharmaceutical coating technology has been shifting away from a sugar base to biopolymer base because of their hydrophobic and low water uptake properties. Many types of polymer have been used for drug coating, such as acrylic derivatives (polyethyl acrylate-co-methacrylate, poly (vinyl acetate)) and cellulose derivatives (ethyl cellulose) [3, 6, 7].

Zein is a natural polymer obtained as a by-product from industrial corn processing [8, 9, 10, 11]. The protein content in different varieties of corn is 6–12% on a dry basis and about 75% of endosperm tissue contains protein [8]. Zein was first extracted from whole corn or dry-milled corn (DMC) using a hydroalcoholic solvent. The total protein content of DMC was 6.8–8.0% of the milled corn. [12]. Zein is a major protein that is found in the endosperm of the corn kernel and it is a class of alcohol-soluble prolamine proteins [8, 13, 14]. Basically, zein is classified into several types based on its solubility in alcohol and sequence homology. Zein consists of three protein fractions: α-zein (75% to 85% of total zein), consisting of two polypeptides with an estimated molecular weight of about 19 kDa and 22 kDa, which is soluble in 95% ethanol; β-zein (10% to 15% of total zein), with a molecular weight of 17 kDa and is soluble in 60% ethanol; and γ-zein (5% to 10% of total zein), with a molecular weight of 27 kDa [8, 16, 17, 18, 19, 20]. Mostly, commercial zein has been used as an aqueous alcohol solution, extensively about 50–90% and containing only α-zein. [8]. In contrast, glacial acetic acid can fully solubilize zein (α-, β-, and γ-zein), resulting in zein comprising α-, β-, and γ-zein [15]. Zein is generally recognized as safe and is biodegradable and biocompatible. [14,21] Due to the alcoholic solubility properties of zein, its thermoplastic character and low water uptake, related to a high content of non-polar amino acids [8], it is useful for tablet coating. Film materials made from zein have relatively high barrier properties compared to other proteins. Films from zein have been applied for the controlled release of compounds in medicinal tablets, for protection of the tablet from moisture, and for masking the taste of bitter orally administered drugs [4]. King *et al*. [15] reported that zein isolation procedures produce zein comprising predominantly α-zein, without β- and γ-zein, which can produce films with elasticity for various applications, whereas zein containing β- and γ-zein content could produce a film only when cast from a solution of aqueous ethanol and perform high stress tolerance with brittle [9, 15]. To improve the film forming capability plasticizers such as polyethylene glycol 400 (PEG 400) and triethylene glycol (TEC) are added [9], which control drug release property effect.

The present work concerns developing coating film formulations using different compositions of the different zein structures (α- and α- + β-zein), with an aim for achieving an improvement in the controlled release of a drug in a medicine tablet model. The findings should also improve the value of the residue products from corn industry waste for use in pharmaceutical applications.

2. Materials and Methods

1. Materials

The corn kernels (strain, Suwan 5) used in this study were obtained from the National Corn and Sorghum Research Center, Pak Chong, Nakhon Ratchasima, Thailand. Commercial zein was purchased from Acros Organics (New Jersey, USA). The organic solvents purchased were ethanol (Chachoengsao, Thailand), isopropanol (J.T.Baker® Chemicals, Avantor's Proven Brands, USA), acetic acid (ACI Labscan, Thailand), and hexane (J.T.Baker® Chemicals, Avantor's Proven Brands, USA). The model drugs used were theophylline (Jilin Shulan Synthetic Pharmaceutical Co., Ltd., China) and chlorpheniramine malate (CPM) (Vankatara Chemicals Ltd., India). The plasticizer used was polyethylene glycol (PEG 400) and triethyl citrate (TEC) (T.C.Sathaporn Group Ltd., PART,

Thailand). Other chemicals used were hydrochloric acid (Qrec, New Zealand), sodium hydroxide (Ajax, Finechem, New Zealand), and sodium lauryl sulfate (T.C. Sathaporn Group Ltd., PART, Thailand).

