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DEVELOPMENT OF WATER IN OIL NANOEMULSION LIPSTICKS

Miss Parichat Promdouang

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Department of Chemical Engineering
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งานวิจัยนี้ถูกออกแบบให้มีการเติมน้ำลงในลิปสติกชนิดไม่มีน้ำเป็นองค์ประกอบในสูตร โดยน้ำถูกเตรียมให้อยู่ในรูปแบบอิมัลชันชนิดน้ำในน้ำมันก่อนเติมลงในเบสลิปสติก เพื่อทำให้เกิดเป็นลิปสติกนาโนอิมัลชันชนิดน้ำในน้ำมัน ในงานวิจัยนี้ได้ศึกษาปัจจัยที่มีผลต่อคุณสมบัติเชิงกายภาพเคมีของลิปสติกอิมัลชัน โดยตัวแปรที่ทำการศึกษาได้แก่ ชนิดอิมัลซิฟายเออร์ ปริมาณน้ำและอิมัลซิฟายเออร์ นาโนอิมัลชันชนิดน้ำในน้ำมันถูกเตรียมโดยใช้เครื่องโฮโมจิไนเซอร์ความเร็วสูงและตามด้วยเครื่องโฮโมจิไนเซอร์ความดันสูง 500 บาร์ จำนวน 3 รอบ จากนั้นนาโนอิมัลชันชนิดน้ำในน้ำมันซึ่งมีปริมาณน้ำ (0.5-10%) และปริมาณอิมัลซิฟายเออร์ (0.5 -2.0%) ที่แตกต่างกันจะถูกนำมาผสมเข้ากับเบสลิปสติกซึ่งประกอบด้วยแวกซ์ชนิดต่าง ๆ นอกจากนั้นแล้วยังได้ทำการศึกษาเปรียบเทียบคุณสมบัติเชิงกายภาพได้แก่ ความแข็งและความลื่นของนาโนอิมัลชันลิปสติกกับลิปสติกอิมัลชันแบบดั้งเดิม โดยลิปสติกอิมัลชันแบบดั้งเดิมถูกเตรียมโดยใช้เครื่องโฮโมจิไนเซอร์ที่ความเร็วรอบ 1000 รอบต่อนาที เป็นเวลา 10 นาที จากผลการทดลองที่ได้แสดงให้เห็นว่าปริมาณน้ำที่เติมลงในเบสลิปสติก ส่งผลต่อความแข็งของลิปสติกนาโนอิมัลชันอย่างเป็นนัยสำคัญ ในขณะที่ปริมาณอิมัลซิฟายเออร์ไม่ส่งผลกระทบต่อความเข็งของลิปสติกนาโนอิมัลชัน นอกจากนั้นแล้วยังพบว่าความแข็งของลิปสติกแบบดั้งเดิมมีความแตกต่างจากลิปสติกนาโนอิมัลชัน ซึ่งเกิดจากปัจจัยของขนาดอนุภาคอิมัลชันที่แตกต่างกัน

ศูนย์วิทยทรัพยากร
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ภาควิชา..... วิศวกรรมเคมี..... ลายมือชื่อนิสิต..... ปาริชาติ พรหมดวง .....

สาขาวิชา..... วิศวกรรมเคมี..... ลายมือชื่อ อ.ที่ปรึกษาวิทยานิพนธ์หลัก.....

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In this study, the anhydrous lipstick base was designed to incorporate with water. The additional water was formulated and prepared into the nanoscale water in oil (w/o) emulsions before mixed with the lipstick base to form w/o nanoemulsion lipsticks. The formulation parameters that are likely to affect the physicochemical properties of lipsticks were evaluated. These parameters included type of emulsifiers as well as dose of water and emulsifiers. The studied nanoemulsions composed of water, isopropyl myristate, mineral oil and PEG-30 dipolyhydroxystearate was prepared in the high speed homogenizer following with the high pressure homogenizer at 500 bars for 3 cycles. The final w/o nanoemulsions with different doses of water and emulsifiers were separately incorporated into the lipstick base. The conventional w/o emulsions with the same formulas that were prepared by the high speed homogenizer at 1000 rpm for 10 min were compared in parallel. The mean particle size, size distribution, stability and physicochemical properties in terms of hardness and spreading ability were operated. Results revealed the water content significantly affected to the hardness of w/o nanoemulsion lipsticks whereas the emulsifier did not. Additionally, the particle size of the preparations also affected to the hardness of lipsticks.

Department :	Chemical Engineering	Student's Signature	Parichat Promdouang
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Academic Year :	2010	Co-advisor's Signature	

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CHAPTER I

INTRODUCTION

1.1 Background

Lipsticks have been used as the color cosmetics for over 5,000 years. The ancient Mesopotamian women used the crushed stone to paint on their lips as lipsticks [1]. De Navarre [2] noted the first true lipstick was introduced in France in 1895 and was called Pomade en Baton. This stick pomade consisted of tallow and beeswax. However, the presently appeared lipstick was introduced about the First World War. Prior to this time, lip coloring was available in pots, jars or bottles as an alkaline solution of cochineal or carmine. The carmine produced a reddish blush color effect. With the development of organic dyes during the late 19^{th} century, the modern lipstick formulations were possibly made.

Nowadays, the function of lipsticks is not only for coloring the lips (make-up) but also for providing the moisturization, protective and therapeutic properties. The conventional lipsticks are generally formulated with hydrophobic ingredients such as oils, waxes, coloring materials and additives. The physical properties of the conventional lipsticks include a melting point, a dropping point and spreading ability that can be modified by varying the proportion and type of oils and waxes in the formulation.

The lipsticks are prepared by mixing the suitable proportions of materials already mentioned mostly being a hydrophobic ingredients. Therefore, the water and/or hydrophilic ingredients are hardly to be added in the formulation. As a result, a number of researchers have intensively studied the incorporation of water and/or water soluble active ingredients into lipstick. The most recent innovation to overcome this problem is to create the w/o emulsion lipsticks.

Conventional lipsticks are generally formulated with hydrophobic ingredients such as oils, waxes, coloring materials and additives. Each formula can be modified by altering the proportions of oils and waxes to adjust to the final product characteristics such as melting points, spreading ability, the long lasting and the amount of film on the lips according to the particularly required characteristics. The wax contributes to the solid form of the lipstick and makes easy in application. Lipsticks can be made from the combination of several waxes such as beeswax, candelilla wax, ceresin wax and carnauba wax [3, 4]. The different types of oils and fats are required to blend intimately with waxes to provide a suitable film and spreading ability when the lipsticks are applied. The usually used oils and fats in lipsticks include castor oil, mineral oil, cocoa butter and petrolatum.

The conventional emulsion lipstick is the type of lipstick formulated from the water or water soluble ingredients in the hydrophobic parts to form the w/o droplets of

various sizes (generally in micro size) for improving the moisturizing and delivery the water soluble active ingredients to the lips. However, several drawbacks of the conventional w/o emulsion lipsticks have been shown, e.g., discoloration of the coloring materials, non-uniform mixing of the coloring materials, undesirable softening, easy breaking, bleeding of water soluble ingredients and difficulty in molding [5]. Therefore, the water or hydrophilic actives are hardly incorporated in the lipsticks. The maximum adding is generally lower than 2%.

The nanoemulsion or submicron emulsion is the emulsion with the very small droplet sizes. Generally is lower than 1 μm and mostly in the nanometer between 20 nm and 200 nm [6]. The particle size of nanoemulsion depends on several factors, for instance, preparation methods, type and concentration of emulsifiers and oils. Unlike the microemulsion, the nanoemulsion is metastable by which the structure depends on its preparation process. The nanoemulstion can be prepared by spontaneous emulsification such as PIT emulsification or phase inversion [7] or by using a high shear device which allows a better control of the droplet size and a large choice of compositions. Nanoemulsions have been used in different applications, e.g. in chemical, cosmetics, food and pharmaceutical industries. Nanoemulsions are kinetically stable against sedimentation or creaming because their very small droplet sizes can reduce the gravitational force. Therefore, the Brownian motion may be sufficient for overcoming

the gravitational forces resulting in non-occurrence of creaming or sedimentation during storage. Due to their small droplet size, nanoemulsions can easily penetrate into the deep skin to render it be used as topical drug delivery systems [8, 9].

Therefore, this study was to incorporate water into anhydrous (waxes and oils) lipstick base. Water was formulated and prepared in the form of w/o emulsions in nanoscale (w/o nanoemulsions) and subsequently w/o nanoemulsions were mixed with the lipstick base to form w/o nanoemulsion lipsticks. The mean droplet size and size distribution of w/o nanoemulsions were measured before incorporated into lipstick base. The effects of formulation parameters on the physicochemical properties of lipsticks including the type of emulsifier and the concentration of water and emulsifier were evaluated. The physicochemical properties including hardness, spreadability, crystallinity and stability of w/o nanoemulsion lipsticks (nanosize range) were investigated in comparison to that of conventional w/o emulsion lipsticks (microsize range) and anhydrous lipsticks, respectively.

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1.2 Objectives

The objectives of this study were as followings:

- To investigate the effect of emulsifier type, amount of water and emulsifier on the physicochemical properties and stability of w/o nanoemulsions as well as w/o nanoemulsion lipsticks

- To compare physicochemical properties including hardness, spreadability, crystallinity and stability of w/o nanoemulsion lipsticks (nanosize range) with conventional w/o emulsion lipsticks (microsize range) and anhydrous lipsticks

1.3 Research scope

The study can be concluded as followings:

1. Select appropriate emulsifiers that have different hydrophile lipophile balance (HLB) values to stabilize w/o nanoemulsions
   - A lipophilic emulsifier = Span83 (HLB 3.7) and PEG-30 dipolyhydroxystearate (HLB 5.5)
   - A hydrophilic emulsifier = Polysorbate 60 (HLB 14.9)
2. Determine the effect of emulsifiers and amount of water (water concentration) on the physicochemical properties and stability of w/o nanoemulsions and w/o nanoemulsion lipsticks.
3. Determine the influence of emulsion droplet size on the physicochemical properties and stability of w/o nanoemulsion lipsticks.

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1.4 Research methodology

The methodology for the present research is shown in Fig. 1

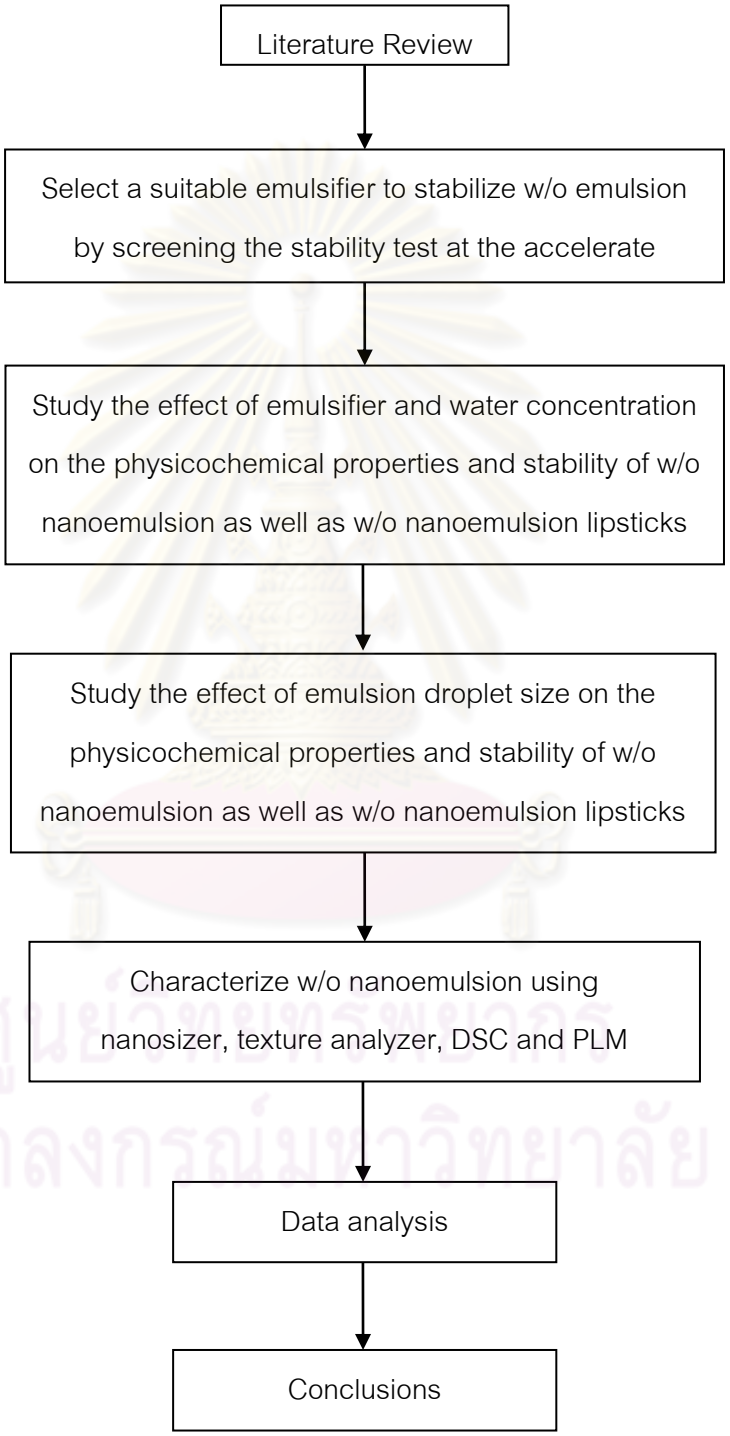

Fig. 1. Research methodology scheme.

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CHAPTER II

THEORY AND LITERATURE REVIEW

2.1 Nanoemulsions

Emulsions are the heterogeneous system of two or more immiscible liquids dispersed in each other in the form of droplets [10, 11]. The International Union of Pure and Applied Chemistry (IUPAC) gives the following definition of an emulsion: “In an emulsion, liquid droplets and/or liquid crystals are dispersed in a liquid” [12]. Emulsions are thermodynamically unstable, the formation mechanism of emulsion is described by Gibbs free energy of formation as shown in the following equation (1).

$$\Delta G_{form} = \gamma \Delta A - T\Delta S \qquad (1)$$

Where γ is the interfacial tension between oil and water phase and ΔA is the change in the interfacial area which significantly increase during the formation of droplets. The interfacial energy term ($\gamma \Delta A$) is the dominant term whereas the change of entropy is insignificantly and consequently to the total free energy of formation of the emulsion is greater than zero. Therefore, the formation of emulsion is non spontaneous and the energy is required. The amount of energy required is given by

$$W = \gamma \Delta A \qquad (2)$$

Three types of emulsions are distinguished in principle, depending on the kind of liquid form in the dispersed and continuous phase.

1. Oil in water (O/W) emulsions, oil droplets dispersed in the water phase.

2. Water in oil (W/O) emulsions, water droplets dispersed in the oil phase.

3. Multiple or complex emulsions including water in oil in water (W/O/W) emulsions or the oil in water in oil (O/W/O) emulsions.

In addition, the emulsions can be classified by their droplet size into 3 main types

1. Macroemulsions

The droplets sizes of dispersed phase in macroemulsions are usually 2-10 μm (generally more than 1 μm). Because their droplet sizes are greater than the wave length of light, the appearance of macroemulsions is opaque.

2. Microemulsions

Microemulsions are equilibrium colloidal system with extremely small droplet sizes of generally 10-75 nm

3. Miniemulsions or nanoemulsions or submicron emulsions

The droplet size of miniemulsions is between the droplet sizes of macroemulsions and microemulsions. Generally, their droplet size is lower than 1 μm (between 20 and 200 nm) [6]. According to the thermodynamic theory, microemulsions

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are thermodynamically stable whereas nanoemulsions are thermodynamically unstable but kinetically stable. The appearance of nanoemulsion is transparent or translucent, depending on the droplet size and the difference between the refractive index of dispersed and continuous phase [13]. As compared to microemulsions, nanoemulsions cannot be formed spontaneously. The formation of nanoemulsions generally requires external energy from mechanical devices or from the chemical potential to produce the small droplets in the nanosize range.

Nowadays, nanoemulsions are intensively studied and widely used in many different applications including chemical, cosmetics, food and pharmaceutical industries. Nanoemulsions are kinetically stable against sedimentation or creaming because of their very small droplet size which reduces the gravitational force. Therefore, the Brownian motion may be sufficient for overcoming the gravitational forces resulting in non-occurrence of creaming or sedimentation during storage. Due to their small droplet size, nanoemulsions can easily penetrate into the deep skin; therefore, they have been used to efficiently deliver active ingredients through the skin surface [14]. In addition, nanoemulsions are used in the pharmaceutical field as drug delivery system [15].

2.2 Preparation of nanoemulsions

Nanoemulsions can be prepared by emulsification methods by means of high and low energy. High energy emulsification methods are the mechanical processes generating nanometric scale emulsions. Several devices have been used to diminish the mean particle size of emulsion including the rotor/stator, ultrasound generator and high pressure homogenizer.

The low energy methods are governed by the intrinsic physicochemical properties and behavior of the systems [16]. These methods make use of changing the spontaneous curvature of the emulsifier. For non ionic surfactants, this can be achieved by changing the temperature of the system, forcing the transition from oil in water (o/w) emulsion at low temperatures to a water in oil (w/o) emulsions at higher temperatures (transitional phase inversion).

As compared to other methods as aforementioned, high pressure homogenization (HPH) has emerged as a reliable and powerful technique; hence, the large scale production of nanoemulsions by means of HPH is possible. HPH has been used and applied in chemical, pharmaceutical and food fields in order to nanoemulsions. The principle of HPH is to force the fluid with high pressure through a narrow gap (order 10-100 μm). The fluid is accelerated in a very short distance to very high velocity (over 1000 km/h) as shown in Fig. 2, causing a very high pressure drop in

the homogenizing valve. The fluid droplets are broken up to the submicron range by the high shear force in the gap [17]. Cavitations, turbulence and impact with solid surfaces take place at the outlet of the valve gap [18].

Ultrasound has been used for many applications and is an alternative method to produce nanoemulsions. The mechanism of emulsification by ultrasound was introduced by Li and Fogler [19]. Emulsification by ultrasound consists of two processes, ultrasonic waves which cause an interfacial instability of the oil–water interface and transient cavitations bubbles which are known to generate micro streaming, high-pressure shock wave of about 100 MPa and high local temperature (4000 K) during their collapse. The advantages of ultrasound include lower energy consumption, use of less surfactant and production of an emulsion more homogeneous compared to a mechanical process [20]. Limitation of ultrasound emulsification method for producing nanoemulsions is suitable for small batch because the distance between the probe tip and the single oil water interface needed for an emulsion production affects the droplet size and size distribution of emulsions [21].

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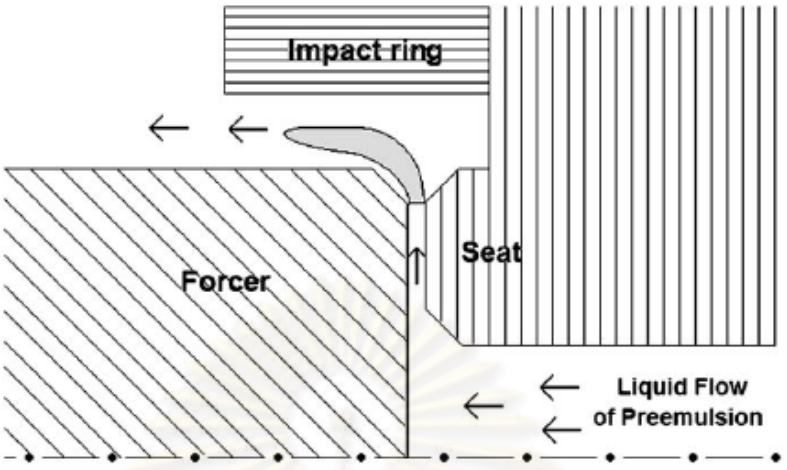

Fig. 2. Schematic of high pressure homogenizer gap region [16].

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2.3 Literature Reviews

Lipstick is the popular color cosmetics for most women. In the recent years, the role of lipstick is beyond the function of coloring the lips but it provides the moisturization, protective and therapeutic effects. Therefore, many innovations and researches on w/o emulsions have been released.

