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Fabrication of delayed release hard capsule shells from zein/methacrylic acid copolymer blends

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ABSTRACT

Hard capsule shells with an inherent delayed release action are useful for oral administration of active ingredients, which are acid-labile and/or enzymatically degradable in the gastric environment, without the need of film coating. The objective of this study was to fabricate delayed release hard capsule shells by the dip coating method. The film coating formulations comprised blends of zein and methacrylic acid copolymer (Eudragit® L100–55), with and without the addition of the plasticizer, polyethylene glycol 1000. The rheology parameters (loss modulus (G'), storage modulus (G") and loss tangent (tan δ, G"/G')) of the film coating solution were measured to investigate the processability. Central composite design was used to investigate the main, interaction and quadratic effects of the proportion of methacrylic acid copolymer, solid content of the film formers and level of polyethylene glycol 1000 on the capsule wall thickness and mechanical strength. Multiple response optimization was further conducted, and the design space was established. The *in vitro* drug release in simulated gastric and intestinal fluids of three different formulations in the design space was compared. The results showed that the tan δ value after the gelation point should be < 0.9 in order to form a thin and sturdy capsule shell. The gelation time and viscosity of the coating solution were related to the thickness of the capsule shell. The study showed that drug release from the capsule with a specified thickness and mechanical strength can be modulated by varying the ratio of zein to methacrylic acid copolymer. The delayed drug release profile was achieved through the capsule shell fabricated from zein to methacrylic acid copolymer at the ratios of 75:25 and 83.2:16.8, with 10% polyethylene glycol 1000.

1. Introduction

Hard capsules are one of the most common dosage forms for oral administration of pharmaceuticals and food supplements. They have an active ingredient encapsulated in two-piece shells, which are conventionally made from gelatin to provide fast disintegration and immediate action of active ingredients (Jones, 2004; Murachanian, 2018). For active ingredients that are delivered to a specific site in the intestine or that are administered for prolonged action, the release from gelatin capsules may be modified by incorporating release rate modifying excipients into the capsule content (Berardi et al., 2017; Ojantakanen et al., 1993; Veski et al., 1994) or by filling the capsule with modified release granules (Cui et al., 2008; Preisig et al., 2021; Siddique et al., 2010; Zakowiecki et al., 2020). Alternatively, it may be modulated through coating the capsule shells with functional films (Burns et al., 1994; Bussemer et al., 2003; Dvorácková et al., 2010; Dvořáčková et al., 2011; Oliveira et al., 2013; Pina et al., 1996). Coating gelatin capsules is challenging, as their smooth surface does not facilitate film adhesion. When the aqueous film coating method is used, they also tend to soften, swell, and stick together (Felton et al., 1996; Thoma and Bechtold, 2018) and they may be brittle during drying due to water loss (Dvorácková et al., 2010). These problems may be overcome by subcoating the gelatin capsules (Dvorácková et al., 2010; Thoma and Bechtold, 2018), or by using the capsules shells made from alternative materials such as hydroxypropyl methylcellulose (Cole et al., 2002; Dvorácková et al., 2010; Dvořáčková et al., 2011; Huyghebaert et al., 2004). However, the additional step of film coating consumes time and cost in development and optimization of coating formulation and

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process to obtain consistent quality and stability products (Fu et al., 2020; Macchi et al., 2016; Murthy et al., 1988; Oliveira et al., 2013; Yang et al., 2018).

Many attempts have been made to develop a capsule shell with inherent modified release properties through chemical treatment of the gelatin shell to reduce its solubility for delayed (Bajpai et al., 2003; Pina et al., 1996) and osmotic controlled release (Monton and Kulvanich, 2019). Modification can be also achieved by blending gelatin with other insoluble materials (Chuenbarn et al., 2021) and the use of non-gelatin materials. Cellulosic materials are often used in the development of capsule shells. Hydroxypropyl methylcellulose (blended with sodium alginate) (Smith et al., 2010), hydroxypropylcellulose (Gazzaniga et al., 2011), hydroxypropyl methylcellulose acetate succinate and hydroxypropyl methylcellulose phthalate (Barbosa et al., 2019) have all been used in developing delayed release capsules; cellulose acetate and ethylcellulose (Wang et al., 2005), also cross-linked methylcellulose (Bhatt and Kumar, 2017) have been used in developing osmotic controlled release capsules. To date, there are commercial capsule shells made from cellulosic materials (i.e., DRcaps® (Capsugel, 2013), enTRinsic™ (Capsugel, 2017), Vcap® enteric capsules (Capsugel, 2018), and Bio--VXR® (BioCaps, 2018)), which are claimed to protect acid labile drugs from the gastric condition. Other non-gelatin materials that have been of interest are zein (Tang, 2015) and methacrylic acid copolymer type A and type B, used for developing delayed release capsules (Barbosa et al., 2019); blends of low and high methoxy pectin, used for colonic delivery (Ponrasu et al., 2021); and blends of inulin and vinyl copolymer (Kollidon® SR) or thermoplastic polyurethane (Carbothane™ PC-3575A), used for developing controlled release capsules (Benzine et al., 2021).

Most capsule shells are commonly prepared by the dip coating technique (Barbosa et al., 2019; Bhatt and Kumar, 2017; Jones et al., 2018; Ponrasu et al., 2021; Smith et al., 2010; Tang, 2015; Zhang et al., 2013). However, some may be made by melting the material, then coating (Lee et al., 2006), 3D-printing (Gaurkhede et al., 2021) or injection molding (Benzine et al., 2021; Gazzaniga et al., 2011; Vilivalam et al., 2000). The selection of materials and film composition for the development of modified release capsule shells should be made taking into account; safety concerns, the technical requirements and the solubility of the material required. Also, in this study, the material must have an ability to form capsules using the dip coating process at an industrial scale. Such manufacturing processes include the steps of; preparation of the polymer solution, dip coating, rotation of the molding pin, drying, stripping, trimming, and joining (Jones et al., 2018). Primarily, solutions of the chosen materials must be able to form a gel on the molding pins under manufacturing conditions. Gelatin and hydroxypropyl methylcellulose generally form a capsule shell on the molding pin through thermal gelation (Jones et al., 2018; Smith et al., 2010; Zhang et al., 2013).

Zein is a corn protein which has been listed as Generally Recognized as Safe (GRAS) by the U.S. Food and Drug Administration. This corn protein contains high contents of hydrophobic amino acid and is insoluble in water. Zein is soluble in alkaline fluid with pH > 11 and aqueous ethanol solution (55–90%v/v) (AbuBaker, 2009; Shukla and Cheryan, 2001). It forms a gel at room temperature (Chen et al., 2013; Nonthanum et al., 2012, 2013). Zein can form a continuous film from concentrated solutions in aqueous ethanol (Bisharat et al., 2018; Kashiri et al., 2017; Lai et al., 1999; Lawton, 2004; Vattanagijyingyong et al., 2021; Xu et al., 2012; Yamada et al., 1995; Yoshino et al., 2000, 2002) or aqueous acetone (Yamada et al., 1995; Yoshino et al., 2000, 2002), as well as from aqueous dispersions (Bisharat et al., 2019; Guo et al., 2008; Li et al., 2010; O'Donnell et al., 1997). Zein-based films have advantages in drug delivery applications (Zhang et al., 2015).

