ผลของค่าเอชแอลบีของทวีน 80/สแปน 80 ต่อระบบไมโครอิมัลชัน สำหรับการนำส่งเมโธเทรกเซต

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิศวกรรมศาสตรมหาบัณฑิต
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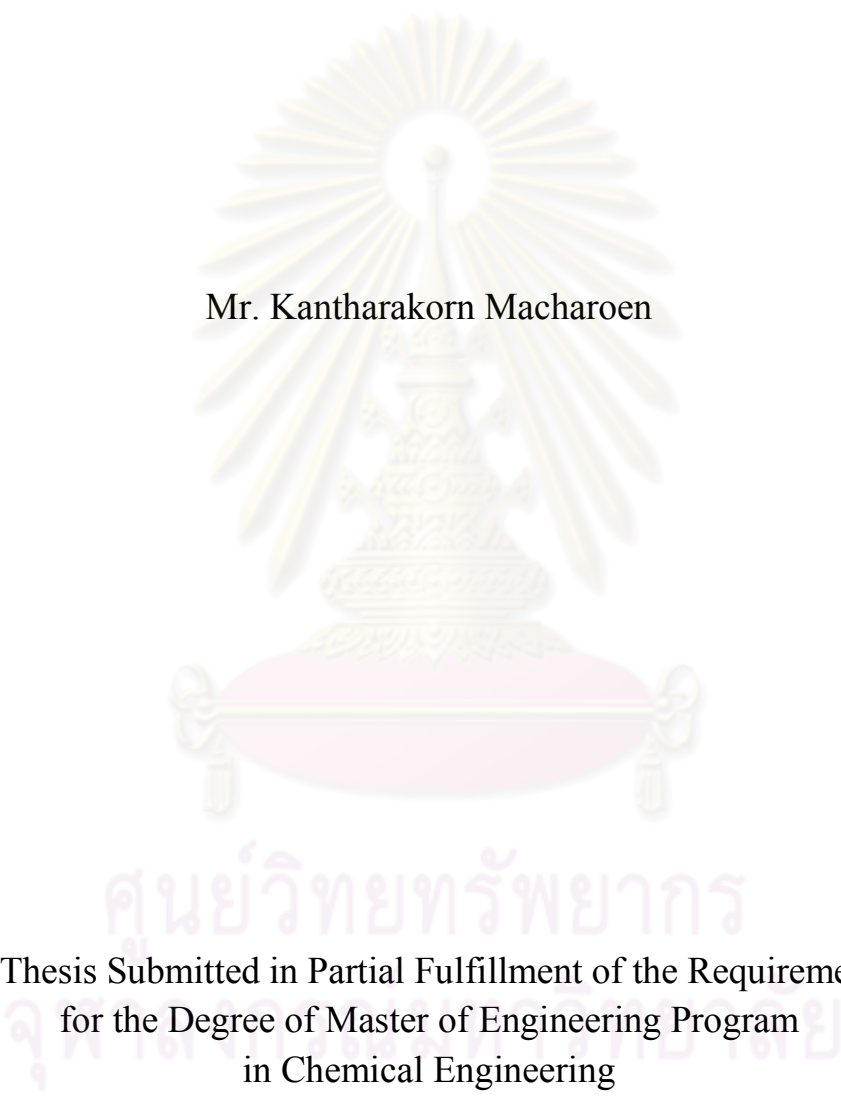คณะวิศวกรรมศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2553

HLB OF TWEEN 80/ SPAN 80 EFFECTS ON MICROEMULSION

SYSTEM FOR METHOTREXATE DELIVERY

Mr. Kantharakorn Macharoen

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Engineering Program
in Chemical Engineering
Department of Chemical Engineering
Faculty of Engineering
Chulalongkorn University
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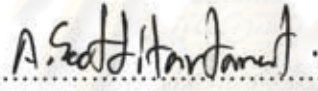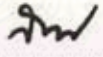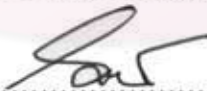| Thesis Title	HLB OF TWEEN 80/ SPAN 80 EFFECTS ON MICROEMULSION SYSTEM FOR METROTHEXATE DELIVERY
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Thesis Advisor	Associate Professor Seeroong Prichanont, Ph.D.
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กันทรากร มาเจริญ : ผลของค่าเอชแอลบีของทวีน 80/ สแปน80 ต่อระบบไมโครอิมัลชัน สำหรับการนำส่งเมโธเทรกเซต (HLB OF TWEEN 80/ SPAN 80 EFFECTS ON MICROEMULSION SYSTEM FOR METHOTREXATE DELIVERY) อ.ที่ปรึกษาวิทยานิพนธ์หลัก : รศ. ดร. สีรุ้ง ปรีชานนท์, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ผศ. ดร. โศรดา กนกพานนท์, 93 หน้า

งานวิจัยนี้เป็นการศึกษาผลของค่า HLB ต่อระบบไมโครอิมัลชัน ทวีน 80/ สแปน 80/ 1,2-ออกเทนไดออล/ ไอโซโพรพิลไมริสเตต/ อะซิเตตบัฟเฟอร์ พีเอช 5.5 สำหรับการนำส่งยาเมโธเทรก เซต พบว่าการเพิ่มค่า HLB จาก 6 ไป 12 ส่งผลให้มีการเพิ่มพื้นที่ของไมโครอิมัลชันชนิดน้ำใน น้ำมัน ในขณะที่ระบบที่มีค่า HLB 14 ไมโครอิมัลชันชนิดน้ำในน้ำมันมีบริเวณน้อยลง แต่การ สังเกตพบบริเวณไมโครอิมัลชันชนิดน้ำมันในน้ำ การทดลองในส่วนที่สองเป็นการนำระบบที่มีค่า HLB 10 12 และ 14 มาศึกษาเมื่อมีการเติมยาเมโธเทรกเซต พบว่าระบบทั้ง 3 ให้บริเวณของไมโคร อิมัลชันน้อยลงเมื่อเทียบกับระบบเดิมที่ไม่มีเมเทรกเซต เมื่อนำระบบที่มีค่า HLB 10 12 และ 14 มา ศึกษาการขนาดและกระจายตัวของอนุภาคพบว่าระบบที่มีค่า การกระจายตัวน้อยที่สุดและขนาด ของอนุภาคลดลงเมื่อค่า HLB สูงขึ้น นอกจากนี้การศึกษาการปลดปล่อยยาจากไมโครอิมัลชัน เครื่องมือ Franz diffusion cell พบว่าระบบ HLB 14 ให้อัตราการปลดปล่อยยาที่ 24 ชั่วโมงมากที่สุด คือ 59.66 % ซึ่งอาจเกิดจากการที่ไมโครอิมัลชันทะลุผ่านรูเมมเบรน ส่วนการปลดปล่อยยาในระบบ HLB 10 และระบบ HLB 12 มีค่าเท่ากับ 4.67 % และ 6.17% ตามลำดับ และการศึกษาจลน์ พลศาสตร์ของการปลดปล่อยยาพบว่าระบบ HLB 10 และ 12 มีการปลดปล่อยยาตามแบบจำลอง Baker – Lonsdale ซึ่งถูกควบคุมโดยการแพร่ของยา

ภาควิชา............วิศวกรรมเคมี.......... ลายมือชื่อนิสิต.......กันทรากร มาเจริญ............

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KEYWORDS: SURFACTANT HLB/ MICROEMULSION/ IN VITRO DRUG RELEASE/ METHOTREXATE

KANTHARAKORN MACHAROEN: HLB OF TWEEN 80/ SPAN 80 EFFECTS ON MICROEMULSION SYSTEM FOR METHOTREXATE DELIVERY. ADVISOR: ASSOC. PROF. SEEROONG PRICHANONT, Ph.D., CO-ADVISOR: ASSIST. PROF. SORADA KANOKPANOT, Ph.D., 93 pp.