The type and ratio of oils and waxes in formulation can influence the perception of consumer after application. The most important ingredients used in lipstick base excluding colorants, preservatives and perfumes are as followings:

Candelilla Wax [22]

This is preferred to the more expensive carnauba wax. This wax is obtained by boiling the leaves with dilute sulfuric acid, and the resulting “cerote” is skimmed from the surface and further processed. It gives high shine, rigidity and increasing hardness without graininess associated with carnauba wax. The melting point of candelilla wax is about 68.5-72.5ºC. Candelilla wax consists of mainly hydrocarbons (about 50%, chains with 29-33 carbons), ester of higher molecular weight (20-29%), free acids (1-9%), free acids (7-9%) and resins (12-14%, mainly triterpenoid ester). This wax is soluble in many organic solvents such as acetone, chloroform, benzene etc.

Fatty acid esters

These are very widely used in classically formulated lipsticks. Those popularly used are isopropyl myristate and/or palmitate. The primary use of fatty acid esters is the reduction of quantity of castor oil applied in lipsticks. These fatty esters are available in varying viscosity. As a result and with their inherent stability, they are reasonable to replace the more sensitive natural oils. The replacement of the natural oils with these esters can provide the formula to change its texture that will enhance the lip adhesion and improve lip spreadability.

Caster oil

Castor oil is used in many lipstick formulations on the account of its unique property. It also functions as a solvent of eosin dyestuffs and as a dispersing agent for insoluble pigments.

Beeswax [22]

Beeswax is a natural wax produced in the bee hive. It is mainly esters of fatty acid and various long chain alcohols. Beeswax is a tough wax formed from a mixture of several compounds. An approximate chemical formula for beeswax is $C_{15}H_{31}COOC_{30}H_{61}$ and has a high melting point of about 62-64^{o}C. Beeswax can be used in the entire range of cosmetic products, for example in W/O and O/W formulations, sticks, salves, make-up

and hair care products. In sticks, beeswax helps improve structure, oil retention, firmness, adhesion, pay-off and mould release. In emulsions, beeswax functions as a consistency regulator and as a co-emulsifier that contributes to skin feel, emolliency, barrier function and appearance.

Microcrystalline wax

Microcrystalline wax is a type of wax produced by de-oiling petrolatum as a part of the petroleum refining process. In contrast to the more familiar paraffin wax which contains mostly unbranched alkanes, microcrystalline wax contains a higher percentage of isoparaffin (branched) hydrocarbons and naphthenic hydrocarbons. It is characterized by the fineness of its crystals in contrast to the larger crystal of paraffin wax. Microcrystalline wax is soluble in warm alcohol, oils and other melted waxes. The melting point of microcrystalline wax is about 63-68ºC. It is used in cosmetics and beauty products as a viscosity agent, binder and emollient, and is often considered an alternative to paraffin wax.

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The production of lipsticks

The steps for basic processing are as followings:

- Pigments are pre-wetted under agitation in twice their weight of the oily portion of the lipsticks

- The wax of lipstick composition such as beeswax, candelilla wax, ceresin wax and carnauba wax are heated while being agitated to a temperature 2-3ºC above the highest melting point of waxes.
- The pre-wetted pigment dispersion is passed through a three-roll mill three times or until there are no agglomerates with full color development is achieved.
- The ground color dispersion paste is added to the molten wax and oil mixture while being agitated under temperature control.
- The remaining oily part is passed through the three roll mill as a rinse and then charged into the batching vessel with the other parts.
- The batch is again returned to temperature and mixed until the batch is uniform and fully de-aerated.
- The batch is passed through a 250 mesh screen to ensure that it is free of foreign matter.

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2.3.1 Process development for emulsion lipsticks

Fujiyama *et al*. [5] produced the cosmetic stick which had an excellent spreading property on the skin in the form of w/o emulsions. The stick contained water from 1 to 50% w/w of lipstick, a gel which was prepared from polyhydroxyl compounds, e.g. glycerol, mannitol, dulcitol and carbohydrates at 1 to 10% w/w of lipstick and the

wax base at 20% w/w of the lipstick. The polyhydroxyl compound was mixed with the nonionic surface agent, e.g. glycerol monooleate, sorbitol monooleate and propylene glycol dioleate to obtain a gel which was effectively emulsified the water in the wax base. The gel was incorporated into the wax base at the temperature of 70°C by stirring. The accelerated stability of emulsion lipsticks was investigated. It was found that the emulsion lipsticks were not change. The rheological property was determined by Ferranti-Shirley cone and plate viscometer. It was reported that the yield stress of the emulsion lipsticks was lower than that of the conventional lipsticks referring that the hardness of emulsion lipstick was lower than that of the conventional lipstick.

Singh [23] prepared the lip care moisturizing product in the form of w/o emulsions in which the water was entrapped in the liposomes made from lecithin. The liposomes were dispersed in the oily/ waxy phase and stabilized with emulsifier system preferably based on behenoyl stearate and sodium borate (borax). The compositions of stable emulsion contain 1 to 35% w/w of water, 0.2 to 30% w/w of liposomes dispersion containing water-glycerin mixture and 5 to 35% w/w of total waxes and oils. In one embodiment, the liposome dispersion contains 82% w/w of liposomes and 18% w/w of a water-glycerin mixture. The size ranges of liposomes are between 25 and 75 nm. Behenoyl stearate emulsifier was very effective to emulsify 33 to 55% w/w of water into 45 to 67% w/w of non polar oils. The best results were obtained by dissolving

approximately 2 to 7.5% w/w of emulsifier in the oil phase, and buffering the system by dissolving up to 4% w/w of borax in the water phase.

In 1997, lipsticks containing encapsulated water in a lamellar lipid vesicle were introduced and patented by Chung *et al.* [24]. The vesicle wall comprises polyoxyethylene fatty acid ether which has a melting point higher than wax and a steroid component. The aqueous phase was prepared separately. Then, the water phase and lipid phase were mixed under high shear condition and subsequently lamellar lipid vesicles were formed. After that it was incorporated into the wax base.

Travkina *et al.* [25] prepared the moisturizing cosmetic stick in the form of w/o emulsion stick. The gelling agents, e.g. polymethacrylates, carbomers, celluloses, water swellable lucentite SWN and Vee gums were dispersed in the water phase at the concentration range from 1% to 8% w/w of the stick. The amount of water was presented from 1 to 35 % w/w of the stick. Polyglyceryl-10 pentastearate/behenyl alcohol was used as an emulsifier to stabilize the emulsion stick. The amount of emulsifier was presented from 1 to 5 % w/w of the stick. The non-aqueous part contained waxes, oils, and a lipophilic polar solvent, e.g. C12-15 alcohol benzoate. Smectite clay was presented as the gelling agent. Lipophilic polar solvent in formulation was used as solvent for gelling Smectite clay. The ratio of water phase to lipophilic phase was 2:3 to 1:5.

Shaikh and Bhise [26] produced the medicated lipstick of allantoin for treatment and UV protection of the lip in the form of emulsion lipstick. Allantoin at the concentration between 0.1 and 2% w/w was dissolved in 0.5% w/w of water containing 0.05% of sodium lauryl sulphate as an emulsifier based on the lipstick formulation. In addition, the natural ingredients, e.g. ghee and honey were incorporated into lipstick as moisturizer and natural emollient for substituting the conventional chemical ingredients, e.g. isopropyl myristate, lanoin and cetyl alcohol. Lipstick containing 5% of honey and 75% of ghee were considered to be optimum for preparing the medicated lipstick containing allantoin. The medicated lipstick was prepared by addition of honey and cow ghee into the molten wax based, after that added the water phase into the wax based. The mixture was stirred until the emulsion was formed. The evaluation parameters, e.g. melting point, softening point, breaking point and stability of medicated lipstick were investigated and it was found that all required aspects, i.e., hardness, spread ability, smoothness, color, taste and odor were achieved.

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2.3.2 Development of the emulsifier of emulsion lipsticks

Dunphy *et al.* [27] produced special mixture of emulsifiers used for stabilized w/o emulsion lipsticks. The emulsifier system was composed of the first emulsifier as phospholipids, e.g. phosphoglycerides, lysophosphoglycerides and sphingomyelins and the second emulsifier having a melting point from -20°C to 80°C. The most preferred

phosphoglyceride was lecithin, particularly soybean lecithin. The amount of phospholipids presented is from 0.2 to 10% w/w preferably from 0.5 to 2% w/w. The second emulsifier having a melting point from -20° C to 80° C, preferably from -5° C to 50° C, e.g. derivatives of glycerol and esters of fatty alcohols with hydroxyl acid such as glyceryl citrate, cetyl citrate and cetyl lactate. The amount of the second emulsifier was from 0.2 to 10 %. The w/o emulsion lipstick in this invention was comprised of 30 to 97 % w/w of oil, 1 to 25 % w/w of waxes and 1 to 20% w/w of water.

In1997, Wang and Lee [28] studied the effect of formulation parameters on the physicochemical properties of emulsion lipsticks. Emulsion lipsticks were prepared by addition of the emulsifier and water to the conventional lipstick. A lipophilic emulsifier, e.g. Span60 (HLB 4.7) and Span80 (HLB 4.3) and a hydrophilic emulsifier, e.g. Tween20 (HLB 16.7) were used as emulsifier systems to stabilize emulsion lipstick. Emulsion lipstick was prepared by conventional stirrer. The waxes and oils were molten at 85°C, followed by the addition of water phase to the mixture of molten waxes and oils. Two phases were mixed together homogeneously by stirrer. The physicochemical properties including hardness and crystallinity of lipstick were investigated. It was found that the hardness of lipstick did not change or slightly decreased as compared to that of conventional lipstick (wax type) after addition of both emulsifiers. Besides, the effect of water phase content (5, 10, 15% water content) on the physical properties of emulsion

lipstick was investigated. The hardness of emulsion lipstick was increased with increasing of the water content. The effect of formulation parameters on crystallization was studied by DSC. The heat of transition of emulsion lipstick was lower than that of conventional lipstick referring to the less crystalline of waxes in emulsion lipstick. When the water content was changed from 5% to 10%, the crystallization slightly decreased. Interestingly, no crystallization peak was observed when water content was increased to 15%. However, the effect of the emulsion droplets size on the lipstick properties was not reported.

2.4 Particle size analysis

Particle size is one of the basic characterization parameters for nanoemulsions. This methodology is a useful tool to confirm if the desired colloidal size range is obtained after preparation, upon storage and further processing, e.g. spray drying, freeze drying or sterilization. Likewise, the data obtained from particle size are crucial information for a formulator to develop and optimize the formulation during preliminary study.

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2.4.1 Photon correlation spectroscopy (PCS)

PCS is the most widely used method to characterize the particle size of nanoparticles. Several advantages of this method are shown such as the requirement of

small amount of sample, the ease to perform and the method's reliability. PCS is also known as dynamic light scattering. It determines the fluctuation of the light intensity scattered from particles caused by the Brownian movement of the particles in the diluted dispersion medium. Compared to the large particles, the smaller particles possess fast intensity fluctuations due to their higher diffusion coefficient. The data obtained from PCS is plotted as a function of scattering intensity and time curve. Afterwards, they are analyzed by an autocorrelation function to obtain effective diameter (z-ave diameter) and polydispersity index (PI), as an indication of the width of the particle size distribution. The z-ave describes an intensity weight. This method covers the particle size in the range of a few nanometers to 6 microns.

PCS is not a direct method for the determination of particle sizes, but it rather determines the particle diffusion coefficient (D) of the particle and subsequently the particle sizes are computed using Stokes-Einstein equation as following:

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$$\mathbf{D} = \frac{\mathbf{kT}}{3\pi\eta\mathbf{d}} \qquad (3)$$

Where D is the diffusion coefficient, k is Boltzmann's constant, T is the absolute temperature, η is the viscosity of the dispersing liquid and d is the particle size diameter. The obtained data from PCS are evaluated based on the assumption that the

particle shape is spherical. Therefore, the data obtained from PCS are meaningful and reliable when the measured sample is spherical with narrow and monomodal particle distribution in the nanometer range. On the other hand, artifacts of the particle sizes measured by PCS occur when the measured samples are broad and the particle size distribution is multimodal, especially containing both nanoparticles and microparticles.

2.4.2 Laser light scattering (LD)

In order to measure the particle sizes of the samples composed of both nanoparticles and microparticles, LD is usually applied. This method is probably a better choice than the PCS for the dispersion containing the particle sizes in the range of upper nanometer and/or the micrometer. LD measures the angular intensity distribution of the light scattered from the particles via an optical arrangement to a series of detectors recording a current proportional to the intensity of the scattered light falling upon them. The higher intensity scattering at high angle is obtained from small particle in comparison to the larger ones. However, the intensity of scattered light decreases tremendously with an increase in the scattering angles. As a result, LD was used to determine the particle size in the range of micrometer to millimeter size. In addition, this method is also used to determine nanoparticles. With using this technique for the measurement of particle in nanosize range, it requires the information of the optical parameters of the dispersed materials, e.g. the real and imaginary refractive indexes,

which can be obtained from the experiment and/or literature. The accuracy of the particle sizes distribution evaluation, therefore, may depend on the used optical parameters.

2.4.3 Light microscopy

In order to distinguish between microparticles and aggregation of nanoparticles, light microscopy is a useful tool. However, light microscopic method cannot be applied to determine the particle size when the size is less than 200 nm due to the limitation of the light source (limit of detection or LOD is approximately 200 nm). The resolution of the microscope is based on the wavelength of the light divided by the numerical aperture of the microscope objective. In general, this method is more reliable to determine the particle size of microparticles ($\geq 1\ \mu$m) than that of nanopaticles.

2.5 Differential Scanning Calorimetry (DSC)

DSC is a technique used to evaluate the difference in heat generated between sample and reference pans (empty pan). The obtained data from DSC reveal phase transitions in the monitored temperature range, for instance, melting point, enthalpy, crystallinity and polymorphism. Generally, two types of DSC instrumentation are available on the market which are power compensated DSC and heat flux DSC. Fig. 3

illustrates schematic diagram of power compensated DSC (a) and heat flux DSC. The power compensated DSC composes of two individual furnaces. Difference in the temperature between the sample and reference are compensated by supplying the heat required to keep both pans at the same temperature. In contrast to power compensated DSC, heat flux DSC comprises a single furnace. Heat flows into both sample and the reference material via an electrically heated constant thermoelectric disk and the difference in output of the two thermocouple junctions is recorded. Fig. 4 shows a DSC curve for an amorphous compound which undergo a glass transition (Tg), crystallization, melting and degradation.

DSC has been frequently used to elucidate and monitor physical changes such as increase in the degree of crystallinity and polymorphism of lipid after production and during storage [29]. This is due to its ability to provide detailed information about both the physical and energetic properties of the substances.

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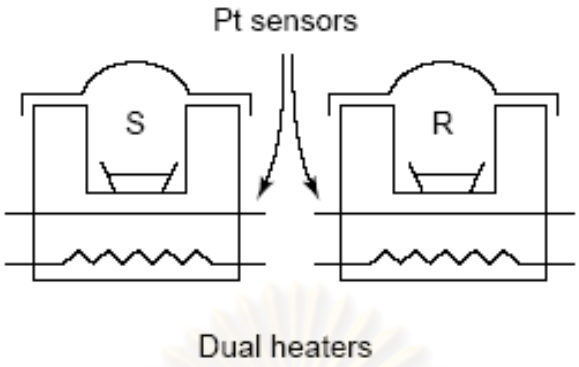
(a)



(b)

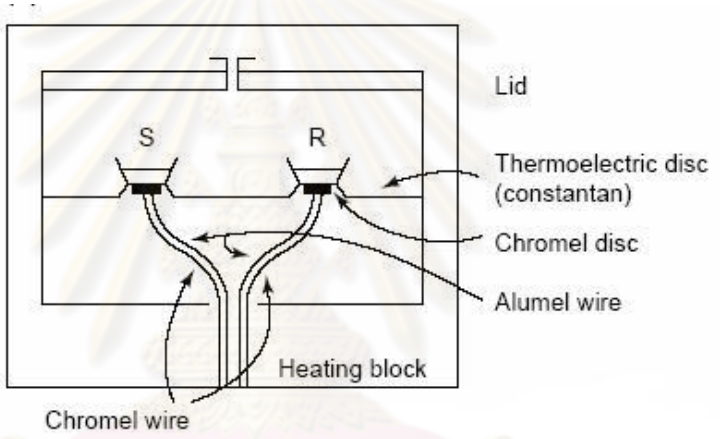


Fig. 3. Schematic diagrams of (a) Power-compensated DSC and (b) Heat flux DSC [30].

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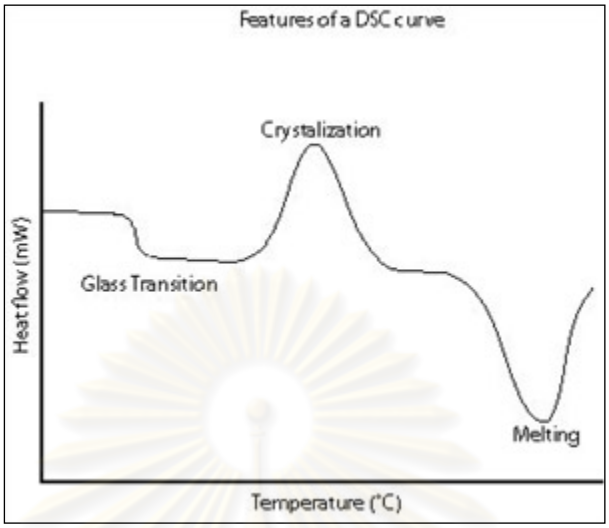

Fig. 4. Typical features of DSC curve [29].

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2.6 Polarized Light Microscopy (PLM)

Polarized light microscopy can mean any of a number of optical microscopy techniques involving polarized light. Simple techniques include illumination of the sample with polarized light. Directly transmitted light can optionally be blocked with a polarizer orientated at 90 degrees to the illumination. There are two polarizing filters in a polarizing microscope - termed the polarizer and analyzer. The polarizer is positioned beneath the specimen stage usually with its vibration azimuth fixed in the left-to-right, or east-west direction, although most of these elements can be rotated through 360°. The analyzer, usually aligned with a vibration direction oriented north-south, but again rotatable on some microscopes, is placed above the objectives and can be moved in and out of the light path as required. When both the analyzer and polarizer are inserted into the optical path, their vibration azimuths are positioned at right angles to each other. In this configuration, the polarizer and analyzer are said to be crossed, with no light passing through the system and a dark view field present in the eyepieces.

For incident light polarized microscopy, the polarizer is positioned in the vertical illuminator and the analyzer is placed above the half mirror. Most rotatable polarizers are graduated to indicate the rotation angle of the transmission azimuth, while analyzers are usually fixed into position (although advanced models can be rotated either 90° or 360°).

The polarizer and analyzer are the essential components of the polarizing microscope, but other desirable features include.

2.7 Texture analyzer

Characterization of texture commonly falls into two main groups, based on sensory and instrumental methods of analysis. Sensory analysis includes the senses of smell, taste, sound and touch. Sometimes the instrumental method is preferable to assess texture rather than sensory analysis because they can be carried out under more strictly defined and controlled conditions. Furthermore, problems of experimental variability are more likely to be caused by sample heterogeneity than by instrumental analysis.

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CHAPTER III

MATERIALS AND METHODS

3.1 MATERIALS

The reagents and substances used in this study were listed as the following:

1. Microcrystalline wax (Sonneborn, USA)
2. Beeswax (Kahl & Co. Vertriebsges, MBH, Germany)
3. Ceresin (Strahl & Pitsch Inc. ,USA)
4. Mineral oil (Sonneborn, USA)
5. Isopropyl myristate (Cognis, Thailand)
6. Dipentaerythrityl hexahydroxystearate (The Nisshin oil mills Ltd., Japan)
7. Hydrogenated polyisobutene (Sophim, France)
8. Tocopheryl acetate (BASF, Netherland)
9. Sorbitan sesquioleate (Span® 83, Kao, Japan)
10. PEG -30 Dipolyhydroxystearate (UNIQEMA, UK)
11. Polysorbate 60 (CRODA, USA)
12. Deionized water

3.2 EQUIPMENTS

1. High pressure homogenizer (APV 2000, Germany)

2. Differential scanning calorimetry (Mettler Toledo, Switzerland)

3. Nanosizer (Malvern Instruments, UK)

4. Laser diffraction particle size analyzer (LA-950V2, HORIBA, USA)

5. Stirrer (Heidon, Japan)

6. Rotor/Stator Homogenizer (Silverson, UK)

7. Texture analyzer (Stable Micro Systems, UK)

8. Camera (Panasonic, Japan)

9. Polarized light microscope (Olympus CX31, Olympus corporate, Japan).

3.3 METHODS

3.3.1 Selection of the emulsifier

Selection of an appropriate emulsifier to stabilize w/o nanoemulsions for further studying was done. Emulsifiers having different HLB values were chosen to study and determine the stability of w/o nanoemulsions. Lipophilic emulsifiers, e.g. Span83 (HLB 3.7) and PEG-30 Dipolyhydroxystearate (HLB 5.5) and a hydrophilic emulsifier, e.g. Polysorbate 60 (HLB 14.9) were selected to prepare w/o nanoemulsions. Span83 and

PEG-30 Dipolyhydroxystearate were added in oil phase containing mineral oil and isopropyl myristate (IPM) whereas polysorbate 60 was added in the water phase. The amount of water and emulsifier in the lipstick formula was constant at 2 and 1% w/w, respectively. The w/o nanoemulsions were prepared by addition of the water phase into the oil phase and then mixed together by rotor/stator homogenizer at a speed of 3000 rpm at 75-80^{o}C for 5 min, then the coarse w/o emulsions were obtained. To reduce the water droplet sizes to nanosize range, w/o coarse emulsions were passed through HPH for 3 cycles at the constant pressure of 500 bar using APV 2000 and temperature was maintained at 75-80^{o}C. The developed w/o nanoemulsions were cool down under ambient condition to room temperature. The compositions of developed w/o nanoemulsions are shown in Table 1. The developed w/o nanoemulsions were stored under solar box and freeze-thaw in order to challenge the system under accelerated condition for predicting long-term stability. The physical stability of developed w/o nanoemulsions was determined by investigation of visual appearance of the samples after storage for 8 hr and 12 days under accelerated condition as mentioned above, respectively. The suitable emulsifier was selected based on the stability of w/o nanoemulsions. The emulsifier providing a good stability of w/o nanoemulsions was selected for further studies.

