การสกัดและการศึกษาคุณลักษณะของเซอินจากกลูเทนข้าวโพดเพื่อใช้เป็นสารก่อฟิล์มในเปลือกแคปซูล

นางสาวอันนา สัณฐิติวณิชย์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
สาขาวิชาเภสัชอุตสาหกรรม ภาควิชาวิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม
คณะเภสัชศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2559
ลิขสิทธิ์ของจุฬาลงกรณ์มหาวิทยาลัย

EXTRACTION AND CHARACTERIZATION OF ZEIN FROM CORN GLUTEN MEAL FOR USE AS
A FILM FORMER IN CAPSULE SHELLS

Miss Unna Santhitiwanich

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science in Pharmacy Program in Industrial Pharmacy
Department of Pharmaceutics and Industrial Pharmacy
Faculty of Pharmaceutical Sciences
Chulalongkorn University
Academic Year 2016

Thesis Title	EXTRACTION AND CHARACTERIZATION OF ZEIN FROM CORN GLUTEN MEAL FOR USE AS A FILM FORMER IN CAPSULE SHELLS
By	Miss Unna Santhitiwanich
Field of Study	Industrial Pharmacy
Thesis Advisor	Jittima Chatchawalsaisin, Ph.D.
Thesis Co-Advisor	Associate Professor Poj Kulvanich, Ph.D.

Accepted by the Faculty of Pharmaceutical Sciences, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

....Dean of the Faculty of Pharmaceutical Sciences

(Assistant Professor Rungpetch Sakulbumrungsil, Ph.D.)

THESIS COMMITTEE

....Chairman

(Associate Professor Parkpoom Tengamnuay, Ph.D.)

....Thesis Advisor

(Jittima Chatchawalsaisin, Ph.D.)

....Thesis Co-Advisor

(Associate Professor Poj Kulvanich, Ph.D.)

....Examiner

(Narueporn Sutanthavibul, Ph.D.)

....Examiner

(Assistant Professor Vorasit Vongsutilers, Ph.D.)

....External Examiner

(Assistant Professor Chutima Wiranidchapong, Ph.D.)

อันนา สัณฐิติวณิชย์ : การสกัดและการศึกษาคุณลักษณะของเซอินจากกลูเทนข้าวโพดเพื่อใช้เป็นสารก่อฟิล์มในเปลือกแคปซูล (EXTRACTION AND CHARACTERIZATION OF ZEIN FROM CORN GLUTEN MEAL FOR USE AS A FILM FORMER IN CAPSULE SHELLS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: อ. ภญ. ดร.จิตติมา ชัชวาลย์สายสินธ์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: รศ. ภก. ดร.พจน์ กุลวานิช, 78 หน้า.

เซอินเป็นโปรตีนที่พบในข้าวโพดซึ่งมีการใช้เป็นสารก่อฟิล์ม สารนี้มีศักยภาพในการเตรียมเปลือกแคปซูลแข็งที่ไม่มีส่วนประกอบซึ่งมาจากสัตว์ วัตถุประสงค์ของการศึกษานี้ คือ เพื่อเตรียมเปลือกแคปซูลแข็งจากเซอินที่มีจำหน่ายในท้องตลาดและเซอินที่สกัดจากกลูเทนข้าวโพดซึ่งเป็นผลพลอยได้จากอุตสาหกรรมแปรรูปข้าวโพดในประเทศไทย โดยศึกษาสมบัติของเซอินสกัดเปรียบเทียบกับเซอินที่มีจำหน่ายในท้องตลาด จากนั้นเตรียมฟิล์มและเปลือกแคปซูลแข็งจากเซอินด้วยสารละลายที่มีเอธานอลร้อยละ 70 โดยปริมาตร และ ศึกษาสมบัติของฟิล์มและเปลือกแคปซูลแข็ง ผลการศึกษาพบว่า สามารถเตรียมเปลือกแคปซูลแข็งจากเซอินได้โดยวิธีการจุ่มในสารละลายเอธานอล ฟิล์มและเปลือกแคปซูลแข็งซึ่งเตรียมจากเซอินสกัดมีสมบัติเชิงกลและให้ลักษณะกราฟการละลายที่แตกต่างจากฟิล์มและเปลือกแคปซูลซึ่งเตรียมจากเซอินที่มีจำหน่ายในท้องตลาด ทั้งนี้เนื่องจากเซอินสกัดมีกรดอะมิโนที่ชอบน้ำมากกว่าซึ่งจะเข้ากันได้กับพลาสทิไซเซอร์ที่ชอบน้ำ ได้แก่ กลีเซอรีน ที่ใช้ในการศึกษานี้ และ พบว่าสูตรตำรับของฟิล์มและเปลือกแคปซูลแข็งต้องมีพลาสทิไซเซอร์เพื่อปรับสภาพยืดหยุ่นและกระบวนการเตรียมต้องการความชื้นสัมพัทธ์ที่เหมาะสม เช่น ความชื้นสัมพัทธ์ร้อยละ 52 เป็นต้น ดังนั้นการศึกษานี้แสดงให้เห็นถึงความเป็นไปได้ในการใช้เซอินเตรียมเปลือกแคปซูลแข็ง

ภาควิชา	วิทยาการเภสัชกรรมและเภสัชอุตสาหกรรม	ลายมือชื่อนิสิต	....
สาขาวิชา	เภสัชอุตสาหกรรม	ลายมือชื่อ อ.ที่ปรึกษาหลัก	....
ปีการศึกษา	2559	ลายมือชื่อ อ.ที่ปรึกษาร่วม	....

5576232733 : MAJOR INDUSTRIAL PHARMACY

KEYWORDS: ZEIN / CORN GLUTEN MEAL / HARD CAPSULE SHELL

UNNA SANTHITIWANICH: EXTRACTION AND CHARACTERIZATION OF ZEIN FROM CORN GLUTEN MEAL FOR USE AS A FILM FORMER IN CAPSULE SHELLS. ADVISOR: JITTIMA CHATCHAWALSAISIN, Ph.D., CO-ADVISOR: ASSOC. PROF. POJ KULVANICH, Ph.D., 78 pp.

Zein, a corn protein, have been used as a film former. It is a potential material for preparation of non-animal derived hard capsule shell. The objectives of this study were to prepare hard capsule shell from a commercial zein and zein extracted from corn gluten meal, a byproduct from corn processing industry in Thailand. The zein extract was characterized, comparing with the commercial zein. Zein films and hard capsule shells were prepared by using 70% v/v ethanol solution. Properties of the film and hard capsule shell were investigated. The results showed that hard capsule shells could be prepared from zein in ethanol solution by dipping method. The film and hard capsule shell made from zein extracted from corn gluten meal showed difference in mechanical properties and dissolution profile, comparing with those made from the commercial zein because it contained more hydrophilic amino acids which were miscible with the hydrophilic plasticizer, glycerol, used in the formulation. The formulation of zein film and hard capsule shell needed a plasticizer to provide elasticity and the preparation process required an optimum relative humidity such as 52% relative humidity in this study. The findings suggested possibility of utilizing zein in preparing hard capsule shell.

Department:	Pharmaceutics and	Student's Signature	....
	Industrial Pharmacy	Advisor's Signature	....
Field of Study:	Industrial Pharmacy	Co-Advisor's Signature	....
Academic Year:	2016		

ACKNOWLEDGEMENTS

CONTENTS

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AOAC	Association of Official Analytical Chemists
ASTM	American Society for Testing and Materials
CGM	Corn gluten meal
cm^{-1}	Reciprocal centimeter
CZ	Commercial zein
°C	Degree Celsius
DLS	Dynamic light scattering
DSC	Differential scanning calorimetry
e.g.	For example
et al.	And others
FTIR	Fourier transform infrared spectroscopy
g	Gram
h	Hour
i.e.	That is
kDa	Kilodalton
L	Liter
M	Molar
mA	Milliampere
mg	Milligram
min	Minute
mL	Milliliter
mm	Millimeter
n	Number
ND	Not detected
no.	Number
ppm	Parts per million
RH	Relative humidity
rpm	Revolutions per minute
s	Second

SD	Standard deviation
SDS	Sodium dodecyl sulfate
SDS-PAGE	Sodium dodecyl sulfate - polyacrylamide gel electrophoresis
SEM	Scanning electron microscope
T_d	Decomposition temperature
T_g	Glass transition temperature
TGA	Thermogravimetric analysis
TMDSC	Temperature modulated differential scanning calorimetry
USP	United States Pharmacopeia
V	Voltage
v/v	Volume by volume
WVP	Water vapor permeability
WVT	Water vapor transmission
w/w	Weight by weight
ZCGM	Zein extracted from corn gluten meal
%	Percentage

CHAPTER I

INTRODUCTION

Zein is a prolamin protein in corn. It can be commonly obtained from byproduct of corn processing using wet milling method. Zein is a non-polar amino acid rich protein (1). Therefore, it is poorly soluble in water but can be dissolved in alcohol, urea, alkali and anionic detergents (1). Zein is polypeptides that have many fractions which are different in solubility, molecular weight, primary structure, chromatographic and immunological properties (2, 3). These fractions are: α-zein (80-85% of total zein), β-zein (10% of total zein), γ-zein (10-15% of total zein), δ-zein (only little amount of total zein). A commercial zein is α-zein which is a hydrophobic fraction and can be extracted by 95% v/v ethanol or 90% isopropanol. The polarity of solvent used in the extraction can affect the yield and purity of α-zein. A more polar solvent i.e. 60% v/v ethanol can co-extract α-zein with more hydrophilic fraction of β-zein or more hydrophilic amino acids.

Zein has a film forming property and have been widely used as a film former in food and pharmaceutical areas (4-6). The zein film is generally brittle and requires addition of a plasticizer to improve film flexibility.

Hard capsule is a common solid dosage from in the market. Hard capsule shells are commercially made from gelatin due to its unique film forming property. However, gelatin has some limitations. It is an animal-derived material which is not favorable for some people. It is a moisture sensitive material and may have quality problem if the capsule was stored under extreme conditions. Also, it is difficult to modify the drug release by coating.

Many film forming materials have been investigated for capsule shell development. To date, commercial non-gelatin capsule shells are made from, for examples, cellulose derivatives (hypromellose), and polysaccharide (pullulan).

Corn is one of major crops in Thailand. The outputs of industrial processing give some valued byproduct e.g. corn gluten meal from wet milling method. Corn gluten meal is protein (zein) rich byproduct and widely used in animal feed. It is of interest to exploit this byproduct of corn processing industry in Thailand through zein extraction. Due to film forming property and hydrophobic character of zein, in this study, the material was applied to hard capsule shell preparation. The property of zein film and hard capsule shell which may be affected by sources of zein, extraction methods, film components and processing parameters were studied. Possibility of preparing of aqueous dispersion of zein was also investigated.

Objective of the study

1. To extract protein (zein) from corn gluten meal, a byproduct of corn processing industry

2. To investigate physicochemical properties and film forming ability of the corn protein

3. To prepare hard capsule shells from the corn protein and evaluate their physical properties

CHAPTER II

LITERATURE REVIEW

1. Zein and corn gluten meal

Corn (*Zea mays* L.) is a cereal grain plant in Gramineae family. Corn has many nutrient components e.g. starch, protein, oil and ash; therefore, it has significantly used to food, animal feed and industrial products (1).

The corn kernel consists of four parts, i.e. endosperm, germ, pericarp and tip and contains about 62% starch, 8-12% protein, and 4% oil. Starch is found mostly in endosperm which is the largest part of the kernel. Proteins are mainly found in endosperm and germ and oil is stored in germ (1, 7). About 75% of total corn proteins are found in endosperm (1).

Generally, grain proteins can be classified into four groups based on their solubility (7). These are albumins (water soluble protein), globulins (aqueous salt solution soluble protein), glutelins (dilute acid or base soluble protein) and prolamins (aqueous alcohol soluble protein) (7, 8). Zein is a prolamin protein which is found in the endosperm of corn (1). It consists of amino acids such as 27% of glutamic acid, 21% of leucine, 11% of proline and 11% of alanine, but does not contain tryptophane and lysine (1). Zein is poor soluble in water due to having high percentage of non-polar amino acid such as leucine, proline and alanine (1, 7). It dissolves in water containing alcohol, urea, alkali or anionic detergents (1).

Esen separated zein into four fractions: α-zein (21-25 or 24-27 kDa polypeptides) which constitutes 80-85% of total zein. β-zein (17 kDa polypeptides) which is methionine-rich polypeptides and constitutes 10% of total zein. γ-zein which is proline rich polypeptides and constitutes 10-15% of total zein. It can be separated to γ-zein1 (27 kDa polypeptides) and γ-zein2 (18 kDa polypeptides). There is also little fraction of δ-zein (10 kDa polypeptides) (2, 3). These fractions are separated based on solubility, molecular weight, primary structure, chromatographic and immunological properties (2, 3).

α-zein is soluble in 50-95% v/v isopropanol and insoluble in 30% isopropanol with 30 mM sodium ethanoate (acetate) at pH 6.0 (2). It is also soluble in 95% v/v ethanol (1). β-zein is soluble in 30-85% v/v isopropanol containing a reducing agent (2) and in 60% v/v ethanol (1). It is not soluble in 90% v/v isopropanol and 30% isopropanol with 30 mM sodium ethanoate at pH 6.0. γ-zein is soluble in 0-80% v/v isopropanol with a reducing agent. It also soluble in 30% isopropanol with 30 mM sodium ethanoate at pH 6.0. The δ-zein's solubility is the same as that of α-zein.

The two major types of zein are α-zein and β-zein. β-zein can be separated from α-zein by 90% isopropanol or 95% ethanol where it is insoluble. α-zein contains some amino acids such as histidine, arginine, proline and methionine less than β-zein, (1, 7). β-zein is quite unstable and tends to precipitate and coagulate. Hence, in commercial zein preparations, α-zein is more favorable (1).

A structural model of zein (molecular weight 19-22 kDa) was proposed by Argos et al. (9). It consists of nine homologous repeating units which are anti-parallel helices within a distort cylinder stabilized with hydrogen bonding of polar residues. It has glutamine rich turns locating between the helices and at the cylindrical caps (9). α-zein contains a high α-helix content (10). Zein shows a globular in non-aqueous solutions. Conformational change is observed when the ethanol concentration is reduce from 80 to 50% ethanol.

Corn can be processed by four methods: dry milling, alkaline processing, wet milling and the dry grind ethanol processing (1). Zein can be extracted from corn and byproduct of corn processing such as corn gluten meal (CGM) obtained from wet milling process, distillers dried grains with solubles (DDGS) from dry-grind ethanol process and defatted corn. However, byproduct which gives highest protein amount (about minimum of 60% dry weight) is CGM. For this reason, CGM is general material to produce commercial zein (CZ) (1, 7).

The production of zein has many factors involved in the process such as raw material, extracting solvent, purification and recovery method (1).

The common process employ polar solvent such as aqueous alcohol for extraction and nonpolar solvent for removal fats and color pigments (1). Extraction

process of zein affects yield, quality and purity. In industrial extraction high quality and purity of zein is compromised with low yield (7).

Zein is a material that is classified as Generally Recognized as Safe (GRAS) by the U.S. Food and Drug Administration. It has benefit properties such as biodegradable, edible and non-toxic (11) so that it has been widely used in food and pharmaceutical industry.

The zein monograph of USP 36 generally consists of identification, impurities, specific tests e.g. loss on drying and protein contents. Identification tests are colorimetry and electrophoresis for determine molecular weight. Impurity tests of zein are applied to inorganic impurities, i.e. residue on ignition and heavy metals, and organic impurities as hexane-soluble matter.

2.   Applications of zein

Proteins from corn have been utilized such as film former (4, 12-14), coating material (6, 15, 16) and drug delivery (5, 6, 17, 18) for food and pharmaceutical applications.

2.1   Film former

Film and coating was investigated and developed from various materials for example gelatin, casein, whey, soy, zein and wheat gluten (11).

Protein can form film because its structure consists of many amino acids that can be interacted and stabilized through electrostatic interactions, hydrogen bonding, van der Waals forces, covalent bonding and disulfide bridges (11). Moreover, the protein chains have various functional groups that can be site of interactions and can be modified for some properties (11).

Film can be generated by several procedures such as solvent casting, spraying and dipping but the preferable preparation method is solvent casting because it is simple, effective and inexpensive. This method form film by spreading film solution or dispersion onto a plate which is then dried under controlled conditions (11).

The three main components of protein-based films and coatings formulations are protein, plasticizer and solvent (11). The final film properties are affected by the properties of film components (intrinsic properties) and processing factors (extrinsic factors). The intrinsic properties which affect protein films are such as amino acid

compositions, crystallinity of the film components, hydrophobicity or hydrophilicity, surface charge, isoelectric point, molecular weight and protein shape. The extrinsic factors are the conditions of film preparation are such as temperature, drying condition, pH, ionic strength, relative humidity (RH) in preparing method and storage (11).

Zein is a good film former which has been used for film and coating (10). Characteristics of zein film is good appearance, glossy, hydrophobicity, antifungal, antibacterial and biodegradable properties (4, 19). The zein film has a barrier properties to moisture and oxygen (20). Therefore, zein coatings are used as moisture and oxygen barrier to preserve products. In pharmaceutical area, zein has beneficial applications such as carrier for drug delivery, film containing active ingredients and coating tablets (5, 18, 21, 22).

The disadvantage of zein is brittle (4). Zein film normally requires a plasticizer to improve functional properties. Glycerol is widely used to plasticize various material including zein for improving flexibility and toughness (23). Miscibility and compatibility of glycerol which the material can be observed through a reduction of glass transition temperature (T_g). Although water can act as a plasticizer for zein but often give negative effects on both mechanical and barrier properties (10). Plasticizers can interact with polymer chains, resulting in increasing space and allowing more movement between protein chains (24). Zhang et al. suggested an ideal plasticizer for zein film that it should be compatible with zein and able to reduce T_g, with no negative effect on barrier properties of film (10).

Zein having polar and non-polar amino acids is not soluble in water but soluble in the mixed solvent of water and an organic solvent (1). The solvent system for zein film preparation are such as aqueous ethanol, aqueous isopropanol and aqueous acetone are shown in Table 1. Several researchers preferred using aqueous ethanol as solvent for zein (4, 19). The aqueous ethanol which is used in zein dispersion preparation is in concentration range of 70-95% (Table 1).

Table 1 The solvent system used as zein film preparation from the previous studied

Solvent system	Solid concentration	Secondary polymer	Plasticizer base on polymer weight	Reference
70% w/v aqueous ethanol	10% w/v corn gluten	-	25% w/w glycerol	(13)
80% v/v aqueous ethanol	1 g zein in 10 mL aqueous ethanol	-	-	(25)
85% v/v aqueous ethanol	10% w/w zein	-	0, 10, 15, 20, 25 and 30% w/w glycerol	(4)
70-90% v/v aqueous ethanol	5 and 15% w/v zein	-	20 and 50% w/w Polyethylene glycol and glycerol	(20)
75, 80, 85, 90, 95% v/v aqueous ethanol	20 g zein in 100 mL aqueous ethanol	70 g Oleic acid	0, 10, 20 and 30% w/w glycerol	(19)
70% v/v aqueous acetone	1 g in 10 mL aqueous acetone	-	-	(25)
85% v/v aqueous isopropanol	10% w/w zein	-	0, 10, 15, 20, 25 and 30% w/w glycerol	(4)

Some researchers avoid using organic solvent by preparing aqueous dispersion of zein (15, 26). The advantage of aqueous dispersion is zein particles distributed in dispersion with low viscosity and therefore coating time is reduced (15).

The zein aqueous dispersion has been previously reported. It was prepared with low concentration of zein and usually used 70% aqueous ethanol for dissolved zein before mixing with water phase as shown in Table 2.

Table 2 Preparation of aqueous dispersion in previous studies

Aqueous dispersion	Solid content	Plasticizer base on polymer weight	Reference
70% aqueous ethanol mixed with same volume of water containing plasticizer and 10% w/w Tween 80 same volume	5% zein	10% w/w polyethylene glycol 400 or 30% w/w glycerol	(21)
70% aqueous ethanol mixed with same volume of water	5% zein	10 and 20% w/w Polyethylene glycol 400	(15)
55-90% aqueous ethanol 15 mL mixed with 40 mL of water	1 g zein in 55 mL		(27)

Characterization of material or film

The remaining water in film affects characteristics of film because water can act as plasticizer for zein. It interacts with protein chains and actually influence on protein structure which can be investigated by Fourier transform Infrared spectroscopy (FTIR) (4). Appearance and morphology can observe by the photographs and scanning electron microscope (SEM) micrographs (19). Hydrophilic or hydrophobic properties of films can be determined by characteristics of water absorption, moisture sorption and water vapor permeability.

Mechanical properties

Mechanical properties are investigated under an applied load. The material behaviors for example strength, hardness, flexibility, and fracture toughness can be determined (28).