This research aimed to studying effects of HLB on microemulsion systems which composed of Tween 80/ Span 80 as surfactants, 1,2-octanediol as a co-surfactant, Isopropyl myristate (IPM) as an oil phase, and acetate buffer pH 5.5 as an aqueous for hydrophilic (methotrexate; MTX) drug delivery. It was found that increasing of HLBs from 6 to 12 resulted in increasing w/o microemulsion regions, while at HLB 14 smaller area of w/o microemulsion than HLB 12 was obtained. However, o/w microemulsion was observed in this system. After that, HLB 10, 12 and 14 which gave large w/o microemulsion area were chosen to study phase behavior and particle size analysis when MTX loaded. Adding MTX into the systems influenced the decrease of w/o microemulsion area in all systems. From size analysis, microemulsion droplets increased with HLB from 14, 12 and 10 respectively with both loaded and unloaded MTX. *In vitro* release study using Franz diffusion cell revealed that HLB 14 had the highest cumulative drug release at 24 hours; 59.66%, which might be due to the leak of microemulsions. The release of MTX of HLB 10 and HLB 12 were 4.67 and 6.17 respectively. The study of kinetics of release found that HLB 10 and 12 followed Baker – Lonsdale model which the release was diffusion controlled.

Department : ......Chemical Engineering..... Student's Signature..กันทรากร..มาเจริญ

Field of Study : ..Chemical Engineering ..... Advisor's Signature.....................

Academic Year : ....2010.............. Co-advisor's Signature.......................

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ศูนย์วิทยทรัพยากร
จุฬาลงกรณ์มหาวิทยาลัย

CHAPTER 1

INTRODUCTION

1.1 Motivation

During the past years, microemulsions have been intensively studied by scientists and technologists because of their great potential in many food and pharmaceutical applications. In the pharmaceutical field, microemulsions are used as transdermal drug delivery vehicles due to their ability to enhance drug solubilization and increase drug penetration rate across the stratum corneum layer. The applications of microemulsion vehicles for cutaneous drug delivery provides high solubilization potential for both lipophilic and hydrophilic drugs (Lawrence et al., 2000; Kogan et al., 2006; Kreilgaard, 2002). As a result, both microemulsions *in vitro* and *in vivo* are used for encapsulating drugs such as 8-methoxalen, diclofenac, diphenylamine, ketoprofen and methotrexate for in vitro (Baroli et al., 2000; Dreher et al., 1997; Schmalfuss et al., 1997; Alvarez-Figueroa et al., 2001; Trotta et al., 1996) and bupranolol, carazolol, methyl nicotinate, piroxicam and timolol for in vivo (Kemken et al., 1991 (a); Kemken et al., 1991(b); Kemken et al., 1992; Bonina et al., 1995; Dalmora et al., 2001). The use of microemulsions, moreover, is advantageous not only due its ease of manufacturing and low cost preparation, but also provides bioavailability (Kogan and Garti, 2006)

Methotrexate (MTX); a folic acid antagonist with antineoplastic activity, is effective for the treatment of psoriasis when administered by the oral or parenteral. The systemic uses of this drug, however, may provoke any of the numerous side effects, notably hepatotoxic effects (Bookbinder et al., 1984; Van Dooren-Greebe et al., 1994). To reduce and avoid such effects, it would clearly be preferable to administer MTX topically (Hwang et al., 1995). The major obstacles are: the drug is insoluble in water, but soluble in mineral acid solution, it has a large structure with high molecular weight (454.56 g/mol), and it is mostly in dissociated form at physiological pH. Thus, its capacity for passive diffusion is limited (Alvarez-Figueroa

and Blanco-Mendez, 2001). However, usage of appropriate vehicles such as microemulsions may enhance transdermal delivery of this drug(Lu and Jun, 1998).

Microemulsions, which consist of oil, water, and surfactant, frequently in combination with a cosurfactant, are clear, isotropic, and thermodynamically stable liquid solution (Danielsson et al., 1981). Normally, for hydrophilic drug, water-in-oil (w/o) microemulsions are suitable, but for lipophilic drug, oil-in-water (o/w) microemulsions are appropriate. The phase behavior of microemulsions depends on many factors; one of the most important factors is the type of surfactants. Polyoxyethylene sorbitan monooleate (Tween80) and sorbitan monooleate (Span 80) are very commonly used surfactants for forming microemulsions as transdermal drug delivery vehicles (Kogan and Garti, 2006). Both of them are non-ionic surfactants which have been reported to have minimal toxicity and appear acceptable for transdermal use (Kibbe, 2000). Furthermore, another benefit of non-ionic microemulsions is the insensitivity to pH and electrolyte concentration relative to their ionic counterparts (Lawrence and Rees, 2000).

Pseudo-ternary phase diagrams are used in cases that four or more components are found in the mixture such as oil, water, surfactant, cosurfactant and/or drug. Apparently, over the whole range of compositions, not every combination of components produces microemulsions (Lawrence and Rees, 2000). In addition, surfactant selection plays a vital role of the formation of microemulsions to be w/o or o/w. Each surfactant, in other words, has its own property of the hydrophile–lipophile balance (HLB) that takes into the relative contribution of hydrophilic and hydrophobic fragments of the surfactant molecule (Carlfors et al., 1991). Surfactants with low HLBs (3-6) are preferred to form w/o microemulsions whereas with high HLBs (8-18) are favored to form o/w microemulsions (Lawrence and Rees, 2000).

Due to the effect of surfactant HLB which leads to different formation of microemulsions, this project aims at studying phase behavior of using blended Tween 80 (HLB 15.0)/Span 80 (HLB 4.3) surfactants which will give HLB 6-14 after blending. Moreover, the HLBs which give the largest region of w/o or o/w microemulsions will be further studied for their phase behavior when MTX loaded and *in vitro* drug release will be conducted.

1.2 Objectives

1. To study phase behavior of blended Tween80/ Span 80 at different ratios (different HLB values, and determine maximum aqueous solution content.

2. To study the effects of mixed surfactants on microemulsion systems with and without the presence of MTX.

3. To study the MTX effects on phase behaviors of the selected systems.

4. To study the *in vitro* drug release through artificial membrane of which microemulsions containing MTX are applied.

1.3 Scope of works

1. Search and study the relevant information.

2. Experiment: plot pseudo- ternary phase diagrams of Tween 80/Span 80/1,2-octanediol, IPM and acetrate buffer pH 5.5 system for HLB 6 – 14.

3. Characterize microemulsions.
   - Type of microemulsions (o/w or w/o) test.
   - Isotropic or anisotropic test using polarized light.
   - System conductance measurement using conductivity meter.
   - Size measurement using dynamic light scattering (DLS) method.

4. Select the systems from No.2 which give large w/o microemulsion areas or o/w microemulsion area to re-plot pseudo-ternary phase diagrams using saturated solution of MTX in acetate buffer pH 5.5 instead of pure buffer.

5. From No.4, select the same aqueous percentages of those microemulsions to study their droplets size.

6. From 5, tests of *in vitro* release of MTX contained microemulsions trough artificial membrane using Franz diffusion cells.

1.4 Expected benefits

1. Obtain an understanding of the effects of HLB of blended surfactants on microemulsion forming.

2. Obtain an understanding of the effect of drug (MTX) on microemulsions with optimal HLB.

3. Obtain an understanding of *in vitro* drug release from different HLB systems.

CHAPTER 2

THEORY

In order to study effects of HLB on microemulsion systems for *in vitro* methotrexate (MTX) release and transdermal delivery, it is necessary to understand the characteristics of surfactants which are used in pharmaceutical applications such as surfactant structure, surfactants" HLB, and types. Furthermore, understanding in pseudo-ternary phase diagram, microemulsion formation, and microemulsion characterization are required for this study. In addition, the research aims to use microemulsions as MTX vehicles for *in vitro* drug release and transdermal delivery; hence, it is important to understand the MTX characteristic and the mechanism of drug release and skin permeation.

2.1 Surfactants

2.1.1 Surfactant structure

Surfactants, surface-active materials, possess a characteristic chemical structure that consists of (1) molecular components that will have little attraction for aqueous phase, normally called the hydrophobic group, and (2) chemical units that have a strong attraction for aqueous phase – the hydrophilic group (Figure 2.1) (Myers, 1996).

