การเตรียมและฤทธิ์ต้านการเกาะกลุ่มแอไมลอยด์ของเคอร์คิวมินที่บรรจุในฟิล์มไฮโดรคอลลอยด์

นางสาวนัทธกานต์ คณะนา

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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PREPARATION AND ANTI-AMYLOID AGGREGATION ACTIVITY OF CURCUMIN LOADED HYDROCOLLOID FILMS

Miss Nattakan Kanana

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
Faculty of Science
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Thesis Title	PREPARATION AND ANTI-AMYLOID AGGREGATION ACTIVITY OF CURCUMIN LOADED HYDROCOLLOID FILMS
By	Miss Nattakan Kanana
Field of Study	Petrochemistry and Polymer Science
Thesis Advisor	Assistant Professor Pattara Thiraphibundet, Ph.D.

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

.....Dean of the Faculty of Science
(Associate Professor Polkit Sangvanich, Ph.D.)

THESIS COMMITTEE

.....Chairman
(Associate Professor Nuanphun Chantarasiri, Ph.D.)

.....Thesis Advisor
(Assistant Professor Pattara Thiraphibundet, Ph.D.)

.....Examiner
(Chatr Panithipongwut Kowalski, Ph.D.)

.....External Examiner
(Associate Professor Boon-ek Yingyongnarongkul, Ph.D.)

นัทธกานต์ คณะนา : การเตรียมและฤทธิ์ต้านการเกาะกลุ่มแอไมลอยด์ของเคอร์คิวมินที่บรรจุในฟิล์มไฮโดรคอลลอยด์ (PREPARATION AND ANTI-AMYLOID AGGREGATION ACTIVITY OF CURCUMIN LOADED HYDROCOLLOID FILMS) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: ผศ. ดร.พัฒทรา ธีรพิบูลย์เดช, 53 หน้า.

แผ่นฟิล์มเคอร์คิวมินเตรียมขึ้นจาก 0.2% เคอร์คิวมิน 3% ไฮดรอกซีโพรพิลเมทิลเซลลูโลส และ 1% พอลิไวนิลพิร์โรลิโดนและทำการศึกษาสมบัติทางกายภาพและเคมีของแผ่นฟิล์ม ภาพถ่ายจากกล้องจุลทรรศน์อิเล็กตรอนแบบส่องกราดที่มีสมรรถนะสูงชนิดฟิลด์อีมิสชัน ข้อมูลจากอินฟาเรดสเปกตรัม DSC และTGA ยืนยันการมีอยู่ของเคอร์คิวมินในแผ่นฟิล์มและการเกิดอันตรกิริยาภายในโมเลกุลระหว่างเคอร์คิวมินและสารก่อฟิล์ม นอกจากนี้แผ่นฟิล์มเคอร์คิวมินมีความเสถียรต่อพีเอชมากกว่าเคอร์คิวมิน สิ่งที่น่าสนใจคือความสามารถในการละลายน้ำของเคอร์คิวมินในแผ่นฟิล์มเคอร์คิวมินมีค่าเท่ากับ 3.1 ไมโครกรัม/มิลลิลิตรซึ่งสูงกว่าการละลายน้ำของเคอร์คิวมินอยู่ประมาณ $2.8X10^3$ เท่า ความคงตัวของอายุการเก็บรักษาของแผ่นฟิล์มเคอร์คิวมินได้รับการประเมินในช่วงเวลา 0, 15, 30, 60, 90 และ 120 วันโดยเก็บในถุงปิดสนิทชนิดโปร่งใสและทึบแสงที่อุณหภูมิห้อง พบว่าปริมาณเคอร์คิวมิน อินฟาเรดสเปกตรัมและTGA เทอร์โมแกรมไม่แสดงถึงการสลายตัวของสารสำคัญและไม่มีความแตกต่างอย่างมีนัยสำคัญระหว่างทั้งสองสภาวะ อย่างไรก็ตามความสามารถในการละลายน้ำและความคงตัวต่อพีเอชลดลงเล็กน้อยตามระยะเวลาการเก็บรักษา

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต .....

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5772026223 : MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
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NATTAKAN KANANA: PREPARATION AND ANTI- AMYLOID AGGREGATION ACTIVITY OF CURCUMIN LOADED HYDROCOLLOID FILMS. ADVISOR: ASST. PROF. PATTARA THIRAPHIBUNDET, Ph.D., 53 pp.

Cur film was fabricated using 0.2% of curcumin, 3% of HPMC and 1% of PVP and characterized their physical and chemical properties. The FE-SEM micrographs, FTIR, DSC and TGA data confirmed the presence of curcumin and intermolecular interaction between curcumin and film-forming polymers. In addition, cur film possessed better pH stability than curcumin. Interestingly, the water solubility of curcumin in the cur film was 3.1 μg/mL which higher than that of curcumin approximately 2.8×10^3 times. The shelf-life stability of cur film was evaluated at the interval times, 0, 15, 30, 60, 90 and 120 days by keeping in transparent and opaque sealed-bags at ambient temperature. It is found that curcumin amount, FTIR and TGA data do not showed the degradation of active compounds and are not significantly different between both storage conditions. However, the water solubility and pH stability slightly reduced as a function of storage time.

Field of Study: Petrochemistry and Polymer Science Student's Signature .....
Advisor's Signature .....

Academic Year: 2016

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LIST OF ABBREVIATIONS

HPMC	hydroxypropyl methyl cellulose
et al.	et alli, and other
PVP	polyvinylpyrrolidone
%	percentage
β	beta position
°C	degree celsius
cur film	curcumin film
cm^{-1}	unit of wavenumber (IR)
ng	nanogram
Fig	figure
ATR-FTIR	attenuated total reflection fourier transform infrared
PEG	polyethylene glycol
MβCD	methyl beta-cyclodextrin
HPβCD	hydroxypropyl-beta-cyclodextrin
CS-G NPs	chitosan-gelatin nanoparticles
WPI	whey protein
ASD	amorphous solid dispersion
CMC	carboxymethyl cellulose
wt	weight
mg	milligram

mL	milliliter
min	minute
nm	nanometer
rpm	revolution per minute
h	hour
FE-SEM	field emission scanning electron microscopy
kV	kilovolt
TGA	thermogravimetric analysis
DSC	differential scanning calorimetry
Aβ	amyloid beta
DMSO	dimethylsulfoxide
μM	micromolar
μL	microliter
ppm	parts per million
mm	millimeter
DPPH	2,2-diphenyl-1-picrylhydrazyl
mM	millimolar
ΔE	total color difference
T-	transparent sealed-bags
O-	opaque sealed-bags
TG	thermogravimetric

DTG	thermogravimetric derivative

CHAPTER I

INTRODUCTION

1.1 Motivation of research

Curcumin is a hydrophobic polyphenol obtained from the rhizomes of *Curcuma longa* L. Curcumin exhibits a broad spectrum of pharmacological activities such as anti-bacterial, anti-inflammatory, anti-amyloid and anti-cancer properties [1]. Nevertheless, curcumin has low bioavailability due to its low water solubility (11 ng/mL). It is also sensitive to pH and organic solvents [2]. The low solubility of curcumin is an important drawback to use in many applications such as in food and pharmaceutical products. To overcome this weakness, curcumin is trapped in vesicles in many forms such as nanoparticles, micelle [3], complexation [4], emulsion [5] and liposome [6]. These vesicles not only enhance the solubility but also protect curcumin from adverse environment [7].

Polymeric encapsulation is commonly used to improve the water solubility of hydrophobic substances. There are many studies reported the improvement of curcumin solubility by this technique. For examples, Cur@EPO was prepared by encapsulation curcumin in the Eudragit® E PO by solid dispersion method. Cur@EPO had not only 7,500-fold higher water solubility than curcumin but also higher stability toward pH and UV irradiation [8]. Curcumin microparticles using polysorbate 80 and soy lecithin can be soluble in water higher than curcumin powder about 2.3×10^5 folds

[9]. These vesicle polymers are normally amphiphilic polymers. Amphiphilic polymers have been known to use as the drug vesicles by forming the fine particle dispersion [10]. The hydrophobic side chains normally attach to the backbone and trap the hydrophobic drug inside while the hydrophilic segments are responsible for the hydration. However, most final products from these technique are in the forms of dry powder and colloid solution. The disadvantage of the powder form is caking or stickiness which caused by the aggregation and degradation of the core substance or vesicles [11]. Emulsion solution is bulky and need large space storage. Moreover, active compound is easily degraded by the emulsion matrix.

Thus, the objective of this research is to entrap curcumin in the edible polymer film by solvent casting method. The HPMC and PVP are used to prepare the encapsulated curcumin particles in an aqueous solution and then casting to obtain curcumin film (cur film). This cur film can re-dissolve in the water and give the curcumin particles which can disperse in the aqueous medium. This is a new platform to prepare water soluble curcumin in place of curcumin powder or emulsion for food and cosmetic applications. Films are easier handle than emulsion and have no caking which is normally observed in powder. Moreover, cur film can be used as curcumin tea for healthy drink. This cur film has higher solubility and pH stability than curcumin powders due to lower surface area per volume. Furthermore, the shelf-life stability of cur film is evaluated for 120 days by keeping film in transparent and opaque seal-bags.

CHAPTER II

THEORY AND LITERATURE REVIEWS

The purpose of this research is to entrap curcumin in the polymer matrix and fabricate in the form of edible film by solvent casting method. The HPMC and PVP are used to prepare the encapsulated curcumin particles in an aqueous solution and then casting to obtain curcumin film (cur film). This cur film can re-dissolve in the water and give the curcumin particles which can disperse in the aqueous medium. This is a new platform to prepare water soluble curcumin in place of curcumin powder or emulsion for food and cosmetic applications. Films are easier handle than emulsion and have no caking which is normally observed in powder. This chapter presents the theory and literature reviews involved in this research.