Due to the solubility of zein, its film is anticipated to resist the acidic pH of gastric fluid and dissolve in the intestine lumen, when it is used for oral administration. However, pure zein film is brittle (Zhang et al., 2015), and is likely to be hydrolyzed by pepsin and denatured by deamidation in the acidic conditions (pH 1–3.5) of the stomach (Soulby et al., 2015; Yong et al., 2004). This causes unreliable gastric resistance. Therefore, zein must be combined with other materials to achieve processability, mechanical properties, and the intended functionality. The addition of a secondary polymer and plasticizer to form composite films is often useful in manufacturing capsule shells. The second polymer is used to aid gelation of the polymer solution on the molding pin during dip coating (Fakharian et al., 2015; Ponrasu et al., 2021; Smith et al., 2010; Zhang et al., 2013) and/or to provide modified release properties to the capsule shell (Chuenbarn et al., 2021; Gaurkhede et al., 2021; Smith et al., 2010). The plasticizer is usually required to improve the film mechanical properties. However, when the film composition contains more than one component, compatibility of all components is important to ensure stability of the capsule shell. Our previous study demonstrated that zein is miscible with methacrylic acid copolymer type C, Eudragit® L100–55 (L100–55), a commercial enteric polymer, and with polyethylene glycol with an average molecular weight of 1000 (PEG1000), used as plasticizer (Vattanagijyingyong et al., 2021). With suitable amounts, the "hard and tough" composite film can be achieved and potentially form capsule shells that are suitable for conventional capsule filling lines. L100–55 is soluble in aqueous fluids at pH above 5.5 (Evonik Nutrition and Care GmbH, 2020; Wulff and Leopold, 2016). It is expected to reduce the risk of protein degradation and maintain the integrity of capsules in the stomach. The dissolution of modified release capsules is, thus, initiated by the pH of intestinal fluid.

In addition to the requirement of film composition, the rheology is a critical quality of the coating solution that needs to be controlled during dip coating. It provides information about gelling performance (e.g., gelation time) which is related to process parameters such as the rotation period of the molding pins (Jones et al., 2018; Yasuda et al., 2004). Several factors, including viscosity and withdrawal speed, have been reported to affect formation of filament and coating layer during dip coating, as well as influencing the capsule shell thickness (Barbosa et al., 2019; Fakharian et al., 2015; Smith et al., 2010; Yasuda et al., 2004; Zhang et al., 2013). The objective of this study was to fabricate delayed release capsule shells based on the composite film of zein and L100–55. Rheological properties of the coating solution were characterized for process design. Central composite designed experiment was employed to attain a design space of delayed release capsules shell with desirable thickness and mechanical strength.

2. Materials and methods

2.1. Materials

Zein (α-zein, 14.0% of total nitrogen, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), methacrylic acid copolymer type C, or methacrylic acid/ethyl acrylate 1:1 copolymer (Eudragit® L100–55, Evonik Röhm GmbH, Darmstadt, Germany, supplied by Jebsen & Jessen Ingredients, Ltd., Bangkok, Thailand), polyethylene glycol with average molecular weight of 1000 (Merck, Darmstadt, Germany), magnesium nitrate hexahydrate (Merck, Darmstadt, Germany), pepsin (632 units/mg solid, Sigma-Aldrich, Missouri, USA) and pancreatin (1x USP specification, 25 units of protease activity, Merck, Germany) were used as received. Ethanol (95%v/v, pharmaceutical grade, Liquor Distillery Organization, Chachoengsao, Thailand) and deionized water were used as solvent. Diltiazem hydrochloride (Piramal Enterprises Ltd., Telangana, India, given by Siam Bheasach, Co., Ltd., Thailand) was used as a model drug in the *in vitro* drug release test. Other reagents used for assay and drug release studies were of analytical grade.

2.2. Methods

2.2.1. Preparation of coating solutions

Zein was dispersed in an aqueous ethanol solution (80%v/v) to obtain 35%w/w dispersion. The dispersion was kept at an ambient temperature (27±2 °C) for 3 h and then the insoluble matter

(approximately of 1%w/w) was separated from the dispersion by centrifuge (refrigerated centrifuge, Himac CR 20B3, Hitachi, Tokyo, Japan) at a speed of 6000 rpm for 30 min. L100–55 was then added to the supernatant; and after that the solution was diluted with the solvent to obtain desired solid contents and plasticized with PEG1000. All formulations studied are shown in Tables 1,2. The solution was equilibrated for 18 h prior to rheological characterization and use as a coating solution for capsule shell preparation.

2.2.2. Rheological measurement of coating solutions

Viscoelastic properties and flow behavior of zein/L100–55 solutions were studied using a rotational rheometer (HAAKE Mars 60, Thermo Scientific, Darmstadt, Germany) with a 35 mm diameter parallel plate geometry. The gap between the parallel plate was 1 mm. After removing the excess sample, the sample was equilibrated at the temperature of 25±0.1°C for 30 s before measurement.

Rheological parameters, in terms of loss modulus (G") and storage modulus (G'), were studied by oscillatory time sweep test at the amplitude of 0.4% (within the linear viscoelastic region) and frequency of 1 Hz for 20 min. Measurements were carried out in triplicate at a temperature of 25±0.1°C. Data were collected and analyzed using RheoWin software version 4.8 (HAAKE, Thermo Scientific, Darmstadt, Germany). The plot of these parameters as a function of time was used to determine gelation time (Nonthanum et al., 2012, 2013).

The flow behavior of coating solutions was determined by a steady shear test. The measurement was carried out in triplicate with varying shear rate from 1 to 100 s^{-1} at 25±0.1°C. Data were collected and analyzed using RheoWin software. A logarithmic plot of shear stress and shear rate was obtained from the measurements. The shear stress versus shear rate relationship was fitted by Ostwald-de Waele (or power-law) and Herschel-Bulkley models according to Eqs. (1) and (2), respectively.

$$\tau = K\dot{\gamma}^n \quad (1)$$

$$\tau = \tau_0 + K\dot{\gamma}^n \quad (2)$$

Where, τ is the shear stress (Pa), τ_0 is the initial shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the flow consistency index ($Pa.s^n$) and n is the flow behavior index that is used to identify the flow behavior: n= 1 for Newtonian, n < 1 for shear-thinning, and n > 1 for shear-thickening (Daubert and Foegeding, 2010). The apparent viscosity, i.e., internal flow resistance, of coating solutions was determined from the slope of the shear stress and shear rate curves. A plot of viscosity versus shear rate was used to examine the flow behavior and discussed together with the n value.