Hydrophilic head   Hydrophobic tail
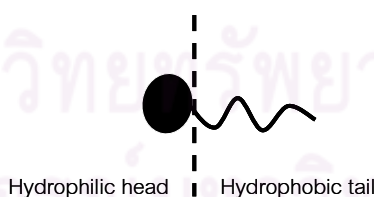
Figure 2.1 The basic structure of surfactant

Materials that possess chemical groups leading to surface activity are generally referred to as being amphiphilic, indicating that they have some affinity for two essentially immiscible phases. When a surface-active material is dissolved in a solvent (whether water or an organic liquid), the presence of the hydrophobic group causes an unfavorable distortion of the liquid structure, increasing the overall free

energy of the system. In an aqueous surfactant solution, for example, such a distortion of the water structure by the hydrophobic group decreases the overall entropy of the system. The surfactant will therefore preferentially adsorb at interfaces, or it may undergo some other process to lower the energy of the system (e.g., micelle formation). Since less work is required to bring the surfactant molecules to an interface relative to solvent molecules, the presence of the surfactant decreases the work required to increase the interfacial area resulting in a decrease in interfacial tension. For example, thermodynamic rationalization of microemulsion when surfactants added is presented below.

$$\Delta G_f = \gamma \, \Delta A \; - \; T \, \Delta S \tag{2.1}$$

where ΔG_f is the free energy of formation, γ is the surface tension of the oil-water interface, ΔA is the change in interfacial area on microemulsification, ΔS is the change in entropy, and T is the temperature. It should be noted that when a microemulsion is formed the change in ΔA is very large due to the large number of very small droplets formed, and γ is very small amounts. Furthermore, entropic distributions arise from other dynamic processes such as surfactant diffusion in the interfacial layer and monomer-micelle surfactant exchange. Thus a negative free energy of formation is achieved (Lawrence and Rees, 2000).

The amphiphilic structure of surfactant molecules not only results in the adsorption of surfactant molecules at interfaces and the consequent alteration of the corresponding interfacial energies, but it will often result in the preferential orientation of the adsorbed molecules such that the hydrophobic groups are directed away from the bulk solvent phase (Fig 2.2) (Myers, 1996)
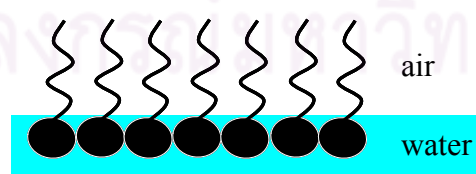
Figure 2.2 The orientation that tends to minimize unfavorable interactions between air and water.

2.1.2 Classification of surfactants

Surfactants may be classified in several ways; one of the most common classifications is charges of surfactants. The four general groups of surfactants are defined as follows (Myers, 1996):

- *Anionic*, with the hydrophilic group carrying a negative charge such as carboxyl ($RCOO^-M^+$), sulfonate ($RSO_3^-M^+$).

- *Cationic*, with the hydrophile bears a positive charge, as for example, the quarternary ammonium halides ($R_4N^+X^-$)

- *Nonionic*, where the hydrophile has no charge but derives its water solubility from highly polar groups such as polyoxyethylene ($-OCH_2-CH_2O-$), sugars or similar groups.

- *Amphoteric* (and zwitterionics), in which the molecule has, or can have, a negative and a positive charge on the principal chain (as opposed to a counterion, M^+ or X^-) such as the sulfobetaines, $RN^+(CH_3)_2CH_2-CH_2SO_3^-$

In this research, polyoxyethylene sorbitan monooleate (Tween 80) and sorbitan monooleate (Span 80) will be used as a mixed surfactant system. Both of them are nonionic surfactants which are insensitive to pH and electrolyte concentration, however, they are sensitive to temperature in terms of forming microemulsions.

Tween 80 (Fig 2.3) is oily amber liquid, fatty odor and with a density of 1.06 - 1.09 g/ml. It is nontoxic water-soluble surfactant, but not soluble in mineral oil and vegetable oil. The hydrophile-lipophile balance (HLB) of Tween 80 is 15, indicates its high water solubility.
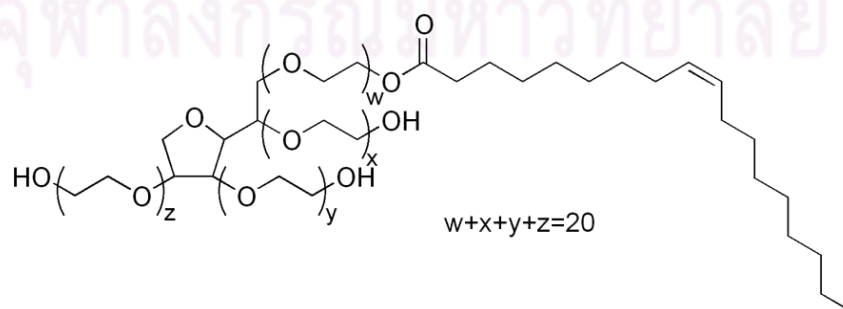
Figure 2.3 Structure of Tween 80.

Span 80 (Fig 2.4) is a nontoxic oil soluble surfactant, and does not dissolve in water. Its density is about 0.995 – 1.05 g/ml, and its HLB is 4.3. Span 80 is a viscous yellow liquid which has higher viscosity than water.
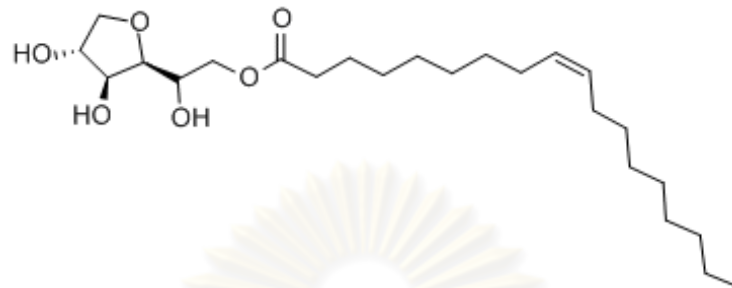
Figure 2.4 Structure of Span 80.

2.1.3 Surfactant's HLB

Selection of a suitable surfactant is the key to the formation of any microemulsion systems. The hydrophile-lipophile balance (HLB) takes into account the relative contribution of hydrophilic and hydrophobic fragments of the surfactant molecules. It is generally accepted that low HLBs (3–6) surfactants are preferred to form w/o microemulsions while surfactants with high HLBs (8–18) are favored for the formation of o/w microemulsion systems. The HLB of nonionic surfactants can be calculated as follows (Griffin, 1954).

$$HLB = 20\,x \left[\frac{relative\ molecular\ mass\ of\ hydrophile\ group}{relative\ molecular\ mass\ of\ surfac\tan t} \right] \qquad (2.2)$$