Mechanical properties including tensile strength, elongation and Young's modulus or elasticity are the important properties of film. The tension test is used to evaluate mechanical properties by applying force to the sample (Figure 1).
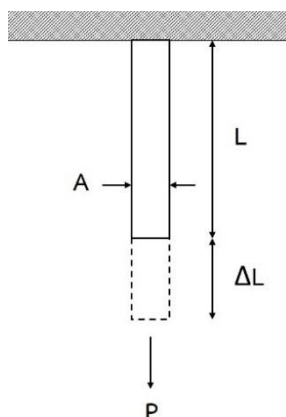
Figure 1 Tension test

Tensile strength (**σ**) is stress which force (P) applied to the cross-section area (A) of the sample. Elongation at break (e) is the strain in the sample, measured as percentage of changed length (ΔL) from initial length (L) on its breakage. Young's modulus is the ratio of stress to strain in the linear elastic region (29). The tensile strength is calculated by the following equation (28):

$$\sigma = \frac{P}{A}$$

where **σ** is tensile strength (Pa), P is force or load (N) and A is the cross-section area (m^2).

Differential scanning calorimetry (DSC)

The DSC is the thermal analysis technique used for measuring the difference in heat flow to the sample and reference at the same temperature by heating or cooling at a constant rate. This tool can detect the transition events such as melting and phase transition also chemical reaction as a function of temperature. DSC is mostly used in pharmaceutical and food industries for studying of material behavior (15, 30).

The glass transition occurs as an endothermic event corresponding to breaking of hydrogen bonds, disulfide bridges, hydrophobic interaction and protein unfolding (4). Therefore, T_g is one of properties of polymer or film demonstrating the polymer transition i.e. softening solid state to rubbery state.

Temperature modulated DSC (TMDSC) is the DSC technique based on stochastic temperature modulation superimposed to a conventional DSC temperature program by applying two simultaneous heating rates to the sample.

These are linear or average heating rate as standard DSC providing **total heat flow rate** and modulated (sinusoidal) heating rate determining the fraction of the total heat flow rate that responds to changing heating rate. This component of heat flow is caused by changes in heat capacity. When there is change in specific heat capacity, the fraction of the total heat flow is called **"reversing heat flow"** or the heat capacity component of the total heat flow. The fraction of total heat flow rate with does not respond to changing heating rate is called **"non-reversing heat flow"** and can be determined by subtracting the heat capacity component (reversing heat flow) which corresponds to specific heat capacity from the total heat flow (31).

Thermogravimetric analysis (TGA)

TGA is the thermal analysis technique determining the amount of weight changes of material with increasing temperature. This technique is used to investigate thermal behavior, for example, the moisture content in material, thermal stability, the onset decomposition temperature (T_d) and weight loss of decomposition (32).

Fourier transform Infrared spectroscopy (FTIR)

Infrared spectroscopic method is used to investigate chemical character in the region of wave number 4000-400 cm^{-1}. The chemical groups of material are vibrated corresponding to absorption of infrared radiation (33). The technique is used to characterize change in chemical character or determine existing interaction in the material.

2.2 Microparticle and nanoparticle

Zein is prepared in small colloidal particles and used as coating dispersion (34). Because of the hydrophobicity of zein, the colloidal particles can be prepared by changing the solubilizing ability of the solvent. By dilution the solvent with non-solvent, zein is precipitated to form particles as the result of solubility limitation in the non-solvent system (34). The major factors affecting the colloidal particles properties are the starting amount of zein and ethanol concentration. It was found that the particle sizes of zein was increased with higher amount of zein, while the particle sizes was smaller when using higher concentration of ethanol (27).

2.3   Other drug delivery system

Zein was used as a carrier for active pharmaceutical ingredients because of edible and safe material as well as its barrier ability and hydrophobicity.

Zein could form agglomerates by lowering its solubility and entrap a solute (18). Besides, zein film can incorporated with active ingredient such as salicylic acid and acetyl salicylic acid (5, 10) and bioactived from natural resource such as curcumin and green tea extract (6, 17).

3.   Hard capsule

Hard capsule is the solid dosage form extensively used in pharmaceuticals. The hard capsule shells consist of two parts called body and cap. While body is for containing active ingredient and excipients, cap is for enclosing the filled material in body. Normally, the materials filled into hard capsules are in either solid form including powder or granule or liquid form (35).

3.1   Hard gelatin capsule

Mostly commercial hard capsules are prepared from gelatin which is protein derived from collagen that found in animal bone and skin (36, 37). Gelatin has the property required for capsule shell preparation such as solubility, viscosity and reversible gelation by heating. Gelatin produces film with beneficial properties including clear, strong and flexible (37).

Gelatin film has some disadvantages. For example, the property of gelatin capsule shell can change if it is stored in different relative humidity (RH). Hard capsules require moisture content of 13-16% (37). When stored under low RH storage conditions, gelatin capsule shell become brittle (38). Also if gelatin capsule is filled with a hygroscopic material, it can change moisture content in the capsule shell. Gelatin has limited use in some group of people as for religious reasons and non-animal foods (37). Therefore, the materials from plants are an alternative film former for use as hard capsule preparation.

3.2   Alternative materials for hard capsule preparation

Many materials which have film forming ability are investigated for gelatin substitution in hard capsule shell. These material include cellulose derivatives (e.g. hydroxypropyl methylcellulose, hydroxypropyl cellulose), starch and modified starch

(e.g. potato, mungbean and waterchestnut starches), and proteins (e.g. zein from corn and gliadin from wheat) (37, 39, 40). The commercially available non-gelatin capsules are made from hydroxypropyl methylcellulose (Capsugel, Shionogi Qualicaps) and Pullulen (Capsugel).

CHAPTER III

MATERIALS AND METHODS

MATERIALS

Ammonium persulfate (Bio-Rad Laboratories, Inc., Hercules, California, USA)

Beta-mercaptoethanol (Bio-Rad Laboratories (Shanghai) Co., Ltd., Shanghai, China)

Bio-Safe™ Coomassie G-250 Stain (Bio-Rad Laboratories, Inc., Hercules, California, USA)

Calcium chloride anhydrous (Laboratory reagent, Ajax Finechem Pty. Ltd., New South Wales, Australia)

Corn gluten meal (Friendship Corn Starch Co., Ltd., Samut Prakan, Thailand)

Deionized water

Ethanol (95%, Pharmaceutical grade, Liquor Distillery Organization, Chachoengsao, Thailand)

Glycerol USP (99.5%, Srichand United Dispensary Co., Ltd., Bangkok, Thailand)

Glycine (Thermo Electron Lls India Pvt. Ltd., Mumbai, India)

Hexane (99.98%, American Chemical Society grade, Honeywell Burdick Jackson®, Ulsan, Korea)

Hydrochloric acid (36%, Analytical reagent, Ajax Finechem Pty. Ltd., New South Wales, Australia)

Isopropyl alcohol (commercial grade)

Laemmli sample buffer (2x, Bio-Rad Laboratories, Inc., Hercules, California, USA)

Magnesium nitrate hexahydrate ($Mg(NO_3)_2 \cdot 6H_2O$, Analytical grade, Ajax Finechem Pty. Ltd., New South Wales, Australia)

N,N'-methylene-bis-acrylamide solution (40%, Bio-Rad Laboratories (Shanghai) Co., Ltd., Shanghai, China)

Potassium chloride (KCl, Analytical reagent, Ajax Finechem Pty. Ltd., New South Wales, Australia)

Potassium dihydrogen orthophosphate (KH_2PO_4, Analytical reagent, Ajax Finechem Pty. Ltd., New South Wales, Australia)

Potassium hydroxide pellets GR (KOH, Analytical reagent, Loba Chemie Pvt. Ltd., Mumbai, India)

Potassium nitrate (KNO_3, Analytical reagent, Ajax Finechem Pty. Ltd., New South Wales, Australia)

Precision Plus Protein™ Dual Color Standards (Bio-Rad Laboratories, Inc., Hercules, California, USA)

Propranolol hydrochloride (99.6%, Jintan Pharmaceutical Factory, Jiangsu, China)

Sodium chloride (NaCl, Analytical reagent, Ajax Finechem Pty. Ltd., New South Wales, Australia)

Sodium dodecyl sulfate (Bio-Rad Laboratories, Inc., Tokyo, Japan)

Sodium hydroxide pellets (American Chemical Society grade, Carlo Erba Reagenti SpA, Rodano, Milan, Italy)

Sucrose ester (DUB SE11S, Stearinerie Duboris Fils, Ciron, France)

N,N,N',N'-tetramethylethylene-diamine (TEMED, Bio-Rad Laboratories, Inc., Hercules, California, USA)

Tris (hydroxymethyl) aminomethane (Bio-Rad Laboratories, Inc., Hercules, California, USA)

Tween 80 (S. Tong Chemicals Co., Ltd., Bangkok, Thailand)

Zein (≥87.5% purity, Lot no. N5JXD, Tokyo Chemical Industry Co., Ltd., Tokyo, Japan)

EQUIPMENT

Analytical balance (A200S, Sartorius, Goettingen, Germany)

Centrifugal vacuum concentrator and freeze dryer (Maxivac, Scanvac, Lynge, Denmark)

Centrifuge (Centrifuge 5810, Eppendorf, Hamberg, Germany)

Desiccator

Differential scanning calorimetry (PB822^e, Mettler Toledo, Schwarzenbach, Switzerland)

Differential scanning calorimetry (DSC1, Mettler Toledo, Schwarzenbach, Switzerland)

Digital caliper (150 mm/0.01 mm, China)

Digital orbital shaker (SHO-1D, Daihan Scientific Co., Ltd., Seoul, Korea)

Dissolution apparatus (VK7000, VanKel, New York City, USA)

Fourier transform infrared spectrometer (Nicolet iS10, Thermo Scientific, Wisconsin, USA)

Hot air oven (Memmert GmbH + Co. KG, Schwabach, Germany)

Magnetic stirrer (Multipoint 6, Variomag, H+P Labortechnik AG, Munich, Germany)

Moisture analyzer (HR83 Halogen Moisture Analyzer, Mettler Toledo, Columbus, USA)

Rotary evaporator (R-200, Büchi Labortechnik AG, Flawil, Switzerland)

Spray dryer (B-290, Büchi Labortechnik AG, Flawil, Switzerland)

Spray dryer (Niro Atomizer, Copenhagen, Denmark)

Teflon plate (B.U.T. Machine Tech Co., Ltd., Chonburi, Thailand)

Thermogravimetric analysis (SDTA851^e, Mettler Toledo, Schwarzenbach, Switzerland)

Universal testing machine (EZ-S 500 N, Shimadzu, Osaka, Japan)

UV spectrometer (UV-1800, Shimadzu, Tokyo, Japan)

Vacuum pump (General electric motor, Arthur H. Thomas Co., Philadelphia, USA)

Vertical electrophoresis (Mini-PROTEAN® Tetra Cell, Bio-Rad Laboratories, Inc., Hercules, California, USA)

Zetasizer (Nano-ZS, Malvern Instruments Ltd., Worcestershire, United Kingdom)

METHODS

## 1.	Preparation of zein from corn gluten meal and characterization

Liquid corn gluten meal (CGM) obtained by wet milling (Friendship Corn Starch Co., Ltd., Samut Prakan, Thailand) was spray dried using spray dryer (B-290, Büchi Labortechnik AG, Flawil, Switzerland; Niro Atomizer, Copenhagen, Denmark) at a spray rate of 20 g/min. The inlet and outlet temperatures were 110°C and approximately 70°C, respectively. The nozzle spray pressure was 2.5 bar. Loss on drying of the spray dried CGM was determined using hot air oven (Memmert GmbH + Co. KG, Schwabach, Germany). The CGM powder was kept in a glass bottle before zein extraction.

The extraction of zein from CGM powder was carried out by weighing 5 g of CGM powder and dispersing it in 45 g aqueous ethanol with varied concentrations i.e. 60, 70, 80, 90% v/v. The dispersion was swirled by digital orbital shaker (SHO-1D, Daihan Scientific Co., Ltd. Seoul, Korea) at 200 rpm for 18 h. Then, the dispersion was centrifuged at 3500 rpm for 30 min. Twenty grams of supernatant was taken. The sediment was mixed with the same solvent and all steps were repeated twice.

A total amount of 60 g supernatant was evaporated by rotary evaporator (R-200, Buchi, Flawil, Switzerland) at 40°C and dried by centrifugal vacuum concentrator and freeze dryer (Maxivac, Scanvac, Lynge, Denmark) at 1300 rpm for 24 h. The extracted zein was ground with liquid nitrogen to small particles by mortar and pestle.

The solvent system was selected based on yield of the extracted zein before grinding.

The extract was then characterized according to USP 36 monograph, and comparing with the commercial zein (≥87.5% purity, Lot no. N5JXD) which was purchased from Tokyo Chemical Industry Co., Ltd., Tokyo, Japan.

### 1.1	Identification of zein

Identification of zein was carried out by two USP 36 methods as the following:

1.1.1 Identification A by colorimetry

The sample solution prepared by dissolving 0.1 g of samples i.e. commercial zein (CZ), zein extracted from CGM (ZCGM) and CGM powder, in 10 mL of 0.1 N sodium hydroxide. The cupric sulfate test solution was prepared by dissolving 12.5 g of cupric sulfate in water to made 100 mL of solution. Few drops of cupric sulfate test solution was dripped into the sample solution, then the solution was heated in a water bath. The acceptance criteria is the sample solution developing purple color. The test is used for detection of protein peptide in the sample by complexing between Cu^{2+} and peptide.

1.1.2 Identification C by sodium dodecyl sulfate - polyacrylamide gel electrophoresis (SDS-PAGE)

The SDS-PAGE technique was carried out on a buffered system using modified method of USP 36. SDS-PAGE was operated in a Bio-Rad Mini-PROTEAN® Tetra Cell apparatus with 16% separating gel and 5% stacking gel. Briefly, 16% Tris-glycine gel was prepared from 1.5 M Tris-HCl pH 8.8, 10% sodium dodecyl sulfate (SDS), 40% acrylamide-bis, 10% ammonium persulfate and water. The 5% stacking gel was made from 1 M Tris HCl pH 6.8, 10% SDS, 40% acrylamide-bis, 10% ammonium persulfate and water. The CZ and ZCGM were dissolved in 55% isopropyl alcohol with 2% beta-mercaptoethanol to prepare a sample stock solution. The sample solutions were heated at 95°C for 10 min. The loading volume of sample was 5 µl in each well. The separation was operated at 100 V. After electrophoresis, the gel sheet was stained with Bio-Safe™ Coomassie Stain for protein band visualization. This test is used for detecting the molecular weight of proteins based on interaction between amine group of protein and sulfonic acid of dye, comparing with standard marker which was 10-250 kDa, in this study.

1.2 Impurities

1.2.1 Inorganic impurities; residue on ignition and heavy metals

Residue on ignition was analyzed by gravimetric method according to the method described in USP 36 at Food Research and Testing Laboratory, Faculty of Science Chulalongkorn University.

Heavy metals were analyzed according to modified method of AOAC (2012), 999.10. The analysis was carried out by Food Research and Testing Laboratory,

Faculty of Science Chulalongkorn University. The quantity of lead, cadmium, mercury and arsenic were determined.

1.2.2 Organic impurities (limit of hexane-soluble matter)

Organic impurities based on hexane-soluble substance were analyzed according to the method described by USP 36. The sample of 15 g was added into 150 mL of aqueous alcohol (17:3, w/w). The mixture was stirred and heated at 30°C to dissolve the solid. Then, the sample solution was transferred to a 500 mL separatory funnel and 60 mL of hexane was added into the funnel. The mixture was shaken well and allowed to separate into two phases. The bottom layer is an alcohol phase and the top layer is hexane phase. The hexane layer was collected in a weighed 500 mL round bottom flask. Sixty milliliters of hexane was added to the alcohol layer and the process of separation was repeated for 4 times.

After the total of 300 mL hexane solution in the round bottom flask was evaporated by rotary evaporator to remove hexane. The weight of flask containing the yellow to reddish oil was recorded. The hexane-soluble matter was calculated using the following equation. The acceptance criteria is not more than 12.5%.

$$Hexane\ soluble\ matter\ = \frac{(W_C - W_F)}{W_S} \times 100$$

where W_C is the weight (g) of the flask containing the oil, W_F is the weight (g) of the 500 mL flask and W_S is the weight (g) of the sample.

1.3 Loss on drying

The sample was dried at 105°C in a hot air oven for 2 h. An initial weight and the weight after drying were recorded. The weight loss was calculated. The acceptance criteria of USP 36 is not more than 8% of a weight.

1.4 Protein content

Protein content was analyzed by nitrogen determination. This was carried out by Kjeldahl method based on AOAC (2010), 991.20 at Food Research and Testing Laboratory, Faculty of Science Chulalongkorn University. The percentage of nitrogen obtained was multiplied by 6.25, resulting in protein content. The acceptance criteria of USP 36 is 81.9%-100% on the dried basis.

2.   Preparation of zein films

CGM powder which was obtained by spray drying two batches of liquid CGM were pooled and kept in a glass bottle placed in desiccator containing silica gel. Zein films were prepared with solvent and aqueous systems. CZ and the zein extracted from CGM (ZCGM) using aqueous ethanol concentration that gave highest solid yield were used for film preparation. An aqueous dispersion of CZ and ZCGM was prepared using the same aqueous ethanol concentration.

2.1   Solvent based film

Zein films were prepared using a procedure previously reported with modification (13, 25).

CZ was dispersed in 70% v/v aqueous ethanol to obtain dispersion with 20% solid content and the dispersion was mixed at 400 rpm for 30 min by magnetic stirrer at an ambient temperature. Then, it was centrifuged (Centrifuge 5810, Eppendorf, Hamberg, Germany) at 3500 rpm for 30 min to separate the insoluble solid. The amount of insoluble solid was used to calculate an actual CZ content in the dispersion. After that the CZ supernatant (19.2% solid content) was heated at 50°C for 10 min. After cooling down to an ambient temperature, the supernatant was subjected to vacuum for 10 min to remove air bubbles.

Subsequently, 5 g of the supernatant was poured onto a Teflon plate with 9 cm in diameter and heated at 50°C for 10 min in a hot air oven. After cooling down, the films were then placed in desiccators which contained $CaCl_2$ anhydrous (dried at 200°C) and saturated salt solutions of KOH, $Mg(NO_3)_2$, NaCl, and KNO_3 to obtain 0, 8, 52, 75, and 92% relative humidity (RH) for 3 days before test.

When the film was casted with plasticizer, glycerol was added at a level of 20% w/w based on the CZ content in supernatant before heating.

For the ZCGM film, ZCGM was extracted from the dispersion of 20% CGM in 70% v/v aqueous ethanol. Then it was centrifuged to separate insoluble solid and the steps were repeated as described above. The ZCGM film was prepared by pouring 14.1 g of the supernatant (6.8% solid content) which had equivalent ZCGM content to the CZ content in the 5 g of the CZ supernatant. The films were heated in hot air oven at 50°C for 30 min before storage in the 52% RH desiccator.

2.2   Aqueous based film

Prior to aqueous based film preparation, the aqueous dispersion of zein was prepared by modified method reported by Li et al. (21) and characterized for particle size and morphology.

Briefly, CZ supernatant and CGM supernatant containing ZCGM was diluted to solid content of 4.8 and 1.7% w/w, respectively. The supernatant was carefully added to the same weight of aqueous solution containing 20% w/w glycerol and varied amounts (10 and 50% w/w) of Tween 80. The weight of glycerol and Tween 80 was calculated based on the CZ or ZCGM contents. Then, the mixture was continuously stirred at 400 rpm for 1 h. The aqueous dispersion was also prepared by adding water phase into 70% v/v aqueous ethanol.

The aqueous based film was casted by pouring 20 g of aqueous dispersion onto Teflon plate and heated at 50°C for at least 3 h in hot air oven.

3.   Characterization of aqueous dispersion

3.1   Particle size distribution

Particle size distribution was measured by dynamic light scattering (DLS) using a Zetasizer (Nano-ZS, Malvern Instruments Ltd., Worcestershire, UK). Freshly prepared samples were diluted to 0.01 g/L with distilled water before measurement. The refractive index of the protein and the medium were 1.45 and 1.33, respectively (41, 42). All measurements were carried out at ambient temperature and the results were an average of three reading.

3.2   Appearance and morphology of zein particles

Appearance of aqueous dispersion was observed. The morphology was studied by scanning electron microscope (SEM) (JSM-6610LV, JEOL, Tokyo, Japan).

Before investigation, a drop of aqueous dispersion was dried on the stub and coated with gold by sputter coater (SCD 040, Balzers, Liechtenstein, Germany) at a condition of 15 mA for 3 min with an argon backfill at 0.05 mbar. Each sample was investigated under SEM at an acceleration voltage of 15 kV with magnifications of 5,000x.

4.   Film characterization

4.1   Appearance and morphology

Physical characters of films were observed. The surface and cross-section morphologies of film were investigated using SEM (JSM-6610LV, JEOL, Tokyo, Japan). Cross-section structure analyses were executed by immersing the sample into liquid nitrogen and breaking the frozen film. Before investigation, small pieces of the film were coated with gold by the same condition with aqueous dispersion. After that, each sample was placed under SEM at acceleration voltage of 15 kV, using different magnifications at 5,000x for films surface and at 3,000x for film cross-section.

4.2   Thickness

Thickness of the sample was determined by digital caliper. Each film was tested in 3 replicates. Ten-positions of each film was measured and the average value were reported.

4.3   Residual moisture and volatile substances

The residual moisture and volatile substances of powder and films were determined at 105°C using moisture analyzer (HR83 Halogen Moisture Analyzer, Mettler Toledo, USA). Films were cut to small size pieces approximately 2-3 mm before test. Each sample was analyzed in 10 replicates, and the average was reported.