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3.3.2 Preparations of w/o nanoemulsions and conventional w/o emulsions

Comparing with other emulsifiers, w/o nanoemulsions which stabilized with PEG 30 dipolyhydroxy stearate showed the highest stability under accelerated storage conditions. Therefore, PEG 30 dipolyhydroxy stearate was chosen as the emulsifier for preparing w/o nanoemulsion and conventional w/o emulsions with the different water contents. The w/o nanoemulsion and conventional w/w emulsions were composed of isopropyl myristate (IPM) and mineral oil as continuous phase. The amount of water phase was in the range of 0-10% w/w. Firstly, an emulsifier was dispersed in the melted lipid phase containing IPM, mineral oil and tocopherol at the temperature of 75-80^{o}C to produce coarse w/o emulsions using rotor/stator homogenizer at a speed of 3000 rpm for 5 min. For preparing w/o nanoemulsions, the hot coarse w/o emulsions were future passed through HPH applying 3 cycles at 500 bar and 75-80^{o}C.

For conventional emulsions, the compositions of the formulations were similar to those of w/o nanoemulsions. However, the procedure for the preparation was different. Briefly, an emulsifier was dispersed in the melted lipid phase containing IPM, mineral oil and tocopherol at the temperature about 75-80^{o}C by rotor/stator homogenizer at 1000 rpm for 10 min in order to obtain w/o emulsion with the droplet size in the micro sized range.

3.3.3 Preparations of w/o nanoemulsions and conventional w/o emulsion lipsticks

The w/o nanoemulsions or conventional w/o emulsions with different water contents were separately incorporated into the lipstick base containing beeswax, candellila wax, ceresin wax, dipentaerythrityl hexahydroxystearate, hydrogenated polyisobutene and polyethylene. The compositions of formulation ingredients were given in Table 1. For producing w/o nanoemulsion or conventional w/o emulsion lipsticks, all ingredients of lipstick base aforementioned above were heated to 85-90^{o}C and subsequently, the w/o nanoemulsions or conventional w/o emulsions were gradually added into the lipstick base and mixed with rotor/stator homogenizer at a speed of 1000 rpm for 5 min. The hot w/o nanoemulsion lipsticks was filled in aluminum mold and subsequently cooled down by storage in the refrigerator for 10 min. The temperature in the refrigerator was set at -2^{o}C. Afterwards, the w/o nanoemulsions lipstick were obtained

3.3.4 Characterization

3.3.4.1 Particle size analysis

The droplet size (z-ave) and polydispersity index (PI) of w/o nanoemulsions were analysed by photon correlation spectroscopy with a Zetasizer NanoZS (Malvern Instrument, UK). PCS yields the mean particle size (z-ave) and the polydispersity index (PI) which is a measure of the width of the size distribution. The z-ave and PI values

were obtained by averaging of 3 measurements at an angle of 173° at 25°C. IPM was used as dispersant media. The refractive index and viscosity of IPM were 1.435 and 9.0 cps, respectively. The refractive index and absorption value of nanoemulsions were set at 1.33 and 0.10, respectively. The droplet size analysis was determined using the Mie theory.

Due to the slow movement and precipitation of large particles; therefore, photon correlation spectroscopy cannot be applied to measure the droplet size of conventional w/o emulsions. To detect the droplet size of conventional w/o nanoemulsions, the laser diffractive technique was applied using LA-950V2, HORIBA, USA. The w/o conventional emulsions were drop gradually into IPM used as the dispersant media. The refractive index of w/o conventional emulsions were set at 1.33-0.1i. The mean particle size were obtained by averaging of 3 measurements.

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Table 1. Compositions of all developed formulations (%w/w)

Compositions	A0	A1	A2	A3	A4	B1	B2	B3
w/o emulsions part								
Water	0.00	0.50	2.00	5.00	10.00	2.00	2.00	2.00
PEG-30	0.00	1.00	1.00	1.00	1.00	0.50	1.50	2.00
Dipolyhydroxy-stearate								
Mineral oil	21.80	21.48	21.15	20.50	19.41	21.26	21.04	20.93
Isopropyl myristate	38.09	37.52	36.95	35.81	33.90	37.14	36.76	36.57
Tocopheryl acetate	0.10	0.10	0.10	0.10	0.09	0.10	0.10	0.10
Lipstick wax base								
Microcrystalline wax	3.97	3.91	3.85	3.73	3.53	3.87	3.83	3.81
Beeswax	4.07	4.01	3.95	3.83	3.62	3.97	3.94	3.91
Hydrogenated polyisobutene	13.20	13.00	12.80	12.40	11.74	12.86	12.73	12.67
Dipentaerthrityl hexahydroxystearate	3.09	3.05	3.00	2.91	2.75	3.02	2.98	2.97
Polyethylene	9.48	9.34	9.20	8.92	8.44	9.25	9.15	9.10
Ceresin wax	6.19	6.09	6.00	5.81	5.51	6.03	5.97	5.94
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

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3.3.4.2 DSC analysis

In order to determine lipstick crystallinity and thermal behavior, DSC was performed using Mettler DSC 1 apparatus (Mettler Toledo, Switzerland). The lipstick samples were weighed approximately 10-20 mg in 40 µl aluminium pan. An empty pan was used as a reference. The samples were heated from 20°C to 100°C and cool down to 20°C at the heating rate or cooling rate of 5°C/min under nitrogen gas flow. The DSC parameters including onset, melting point and enthalpy were evaluated using STAR^{e} software 9.3

3.3.4.3 Hardness and spreadability measurement

The hardness and spreading ability of lipsticks were determined by using texture analyzer (Stable Micro Systems, UK). The lipstick with 13 mm in diameter and 20 mm in length were prepared. The lipstick samples were then held at 25±0.1°C for 24 hr before measuring. To study the hardness of lipsticks, a needle probe was selected for the measurement. Briefly, the needle probe was pressed into the lipsticks at the distance of 10 mm from the top of flat surface lipsticks with the constant velocity of 1.0 mm/s. The force-displacement curve was obtained and the maximum force in the force-displacement curve was determined as the hardness. The spreading ability of lipsticks was performed using a spherical stainless probe. The constant force of 30 g was applied for penetrating the probe into the sample at constant velocity of 1.0 mm/s. When

the 30 g load was reached, the distance traveled by 30g load was measured over a 5 sec period and was determined as the spreading ability. The experiment was done triplicate for each formulation.

3.3.4.4 Microstructure determination

The microstructures of lipsticks were observed using polarized light microscopy (PLM). The lipstick sample was placed on a glass slide and then the glass cover slip was placed over the sample. The lipstick samples were viewed using Olympus CX31 polarized light microscope (Olympus corporate, Japan). The images of all lipstick samples were recorded using an Olympus DP20 camera (Olympus corporate, Japan). The pictures were taken at the magnitude of 20X.

3.3.4.5 Stability test

- **Accelerated stability test**

The w/o nanoemulsions were kept under accelerating conditions, e.g., solar exposing condition for 8 hr and freeze-thaw cycles for 6 cycles. The irradiation range in solar box is 295-800 nm and the average radiation received in solar box 550 W/m^2. In a cycle of the freeze-thaw condition, the sample were frozen at 5^{o}C with the freezing time was 24 hr. Thawing was carried out at temperature of 50^{o}C for 24 hr. After a certain time, the appearance of the stored samples were investigated.

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- **Long-term stability test**

The w/o nanoemulsions (dispersions), lipstick base and lipstick containing w/o nanoemulsions were kept at the different temperatures (4°C, 25°C and 40°C) for 3 months. After a certain time, the physicochemical properties including mean droplet size, size distribution, and appearance were determined.

3.3.4.6 Statistics

The reported data were presented as mean value±standard deviation (S.D.). The significant of difference was evaluated using one way ANOVA at the probability level of 0.05.

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CHAPTER IV

RESULTS AND DISCUSSION

4.1 Selection of an appropriate emulsifier

The emulsifiers are the most important composition of emulsions. They play a major role in stabilize emulsion formulations by being adsorbed onto the oil droplet surface. Therefore, the selection of an appropriate emulsifier to stabilize w/o nanoemulsions was firstly examined in the present study.

Fig. 5 shows the appearance of w/o nanoemulsions prepared by different types of emulsifiers including span 83, PEG 30 Dipolyhydroxy stearate and polysorbate 60. These selected emulsifiers are nonionic surfactants generally used in cosmetics and their HLB values are 3.7, 5.5 and 14.9, respectively. The w/o nanoemulsions containing 2% w/w of water with 1% w/w of each emulsifier were prepared and stored in the solar box (representative of sunlight exposure) and freeze-thaw cycles as the accelerated conditions for the primary prediction of the product stability. The obtained results showed that the w/o nanoemulsions emulsified by span 83 and polysorbate 60 were not stable because the phase separation was observed under solar box and also under the freeze-thaw cycles. On the contrary, the w/o nanoemulsions samples emulsified by PEG

30 Dipolyhydroxystearate was observed of no separated phase under either aforementioned condition or room temperature indicated the preparations were stable.

According to above results, it could be explained by the HLB theory as described by Griffin [31]. HLB value is the function of the weight percentage of hydrophilic portion of the molecules of non-ionic surfactant for determining the HLB of emulsifiers. Low HLB values are classified as lipophilic emulsifiers, whereas emulsifiers with high HLB values are considered as hydrophilic emulsifiers. With regard to w/o emulsion system, the suitable HLB values are in the range of 3-8. Concerning the HLB value of Polysorbate 60, it is 14.9 which acts as o/w emulsifier instead of w/o emulsifier. Generally, this nonionic emulsifier adsorbs onto the emulsion droplets and, although it generally reduces zeta potential, it maintains stability by creating a hydrated layer on the hydrophobic particle in o/w emulsions. Due to the high HLB value of Polysorbate 60, it could not stabilize the w/o emulsion system. This can be explained by the theory of required HLB. In general, stable emulsions can be prepared by selecting emulsifiers having HLB close to the required HLB value (rHLB) of the oils. The rHLB values for both o/w and w/o emulsions have been determined empirically for a number of oils and oil-like substances. Based on composition of the w/o emulsions, oils and oil-like substances composed of mineral oil and isopropyl myristate. The rHLB of mineral oil is in the range of 5-7 for w/o emulsions whereas that of isopropyl myristate is not available for w/o

emulsions. However, we estimated that the rHLB value of isopropyl myristate for w/o emulsions might be in the range of 5-7 as well. Therefore, the rHLB of developed w/o emulsions should be in the range of 3-8.

Concerning the stability data of w/o emulsion stabilized with Span 83, instability of system was also observed. Because of invalid information of rHLB of isopropyl myristate, two possibilities used to explain instability of w/o emulsion stabilized with Span 83 are proposed. Firstly, the HLB value of Span 83 is not suitable (too low). Secondly, the amount or concentration of emulsifier in the formulation is not enough. According to rHLB calculation, it is possible to establish an HLB range for optimum formulation but the total amount of emulsifier phase may not be reached. The optimum amount of emulsifier could be obtained from trial and error by increasing the amount of emulsifier. Usually, the minimum concentration to give the desired degree of physical stability was chosen.

Regarding to the stability study, the w/o emulsion emulsified with PEG 30 Dipolyhydroxystearate was finally selected to prepare w/o nanoemulsion for further investigation.

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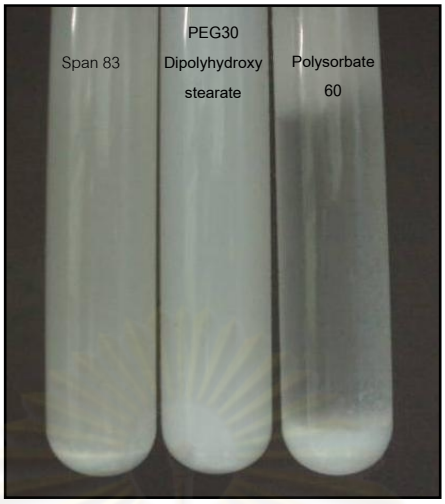

(b)

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Fig. 5. Separation of w/o nanoemulsions emulsified with Span 83, PEG 30 Dipolyhydroxystearate and Polysorbate 60 after keeping at the accelerated conditions (a) solar box for 8 hr and (b) freeze-thaw for 6 cycles.

4.2 Effect of water content on the particle size and polydispersity index of w/o nanoemulsions

To investigate the influence of water content on the particle size and size distribution of w/o nanoemulsions, all developed formulations were emulsified with PEG 30 Dipolyhydroxystearate. The dose of this emulsifier was kept constantly at 1 % w/w while the water content was varied from 0.5% to 10% w/w. The mean particle size (z-ave) of all developed formulations after preparation is shown in the Fig. 6. The z-ave of all developed formulations was found to be in the range of 70-160 nm. It was noticeable that the water content (dispersed phase) significantly affected the z-ave ($p<0.05$). In comparison the z-ave of w/o nanoemulsions containing 0.5 and 2% of water, there was no significant difference was observed, whereas the significant difference of this z-ave was observed in w/o nanoemulsion containing 5 and 10% of water. The similar finding has been reported by Teeranachaideekul *et al* [32]. This study is likely to conclude that the increase of water tends to increase the z-ave. Concerning the PI values, all developed formulations showed the PI values of lower than 0.3 indicating a relative narrow size distribution.

Fig. 7 shows the relation between the ratio of water and PEG 30 dipolyhydroxy stearate on the z-ave. The results indicate that the proportion of water to emulsifier at 2:1 did not affect the z-ave but instead, the proportion of water and emulsifier to 10:1

increased this value. This may be due to the increase in surface area of dispersed phase (with constant emulsifier).

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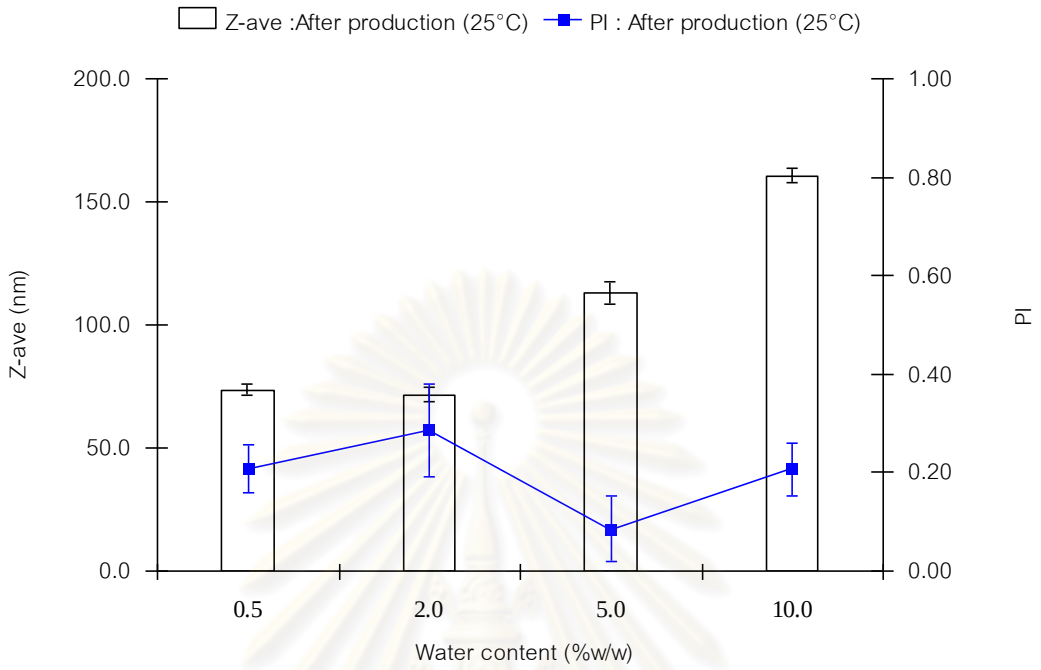

Fig. 6. Mean particle size (z-ave) and Polydispersity Index (PI) of w/o nanoemulsions with different water compositions.

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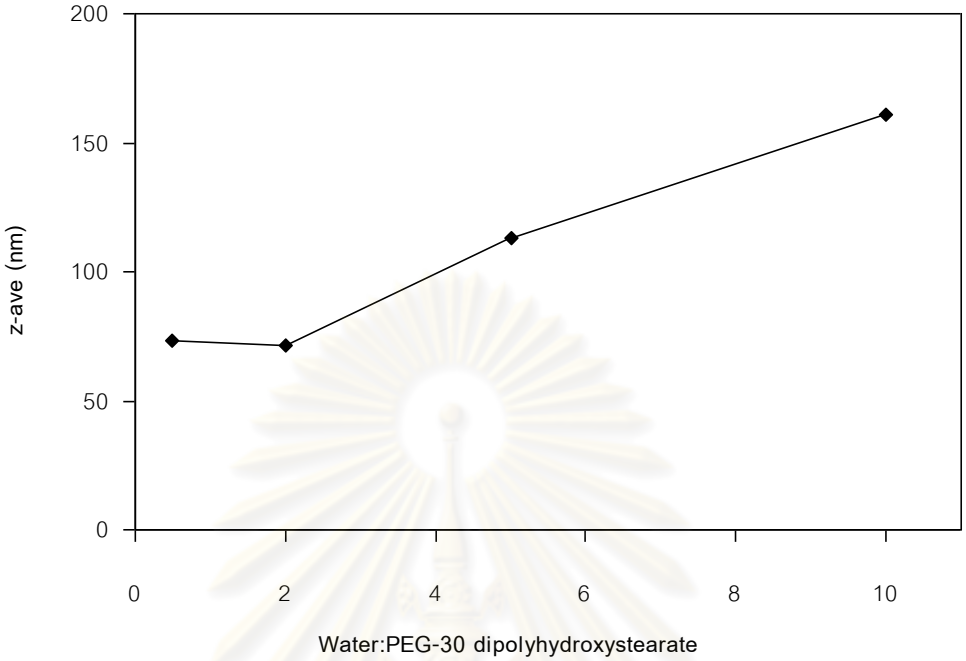

Fig. 7. Particle sizes of nanoemulsions prepared from different contents of water with constant dose of PEG 30 Dipolyhydroxystearate.

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4.3 Effect of emulsifier concentration on the particle size and polydispersity index of w/o nanoemulsions

To investigate the effects of the emulsifier concentration on the particle size of w/o nanoemulsions, the w/o nanoemulsion samples were prepared from 2% w/w of water with different doses varied from 0.5-2% w/w of PEG 30 Dipolyhydroxystearate. Results in Fig. 9 showed that the emulsifier concentration significantly influenced the z-ave. The w/o nanoemulsion emulsified with 0.5% w/w of PEG 30 Dipolyhydroxystearate showed a bigger particle size than those emulsified with 1, 1.5 and 2%. It is probable that the increasing of emulsifier concentration would reduce the interfacial tension between water and oils phases [33, 34]. Consequently, the water droplets can be easily reduced during the homogenization process. However, the increasing amount of PEG 30 dipolyhydroxystearate from 1 to 2% did not have any effect on the z-ave ($p>0.05$). Fig. 7 showed the relation between the ratio of water to emulsifier and the mean particle size. The optimum amount of emulsifier required to fully cover the surface of water droplet was 2%.