2.2.3. Preparation of hard capsule shells

Stainless-steel molding pins of standard size 0 capsule (supplied by Capsule Products Co., Ltd.) were lubricated and manually immersed into the coating solution. The immersed length from the pin tip and the dwell time were kept at 25–28 mm and 5 s, respectively. Then, the coated pin was withdrawn from the bath of coating solution at a rate of 2.8–3.5 mm/s, rotated about 180° several times, and inverted. These steps mimic dip coating in the conventional manufacturing process. The molding pins and the polymer solution were at ambient temperature during the coating process. Solvent evaporation allowed gelation of the coating solution to occur during pin rotation. The coating layer was further dried in a hot air oven at a temperature of 48±2 °C and relative humidity of 20±2% for 2 h and the moisture content (%MC) was analyzed by a moisture analyzer (HR83 Halogen, Mettler Toledo, Columbus, USA) in the range of 5–8%. The capsule shell was stripped off the pin and trimmed. For each formulation, the shell (wall) thickness of eight capsule caps and bodies were measured by 0.01 mm resolution digital caliper (Pittsburgh, Pennsylvania, USA) at three points for each section and the average values are reported. The capsule shells were then kept in the desiccator containing a saturated solution of magnesium nitrate to provide a relative humidity of 53±5% of at ambient temperature for 1 week before characterization.

Table 1
Compositions of the coating solution for rheological study.

Ratios of zein/L100–55	Solid polymer content (%w/w)	PEG1000 (%w/w)[a]
100:0	27	0
100:0	31	0, 10, 20
100:0	35	0, 10, 20
75:25	27	0
75:25	31	0, 10, 20
75:25	35	0, 10, 20
0:100	27	0
0:100	31	0
0:100	35	0

[a] based on weight of solid polymer.

Table 2
Formulation variables and responses with target values of capsule shells in the inscribed central composite design.

Formulation variables	Coded levels				
	−1	−1/α	0	+1/α	+1
X_1: L100–55 proportion	0	5.0675	12.5	19.9325	25
X_2: Solid polymer content (% w/w)	32	32.4054	33	33.5946	34
X_3: PEG1000 level (% w/w)[a]	10	12.0270	15	17.9730	20
Responses	**Target**				
Y_1: Thickness (mm)	0.09–0.11 mm				
Y_2: F_{max} (N)	Maximize				
Y_3: e_{max} (mm)	Maximize				

α=1.6818.
[a] based on weight of solid polymer.

2.2.4. Mechanical property measurement of hard capsule shells

A compression test was conducted to measure mechanical properties, i.e., maximum force (F_{max}) and displacement (e_{max}), at the point where the empty hard capsule could withstand the applied force before breaking. These parameters represent the hardness and flexibility of hard capsule shells, respectively (Pinto et al., 2020). The test was carried out by a universal testing machine (EZ-S 500 N, Shimadzu, Osaka, Japan) with 500 N load cell, equipped with a 25.4 mm diameter cylinder plunger. The empty capsule shell was placed horizontally, perpendicular to the plunger and compressed at a rate of 5 mm/s. In case that the shell was highly flexible, the maximum displacement was kept at 5.1 mm to avoid load cell damage due to the plunger coming in contact with hard surface of the machine, and F_{max} was recorded. The measurement was carried out for five capsule shells of each formulation at a temperature of 25±2°C controlled by Trapezium software version 2 (Shimadzu, Osaka, Japan).

2.2.5. Experimental design and data analysis

A response surface design, i.e., inscribed central composite design (CCD), was used to investigate the main, interaction and quadratic effects of formulation variables (i.e., L100–55 proportion (X_1), solid content of polymers (X_2) and PEG1000 level (X_3)) on the responses (i.e., capsule shell thickness (Y_1) and mechanical properties: F_{max} (Y_2) and e_{max} (Y_3)). The values of formulation variables were selected based on the miscibility results of our previous study (Vattanagijyingyong et al., 2021) and the results of rheological measurement. The coded level of axial points in the design are −1 and +1; and the coded level of factorial points are −1/α and +1/α, as shown in Table 2. The center point was repeated for six times. The designed formulations in Table A.1 were used to prepare hard capsule shells by the dip coating method in a random sequence. The response values were statistically analyzed by multiple regression analysis (Minitab software version 19, Minitab, LLC., Pennsylvania, USA). Backward elimination was employed to define the final

models at the significant level (P-value) of 0.05.

Contour diagrams were plotted and superimposed using the obtained models. Multiple response optimization was conducted to establish a design space where the optimal setting of formulation variables simultaneously produced capsule shells with a specified thickness (0.09–0.11 mm) and maximized F_{max} and e_{max} (Table 2).

2.2.6. *In vitro drug release*

Three formulations with different proportions of L100–55 in the established design space were selected to study *in vitro* drug release and compared with pure zein capsule shells. For each formulation, three capsules were filled with 100 mg of diltiazem hydrochloride (DTZ) and tested using USP dissolution apparatus I (VK7000, Vankel, North Carolina, USA) at a rotation speed of 100 rpm. The drug release was studied in 900 ml simulated gastric fluid (SGF, pH 1.2) for 2 h, followed by in simulated intestinal fluid (SIF, pH 6.8) for 10 h at 37±2°C. SGF and SIF with and without pepsin and pancreatin were prepared according to USP (The United States Pharmacopeial Convention, 2019).

A 10 ml sample was taken at 0.5, 1 and 2 h in SGF, and at 0.25, 0.5, 1, 2, 4, 6 and 10 h in SIF. Fresh medium was replaced into the vessel after sampling. The sample was filtered through a 0.45 μm nylon filter and the amount of drug released was analyzed by HPLC (LC-20AD, Shimadzu, Osaka, Japan) with a UV detector at 240 nm (SPD-M20A, Shimadzu, Osaka, Japan). The analytical method was modified from the USP monograph of DTZ (The United States Pharmacopeial Convention, 2019). Briefly, the chromatographic conditions were as follows: flow rate of 1.5 ml/min, injection volume of 20 μl, run time of 15 min and oven temperature of 25±2°C. The test column was C18 of 250 mm length x 4.6 mm diameter and 5 μm packing (Kitenex®, Phenomenex, California, USA). The mobile phase was a solution of 30:25:45 acetronitrile/methanol/solution A. Solution A was prepared by dissolving d-10-camphorsulfonic acid in 0.1 M sodium acetate trihydrate to obtain a concentration of 1.16 mg/ml; then, the pH of solution was adjusted by addition of 1 N sodium hydroxide to pH 6.2. The accuracy, precision, linearity and specificity of the method was verified before analysis. The standard solution of DTZ was prepared by dissolving DTZ working standard in SGF or SIF and diluted to a concentration of 120 μg/ml. It was used to standardize the area under the curve of sample for calculation of the drug release. To ensure dissolution sink condition during the test, the solubility of DTZ was determined by the USP saturation shake-flask method (The United States Pharmacopeial Convention, 2019).

The remaining capsule shell after 12 h *in vitro* drug release test was dried at the same condition as the capsule shell. The sample was gold coated by gold sputter coater (SCD 040, Balzers Union, Liechtenstein, Germany); then surface and cross-sectional images were examined by scanning electron microscope (SEM) (JSM-IT300, Jeol, Tokyo, Japan) at a magnification of 5000x and 3000x, respectively, using an accelerating voltage of 15.0 kV.

3. Results and discussion

3.1. *Rheological measurement of coating solutions*

Processability of capsule shells was screened by single dipping the capsule molding pins into a solution containing zein and L100–55, with varying solid contents. The L100 proportion and solid polymer content that gave capsule shells with no visual defect, and which could be stripped off the pin were chosen for further study. The chosen formulation was then plasticized by PEG1000 in order to achieve “hard and tough” films (Vattanagijyingyong et al., 2021). The rheological parameters (i.e., loss modulus (G") and storage modulus (G’), which represent the viscous and elastic portion, respectively) of coating solutions (Table 1) were measured in order to understand their impact on capsule formation.