4.4   Water solubility

Water solubility of film was determined by weight loss due to that film water soluble components was dissolved after immersion in water for 24 h. The method was modified from Pena-Serna et al. (19).

CZ and ZCGM films were cut into three 1×2 cm^2 pieces. Then, the sample was dried in hot air oven at 50°C for 24 h. After cooled down in a desiccator, the sample was weighed and the weight was recorded as W_i. Subsequently, the sample was soaked in distilled water at ambient temperature for 24 h. Then, it was taken from the water using forcep, and excess water was removed with filter paper. After that, the sample was then dried again at 50°C for 24 h. After cooled down, its weight was recorded as W_f. The result of each film was average from 3 replicates. The equation used is as follows (43):

$$Water\ solubility\ (\%) = \frac{(W_i - W_f)}{W_i} \times 100$$

4.5 Moisture sorption

CZ and ZCGM films were cut into 1×1 cm² pieces and equilibrated in 0% RH desiccator until the weight was constant (2 weeks). The weight was recorded as initial weight (W_i). Then the sample was stored in varied relative humidity desiccators i.e. 8, 52, 75 and 92% RH (44). The samples were weighed again after storage for 2 weeks (W_f). Moisture sorption was calculated as a percentage ratio of weight gain to initial weight. The result of each specimen was average from 3 replicates. The equation used is as follows:

$$Moisture\ sorption\ (\%) = \frac{W_f - W_i}{W_i} \times 100\%$$

4.6 Water vapor permeability

The water vapor permeability (WVP, g mm/h m² kPa) was determined by modified ASTM E96 method by measuring weight gain of the film that was placed in between two different humidity chambers. Briefly, the film was cut into a round shape with an approximately 20 mm in diameter. Then, it was attached on mouth of 10 mL glass bottle (12.2 mm air-gap) which contained saturated $Mg(NO_3)_2$ solution, i.e. 52% RH. The glass bottle was placed in the desiccator containing saturated KNO_3 solution, i.e. 92% RH. The glass bottle was taken and weighed at 1, 2, 3, 24, 48, 72, 96, 120, 144 and 168 h. The WVP of films was calculated as follows (13):

$$WVP = \frac{WVT}{S(R_1 - R_2)} \times L$$

where WVT (g/h m²) is rate of water vapor transmission; S is saturation vapor pressure at test temperature (3.567 kPa at approximately 27°C) (45); R_1 and R_2 are RH in the desiccator and in the glass bottle express as a fraction, respectively; L is thickness of film (mm) and calculated according to

$$WVT = \frac{G}{t \times A}$$

where G is a weight increase (g) of glass bottle after time t; t is measuring time (h); A is measuring area (1.2×10^{-4} m^2); G/t is slope of the straight line (g/h).

4.7 Mechanical properties

The film was cut into rectangular shape with 10 mm x 50-60 mm width x length. The mechanical strength, in terms of tensile strength, elongation and elastic of films were determined using a Universal Testing Machine (Model EZ-S 500 N, Shimadzu, Osaka, Japan) using a load cell of 500 N. The pulling rate was 5 mm/min. An initial separation of 35 mm was used. For each film, the measurement was carried out in ten replicates and an average was reported.

4.8 Differential scanning calorimetry (DSC)

Glass transition temperatures (T_g) of CZ and ZCGM powder were measured using DSC (PB822e, Mettler Toledo, Schwarzenbach, Switzerland). An approximately 3-4 mg of powder was weighed into an aluminum pan and sealed hermetically. The sample was heated from 0 to 300°C at a heating rate of 10°C/min and nitrogen gas purge of 30 mL/min.

T_g of films was determined by Temperature modulated differential scanning calorimetry (TMDSC) (DSC1, Mettler Toledo, Schwarzenbach, Switzerland). The film of approximately 1-7 mg was placed in aluminum pan and sealed hermetically. The experiment was carried out by heating the sample from -30 to 180°C at a heating rate of 2°C/min. The period and the amplitude of modulation were 60 s and 0.5°C, respectively.

4.9 Thermogravimetric analysis (TGA)

The thermal property of powder and films were measured by TGA (SDTA851[e], Mettler Toledo, Schwarzenbach, Switzerland). TGA was used to find the decomposition temperature (T_d). The sample was heated from 25 to 800°C at the rate of 10°C/min and nitrogen gas purge of 30 mL/min. The measurement was carried out in two replicates were measured.

4.10 Fourier transform Infrared spectroscopy (FTIR)

FTIR spectra of CZ, ZCGM, glycerol also films were examined with a Fourier transform infrared spectrometer (Nicolet iS10, Thermo Scientific, Wisconsin, USA). Each sample was measured with 32 scans at 4 cm^{-1} resolution. The sample was placed on the window of ATR sampling accessory and scanned in a range of 4000-600 cm^{-1} at ambient temperature (4). Five measurements of FTIR were carried out for each sample.

5. Hard capsule preparation

Capsule shells made by dipping cap and body pins of capsule No. 0, into CZ and CGM supernatant containing ZCGM.

Initially, the pin was lubricated with sucrose ester (DUB SE11S, Stearinerie Dubois, Boulogne Billancourt, France) before dipping into the supernatants containing the same solid content (19.2% in CZ supernatant and 6.8% in CGM supernatant) and glycerol as described for film preparation (section 2.1). Then, it was lifted up from the supernatant and immediately turned round half cycle to avoid the liquid from dripping. After that, the pin was heated at 50℃ for 10 min in a hot air oven. The dipping process was repeated 3 and 8 times for CZ and ZCGM, respectively, to obtain the same thickness. The length of the cap (10.8 mm) and body (18.5 mm) was trimmed to the same length of commercially available gelatin capsules.

The dried capsule shell was allowed to dry at ambient temperature in 52% RH controlled environment chamber. Finally, the capsule shell was peeled off.

6. Characterization of hard capsule shells

6.1 Appearance, weight and thickness

Appearance of the capsule shell was visually observed and morphology was investigated using SEM as described in section 4.1 at magnification of 3,000x and 5,000x. Ten capsules shells i.e. cap and body were weighed. Thickness of 5 pairs of cap and body were determined by digital caliper. For each cap and body, the measurement was done for 5 points on capsule shell. The average values and standard derivation were calculated.

6.2 Residual moisture and volatile substances

The residual moisture and volatile substances of the capsules was determined at 105°C using moisture analyzer. Capsules shells were cut to small pieces of approximately 3x3 mm^2 before test. Each sample was analyzed in 5 replicates; and the average values and standard derivation were reported.

6.3 Mechanical properties

The mechanical properties of capsule were measured using universal testing machine in compression mode. The capsule was horizontally placed on the plate and a cylindrical probe of 30 mm diameter was moved down until contact to the capsule. The loading rate was 5 mm/min. The compression force was set upto 75% of the capsule external diameter. Five capsules were tested.

6.4 Dissolution of hard capsule

Forty milligrams of propranolol hydrochloride, was filled into capsule. The dissolution of hard capsule was performed using VK7000 (VanKel, NC, USA) apparatus I (basket) in 37°C, 900 mL of hydrochloric acid buffer solution pH 1.2 for 1.5 h and subsequently in 900 mL of phosphate buffer solution pH 6.8 for further 6.5 h. The basket was rotated at 100 rpm. Ten milliliter of dissolution medium was taken at 0.25, 0.5, 1.5, 2, 3, 5 and 8 h and analyzed using UV spectrometer (UV-1800, Shimadzu, Tokyo, Japan) at the maximum wavelength of 289 nm.

CHAPTER IV
RESULTS AND DISCUSSION

1.   Characterization of zein

The spray dried CGM appeared as yellow and fine powder (Figure 2a). The yield of CGM powder was about 10-14% of the CGM liquid obtained from the company. Loss on drying of CGM powder varied between 3.9-6.3%, as shown in Table 3. The protein in spray dried CGM was identified using colorimetry which is USP 36 identification method A and quantified by Kjeldahl method. The protein content in spray dried CGM of each batch was approximately 70% of CGM powder as shown in Table 3.

Table 3 Yield, loss on drying and protein content of spray dried CGM

Batch no.	CGM liquid (kg)	CGM powder (kg)	Yield (%)	Loss on drying (%, mean, n=3)	Protein content (calculated on a dry basis, %, mean, n=2)
150113	1.80	0.18	10.0	6.27	70.69
280813	8.57	1.18	13.7	3.85	70.01
241214	19.58	2.41	12.3	4.84	68.85

a   b
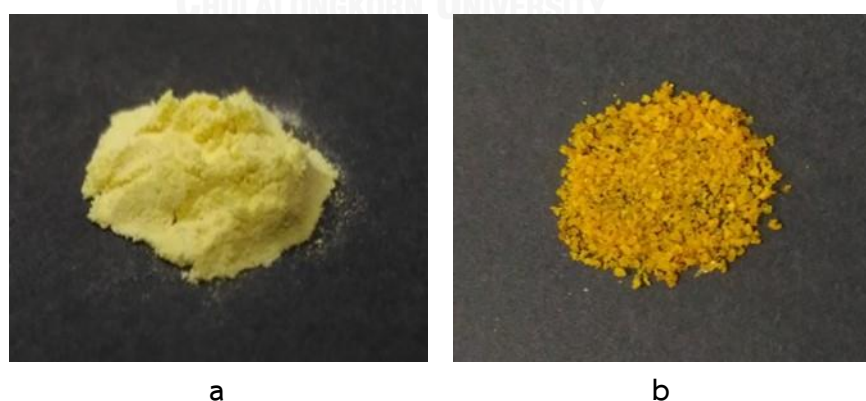
Figure 2 Photographs of CGM powder (a) and the zein extracted from CGM using 70% aqueous ethanol (b)

The yield of protein extracted from CGM was varied between batches and the solvent used in extraction as shown in Table 4. Overall a high yield (19-20% w/w) of ZCGM was obtained from 70-80% v/v aqueous ethanol. The concentration of 70% v/v aqueous ethanol was selected as the solvent for zein extraction as well as for film and capsule preparations due to more environmentally-friendly processing.

Table 4 Yield (% w/w) of the zein extracted from CGM powder with varied concentrations of aqueous ethanol; only two batches were studied

Aqueous ethanol (% v/v)	60	70	80	90
Batch no. 280813	12.07	23.27	20.03	14.22
	15.60	22.00	19.89	13.99
	17.30	26.23	20.44	13.17
	11.54	19.08	21.41	11.28
	14.53	22.58	20.20	11.68
	14.38	23.13	21.38	11.62
Batch no. 241214	17.69	16.70	19.16	10.33
	19.35	13.87	19.66	10.71
	19.92	21.67	18.76	10.65
	18.14	17.50	15.96	9.93
	17.38	16.08	17.61	9.76
	18.64	16.83	16.94	9.32
mean	16.38	19.91	19.29	11.39
SD	2.63	3.58	1.63	1.56

The protein extracted from CGM which was obtained in 60, 70 and 80% v/v of aqueous ethanol were red-yellow, dense solid aggregates (Figure 2b). The extract of 90 and 95% v/v aqueous ethanol could not be completely dried and it occurred as an orange colored, viscous liquid with little sediment. This could be oil or fat remaining in CGM.

The protein extracted from CGM were characterized according to zein monograph of USP 36 as shown in Table 5. The extract complied with zein's USP

specification and it qualities were similar to the commercial zein (CZ).   The acceptance criteria of identification C (SDS-PAGE) in USP 36 is that there are two major bands i.e. α-band at 21-25 kDa and β-band at 17-18 kDa, whereas USP 39 specification states that two major bands of 15-26 kDa for α-zein (Figure 3).

Table 5 Characteristics of commercial zein (CZ), zein extracted from CGM using 70% aqueous ethanol

Tests	USP 36 Specifications	CZ	Extract from CGM batch no.		
			150113	280813	241214
Identification A (colorimetry)	Purple color develops	yes	yes	yes	yes
Identification C (SDS-PAGE)	Two major bands α: 21-25 kDa, β: 17-18 kDa	yes	yes	yes	yes
Residue on ignition	NMT 2.0% 800±25℃	0.66%	-	-	1.22%
Heavy metal	NMT 20 ppm	< 1.03 ppm	-	-	< 1.09 ppm
Organic (limit of hexane soluble matter)	NMT 12.5%	6.07%	-	-	6.67%
Loss on drying	NMT 8.0%	1.29% COA[*]: 0.2%	5.43%	6.04%	5.53%
Protein content (calculated on a dry basis)	81.9-100%	91.32% COA[*]: 87.50%	84.64%	87.54%	84.93%

[*]COA, Certification of analysis of CZ (Tokyo Chemical Industry Co., Ltd.)

Figure 3 SDS-PAGE result; standard marker (a); commercial zein (b); zein extracted from CGM batch no. 150113 (c), 280813 (d), 241214 (e, g) using 70% v/v aqueous ethanol; zein extracted from CGM batch no. 241214 using 60% v/v (f) and 80% v/v (h) aqueous ethanol

It has been known that commercial extraction method gave more purity of α-zein when using higher alcohol concentration such as 95% ethanol or 88% isopropyl alcohol (1, 8).

In this study, extraction using the lower amount of alcohol in may extract other all zein fractions from CGM resulting in co-extraction of α-zein with β-zein or more polar amino acids which are soluble in approximately 60% ethanol (1). β-zein is easily precipitated and coagulated, hence it is unstable in solutions comparing with α-zein. Therefore, although the aqueous ethanol concentration selected in this study could provide an increased yield, the zein extracted from CGM (ZCGM) may be relatively unstable comparing with CZ.

2.   Preparation of zein films and characterization

After centrifugation, the CZ and ZCGM content in the solvent system was found to be 19.2 and 6.8% w/w, respectively due to removal of insoluble solid in 70% aqueous ethanol.

2.1   Appearance and morphology

2.1.1   Film casted by solvent system

The CZ and ZCGM films could be prepared with solvent system using 70% v/v aqueous ethanol. Similar appearance of smooth surface was observed for CZ film when equilibrated at various relative humidity; an example is shown in Figure 4.

| a | b |

Figure 4 Photographs of CZ film; films prepared without glycerol equilibrated at 52% RH (a), and with glycerol equilibrated at 52% RH (b)
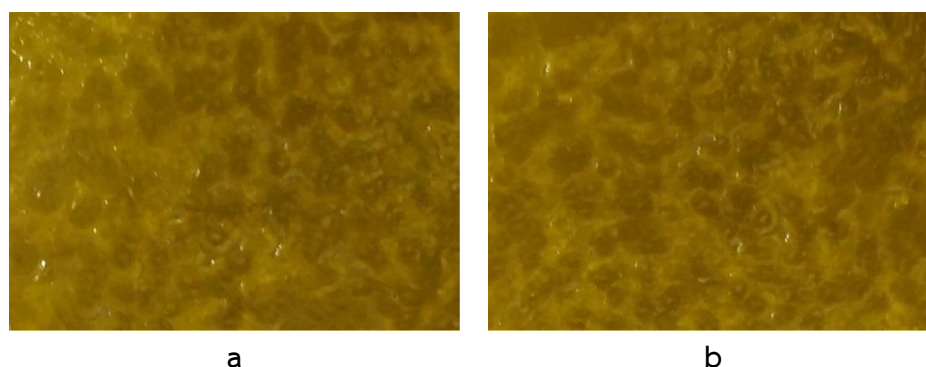

| a | b |

Figure 5 Photographs of ZCGM films without glycerol (a) and ZCGM films with glycerol (b) equilibrated at 52% RH

CZ films prepared without glycerol were more brittle when it was equilibrated at the low RH (0 and 8%RH). Flexibility of these films were slightly improved under relatively high RH but the appearance was more opaque at 92% RH.

CZ films prepared with glycerol were opaque comparing with the films without glycerol, particularly at high RH. The surface of 92% RH equilibrated film was tacky, perhaps owing to excess water in the film dried slowly under high RH conditions. Overall results showed that the equilibrating RH at 52% provided the film with desired appearance. Therefore, this RH was employed to equilibrate ZCGM films for comparison.

The ZCGM films without/with glycerol were yellowish and had rough and discontinuous surface (Figure 5). This may be that at the same solid content, the formulation of ZCGM films took longer time to evaporate and this allowed protein to precipitate and coagulate, instead of forming network structure. Yoshino et al. (46) reported surface microstructure of zein film depends on the rate of evaporation, especially humidity during drying.

SEM micrographs of surface and cross section of CZ films are shown in Figure 6 and Figure 7. It is evident that addition of glycerol exhibited a significant homogeneous especially in the films equilibrated at 52% and 75% RH. The SEM micrographs of CZ film without glycerol equilibrated at low RH showed cracks and cavity on the surface and pores when the film was equilibrated at high RH conditions. The cracks on the film surface was diminished with increasing relative humidity and added glycerol. Glycerol molecules could interpose themselves and introduce space between protein chains (20). This results in reduced attractive force between protein chains and allowed them to form meshwork easier.

White particles on the film surface was believed to be some constituents becoming insoluble and precipitate excluded from the film during evaporation (Figure 6j) (19, 20).

The morphologies of cross-sectioned CZ films display different film structure equilibrated at varied RH. Film's porous structure was obvious at the higher RH especially at the basal side. The similar result was previously reported by Yoshino (25). It is probable that at higher RH, a slow rate of solvent evaporation allowed

protein to move and form more adhesive network structure at the air side of the film.

The SEM micrographs of ZCGM films surface are shown in Figure 8. The films equilibrated at 52% RH showed heterogenous surface with some particle and cavity. Whereas films of cross-section exhibited denser structure with little porous (Figure 9).