2. Methods

2.1 Corn powder preparation

Zein protein was extracted from corn kernels. First, corn kernels (strain, Suwan 5) were ground and the sample was extracted with hexane to remove the oil. The extraction process produced a slurry contaminated with oil. The slurry was then filtered using an aspirator pump (EYELA, Aspirator A-1000S Pump, Shanghai, China) with a Whatman No.1 filter. The defatted powder corn kernel was dried in a hood overnight and then ground.

2.2 Zein protein extraction process

Defatted powdered corn kernel was extracted for 45 min with three different organic solvents (ethanol, isopropanol, and acetic acid). The concentration in each solvent was 70%, 80%, and 90%. The mixture was stirred in a beaker using an overhead stirrer (IKA, RW 20 Digital, USA) and the beaker was covered with aluminium foil. Then, the slurry was filtered to separate the extract from the other components, and the extract containing the dissolved zein protein was vaporized in a rotary evaporator (EYELA, Tokyo Rikakikai Co., Ltd., Japan) to remove the organic solvent and obtain zein protein. The precipitate zein protein was cooled with cool distilled water (4–10 °C) and the acidity was adjusted to approximately pH 6.2. The zein protein was then washed with distilled water. Finally, the zein protein was dried, ground, and analysed for quality and quantity.

2.3 Characterization of the extracted zein protein

- Protein analysis

The moisture content of all the samples was determined by drying the samples at 105 °C for 2 h [22]. Protein content analysis was conducted using the Kjeldahl method [23]. The % yield was calculated as the amount of protein in the ground corn.

- Fourier transform infrared spectroscopy (FT-IR)

The infrared spectra of commercial zein and zein protein extracted from the corn kernel (strain, Suwan 5) in different organic solvents and at different concentrations were obtained using an FT-IR spectrometer (Bruker Tenser 27, USA) in the attenuated total reflection (ATR) mode. The protein sample was placed on the ATR crystal to cover the crystal surface. The FT-IR data were collected in the region 400–4000 cm-1 [24].

- Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE was used to classify the zein protein composition. The protein concentration of each sample was determined using the Bradford reagent [24]. SDS-PAGE was prepared using 12% separating gel and 4% stacking gel according to the Laemmli method. A prepared sample buffer (2X Laemmli buffer system, Biorad, USA) was diluted with deionized water and mixed with 2-mercaptoethanol. The protein samples were prepared by dissolving them in 2% sodium dodecyl sulfate using a sonicator; then they were mixed in with the sample buffer, heated in boiling water (96 °C) for 4 min, and then loaded into the sample well. Electrophoresis was conducted at 150 V and 35 mA for 50 min. The gels (Mini-Protean® Tetra Cell, Biorad, China) were stained with a Coomassie stain (Biorad, USA). A molecular weight standard of 10–250 kDa was used (Precision Plus ProteinTM All Blue Standards, Biorad, USA) [13,25].

2.4 Determination of the physical properties of the extracted zein films

Zein films were obtained by dissolving 5% zein (two samples from the extraction experiment, with the first sample consisting of only α-zein and the second sample consisting of α-zein and β-zein) and 2% sodium dodecyl sulphate (w/w) into an 80% concentration of isopropanol, which was then mixed until it homogenized. Then, 20% plasticizer (PEG 400 or TEC) was added to the solution and stirred using an overhead stirrer for 30 min. After cooling, the films were formed in a Teflon tray (5 x 5 cm) and dried at 50 °C until the solvent had completely evaporated [9, 26].

The mechanical properties of the zein films containing the different amounts of extracted zein and different types of plasticizer were measured in the tensile and elongation modes using a Texture Analyzer (Lloyd Material Testing, Lloyd TA1, USA) with a 5 kg load cell. The speed was kept constant at 5 mm/sec and 2.5 cm distance. In order to measure the tensile properties, a film with a well-defined geometry was used. Film strips (5 cm long and 1 cm wide) were cut (adapted from [27]). The thickness of the film was measured by using a thickness gauge tester (Mititoyo Absolute, USA). All the extracted zein samples were compared with the film from a commercial zein sample. The results were reviewed according to two parameters: elongation (calculated using equation 1) and tensile strength (calculated using equation 2).