The change in the modulus behavior was dependent on the solution composition (Figs. 1 and A.1–A.6). It typically divided into three regions: G" > G’, G’=G", and G’ > G″ and could be related to gelling performance of the coating solution and the quality of capsule shells. G" was initially greater than G’; then, the modulus rapidly increased until the curves cross. The intersection is the point at which the viscous liquid turns to elastic solid. The time from the start of the test to the gelation point is considered to be the gelation time of the coating solution (Nonthanum et al., 2013; Tung and Dynes, 1982). After the point of intersection, G’ increased to be higher than G" because the elastic property dominated the gel structure. The modulus continually increased until a plateau was reached, as a result of complete gel formation (Tung and Dynes, 1982). The great difference between G’ and G" (G’ >> G″) and the high values of both moduli was attributed to highly strengthening network formation and a strong gel structure (Nonthanum et al., 2012; Smith et al., 2010; Zarzycki et al., 2019). For pure L100–55 solutions, the viscoelastic behavior was different. The rapid increase in the modulus was not observed and the solution took longer to gel. In addition, after the gelling point, the difference between the G’ and G" values of the L100–55 solution was relatively small. However, the blend of 75:25 zein/L100–55 could decrease gelation time and increase the modulus values of the coating solution. With relatively high solid contents, the solution took less time before an initial increase in the modulus; and hence the gelation time was generally shorter. The addition of PEG1000 could further decrease the gelation time but did not markedly change the modulus values of coating solutions (Figs. A.3–A.6 and Table 3).

Gelation of zein occurs by unfolding of peptide chains, followed by entanglement of the chain (Chen et al., 2013; Nonthanum et al., 2013). Gelation of L100–55 is induced by solvent evaporation (Siepmann et al., 2008). In this study, greater differences between G’ and G" values resulted in higher values of both moduli after gelation, as can be seen in Fig. 1a compared to Fig. 1e. This suggests that the gel network of zein peptide chains was stronger than that of L100–55 polymer chains. With relatively high solid polymer contents in the formulations, the close packing of polymer aided network formation, reduced gelation time, and increased the modulus values. The effect of L100–55 and PEG1000 on strengthening the gel network may be caused by hydrogen bonding between zein, L100–55 and PEG1000 molecules which was reported in our previous study (Vattanagijyingyong et al., 2021).

In the dip coating process, rotation of the molding pins after withdrawal from the coating solution bath allows the coating solution to be evenly distributed before setting and gelation. Then, solvent evaporation continues until the gel layer finally forms a continuous film on the surface of the molding pins, providing sturdy capsule shells. Determination of gelation time is useful to suggest the time required for the rotation step before keeping the pin in an inverted position to avoid drainage of the coating solution. A coating solution that took longer time to form gel would require a longer period of rotation. Other factors, e.g., viscosity and evaporation rate of the coating solution, should also be controlled when the rotation period is estimated through gelation time measured by the oscillatory time sweep test.

The gelation time, however, could not explain the presence of a horizontal band of thicker film observed under some conditions. For the pure L100–55 solution, the solution evenly deposited on the pin surface over the period of gelling time of 8–15 min (Table 3). But the coating layer on the surface of molding pins in the inverted position grew gravitationally during drying, causing a thick horizontal ‘ring’ (Fig. 1f). This was observed within a short period of time for the solution having the higher solid polymer content. Flowable behavior after gelation of the L100–55 solution resulted from insufficient strength of gel structure (having small differences between G’ and G" in Fig. 1e and low values of both moduli after gelation) when compared to other zein/L-100 ratios. The value of loss tangent (tan δ), i.e., G"/G’, at the first time point after the gelation point (i.e., G"=G’ and tan δ = 1) was found to be useful to identify an appropriate gel strength for capsule formation. When the tan δ is close to 1, viscous liquid and elastic solid behaviors were almost in

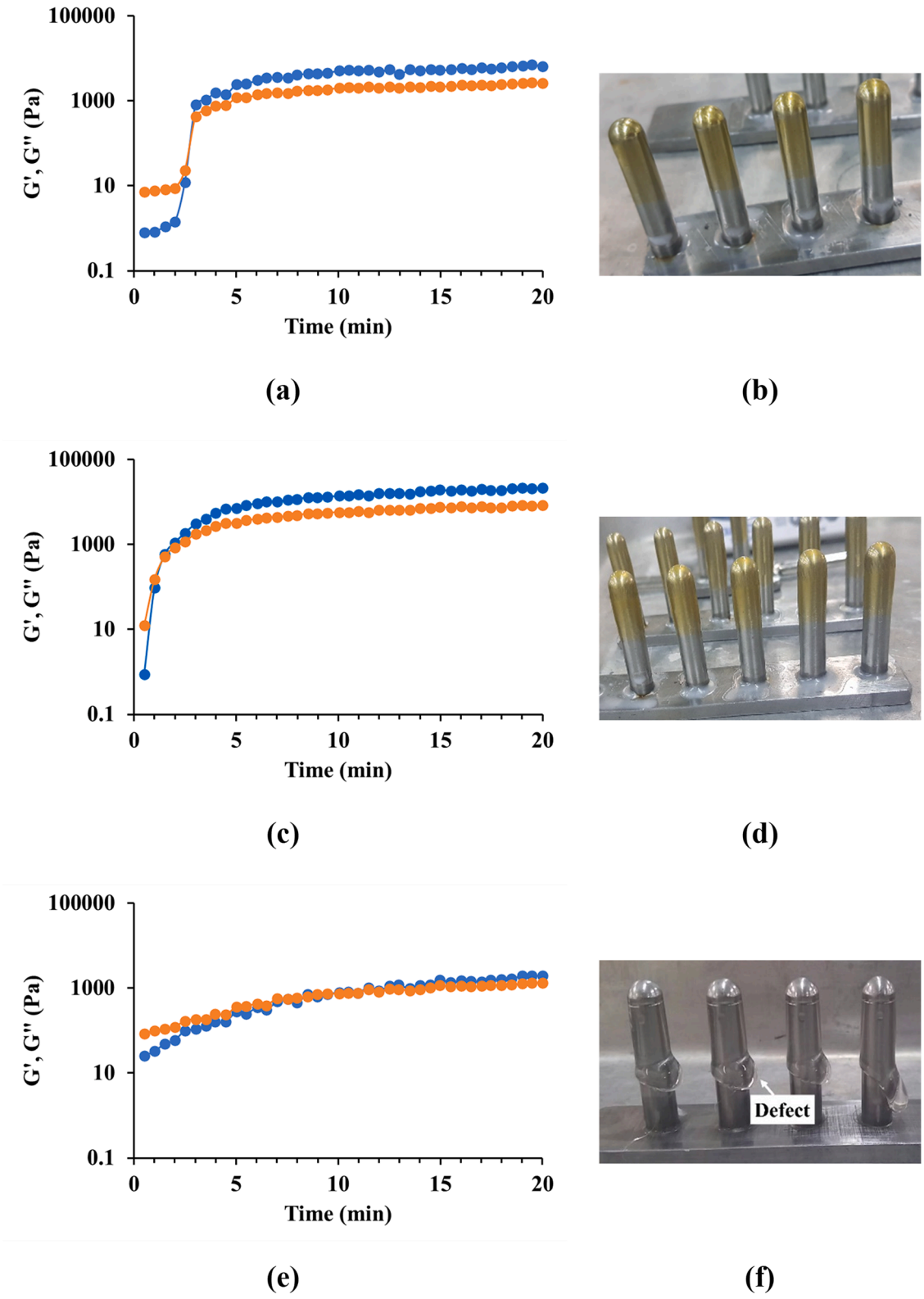

Fig. 1. Examples of the plot of loss modulus (G", orange line) and storage modulus (G', blue line) as a function of time and the photograph of the coating layer on the molding pin for the capsule shell formulation with 35% solid polymer content, having zein/L100–55 at the ratios: (a, b) 100:0, (c, d) 75:25, and (e, f) 0:100; 0% PEG1000.