HLB calculation of mixed surfactant system is defined as (Griffin, 1954)

$$HLB = \Sigma\,(HLB_i\,x\,f_i\,) \qquad (2.3)$$

Where HLB_i is HLB value of surfactant i

f_i is mass or weight fraction of surfactant i

2.2 Three components system

2.2.1 Phase diagram

A simple ternary phase diagram is generally composed of oil, water, and amphiphile. However, it is called pseudo-ternary phase diagram ib case that amphipile are surfactant and cosurfactant. Each of the ingredients occupies one of the triangle corners which are 100% by weight of the particular components. The surfactant (and co-surfactant) is mixed with one of the phase at various ratios, and the mixture is titrated with the other immiscible phase. In Figure 2.5, a hypothetical pseudo-ternary phase diagram shows the existence regions of micelles, reverse micelles, w/o micro-emulsions, and o/w microemulsions which are formed, along with the bicontinuous phase.
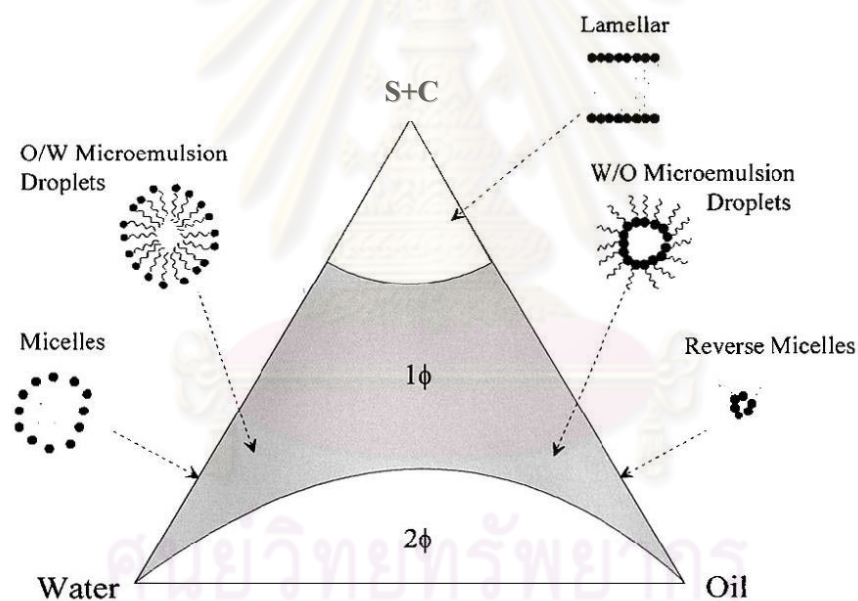
Figure 2.5 A hypothetical pseudo-ternary phase diagram of an oil/surfactant/water system with emphasis on microemulsion and emulsion phases (Lawrence and Rees, 2000).

From Figure 2.5, in addition, shows the liquid crystalline phase (or lamellar phase or gel phase) which is located at the surfactant rich corner. And the two-phase region is located between oil and water corners.

According to Winsor notation, different cases of phase behavior and self-assembly structures of systems containing an amphiphile such as alkyl sulfate or alkyl ammonium chloride, a hydrocarbon, an aqueous solution of electrolyte, and often a nonionic additive such as glycol, hexanol, or amine are discovered (Fig. 2.6)
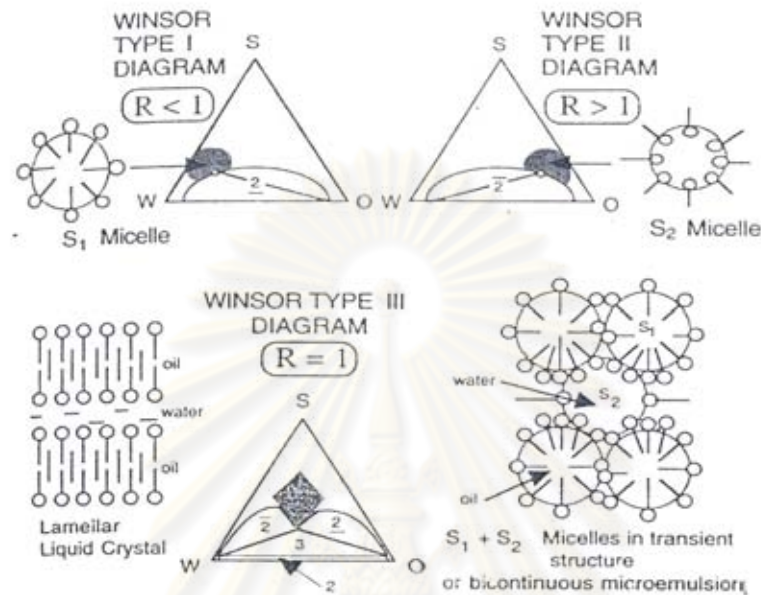
Figure 2.6 Phase behavior and types of structure according to Winsor notation (Kumar and Mittal, 1999)

In type I phase behavior, an S_1 type of aqueous micellar system (and its extension to an o/w microemulsion when swollen micelles occur or to a percolated microemulsion if a large amount of oil is solubilized in the micellar core) is in equilibrium with almost pure oil. This phase behavior exhibits in the polyphasic region of the so-called Winsor I ternary diagram and has also been label $\underline{2}$, since it appears as two phases with the surfactant-rich phase being the water or lower phase.

Winsor II diagram and phase behavior (also noted $\overline{2}$), conversely, corresponds to the opposite performance. The polyphasic equilibrium consists of an inverse micellar organic solution S_2 (that eventually solubilizes enough water to become a microemulsion) in equilibrium with an essentially pure aqueous phase.

In Winsor III, the polyphasic region contains a three-phase zone surrounded by three two-phase zones. Systems which compositions lie in the three-phase zones separate into a surfactant-rich phase that is in the middle of the diagram at the

boundary of the microemulsion single-phase zone (shaded), and two excess phases, which are essentially pure aqueous phase and pure oil. Since it is at equilibrium with both excess phases, it cannot be diluted by either water or oil, and it is thus neither water nor oil-continuous but bicontinuous.

Winsor IV phase behavior is a single-phase region contained liquid crystalline phase with lamellar or other structure (Kumar and Mittal, 1999)

2.2.2 Microemulsions

2.2.2.1 Properties and structures of microemulsion

Emulsions are suspensions of oil and water in the presence of a small amount of surfactants. Most emulsions are metastable colloids with droplet sizes generally larger than 1 μm. Because of this large droplet size, emulsions drops will eventually separate into oil and water phases over a period of time. Emulsions are optically turbid dispersion and can only be obtained by mechanical mixing of the components because of their thermodynamic instability (Bagwe et al., 2001).

Under certain conditions, the oil droplets in emulsion can be made small enough that they do not scatter light, hence forming a transparent dispersion called microemulsion, which its droplet size is generally smaller than 100 nm (Benita, 2005). Microemulsion is defined as a system of water, oil, and surfactant (or amphiphile) which is a single optically isotropic and thermodynamically stable solution. It is well established that microemulsions can appear in at least three major microstructure: water-in-oil (w/o), bicontinuous structure, and oil-in-water (o/w) (Benita, 2005).

Figure 2.7 shows schematic representations of the three types of microemulsions which are most preferably to be formed depending on composition.

(1) Micellar („o/w") droplet structure: water diffusion will be rapid, in the same order of magnitude as that of neat water. Oil and surfactant diffusion will be slow and within the same order of magnitude.

(2) Inverted micellar („w/o") droplet structure: oil diffusion will be rapid, in the same order of magnitude as that of neat oil. Water and surfactant diffusion will be slow and within the same order of magnitude.