Tensile strength = (load at break)/ (original width) (original thickness) (1)

Percent elongation = (elongation at rupture) x 100 /(initial gauge length) (2)

2.5 Preparation of a coated tablet from zein

Two model drugs with different solubility, namely theophylline and chlorpheniramine maleate (CPM), were used. Core tablets containing a model drug were prepared by wet granulation. The model drug (100 mg) was added into each core tablet (300 mg), using lactose as a filler, magnesium stearate as a lubricant, and corn starch as a disintegrant. Core tablets with hardness higher than 7–8 kg were required

Zein protein (5% w/w or 10% w/w) with 2% w/w sodium lauryl sulphate (calculated from the polymer weight) was dispersed in 80% v/v isopropanol, and then a plasticizer polyethylene glycol 400 (PEG 400) was added to the solution at a concentration of 20% w/w (calculated from the polymer weight). The solution was mixed using a magnetic stirrer. The coated tablets were manually obtained using a dip-coating technique. The film coating solution was cast onto a Teflon tray and dried in a hood overnight, followed by drying at 0°C in a hot-air oven for 2 h.

2.6 In vitro drug control release properties

Drug release from the zein-coated tablets was studied using USP dissolution apparatus 2 (Varian VK7010 Dissolution Apparatus, USA). An amount (900 ml) of 0.1 N HCl or phosphate buffer pH 6.8 at 37±0.5 °C was used. The sample was determined using a UV-VIS spectrophotometer (Agilent Technologies, Varian Cary® 50 UV-Vis Spectrophotometer, USA) at 272 nm and 265 nm for theophylline and CPM, respectively. This experiment was conducted in triplicate.

2.7 Statistical analysis

Statistical analyses were performed using the SPSS software (version 19). Duncan's multiple range test ($P < 0.05$) was used to compare significant differences of the means of the % yield, protein content, and the mechanical properties in the zein film experiments.

3. Results and Discussion

3.1 Percentages of the yield and protein content

The zein extraction from corn was focused on two factors: the type of solvent and the concentration. Ethanol, isopropanol, and acetic acid at concentrations of 70%, 80%, and 90% were used to extract the zein from corn and its coproducts. The performance of zein extraction under each condition was determined based on the % yield and % protein. The results showed that isopropanol at 70% had the highest yield and protein content with amounts of 85.33% and 73.64%, respectively, followed by acetic acid at 90%, which had a yield and protein content of 66.23% and 69.87%, respectively, as seen from Table 1. Moreover, the protein yields of the extracted zein decreased with increasing the solvent concentration, because a high concentration of solvent came with high hydrolytic properties such that the protein was hydrolysed into corn as small molecules of amino acid. Thus, the optimum suitable condition was a low concentration of solvents [28]. Selling and Wood [29] obtained the same results after using acetic acid as the solvent for zein extraction and reported that acetic acid removed zein with a higher yield and more effectively compared with ethanol solvents, whereas the structure of the extracted zein from acetic acid and ethanol had similar structures based on SDS-PAGE results.

Table 1. % Yield and protein content of extracted zein protein by using different solvent and concentration

Properties	Ethanol (%, v/v)			Isopropanol) (%, v/v)			Acetic acid) (%, v/v)		
	70	80	90	70	80	90	70	80	90
Yield (%)	50.61± 1.3^{bc}	52.90 ±3.1^{bc}	20.88± 6.6^{d}	85.33± 14.9^{ab}	48.40± 4.2^{bc}	43.34± 17.4^{c}	64.15± 11.9^{ab}	58.85± 3.6^{abc}	66.23± 0.1^{a}
Protein (%)	56.65± 6.0^{bc}	43.07 ±2.2^{de}	33.87± 13.7^{e}	73.64± 0.6^{ab}	52.20± 1.3^{cd}	43.14± 4.15^{de}	63.46± 0.5^{abc}	72.11± 17.9^{ab}	69.87± 11.3^{a}

a – d Means± SD followed by difference letters in each column were significantly difference at $P<0.05$