2.1 Curcumin

Curcuminoids are hydrophobic yellow-orange polyphenolic compounds extracted from the rhizomes of *Curcuma longa* L (Zingiberaceae). Curcuminoids are the mixture of curcumin, demethoxycurcumin, and bisdemethoxycurcumin (Fig 2.1) which curcumin is the main component (75%) [12]. Curcumin is used as an herbal medicine in many Asian countries for thousands of years [13]. Curcumin has a broad spectrum of bioactivities such as anti-inflammatory, anti-bacterial, anti-oxidant, antimicrobial, and anti-cancer properties [14]. However, curcumin has low water solubility (11 ng/mL), low bioavailability, be sensitive to light, pH, organic solvent and

oxygen**.** Curcumin is stable in acidic condition due to the conjugated diene structure. Curcumin degrades quickly in neutral or alkaline buffer solution. When pH is basic condition, the protons are removed from the phenolic group and leading to structural damage. The major degradation product of curcumin is *trans*-6-(4'-hydroxy-3'-methoxyphenyl)-2,4-dioxo-5-hexenal and minor degradation products are vanillin, ferulic acid and feruloyl methane (Fig 2.2). Curcumin rapidly degraded into vanillin, vanillic acid, ferulic aldehyde and ferulic acid upon exposure to light [15] (Fig 2.2). This limits its applicability on industrial scale and its expectation on the shelf-life products. Moreover, curcumin has low bioavailability due to low solubility, poor absorption, rapid metabolism, inactivity of metabolites, and finally rapid clearance outside the body.

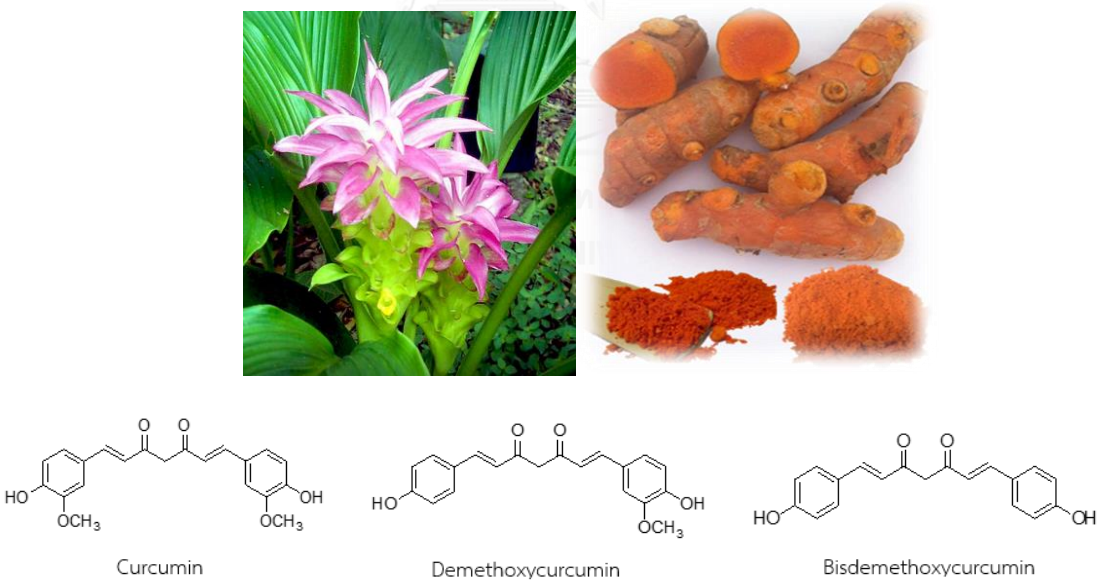


Figure 2.1 The flower and rhizome of *Curcuma longa* Linn., and chemical structures of main components.

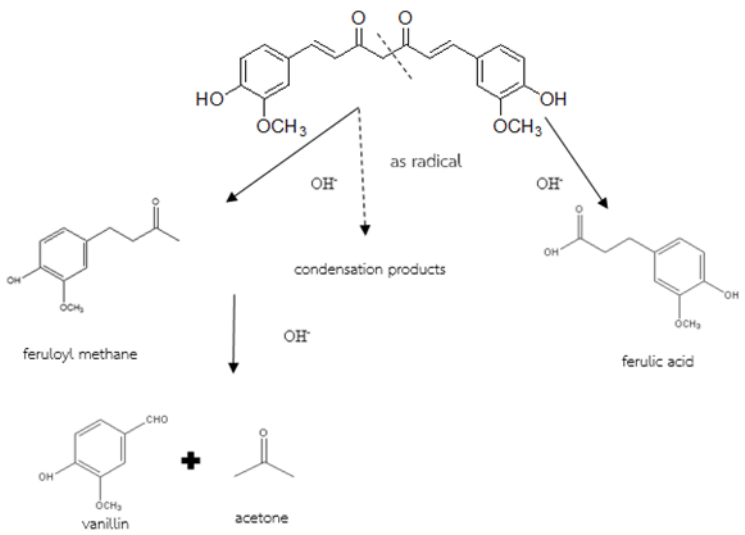


Figure 2.2 Chemical structures of degraded products of curcumin in alkaline buffer solution [16]

2.2 Improvement water solubility of curcumin by encapsulation

Encapsulation technology has received considerable interest in wide range of disciplines and in numerous fields of applications such as pharmaceutical, food, cosmetic, agricultural, electronic, and molecular diagnostic applications. Encapsulation may be defined as a process to entrap one substance (active agent) within another substance (wall material). The encapsulated substance can be called the core, fill, active, internal or payload phase. The substance that is encapsulating is often called the coating, membrane, shell, capsule, carrier material, external phase, or matrix [17-19]. The utilization of encapsulation is to protect of core material from adverse environmental conditions, and including enhance the solubility. The low water solubility of curcumin is an important drawback to use in food industry and pharmaceutical application. To overcome this limitation, curcumin encapsulation had

been reported in many studies. Table 2.1 summarized some reports that studied the ways to increase curcumin solubility. The efficacy of each method not only depends on the ways to encapsulation but also the kinds of matrix.

Table 2.1 The result of curcumin solubility enhancement obtained by the different methods.

Method	Substances	Curcumin solubility enhancement comparing to native curcumin (times)	Reference
Solid dispersion	Hydroxypropyl methylcellulose acetate succinate (HPMCAS)	12	[20]
Solid dispersion	Hydroxypropyl methyl cellulose (HPMC), lecithin and isomalt	>1000	[21]
Co-precipitation, freeze-drying and solvent evaporation	Beta-cyclodextrin	31	[22]

Spray drying	Whey protein isolate	11,355	[23]

Table 2.1 The result of curcumin solubility enhancement obtained by the different methods. (continue)

Method	Substances	Curcumin solubility enhancement comparing to native curcumin (times)	Reference
Nanoprecipitation	Eudragit® EPO	20,000	[24]
Nanosuspensions method	D-α-Tocopherol polyethylene glycol 1000 succinate (TPGS)	400	[25]
Kneading method	Methyl beta-Cyclodextrin (MβCD) and hydroxypropyl-beta-Cyclodextrin (HPβCD) complexes	202	[26]
Solid dispersion	Alginate and gelatin	10,000	[27]
Solid dispersion	Polyethylene glycol (PEG) and encapsulated into	2,000	[28]

	chitosan-gelatin nanoparticles (CS-G NPs).		
Nanosuspension	Tween 80	216	[29]

Table 2.1 The result of curcumin solubility enhancement obtained by the different methods. (continue)

Method	Substances	Curcumin solubility enhancement comparing to native curcumin (times)	Reference
Nanosuspension	Tween 80	216	[29]
Facile solution mixing	starch nanoparticles (cur@star)	715	[30]
Antisolvent precipitation	Polyvinylpyrrolidone (K30)	20	[31]
Solid dispersion	Soy protein isolate	812	[32]
Derivative synthesis	Hyaluronic acid	360	[33]

	3-dicyclohexylcarbodiimide (DCC) 4-dimethylaminopyridine (DMAP)		
Solid dispersions	Polyethylene glycol Polyvinylpyrrolidone (K30)	1,000	[34]
Electrohydrodynamic atomization	Gelatin	38.6	[35]

Table 2.1 The result of curcumin solubility enhancement obtained by the different methods. (continue)

Method	Substances	Curcumin solubility enhancement comparing to native curcumin (times)	Reference
Solid dispersion	Cyclodextrin complex	10,000	[36]
Microemulsification	Polysorbate 80 and soy lecithin	2.3×10^5	[9]

Solid dispersion	Eudragit®E PO polymer	7,500	[8]
Solid dispersion	Sodium caseinate (NaCas)	40	[37]
Solid dispersion	γ-cyclodextrin (γ-CD) and hydroxypropyl-γ -cyclodextrin (HP-γ -CD)	60	[38]

2.3 Hydrocolloid film

Hydrocolloids are hydrophilic polymers which are obtained from animal, vegetable, microbial or synthetic origin such as alginate, pectin, gum arabic, gelatin, carrageenan, methyl cellulose, carboxymethylcellulose, hydroxypropyl methylcellulose, gellan gum, pectin and xanthan gum. Nowadays, they are widely used for performing and controlling the texture, flavour, and shelf-life of many kinds of foods [39]. Moreover, hydrocolloidal materials such as proteins and polysaccharides are used extensively for the formation of edible films and fruit/vegetable coatings [40].

In this work, hydroxypropyl methyl cellulose (HPMC) and polyvinylpyrrolidone (PVP) were used as film forming agents. Thus, we gave the information and some previous reports of these hydrocolloids.