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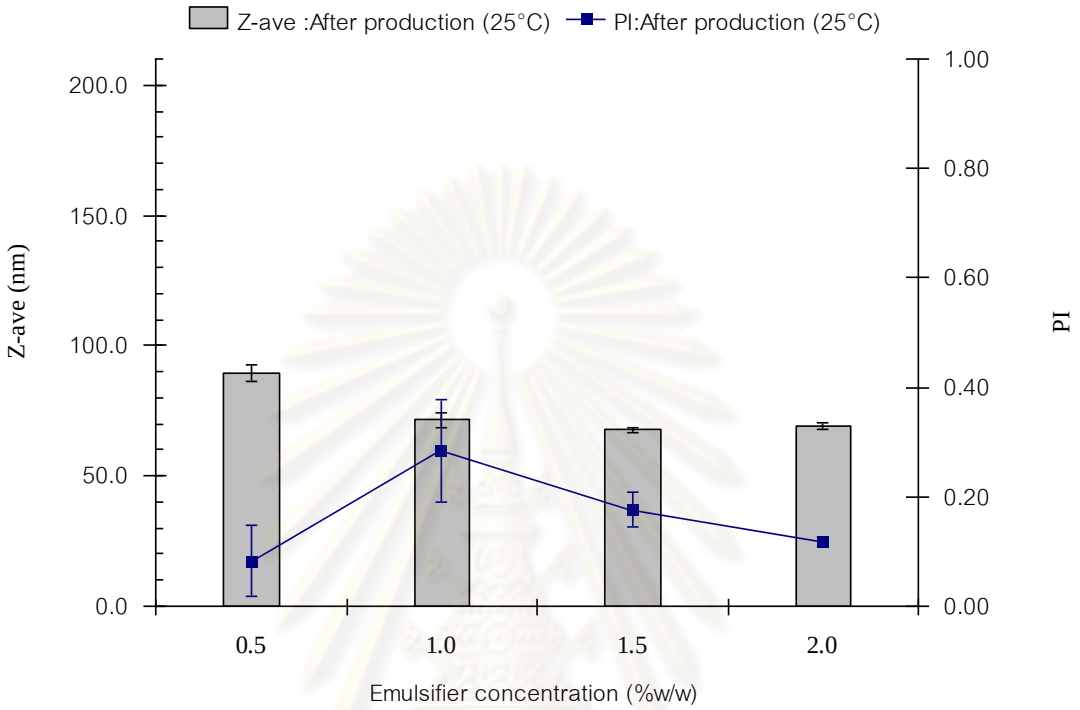

Fig. 8. Mean particle size (z-ave) and polydispersity index of w/o nanoemulsions at the constant water content 2% w/w and varied concentrations of PEG 30 Dipolyhydroxystearate.

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4.4 Effect of water content on the microstructure of w/o nanoemulsion lipsticks

The microstructures of lipsticks were performed by using PLM. Fig. 9 shows the microstructure of conventional lipsticks and w/o nanoemulsion lipsticks containing various amounts of water content (a) 0%, (b) 0.5%, (c) 2% and (d) 10 % w/w. It was found that the crystal size of waxes depended on the amount of water in the formulation. The higher the water content, the higher the crystal size was observed. The w/o nanoemulsions lipsticks containing 10% of water showed the highest crystal size, followed by w/o emulsion lipstick containing 2% and 0.5%, respectively. Increasing the water content in w/o nanoemulsion or lipstick formulation may accelerate the crystallization of waxes leading to the formation of large crystal during molding lipstick at the ambient condition. This also affected the hardness of lipsticks.

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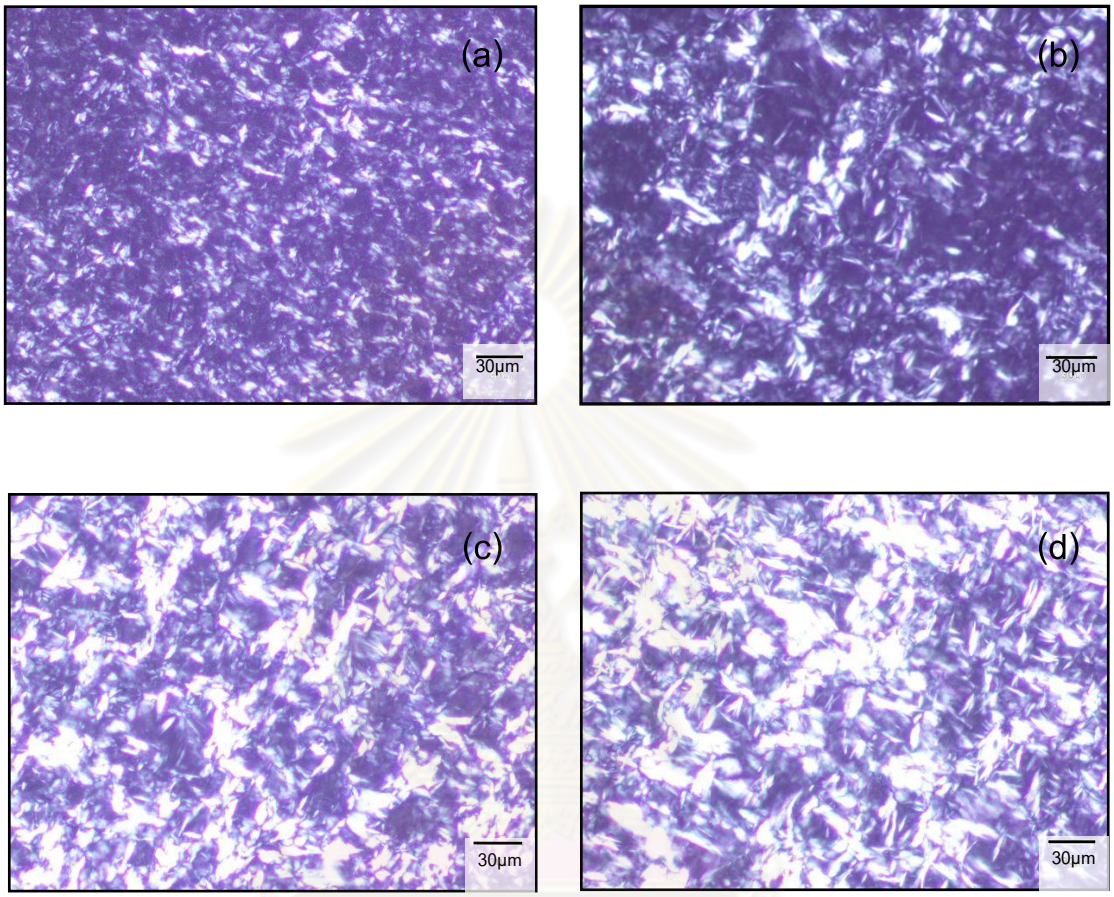

Fig. 9. Polarized light micrograph of (a) conventional lipsticks, (b) w/o nanoemulsion lipsticks containing water 0.5%, (c) 2.0% and (d) 10% w/w.

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4.5 Effect of water and emulsifier concentration added in the w/o nanoemulsions on the hardness and spreadability of lipsticks

The hardness and spreadability are the important physical properties to identify and characterize fat products, e.g., margarine, butter, shortening and chocolate. A number of studies revealed the physicochemical properties of fat was affected by the crystallization process influenced by several factors including solid fat content (SFC), polymorphism and microstructure of crystal network [35, 36] and a cooling rate [37, 38].

The influence of water content on hardness and spreadability is shown in Figs. 10 and 11, respectively. It was observed that the hardness and spreadability of emulsion lipsticks slightly increased with the increasing amount of water content. It could be due to the fact that the increase of water in the formulations might accelerate the crystallization of solid lipid in the lipstick formulation, as confirmed by PLM image as said above, resulting in the increase in the lipstick hardness. On the other hand, the increasing amount of emulsifiers did not significantly affect the hardness and spreadability of w/o nanoemulsion lipsticks (p>0.05) as shown in Figs. 12 and 13, respectively. Regarding the chemical structure of PEG 30 dipolyhydroxystearate, it composes of hydrophobic group (hydroxystearate) and hydrophilic group (PEG-30). The hydrophobic part of PEG 30 dipolyhydroxystearate (hydrocarbon chain) protrudes into oily continuous phase. This leads to more imperfection in crystal or retards/disturbs the

crystallization of solid lipids (waxes). In previous study, some research group reported that the emulsifier can accelerate the nucleation rate but reduced the rate of growth of crystallization process [39] which effect to the hardness of fat. However, the effect of emulsifier on the hardness and spreadability of lipsticks in this study was neglectful. This might be due to the low amount of an emulsifier added to the system (0.5-2.0% w/w).

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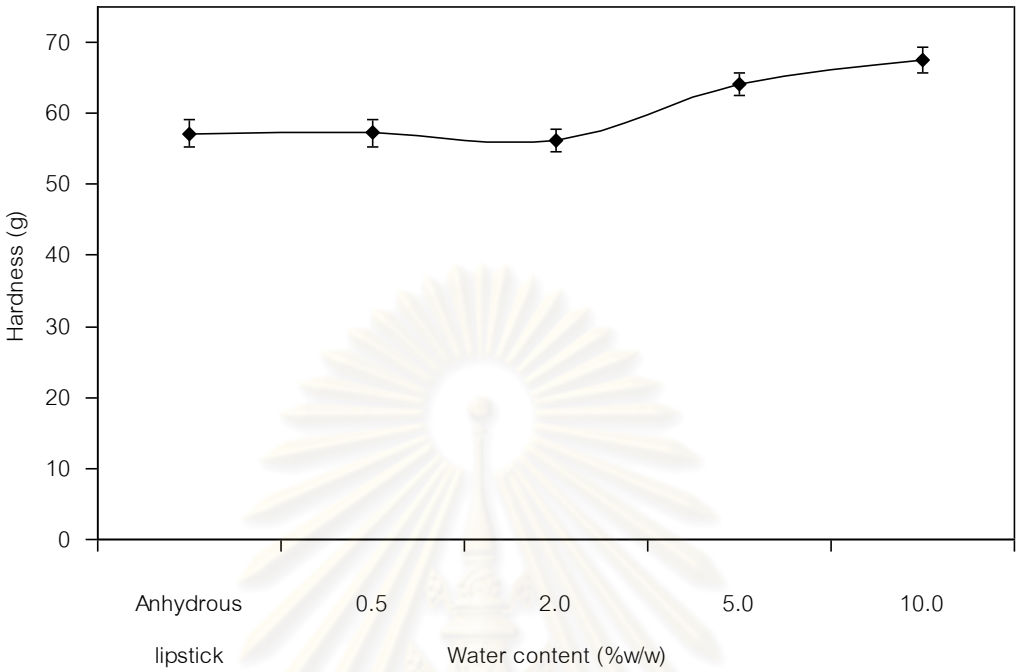

Fig. 10. Hardness of w/o nanoemulsion lipsticks prepared from different water contents.

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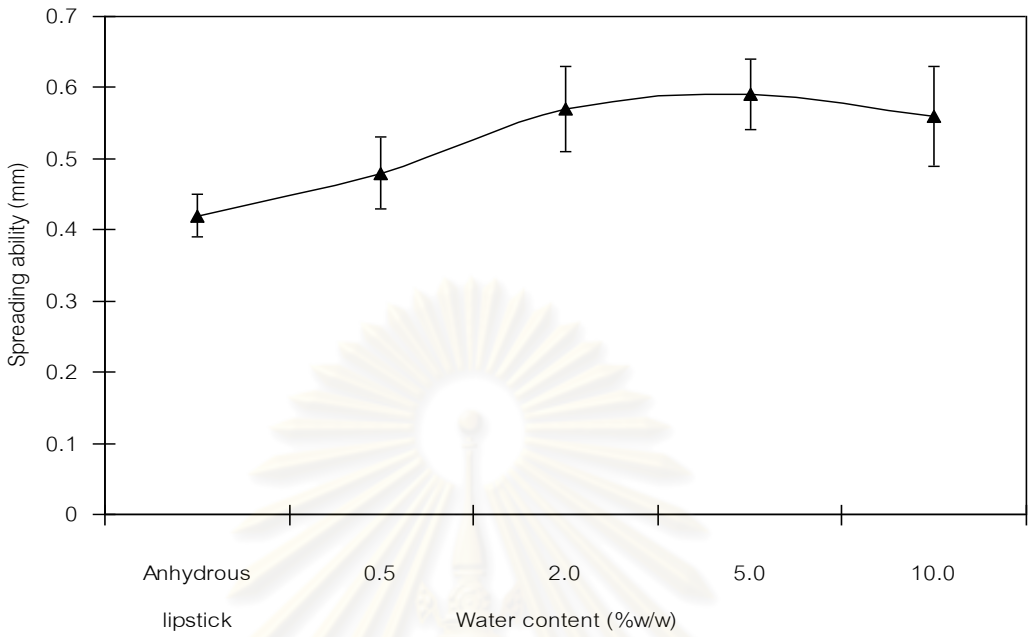

Fig. 11. Spreadability of w/o nanoemulsion lipsticks prepared from different water contents.

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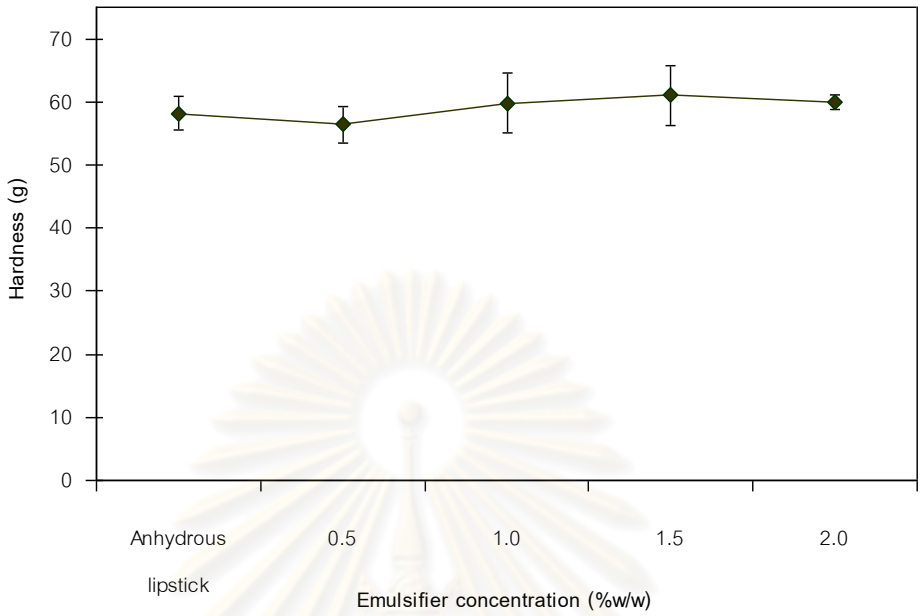

Fig. 12. Hardness of w/o nanoemulsion lipsticks prepared from different emulsifier contents.

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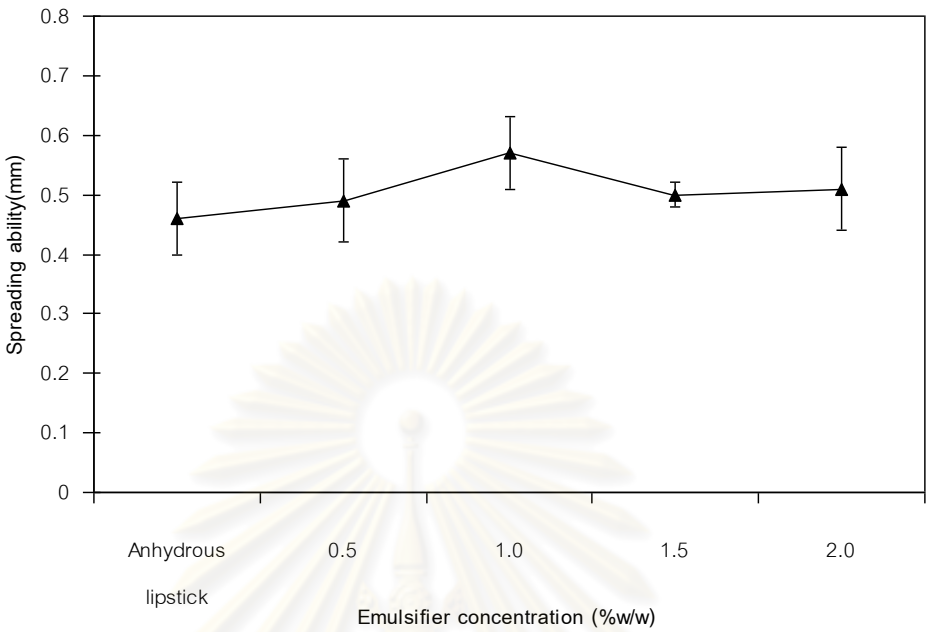

Fig. 13. Spreadability of w/o nanoemulsion lipsticks prepared from different emulsifier contents.

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4.6 Effects of water and emulsifier concentration on the crystallization of w/o nanoemulsion lipsticks

DSC was performed to investigate the influence of water and emulsifier concentration on the crystallization of w/o nanoemulsion lipsticks. Tables 2 and 3 show the DSC parameters. These parameters included melting point, onset and enthalpy of conventional and w/o nanoemulsion lipsticks. It was found that the presence of water affected the crystallization of solid lipid as indicated by the enthalpy of w/o nanoemulsion lipsticks. The enthalpy of w/o nanoemulsions containing water 0.5, 2, 5 and 10% was higher than that of the conventional lipsticks without any water. Besides, the higher the water content the higher the enthalpy of w/o nanoemulsion lipsticks was obtained. It might be explained that the increasing of water content might accelerate the crystallization of solid lipid in the lipstick formulation as already described in the section 4.5. The data from DSC are correlated with the data obtained from PLM, hardness and spreadability.

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Concerning the effect of emulsifier concentration on the crystallization of lipsticks, the varied emulsifier concentration from 0.5 to 2%w/w did not significantly affect the enthalpy of w/o nanoemulsion lipsticks; the enthalpy of lipsticks was almost constant although the concentration of emulsifier was increased. Therefore, the increase in enthalpy of w/o nanoemulsion lipsticks was not due to the effect of emulsifier

concentration but it would rather dominated by the concentration of water in the lipstick formulations. Concerning the effects of water and emulsifier concentration on melting point of lipsticks, it was found of no correlation. Because the lipstick formulation composed of many types of wax which has the different melting point, therefore the melting point and onset of lipsticks were fluctuated in a broad range.

Table 2. Melting point, onset and enthalpy of convention and w/o nanoemulsion lipsticks prepared from different water content detected by DSC

Formulated water (% w/w)	Melting point (^{o}C)	Onset (^{o}C)	Enthalpy (J/g)
Conventional lipsticks (Nanoemulsified lipsticks)	78.65	78.25	7.46
0.5	64.82	43.45	9.74
2.0	55.08	43.84	10.22
5.0	55.23	44.02	10.42
10.0	84.51	78.87	12.79

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Table 3. Melting point, onset and enthalpy of w/o nanoemulsion lipsticks prepared from different emulsifier concentration after preparation detected by DSC

Formulated mulsifier (% w/w)	Melting point (^{o}C)	Onset (^{o}C)	Enthalpy (J/g)
0.5	65.55	63.71	13.71
1.0	70.13	69.23	13.77
1.5	68.20	66.40	13.66
2.0	64.63	59.70	13.70

4.7 Long-term physical stability of w/o nanoemulsions and w/o nanoemulsion lipsticks

To investigate the long-term physical stability of the w/o nanoemulsions and w/o nanoemulsion lipsticks, the developed formulations were stored for a period of 3 months. The effects of formulation parameters including the concentration of water and emulsifier of w/o nanoemulsion lipsticks were investigated. In addition, the physical properties in terms of the mean particle size and appearance of w/o nanoemulsion were elucidated.

4.7.1 Effects of water and emulsifier concentration on the long-term physical stability of particle size and size distribution of w/o nanoemulsions

Figs. 14 and 15 depict the particle size (z-ave) of w/o nanoemulsions prepared at various concentrations of water and emulsifier (PEG 30 Dipolyhydroxystearate). The z-ave was analysed by dynamic light scattering technique after preparation and storage at 4, 25 and 40°C for 3 months. Results showed that the z-ave of all developed formulations were in the nanosize of less than 200 nm with the PI value of lower than 0.3, indicating a good physical stability of these colloidal systems.

4.7.2 Effects of water and emulsifier concentration on the long-term physical stability of the appearance of w/o nanoemulsions

Figs. 16 and 17 show the appearance of w/o nanoemulsions after storage at 4, 25 and 40°C for 3 months. It was found that the w/o nanoemulsions were homogeneous. There was no phase separation in all developed formulations. This result agreed with the particle size of w/o nanoemulsions as mentioned above.