3.2 Structural analysis

Zein protein: Size analysis was carried out using two main methods: SDS-PAGE and size exclusion chromatography. The SDS-PAGE patterns (Figure 1) of the zein samples exhibited two major broad bands at 19–22 kDa and 23–24 kDa, indicating the presence of α-zein [30, 31]. A thinner band below the α-zein band was observed at 16–20 kDa, indicating the presence of β-zein [31]. Another light band above the β-zein appeared at 37 kDa, representing a zein dimer of γ-zein. Zein with ethanol and isopropanol (Lanes 1–6) showed similar patterns, with broad bands at 19–24 kDa representing α-zein. On the other hand, zein with acetic acid extraction (Lanes 7–9) showed a small

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band beside the main bands at 17 kDa, which could have represented β-zein. Additionally, dimers were represented by thin bands at 37 kDa and 47–48 kDa. Zhu, Kale, and Cheryan [32] obtained the same results with extracted zein from whole corn with minor amounts of δ- and β-zein at low molecular weights compared with commercial zein, which only presented α zein. FT-IR was used to probe the zein-corn structure under different conditions of solvent extraction (Figure 2). The FT-IR spectra from 4000 to 400 cm-1 of corn zein using the different extraction solvents showed the typical protein sequence absorption bands, consisting of amide A (3600–3100 cm^{-1}), amide I (1700–1600 cm-1), and amide II (1575–1480 cm^{-1}) [13, 33, 34]. Each sample's zein spectrum had a similar pattern to the commercial spectrum. According to Forato [33], β-zein could be detected by FT-IR by the presence of a peak at 1656 cm-1. However, the quantity of β-zein was low, and the peak could not be clearly seen in FT-IR chart. The only difference between the sheet structures resulted from fatty acid contamination, as seen in the peak at 2980–2850 cm-1. This was found as a result of the high amount of fatty acid in zein from extraction using a high concentration of solvent [31]. Zein with acetic acid extraction showed such a pattern in its spectrum, because the structure of protein had already been hydrolyzed to small molecules of amino acid and fatty acid [11]. According to a study by King [15], glacial acetic can fully solubilize zein (α-, β-, and γ-zein). Thus, zein extracted with acetic acid could contain both α- and β-zein. Both extracted zein samples with 70% isopropanol (consisting of α-zein) and 70% acetic acid (consisting of α- and β-zein) were selected for further study of their film properties and drug control release properties.

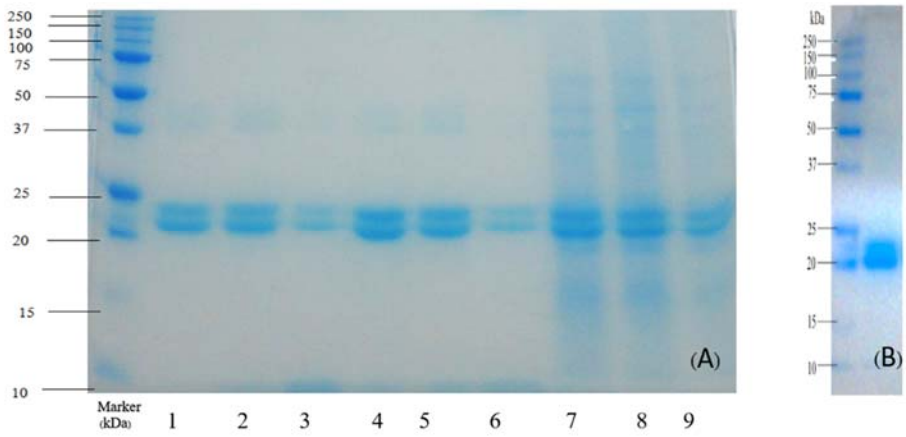

Figure 1. SDS-PAGE of (A) zein proteins extracted from corn kernel Suwan 5 by using ethanol (lane 1, 2, 3; isopropanol (lane 4, 5, 6) acetic acid (7, 8, 9) at concentration 70%, 80%, 90%, respectively and (B) commercial zein