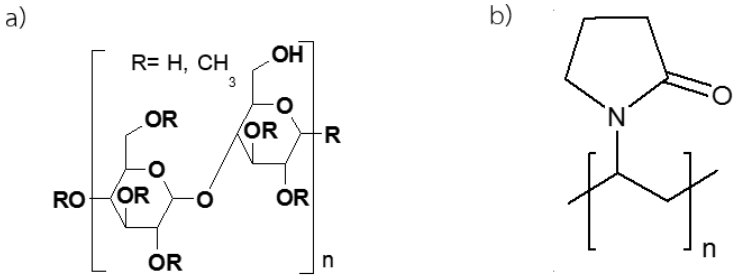


Figure 2.3 Chemical structures of a) hydroxypropyl methyl cellulose and b) polyvinylpyrrolidone

Hydroxypropyl methyl cellulose or HPMC (Fig.2.3a) is a water-soluble cellulose which has good biocompatibility, biodegradability and film forming properties. HPMC is used as a film former, emulsifier, stabilizer or thickener in food and pharmaceutical industries [41]. In addition, HPMC have been used for fabricating the edible food packaging and preparing micro- or nano-particles for drug delivery system. The collagen/HPMC blend film had higher thermal and mechanical properties than collagen film [42]. Adding HPMC in whey protein (WPI) edible film improved 15 folds of the tensile strength and 29 folds of elastic modulus [43]. Jefferson and coworkers studied mechanical and thermal properties of chitosan–HPMC films plasticized with sorbitol. The higher proportion of HPMC in the chitosan–HPMC films enhanced thermal properties but mechanical properties was not significantly different from chitosan film [44]. Gabriel and coworker used HPMC and β-cyclodextrin to improve the water solubility of norfloxacin. The addition of HPMC/β-cyclodextrin enhanced the water solubility of norfloxacin but more HPMC addition above 5% (w/w) reduced norfloxacin

solubility [45]. Chuah et al. prepared an amorphous solid dispersion (ASD) of curcumin using HPMC, lecithin and isomalt. ASD curcumin had the higher water solubility about 1000 times than pure curcumin. The water-soluble enhancement of ASD curcumin was found to increase bioavailability and bio- efficiency [21].

Polyvinylpyrrolidone or PVP (Fig.2.3b) is a nontoxic polymer, non-ionic, water soluble with an excellent transparency, biocompatibility and film-forming ability [46]. PVP is used in food, pharmaceutical, cosmetic and other industries. The PVP/ carboxymethyl cellulose (CMC) hydrogel film is transparent and flexible. This film showed good mechanical properties as well as biodegradability [47]. Zivanovic and colleagues studied the effect of PVP and polyethylene oxide (PEO) to the properties of chitosan films. The increasing of PVP content did not significantly enhance the elasticity of the blend film. In contrast, PEO barely altered on the flexibility but largely affected the elasticity of the chitosan/PEO film [48]. The HPMC/PVP blend film is flexible and can be used as food packaging as well as drug delivery, biomaterial and tablet coating [49-51]. Modasiya and Patel enhanced the water solubility of curcumin using polyethylene glycol and PVP by different solid dispersion techniques. It was found that the hot melt method gave the best improvement in which the water solubility of curcumin increased by 1000 folds [34].

CHAPTER III

EXPERIMENTALS

3.1 Chemicals and Materials

Curcumin with 95% purity was purchased from Welltech Biotech Company (Yannawa, Bangkok). Hydroxypropyl methylcellulose (HPMC) was purchased from a weikem company (Jiang, China). Polyvinylpyrrolidone (PVP) and xanthan gum (XG) were purchased from Ruam chemical 1986 Co., Ltd. (Pranakhon, Bangkok), respectively. All organic solvents were AR grade.

3.2 Preparation of curcumin film

From the preliminary test, the best ratio of film-forming polymers was 3%wt of HPMC and 1%wt of PVP. Film had smooth surface and be easily peeled off. Moreover, it can trap curcumin and gave homogeneous film. Thus, this ratio of film-forming polymers was then used for further study.

The film-forming solution was prepared by dissolving 3% HPMC and 1% PVP in distilled water at 30 °C. Curcumin solution was prepared by dissolving curcumin in ethanol in the ratio of 50 mg:1 mL prior to add in the film-forming solution. Four different concentrations of curcumin, 0.05, 0.1, 0.2 and 0.5%wt, were studied. The mix solution was then homogenized at 3500 rpm for 3 minutes. The final mixture was poured in a silicone plate (10 cm ×17 cm) and dried at 50 °C in an oven for 14 h to obtain cur film. Then, 20 mg of each cur film were dissolved in 30 mL of distill water

at room temperature with magnetic stirring for 24 h. The solution was centrifuged at 4000 rpm for 10 min. The supernatant was analyzed the absorbance at 420 nm using UV -Vis spectrophotometer. The cur film which gave the highest absorbance intensity will be further study and characterization. Moreover, the 3% HPMC and 1% PVP film without curcumin was prepared as control film.

3.3 Characterization of films

3.3.1 Water solubility

Three square pieces (5×5 cm^2) were cut and dried at 105 ºC for 24 h. The samples were weighed to gain the initial weight (W_i). Then, samples were immersed in 30 mL of distilled water with mild stirring at room temperature for 24 h. After filtration, the undissolved samples were dried at 105 ºC for 24 h to obtain the dry matter weight (W_f). The water solubility of film was determined by the following expression:

$$\%\text{water solubility} = [W_i - W_f / W_i] \times 100 \quad (3.1)$$

Where W_i is the initial weight of the dry matter and W_f is the dry matter weight of the dispersion process after 24 h.

3.3.2 Moisture content

Moisture content of films was determined by the loss of weight of the film after drying at 105 ºC for 24 h. The weight of film before and after drying was calculated for the moisture content using equation (3.2).

$$\%\text{ moisture content} = [W_0 - W_1 / W_0] \times 100 \quad (3.2)$$

Where W_0 and W_1 were the weight of film before and after drying, respectively.

3.3.3 Thickness

Film thickness was determined with a digital electronic vernier caliper micrometer (Mituto, Tokyo, Japan) with a sensitivity of 0.01 mm. Each film sheet was measured at different points at least five random locations and results reported as mean and standard deviation.

3.3.4 Attenuated total reflection fourier transform infrared (ATR-FTIR) spectroscopy

The ATR-FTIR spectra of curcumin and films were recorded at wavenumber range of 800 – 4000 cm^{-1} at resolution of 4 cm^{-1} using a PerkinElmer Spectrum 100 spectrophotometer. Each spectrum was obtained by averaging 16 scans.

3.3.5 Field emission scanning electron microscopy (FE-SEM)

The morphological examination of films was performed by field emission scanning electron microscopy JSM – 7610F (FE-SEM, JEOL, Tokyo, Japan) at an accelerating voltage of 2 kV mode GB high.

3.3.6 Thermogravimetric analysis (TGA)

The thermogravimetric characteristic of films was measured by TGA (SDTA851e, Mettler Toledo, Columbus, USA). This technique was used to determine the onset temperature of overall thermal degradation (T_d) of films. The sample was heated from 30 to 600 °C at the rate of 10 °C /min with nitrogen gas purged at 30 mL/min.

3.3.7 Differential scanning calorimetry (DSC)

Thermal transition properties of films were measured by a model Q20 differential scanning calorimeter (TA Instrument, New Castle, USA). Sample powder (about 8.0 mg) was weighted and sealed in an aluminum DSC pan. DSC scanning was performed from 30 °C to 300 °C at a heating rate of 10 °C /min under dry nitrogen.

3.3.8 Color

The color of the films was determined using a colorimeter (Minolta Chroma Meter CR-400 Japan), at five random locations of the films. A colorimeter was used to measure CIE L* a* b*. Results were expressed in terms of L* value, that indicates the lightness [black (L*=0) and white (L*=100)], "a*" value that indicates redness–greenness [total red (a*=100) and total green (a*=-100)], and "b*" value that indicates yellowness–blueness [total yellow (b*=100) and total blue (b*=-100)]. The measurements were taken on white standard backgrounds (L* = 90.45, a* = -0. 61 and b* = -5.00). Total color difference (ΔE) was calculated using the equations (3.3).

$$\Delta E = \sqrt{(L^* - L)^2 + (a^* - a)^2 + (b^* - b)^2} \qquad (3.3)$$

Where L*, a*, and b* are the color parameter values of the film and L, a, and b are the color parameter values of the standard.

3.3.9 pH stability

Preparing curcumin and cur film in the water: ethanol (70: 30) solution. The sample solution was adjusted to the pH 4, 7 and 10 using phosphate buffer solutions.

The absorbance at 420 nm of each sample was determined at the interval times, 0, 1, 1.5, 2, 24 and 48 h.

3.4 Water solubility of curcumin incorporated in cur film

Cur film was dissolved in distilled water at room temperature to obtain stock solutions with the concentrations of 10, 20, 30, 40, 50 and 60 ppm. These solutions were centrifuged at 5000 rpm for 10 min to allow the sediment of undissolved curcumin. The supernatant was analyzed for curcumin amount using UV- visible spectrophotometer at 420 nm. Then plot the relationship between the concentration and absorbance of curcumin to obtained the maximum water solubility of curcumin particles.

3.5 Bioactivity tests.

3.5.1 amyloid beta (1-42) aggregation assay

Preparation amyloid peptides

Amyloid beta (1-42) human peptide was prepared by the method described in the previous report [52]. The Aβ (1-42) human peptide powder was dissolved in 100% of 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and incubated at room temperature for 1 h. Next, sonicated in a water bath for 10 min to obtain the concentration of 1 mg/mL. The HFIP solution was dried under a gentle stream of nitrogen gas. Then, this HFIP-treated amyloid β (1-42) was dissolved in dimethylsulfoxide (DMSO) to adjust the concentration as 1 mg/mL and store at -20 °C. For a working solution, add 1x PBS

(phosphate buffer saline, pH 7.4) to final concentration at 20 μM to the peptide stock solution and mixed with sample before incubating at 37 °C for 28 h.