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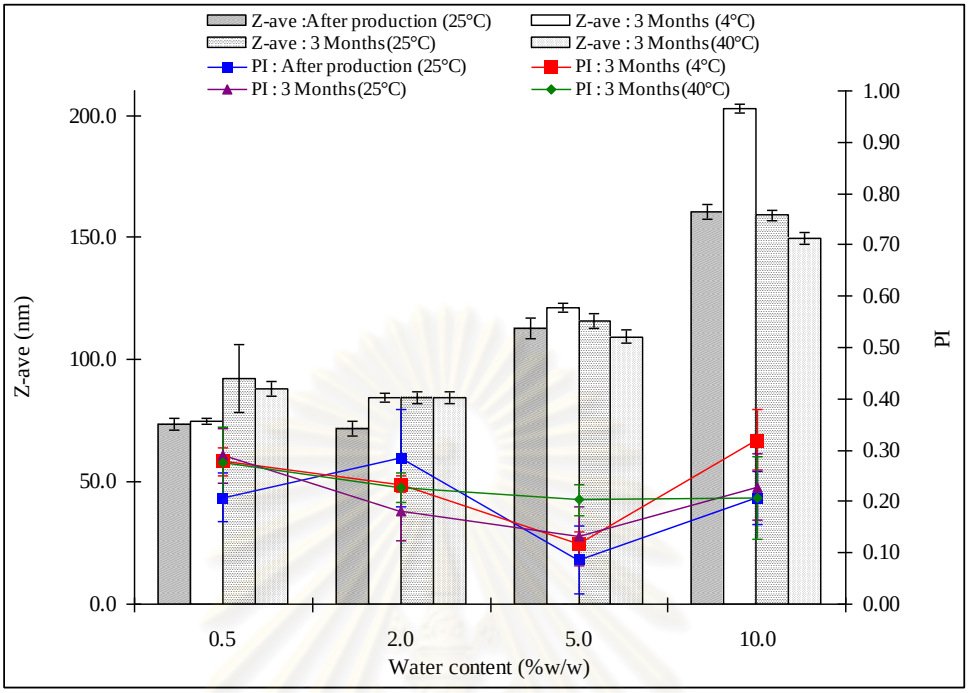

Fig. 14. Mean particle size of w/o nanoemulsions formulated with different water contents after being kept at 4, 25, 40°C for 3 months compared to the particle size after freshly prepared.

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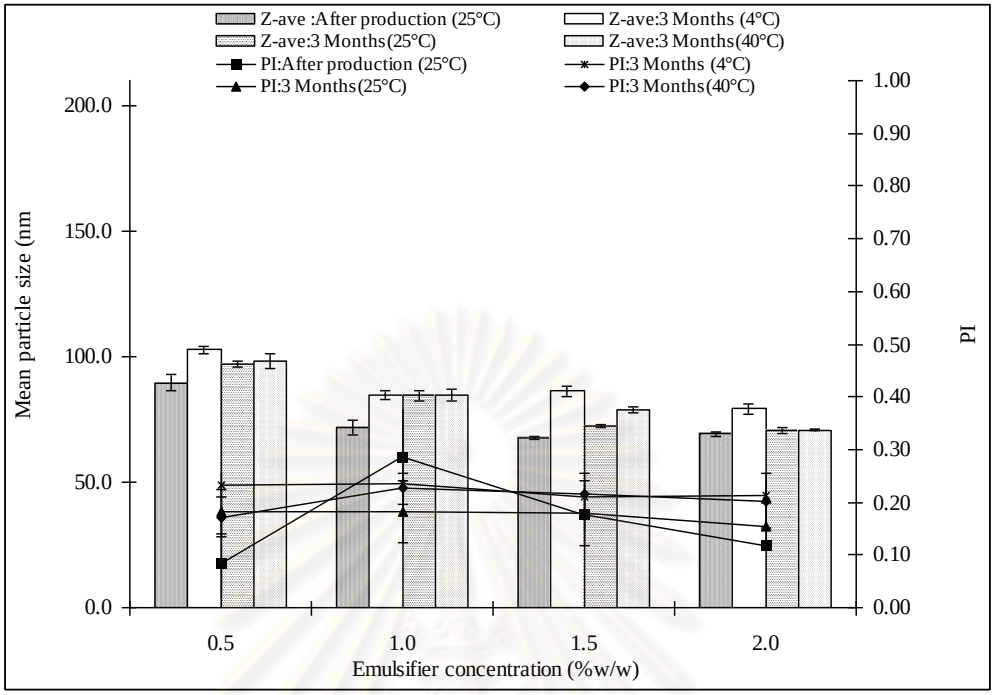

Fig. 15. Mean particle size of w/o nanoemulsions formulated with different emulsifier concentration contents after being kept at 4, 25, 40°C for 3 months compared to the particle size after freshly prepared.

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(a)

(b)

(c)

Fig. 16. Appearance of w/o nanoemulsions formulated with different emulsifier concentrations (0.5, 1, 1.5 and 2%w/w) after being kept at (a) 4°C, (b) 25°C and (c) 40°C for 3 months.

(a)

(b)

(c)

Fig. 17. Appearance of w/o nanoemulsions formulated with different water contents (0.5, 20, 5 and 10%w/w) after being kept at (a) 4°C, (b) 25°C and (c) 40°C for 3 months.

4.7.3 Effects of water and emulsifier concentration on the long-term physical stability of the hardness of w/o nanoemulsion lipsticks

The effects of water and emulsifier concentrations on the hardness of w/o nanoemulsion lipsticks after being kept at 25°C for 3 months were investigated. The results revealed that the hardness of w/o nanoemulsion lipsticks after 3-month storage at 25°C increased (Fig. 18 and 19). This would be due to the recrystallization of waxes during storage as confirmed by increasing enthalpy in DSC detection as shown in Table 4.

Table 4. Comparison of enthalpy values of w/o nanoemulsion lipsticks formulated with different water contents after preparation and having been kept at 25°C for 3 months

Formulated lipstick	Enthalpy (J/g)	
with water (%w/w)	Freshly prepared	3-minth kept
0	7.46	13.36
0.5	9.74	13.47
2.0	10.22	13.59
5.0	10.42	15.13
10.0	12.79	16.63

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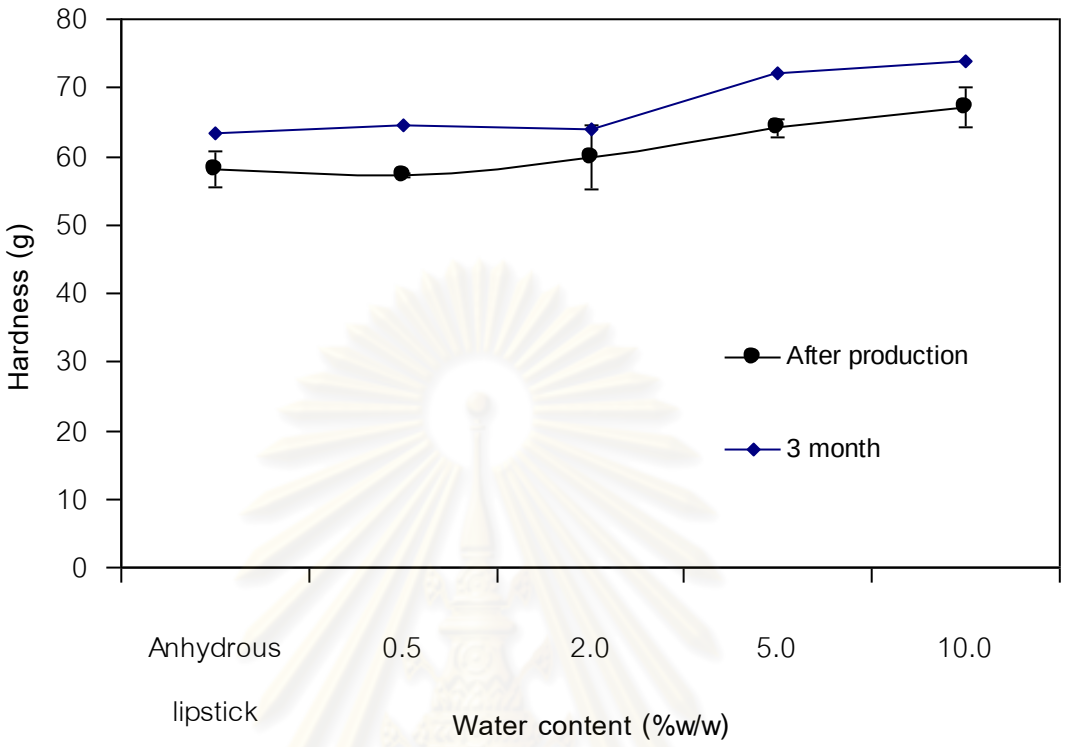

Fig. 18. Hardness of w/o nanoemulsion lipsticks formulated with different water content after 3-month storage at 25°C compared to the freshly prepared preparation.

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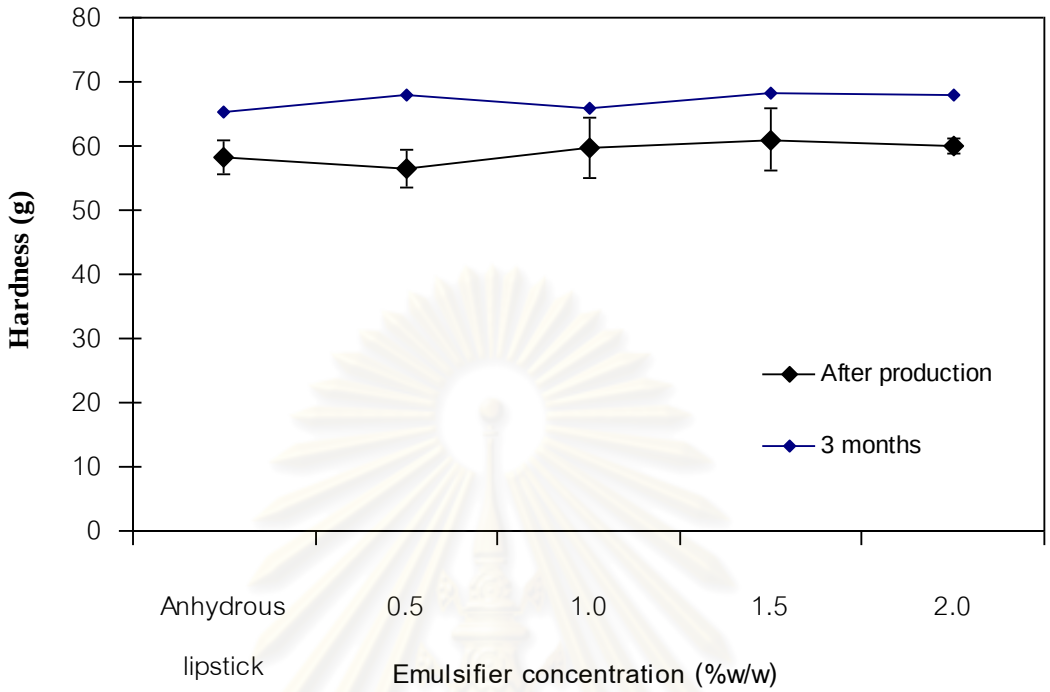

Fig. 19. Hardness of w/o nanoemulsion lipsticks formulated with different emulsifier concentrations after 3-month storage compared to the freshly prepared preparation.

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4.7.4 Effects of water and emulsifier concentration on the long-term physical stability of the appearance of w/o nanoemulsion lipsticks

To investigate the long-term physical stability, the appearance of w/o nanoemulsion lipsticks was evaluated. It was found that the appearance of all developed w/o nanoemulsion lipsticks did not change. No oil bleeding/sweating was observed. The images of developed w/o nanoemulsion lipsticks are shown in Figs. 20 and 21.

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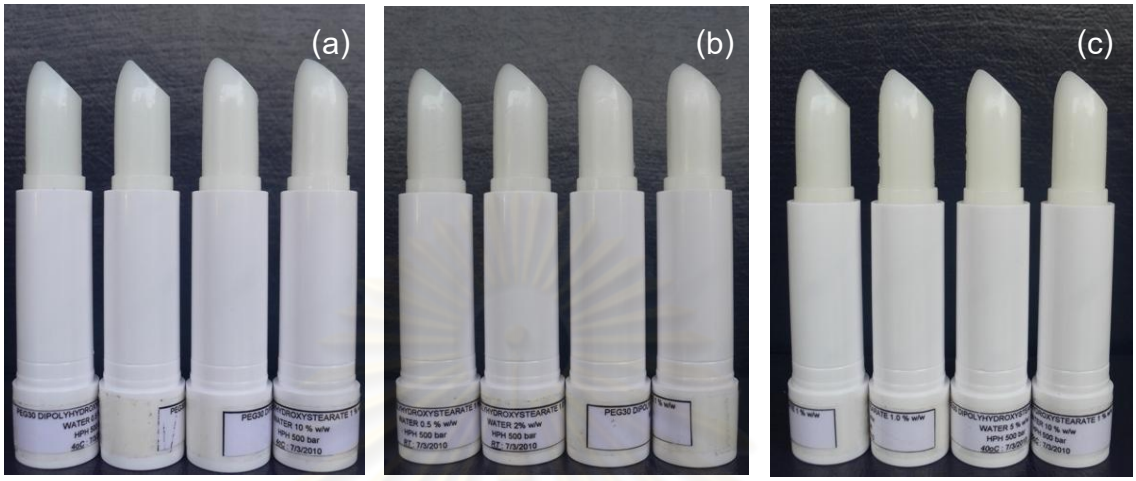

Fig. 20. Appearance of w/o nanoemulsion lipsticks formulated with different water contents (0.5, 2, 5 and 10%w/w) after 3-month storage at (a) 4°C, (b) 25°C and (c) 40°C.

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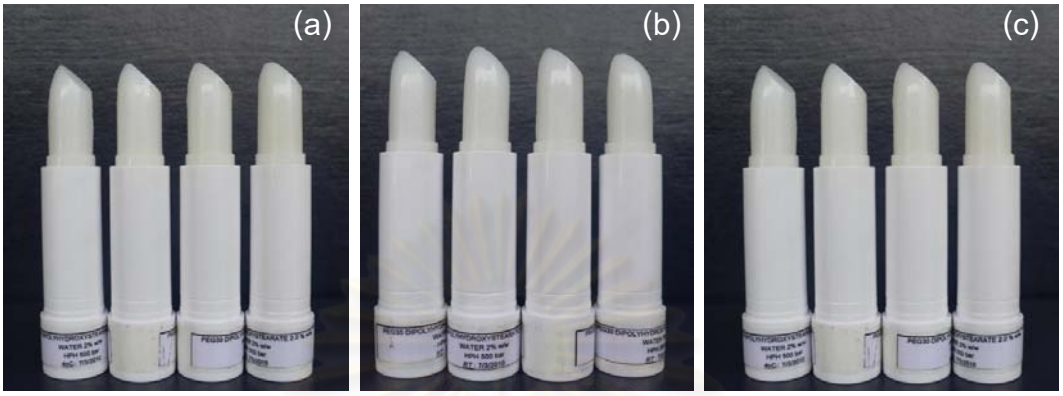

Fig. 21. Appearance of w/o nanoemulsion lipsticks formulated with different emulsifier concentrations (0.5, 1, 1.5 and 2%w/w) after 3-month storage at (a) 4°C, (b) 25°C and (c) 40°C.

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4.8 Effect of particle size on the physicochemical properties of conventional w/o emulsion lipsticks

In order to investigate the influence of particle size on the physical properties of lipsticks in terms of hardness, spreadability, thermal behavior and microstructure, the conventional w/o emulsions were prepared with the similar compositions of w/o nanoemulsion but the preparation method was different. They were prepared by high speed homogenizer to get the bigger particle size than the w/o nanoemulsions.

4.8.1 Particle size of conventional w/o emulsions

Fig. 23 shows the average particle size of w/o conventional emulsions analyzed by volume- and number distribution-based techniques after freshly prepared and 3-month storage at 4, 25 and 40°C. The diameter of the particle size of the above emulsion analyzed by these two techniques was found to be 21 μm and 3 μm, respectively. In comparison of the mean particle between the convention w/o emulsions and w/o nanoemulsions, the volume distribution diameter was used to compare with the z-ave in case of w/o nanoemulsions.

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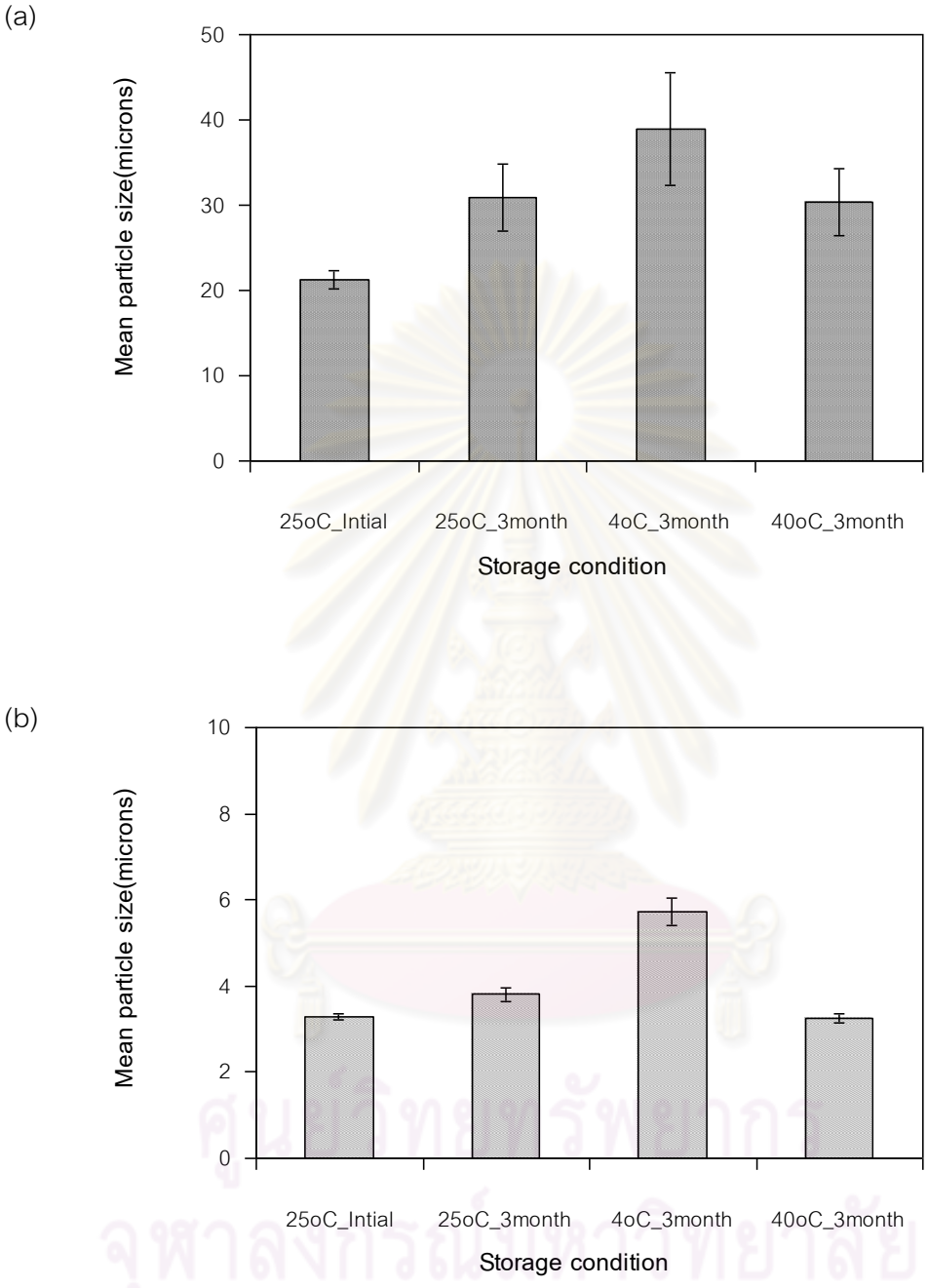

Fig. 22. Particle size distribution of conventional w/o emulsions analyzed by (a) volume-based and (b) number distribution-based techniques after freshly prepared and 3-month storage at 4, 25 and 40°C.