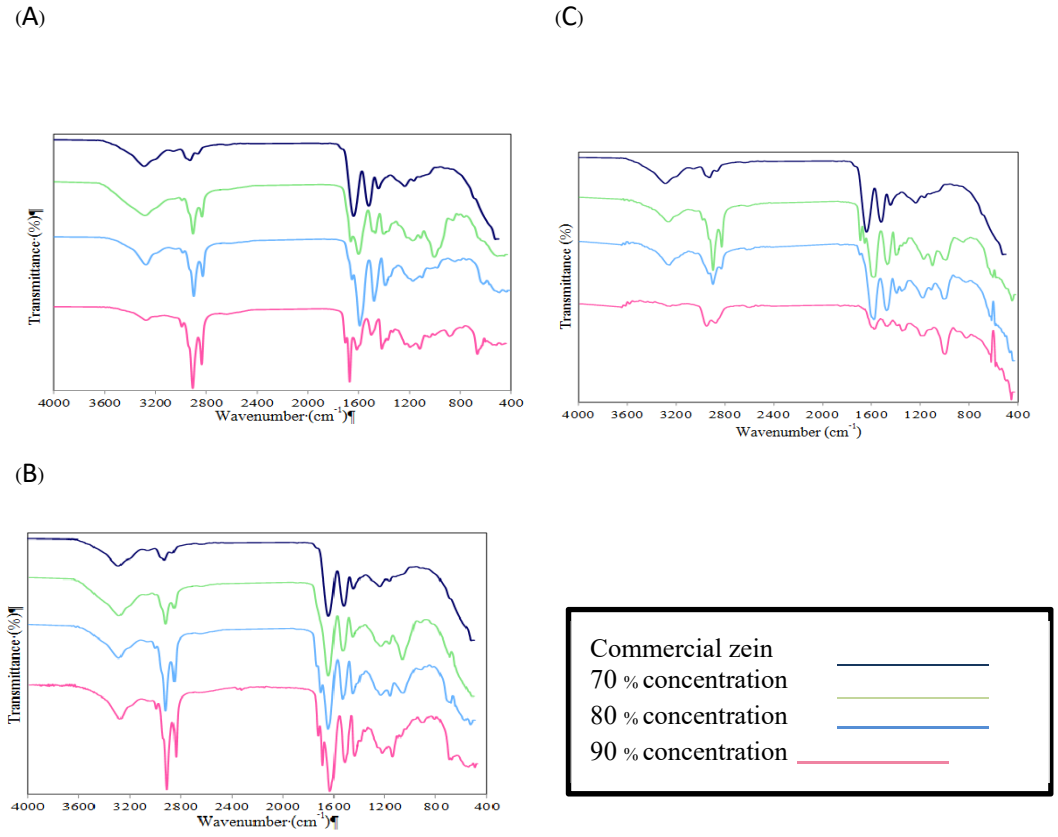

Figure 2. Fourier transform infrared spectra of the zein protein extracted by using (A) ethanol, (B) isopropanol and (C) acetic acid at concentration 70%, 80% and 90% comparing with commercial zein.

3.3 Mechanical properties of the zein films

Based on the study of the zein structure (SDS-PAGE and FT-IR), zein films were prepared using two types of extracted zein. The first consisted of only α-zein (70% isopropanol solvent) (zein 1), while the second consisted of α- and β-zein structures (70% acetic acid solvent) (zein 2). Basically, the moisture barrier properties and control drug release were the main features of the protein-based film used for the tablet film coating. However, the mechanical properties of biopolymers film are equally important to maintain the structural integrity of the tablet as well as providing protection. The tensile strength and elongation at failure and the modulus of the commercial and extracted zein films with/without plasticizers are shown in Table 2. Both the 10% and 20% concentrations of zein without a plasticizer had a low tensile strength of 0.49 N and 0.67 N, respectively, and elongation at 4.36% and 8.56%, respectively, which are quite low because of the brittle nature of the zein film free of plasticizer [27]. Plasticizers, such as TEC or PEG 400, are usually added to modify the mechanical properties of zein film because the plasticizer can embed itself between the polymer chains and reduce the intermolecular and intramolecular interactions of polymer molecules, thus lowering the glass transition temperature (Tg) of the film and improving the flexibility [14, 35]. Consequently, a comparison was then made between commercial zein film with added TEC and