Preparation of test samples

The samples were weighed 1 mg of pure curcumin or 20 mg of cur film (containing 1 mg of curcumin). Curcumin was dissolved in DMSO. Cur film dissolved in distilled water and DMSO. The concentration of test sample was 100 μM.

Assay

Thioflavin T (ThT) dye was used to determine the presence of amyloid-like aggregates. The fluorescence emission of ThT is shifted when ThT binds to β-sheet aggregate structures. The amyloid β (1-42) aggregation potential of samples was determined by the method described in a previous report [52]. Briefly, 80 μL of the incubated samples (the HFIP-treated Aβ 42 mixed with sample and incubated at 37 °C for 28 hours) and 20 μL of 50 μM ThT in 500 glycine-NaOH buffer (pH 8.5) were added in 96-well plates. Then the measurement of fluorescence intensity was carried out (λ_{exc}= 450 nm, λ_{em}= 490 nm) by microplate reader (Cary Eclipse Fluorescence Spectrophotometer) and values at the plateau were calculated after subtraction of the background fluorescence of the 50 μM thioflavin T solution. Each experiment was done in triplicate. The percentage inhibition was calculated according to the equations (3.4.)

$$\% \text{ Inhibition} = [(A_{A\beta} - A_{sample}) / A_{A\beta}] \times 100 \qquad (3.4)$$

Where $A_{A\beta}$ is the absorbance of amyloid beta (1-42) human peptide and A_{sample} is absorbance of the sample.

3.5.2 DPPH radical-scavenging activity

Preparation sample

The 1.4 mg of pure curcumin and 30 mg of cur film (containing 1.4 mg of curcumin) were weighed. Curcumin was dissolved in 10 mL of methanol and cur film dissolved in 10 mL of distilled water. The concentration of curcumin was 140 ppm.

Assay

The radical scavenging activity of curcumin and cur films were measured by the method of [53]. This test is based on the hydrogen atom or electron donation abilities, and is evaluated by measuring the colorimetric changes (from deep-violet to light-yellow) on methanol solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH). Briefly, 20 μL of sample solution was added in a 96 wells microplate. Then, 100 μL of 0.2 mM DPPH solution in methanol was added and incubated in a dark place at ambient temperature for 60 min. The absorbance was measured against methanol (blank) at 517 nm and the percentage of DPPH radical scavenging activity was achieved by following equation:

$$\% \text{ DPPH scavenging activity} = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100 \qquad (6)$$

Where A_{blank} is the absorbance of blank solution and A_{sample} is the absorbance of sample.

3.6 Shelf stability

Cur films were kept in the transparent and opaque sealed-bags at 40ºC for 120 days. The chemical and physical properties of samples were characterized at any times that showed in Table 3.1.

Table 3.1 Characterization of the cur films kept in the T- and O- sealed- bags

Characterization	Storage time (day) at 40ºC					
	0	15	30	60	90	120
Thickness	√	√	√	√	√	√
Moisture content	√	√	√	√	√	√
Solubility in water	√	√	√	√	√	√
Color	√	√	√	√	√	√
curcumin amount	√	√	√	√	√	√
FE-SEM	√	-	√	-	-	√
TGA	√	-	√	-	-	√
DSC	√	-	√	-	-	√
FTIR	√	-	√	-	-	√
DPPH	√	-	-	-	-	√
anti-amyloid	√	-	-	-	-	√
pH stability	√	√	√	-	-	√

√ = Evaluated - = Not evaluated

3.7. Statistical Analysis

Quantitative data were reported as means ± standard deviations, where indicated. Statistical analysis was performed using a one-way Anova analysis, followed by the Turkeys HSD for multiple comparisons. A p-value <0.05 was considered statistically significant.

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Thickness, moisture content and water solubility of film

From the preliminary test, the best ratio of film-forming polymers was 3% HPMC and 1% PVP which gave a smooth and homogeneous film. Then, several concentrations of curcumin were incorporated in film which was further determined the curcumin amount in the re-dissolved cur film solution. It was found that the 0.05, 0.1, 0.2 and 0.5% cur films gave the 0.756 ± 0.09, 0.809 ± 0.02, 0.972 ± 0.08 and 0.583 ± 0.14 absorbance intensity, respectively. Due to the highest intensity, the 0.2% cur film was chosen for further study. We will use “cur film” instead of 0.2% cur film in the following study.

The thicknesses of the control film and the cur film were 0.19±0.03 mm and 0.27±0.06 mm respectively. Curcumin was entrapped into the networks of film-forming polymers resulted in the increasing of film thickness. The moisture contents of the control film and the cur film were 12.7% and 4.1%, respectively. Most residues in dry cur film were curcumin which has higher boiling point than water. The water solubility of cur film was 97.5±1.25% which was slightly higher than that of the control film (93.1% ±3.25). These results suggested that the incorporating hydrophobic substances interrupted the intermolecular association of polymer chains in the films and brought about the faster water solubility [54].

4.2 Attenuated total reflection fourier transform infrared (ATR-FTIR) spectroscopy

The FTIR spectra of the control film (Fig.4.1a) revealed the C=O band at 1649 cm^{-1}, the C-H stretching band at 2919 cm^{-1} and the OH stretching broad band at 3443 cm^{-1} in the agreement of the previous study [50]. From the cur film spectra (Fig.4.1b), the enol-carbonyl stretching peak of curcumin shifted to 1602 cm^{-1} and the significant bands of film-forming polymers also shifted to 2916 and 1652 cm^{-1}. The FTIR spectra of curcumin (Fig.4.1c) showed the enol-carbonyl stretching vibration peak at 1623 cm^{-1}, anti-symmetrical CH_2 stretching peak at 2942 cm^{-1} and broad phenolic –OH stretching vibration peak at ~3506 cm^{-1}.This could be confirmed the incorporation of curcumin in film and the interaction between HPMC/PVP polymers and curcumin.

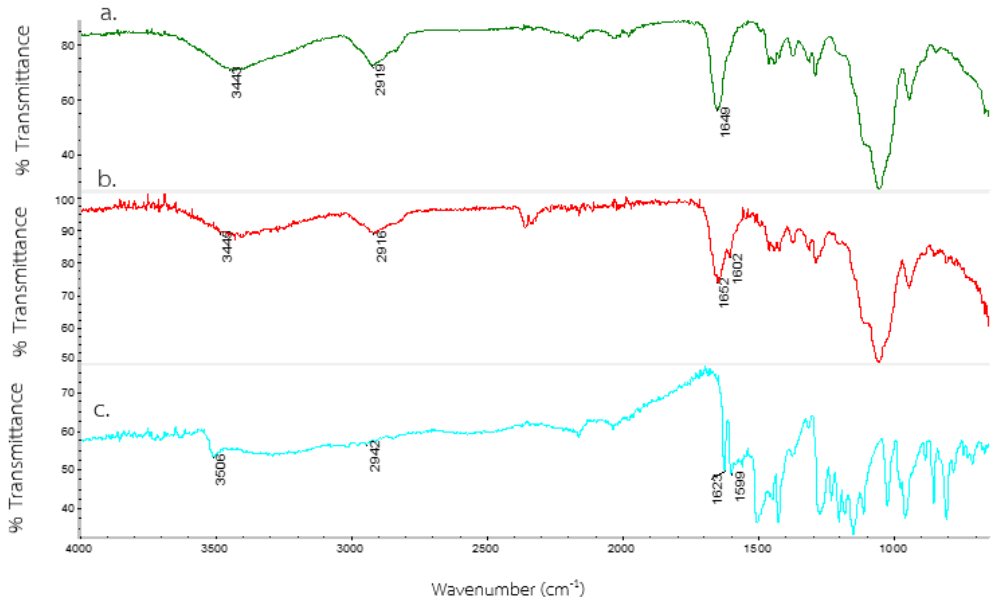


Figure 4.1 FTIR spectrum of a) control film, b) cur film and c) curcumin

4.3 Field emission scanning electron microscopy (FE-SEM)

Analysis the surface and cross-section of films by FE-SEM, the control film by were smooth and homogeneous (Fig.4.2a) while those of cur film was rough, heterogeneous and some curcumin aggregation was found on the surface (Fig.4.2b). After dissolving the cur film in water, we found that the curcumin particles can well disperse in the water which was different from curcumin that is immiscible in the water as showed in Figure 4.3.

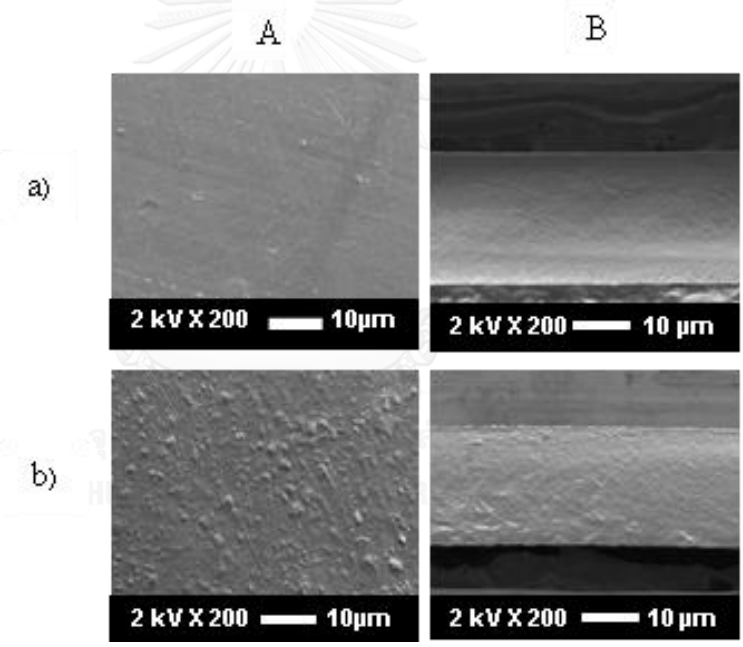


Figure 4.2 FE-SEM micrographs of surfaces (left, A) and cross-section (right, B) of a) control films and b) cur film

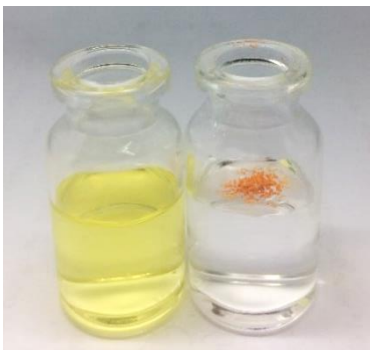


Figure 4.3 The water solubility of cur film (left) and curcumin (right)

4.4 Thermal stability

The weight loss and first derivative of thermal gravity curves of samples were shown in Fig. 4.4. The control and cur films showed weight loss of moisture at ~100 ℃ and thermal degradation of film-forming composition from 300 to 450℃. The mass loss evolution of cur film was 2.3% higher than that of control film which confirmed the presence of curcumin mass in film. Moreover, the thermal degradation of curcumin was absence which indicated the homogeneous phase between curcumin and film-forming polymers.