Figure 6 SEM micrographs of CZ film surface at the magnification of 5,000x; films prepared without glycerol equilibrated at 0% RH (a), 8% RH (c), 52% RH (e), 75% RH (g) and 92% RH (i) and with glycerol equilibrated at 0% RH (b), 8% RH (d), 52% RH (f), 75% RH (h) and 92% RH (j)
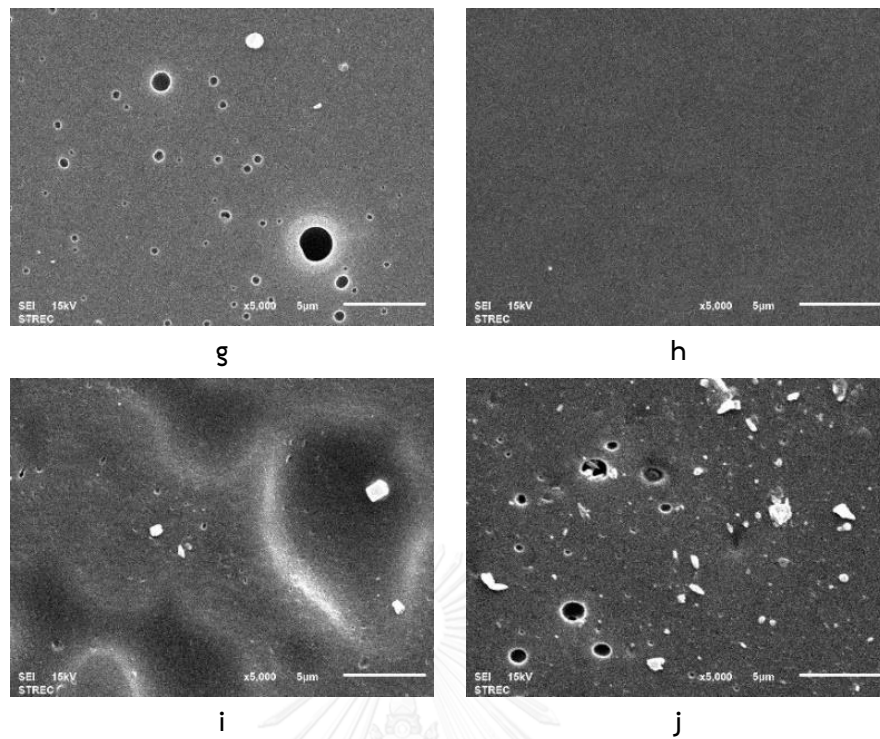
Figure 6 (continue) SEM micrographs of CZ film surface at the magnification of 5,000x; films prepared without glycerol equilibrated at 0% RH (a), 8% RH (c), 52% RH (e), 75% RH (g) and 92% RH (i) and with glycerol equilibrated at 0% RH (b), 8% RH (d), 52% RH (f), 75% RH (h) and 92% RH (j)
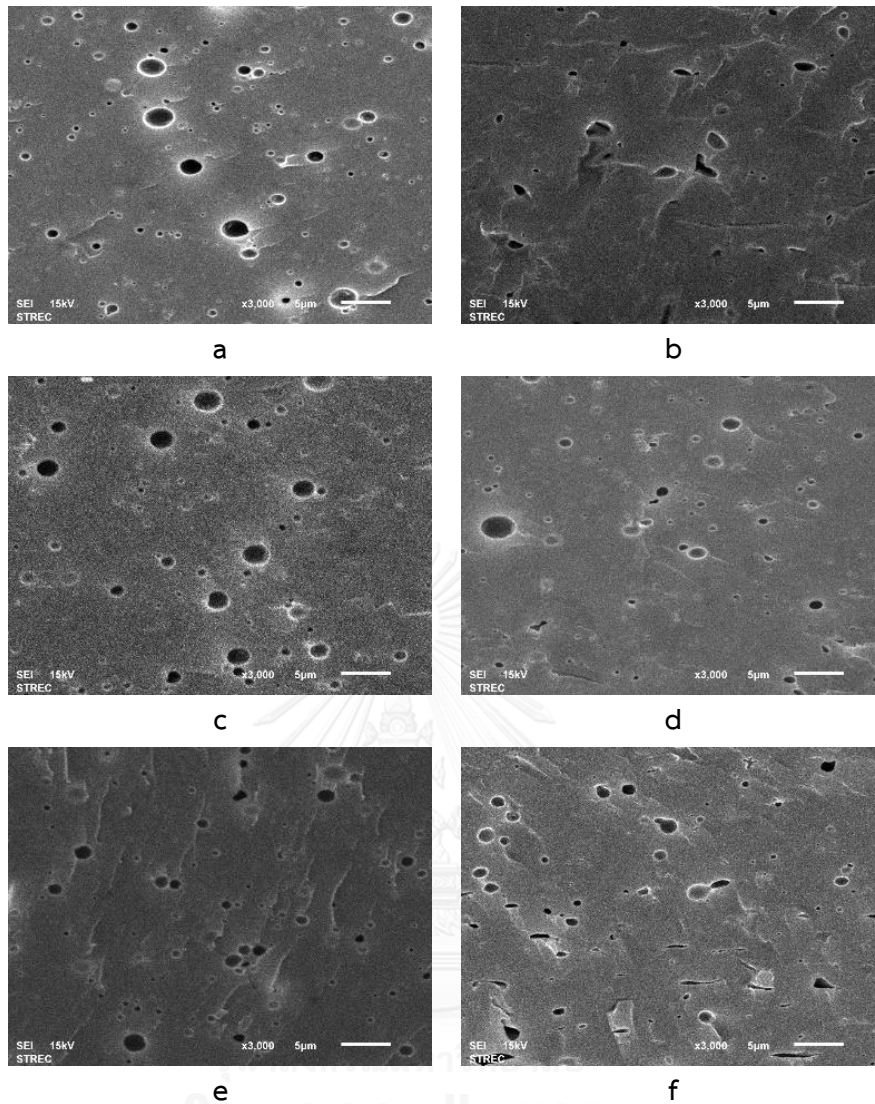
Figure 7 SEM micrographs of CZ film cross-section at the magnification of 3,000x; films prepared without glycerol equilibrated at 0% RH (a), 8% RH (c), 52% RH (e), 75% RH (g) and 92% RH (i) and with glycerol equilibrated at 0% RH (b), 8% RH (d), 52% RH (f), 75% RH (h) and 92% RH (j)
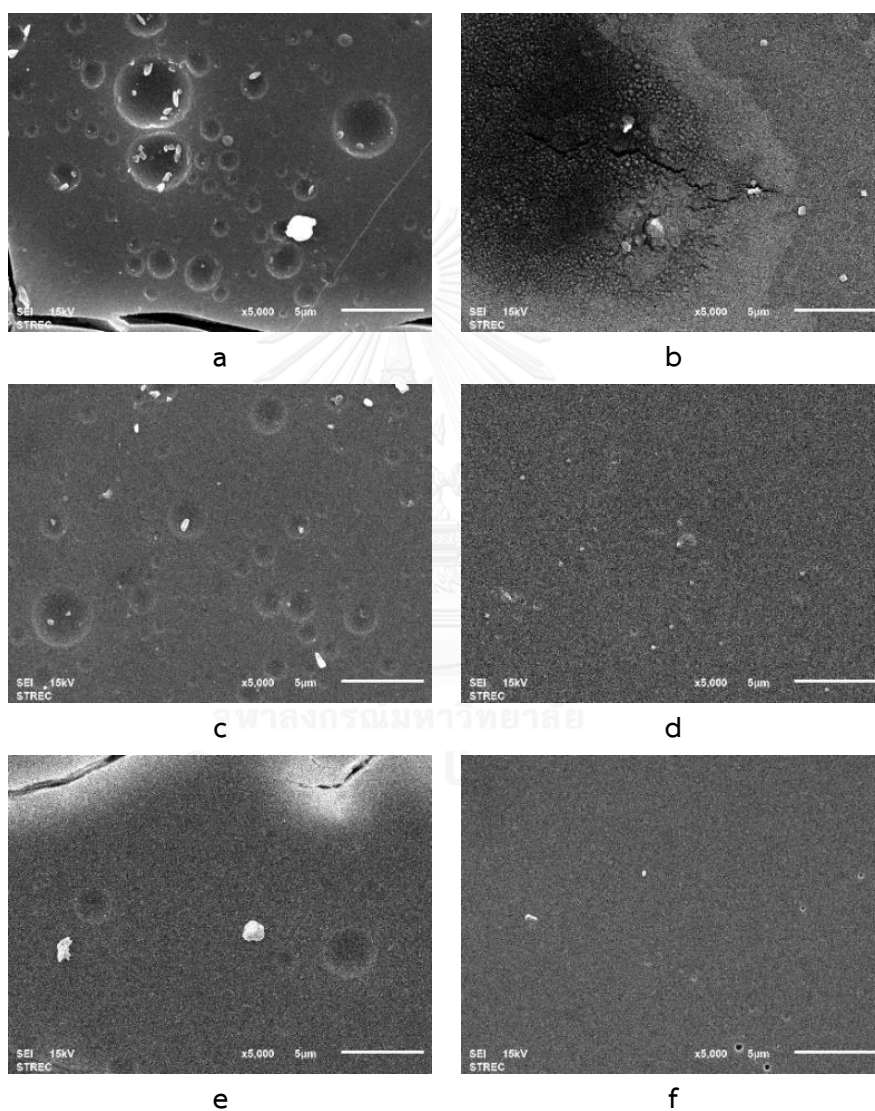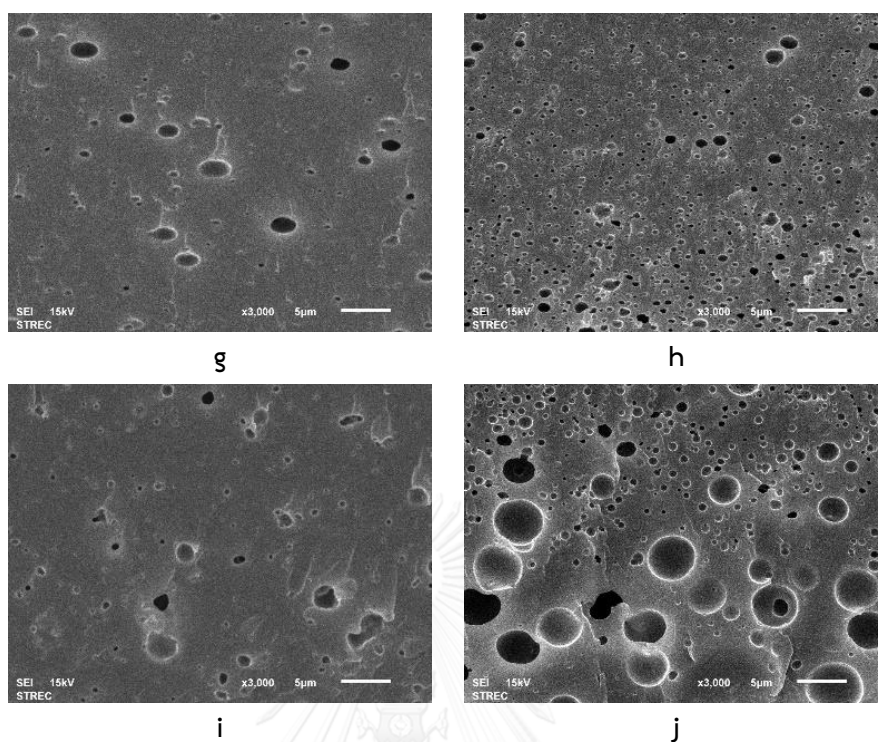
Figure 7 (continue) SEM micrographs of CZ film cross-section at the magnification of 3,000x; films prepared without glycerol equilibrated at 0% RH (a), 8% RH (c), 52% RH (e), 75% RH (g) and 92% RH (i) and with glycerol equilibrated at 0% RH (b), 8% RH (d), 52% RH (f), 75% RH (h) and 92% RH (j)
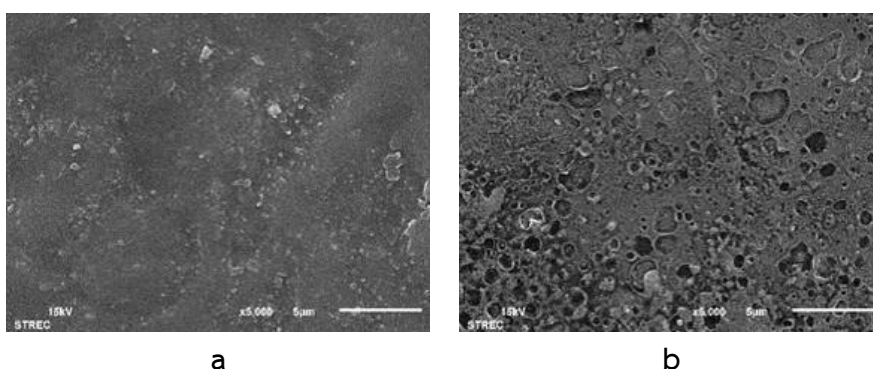
Figure 8 SEM micrographs of ZCGM film surface at the magnification of 5,000x; films prepared without (a) and with glycerol (b) equilibrated at 52% RH.
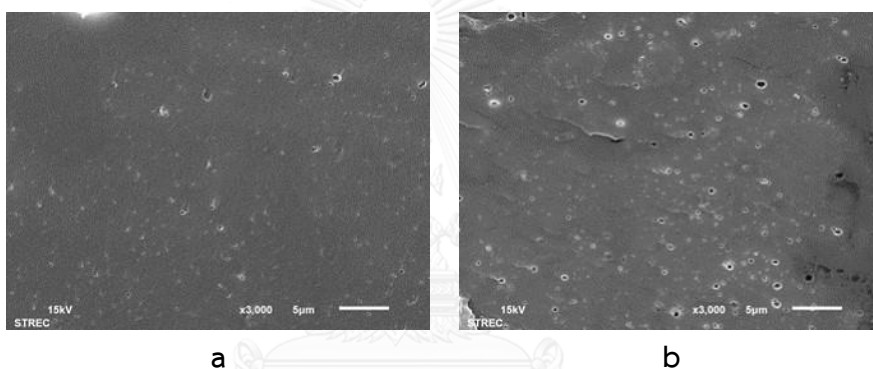
Figure 9 SEM micrographs of ZCGM film cross-section at the magnification of 3,000x; films prepared without (a) and with glycerol (b) equilibrated at 52% RH.

2.1.2 Film casted by aqueous dispersion

For CZ aqueous dispersion, it could be obtained by either adding 70% v/v ethanol phase of CZ into the water phase (Figure 10a-b) or the water phase into 70% v/v ethanol phase (Figure 10c-d). The dispersion obtained by adding 70% v/v ethanol phase of CZ into the water phase was cloudier and more stable dispersion (Figure 10).

For ZCGM, the aqueous dispersion could not be formed perhaps due to more polar amino acids in the preparation which were more favorable to aqueous medium.

The particle size of CZ in the aqueous dispersion are shown in Table 6. Adding aqueous ethanol phase into water phase gave relatively bigger size and narrower size

distribution of CZ particles. The particle size of this dispersion was more homogeneous around 1,000 nm with little amount of small aggregates in the tails (Figure 26-27 in Appendix). The dispersions obtained by adding water phase to aqueous ethanol phase showed wide distribution of particle size (Figure 28 in Appendix). The size of CZ particles was dependent on the amount of Tween 80 in the formulation as shown in Table 6. More Tween 80 in the formulation gave bigger size of CZ particles and hardly reproducible (Figure 29 in Appendix). Solubilization of zein in alcoholic dispersion was rapidly decreased when it was added into water phase. The protein chains were then packed into particles and stabilized by the surfactant in the dispersion.

The SEM images show that these particles coalesced to form a layer of film upon drying during sample preparation (Figure 11a-b). The SEM results corresponded to the particle size determined by dynamic light scattering.

The only few CZ particles were obtained by adding water phase into 70% v/v ethanol phase (Figure 11).
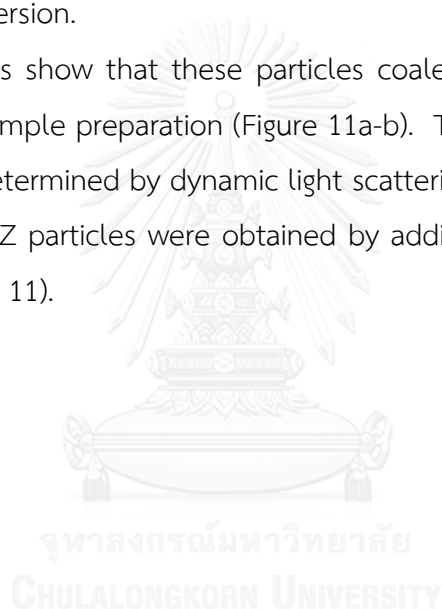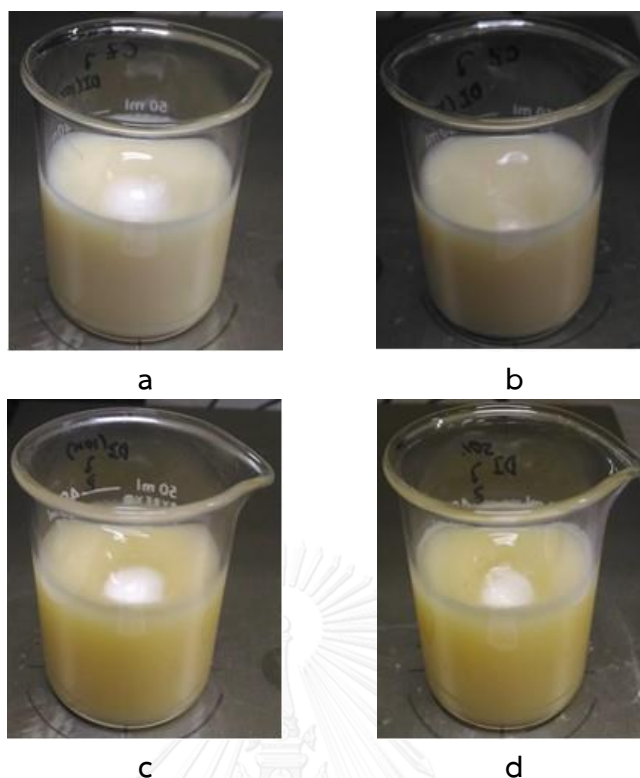
Figure 10 Photographs of CZ aqueous dispersions prepared by adding 70% v/v ethanol phase into water phase with Tween 80 of 10% w/w (a) and 50% w/w (b); the dispersions prepared by adding water phase with Tween 80 of 10% w/w (c) and 50% w/w (d) into 70% v/v ethanol phase.

Table 6 The particle size of CZ in the aqueous dispersions

	Adding 70% v/v ethanol phase into water phase		Adding water phase into 70% v/v ethanol phase	
	Z-average (nm)	PdI	Z-average (nm)	PdI
10% of Tween 80	922.2	0.4	590.8	0.7
50% of Tween 80	1,095.3	0.3	792.2	0.8

*PdI: Polydispersity index
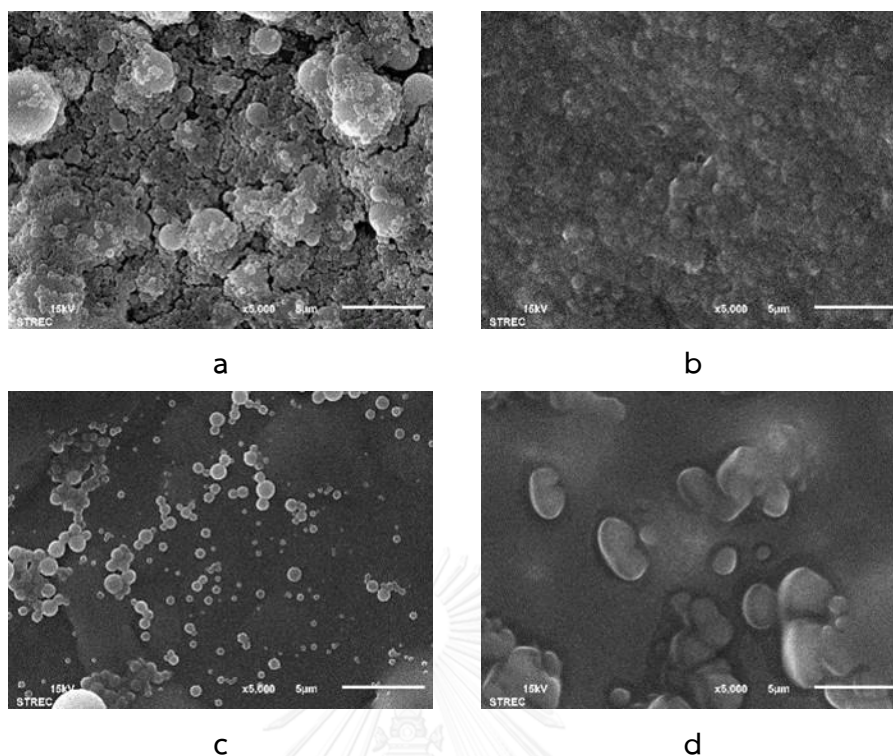
Figure 11 SEM micrographs of dried CZ aqueous dispersions at magnification of 5,000x; the dispersions prepared by adding 70% v/v ethanol phase into water phase with Tween 80 of 10% w/w (a) and 50% w/w (b); the dispersions prepared by adding water phase with Tween 80 of 10% w/w (c) and 50% w/w (d) into 70% v/v ethanol phase.

The film casted from aqueous dispersion could not be peeled off from the Teflon plate due to insufficient solid content and thin film was formed. Moreover, the film containing Tween 80 of 50% w/w had excess liquid on the film surface (Figure 30 in Appendix). Accordingly, the aqueous dispersions of CZ and ZCGM were not further used for film preparation.

2.2 Thickness

The thickness of CZ and ZCGM films are shown in Table 7. The thickness of CZ films was not much different between films formed without or with glycerol. They were likely to increase with increasing storage RH and with presence of glycerol.

The thickness of ZCGM films were more than CZ film equilibrated at the same RH (52%) because the ZCGM film was not continuously formed as observed in Figure 5.

Table 7 Thickness (mm) of films with/without added glycerol in different relative humidity conditions; data shown are mean (SD), n=3

RH (%)	Films without glycerol	Films with glycerol
0% RH CZ film	0.14 (0.01)	0.15 (0.02)
8% RH CZ film	0.14 (0.02)	0.16 (0.02)
52% RH CZ film	0.15 (0.02)	0.15 (0.01)
75% RH CZ film	0.17 (0.01)	0.18 (0.02)
92% RH CZ film	0.18 (0.02)	0.20 (0.02)
52% RH ZCGM film	0.24 (0.02)	0.25 (0.02)

2.3   Residual moisture and volatile substances

Residual moisture and volatile substances in the zein films formed without and with glycerol are shown in Figure 12. Generally, the storage humidity could affect the content of residual moisture and volatile substances in the films, ranging between 3.7% of CZ film prepared without glycerol at 0% RH to 23.4% of CZ film prepared with glycerol at 92% RH, respectively. The residual moisture and volatile substances of films was increased with increasing storage RH. The residual moisture and volatile substances of zein films prepared with glycerol were likely to be higher than that prepared without the plasticizer but the difference was insignificant.

Glycerol is a polyol material, which is soluble in water and hygroscopic (23). It could adsorb more water into the film. The results agreed with the work carried out by Lawton (47) indicating that the zein film containing glycerol had higher moisture content comparing with the film without glycerol.
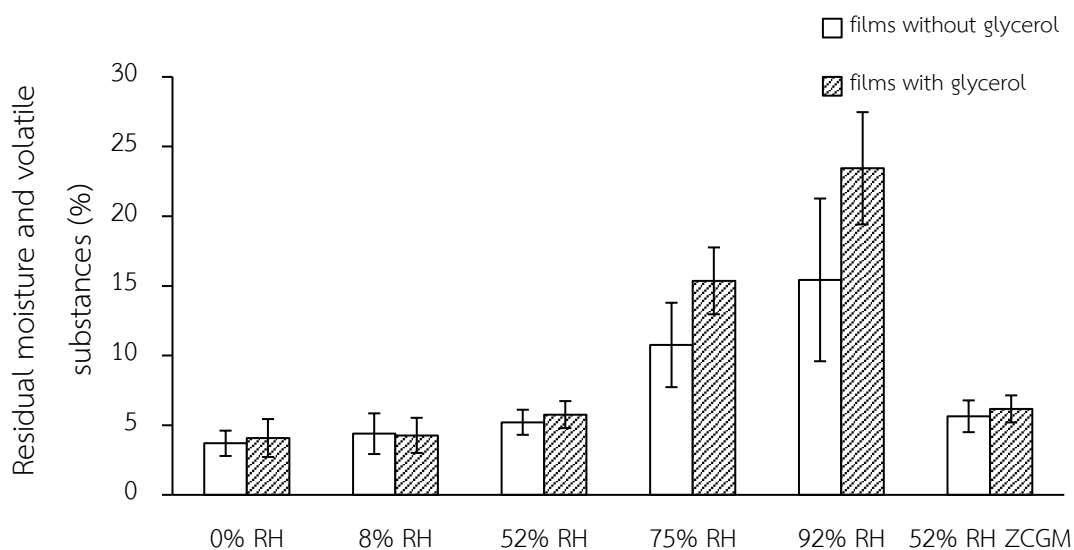
Figure 12 Residual moisture and volatile substances of CZ and ZCGM films equilibrated at varied relative humidity levels; film prepared without glycerol (white) and with glycerol (grid)

2.4   Water solubility

Water solubility of CZ films prepared without and with glycerol are shown in Figure 13.   The equilibrating condition and addition of plasticizer could affect the water solubility of the films. The film prepared without glycerol was more soluble when it was equilibrated at low RH (0, 8, 52% RH) and less soluble when it was equilibrated at the relatively high humidity (75 and 92% RH).