PEG 400 for both levels of zein contents, and they showed significant differences in the % elongation at 7.4% and 35.2% in the 5% zein content and 7.84% and 38.52 % in the 10% zein content films, respectively. Basically, zein is a non-polar protein and contains a lot of hydrophobic amino acids, which causes a low vapor permeability in the zein structure [6]. Zein film added with PEG 400, which is highly polar and has a higher molecular weight than TEC, could reduce the number of hydrogen bonds and prolong the distance between zein molecules, resulting in the zein film structure being more flexible and moveable. Thus, PEG 400 was more suitable to apply in the tablet coating than TEC in our further study. Film coatings using extracted zein 1 and extracted zein 2 with added PEG 400 were compared based on their mechanical properties. The film with extracted zein 2 had a higher % elongation than the film with extracted zein 1 (144.83% and 84.4%, respectively), because the short chains of β-zein in extracted zein 2 increased the distance between the α-zein molecules. Thus, the film with extracted zein 2 was more flexible at the molecular level [35].

Table 2. Mechanical properties (tensile strength and elongation) of zein films

Type of zein	Plasticizers	Film thickness (mm)	Tensile strength (N)	Elongation (%)
5% Commercial zein	None	0.49 ± 0.11^{bc}	10.1 ± 4.4^{a}	4.36
	TEC	0.31 ± 0.06^{d}	3.73 ± 0.9^{b}	7.4
	PEG 400	0.41 ± 0.10^{cd}	2.49 ± 1.98^{bc}	35.2
10% Commercial zein	None	0.67 ± 0.20^{a}	4.52 ± 1.60^{b}	8.56
	TEC	0.56 ± 0.18^{ab}	12.73 ± 4.6^{a}	7.84
	PEG 400	0.57 ± 0.13^{ab}	4.45 ± 2.87^{b}	38.52
10% Extracted zein 1*	PEG 400	0.31 ± 0.08^{cd}	2.60 ± 2.11^{bc}	84.4
10% Extracted zein 2**	PEG 400	0.28 ± 0.01^{d}	0.18 ± 0.09^{c}	144.83

* Extracted zein 1 is zein from 70% isopropanol solvent

**Extracted zein 2 is zein from 70% acetic acid solvent

a – d Means± SD followed by difference letters in each column were significantly difference at $P<0.05$

3.4 Drug release from tablets coated with zein

The release of theophylline and CPM demonstrated different release kinetics. The release from the tablets without a coating was rapid, reaching 90% within 45 min (Figures 4 and 5). With commercial zein, the drug release was slow and the cumulative drug release was less than 5% after 30 min, except for the coated CPM tablets in 0.1 N HCl because of the high solubility of the weak basic drug in the acidic solution. The pulsatile release from the theophylline and CPM tablets coated with the commercial available zein are presented in Figures 4 and 5. The pulsatile release could be explained by the penetration of solvent into the dosage forms and as the drug release started after there had been sufficient water adsorption to break the films. Surprisingly, the drug release of the tablets coated with the extracted zein showed zero-order release profiles (Figures 4 and 5). A linear relationship of the drug release over time was achieved. Zero-order release profiles from coated tablets prepared by both extracted zeins occurred regardless of the model drug and the release medium used in the study. The solvent penetration into the core tablet retarded the drug release [1].

The low tensile strength and the greater elongation, which represent good mechanical properties of the extracted zein films, helped the films to remain intact. The extracted films could control drug release over a period of 4 h. However, the stability of the tablets was a concern. Batch-to-batch variation of the extracted zein might be further studied to ensure the stability of the coated tablets using extracted zein.