(3) Bicontinuous structures: both water and oil diffusion will be rapid, only slightly slower than those of the neat liquids. Surfactant diffusion will be low due to the constitution of the interfacial film, but slightly higher than (1) and (2) as the interface is fluctuating and less restricted than in the micellar shapes (Kreilgaard, 2002).
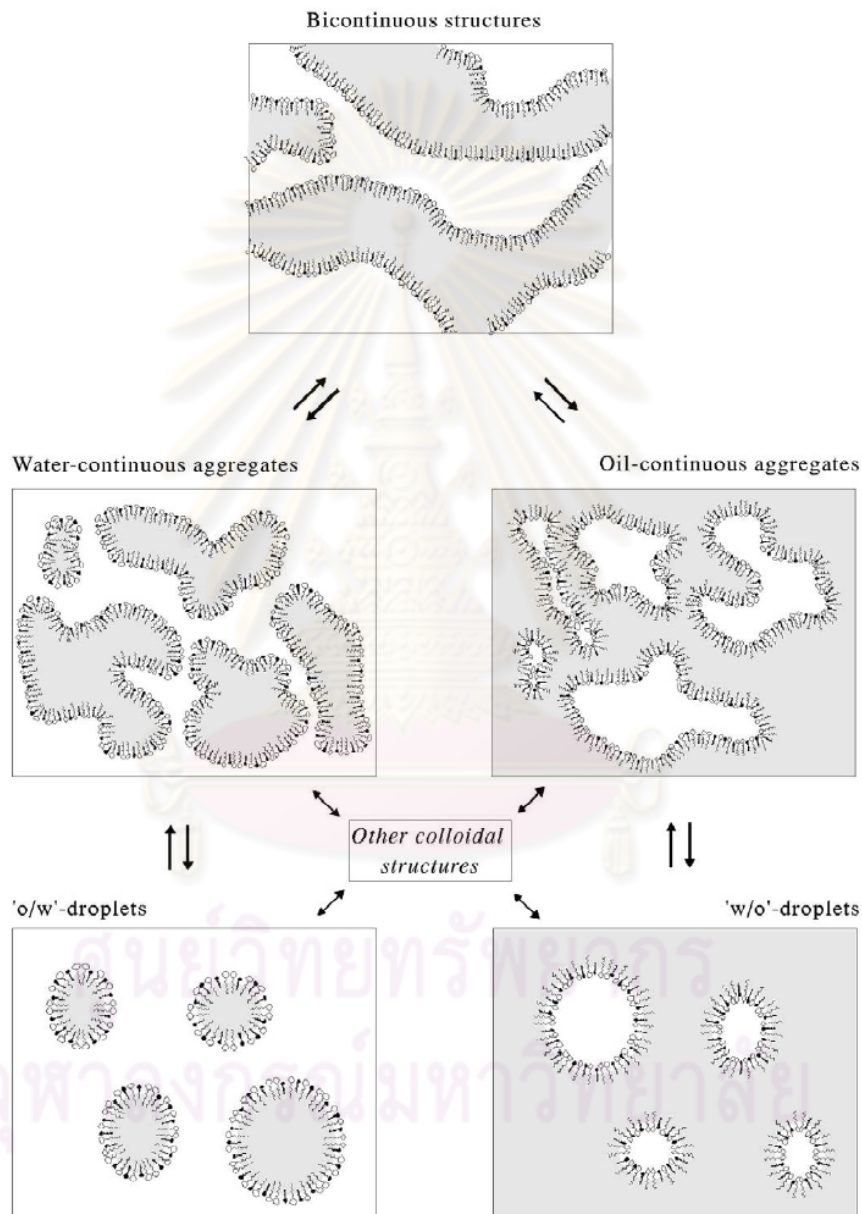
Figure 2.7 Basic dynamic microemulsion structures formed by oil phase (grey), aqueous phase (white) and surfactant / co-surfactant interfacial film, and plausible transitions between the structures (indicated by arrows) (Kreilgaard, 2002)

2.2.2.2 Factors affecting microemulsion formation

1. Shape of the surfactant aggregate formed

The surfactant (or packing) parameter, p, which defines the spontaneous curvature of the interface, is fixed by geometrical packing and interfacial forces (Kumar and Mittal, 1999).

$$p = \frac{V}{a_0 l_c} \qquad (2.4)$$

when V is the volume of the hydrocarbon per amphiohile

   a_0 is the optimal area per polar head

   l_c is the length of extended (all-trans) alkyl chain
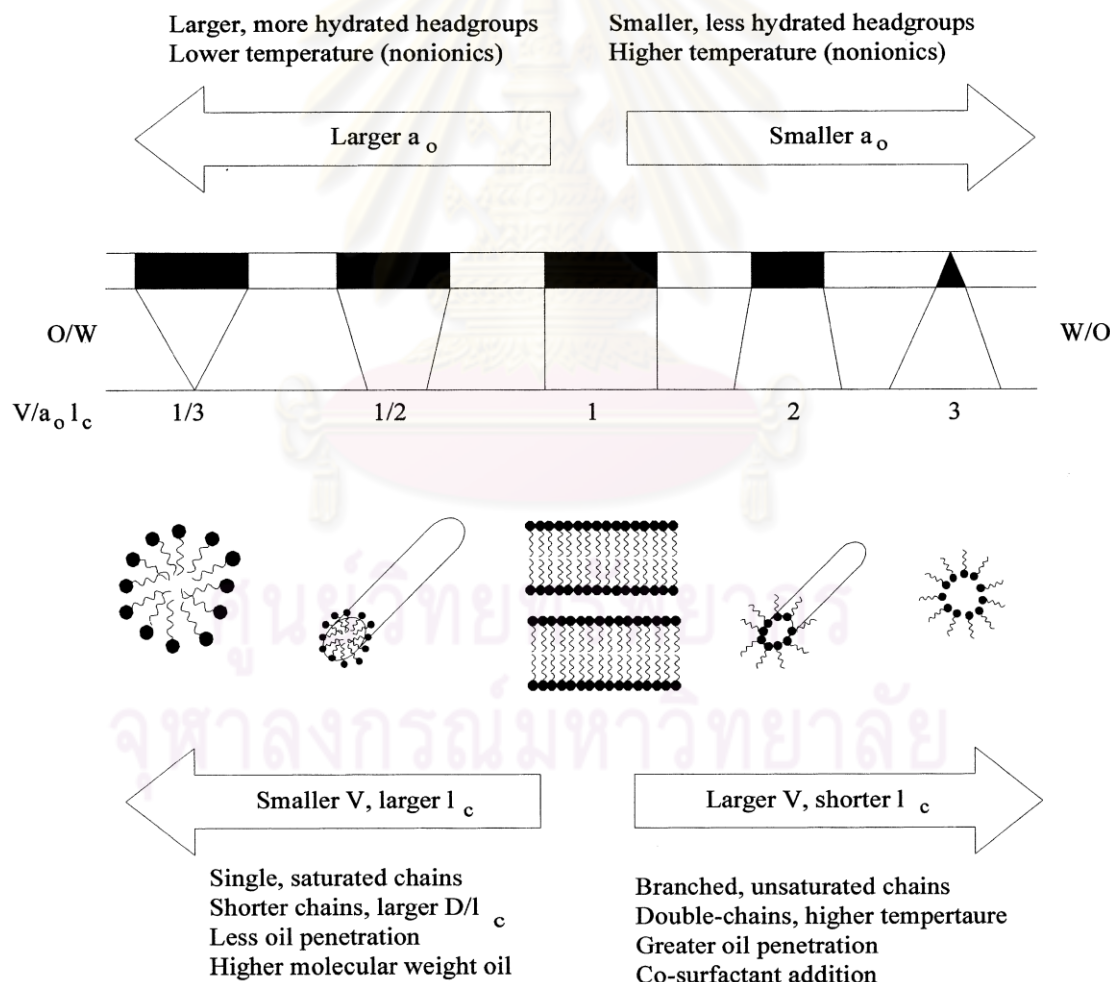
Figure 2.8 The packing parameter governs the shape of the surfactant aggregate formed in solution (Lawrence and Rees, 2000).

The packing parameter may serve as a guide to the shape of that aggregates that will form spontaneously (Fig. 2.8). For example, if $p < 1/3$, the formation of spherical micelles in water is observed, p is between 1/3 and 1/2 rod shaped micelle will be formed, p is between 1/2 and 1 lamellar is preferably formed, and p is greater than 1 molecules will formulate to reverse micelle.

However, the calculation of p for multicomponent systems is not simple; oil may penetrate into the hydrophobic core and increase V; alcohol (cosurfactant) may absorb at the interface and increase a_0. The area a_0 is also sensitive to ionic strength for ionic surfactants and to temperature in the case of nonionic surfactants (Kumar and Mittal, 1999).

2. Type of the surfactants

Hydrophobic surfactants will be proper for the formation of w/o microemulsions, and the hydrophilic surfactants will form o/w microemulsions. Nevertheless, there are many examples in which hydrophilic surfactants will form w/o microemulsions in the presence of a cosurfactant (Benita, 2005).

Ionic surfactants generally do not lead to microemulsions at the ambient temperature, but to liquid crystal, which are organized system. To produce a microemulsion, it is often required to introduce disorder either by increasing temperature (up to 50 ˚C) or by adding a cosurfactant, generally an alcohol (Kumar and Mittal, 1999).