balance. The smaller tan δ indicates that more elastic solid behavior dominated in gel network. The gel which was insufficiently strong and flowable after gelation had the tan $\delta > 0.9$ (Table 3). Therefore, the value of tan $\delta < 0.9$ in the study was a useful criterion for selection of coating composition for preparing thin and firm film of capsule shells. It also suggested that pure L100–55 was unlikely to form capsule shells by the dip coating process under the condition studied unless it was blended with other polymers such as zein.

Table 3
Results of rheological measurement, capsule defects and shell thickness (average±SD).

Ratios of zein/L100–55	Solid polymer content (%)	PEG1000 (%)	Time to G'=G" (min)	Tan δ[a]	Shell thickness (mm)	Capsule Defects
100:0	27	0	15.84±1.84	0.84±0.08	0.02±0.00	No
100:0	31	0	9.97±2.12	0.84±0.08	0.05±0.00	No
100:0	35	0	2.56±1.15	0.56±0.06	0.11±0.01	No
75:25	27	0	6.94±1.40	0.82±0.05	0.06±0.00	No
75:25	31	0	2.33±0.27	0.70±0.16	0.10±0.00	No
75:25	35	0	1.37±0.44	0.87±0.05	0.17±0.02	No
0:100	27	0	14.71±2.74	0.94±0.06	0.10±0.01[b]	Yes
0:100	31	0	12.64±2.28	0.98±0.01	0.16±0.00[b]	Yes
0:100	35	0	8.28±1.17	0.97±0.04	0.18±0.00[b]	Yes
100:0	31	10	3.82±0.35	0.86±0.07	0.03±0.01	No
100:0	35	10	2.01±0.57	0.86±0.10	0.09±0.02	No
75:25	31	10	1.46±0.07	0.79±0.10	0.09±0.02	No
75:25	35	10	1.20±0.23	0.75±0.17	0.16±0.03	No
100:0	31	20	4.40±1.81	0.85±0.05	0.03±0.01	No
100:0	35	20	1.28±0.07	0.74±0.09	0.09±0.02	No
75:25	31	20	1.89±0.76	0.79±0.07	0.09±0.03	No
75:25	35	20	1.06±0.19	0.82±0.07	0.17±0.03	No

[a] Tan δ value at the first time point after gelation (G'=G").
[b] The defect (stripe or horizontal band of thicker film) was trimmed before measurement of capsule shell thickness.

The viscosity of the coating solution is also an important attribute, relating to processability and capsule shell thickness as was previously reported (Barbosa et al., 2019; Fakharian et al., 2015; Smith et al., 2010; Zhang et al., 2013). Plots of the viscosity versus the shear rate of zein/L100–55 solutions show a decrease in the viscosity with an increase in the shear rate (Fig. 2). This indicates non-Newtonian and shear-thinning characteristics. The shear-thinning behavior was further confirmed by fitting a plot of shear stress and shear rate with Herschel-Bulkley and Ostwald-de Waele (power-law) models. The results are shown in Table 4. The Herschel-Bulkey model is used to fit the shear stress and shear rate curve with an initial shear stress (Daubert and Foegeding, 2010). In this study, the Herschel-Bulkley model gave a higher coefficient of determination (R^2) than the Ostwald-de Waele model. However, it was not useful for determining the behavior of the coating solution as the calculated initial stress of the formulation, containing relatively low solid content, was negative. The flow behavior index (n) of the formulation containing 35% w/w solid content which was > 1, indicating shear-thickening (Table 4) which did not agree with the shear-thinning behavior observed in Fig. 2. The Ostwald-de Waele model gave an n value < 1 in agreement with the shear-thinning behavior of viscosity profiles in Fig. 2. The n value of the Ostwald-de Waele model decreased; while the K value increased, as the solid content increased (Table 4), suggesting more pronounced shear-thinning behavior. The change of n and K values agree with an increase in the modulus values and short gelation time observed in Figs. 1, A.1–A.6, and Table 3. The high K value is attributed to stronger intermolecular interaction in the gel structure (Nonthanum et al., 2013; Zarzycki et al., 2019).

Shear-thinning could facilitate the dip coating process. Where high shear rates occur (during immersion, dwelling and withdrawal of molding pins) a coating solution of low viscosity could be easily distributed, providing a uniform layer on the molding pin. Where low shear rates occur (during gelation and film formation), the high viscosity provides good adhesion of the coating layer on the surface of molding pins (Yasuda et al., 2004).

The viscosity of coating solutions was markedly increased in the blend of zein/L100–55, in particular, with higher solid contents (Fig. 2). There was also an effect of PEG1000. An increase in the solution viscosity was caused by intermolecular interaction, i.e., hydrogen bonding among zein, L100–55, PEG1000 and the solvent (Ma et al., 2018; Vattanagijyingyong et al., 2021; Zarzycki et al., 2019).

Coating solutions with a short gelation time and high viscosity provided thicker capsule shells (Fig. 3). The viscosity under the high shear rate (i.e., 89 s^{-1}), presumably during immersion and withdrawal of molding pins in the coating process, was clearly related to the capsule wall thickness. The fact that higher viscosity produces thicker capsule was also previously reported (Barbosa et al., 2019; Fakharian et al., 2015; Smith et al., 2010; Zhang et al., 2013). In our study, capsules with shell thickness of less than 0.06 mm were too thin and too fragile to be stripped off the pin and that of more than 0.11 were too thick to join the