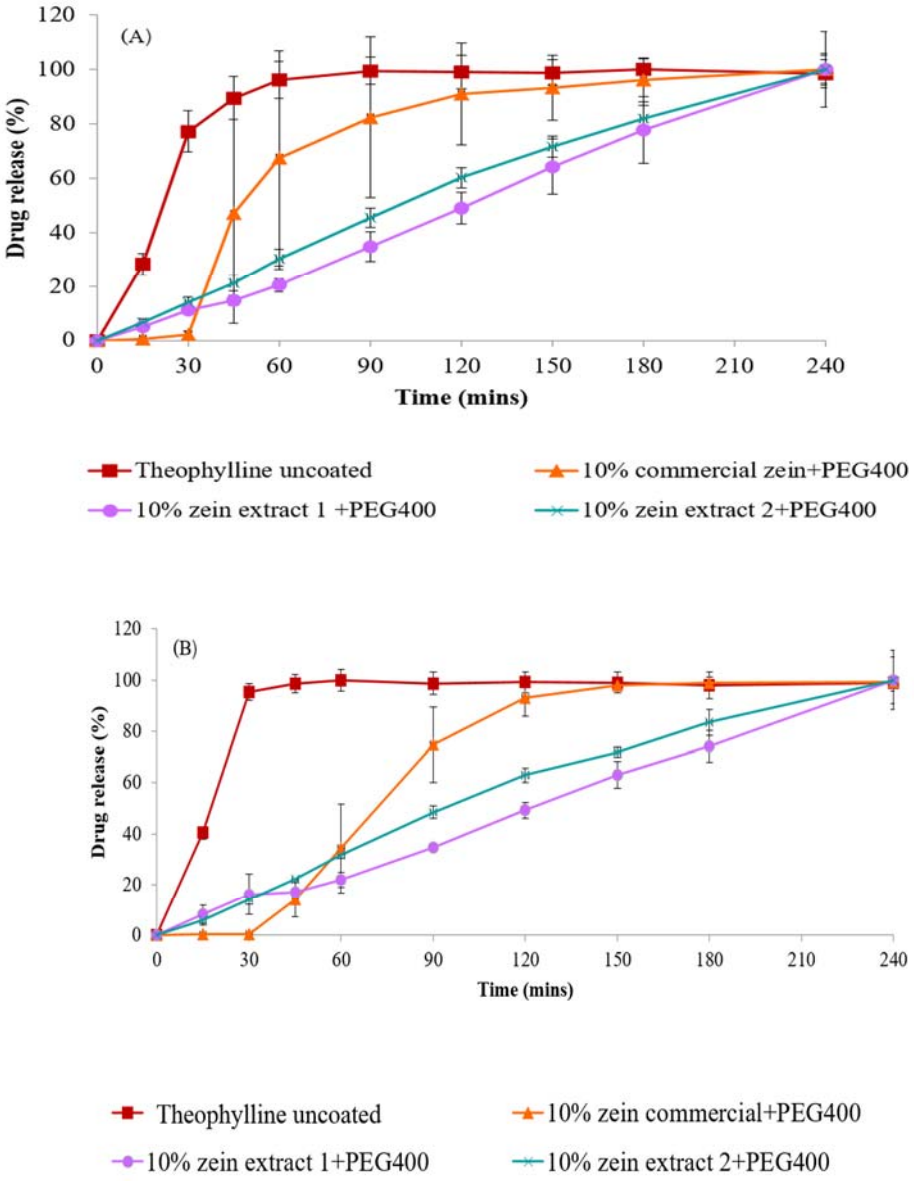

Figure 3. Dissolution profiles of tablets containing Theophylline; uncoated tablets, 10% commercial zein coating with PEG 400, 10% extracted zein 1 with PEG 400 and 10% extracted zein 2 with PEG 400; (A) 0.1 N HCl and (B) phosphate buffer pH 6.8