Nonionic surfactants such as polyoxyethylene sorbitan n-acylesters (Tweens), have been reported to have minimal toxicity. They are very often used in pharmaceutical microemulsion formation. Besides, Tweens are water-soluble and have high HLB values and, therefore, are used mainly for making o/w microemulsions.

3. Cosurfactant

Cosurfactant helps the surfactant to reduce the interfacial tension to very low values to achieve thermodynamic stability. They both modify the curvature of the interface by incorporating additional apolar groups and provide more fluidity to the

film, preventing crystallization of the tails of the surfactants, which could result in the formation of the liquid crystal (Benita, 2005).

4. Temperature

Microemulsions stabilized by nonionic surfactants, especially those based on polyoxyethylene, are very susceptible to temperature. A decrease in surfactant solubility occurs with increasing temperature, and as a result systems stabilized by nonionic surfactants or mixtures. They often have characteristic phase inversion temperatures (PITs), with the PIT of the microemulsion varying with a range of experimental factors including the amount and nature of the oil present and the nature of the surfactant(s) present (Lawrence and Rees, 2000).

5. pH of aqueous phase

Acidity of aqueous plays an important role to microemulsion system which occupy ionic surfactant (Benita, 2005).

6. Additive such as electrolyte and drug

Additive may affect to microemulsion system by increasing microemulsion region (Trotta, 1996; Baroli et al., 2000; Zhu et al., 2006).

2.2.2.3 Microemulsion characterization

There are several techniques to characterize microemulsions. Generally, microemulsions are characterized within macroscopic level and microscopic level. At the macroscopic level viscosity and conductivity methods provide useful information (Lawrence and Rees, 2000).

Macroscopic level

1. Viscosity

Viscosity measurements, for example, can indicate the presence of rod-like or worm-like reverse micelles. The change of viscosity means changing in microemulsions structure, for instant, increasing volume of water will increase w/o microemulsion viscosity because of more water increases dispersed droplet size (Moulik, 1998)

2. Polarized light analysis

Since microemulsions are translucent isotropic, hence, they will not scatter the polarized light. This can separate others transparent or translucent anisotropic structures (i.e. lamellar, macroemulsions, hexagonal) from microemulsions. (Friberg, 1990)

3. Conductivity

Conductivity measurements provide a means of determining whether a microemulsion is oil-continuous or water-continuous. For example, o/w microemulsions will give the same conductivity to water which can conduct the electricity. In the other words, w/o microemulsions give very small conductivity (Djordjevic et al, 2004).

Microscopic level

1. Small-Angle Scattering

The techniques of small-angle scattering (SAS), in particular, small-angle X-ray scattering (SAXS) and small-angle neutron scattering (SANS), probe the pertinent colloidal length scales of 1 to 100 nm. Therefore, they are used to obtain information on the size, shape, and internal structure of colloidal particles (Benita, 2005).

2. Dynamic light-scattering (DLS)

DLS analyzes the fluctuation in scattering intensity that occurs over very short time intervals resulting from the Brownian motion of the particles. The diffusion coefficient of the scattering centers may be calculated from decay of the correlation function, and in the absence of interparticle interference, the hydrodynamic radius of the particle r_H can be determined from the diffusion coefficient D using the Stokes-Einstein relationship

$$D = \frac{k_B T}{6\pi\eta\, r_H} \qquad (2.5)$$

where k_B is the Boltzmann constant, T is absolute temperature, and η is viscosity of the solvent (Sjoblom and Friberg, 1978) .

3. Freeze-Fracture transmission electron microscope (FFTE)

In this technique, the microemulsion must be frozen rapidly enough to avoid phase separation or crystallization. The sample is subsequently fractured, and its visibility is enhanced by depositing a platinum carbon *in vacuo*. The microemulsion sample, mounted on a support film or grid, is replicated by breaking apart the film, and the replica is then viewed by TEM and assumed to be representative of bulk microemulsion.

2.3 Methotrexate

Methotrexate (MTX); a folic acid antagonist with antineoplastic activity, is effective for the treatment of psoriasis when administered by the oral or parenteral. The systemic use of this drug, however, may provoke any of the numerous side effects, notably hepatotoxic effects (Bookbinder et al., 1984; Van Dooren-Greebe et al., 1994). To reduce and avoid such effects, it would clearly be preferable to administer MTX topically (Hwang et al., 1995).

The chemical name of MTX is N-[4-[[(2,4-diamino-6-pteridinyl)methyl] methyl amino] benzoyl]-l-glutamic acid , and its chemical formula is $C_{20}H_{22}N_8O_5$. There are several trading names of MTX, for example, amethoptherin, antifolan, emtexate, ledertrexate, metatrexan, rheumatrex methylaminopterin, and mexate. MTX is practically insoluble in water, in ethanol, in chloroform, in 1,2-dichloro-ethane, and in ether. It dissolves in solution of mineral acids and in dilute solutions of alkali hydroxides and carbonates. It is slightly soluble in 6M hydrochloric acid (Lund, 1994).
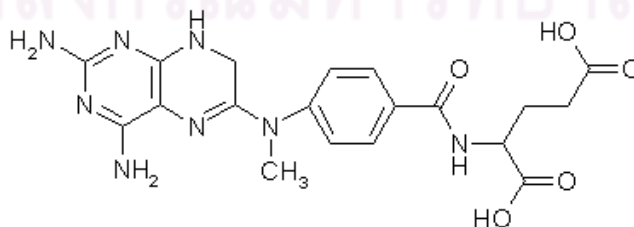
Figure 2.9 Structure of methotrexate (www.steve.gb.com)

These are some properties of MTX (Lund, 1994 and www.chemicalland21.com)

Appearance	yellow to orange-brown crystalline powder
Molecular weight	454.44 g/mol
Melting point	195 ˚C
Dissociation constant	pKa 3.8, 4.8, 5.6
Stability	normally stable, but sensitivity to light
Side effects	severe effects to bone marrow, liver and kidney

2.4 *In vitro* drug release

The main objective of the *in vitro* experimentation is to simulate diffusion conditions in human (Twist and Zatz, 1986), thus obviating the requirement for *in vivo* research using humans or animals. Although, skin permeation experiments give interesting and valuable information but skin availability and the risks related with its use is a motivation to make preliminary studies with artificial membranes. Therefore, *in vitro* experiment procedures have become increasingly important in this field because of the multitude of problems associated with *in vivo* protocols.

2.4.1 *In vitro* apparatus: Diffusion cells

In vitro skin permeation studies are most often carried out using diffusion cells (e.g., glass, Teflon, stainless steel). The various types of diffusion cells have recently been reviewed (Franz, 1990). A wide variety of diffusion cell apparatus has been employed to measure drug release and skin permeation *in vitro*. Despites variations in design, most cells have several common features. They generally employ two chambers, one containing the active formulation, and the other, stirred, chamber containing the receptor solution. The two chambers are separated by the rate-limiting barrier, usually excised skin or synthetic membrane.

The vertical Franz diffusion cell (Franz, 1975) is a commonly used apparatus for evaluating transdermal delivery. It is composed of two major compartments, a donor chamber and a receptor chamber (Fig. 2.10).
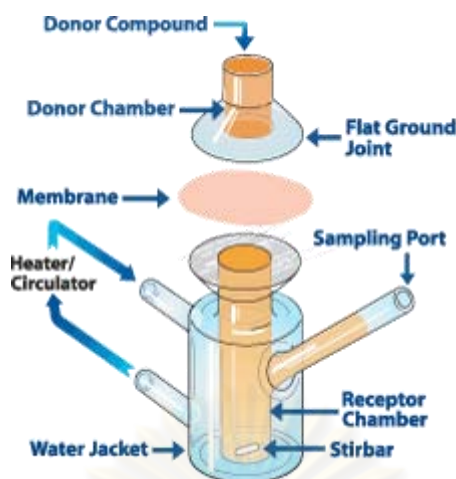
Figure 2.10 A side view of a modified Franz diffusion cell (adapted from Franz, 1990, http://www.permegear.com/franz.htm).