After the film was immersed in water for 24 h, weight loss of the films prepared with glycerol were higher than films without glycerol.   However, the weight loss which was about 20% of initial weight was mainly due to presence of 20% glycerol based on solid film, i.e. 16.7% in the film (Table 18 in Appendix). Glycerol has three hydrophilic hydroxyl groups providing hydrophilic nature. It could be dissolved from the film easily. Consequently, the CZ films prepared with glycerol were less soluble in water, i.e. only approximately 4% of zein was dissolved in water at 24 h. It was possible that the meshwork structure of these films were well established due to plasticizing effect of sufficient moisture and glycerol.

The water solubility of films associated with hydrophilic and hydrophobic properties (4). However, difference between the solubility of ZCGM film which contained more polar amino acids and CZ film was not observed.

Figure 13 Water solubility (%) of CZ and ZCGM films equilibrated at varied relative humidity levels prepared without glycerol (white) and with glycerol (grid)

2.5   Moisture sorption

The moisture sorption of zein films equilibrated at various RH are shown in Figure 14 and Figure 15. The moisture sorption was increased with increasing storage RH, as also observed by Gillgren et al. (48). Generally, moisture sorption was greater in the films prepared with glycerol. The films prepared with glycerol showed outstanding moisture sorption especially at the storage RH at 52% RH up to 92% RH. It was possible that glycerol molecules which has hydrophilic and hygroscopic properties promoted moisture sorption. In addition, presence of glycerol increased the free volume of the molecular network and hence increased mobility of polymer chain (44). This agreed with previous report (44) which suggested that addition of glycerol in the film had impact on moisture sorption.
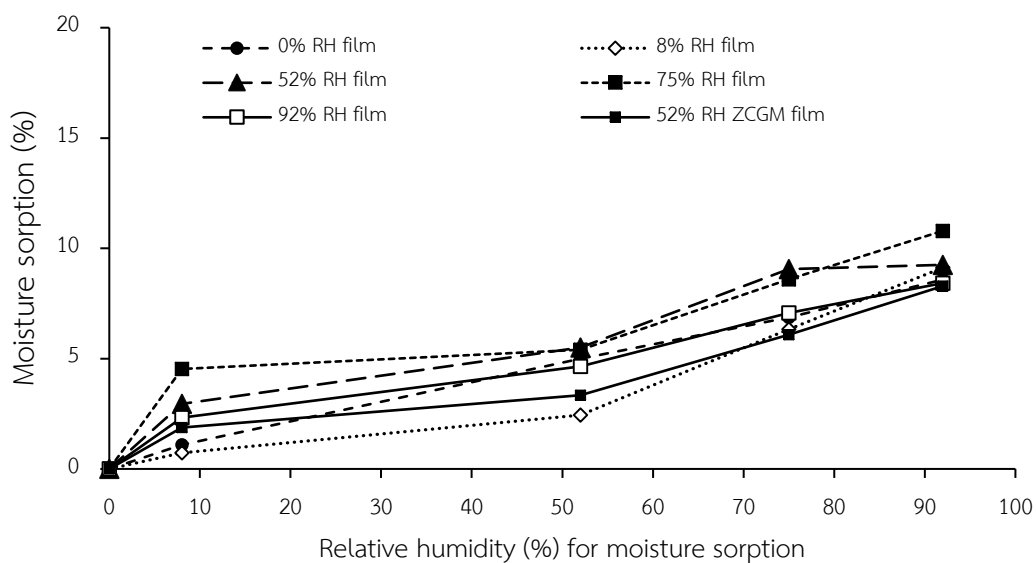
Figure 14 Moisture sorption of CZ films without glycerol at varied relative humidity levels and ZCGM film equilibrated at 52% RH
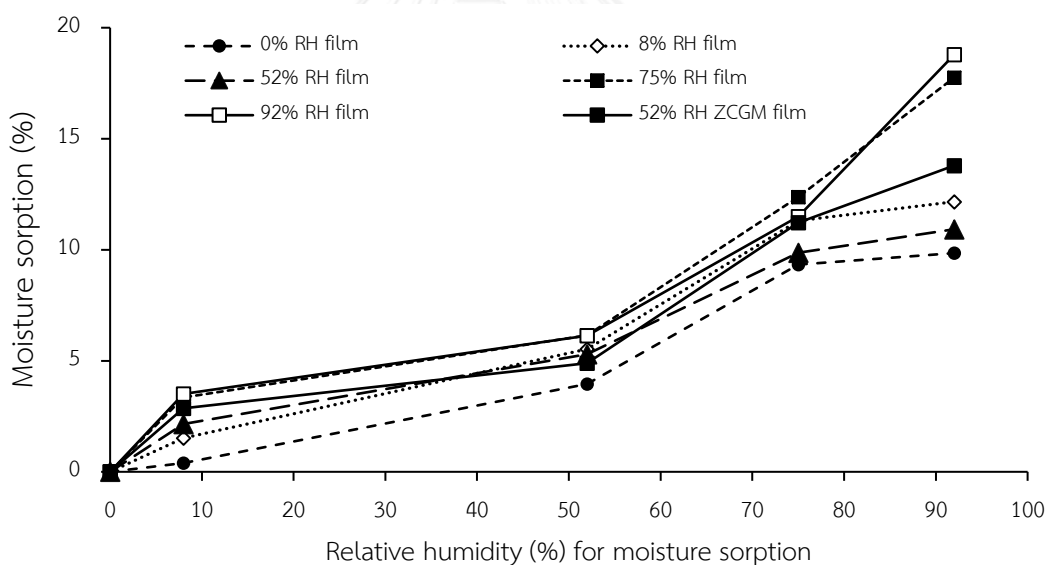
Figure 15 Moisture sorption of CZ films with glycerol at varied relative humidity levels and ZCGM film equilibrated at 52% RH

2.6 Water vapor permeability (WVP)

Before the total time of study (168 h), it was found that the weight gain of the tested film was considerably constant after 48 h and during the subsequent 120 h.

The results of WVP are shown in Figure 16. WVP of CZ film was increased as increasing glycerol content. The maximum of WVP was observed for CZ film with glycerol equilibrated at 92% RH. Because of hygroscopic and hydrophilic nature, glycerol could allow water molecules to permeate and adsorb water through hydrogen bonding (43). The WVP was tended to be higher as increasing environmental RH. This suggested that adsorption of vapor moisture onto the zein film surface followed by hydrophilic association leading to high water vapor permeability.
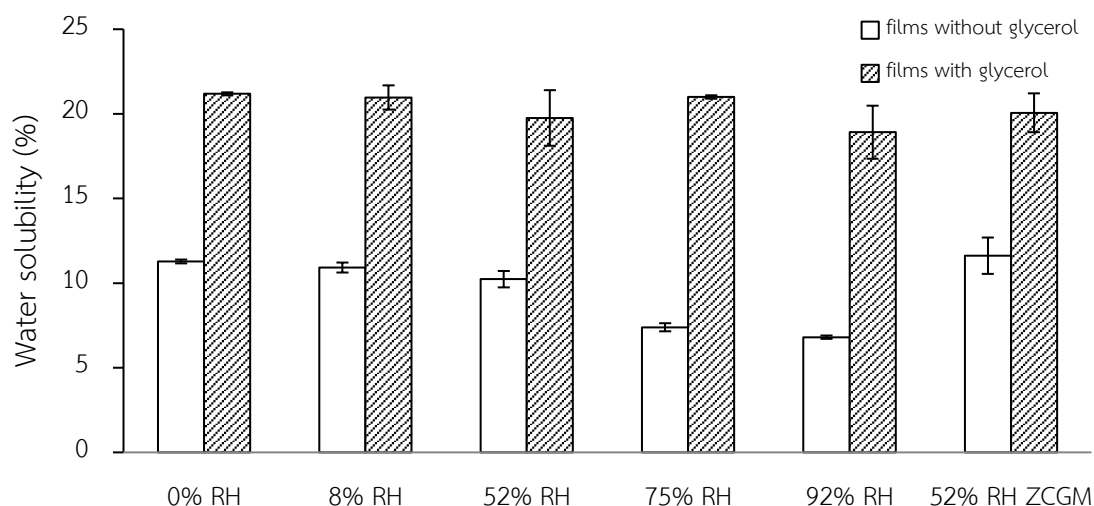

Figure 16 WVP (g mm/h m^2 kPa) of CZ and ZCGM films equilibrated at varied relative humidity levels prepared without glycerol (white) and with glycerol (grid)

2.7 Mechanical properties

The mechanical properties of CZ films without and with glycerol are shown in Figure 17. It is evident that equilibrating conditions of the films and presence of glycerol was likely to affect on the mechanical properties of films, i.e. tensile strength, elongation at break and Young's modulus. This effect was marked observed for the CZ films with glycerol equilibrated at the high relative humidity levels (75 and 92%RH). The tensile strength of these films were considerably reduced, indicating weak structure. Glycerol can form hydrogen bond with water molecules and with amino acids, resulting in reduction of the attractive force between polypeptide chains and promoting intermolecular mobility. Yoshino et al.

(25) demonstrated that when the increasing drying rate of ethanol zein film, the tensile strength was higher. Chen et al. also indicated that film tensile strength was decreased with raising RH because of the high amount of moisture content in film gave great intermolecular mobility (43).

The CZ films were likely to possess more elongation ability and more elasticity (i.e. reduction of Young's modulus) when they were equilibrated at the higher RH and particularly those were prepared with glycerol (Figure 17b-c). The effect of glycerol was more pronounced.

Lawton reported that the elongation of film was not increased with increasing studied RH (3-93%) of zein film containing glycerol or no plasticizer (47). Also, Yoshino et al. found that the elongation of zein film was not related to drying rate under various relative humidity (5 and 90%) of drying conditions (46). They concluded that elongation was different because of initial zein concentration; the film formed with high concentration showed low elongation (46).

The ZCGM films prepared with glycerol clearly showed higher tensile strength, more elongation and lower Young's modulus comparing with the CZ film prepared at the same RH condition. The ZCGM film was therefore tougher and more elastic than the CZ films. This because glycerol was more compatible with more polar amino acids in ZCGM extract.

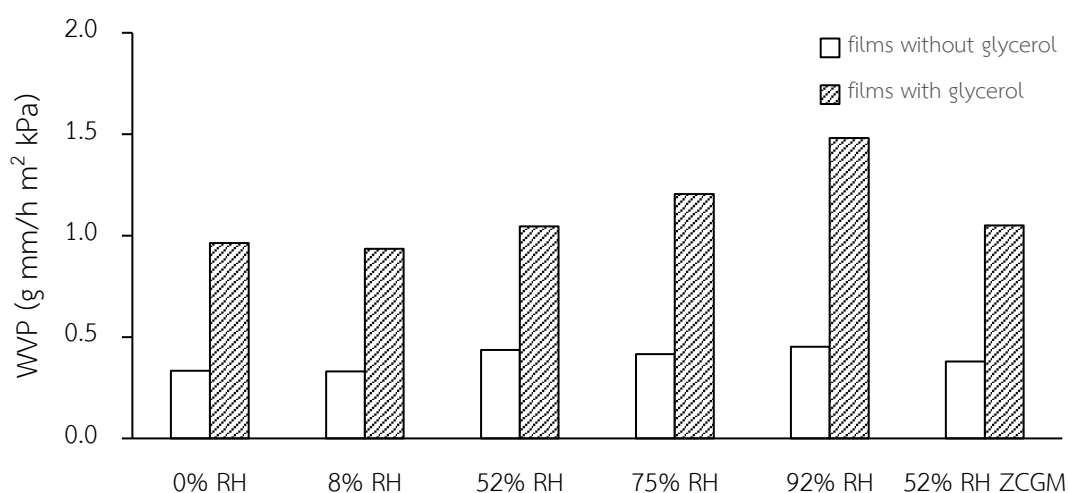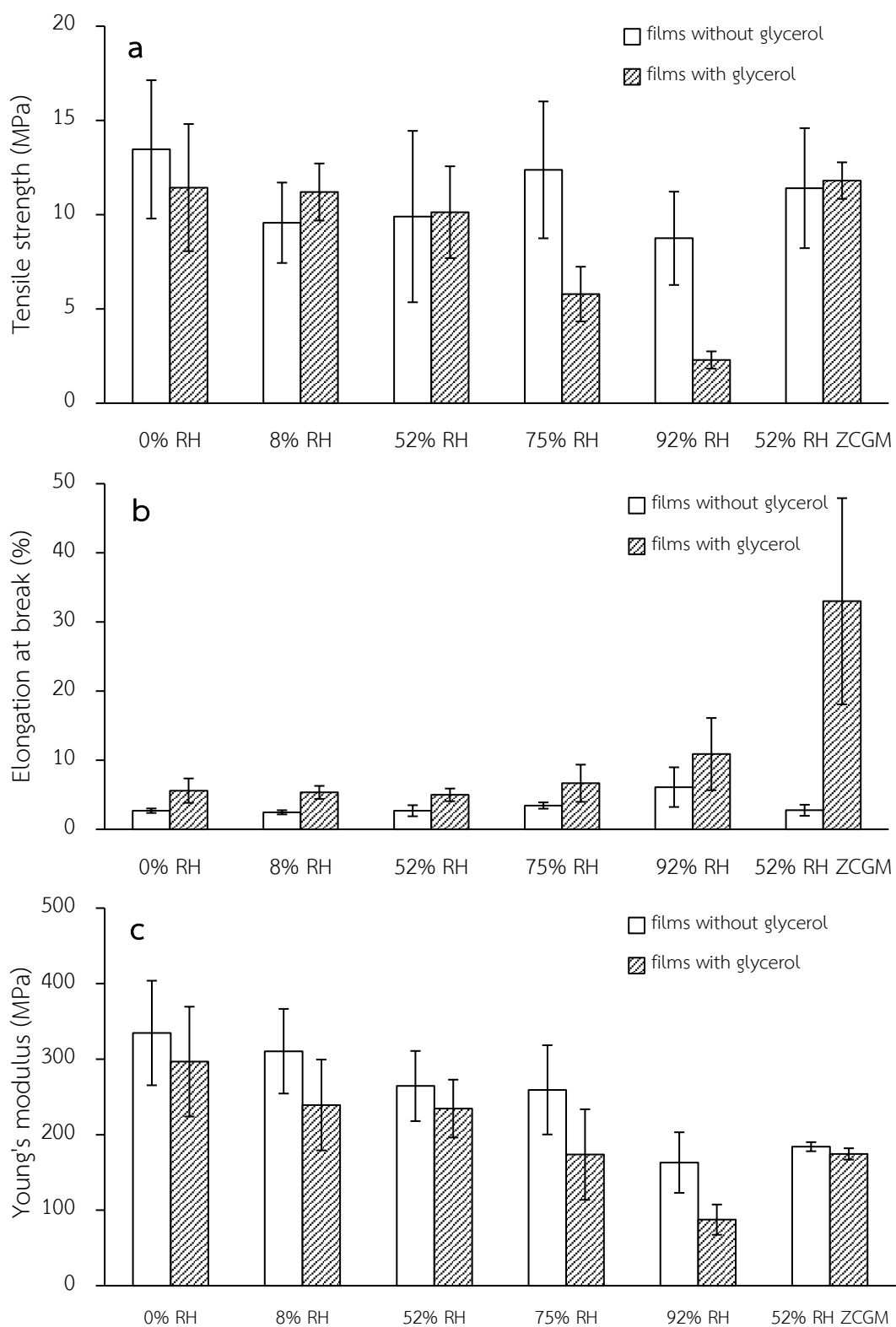Figure 17 Mechanical properties: (a) tensile strength, (b) elongation at break and (c) Young's modulus of CZ and ZCGM films equilibrated at different relative humidity levels prepared without glycerol (white) and with glycerol (grid)

2.8   Differential scanning calorimetry (DSC)

Basically, the miscibility and compatibility of plasticizer to the film former is indicated by reduction of T_g. The T_g of ZCGM extract (195.0°C) was slightly higher than that of CZ powder (155.2°C). This was attributed to different amino acid residues in the ZCGM extract which was less purity of α-zein fraction (1). The actual T_g of zein was unclear and perhaps varied depending on the types and amounts of amino acids resulted from extraction methods. The reported T_g of zein was inconsistent e.g. 170.8 °C (15) and could be two values at 180.1 and 200.1°C (49).

It has been reported that presence of moisture reduced T_g of zein and the reduction of T_g responded to increasing moisture content (50). However, in this study, the presence of moisture in the films equilibrated at varied RH did not systematically affect the T_g of the films either with or without glycerol (Table 8). However, there appeared to be second T_g existing at a lower temperature for the films without glycerol equilibrated at 75 and 92% RH. This was possibly due to zein is mainly composed of hydrophobic amino acids which was not compatible with water. At relative high moisture content, there was sufficient water molecules to hydrate hydrophilic amino acids in the CZ film, resulting in the second T_g at the lower temperature.

Glycerol was shown to be a candidate plasticizer for zein films as evidenced by a marked reduction of T_g in the films equilibrated at all conditions. This results agreed with previous work reported by Lawton (47). The T_g of ZCGM film (52°C) prepared with the same amount glycerol was higher than that of CZ film, suggesting that the polypeptide chains in ZCGM extract was more cohesive as evidenced by higher T_g.

Table 8 T_g (°C) of films equilibrated at different relative humidity levels prepared without glycerol and with glycerol, n=1

	Films without glycerol	Films with glycerol
0% RH CZ film	142.5	20.5
8% RH CZ film	128.2	22.7
52% RH CZ film	142.5	18.8
75% RH CZ film	61.5, 116.3	22.8
92% RH CZ film	39.4, 149.0	16.1
52% RH ZCGM film	122.3	48.7

Remarks: T_g of CZ powder and ZCGM extract were 155.2 (2.8) °C (n=3) and 195.0 (5.7) °C (n=3), respectively.

2.9 Thermogravimetric analysis (TGA)

The weight loss of films was increased with increasing RH of drying environment as shown in Table 9. The results of TGA agreed with the results of residual moisture and volatile substances in section 2.3. The TGA curves of samples exhibited two stages of weight loss as shown in Figure 18. The first stage mostly responded to evaporation of adsorbed water and other volatile constituents from the material, where the plateau was shown about 150°C. Is was also possible that some amino acids e.g. asparagine (T_d = 77°C) and cysteine (T_d = 120°C) were decomposed (51). The minimum and maximum weight loss up to 150°C were about 5.3% for CZ powder and 18.1% for CZ film with glycerol equilibrated at 92% RH.

The second stage of weight loss occurred after 150°C was related with thermal degradation of amino acids and breakage of covalent peptide bonds. The T_d of glutamic acid, leucine, alanine and proline began at 186, 207, 217, 218°C, respectively (51). The significant weight loss began at 285 and 284°C for CZ and ZCGM powders, respectively; and this appeared at slightly lower temperatures for the films, especially films with glycerol and equilibrated at various RH. The T_d of films prepared with glycerol were lower than that of films prepared without glycerol. Although, the T_d of ZCGM film was comparable to that of CZ films when they were prepared without glycerol, overall results of the ZCGM film suggested that ZCGM

provided more stable films. This may be due to that β-zein in ZCGM extract contained more amino acids such as proline (T_d = 218°C), methionine (T_d = 236°C), arginine (T_d = 244°C) which have thermal stability (51).