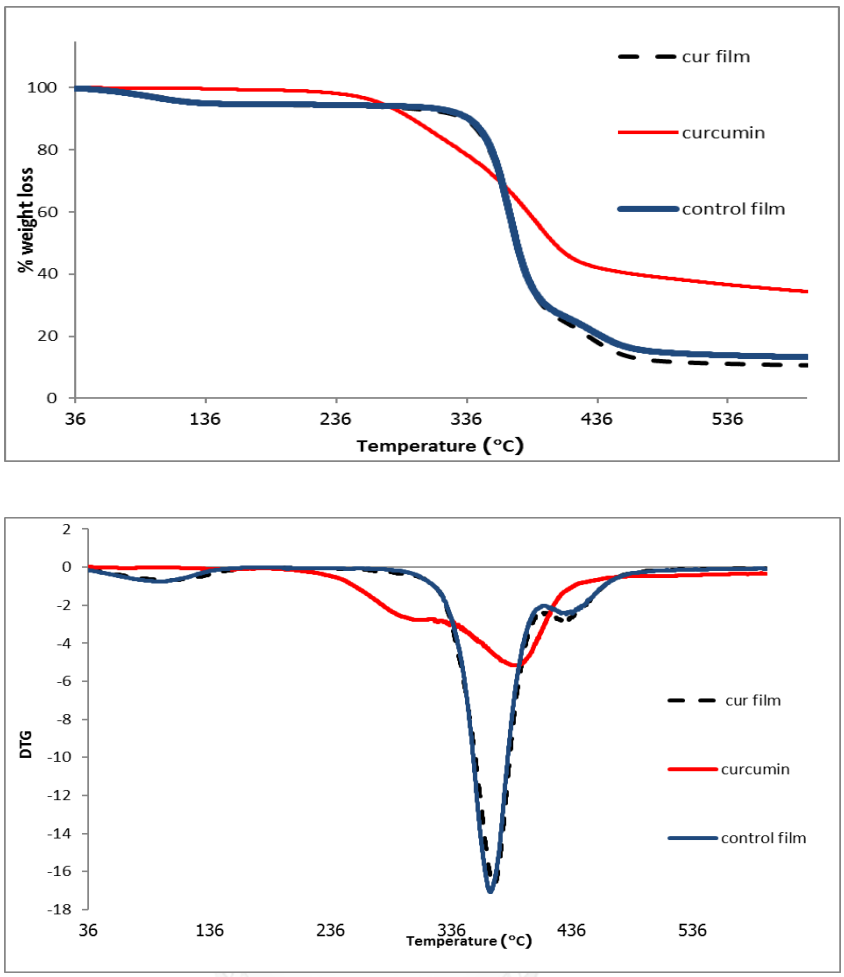


Figure 4.4 TG and DTG curves of cur film, curcumin and control film

Figure 4.5 represented the DSC thermograms of cur film, curcumin and control film. Curcumin showed an endotherm peak (Tm) at 175.4°C. The Tm of the cur film (93.5°C) was slightly higher than that of the control film (91.5°C) which indicated the weak interaction between curcumin and film-forming polymers.

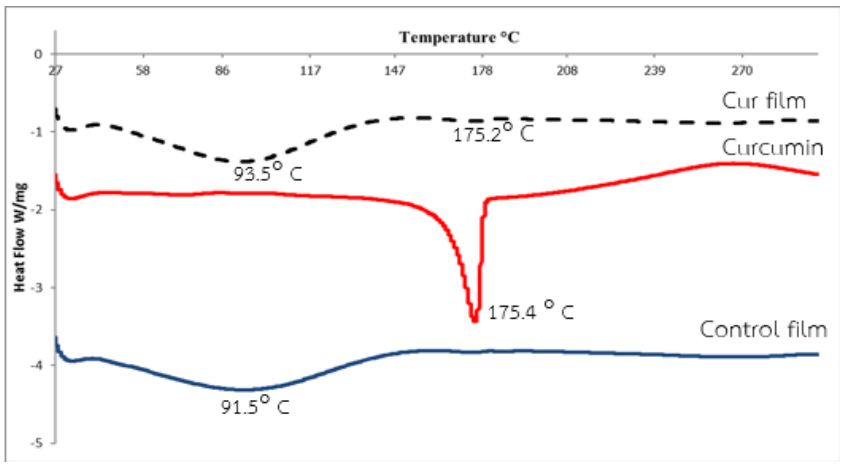


Figure 4.5. Differential scanning calorimetry (DSC) results of cur film, curcumin and control film

4.5 Appearance and color

The control film was transparent and colorless whereas the cur film was yellow due to the color of curcumin (Fig.4.6). The film color was detected using Hunter L*, a*, b* scale. The L* value of the control film was 84.34±0.89 and that of the cur film was 67.39±1.46. This indicated the more transparency of the control film over that of the cur film. The yellowness +b* value of cur film was 66.00±0.91 while that of the control film was 5.54±1.29. And the greenness +a* value of the cur film (21.21±0.053) was slightly higher than that of the control film (0.58 ± 0.09).

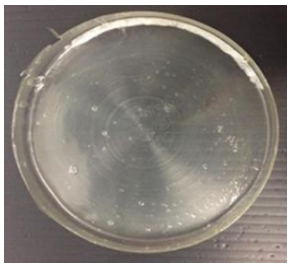
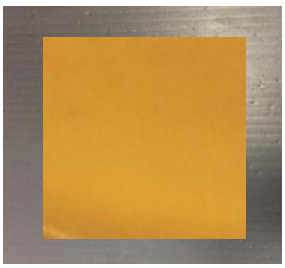
a.  b. 

Figure 4.6 The appearance of a) control film and b) cur film

4.6 pH stability

It has been known that curcumin degrades quickly in neutral or alkaline buffer solution [22]. In this study, we expected that the film-forming polymers can protect curcumin from the adverse condition. The cur film was dissolved in the water: ethanol (70:30) solution and evaluated the pH stability at the pH 4, 7 and 10 comparing to the curcumin solution (Fig.4.7). The absorbance of these solutions was measured at 420 nm. Although curcumin has been reported to be chemically stable under acidic conditions, the degradation of curcumin solution at pH 4 was observed after the first 2 h (Fig.4.7a). At this pH, the cur film solution showed the good stability until 48 h. The cur film solution showed significantly higher stability than curcumin solution at pH 7 and 10. At pH 7, the curcumin solution began being damage after 1 h incubation whereas the cur film solution degraded after 2 h (Fig.4.7b). Both the curcumin and cur film solutions degraded immediately at pH 10 which could be observed by the color change of solution from yellow to red, however, the cur film solution showed better stability (Fig.4.7c). In basic solution, the protonation at the carbonyl groups of curcumin leads to structural damage. The results indicated the potential of HPMC and PVP on

the pH stability of curcumin by retarding and shielding curcumin molecule from the alkaline environment.