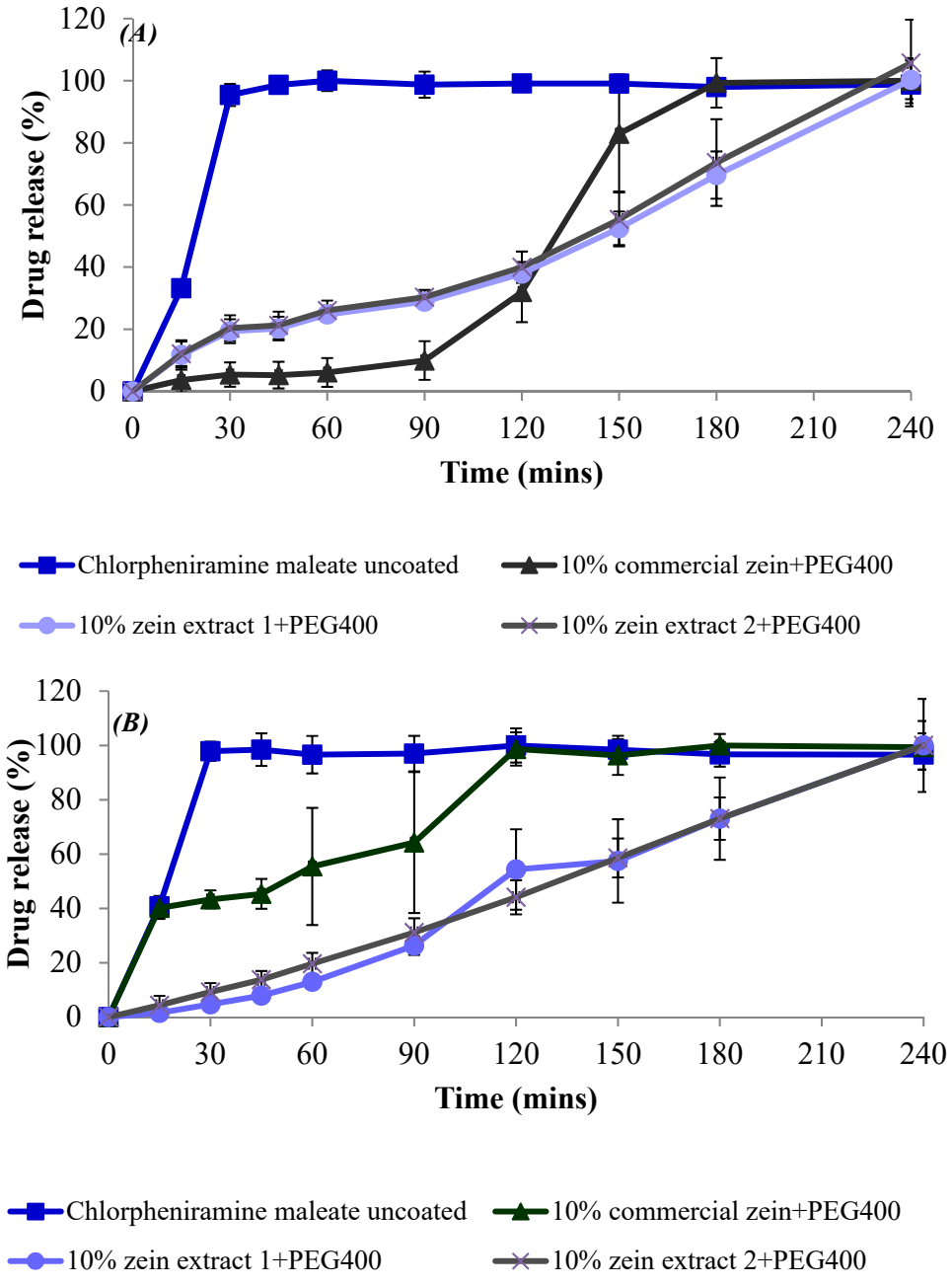

Figure 4. Dissolution profiles of tablets containing CPM; uncoated tablets, 10% commercial zein coating with PEG 400, 10% extracted zein 1 with PEG 400 and 10% extracted zein 2 with PEG 400; (A) 0.1 N HCl and (B) phosphate buffer pH 6.

4. Conclusions

Based on the results of this study, the most suitable solvent for zein extraction was 70% isopropanol, which had the highest % yield and protein content. However, we found that the extracted zein from acetic acid consisted of β-zein and α-zein compared with the extracted zein from 70% isopropanol having only α-zein. Thus, acetic acid could extract more of the smaller molecular weight β-zein than the alcohol solvents due to their hydrolytic property. The structures of both extracted zeins showed similar patterns. In this study, the two extracted zeins were applied in a tablet coating. PEG 400 was more effective as a plasticizer for improving the elongation properties than TEC, due to its high molecular weight and polarity, and thus the addition of PEG 400 in both types of extracted zein film could improve their elongation more than in the commercial zein film, especially for the zein extracted using the acetic acid solvent. The drug release of coated tablets from extracted zein showed a zero-order release profile with a linear relationship between time and drug release for both drug models.

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