Table 9 Weight loss (%) at 150°C, onset degradation temperature (T_d) of CZ and ZCGM films without/with glycerol determined by TGA; data shown are average, n=2

RH (%)	Films without glycerol		Films with glycerol	
	Weight loss (%) at 150°C	T_d (°C)	Weight loss (%) at 150°C	T_d (°C)
0% RH CZ film	5.4	283	6.1	267
8% RH CZ film	6.3	283	8.2	267
52% RH CZ film	7.4	284	9.0	267
75% RH CZ film	9.4	282	12.2	267
92% RH CZ film	9.8	283	18.1	268
52% RH ZCGM film	5.7	282	8.2	276

Remark: T_d of CZ and ZCGM raw material were 285 and 284°C, respectively. Weight loss at at 150°C of CZ and ZCGM raw material were 5.3 and 5.6%, respectively
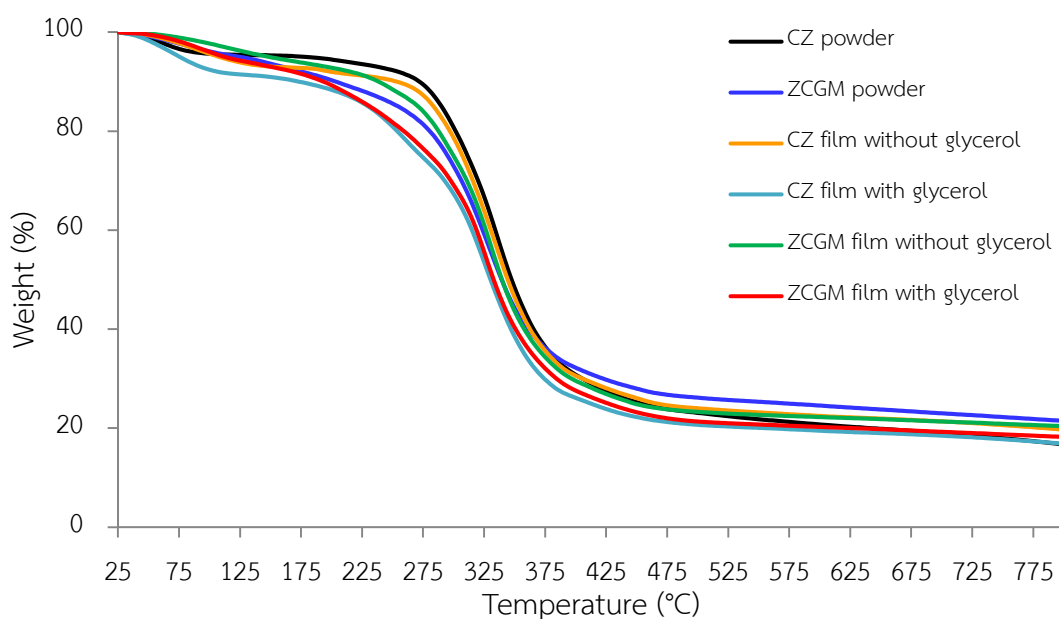
Figure 18 TGA curves of CZ powder, ZCGM extract and the films equilibrated at 52% RH

2.10 Fourier transform infrared spectroscopy (FTIR)

FTIR was used to identify structural changes though shift in characteristic peaks of functional group (52). The infrared spectra of CZ and ZCGM samples, i.e. powders and films, exhibited the characteristic bands in the amide I region at 1700-1600 cm^{-1} and the amide II region at 1600-1500 cm^{-1}. The amide I band is the most intense absorption band in proteins, mainly corresponding to stretching vibrations of the carbonyl (C=O), whereas the amide II band corresponds to the N-H bending and C-N stretching. In addition, the band appears at about 3500-2800 cm^{-1} of O-H and N-H stretching of amino acids, indicating presence of intermolecular bonded hydroxyl groups and thiol groups (4, 53).

A zein film could be formed through agglomeration of the protein and occurrence of hydrogen bonding, disulfide bonding and hydrophobic interactions during evaporation (54). The amide I band of CZ powder appeared at 1644 cm^{-1} which was slightly shifted to lower wavenumbers as increasing RH of equilibrating conditions to about 1643, 1641 and 1639 cm^{-1} for the CZ films without glycerol equilibrated at 52, 75 and 92% RH, respectively. The amide II band of CZ powder

was also shifted from 1516 cm^{-1} to higher wavenumber of 1530, 1535, 1535, 1535 and 1538 cm^{-1} for the CZ films without glycerol equilibrated at 0, 8, 52, 75 and 92% RH, respectively. In case of CZ films with glycerol, the amide I band was changed in the same manner with the CZ films without glycerol, existing at 1647, 1647, 1644, 1644 and 1638 cm^{-1} for the films equilibrated at 0, 8, 52, 75 and 92% RH, respectively. While the amide II band of the CZ film with glycerol was shifted from 1516 cm^{-1} of CZ powders to 1539 cm^{-1} for CZ film equilibrated at 0% RH, and to 1538 cm^{-1} for the films equilibrated at 8, 52, 75 and 92% RH.

The amide I and amide II bands of ZCGM appeared at 1640 cm^{-1} and 1516 cm^{-1}, respectively. The amide I band was shifted to 1641 cm^{-1} for ZCGM film without glycerol and to 1649 cm^{-1} for ZCGM film with glycerol. The amide II band occurred at 1539 cm^{-1} for ZCGM films either with or without glycerol.

The shift in amide I band (i.e. stretching vibration of C=O in the peptide bonds) may be related to change in its secondary structure. The amide II band is sensitive to change in environment of N-H group. Water may influence intermolecular bonding in the film equilibrated at various RH (48). The water molecules interacted with amide groups of protein and modified protein network structure (55).

The band at approximately 1041-1047 cm^{-1} in the spectra of CZ and ZCGM films without glycerol and about 1040-1044 cm^{-1} in the films prepared with glycerol. This band was also found at 1030 cm^{-1} for glycerol. The band is a character of C-O stretching which was reported at 1015-1200 cm^{-1} (56). It was slightly shifted but could not be explained by varied RH of equilibrating conditions. Generally, its intensity was increased with presence of glycerol. The peak about 1045-1044 cm^{-1} was also reported with the presence of glycerol in the film sample and its intensity was increased when the glycerol content was increased (4, 57).
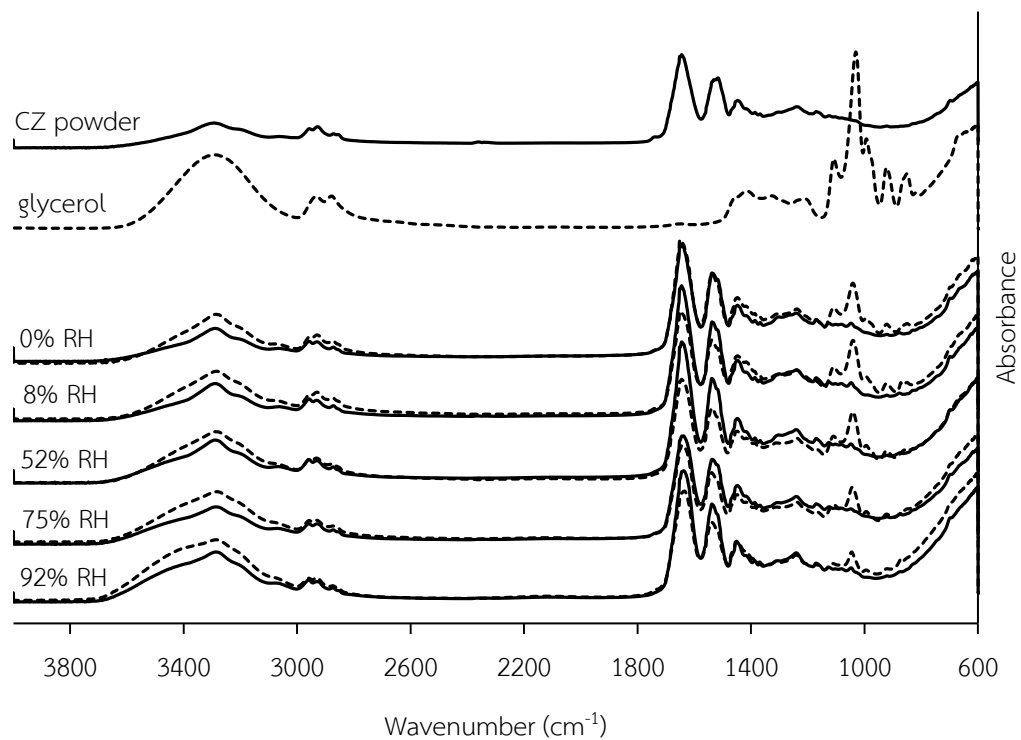
Figure 19 FTIR spectra of CZ powder, glycerol, CZ films without glycerol (solid line) and CZ films with glycerol (dot line) equilibrated at varied RH
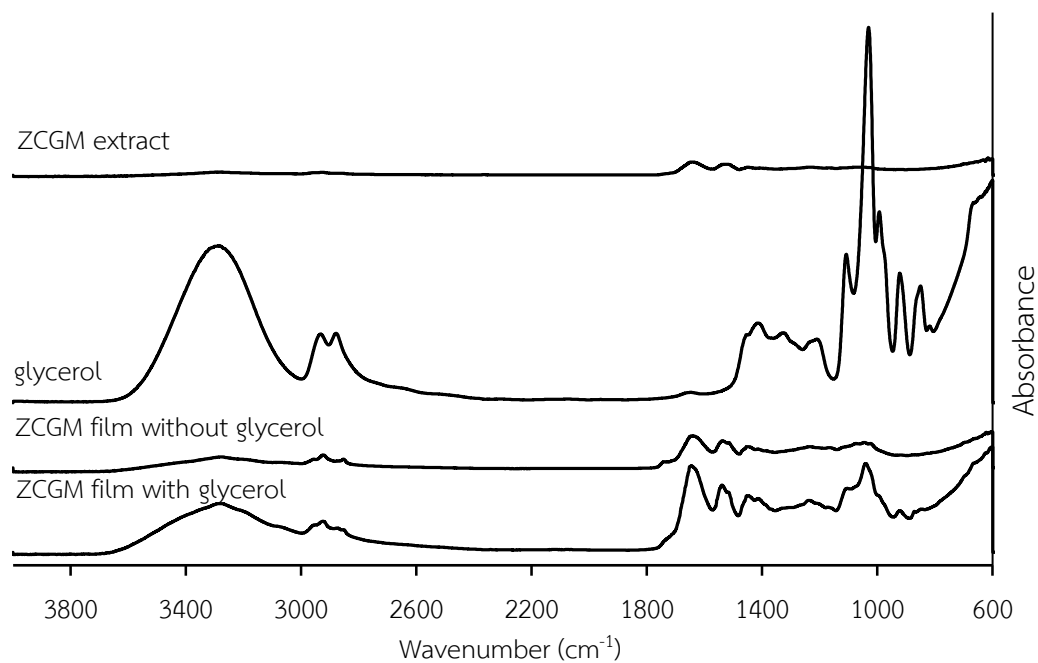
Figure 20 FTIR spectra of ZCGM extract, glycerol, ZCGM films without and with glycerol equilibrated at 52% RH

3. **Preparation of hard capsule and characterization**

The film equilibrated at 52% RH possessed desired properties e.g. appearance and mechanical properties; therefore, this condition was used for equilibrating capsules.

3.1 **Appearance, weight, thickness and residual moisture and volatile substances**

The capsule shells could be formed from both CZ and CGM solvent system after equilibration at 52% RH and ambient temperature, as shown in Figure 21. They were easily peeled off. Capsule shells were yellowish, translucent, flexible and had smooth surface. The cap and body could be joined together.

Figure 21 Photographs of CZ (a) and ZCGM (b) capsule shells

The CZ and ZCGM capsules prepared with glycerol as the plasticizer had similar content of residual moisture and volatile substances to that of commercial gelatin capsules. It is known that water is the plasticizer for gelatin capsules where the moisture content is critical to maintain flexibility of gelatin capsule shells. Common specification of moisture content for hard gelatin capsules is 13-16% w/w (37). In this study, measured moisture content of gelatin capsules was 7.43%. CZ and ZCGM capsules required glycerol to be plasticizer and hydrophilicity of glycerol could adsorb and retain water to an optimum moisture content (Table 10).

Table 10 The properties of capsule shells; data shown are means (SD)

Capsule shells	Weight (mg), n=10	Thickness (mm), n=5		Residual moisture and volatile substances (%), n=5
		body	cap	
Gelatin	94.69 (1.76)	0.12 (0.01)	0.12 (0.01)	7.43 (0.62)
CZ with glycerol	135.28 (2.37)	0.13 (0.01)	0.12 (0.01)	7.26 (0.20)
ZCGM with glycerol	143.09 (4.27)	0.13 (0.01)	0.12 (0.01)	7.18 (0.60)

3.2   Mechanical properties

Gelatin capsule required higher maximum stress (12 MPa) to compress upto 75% of shell diameter and possessed more elasticity (16 MPa) than zein capsules (Table 11). This suggested that gelatin capsules were significantly stronger and more elastic.

ZCGM capsules were also slightly stronger than CZ capsules due to denser structure as shown in Figure 22.   However, the surface of ZCGM capsules was relatively rough (Figure 23).

Table 11 Mechanical properties of capsule shells in terms of maximum stress at 75% of capsule diameter and elasticity; data shown are means (SD), n=5

Capsule shells	Maximum stress (MPa)	Elasticity (MPa)
gelatin	11.99 (2.93)	15.61 (1.95)
CZ with glycerol	2.36 (0.37)	6.13 (0.78)
ZCGM with glycerol	4.96 (2.51)	9.39 (1.15)

3.3   Dissolution

The dissolution profiles of CZ and ZCGM capsules containing propranolol hydrochloride are shown in Figure 24. The average drug release at 8 h of CZ capsule was about 87% w/w which higher than ZCGM capsule (59% w/w). The release drug of CZ capsule was markedly increased with high variation after changing the medium from pH 1.2 hydrochloric acid buffer solution to pH 6.8 phosphate buffer solution. While, the ZCGM capsules gradually released the drug at early time points and showed relatively high variation at late time points. The behavior of CZ capsules

was delayed release; while ZCGM capsules tended to extend the drug release. This could be explained by denser film of ZCGM capsule shells.

After dissolution study of in two dissolution media, the CZ capsules were in pale color, swollen and deformed, while ZCGM capsules showed a slight change in color and shape.

The SEM micrographs (Figure 22c-d, Figure 23c-d) shows the morphology of both types of capsules was different after dissolution study. There appeared to be a larger pore on the shell of CZ capsules.
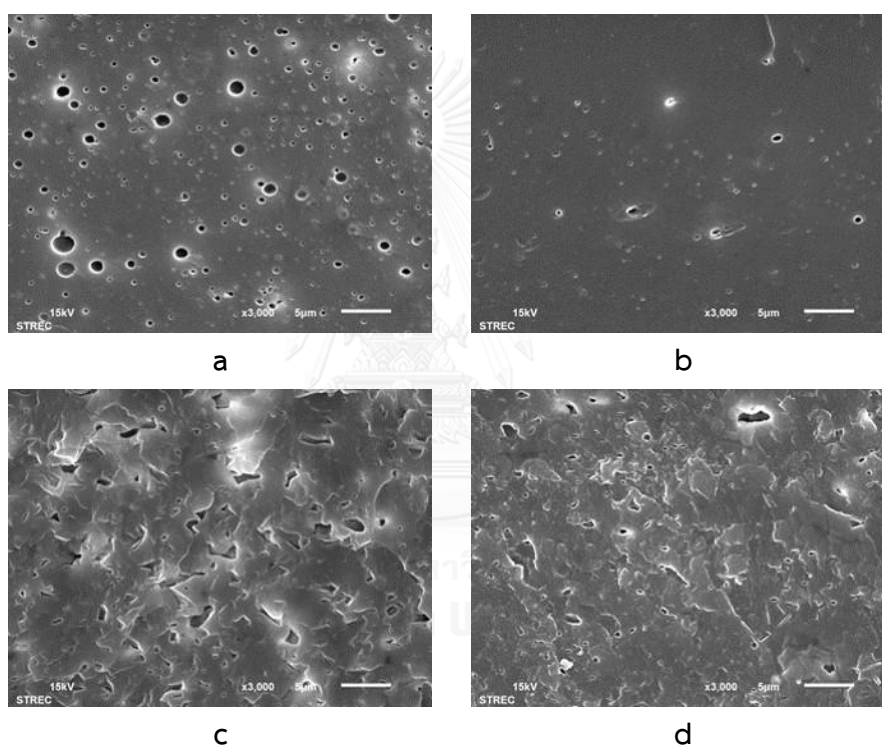
Figure 22 SEM micrographs of zein capsule with glycerol cross-section at the magnification of 3,000x; CZ capsule (a) and ZCGM capsule (b) equilibrated at 52% RH before dissolution; CZ capsule (c) and ZCGM capsule (d) after dissolution

Figure 23 SEM micrographs of zein capsule with glycerol surface at the magnification of 5,000x; CZ capsule (a) and ZCGM capsule (b) equilibrated at 52% RH before dissolution; CZ capsule (c) and ZCGM capsule (d) after dissolution
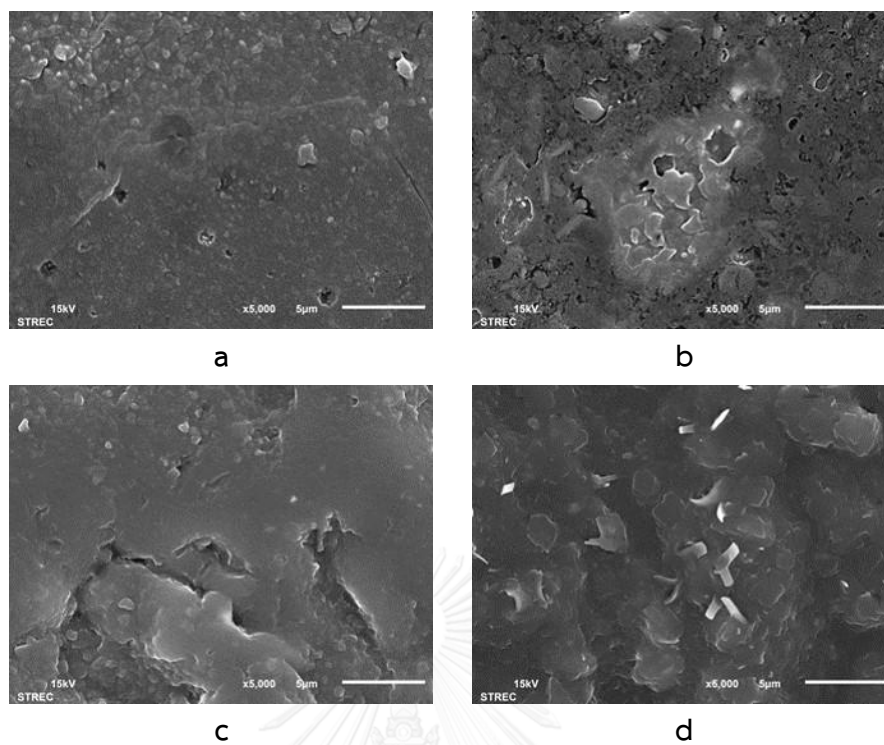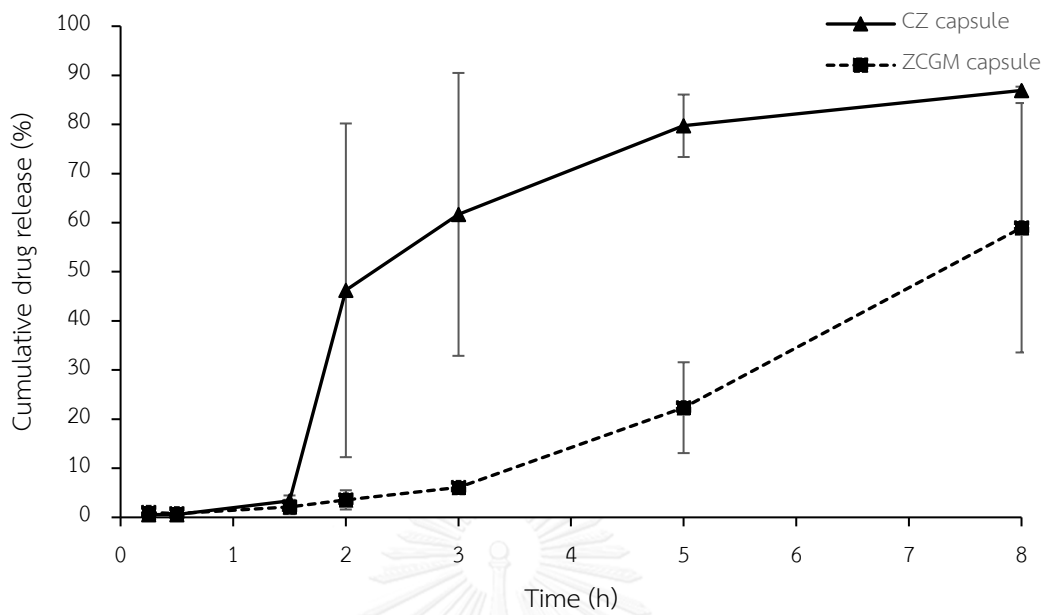
Figure 24 The cumulative drug release (%) curves of CZ capsules and ZCGM capsule containing propranolol hydrochloride in pH 1.2 hydrochloric acid buffer solution (upto 1.5 h) and pH 6.8 phosphate buffer solution (after 1.5 h)

CHAPTER V

CONCLUSIONS

In the present study, attempts were made to extract zein from corn gluten meal, a byproduct from corn processing industry in Thailand. The extract was characterized and compared with a commercial zein. Possibility of preparing hard capsule shells from zein was investigated. The conclusions of the study are as follows:

- Zein extracted from corn gluten meal had quality complies with USP 36 specification and comparable to commercial zein. The solvent used in extraction was crucial for the yield and purity of zein. A more polar solvent, such as 70% v/v ethanol in this study may give more yield but co-extracted non-polar and polar fractions of zein.

- It was possible to form film and prepare hard capsule shell from both the commercial zein and the extracted zein. Although the fraction of polar or hydrophilic protein have not be identified, the extracted zein was more compatible with the hydrophilic plasticizer (glycerol) and had impact on overall characteristics of film and capsule shell, including more elasticity, higher thermal stability and altered dissolution profile. The capsule shells made from the extracted zein plasticized with glycerol was likely to extend the drug release, while those made with the commercial zein showed the dissolution which depended on pH of medium.