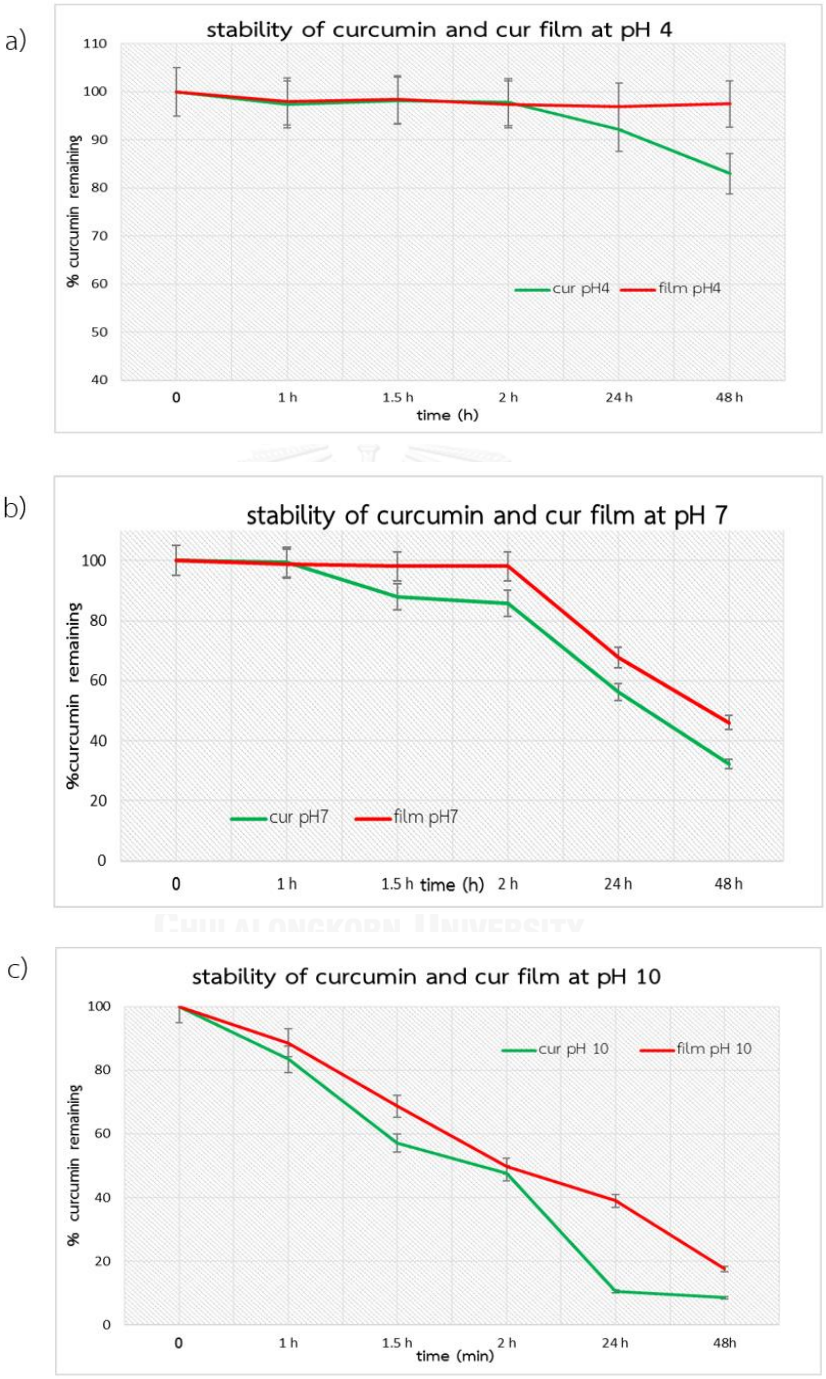


Figure 4.7 Cur film and curcumin stability at a) pH4, b) pH7 and c) pH10

4.7 Water solubility of curcumin

Figure 4.8 showed the highest absorbance of cur film solution at 31 ppm or 3.1 μg/mL which was 2,810-fold higher than the water solubility of curcumin (11 ng/mL) [13].

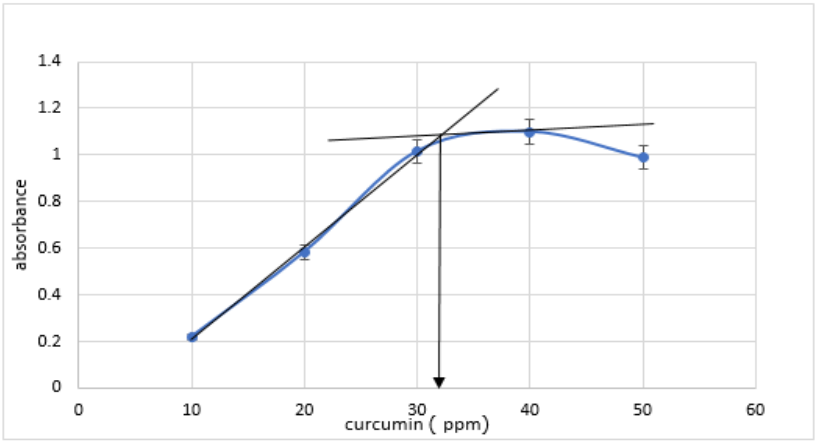


Figure 4.8 The maximum water solubility of curcumin incorporated in cur film

4.8 Shelf-life stability

The cur films were studied the shelf-life stability by keeping in transparent and opaque sealed-bags (T- and O-bag) at ambient temperature for 120 days.

4.8.1 Thickness, moisture content and water solubility of film

The thicknesses of all T- and O- bags cur film were not significantly different ($p<0.05$) in the period of 120 days (Table 4.1). However, the moisture contents of the cur film slightly increased during the storage by a function of time but not significantly different between T- and O-bags. Thus, this film should be kept in vacuum sealed-bag to defend the moisture upsurge. The water solubility of the cur film was gradually

decreased during the storage by a function of time but not significantly different between T- and O- bags. This might be caused by the collapse of film-forming networks.

Table 4.1 Film thickness, moisture content and water solubility of the cur films at 0, 15, 30, 60, 90 and 120 days in T- and O-bags

Cur film (Day)	Thickness (mm)		Moisture content (%)		Water solubility (%)	
	T-bags	O-bags	T-bags	O-bags	T-bags	O-bags
0	0.23 ± 0.03	0.27 ± 0.01	4.13 ± 1.15	4.27 ± 1.45	97.50 ± 1.25	97.80 ± 2.45
15	0.20 ± 0.02	0.27 ± 0.01	3.26 ± 1.20	3.16 ± 0.58	93.67 ± 5.48	96.86 ± 2.74
30	0.20 ± 0.01	0.28 ± 0.03	3.45 ± 0.79	3.83 ± 1.29	93.80 ± 2.45	96.34 ± 4.72
60	0.22 ± 0.02	0.28 ± 0.02	4.16 ± 0.34	4.05 ± 0.53	86.37 ± 4.19	87.31 ± 4.83
90	0.22 ± 0.02	0.29 ± 0.01	4.27 ± 1.55	4.53 ± 0.80	88.50 ± 2.73	81.67 ± 2.56
120	0.23 ± 0.05	0.30 ± 0.01	9.86 ± 1.47	9.21 ± 0.32	86.85 ± 4.80	86.51± 1.93

4.8.2 Attenuated total reflection fourier transform infrared (ATR- FTIR) spectroscopy

The FTIR spectrum of 0-, 30- and 120-day cur films in O- and T-bags were showed in Fig. 4.9. All cur films showed similar IR spectra and intensities with the existence of the enol-carbonyl stretching peak of curcumin at 1602 cm^{-1}.

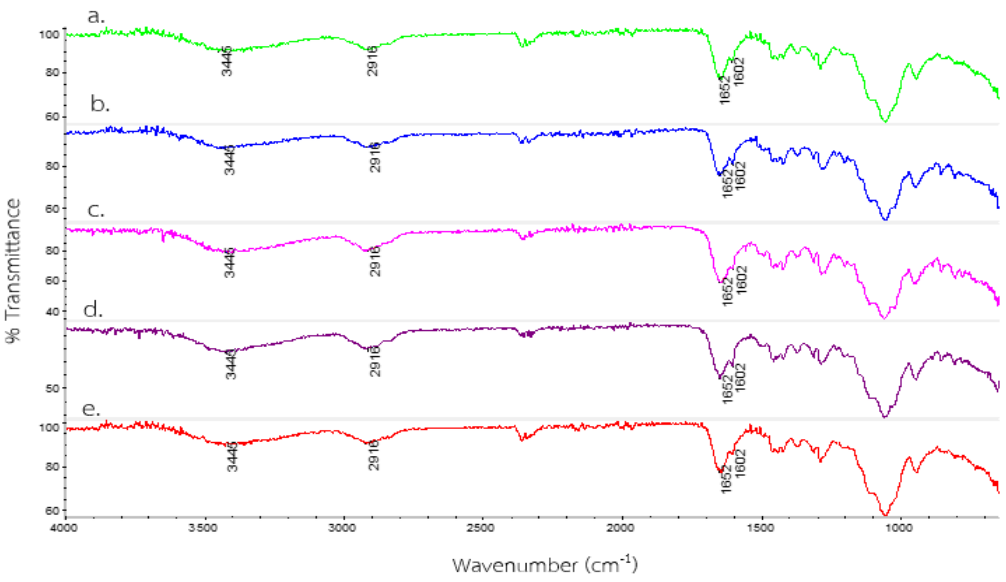
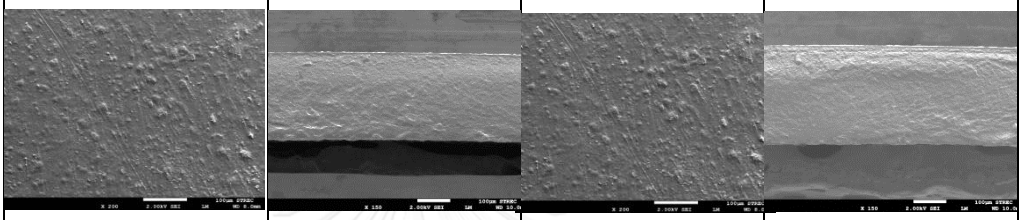
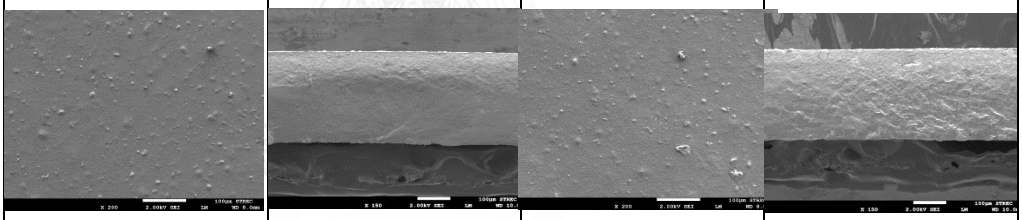
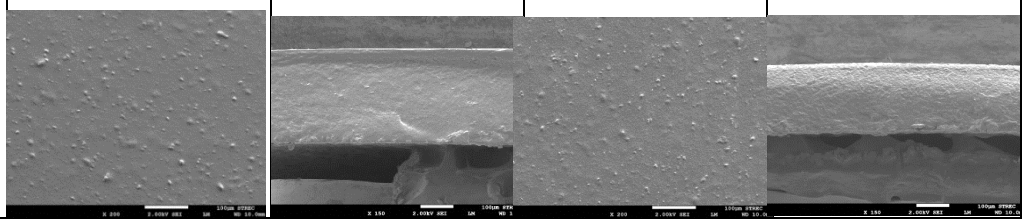


Figure 4.9 FTIR spectrum of cur film at a) 0 day, b) 30 day in T-bag, c) 120 day in T-bag, d) 30 day in O-bag and e) 120 day in O-bags

4.8.3 Field emission scanning electron microscopy (FE-SEM)

The surface and cross-section images of cur films at 0, 30 and 120 days were determined by FE-SEM. The images of all samples were shown in Table 4.2. There was no noticeable distinction between all samples.