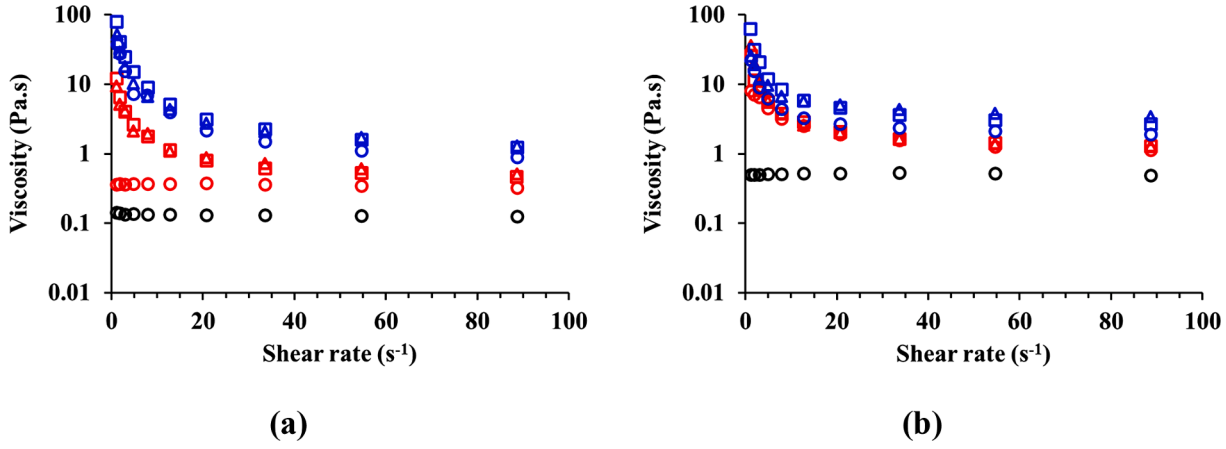

Fig. 2. Examples of the plot between the viscosity and shear rate of the coating solutions, having the zein/L100–55 ratios: (a) 100:0 (zein) and (b) 75:25; and solid polymer content of 27% (black), 31% (red) and 35% (blue); with PEG1000 level of 0% (circle), 10% (triangle) and 20% (square).

Table 4
Herschel-Bulkley and Ostwald-de Waele equation parameters for zein/L100–55 solutions (average±SD, $n = 3$).

Ratios of zein/ L100–55	Solid polymer content(%)	PEG (%)	Herschel-Bulkley model				Ostwald-de Waele model		
			K (Pa.s^n)	n	R^2	τ_0	K (Pa.s^n)	n	R^2
100:0	27	0	0.19±0.04	0.94±0.02	0.9999–1.0000	−0.08±0.05	0.18±0.03	0.95±0.01	0.9998–1.0000
100:0	31	0	0.54±0.01	0.89±0.00	0.9999	−0.35±0.01	0.46±0.01	0.92±0.01	0.9997–0.9998
100:0	35	0	0.01±0.02	2.05±0.61	0.7705–0.9025	44.88±1.25	37.74±2.96	0.12±0.04	0.4623–0.8128
75:25	27	0	1.43±1.30	0.85±0.14	0.9910–0.9999	−1.83±2.42	0.88±0.49	0.92±0.07	0.9872–0.9998
75:25	31	0	3.64±2.10	0.76±0.11	0.9802–0.9971	8.05±10.93	7.79±3.28	0.59±0.12	0.9614–0.9981
75:25	35	0	2.09±0.43	0.97±0.04	0.9990–0.9995	22.01±2.90	9.95±1.85	0.64±0.05	0.9809–0.9841
100:0	31	10	−981.20±308.84	−0.01±0.00	0.6913–0.8657	990.20±301.66	9.93±6.83	0.35±0.16	0.7578–0.9634
100:0	35	10	13.34±22.06	0.68±0.41	0.9945–0.9967	−3.13±21.26	54.25±16.51	0.13±0.08	0.3533–0.9620
75:25	31	10	3.33±2.47	0.83±0.22	0.9961–0.9987	16.97±8.26	12.13±5.94	0.52±0.13	0.9590–0.9965
75:25	35	10	1.02±0.35	1.18±0.09	0.9894–0.9972	54.25±3.97	24.42±4.80	0.50±0.05	0.9212–0.9514
100:0	31	20	0.95±1.58	1.58±0.95	0.8578–0.9917	60.51±15.58	8.46±5.30	0.42±0.06	0.9640–0.9929
100:0	35	20	−2343.33±653.50	−0.01±0.00	0.7541–0.9372	2374.67±641.51	33.08±9.08	0.24±0.07	0.8247–0.9840
75:25	31	20	2.14±1.15	0.91±0.13	0.9936–0.9998	15.74±19.29	14.69±8.92	0.49±0.19	0.8692–0.9946
75:25	35	20	4.23±2.14	0.94±0.11	0.9960–0.9997	29.80±12.75	14.77±3.01	0.66±0.05	0.9685–0.9957

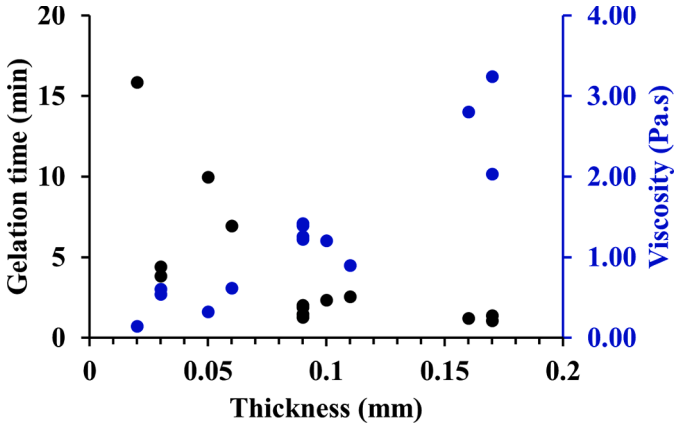

Fig. 3. The plots between capsule shell thickness versus gelation time (black) and viscosity at the high shear rate (89 s^{-1}) (blue). The capsule thickness and viscosity value of pure L100–55 formulation was excluded due to the presence of capsule defects.

cap with the body of capsule. The range of 0.06–0.11 mm corresponded to the solution viscosity determined at the high shear rate in a range of 0.62–1.42 Pa.s.

3.2. Optimization of capsule shell formulations using inscribed central composite design

The range of solid polymer content, i.e., 32–34%w/w was justified to ensure the processability of the coating solution. The rotation period of pin, estimated based on the gelation time, was 5 min. The acceptable range of capsule shell thickness was 0.09–0.11 mm, with a target of 0.10 mm (comparable to the thickness of commercial gelatin capsule shells). F_{max} and e_{max} were to be maximized to obtain hard and tough capsule shells.

From the designed experiment, the capsule shell thickness was in a range of 0.08–0.12 mm. F_{max} was in the range of 6.79–14.15 N; and e_{max} was in the range of 4.91–5.10 mm. The e_{max} value of 5.10 mm was the maximum displacement allowed in the test condition.

Multiple regression analysis using the second-order polynomial model gave the capsule shell thickness, F_{max} and e_{max} as tabulated in Table 5. The model for capsule shell thickness (Eq. 3) demonstrates that the formulation variables which significantly affect capsule shell thickness were L100–55 proportions (X_1) and solid polymer contents (X_2). An increase in the level of these factors increased the capsule shell thickness. This was associated with the increase in viscosity of the zein/L100–55 solutions, resulting in thicker capsules as observed in the rheological results. The model for F_{max} (Eq. 4) suggests that all formulation variables affected F_{max}. L100–55 proportions and solid polymer contents provided positive effects. By increasing these variables, F_{max} was increased. On the other hand, PEG1000 levels provided a negative effect. The plasticizing effect of PEG1000 induced a decrease in the film strength and an increase in the film flexibility as previously reported (Vattanagijyingyong et al., 2021). There was also quadratic effect of L100–55 on F_{max}. The models could explain 52.51% and 83.79% of variation in capsule shell thickness and F_{max}, respectively. The model for e_{max} (Eq. 5) was not fitted to data (P-value of lack of fit < 0.05). Also, it could explain only 30.46% of the variation of e_{max}, possibly due to the limitation of displacement distance in the test.