4.8.2 Microstructure of conventional w/o emulsion lipsticks

The crystallization of waxes in the conventional w/o emulsion lipsticks was influenced by particle size. The amount and size of crystals of conventional w/o emulsions and w/o nanoemulsions were determined by using PLM. Fig. 23 shows the difference in amount and size of crystals of lipsticks containing the conventional w/o emulsions and w/o nanoemulsions. Generally, the microstructure of continuous phase (waxes) affects the performance of texture [40]. If it contains a lot of large crystals, the texture of waxes will be hard and brittle. On the other hand, if the microstructure of continuous phase contains a lot of small crystals, its texture will be soft, sloppy and oily [41,42]. In the present study, the microstructure of conventional w/o emulsions contained a lot of small and large crystals of waxes while that of the w/o nanoemulsion contained mostly large crystals. As a result, a strong crystal network of conventional w/o emulisions was predominant, as compared to w/o nanoemulsions, resulting in a higher value of hardness. There was a study revealed the forces used to stabilize a wax crystal network were van der Waals forces [43]. However, the explanation why the microstructure of the conventional w/o emulsion lipsticks contained a lot of small and large crystals needed to be studied more as it could not be concluded in this study.

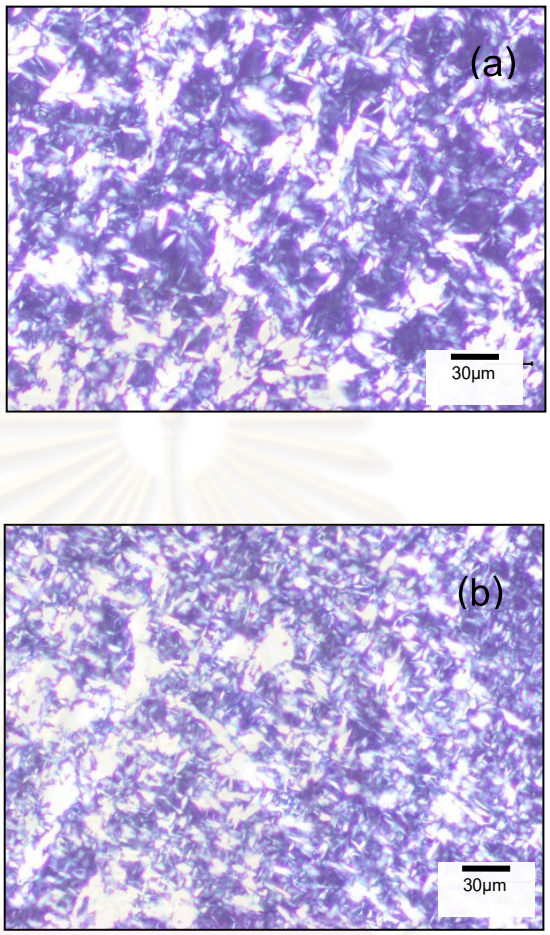

Fig. 23. Comparison of microstructure between (a) w/o nanoemulsion lipsticks and (b) conventional w/o emulsion lipsticks.

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4.8.3 The hardness and spreadability of conventional w/o emulsion lipsticks

To compare the physical properties of lipsticks in terms of hardness and spreadability among anhydrous lipsticks, conventional w/o emulsion lipsticks (microsize range) and w/o nanoemulsion lipsticks (nanosize range), the texture analyzer was used in the analysis. The results showed that the hardness of conventional w/o emulsion lipsticks was higher than of w/o nanoemulsion and anhydrous lipsticks, respectively. In contrast, the spreadability of w/o nanoemulsion lipsticks was better than that of the conventional w/o emulsion and the anhydrous lipsticks, respectively (Table 5).

Table 5. Comparison of mean particle size, hardness and spreadability of lipstick formulated with 2% w/w water and 1% w/w PEG 30 Dipolyhydroxystearate

Formulated lipstick	Mean particle size (nm)	Hardness (g)	Spreadability (mm)
Convention	ND*	58.15±2.56	0.46±0.06
Convention w/o emulsion	20,101±1561**	69.23±2.69	0.47±0.01
w/o nanoemulsion	71.56±2.98	59.85±4.69	0.57±0.06

* Not determined

**LD method

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4.8.4 Thermal behavior of conventional w/o emulsion lipsticks

The effect of particle size on the melting behavior of the conventional w/o emulsion lipsticks and w/o nanoemulsions was compared using DSC method. The results revealed that the enthalpy of w/o nanoemulsion lipsticks was lower than that of conventional w/o emulsion lipsticks as shown in Table 6. The data from DSC agreed with the data from PLM and hardness.

Table 6. DSC parameters of conventional w/o emulsion lipsticks and w/o nanoemulsion lipsticks having the same formula but different preparation processes

Formulated lipstick	Melting point (^{o}C)	Onset (^{o}C)	Enthalpy (J/g)
Convention	78.65	78.25	7.46
Conventional w/o emulsion	68.65	67.73	14.58
W/O nanoemulsion	55.08	43.84	10.22

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4.8.5 Effects of particle size on the long-term physical stability test of conventional w/o emulsions

Fig. 24 shows the appearance of conventional w/o emulsions after being kept at 4°C, 25°C and 40°C for 3 months. It was found the emulsion separation of the formulation at storage conditions. This occurrence correlated with the particle size from analysis as shown in Fig. 23 By which the particle size of the conventional w/o emulsion became bigger and the uneven distribution was observed after 3-month storage at all studied conditions. The change of the particle size was highest after being kept at 4°C. This might be due to the precipitation of emulsifier from the system as shown in Fig. 25. This might be due to the low solubility and recrystallization of PEG 30 Dipolyhydroxystearate in oil phase (mineral oil and isopropyl myristate) at 4°C. However, the conventional w/o emulsion lipsticks showed a good stability. No oil bleeding/sweating was observed at all storage conditions (Fig not shown). Fig. 26 demonstrates the appearance of 3-month storage at 25°C of conventional w/o emulsion and w/o nanoemulsion prepared with the same formula but different processes that gave the different particle size.

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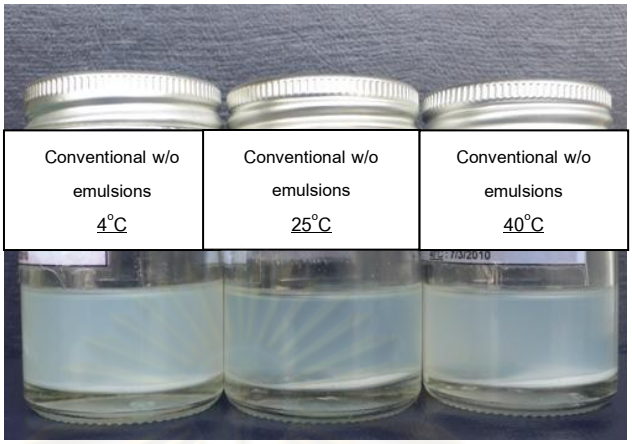

Fig. 24. Appearance of conventional w/o emulsions after 3-month storage at 4, 25 and 40°C.

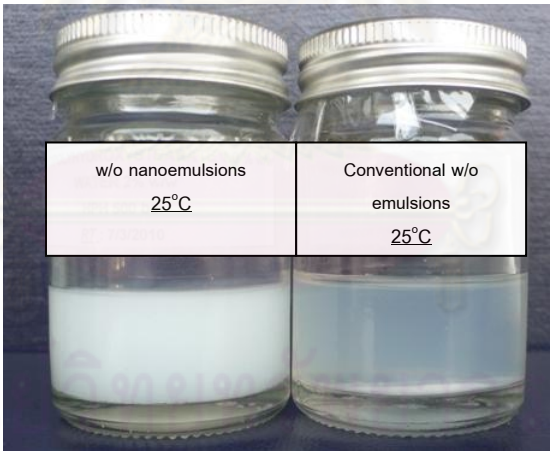

Fig. 25. Comparison of the appearance of conventional w/o emulsions and w/o nanoemulsions after 3-month storage at 25°C.

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CHAPTER V

CONCLUSION AND RECOMMENDATION

5.1 Conclusions

The present study can be concluded as followings:

1. The mean particle size and particle size distribution of w/o nanoemulsions varied with the ratio of water to emulsifier concentrations.

2. The water concentration in the w/o nanoemulsion significantly affected the hardness of w/o nanoemulsion lipsticks.

3. The emulsifier concentration in the w/o nanoemulsion did not significantly affect the hardness, spreading ability and long-term physical stability of the lipsticks prepared with this emulsion

4. The w/o nanoemulsion lipsticks containing water up to 10% w/w was stable for at least 3 months under storage at 4, 25 and 40°C

5. The particle size of w/o emulsion affected the hardness, spreadability and long-term physical stability of lipsticks containing this emulsion.

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5.2 Recommendations

From the previous conclusions, the following recommendations for future studies can be proposed.

1. According to the results of formula parameter, the amount of PEG 30 Dipolyhydroxystearate which used as the emulsifier did not affected to the physical properties of emulsion lipsticks. It is interesting to study the effect of emulsifier type on the physical properties of w/o emulsion lipsticks by selection another emulsifier that can stabilize w/o emulsions.

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REFFERENCES

[1] Johnson, R. Chemical & Engineering News. 77. 28. USA : CENEAR, 1999.

[2] Berg, R. Beauty: The New Basics. New York : Workman Publishing, 2001.

[3] Williams, D.F., and Schmitt, W.H. Chemistry and Technology of the Cosmetics and Toiletries Industry. London : Chapman & Hall, 1996.

[4] Poucher, W.A. Perfumes, Cosmetics and Soaps. 7. London : Chapman & Hall, 1959.

[5] Fujiyama, Y., Kanda, Y., and Matsuda, H. Cosmetic stick comprising water-in-oil emulsion. United States Patent 3,957,969. 1976.

[6] Solan, C., Esquena, J., Forgiarini, A.M., Uson, N., Morales, D., and Izquierdo, P. Nano-emulsion: formation, properties and applications. Surfactant Science Series 109 (2003) : 525-554.

[7] Izquierdo, P., Esquena, J., Tadros, Th.F., Dederen, J.C., Feng, J., and Garcia, M.J. Phase behavior and nano-emulsion formation by the phase inversion temperature method . Langmuir 20 (2004) : 6594-6598.

[8] Izquierdo, P., Esquena, J., Tadros, Th.F., Dederen, C., and Garcia, M.J. Formation and stability of nano-emulsions. Advances in Colloid and Interface Science 108-109 (2004) : 303-318.

[9] Sonnevile-Aubrun, O., Simonnet, J.T., and L'Alloret, F. Nanoemulsions a new vehicle for skincare products. Advance in Colloid and Interface Science 108-109 (2004) : 145-149.

[10] Gutierrez, J.M., Gonzalez, C., Maestro, A., Sole, I., Pey, C.M., and Nolla, J. Formation and stability of nano-emulsions prepared using the phase inversion temperature method. Langmuir 18 (2002) : 26-30.

[11] Solans, C., Izquierdo, P., Nolla, J., Azemar, N., and Garcia, M.J. Nano-emulsions. Current Opinion in Colloid & Interface Science 10 (2005) : 102-110.

[12] International Union of Pure and Applied Chemistry. Manual of Colloid Science. London : Butterworth, 1972.

[13] Anton, N., Benoit, J., and Saulnier, P. Design and production of nanoparticles formulated from nano-emulsion templates-A review. Journal of Controlled Release 128 (2008) : 185-199.

[14] Gutierrez, J.M., Gonzalez, C., Maestro, A., Sole, I., Pey, C.M., and Nolla, J. Nano-emulsions and optimization of their preparation. Current Opinion in Colloid and Interface Science 13 (2008) : 245-251.

[15] Teeranachaideekul, V., Souto, E.B., Junyaprasert, V.B., and Muller, R.H. Cetyl palmitate-based NLC for topical delivery of coenzyme Q10: development, physicochemical characterization and *in vitro* release studies. European Journal of Pharmaceutics and Biopharmaceutics 67 (2007) : 141-148.

[16] Uson, N., Garcia, M.J., and Solans, C. Formation of water in oil (W/O) nano-emulsionns in a water/mixed non ionic surfactant/oil systems prepared by low-energy emulsification methods. Journal of Colloid and Interface Science A: Physicochemical and Engineering Aspect 250 (2004) : 415-421.

[17] Tragardh, I.F. Visualixation of drop de formation and break-up process in a high pressure homogenizer. Chemical Engineering & Technology 28 (2005) : 882-891.

[18] Deils, A., Callewaert, L., Wuytack, E., and Masschalc, B. Interactivation of Escherichia coli by high-pressure homogenization is influenced by fluid viscosity but not by water activity and product composition. International Journal of Food Microbiology 101 (2005) : 281-291.

[19] Li, M.K., and Fogler, H.S. Acoustic emulsification. Part 2: Breakup of the large primary oil droplets in a water medium. Journal of Fluid Mechanics 88 (1978) : 513-528.

[20] Abismail, B., Canselier, J.P., Wihelm, A.M., Delmas, H., and Gourdon, C. Emulsification by ultrasound: drop size distribution and stability. Ultrasonics Sonochemistry 6 (1999) : 75-83.

[21] Cucheval, A., and Chow, R.C.Y. A study on the emulsification of oil by power ultrasound. Ultrasonic Sonochemistry 15 (2008) : 916-920.

[22] Wolfmeier, U. et al. "Waxes" in Ullmann's Encyclopedia of Industrial Chemistry. Weinheim : Wiley-VCH, 2002.

[23] Singh, M. Lip care moisturizer. United State Patent 6,663,853 B2. 2003.

[24] Chung, K., Keeler, T., and Tan, M. Water containing wax-based. United State Patent 6,039,960. 1997.

[25] Travkina, I., Raouf, M., and Pahlck, H. Cosmetic stick. United State Patent 1, 018, 333

A2. 2000.

[26] S. Shaikh, S., and Bhise, K. Formulation and evaluation of medicated lipstick of allantoin. Asian Journal of Pharmaceutics (2008) : 91-95.

[27] Dunphy, P.J., Meyers, A.J., and Rigg, R.T. Cosmetic water-in-oil emulsion lipstick comprising a phospholipids and glycerol fatty acid esters emulsifying system. United State Patent 5, 085, 856. 1992.

[28] Wang, T.S., and Lee, G. The effect of formulation on the hardness and crystallization of emulsion lipsticks. Journal of Cosmetic 48 (1997) : 51-76.

[29] O'Neill, M.J. The analysis of a Temperature-Controlled Scanning Calorimeter. Analyical Chemistry 36 (1964) : 1238-1245.

[30] Saito, Y., Saito, K., and Atake, T. Theoretical analysis of heat-flux differential scanning calorimetry based on a general model. Thermochimica Acta 99 (1986) : 299-307.

[31] Griffin, W.C. Classification of the surface-active agents by HLB. Journal of Society of Cosmetic Chemists 1(1949) : 311-326.

[32] Teeranachaideekul, V., Junyaprasert,V.B., Souto, E.B., and R.H. Muller. Development of ascorbyl palmitate nanocrystals applying the nanosuspension technology. International Journal of Pharmaceutics 354 (2008) : 227-234.

[33] Santini, E., Liggieri,L., Sacca, L., Clausse, D., and Ravera, F. Interfacial rheology of Span 80 adsorbed layers at paraffin oil-water interface and correlation with the corresponding emulsion properties. Journal of Colloid and Interface Science A: Physicochemical and Engineering Aspect 309 (2007) : 270-279.

[34] Peng, L.C., Liu, C.H., Kwan, C.H., and Huang, K.F. Optimization of water-in-oil nanoemulsions by mixied surfactants. Journal of Colloid and Interface Science A: Physicochemical and Engineering Aspect 370 (2010) : 136-142.

[35] Sabine, B.D., and Claude, D. Influence of SFC, microstructure and polymorphism on texture (hardness) of binary blends of fats involved in the preparation of industrial shortenings. Food Research International 37 (2004) : 941-948.

[36] Vereecken, J., Meeussen, W., Lesaffer, A., and Dewettinck, K. Effect of water and monoglyceride concentration on the behaviour of monoglyceride containing fat systems. Food Research International 43 (2010) : 872-881.

[37] Tippets, M., and Martini, S. Effect of cooling rate on lipid crystallization in oil-in-water

emulsions. Food Research International 42 (2009) : 847-855.

[38] Campos, R., Narine, S.S., and Marangoni, A.G. Effect of cooling rate on the structure and mechanical properties of milk fat and lard. Food Research International 35 (2002) : 971-981.

[39] Kaneko, N., Horie, T., Ueno, S., Yano, J., Katsuragi, T., and Sato, K. Impurity effects on crystallization rates of n-hexadecane in oil-in-water emulsions. Journal of Crystal Growth 197 (1999) : 263-270.

[40] Buchheim, W. and Dejmek, P. Food emulsions (3rd edition). New York : S.E. Friberg and K. Larsson, 1997.

[41] Haighton, A.J. Blending, Chilling, and Tempering of Margarines and Shortenings. Journal of American Oil Chemists's Society 53 (1976) : 397-399.

[42] Wright, A.J., and Marangoni, A.G. Crystallization and Rheological Properties of Milk Fat. Advance Dairy Chemistry, 245-291. New York : Springer,2006.

[43] DeMan, L., DeMan, J.M., and Blackman, B. Physical and textural characteristics of some North American shortenings. Journal of the American Oil Chemist's Society 68 (1991) : 63-69.

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APPENDICES

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APPENDIX A

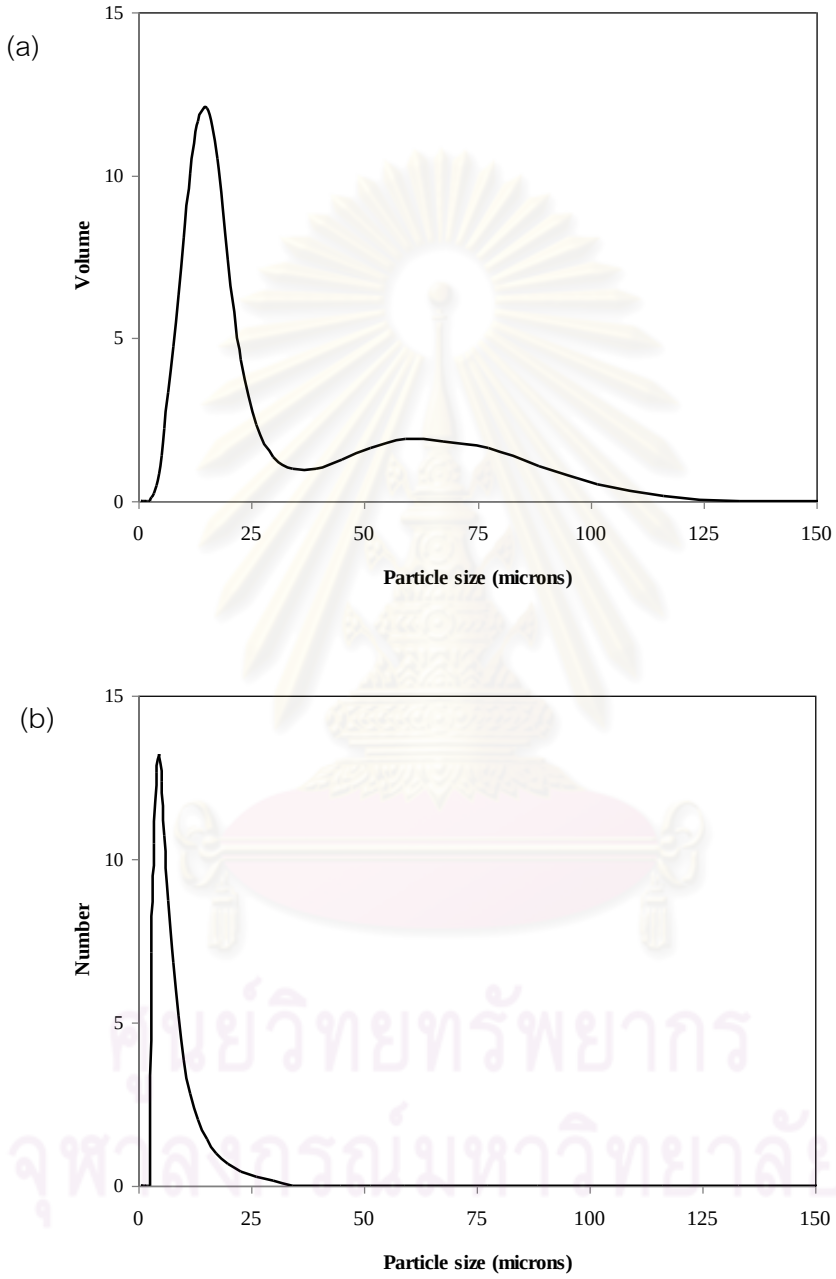
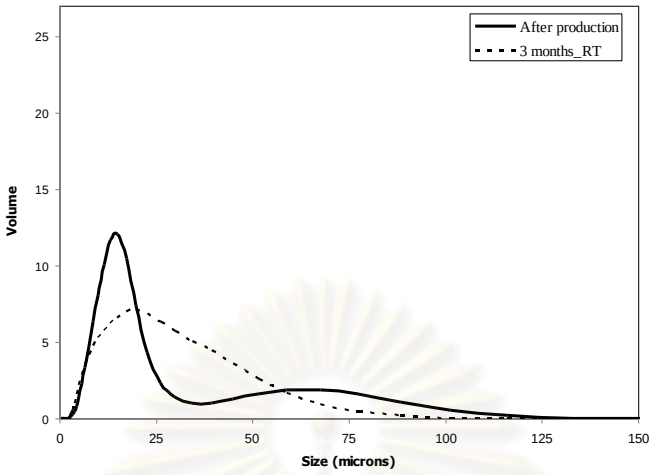
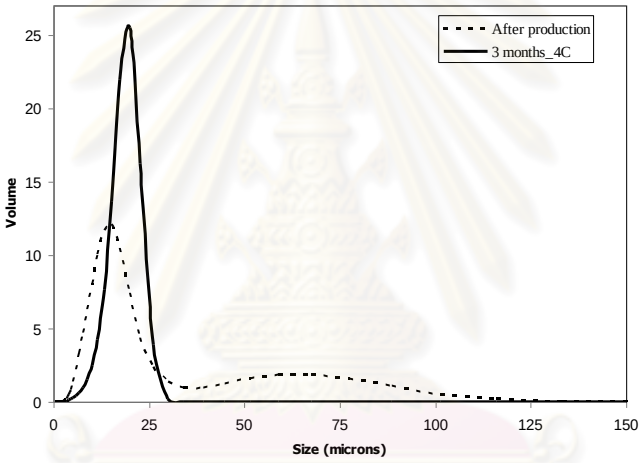


Fig. A.1. The particle size distribution of conventional w/o emulsions (microsize range) in term of (a) volume and (b) number distribution after preparation.