Table 4.2 FE-SEM micrographs of cur films at 0, 30 and 120 days in T- and O-bags

Cur film (Day)	T-bags		O-bags	
	Surface	Cross-section	Surface	Cross-section
0				
30				
120				

4.8.4 Thermal stability

The TGA and DTG curves of 0, 30 and 120 days were shown in Fig.4.10 and the percent weight loss of the cur films at a range of 300 to 450 °C was shown in the Table 4.3. The % weight loss of all samples in T-and O-bags at any times was not

significantly different. However, the DSC chromatogram (Fig. 4.11) displayed the increasing of T_m by a function of times and the T_m of cur film stored in T-bags raised more than that stored in O-bags (Fig.4.11). Curcumin in T-bags may slightly degrade by light and resulting in the T_m change.

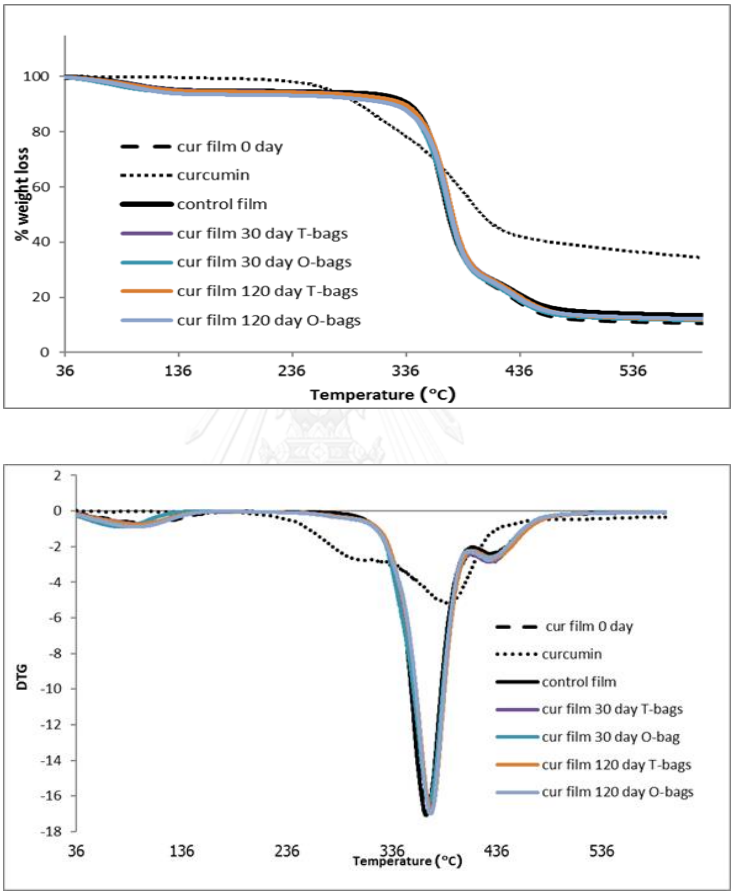


Figure 4.10 TG and DTG curves of the cur films at 0, 30 and 120 days in T- and O-bags

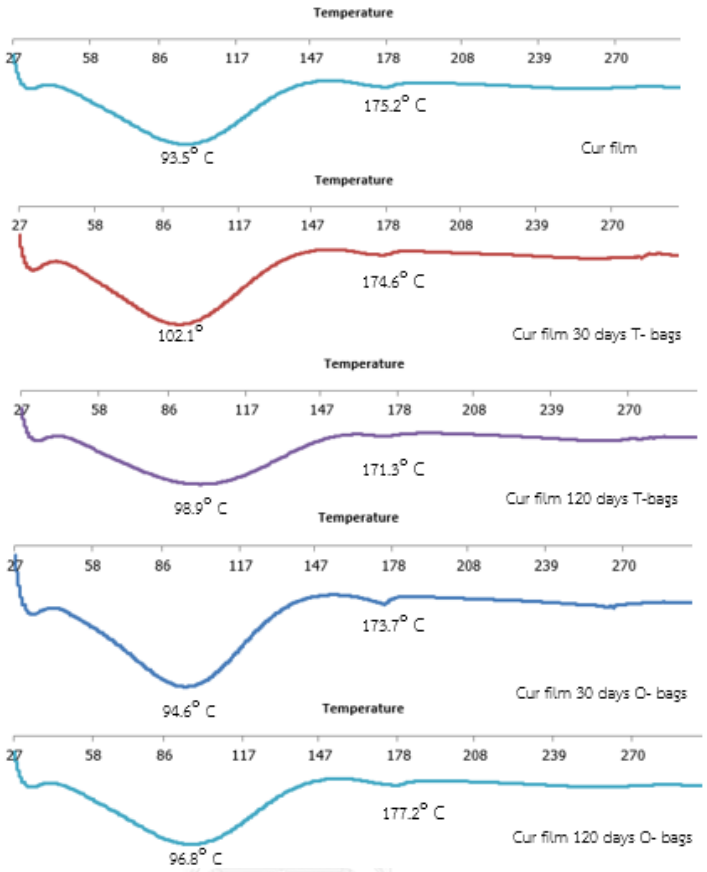


Figure 4.11 DSC curves of the cur films at 0, 30 and 120 days in T- and O-bags

Table 4.3 The percent weight loss of the cur films at 0, 30 and 120 days T-and O-bags

Cur film (Day)	%weight loss	
	T-bags	O-bags
0	83.44	83.44
30	82.33	82.79
120	82.57	82.57

4.8.5 Appearance and color

The photograph images of the stored cur films were summarized in Table 4.4. The result showed that the brown-yellow color of cur film was gradually increase as a function of time corresponding to the rise of b* values (Table 4.5). The color was much different between T- and O-bags at 120-day by naked eye detection. This confirmed the light effect toward the curcumin stability. However, the transparency (L* value) of the cur films in T- and O-bags was not significantly different.

Table 4.4 The photograph images of the stored cur films

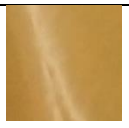
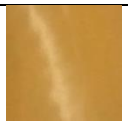
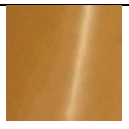
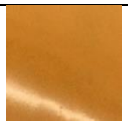
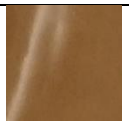
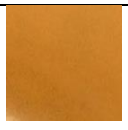
Cur film (Day)	Appearance	
	T-bags	O-bags
0		
15		
30		
60		
90		
120		

Table 4.5 Color of the cur films in T- and O- bags

Cur film (Day)	L*		b*	
	T-bags	O-bags	T-bags	O-bags
0	67.40 ± 1.46	67.40 ± 1.46	66.00 ± 0.91	66.00 ± 0.91
15	67.59 ± 0.72	67.41 ± 0.35	67.64 ± 3.34	67.15 ± 0.84
30	66.65 ± 1.62	67.92 ± 2.11	67.26 ± 3.32	67.65 ± 2.46
60	69.75 ± 1.04	69.84 ± 0.63	69.38 ± 1.73	69.10 ± 1.12
90	67.24 ± 0.99	69.32 ± 0.86	69.88 ± 2.30	69.02 ± 1.82
120	68.44 ± 1.50	69.94 ± 0.80	72.63 ± 1.72	69.77 ± 1.52

4.8.6 Curcumin amount

The absorbance values of the cur films at 420 nm stored for 120 days were showed in Table 4.6. The absorbance values of all stored samples in both T- and O-bags were the same as that of the 0-day cur film.

Table 4.6 The absorbance of the cur films in T- and O-bags

Cur film (Day)	Absorbance	
	T- bags	O- bags
0	0.63 ± 0.01	0.63 ±0.01
15	0.62 ± 0.02	0.61 ± 0.03
30	0.60 ± 0.02	0.64 ± 0.01

60	0.60 ± 0.03	0.63 ± 0.02
90	0.63 ± 0.02	0.63 ± 0.01
120	0.59 ± 0.04	0.60 ± 0.01

4.8.7 pH stability

Fig.4.12 showed results of the pH stability of cur film which kept for 15, 30, 120 days at pH 4. The 15-day cur film showed good stability liked the 0-day cur film. After 30-day storage, the stability slightly decreased as a function of time and films kept in T-bags had a little higher than films kept in O-bags.

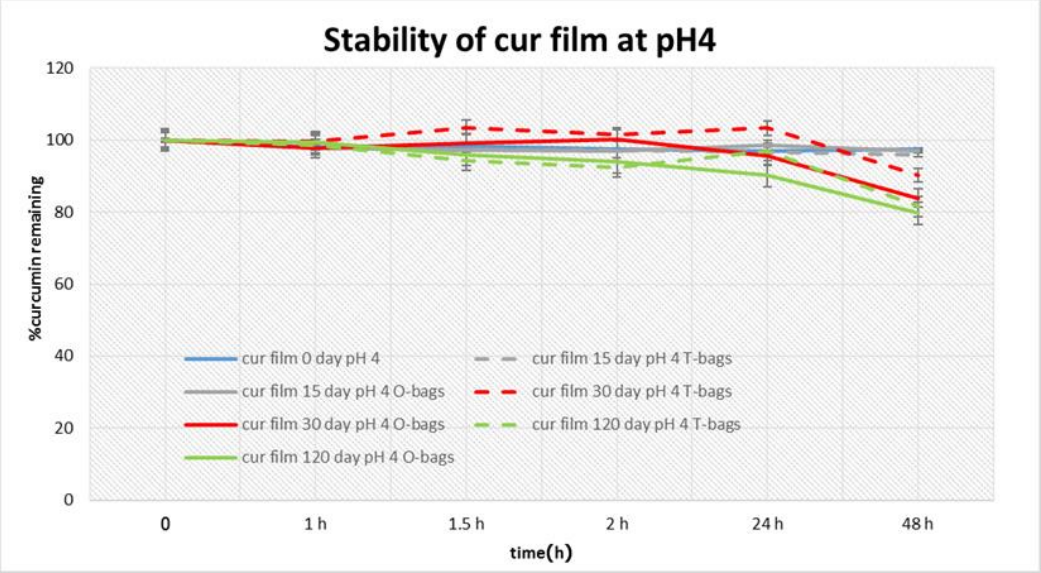


Figure 4.12 Cur film 0, 15, 30, 120 days T-bag and O-bags at pH4

4.9 Bioactivity tests.

4.9.1 The anti-amyloid aggregation

The anti-amyloid aggregation percentages of curcumin and the cur film were showed in Table 4.7. The activities of curcumin, the 0- and the 120-day cur films of both conditions were not different. However, the cur films can dissolve in both DMSO and water while curcumin can dissolve only in DMSO.