The design space was established by multiple response optimization of the capsule shell thickness (Eq. 3) and F_{max} (Eq. 4) at different levels of PEG1000. The greatest area of design space that could produce desired properties of capsule shells was obtained from 10% PEG1000 (Figs. 4, A.7). Three formulations from this space (C_1, C_2 and C_3; Table 6) were selected to prepare capsule shells.

Varying zein/L100–55 ratios was expected to delay the drug release

Table 5
ANOVA table and the response surface mathematic models for capsule shell thickness, F_{max} and e_{max}.

Responses	Terms	Adj SS	Adj MS	F-value	P-value*	R^2	Equation	
Y_1 (Thickness)	Model	0.002125	0.001062	11.50	0.001	52.51%	$Y_1 = -0.409 + 0.001187(X_1) + 0.01483(X_2)$	(Eq. 3)
	Lack-of-fit	0.001287	0.000107	1.89	0.249			
Y_2 (F_{max})	Model	73.713	18.4283	25.55	0.000	83.79%	$Y_2 = -46.4 + 0.504(X_1) + 1.721(X_2) - 0.2406(X_3) - 0.01008(X_1)^2$	(Eq. 4)
	Lack-of-fit	7.874	0.7874	1.34	0.394			
Y_3 (e_{max})	Model	0.018378	0.006126	3.77	0.032	30.46%	$Y_3 = -4.63 + 0.290(X_2) + 0.602(X_3) - 0.01799(X_2{*}X_3)$	(Eq. 5)
	Lack-of-fit	0.025969	0.002361	3067.27	0.000			

Adj SS, adjusted sum of squares; Adj MS, adjusted mean square; *at the significance level of 0.05.
X_1, L100–55 proportion; X_2, solid polymer content; X_3, PEG1000 level.

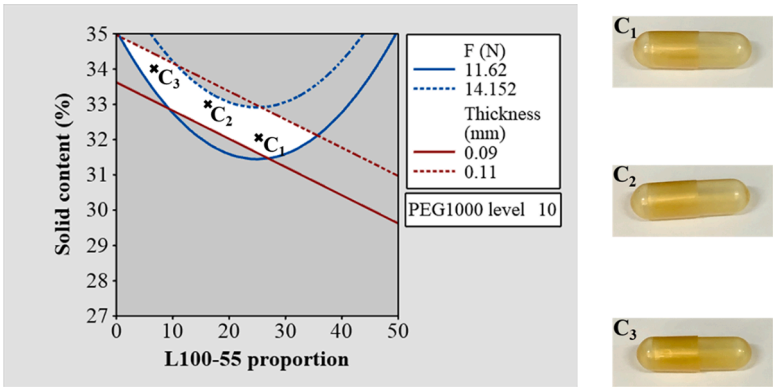

Fig. 4. The overlaid contour plot demonstrating the design space (white region) at the PEG1000 level of 10%. C_1, C_2 and C_3 were selected capsule shells for dissolution study.

Table 6
Observed values of the selected capsule shell formulations in the design space.

Formulation					Observed values (average±SD)	
	X_1	X_2	X_3	Desirability	Y_1	Y_2
C_1	25	32.2	10	0.6579	0.11±0.03	11.5730±1.5260
C_2	16.8	32.9	10	0.8100	0.11±0.02	12.5423±2.9044
C_3	8.6	34	10	0.5343	0.10±0.02	10.7966±1.2347
Pure zein	0	35	0	–	0.11±0.02	6.9875±1.7895

X_1, L100–55 proportion; X_2, solid polymer content (%); X_3, PEG1000 level (%). Y_1, Thickness (mm); Y_2, F_{max} (N).

in the gastrointestinal tract differently, based on the difference in the solubility of zein (soluble at pH > 11) and L100–55 (soluble at pH > 5.5). The difference in solid content of the selected formulations was to control the capsule thickness in the range of 0.10–0.11 mm (Table 6). The capsules were successfully prepared as shown in Fig. 4. They were not physically different, except for pure zein capsules which were not in the design space.

3.3. *In vitro drug release*

The solubility of DTZ in SGF without and with pepsin was 511.2 and 500.4 mg/ml, respectively. The solubility of DTZ in SIF without and with pancreatin was 595.4 and 603.0 mg/ml, respectively. Therefore, a sink condition was maintained during the test. Delayed release action could be achieved from the C_1 and C_2 formulations. After 2 h in SGF, the cumulative of DTZ release from C_1 and C_2 capsules was less than 10%, being 8.7 ± 8.1% and 1.4 ± 1.9%, respectively. Then, after 2 h in SIF, it reached 70%, being 75.5 ± 11.6% and 70.0 ± 26.8%, respectively (Fig. 5a). Pure zein and C_3 formulations could not provide a delayed release capsule. More than 80% of DTZ was released from the capsule after 2 h in SGF (Fig. 5b). Under the gastric condition, a protein could be degraded by pepsin hydrolysis of amide bonds and deamidation of glutamine and asparagine, resulting in unfolding of the protein structure (Blanco and Blanco, 2017; Riha et al., 1996; Soulby et al., 2015; Zhang et al., 2011). The unfolding protein caused the drug release of more than 80% from the capsule containing high zein content. The resistance to gastric condition was significantly improved when zein was blended with L100–55 at appropriate proportion. This can be explained by hydrogen bond formation between carboxyl groups of L100–55 and amide groups of zein, especially the β-turn region which is rich in glutamine (Argos et al., 1982; Momany et al., 2006; Vattanagijyingyong et al., 2021). The intermolecular interaction could suppress the unfolding of zein, resulting in a decrease in the drug release from the capsules.

The effects of enzymatic degradation and deamidation were investigated by comparing the drug release profile in SGF with and without pepsin. Pepsin was unlikely to influence the drug release. The effect of pepsin hydrolysis on unfolding of zein structure was insignificant (Lee and Hamaker, 2006). The main degradation of zein under the gastric condition, therefore, was attributed to deamidation of protein.

The release profile of C_1 and C_2 capsules showed that the drug was markedly released under the intestinal condition. The drug release was likely to be triggered by the solubility of L100–55 (which dissolves at pH > 5.5), rather than zein (which dissolves at pH > 11). L100–55 was