- Formulation variables (e.g. presence of glycerol) and process variables (e.g. relative humidity during drying film) have been proved to affect the physical properties of zein film. The zein film and hard capsule shell formulations required 20% glycerol based on solid zein. The optimum relative humidity for drying in this study was 52%.

In further work, zein extraction method and hard capsule shell preparing technique shall be refined. An aqueous dispersion of zein needs more development

to increase solid content so that it can be efficiently replaced the solvent based coating.

REFERENCES

1.   Shukla R, Cheryan M. Zein: the industrial protein from corn. Industrial Crops and Products. 2001;13(3):171-192.

2.   Esen A. A proposed nomenclature for the alcohol-soluble proteins (zeins) of maize (*Zea mays* L.). Journal of Cereal Science. 1987;5(2):117-128.

3.   Esen A. An immunodominant site of γ-zein1 is in the region of tandem hexapeptide repeats. Journal of Protein Chemistry. 1990;9(4):453-460.

4.   Chen Y, Ye R, Liu J. Effects of different concentrations of ethanol and isopropanol on physicochemical properties of zein-based films. Industrial Crops and Products. 2014;53:140-147.

5.   Singh N, Georget DMR, Belton PS, Barker SA. Physical properties of zein films containing salicylic acid and acetyl salicylic acid. Journal of Cereal Science. 2010;52(2):282-287.

6.   Yilmaz A, Bozkurt F, Cicek PK, Dertli E, Durak MZ, Yilmaz MT. A novel antifungal surface-coating application to limit postharvest decay on coated apples: Molecular, thermal and morphological properties of electrospun zein–nanofiber mats loaded with curcumin. Innovative Food Science & Emerging Technologies. 2016;37, Part A:74-83.

7.   Anderson TJ, Lamsal BP. Zein extraction from corn, corn products, and coproducts and modifications for various applications: a review. Cereal Chemistry. 2011;88(2):159-173.

8.   Anderson TJ, Lamsal BP. Development of new method for extraction of α-zein from corn gluten meal using different solvents. Cereal Chemistry. 2011;88(4):356-362.

9.   Argos P, Pedersen K, Marks MD, Larkins BA. A structural model for maize zein proteins. J Biol Chem. 1982;257(17):9984-9990.

10.   Zhang Y, Cui L, Che X, Zhang H, Shi N, Li C, et al. Zein-based films and their usage for controlled delivery: origin, classes and current landscape. Journal of Controlled Release. 2015;206:206-219.

11.   Dangaran K, Tomasula PM, Qi P. Structure and function of protein-based edible films and coatings. In: Huber KC, Embuscado ME, editors. Edible films and coatings for food applications. New York, NY: Springer New York; 2009. p. 25-56.

12.   Shi K, Kokini JL, Huang Q. Engineering zein films with controlled surface morphology and hydrophilicity. Journal of Agricultural and Food Chemistry. 2009;57(6):2186-2192.

13.   Escamilla-García M, Calderón-Domínguez G, Chanona-Pérez JJ, Farrera-Rebollo RR, Andraca-Adame JA, Arzate-Vázquez I, et al. Physical and structural characterisation of zein and chitosan edible films using nanotechnology tools. International Journal of Biological Macromolecules. 2013;61:196-203.

14.   Ghanbarzadeh B, Oromiehi AR. Biodegradable biocomposite films based on whey protein and zein: barrier, mechanical properties and AFM analysis. International Journal of Biological Macromolecules. 2008;43(2):209-215.

15.   Guo HX, Heinämäki J, Yliruusi J. Stable aqueous film coating dispersion of zein. Journal of Colloid and Interface Science. 2008;322(2):478-484.

16.   Hsu B-L, Weng Y-M, Liao Y-H, Chen W. Structural investigation of edible zein films/coatings and directly determining their thickness by FT-raman spectroscopy. Journal of Agricultural and Food Chemistry. 2005;53(13):5089-5095.

17.   Donsì F, Voudouris P, Veen SJ, Velikov KP. Zein-based colloidal particles for encapsulation and delivery of epigallocatechin gallate. Food Hydrocolloids. 2017;63:508-517.

18.   Sousa FFO, Luzardo-Álvarez A, Blanco-Méndez J, Martín-Pastor M. NMR techniques in drug delivery: application to zein protein complexes. International Journal of Pharmaceutics. 2012;439(1–2):41-48.

19.   Pena-Serna C, Lopes-Filho JF. Influence of ethanol and glycerol concentration over functional and structural properties of zein–oleic acid films. Materials Chemistry and Physics. 2013;142(2–3):580-585.

20.   Tihminlioglu F, Atik İD, Özen B. Water vapor and oxygen-barrier performance of corn–zein coated polypropylene films. Journal of Food Engineering. 2010;96(3):342-347.

21.   Li XN, Guo HX, Heinamaki J. Aqueous coating dispersion (pseudolatex) of zein improves formulation of sustained-release tablets containing very water-soluble drug. Journal of Colloid and Interface Science. 2010;345(1):46-53.

22.   Guo HX, Shi YP. A novel zein-based dry coating tablet design for zero-order release. International Journal of Pharmaceutics. 2009;370(1–2):81-86.

23.   Pagliaro MR, Michele. The future of glycerol: new user of a versatile raw material. Rossi M, editor: RSC Green Chemistry; 2008. 104 p.

24.   Vieira MGA, da Silva MA, dos Santos LO, Beppu MM. Natural-based plasticizers and biopolymer films: a review. European Polymer Journal. 2011;47(3):254-263.

25.   Yoshino T, Isobe S, Maekawa T. Influence of preparation conditions on the physical properties of zein films. Journal of the American Oil Chemists' Society. 2002;79:345-349.

26.   O'Donnell PB, Wu C, Wang J, Wang L, Oshlack B, Chasin M, et al. Aqueous pseudolatex of zein for film coating of solid dosage forms. European Journal of Pharmaceutics and Biopharmaceutics. 1997;43(1):83-89.

27.   Zhong Q, Jin M. Zein nanoparticles produced by liquid–liquid dispersion. Food Hydrocolloids. 2009;23(8):2380-2387.

28.   Balani K, Verma V, Agarwal A, Narayan R. Physical, thermal, and mechanical properties of polymers. Biosurfaces: John Wiley & Sons, Inc; 2015. p. 329-344.

29.   Pelleg J. Mechanical testing of materials. Mechanical properties of materials. Dordrecht: Springer Netherlands; 2013. p. 1-84.

30.   Almutawah A, Barker SA, Belton PS. Hydration of gluten: a dielectric, calorimetric, and fourier transform infrared study. Biomacromolecules. 2007;8(5):1601-1606.

31.   Lacey AA, Price DM, Reading M. Theory and practice of modulated temperature differential scanning calorimetry. In: Reading M, Hourston DJ, editors. Modulated temperature differential scanning calorimetry: theoretical and practical applications in polymer characterisation. Dordrecht: Springer Netherlands; 2006. p. 1-81.

32.   Thermogravimetry (TG). In: Brown ME, editor. Introduction to thermal analysis: techniques and applications. Dordrecht: Springer Netherlands; 2001. p. 19-54.

33.   FTIR spectroscopy. In: Roberts GCK, editor. Encyclopedia of biophysics. Berlin, Heidelberg: Springer Berlin Heidelberg; 2013. p. 854-854.

34.   Patel AR, Velikov KP. Zein as a source of functional colloidal nano- and microstructures. Current Opinion in Colloid & Interface Science. 2014;19(5):450-458.

35.   Machado AHE, Kokubo T, Dujovny G, Jones B, Scialdone C, Bravo R, et al. A microstructural study of water effects in lipid-based pharmaceutical formulations for liquid filling of capsules. European Journal of Pharmaceutical Sciences. 2016;90:64-75.

36.   Lowry WT, Garriott JC. Pharmaceutical dosage forms. Forensic toxicology: controlled substances and dangerous drugs. Boston, MA: Springer US; 1979. p. 9-16.

37.   Podczeck F, Jones BE. Pharmaceutical capsules 2nd ed. London: Pharmaceutical Press; 2004.

38.   Kontny MJ, Mulski CA. Gelatin capsule brittleness as a function of relative humidity at room temperature. International Journal of Pharmaceutics. 1989;54(1):79-85.

39.   Bae HJ, Cha DS, Whiteside WS, Park HJ. Film and pharmaceutical hard capsule formation properties of mungbean, waterchestnut, and sweet potato starches. Food Chemistry. 2008;106(1):96-105.

40.   Cuihua L, Meize J, Yunfei D, inventors; Cuihua L, assignee. Hard prolamin capsule and preparation method. China 2015.

41.   Sun C, Dai L, Liu F, Gao Y. Dynamic high pressure microfluidization treatment of zein in aqueous ethanol solution. Food Chemistry. 2016;210:388-395.

42.   Hu K, McClements DJ. Fabrication of surfactant-stabilized zein nanoparticles: a pH modulated antisolvent precipitation method. Food Research International. 2014;64:329-335.

43.   Chen Y, Ye R, Li X, Wang J. Preparation and characterization of extruded thermoplastic zein–poly(propylene carbonate) film. Industrial Crops and Products. 2013;49:81-87.

44.   Su J-F, Huang Z, Zhao Y-H, Yuan X-Y, Wang X-Y, Li M. Moisture sorption and water vapor permeability of soy protein isolate/poly(vinyl alcohol)/glycerol blend films. Industrial Crops and Products. 2010;31(2):266-276.

45.   Lide DR. CRC handbook of chemistry and physics. 84th ed. Boca Raton, Florida: CRC Press; 2004.

46.   Yoshino T, Isobe S, Maekawa T. Physical evaluation of pure zein films by atomic force microscopy and thermal mechanical analysis. Journal of the American Oil Chemists' Society. 2000;77:699-704.

47.   Lawton JW. Plasticizers for zein: their effect on tensile properties and water absorption of zein films. Cereal Chemistry. 2004;81:1-5.

48.   Gillgren T, Barker SA, Belton PS, Georget DM, Stading M. Plasticization of zein: a thermomechanical, FTIR, and dielectric study. Biomacromolecules. 2009;10(5):1135-1139.

49.   Wu Q, Yoshino T, Sakabe H, Zhang H, Isobe S. Chemical modification of zein by bifunctional polycaprolactone (PCL). Polymer. 2003;44(14):3909-3919.

50.   Madeka H, Kokini JL. Effect of glass transition and cross-linking on rheological properties of zein: development of a preliminary state diagram. Cereal Chemistry Journal. 1996;73(4):433-438.

51.   Rodante F. Thermodynamics and kinetics of decomposition processes for standard α-amino acids and some of their dipeptides in the solid state. Thermochimica Acta. 1992;200:47-61.

52.   Georget DM, Belton PS. Effects of temperature and water content on the secondary structure of wheat gluten studied by FTIR spectroscopy. Biomacromolecules. 2006;7(2):469-475.

53.   Corradini E, Curti PS, Meniqueti AB, Martins AF, Rubira AF, Muniz EC. Recent advances in food-packing, pharmaceutical and biomedical applications of zein and zein-based materials. Int J Mol Sci. 2014;15(12):22438-22470.

54.   Guo Y, Liu Z, An H, Li M, Hu J. Nano-structure and properties of maize zein studied by atomic force microscopy. Journal of Cereal Science. 2005;41(3):277-281.

55.   Mujica-Paz H, Gontard N. Oxygen and carbon dioxide permeability of wheat gluten film: effect of relative humidity and temperature. Journal of Agricultural and Food Chemistry. 1997;45(10):4101-4105.

56.   Pelletier MJ, Pelletier CC. Spectroscopic theory for chemical imaging. Raman, infrared, and near-infrared chemical imaging: John Wiley & Sons, Inc.; 2010. p. 1-20.

57.   Wongsasulak S, Tongsin P, Intasanta N, Yoovidhya T. Effect of glycerol on solution properties governing morphology, glass transition temperature, and tensile properties of electrospun zein film. Journal of Applied Polymer Science. 2010;118(2):910-919.

APPENDIX

Table 12 Residue on ignition of commercial zein (CZ) and extract (ZCGM) from CGM batch no. 241214

	Sample weight (g)	Ash weight (g)	Ash (%)	mean
CZ sample 1	1.2710	0.0084	0.66	0.66
CZ sample 2	1.0736	0.007	0.65	
ZCGM sample 1	1.6054	0.0165	1.03	1.22
ZCGM sample 2	1.8779	0.0266	1.42	

Table 13 Heavy metal (ppm) of commercial zein (CZ) and extract (ZCGM) from CGM batch no. 241214

	Lead	Cadmium	Mercury	Arsenic	total
USP 36 limit of detection	0.32	0.35	0.02	0.34	1.03
CZ	ND	ND	ND	ND	<1.03
ZCGM	0.38	ND	ND	ND	<1.09

*ND: Not detected

Table 14 Hexane soluble matter (%) of commercial zein (CZ) and extract (ZCGM) from CGM batch no. 241214

	Solid (g)	Hexane soluble matter (g)	Hexane soluble matter (%)
CZ	15.0004	0.91	6.07
ZCGM	15.0002	1.00	6.67

Table 15 Loss on drying (%) of commercial zein (CZ), extract (ZCGM) from CGM and CGM

	LOD (%)			mean	SD
	1	2	3		
CZ	1.89	1.24	0.73	1.29	0.58
ZCGM batch no. 150113	5.34	5.59	5.37	5.43	0.13
ZCGM batch no. 280813	5.62	6.35	6.11	6.03	0.37
ZCGM batch no. 241214	5.57	5.56	5.45	5.53	0.07
CGM batch no. 150113	6.09	6.45	6.27	6.27	0.18
CGM batch no. 280813	3.87	3.91	3.77	3.85	0.07
CGM batch no. 241214	4.67	4.63	5.23	4.84	0.33

Table 16 Protein content (%) of commercial zein (CZ) and extract (ZCGM) from CGM and CGM

|	Nitrogen (%)	Protein content (%) (N x 6.25)	mean
CZ sample 1	14.6027	91.27	91.32
CZ sample 2	14.6207	91.38	
ZCGM batch no. 150113 sample 1	13.4737	84.21	84.64
ZCGM batch no. 150113 sample 2	13.6124	85.08	
ZCGM batch no. 280813 sample 1	13.9681	87.30	87.54
ZCGM batch no. 280813 sample 2	14.0461	87.79	
ZCGM batch no. 241214 sample 1	13.5709	84.82	84.93
ZCGM batch no. 241214 sample 2	13.6070	85.04	
CGM batch no. 150113 sample 1	11.3031	70.64	70.69
CGM batch no. 150113 sample 2	11.3166	70.73	
CGM batch no. 280813 sample 1	11.2946	70.59	70.01
CGM batch no. 280813 sample 2	11.1085	69.43	
CGM batch no. 241214 sample 1	10.9805	68.63	68.85
CGM batch no. 241214 sample 2	11.0509	69.07	
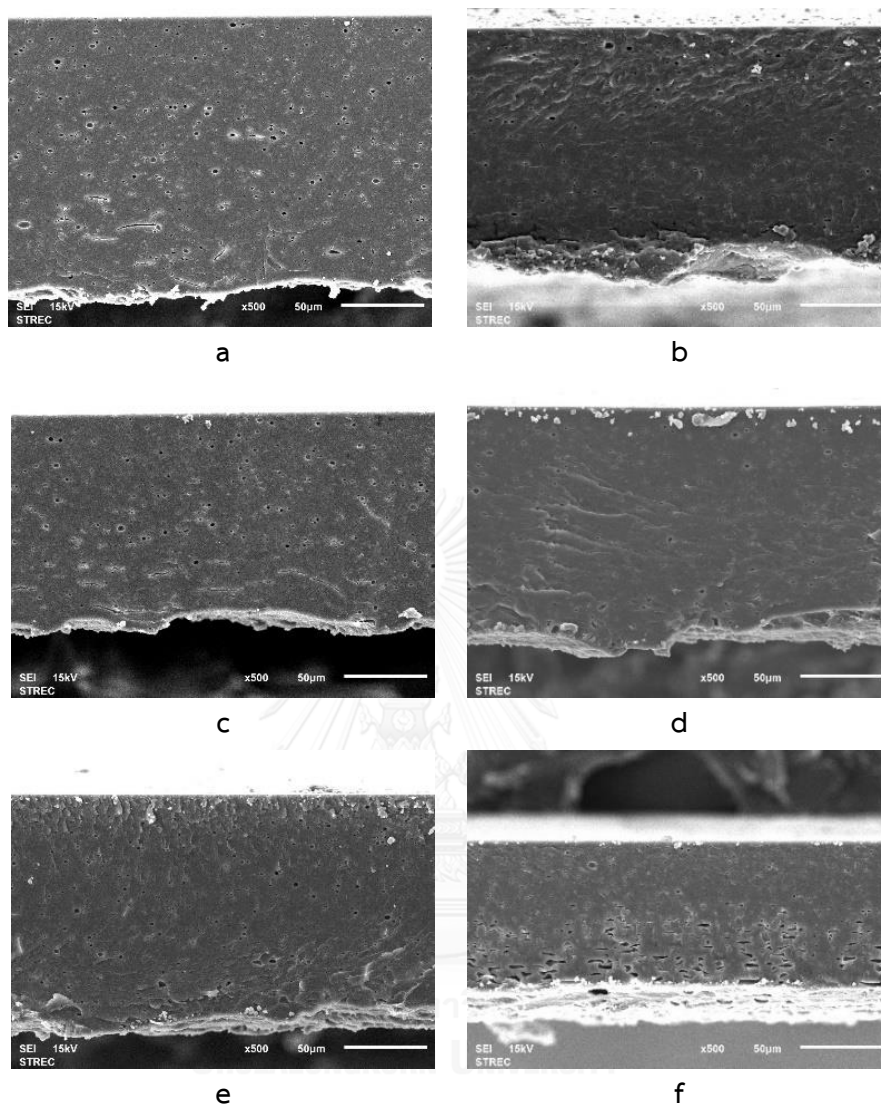
Figure 25 SEM micrographs of CZ film cross-section at the magnification of 500x; films prepared without glycerol equilibrated at 0% RH (a), 8% RH (c), 52% RH (e), 75% RH (g) and 92% RH (i) and with glycerol equilibrated at 0% RH (b), 8% RH (d), 52% RH (f), 75% RH (h) and 92% RH (j)
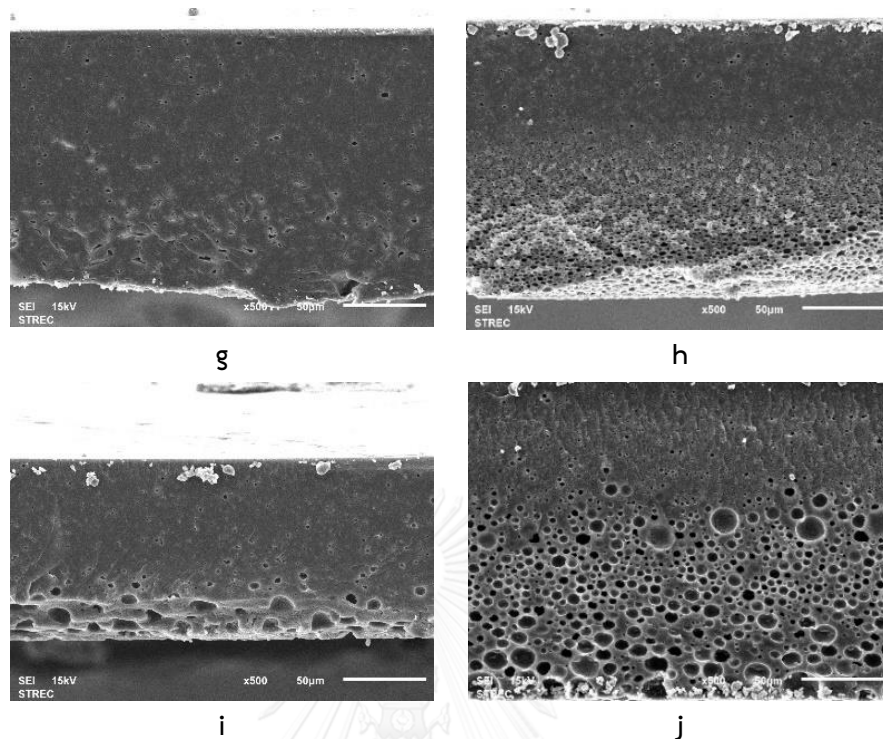
Figure 25 (continue) SEM micrographs of CZ film cross-section at the magnification of 500x; films prepared without glycerol equilibrated at 0% RH (a), 8% RH (c), 52% RH (e), 75% RH (g) and 92% RH (i) and with glycerol equilibrated at 0% RH (b), 8% RH (d), 52% RH (f), 75% RH (h) and 92% RH (j)
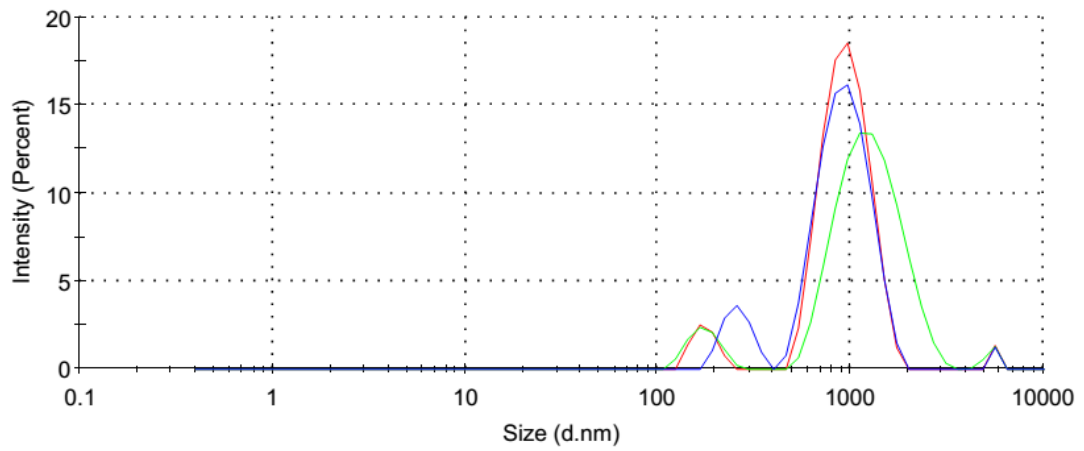
Figure 26 Size distribution of the dispersion prepared by adding 70% v/v ethanol phase into water phase with Tween 80 of 10% w/w
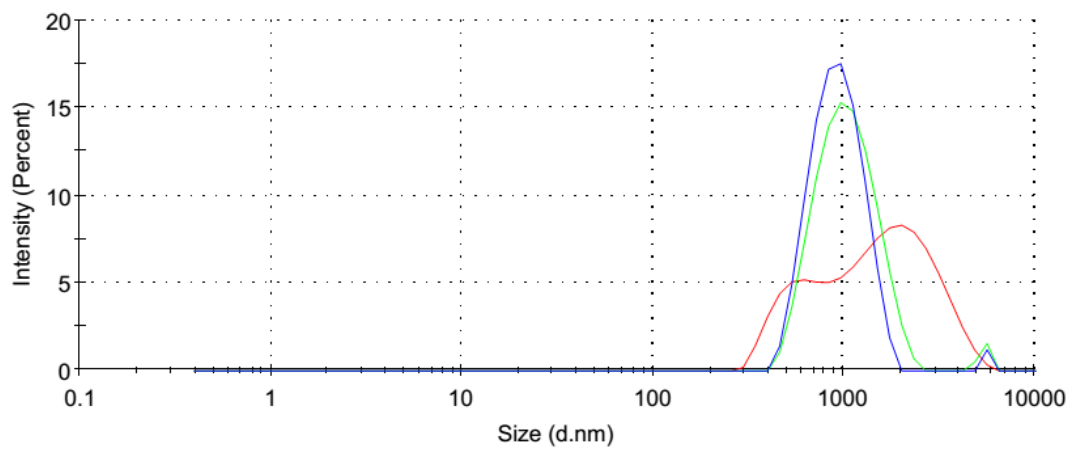