Stirring of the receptor cell is essential to remove or minimize the unstirred static diffusion boundary layers and to avoid high local concentrations of drug. It often carried out by using magnetic bar coupled with magnetic stirrer (Fig. 2.11). Maintenance of sink conditions is also paramount for accurate *in vitro* permeation studies.

Figure 2.11 A side view of 6-stationed stand stirrers.

Temperature control is essential for accurate *in vitro* studies, and is usually maintained by the use a controlling temperatures water-bath flow-through the surrounding water-jackets diffusion chambers or immersion in the water bath.

2.4.2 Release models

There are several models, which can be used for description of the release profiles from controlled release systems. The choice of a specific model for the data set from a particular controlled release formulation depends on shape of the graphics and the underlying controlling mechanism.

2.4.2.1 Hixon and Crowell equation

Hixon and Crowell originally derived the following equation to describe the dissolution of solid particles (Hixon and Crowell, 1931).

$$(1-F)^{1/3} = -kt \qquad (2.8)$$

Where,

F is the fraction release at time t, equal to Q_t/Q_0.

Q_t is initial amount of drug in microemulsion.

Q_0 is amount of drug release at time t.

k is a constant.

This equation has been used by some researchers to describe the release of drugs from spherical matrices that have been compressed into tablets and satisfactory results were obtained (Touitou and Donbrown, 1982; and Franz et al., 1987). The use of this model is based on the assumption that the rate of release is limited by the rate of dissolution of the drug particles and not by diffusion through the polymer matrix.

2.4.2.2 Higuchi equation of square root of time

The following equations was derived by Higuchi (1961) to describe the release of drug diffusing through a planar system and it has occasionally been used to fit release data from microsphere formulations.

$$F = k\sqrt{t} \qquad (2.9)$$

Where,

F is the fraction of drug released at any time t.

k is the constant that equal to $(2ADC_s)^{1/2} / M_0$.

A is the surface area of the device.

D is the drug diffusivity in the microemulsion.

C_s is the solubility of the drug in themicroemulsion.

M_0 is the amount of drug per unit area present initially in the system.

Although the use of this equation for spherical matrices does not reflect any single release mechanism, some data showed good fitting (Mortada et al., 1988; and Špiclin et al., 2003). This may indicate the possibility of superposition of two mechanisms or more. In this case, it may correspond to an exponent n = 0.5 in the Korsmeyer – Peppas equation. A square root model may also describe the release from monolithic solution in a spherical device, where the early time approximation results in a square root of time equation (Baker and Lonsdale, 1974).

2.4.2.3 Baker and Lonsdale equation

The Baker and Lonsdale equation (Baker and Lonsdale, 1974) which was derived from Higuchi"s model (Higuchi, 1963) depicted a matrix system wherein dispersed drug phase released by diffusion through the polymer. And drug particles are assumed to be small compared with the particle radius:

$$\frac{3}{2}\left[1-(1-F)^{2/3}\right]-F = kt \tag{2.6}$$

Where,

F is the fraction of drug released at any time t.

k is the constant that equal to $3DC_s / r_0^2 C_0$.

D is the diffusion coefficient of drug in microemulsion.

C_s is the drug solubility in microemulsion.

r_0 is the radius of microemulsion.

C_0 is the initial concentration of the drug in the polymer matrix.

The equation has been fitted to release data from various microspheres formulations (Jun and Lai, 1983; Leelarasamee et al., 1986; Chang et al., 1986; Shukla and Price, 1989, 1991; Duberuet et al., 1990; and Radin, 2009). Linear fitting was usually used after calculating the quantity corresponding to the expression on the left side of the equation at each time point.

2.4.2.4 Korsmeyer – Peppas   equation

The Korsmeyer – Peppas equation (Korsmeyer et al., 1983) represents a general data fitting approach for drug release:

$$F = kt^n \qquad\qquad (2.7)$$

Where,

F is the fraction release at time t.

k is a constant incorporating structural and geometric characteristics of the controlled release device.

n is the release exponent, that may be used to indicate the mechanism of   drug release.

The general semi-empirical equation is not based on a certain model, certain geometry or a single mechanism. It is usually used to analyze release data from polymeric devices, when the mechanism of release is not well know or when more than one type of release may be involved (Franz et al., 1987; Orienti and Zecchi, 1993; and Mehta et al., 2007)

2.4.2.5 First order equation

Occasionally, drug release data are fitted to the first-order decline model (Shah et al., 1987; Mortada et al., 1988). Since most microspheres consist of drug particles embedded in a polymer matrix, a first order release does not conform with a known mechanism for drug release from spherical matrices. One situation that can be described by the first-order release kinetics is the non-constant activity reservoir spherical device, where the drug solution is enclosed within a porous membrane through which diffusion occurs (Baker and Lonsdale, 1987).

For the fitting purpose, the first order release equation is:

$$\ln(1 - F) = -kt \qquad\qquad (2.10)$$

Where,

F is the fraction dissolved at time t.

k is a constant.

2.5 Transdermal drug delivery

The skin covers a total surface are about 1.8 m^2 and provides the contact between the human body and its external environment (Shaefer and Redelmeier, 1996). The skin itself has two main layers: the epidermis, which is the outermost layer of the skin, covering the dermis that is the active part of the skin, holding the hair muscles, blood supply, sebaceous glands, and nerve receptors (Fig. 2.10). There is a fat layer underneath the dermis. The skin is a very heterogeneous membrane and has a variety of cell types, but the layer that controls the penetration of drugs is called the stratum corneum and, despite its thickness of only 15–20 μm, it provides the main barrier to penetration. The permeation of the drug through the stratum corneum has several routes: transcellular, intercellular, and appendageal (through eccrine (sweat) glands or hair follicles) (Fig. 2.11). However, the appendages occupy a very small surface areas, this means of permeation is less significant under normal conditions (Hadgraft, 2001).

Apparently, the two major routes of transdermal drug delivery are transcellular route, crossing through the corneocytes and intercellular route, along the lipid domains between the corneocytes. The intercellular route has been thought to be the route of preference for most drug molecules (Potts and Guy, 1992).

Focus on colloidal vehicles, drug delivery systems are, for example, micelles, microemulsions, macroemulsions (or emulsion such as cream, gel and lotion), niosomes, liposomes, and nanoparticles. For MTX transdermal delivery, microemulsions may be of value for the topical administration (Alvarez-Figueroa and Blanco-Mendez, 2000).
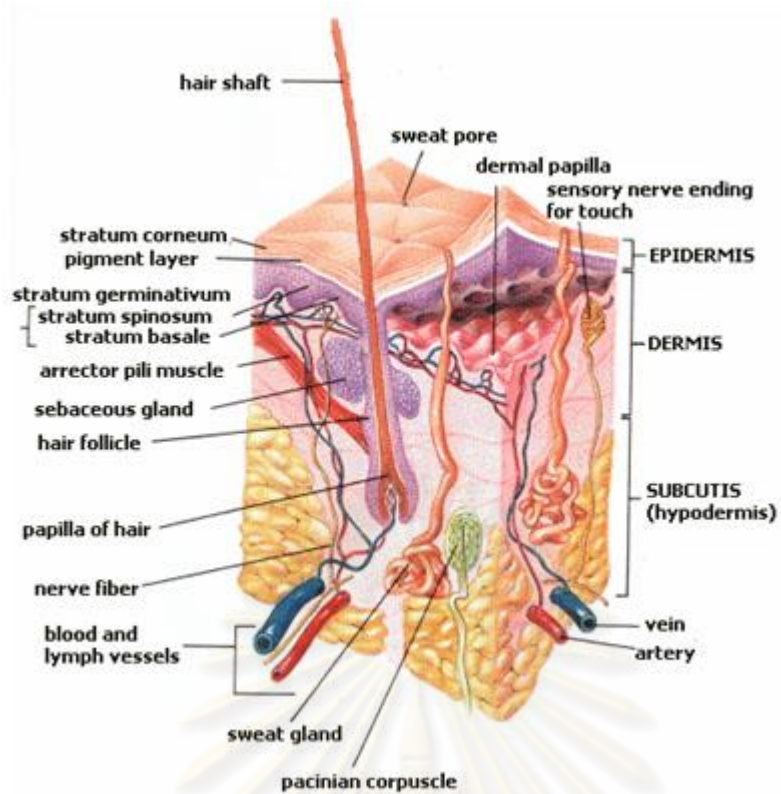
Figure 2.12 Human skin structure (http://training.seer.cancer.gov/ss_module14_
melanoma/unit02_sec01_anatomy.html).
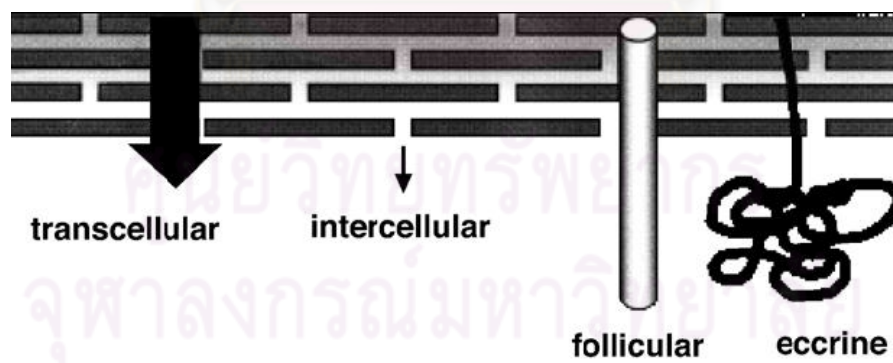
Figure 2.13 Schematic representation of the different possible routes of penetration
through the skin (Kogan and Garti, 2006).