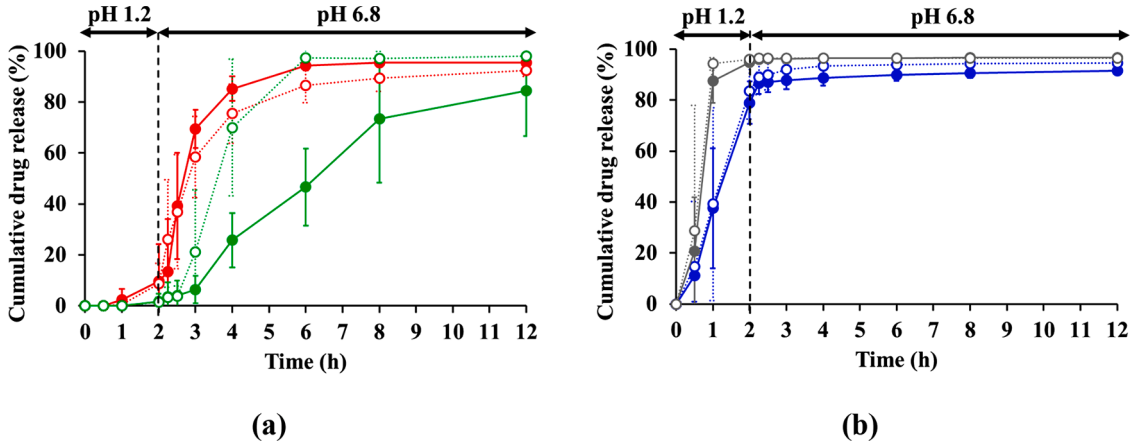

Fig. 5. Drug release profiles of selected capsule shell formulations: (a) C_1 (red) and C_2 (green), and (b) C_3 (blue) and pure zein (gray) in SGF 2 h, followed by SIF 10 h; without enzyme (solid circle and solid line) and with enzyme (blank circle and dash line); $n = 3$.

readily soluble in the medium, resulting in pore formation in the capsule shells (Fig. 6), followed by disruption of the capsule structure, allowing the drug to be released from the capsule. Thus, faster drug release was observed for C_1 capsules having higher L100–55 content (i.e., 75:25 zein/L100–55); and an initial lag time was only observed for the C_2 capsules. Zein is digested by pancreatin. Thus, the drug was released more rapidly in SIF with pancreatin than in SIF without pancreatin (Fig. 5a).

Despite these findings, the drug release performance of capsules may be further modified within the design space at the high level of PEG1000 (Fig. A.7). PEG1000 can affect F_{max} of capsules and its hydrophilicity may affect the drug release.

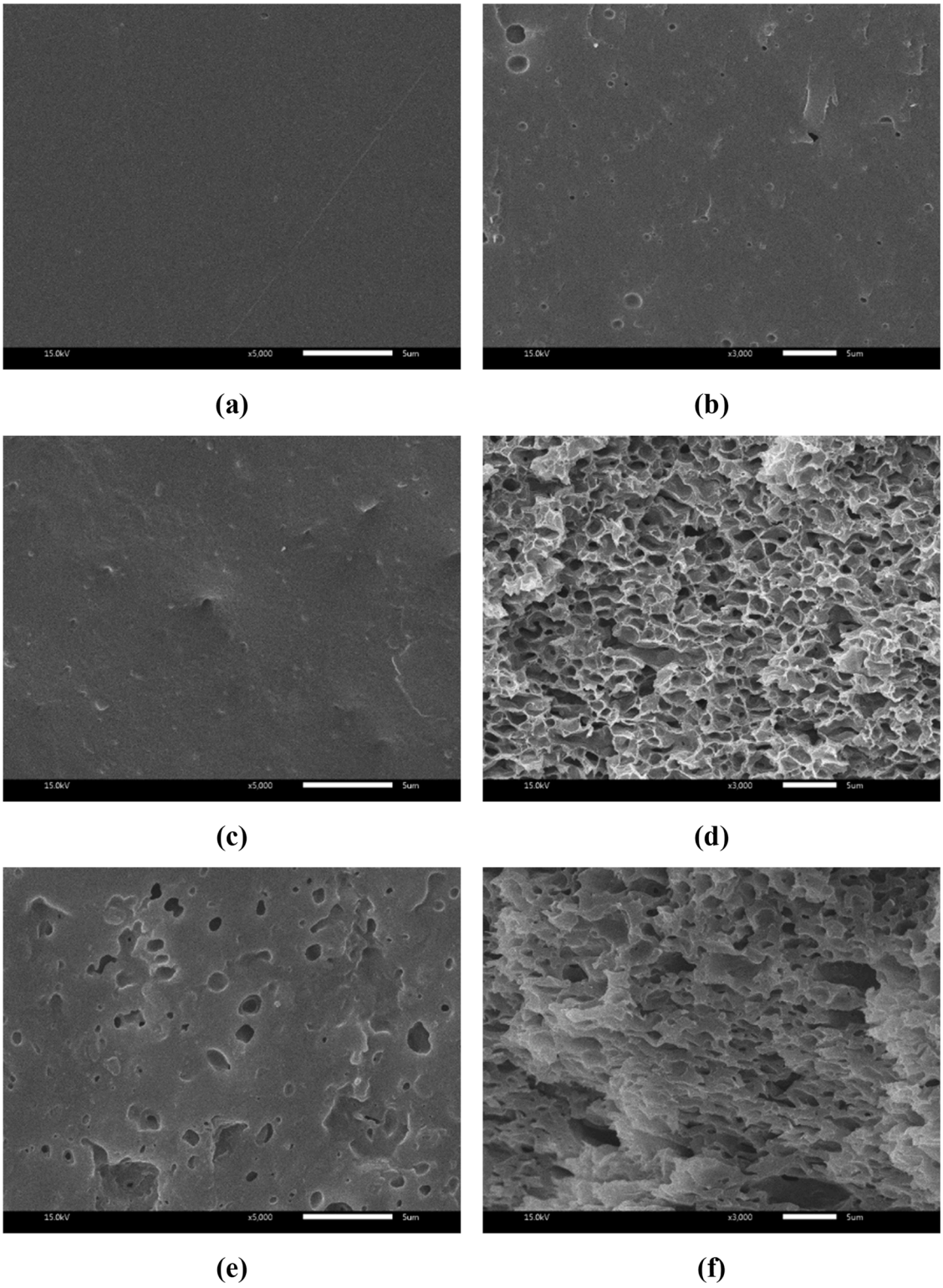

Fig. 6. SEM images of C_1 hard capsules; (a) surface and (b) cross-section before *in vitro* drug release test; (c) surface and (d) cross-section after 12 h in the media without enzymes; (e) surface and (f) cross-section after 12 h in the media with enzymes.

4. Conclusions

Single layer, delayed-release hard capsule shells could be developed from blends of zein and L100–55 using the dip coating method. Zein was used as the primary film former, and L100–55 was used as the second polymer to modulate drug release. Gelation and film formation of the coating solution was facilitated by solvent evaporation at the ambient temperature. The processability of coating solutions was related to the gel strength after the point of gelation. In this study, the gel strength of L100–55 solution alone was insufficient to form capsule. But solutions of pure zein and polymer blend were able form capsule shells. The thickness and hardness of capsule shells were significantly affected by the L100–55 proportion and solid polymer content in the film formulation. The drug release performance could be adjusted by varying the ratio of zein to L100–55 within the design space that gave capsule shells with specified thickness and hardness. Higher sensitivity to enzymatic degradation in the intestinal fluid was caused by higher zein content in the capsules. The design space and guides to process design, e.g., rotation period of molding pins, still need further verification under the controlled condition of capsule manufacturing machine in industrial scale.

CRediT authorship contribution statement

Yada Vattanagijyingyong: Methodology, Formal analysis, Investigation, Writing – original draft. **Poj Kulvanich:** Conceptualization, Writing – review & editing. **Jittima Chatchawalsaisin:** Conceptualization, Methodology, Writing – review & editing, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ejps.2022.106124.

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