(a)



(b)



(c)

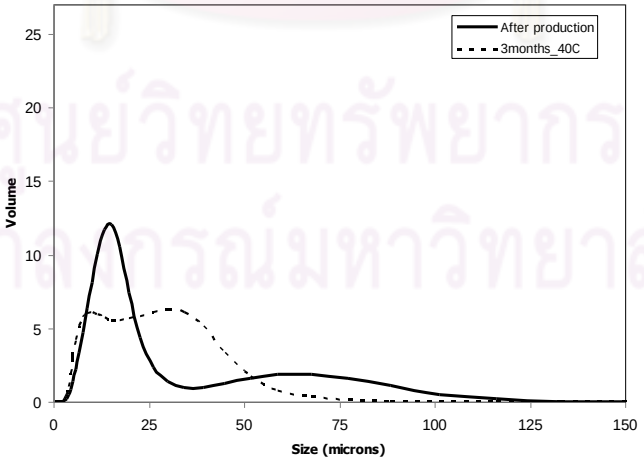


Fig. A.2. Mean particle size by volume of w/o microsize range after 3 month at (a) 25°C, (b) 4°C and (c) 40°C.

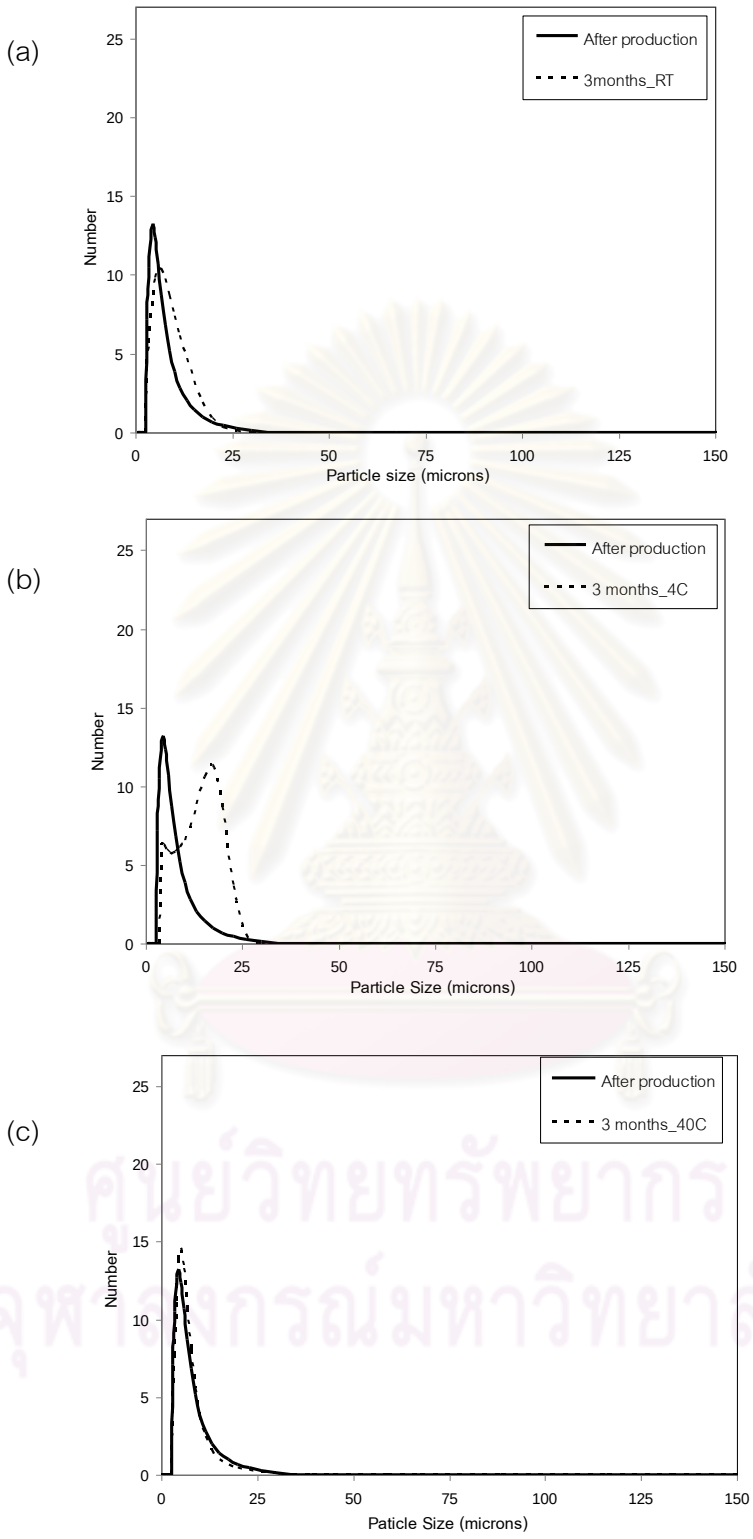


Fig. A.3. Mean particle size by number of conventional w/o emulsions after 3 months at (a) 25°C, (b) 4°C and (c) 40°C.

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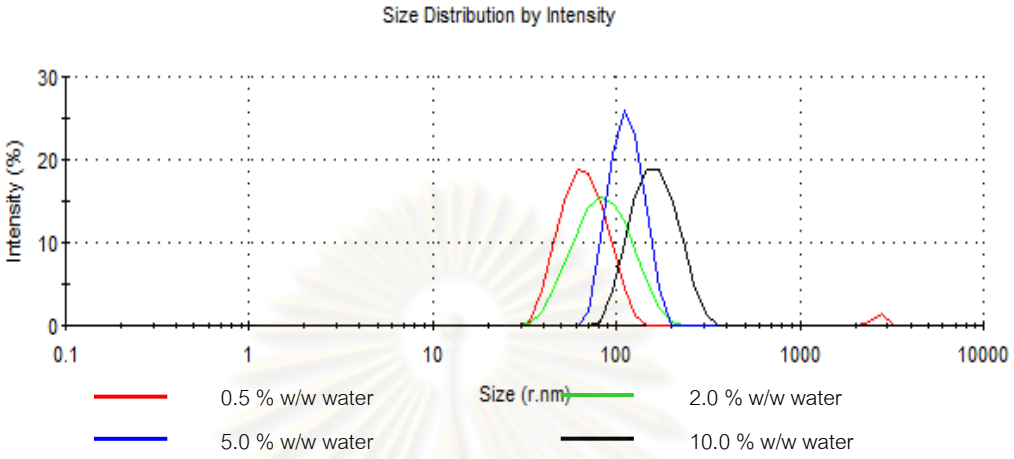

Fig. A.4 Particle size and size distribution of w/o nanoemulsion composed of 1 %w/w of PEG 30 Dipolyhydroxystearate and different water content (0.5, 2.0, 5.0 and 10.0% w/w) after preparation (initial) at 25°C.

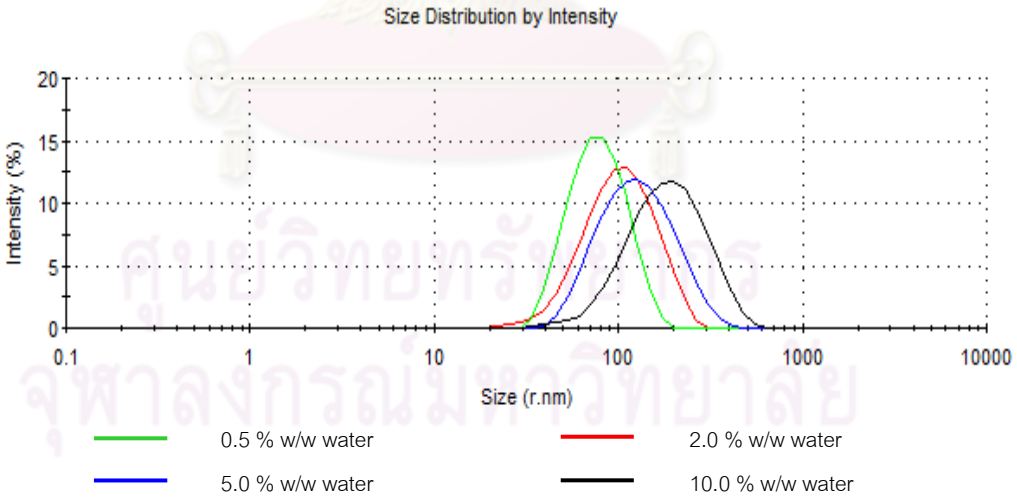

Fig. A.5 Particle size and size distribution of w/o nanoemulsion composed of 1 %w/w of PEG 30 dipolyhydroxystearate and different water content (0.5, 2.0, 5.0 and 10.0% w/w) 3-month after preparation at 25°C storage condition.

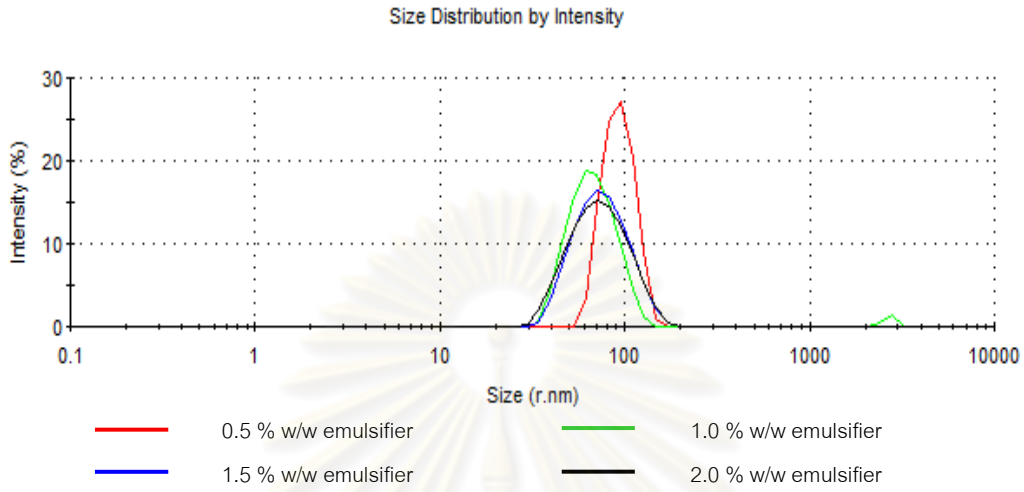

Fig. A.6 Particle size and size distribution of w/o nanoemulsion composed of 2 %w/w of water content with different emulsifier concentration (0.5, 1.0, 1.5 and 2.0% w/w) after preparation (initial) at 25°C.

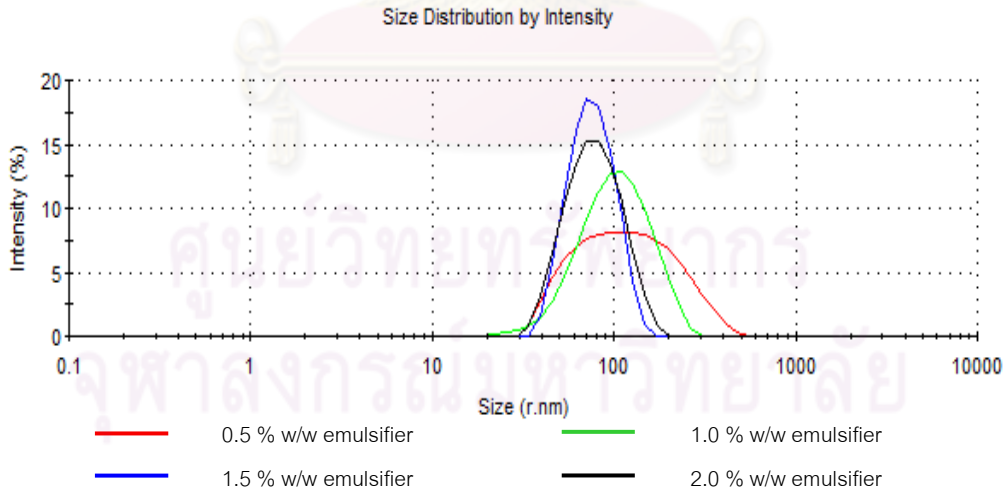

Fig. A.7 Particle size and size distribution of w/o nanoemulsion composed of 2 %w/w of water content with different emulsifier concentration (0.5, 1.0, 1.5 and 2.0% w/w) 3 months after preparation at 25°C storage condition

APPENDIX B

TABLE B-1

Statistic data of studying the effect of water content on the particle size of w/o nanoemulsions prepared at the constant concentration of PEG 30 Dipolyhydroxy stearate 1% w/w after preparation

(I) Water content	(J) water content	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.5	2	2.05000	2.67418	.465	-4.1167	8.2167
	5	-39.09333*	2.67418	.000	-45.2600	-32.9267
	10	-87.02667*	2.67418	.000	-93.1933	-80.8600
2	0	-2.05000	2.67418	.465	-8.2167	4.1167
	5	-41.14333*	2.67418	.000	-47.3100	-34.9767
	10	-89.07667*	2.67418	.000	-95.2433	-82.9100
5	0	39.09333*	2.67418	.000	32.9267	45.2600
	2	41.14333*	2.67418	.000	34.9767	47.3100
	10	-47.93333*	2.67418	.000	-54.1000	-41.7667
10	0	87.02667*	2.67418	.000	80.8600	93.1933
	2	89.07667*	2.67418	.000	82.9100	95.2433
	5	47.93333*	2.67418	.000	41.7667	54.1000

*. The mean difference is significant at the 0.05 level.

TABLE B-2

Statistic data of studying the effect of water content on the particle size of w/o nanoemulsions prepared at the constant concentration of PEG 30 Dipolyhydroxy stearate 1% w/w after 3 months at 25°C

(I) Water content	(J) Water content	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.50	2.00	7.74000	5.94700	.229	-5.9738	21.4538
	5.00	-23.54000*	5.94700	.004	-37.2538	-9.8262
	10.00	-65.77333*	5.94700	.000	-79.4871	-52.0595
2.00	.50	-7.74000	5.94700	.229	-21.4538	5.9738
	5.00	-31.28000*	5.94700	.001	-44.9938	-17.5662
	10.00	-73.51333*	5.94700	.000	-87.2271	-59.7995
5.00	.50	23.54000*	5.94700	.004	9.8262	37.2538
	2.00	31.28000*	5.94700	.001	17.5662	44.9938
	10.00	-42.23333*	5.94700	.000	-55.9471	-28.5195
10.00	.50	65.77333*	5.94700	.000	52.0595	79.4871
	2.00	73.51333*	5.94700	.000	59.7995	87.2271
	5.00	42.23333*	5.94700	.000	28.5195	55.9471

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TABLE B-3

Statistic data of studying the effect of water content on the particle size of w/o nanoemulsions prepared at the constant concentration of PEG 30 Dipolyhydroxy stearate 1% w/w after 3 months at 4°C

(I) water content	(J) Water content	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.50	2.00	-9.86667*	1.41737	.000	-13.1351	-6.5982
	5.00	-46.42000*	1.41737	.000	-49.6885	-43.1515
	10.00	-127.85333*	1.41737	.000	-131.1218	-124.5849
2.00	0.50	9.86667*	1.41737	.000	6.5982	13.1351
	5.00	-36.55333*	1.41737	.000	-39.8218	-33.2849
	10.00	-117.98667*	1.41737	.000	-121.2551	-114.7182
5.00	0.50	46.42000*	1.41737	.000	43.1515	49.6885
	2.00	36.55333*	1.41737	.000	33.2849	39.8218
	10.00	-81.43333*	1.41737	.000	-84.7018	-78.1649
10.00	0.50	127.85333*	1.41737	.000	124.5849	131.1218
	2.00	117.98667*	1.41737	.000	114.7182	121.2551
	5.00	81.43333*	1.41737	.000	78.1649	84.7018

*. The mean difference is significant at the 0.05 level.

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TABLE B-4

Statistic data of studying the effect of water content on the particle size of w/o nanoemulsions prepared at the constant concentration of PEG 30 Dipolyhydroxy stearate 1% w/w after 3 months at 40°C

(I) water content	(J) water content	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.50	2.00	3.27333	2.16566	.169	-1.7207	8.2674
	5.00	-21.39333*	2.16566	.000	-26.3874	-16.3993
	10.00	-61.79333*	2.16566	.000	-66.7874	-56.7993
2.00	0.50	-3.27333	2.16566	.169	-8.2674	1.7207
	5.00	-24.66667*	2.16566	.000	-29.6607	-19.6726
	10.00	-65.06667*	2.16566	.000	-70.0607	-60.0726
5.00	0.50	21.39333*	2.16566	.000	16.3993	26.3874
	2.00	24.66667*	2.16566	.000	19.6726	29.6607
	10.00	-40.40000*	2.16566	.000	-45.3940	-35.4060
10.00	0.50	61.79333*	2.16566	.000	56.7993	66.7874
	2.00	65.06667*	2.16566	.000	60.0726	70.0607
	5.00	40.40000*	2.16566	.000	35.4060	45.3940

*. The mean difference is significant at the 0.05 level.

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TABLE B-5

Statistic data of studying the effect of emulsifier concentration on the particle size of w/o nanoemulsions prepared at the constant water content 2% w/w after preparation

(I) Emulsifier content	(J) emulsifier content	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.50	1.00	18.09000*	1.85722	.000	13.8072	22.3728
	1.50	22.05333*	1.85722	.000	17.7706	26.3361
	2.00	20.44333*	1.85722	.000	16.1606	24.7261
1.00	0.50	-18.09000*	1.85722	.000	-22.3728	-13.8072
	1.50	3.96333	1.85722	.065	-.3194	8.2461
	2.00	2.35333	1.85722	.241	-1.9294	6.6361
1.50	0.50	-22.05333*	1.85722	.000	-26.3361	-17.7706
	1.00	-3.96333	1.85722	.065	-8.2461	.3194
	2.00	-1.61000	1.85722	.411	-5.8928	2.6728
2.00	0.50	-20.44333*	1.85722	.000	-24.7261	-16.1606
	1.00	-2.35333	1.85722	.241	-6.6361	1.9294
	1.50	1.61000	1.85722	.411	-2.6728	5.8928

* The mean difference is significant at the 0.05 level.