Figure 27 Size distribution of the dispersion prepared by adding 70% v/v ethanol phase into water phase with Tween 80 of 50% w/w
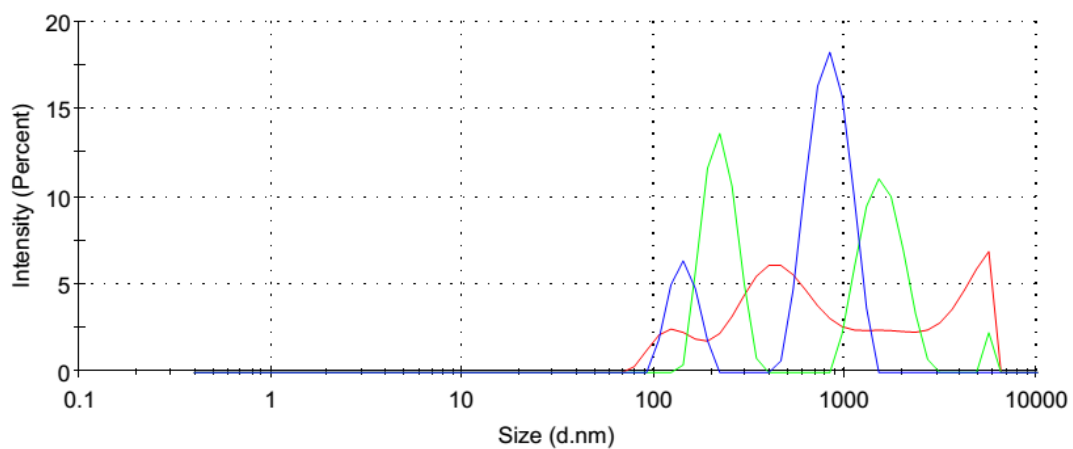
Figure 28 Size distribution of the dispersion prepared by adding water phase with Tween 80 of 10% w/w into 70% v/v ethanol phase.
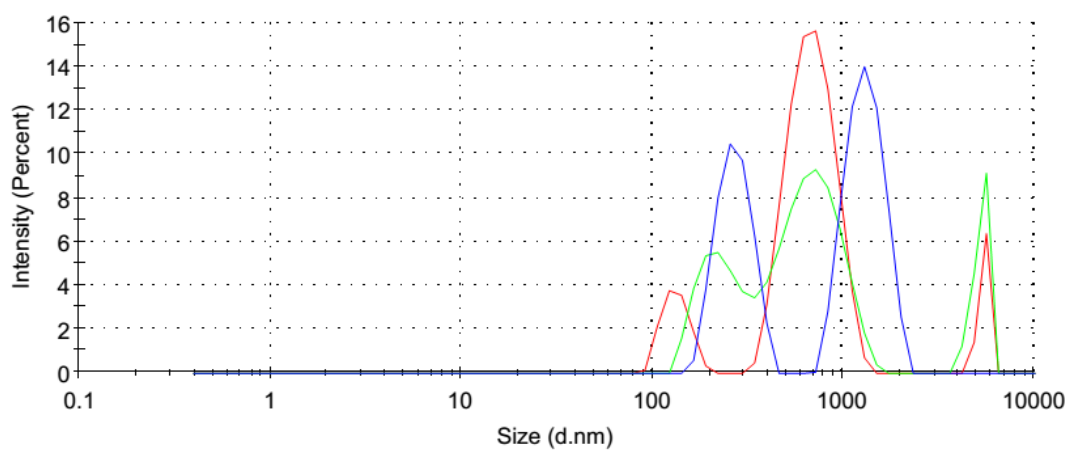

Figure 29 Size distribution of the dispersion prepared by adding water phase with Tween 80 of 50% w/w into 70% v/v ethanol phase.
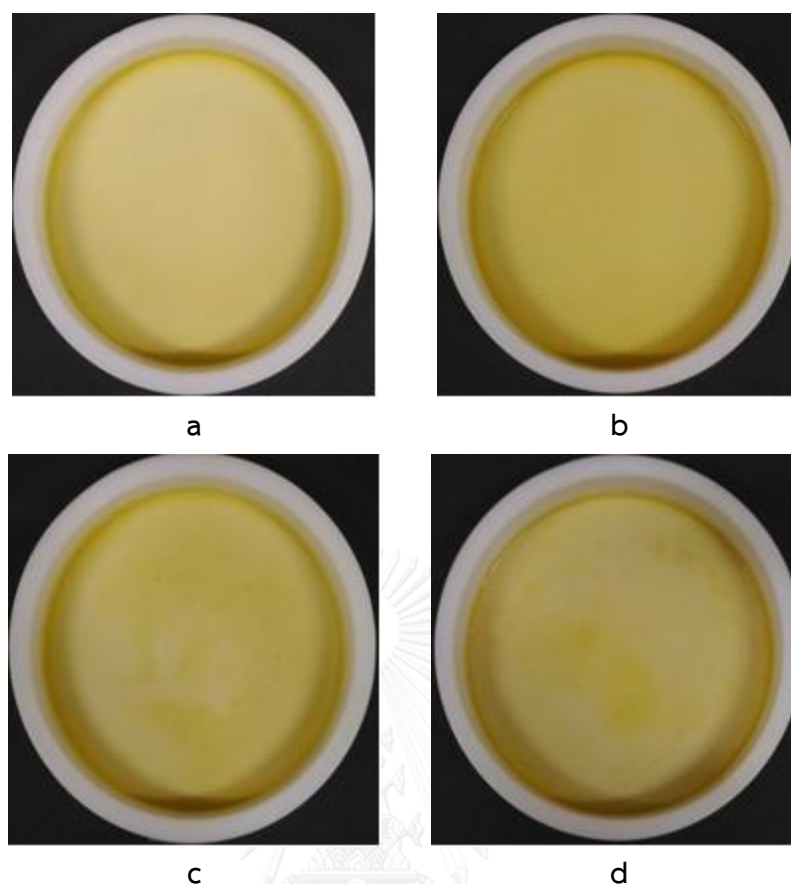
Figure 30 Photographs of casted films from the dispersions prepared by adding 70% v/v ethanol phase into water phase with Tween 80 of 10% w/w (a) and 50% w/w (b); the dispersions prepared by adding water phase with Tween 80 of 10% w/w (c) and 50% w/w (d) into 70% v/v ethanol phase.

Table 17 Residual moisture and volatile substances (%) of films without/with added glycerol in different relative humidity conditions; data shown are mean (SD), n=10

RH (%)	Films without glycerol	Films with glycerol
0% RH CZ film	3.70 (0.91)	4.08 (1.36)
8% RH CZ film	4.39 (1.46)	4.27 (1.26)
52% RH CZ film	5.21 (0.90)	5.76 (0.97)
75% RH CZ film	10.76 (3.03)	15.36 (2.40)
92% RH CZ film	15.43 (5.84)	23.44 (4.03)
52% RH ZCGM film	5.64 (1.14)	6.17 (0.97)

Table 18 Water solubility (%) of films without/with added glycerol in different relative humidity conditions; data shown are mean (SD), n=3

RH (%)	Films without glycerol	Films with glycerol
0% RH CZ film	11.28 (0.11)	21.18 (0.09)
8% RH CZ film	10.92 (0.29)	20.97 (0.72)
52% RH CZ film	10.23 (0.48)	19.76 (1.64)
75% RH CZ film	7.39 (0.24)	20.10 (0.10)
92% RH CZ film	6.81 (0.10)	18.92 (1.57)
52% RH ZCGM film	11.62 (1.07)	20.07 (1.15)

Table 19 moisture sorption (%) of films without glycerol in varied relative humidity desiccator; data shown are mean (SD), n=3

Films without glycerol	relative humidity for moisture sorption			
	8% RH	52% RH	75% RH	92% RH
0% RH CZ film	1.09 (0.36)	5.00 (3.13)	6.87 (0.26)	8.58 (1.16)
8% RH CZ film	0.73 (0.25)	2.44 (0.45)	6.32 (0.22)	9.12 (1.76)
52% RH CZ film	2.95 (0.55)	5.51 (0.31)	9.06 (0.64)	9.25 (1.55)
75% RH CZ film	4.53 (3.17)	5.40 (0.09)	8.59 (1.44)	10.79 (1.58)
92% RH CZ film	2.33 (0.25)	4.65 (0.12)	7.07 (0.37)	8.42 (0.65)
52% RH ZCGM film	1.88 (0.28)	3.35 (0.27)	6.09 (0.21)	8.29 (2.35)

Table 20 moisture sorption (%) of films with glycerol in varied relative humidity desiccator; data shown are mean (SD), n=3

Films with glycerol	relative humidity for moisture sorption			
	8% RH	52% RH	75% RH	92% RH
0% RH CZ film	0.39 (0.72)	3.96 (0.07)	9.34 (2.01)	9.85 (3.12)
8% RH CZ film	1.52 (1.38)	5.53 (1.27)	11.30 (0.95)	12.16 (3.90)
52% RH CZ film	2.15 (0.21)	5.30 (0.35)	9.87 (1.12)	10.92 (2.77)
75% RH CZ film	3.35 (0.42)	6.15 (0.41)	12.38 (0.57)	17.75 (3.98)
92% RH CZ film	3.50 (0.43)	6.13 (0.62)	11.49 (1.32)	18.79 (2.99)
52% RH ZCGM film	2.85 (0.35)	4.89 (0.28)	11.22 (0.74)	13.79 (1.59)

Table 21 Mechanical properties of CZ and ZCGM films without/with glycerol in different relative humidity conditions; data shown are mean (SD), n=10

| RH (%)	Tensile strength (MPa)		Elongation at break (%)		Young's modulus (MPa)	
	Film without glycerol	Film with glycerol	Film without glycerol	Film with glycerol	Film without glycerol	Film with glycerol
0% RH CZ film	13.46 (3.67)	11.44 (3.37)	2.69 (0.33)	5.59 (1.77)	334.65 (69.22)	296.73 (72.77)
8% RH CZ film	9.57 (2.13)	11.20 (1.51)	2.46 (0.30)	5.34 (0.95)	310.53 (56.03)	239.37 (60.15)
52% RH CZ film	9.90 (4.55)	10.13 (2.44)	2.69 (0.80)	4.98 (0.92)	264.42 (46.44)	234.57 (38.30)
75% RH CZ film	12.38 (3.63)	5.79 (1.45)	3.44 (0.45)	6.67 (2.69)	259.36 (59.07)	173.70 (59.94)
92% RH CZ film	8.75 (2.47)	2.29 (0.46)	6.11 (2.87)	10.88 (5.23)	163.10 (40.11)	87.50 (20.03)
52% RH ZCGM film	11.41 (3.18)	11.81 (0.97)	2.75 (0.80)	32.99 (14.92)	184.10 (6.02)	174.48 (7.54)
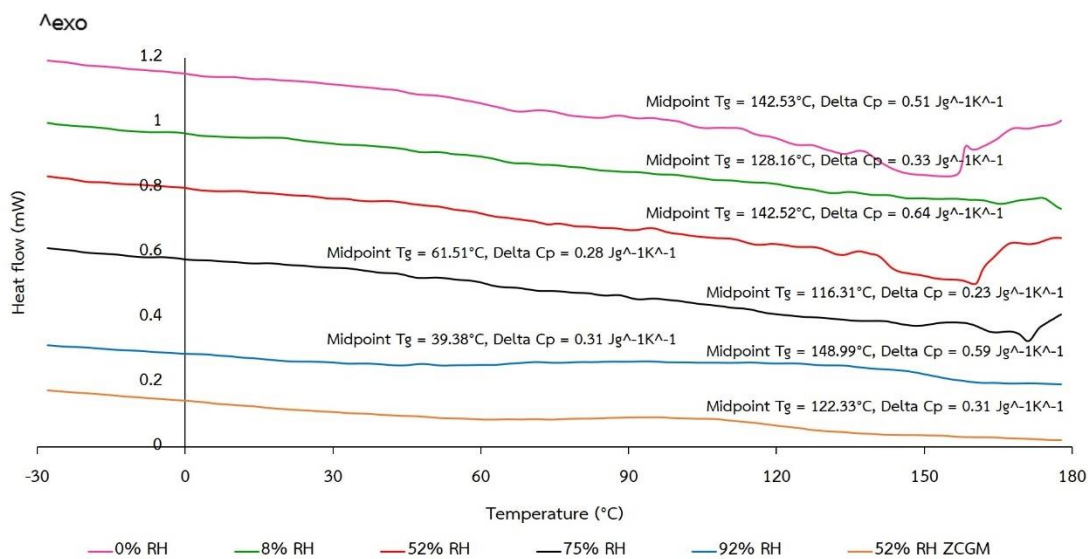
Figure 31 DSC thermograms of CZ films without glycerol in different relative humidity conditions and ZCGM film equilibrated at 52% RH, n=1
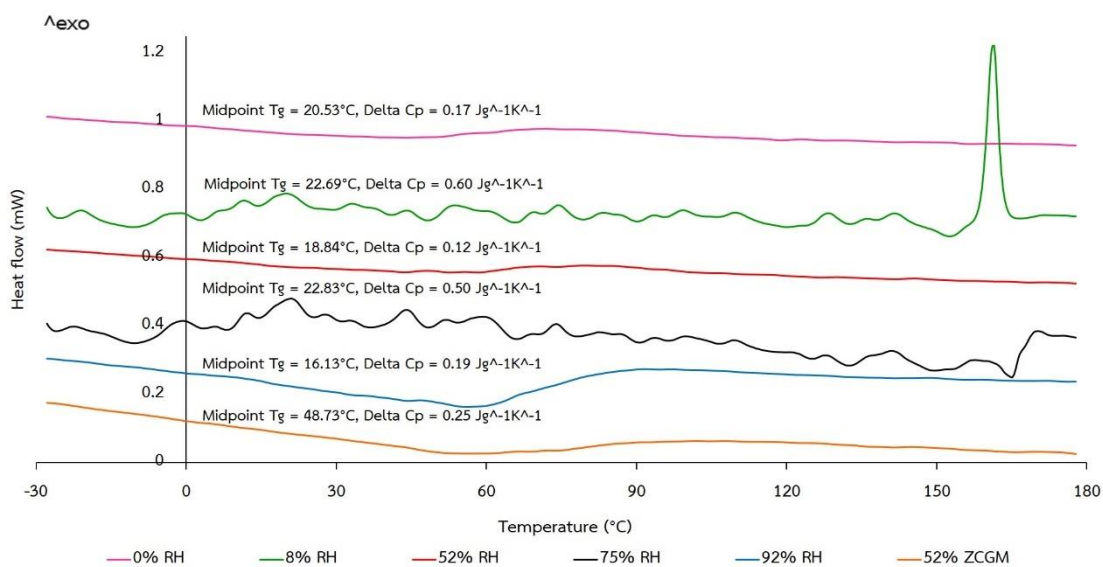

Figure 32 DSC thermograms of CZ films with glycerol in different relative humidity conditions and ZCGM film equilibrated at 52% RH, n=1

Table 22 Propranolol hydrochloride release (%) of dissolution test

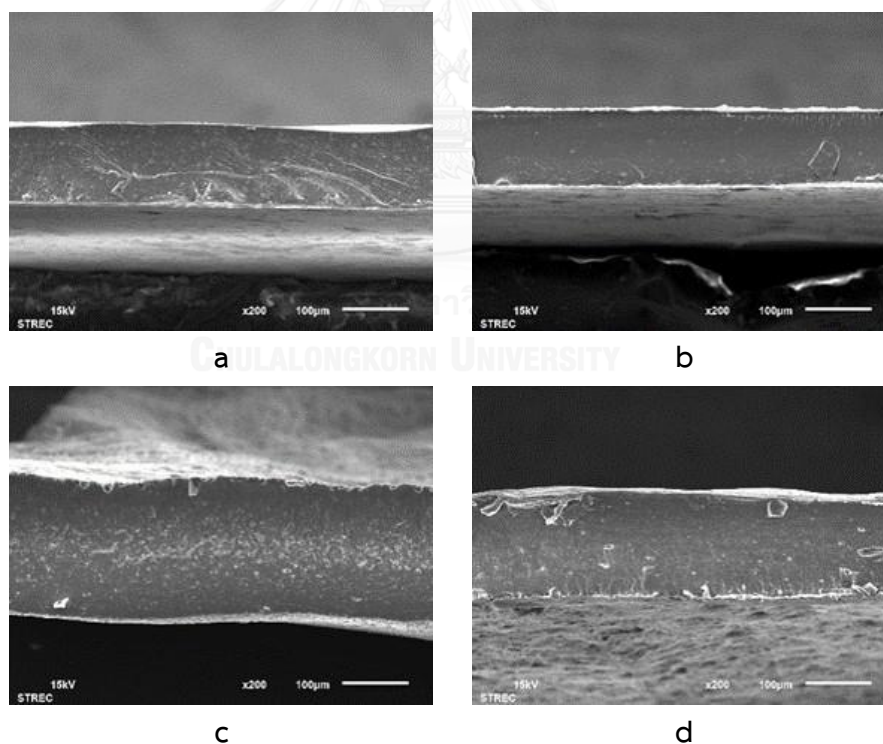| Time (h)	CZ capsule		ZCGM capsule	
	mean	SD	mean	SD
0.25	0.59	0.13	1.01	0.34
0.5	0.58	0.10	0.76	0.34
1.5	3.37	1.09	2.12	1.46
2	46.23	33.97	3.56	1.96
3	61.69	28.80	6.07	1.06
5	79.73	6.36	22.33	9.24
8	86.92	0.77	58.97	25.39

a

b

c

d

Figure 33 SEM micrographs of zein capsule with glycerol cross-section at the magnification of 200x; CZ capsule (a) and ZCGM capsule (b) equilibrated at 52% RH before dissolution; CZ capsule (c) and ZCGM capsule (d) after dissolution

VITA

Miss Unna Santhitiwanich was born in Bangkok, Thailand, on August 11th, 1989. She received her Bachelor of Science in Pharmacy in 2012 from the Faculty of Pharmaceutical Sciences, Chulalongkorn University, Bangkok, Thailand.   She presented a poster on the title of "Effect of moisture content on mechanical properties of corn protein films" in the 5th Burapha University International Conference on July 28-29, 2016 at Bangsaen, Chonburi, Thailand.

จุฬาลงกรณ์มหาวิทยาลัย
CHULALONGKORN UNIVERSITY