Table 4.7 The anti-amyloid aggregation of curcumin and 0- and 120-day cur films

Sample	%Inhibition
DMSO	10.3 ± 1.6
Curcumin dissolve in DMSO	43.3 ± 2.5
Cur film 0-day dissolve in DMSO	42.1 ± 2.7
Cur film 120-day T-bags dissolve in DMSO	42.0 ± 5.5
Cur film 120-day O-bags dissolve in DMSO	43.0 ± 2.2
Cur film 0-day dissolve in water	31.4 ± 2.2
Cur film 120-day T-bags dissolve in water	35.5 ± 2.0
Cur film 120-day O-bags dissolve in water	35.3 ±2.4

4.9.2 DPPH scavenging activity

The DPPH radical scavenging assay was used to determine the antioxidant activity. It has been known that curcumin has a good potential to destroy free radical

as showed in Fig.4.13. Table 4.8 showed the DPPH scavenging activity of curcumin and stored cur film at the curcumin concentration of 140 ppm. The control film had low activity (3.23%). The 0- and 120-day cur films exhibited the same activity as curcumin. Moreover, cur films kept in T- and O-bags possessed the same inhibition percentages.

Table 4.8 The DPPH scavenging activity of curcumin and cur film

Sample	DPPH scavenging activity (%)
Control film	3.2 ± 1.8
Curcumin	76.6 ± 2.9
Cur film 0 day	76.6 ± 1.3
Cur film 120 days O- bags	76.1 ± 0.7
Cur film 120 days T- bags	76.3 ± 1.6

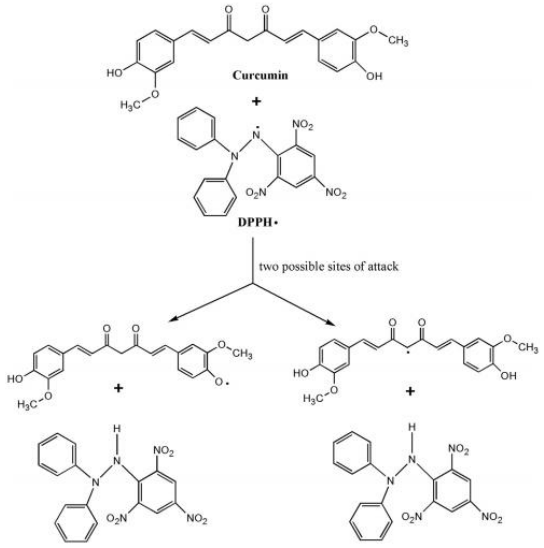


Figure 4.13 Chemical reaction between DPPH and curcumin [55]

From all results of shelf-life stability, cur films kept in T- and O-sealed bags gave the similarity outcomes excepted for color and DSC chromatogram. However, vacuum packaging was suggested to protect the increase of moisture content. The reduction of water solubility and pH stability were found and are interesting for further study.

CHAPTER V

CONCLUSION

This study reported the preparation of cur film expected to use in food and cosmetic applications. Cur film was fabricated using 0.2% of curcumin, 3% of HPMC and 1% of PVP. The cur film was characterized the physical and chemical properties. The FE-SEM micrographs revealed the rough and some curcumin aggregation on the surface of cur film. Cur film can re-dissolve in the water and gave the curcumin particles dispersing in the aqueous medium. The FTIR, DSC and TGA data confirmed the presence of curcumin and intermolecular interaction between curcumin and film-forming polymers. Moreover, cur film exhibited better stability than curcumin at pH 4, 7 and 10. At pH4, the degradation of cur film was not found within 48 hours. At pH7 and 10, cur film degraded slower than curcumin. Interestingly, curcumin in the cur film had higher water solubility than of curcumin approximately 2.8×10^3 times.

The shelf-life stability of cur film was evaluated at the interval times, 0, 15, 30, 60, 90 and 120 days by keeping in transparent and opaque sealed-bags (T- and O-bags) at ambient temperature. The result showed that the brown-yellow color of cur film gradually increased as a function of time. However, curcumin amount, FTIR and TGA data were not presence the different for all samples. The water solubility and pH stability were reduced if strorage for longer time. Most data indicated insignificantly

different between storage cur film in T- and O- bags. However, DSC data revealed some degradation of curcumin.

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APPENDIX

Appendix A

Table A1 the color of cur film in O-sealed bags within 120 days

0 day white paper				15 day white paper			
sample	L*	a*	b*	sample	L*	a*	b*
0 day white paper	67.13	21.72	65.95	15 day white paper	67.21	20.67	68.39
	67.46	21.83	65.34		67.86	20.24	66.63
	68.58	20.98	66.06		67.58	20.08	66.6
	68.73	20.62	67.48		66.96	20.7	66.46
	65.09	20.89	65.17		67.46	20.1	67.65
Avg. 0 day white paper	67.398	21.208	66	Avg. 15 day white paper	67.414	20.358	67.146
sd	1.464	0.536	0.911	sd	0.345	0.305	0.842

30 day white paper				60 day white paper			
sample	L*	a*	b*	sample	L*	a*	b*
30 day white paper	67.69	17.08	68.72	60 day white paper	70.24	15.18	68.69
	64.92	13.99	64.77		69.07	14.82	67.72
	67.42	16.42	65.82		70.39	15.77	70.82
	68.86	14.28	67.94		69.24	15.1	69.12
	70.69	15.52	71.02		70.27	15.26	69.16
Avg. 30 day white paper	67.916	15.458	67.654	Avg. 60 day white paper	69.842	15.226	69.102
sd	2.113	1.333	2.461	sd	0.633	0.346	1.122

90 day white paper				120 day white paper			
sample	L*	a*	b*	sample	L*	a*	b*
90 day white paper	69.31	15.14	68.52	120 day white paper	70.95	22.77	71.98
	68.1	15.47	68.64		70.58	23.12	67.81
	69.47	14.5	66.49		69.04	22.21	69.77
	69.18	15.98	71.25		69.47	21.47	69.13
	70.52	15.64	70.21		69.64	21.74	70.14
Avg. 90 day white paper	69.316	15.346	69.022	Avg. 120 day white paper	69.936	22.262	69.766
sd	0.862	0.562	1.816	sd	0.799	0.689	1.523

Table A2 the color of cur film in T-sealed bags within 120 days

0 day white paper				15 day white paper			
sample	L*	a*	b*	sample	L*	a*	b*
0 day white paper	67.13	21.72	65.95	15 day white paper	67.1	19.22	63.84
	67.46	21.83	65.34		67.42	19.39	64.16
	68.58	20.98	66.06		68.6	19.87	70.48
	68.73	20.62	67.48		68.01	19.97	69.92
	65.09	20.89	65.17		66.81	19.68	69.81
Avg. 0 day white paper	67.398	21.208	66	Avg. 15 day white paper	67.59	19.63	67.64
sd	1.464	0.536	0.911	sd	0.72	0.32	3.34

30 day white paper				60 day white paper			
sample	L*	a*	b*	sample	L*	a*	b*
30 day white paper	66.37	20.86	66.03	60 day white paper	69.51	16.55	69.09
	63.94	20.74	61.97		68.75	16.56	70.01
	67.39	20.2	69.05		71.51	17.48	71.4
	67.85	20.57	69.31		69.57	15.25	69.7
	67.69	20.32	69.94		69.4	16.21	66.68
Avg. 30 day white paper	66.648	20.538	67.26	Avg. 60 day white paper	69.748	16.41	69.376
sd	1.620	0.277	3.320	sd	1.038	0.802	1.729

90 day white paper				120 day white paper			
sample	L*	a*	b*	sample	L*	a*	b*
90 day white paper	67.19	20.05	71	120 day white paper	66.44	18.05	70.34
	66.21	20.46	66.41		67.57	18.06	71.24
	66.36	19.88	69.06		69.86	20.5	74.09
	67.88	20.15	70.45		68.37	19.96	73.76
	68.54	19.04	72.49		69.94	20.43	73.74
Avg. 90 day white paper	67.236	19.916	69.882	Avg. 120 day white paper	68.436	19.40	72.634
sd	0.992	0.533	2.297	sd	1.502	1.245	1.719

VITA

Name : Miss Nattakan Kanana

Date of birth : August 19, 1991

Nationality : Thai

Address : 11/10, Moo. 12, Thasala, Thasala, Nakhonsrithammarat, 80160

University Education : Bachelor's Degree from Department of Material Science, Faculty of Engineering, Silpakorn University, 2010-2014. Master's Degree from Program in Petrochemistry and polymer science, Faculty of science, Chulalongkorn University.

Conference attendance : Poster presentation "Curcumin film for aqueous based application " at Pure and Applied Chemistry International Conference 2017 (PACCON 2017), Centra Government Complex Hotel & Convention Centre Chaeng Watthana, Bangkok , Thailand, 2-3 February 2017.