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TABLE B-6

Statistic data of studying the effect of emulsifier concentration on the particle size of w/o nanoemulsions prepared at the constant water content 2% w/w after 3 months at 25°C storage condition

(I) emulsifier content	(J) emulsifier content	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.50	1.00	12.81333*	1.16635	.000	10.1237	15.5029
	1.50	25.01333*	1.16635	.000	22.3237	27.7029
	2.00	26.81333*	1.16635	.000	24.1237	29.5029
1.00	0.50	-12.81333*	1.16635	.000	-15.5029	-10.1237
	1.50	12.20000*	1.16635	.000	9.5104	14.8896
	2.00	14.00000*	1.16635	.000	11.3104	16.6896
1.50	0.50	-25.01333*	1.16635	.000	-27.7029	-22.3237
	1.00	-12.20000*	1.16635	.000	-14.8896	-9.5104
	2.00	1.80000	1.16635	.161	-.8896	4.4896
2.00	0.50	-26.81333*	1.16635	.000	-29.5029	-24.1237
	1.00	-14.00000*	1.16635	.000	-16.6896	-11.3104
	1.50	-1.80000	1.16635	.161	-4.4896	.8896

*. The mean difference is significant at the 0.05 level.

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TABLE B-7

Statistic data of studying the effect of emulsifier concentration on the particle size of w/o nanoemulsions prepared at the constant water content 2% w/w after 3 months at 4°C storage condition

(I) Emulsifier content	(J) Emulsifier content	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.50	1.00	18.07333*	2.77798	.000	11.6673	24.4794
	1.50	16.48000*	2.77798	.000	10.0740	22.8860
	2.00	23.47667*	2.77798	.000	17.0706	29.8827
1.00	0.50	-18.07333*	2.77798	.000	-24.4794	-11.6673
	1.50	-1.59333	2.77798	.582	-7.9994	4.8127
	2.00	5.40333	2.77798	.088	-1.0027	11.8094
1.50	0.50	-16.48000*	2.77798	.000	-22.8860	-10.0740
	1.00	1.59333	2.77798	.582	-4.8127	7.9994
	2.00	6.99667*	2.77798	.036	.5906	13.4027
2.00	0.50	-23.47667*	2.77798	.000	-29.8827	-17.0706
	1.00	-5.40333	2.77798	.088	-11.8094	1.0027
	1.50	-6.99667*	2.77798	.036	-13.4027	-.5906

*. The mean difference is significant at the 0.05 level.

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TABLE B-8

Statistic data of studying the effect of emulsifier concentration on the particle size of w/o nanoemulsions prepared at the constant water content 2% w/w after 3 months at 40°C storage condition

(I) Emulsifier content	(J) Emulsifier content	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
0.50	1.00	13.57000*	1.69379	.000	9.6641	17.4759
	1.50	19.46333*	1.69379	.000	15.5575	23.3692
	2.00	27.44333*	1.69379	.000	23.5375	31.3492
1.00	0.50	-13.57000*	1.69379	.000	-17.4759	-9.6641
	1.50	5.89333*	1.69379	.008	1.9875	9.7992
	2.00	13.87333*	1.69379	.000	9.9675	17.7792
1.50	0.50	-19.46333*	1.69379	.000	-23.3692	-15.5575
	1.00	-5.89333*	1.69379	.008	-9.7992	-1.9875
	2.00	7.98000*	1.69379	.002	4.0741	11.8859
2.00	0.50	-27.44333*	1.69379	.000	-31.3492	-23.5375
	1.00	-13.87333*	1.69379	.000	-17.7792	-9.9675
	1.50	-7.98000*	1.69379	.002	-11.8859	-4.0741

*. The mean difference is significant at the 0.05 level.

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TABLE B-9

Statistic data of studying the effect of water content on the hardness of w/o nanoemulsions lipsticks formulated with the different water content after preparation

(I) water content	(J) water content	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
.00	.50	-.01525	1.25815	.990	-2.6969	2.6664
	2.00	1.08350	1.25815	.403	-1.5982	3.7652
	5.00	-6.86150*	1.25815	.000	-9.5432	-4.1798
	10.00	-10.25975*	1.25815	.000	-12.9414	-7.5781
.50	.00	.01525	1.25815	.990	-2.6664	2.6969
	2.00	1.09875	1.25815	.396	-1.5829	3.7804
	5.00	-6.84625*	1.25815	.000	-9.5279	-4.1646
	10.00	-10.24450*	1.25815	.000	-12.9262	-7.5628
2.00	.00	-1.08350	1.25815	.403	-3.7652	1.5982
	.50	-1.09875	1.25815	.396	-3.7804	1.5829
	5.00	-7.94500*	1.25815	.000	-10.6267	-5.2633
	10.00	-11.34325*	1.25815	.000	-14.0249	-8.6616
5.00	.00	6.86150*	1.25815	.000	4.1798	9.5432
	.50	6.84625*	1.25815	.000	4.1646	9.5279
	2.00	7.94500*	1.25815	.000	5.2633	10.6267
	10.00	-3.39825*	1.25815	.016	-6.0799	-.7166
10.00	.00	10.25975*	1.25815	.000	7.5781	12.9414
	.50	10.24450*	1.25815	.000	7.5628	12.9262
	2.00	11.34325*	1.25815	.000	8.6616	14.0249
	5.00	3.39825*	1.25815	.016	.7166	6.0799

*. The mean difference is significant at the 0.05 level.

TABLE B-10

Statistic data of studying the effect of particle size on the hardness of w/o nanoemulsions lipsticks formulated with the same composition

(I) Formulas	(J) Formulas	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Conventional lipsticks	Conventional emulsion lipsticks	-12.79375*	1.38490	.000	-15.9266	-9.6609
	Nanoemulsion lipsticks	1.08350	1.38490	.454	-2.0494	4.2164
Conventional emulsion lipsticks	Conventional lipsticks	12.79375*	1.38490	.000	9.6609	15.9266
	Nanoemulsion lipsticks	13.87725*	1.38490	.000	10.7444	17.0101
Nanoemulsion lipsticks	Conventional lipsticks	-1.08350	1.38490	.454	-4.2164	2.0494
	Conventional emulsion lipsticks	-13.87725*	1.38490	.000	-17.0101	-10.7444

*. The mean difference is significant at the 0.05 level.

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APPENDIX C

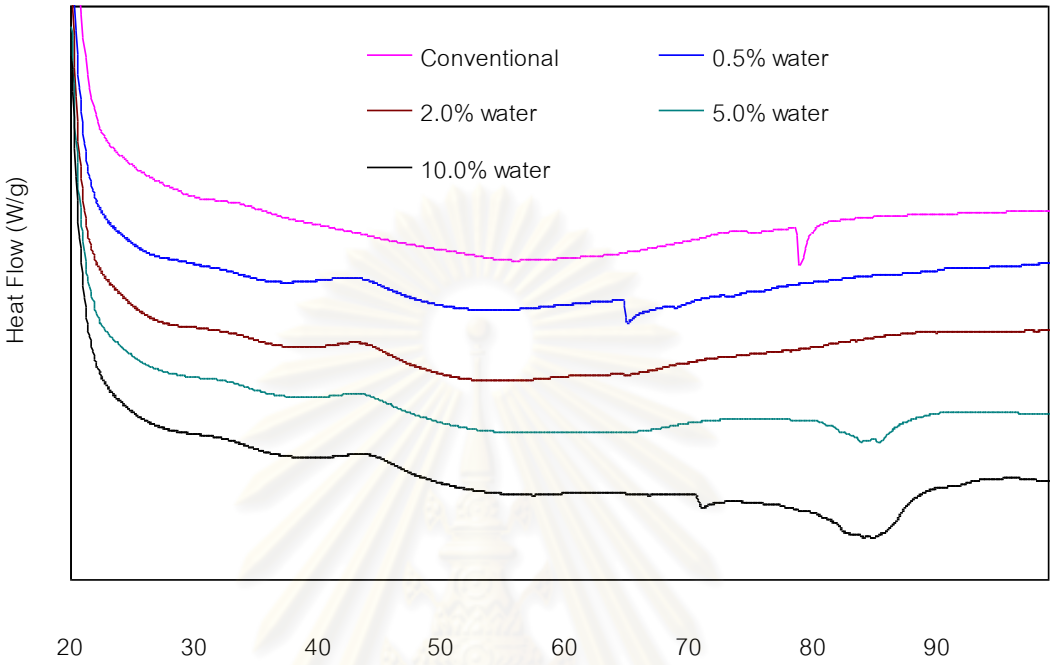

Fig. C.1. The DSC thermogram of w/o nanoemulsion lipsticks composed of 1 %w/w of PEG 30 Dipolyhydroxystearate and different water content (0.5, 2.0, 5.0 and 10.0% w/w) after preparation (initial).

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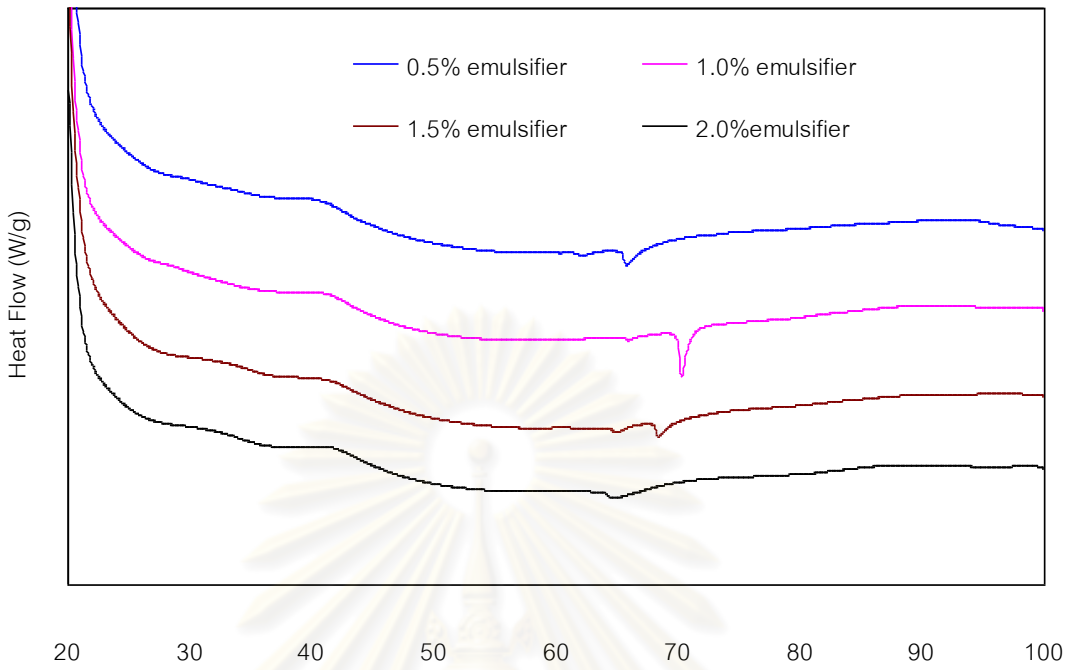

Fig. C.2. The DSC thermogram of w/o nanoemulsion lipsticks composed of 2 %w/w of water content with different emulsifier concentration (0.5, 1.0, 1.5 and 2.0% w/w) after preparation (initial).

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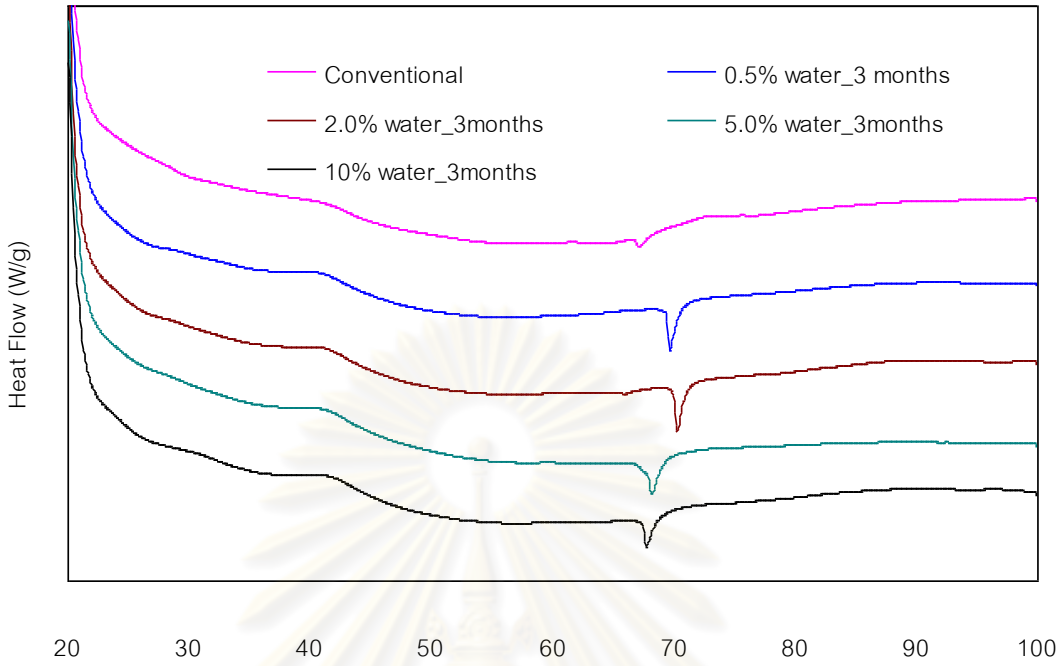

Fig. C.3. The DSC thermogram of w/o nanoemulsion lipsticks composed of 1 %w/w of PEG 30 Dipolyhydroxystearate and different water content (0.5, 2.0, 5.0 and 10.0% w/w) after being kept at 25°C for 3 months.

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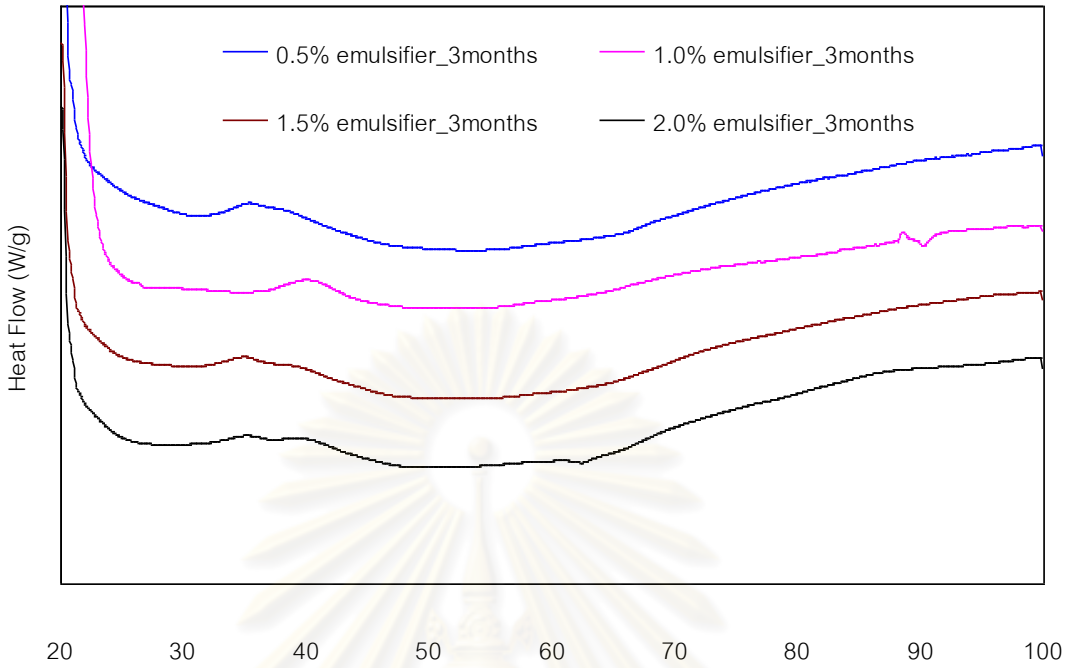

Fig. C.4. The DSC thermogram of w/o nanoemulsion lipsticks composed of 2 %w/w of water content with different emulsifier concentration (0.5, 1.0, 1.5 and 2.0% w/w) after being kept at 25°C for 3 months.

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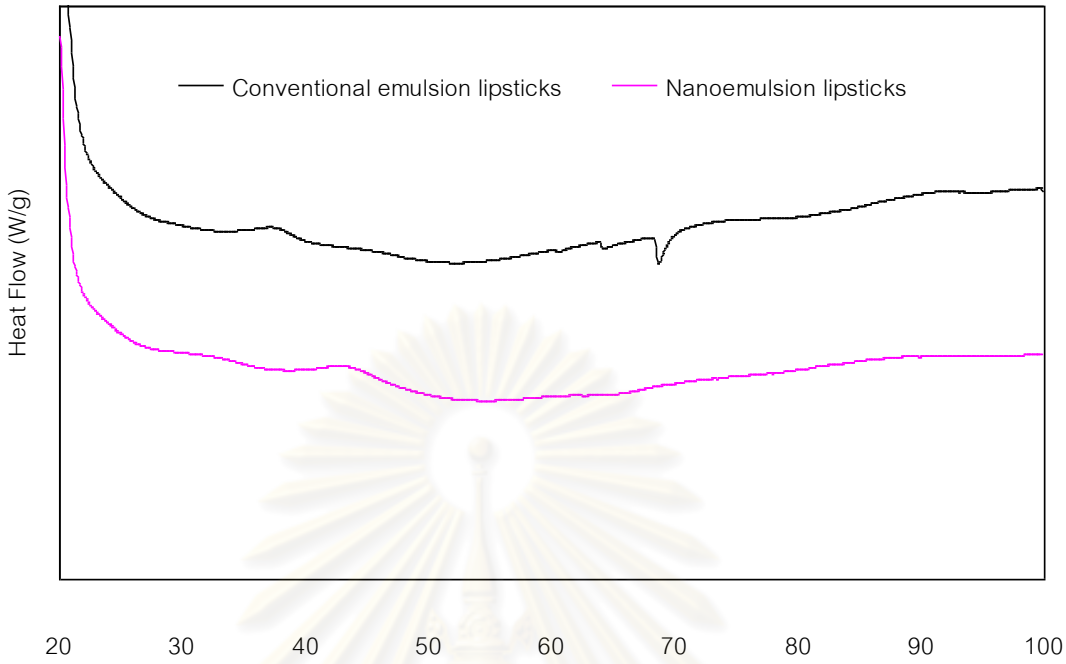

Fig. C.5. The DSC thermogram of conventional w/o emulsion lipsticks and w/o nanoemulsion lipsticks composed of 1 %w/w of PEG 30 Dipolyhydroxystearate and 2% w/w after preparation (initial).

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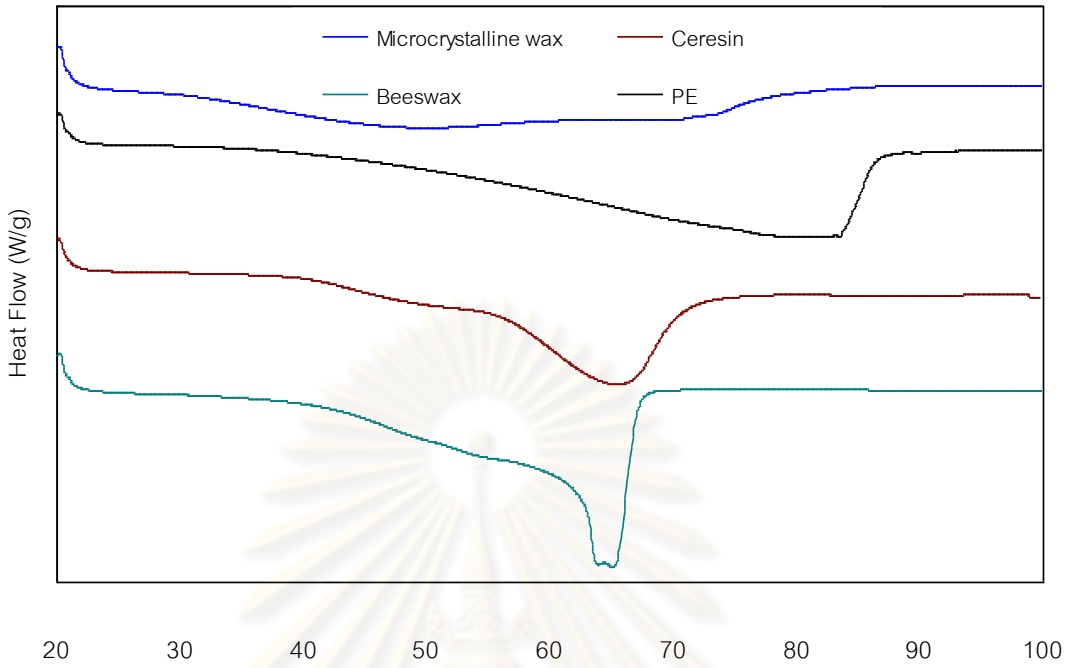

Fig. C.6. The DSC thermogram of microcrystalline wax, ceresin wax, beeswax and polyethylene wax.

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VITAE

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