The rationale for using vesicles in dermal and transdermal drug delivery is manifold (Schreier and Bouwstra, 1994 and Martin and Lloyd, 1992)

1. Vesicles might act as drug carriers to deliver entrapped drug molecules into or across the skin

2. Vesicles might act as penetration enhancers owing to the penetration of the individual lipid components into the stratum corneum and subsequently the alternation of the intercellular lipid lamellae within the skin layer.

3. Vesicles might act serve as a rate-limiting membrane barrier for the modulation of systemic absorption, hence providing a controlled trandermal delivery system.

4. The individual components of vesicles might have additional useful properties.

5. Vesicles are biodegradable, minimally toxic, and relatively nonimmuno-genic.

Advantages of transdermal drug delivery (Guy 1996, Delgado-Charro and Guy, 2001)

1. Avoid drug destruction from enzyme and acidity in gastrovascular.

2. Decrease variance of drug in blood circular.

3. Remain stability of drug dose for treatment and decrease drug side effects.

Disadvantages of transdermal drug delivery (Delgado-Charro and Guy, 2001)

1. Only strong activity and proper physicochemistry drugs are used. The drugs should be able to dissolve enough in fat and water ambient.

2. It is not suitable for skin irritation drugs or allergic drugs.

CHAPTER 3

LITERATURE REVIEW

The study of pseudo ternary phase behavior of blended Tween 80/Span 80 as surfactants, isopropyl myristate (IPM) as oil phase ,and acetate buffer pH 5.5 as aqueous phase requires many aspects of the information such as calculation of HLB of blended surfactants, and effect of HLB on microemulsion systems. In addition, the use of Tween 80/Span 80 as surfactants to form microemulsions, and systems for Methotrexate (MTX) transdermal delivery will be reviewed.

3.1 Blended surfactants in microemulsion systems

3.1.1 Phase behavior of blended surfactants

Pseudo ternary phase diagram is used to study phase behavior of micro-emulsion systems. Baroli et al. (2000) prepared microemulsions by using water, isopropyl myristate (IPM) and Tween 80: Span 80: 1,2-Octanediol (3:1:1.2 w/w). The phase behavior is shown in figure 3.1.
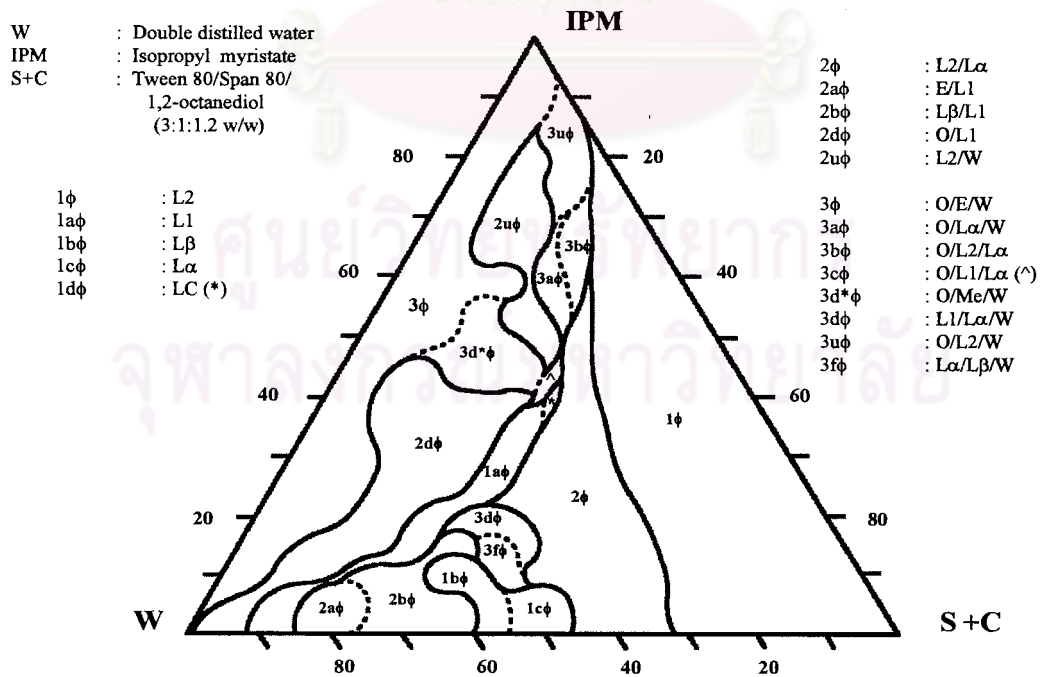
Figure 3.1 Pseudo ternary diagram of the W:IPM:S+C system at 25 °C (Baroli et al, 2000).

The diagram is characterized by the presence of regions of one, two or three phases (1f, 2f, 3f). L2 and L1 phases represent the regions of W/O and O/W microemulsions, respectively. Lα and Lβ correspond to lamellar-liquid crystals and lamellar-gel phases. LC phase is an anisotropic region of liquid crystals. Considering microemusion regions, o/w miroemulsion takes larger zone than w/o. It could be explained that the ratio of Tween 80 is greater than Span 80 (3:1 w/w), therefore, effects of hydrophilic from Tween 80 plays an important role to w/o microemulsion formulation preferably.

Kreilgaard et al.(2000) studied about the different ratios of Labrasol and Plurol isosterique; surfactant and cosurfactant, with isostearylic isostearate as oil phase and water. Figure 3.2, the large microemulsion regions of existence found with the present components, enabled a broad variety of possible formulation compositions with different internal structure and solubility properties for lipo- and hydrophilic drugs. Based on the phase diagrams, seven microemulsion compositions (referred to as system A–G, Table 3.1) were selected from the regions of existence. All microemulsion compositions studied maintained microemulsion characteristics after addition of lidocaine (lipophilic) or prilocaine hydrochloride (hydrophilic) in the concentrations of interest in the temperature range of 24–32°C.

Table 3.1 Microemulsions composition (Kreilgaard et al., 2000)

System	% Water	% Isostearylic isosterate	% Labrasol	% Plurol Isostearique
A	20	10	35[a]	35[a]
B	20	10	47[b]	23[b]
C	20	10	53[c]	17[c]
D	7	70	11.5[a]	11.5[a]
E	11	26	42[b]	21[b]
F	55	8	25[b]	12[b]
G	65	3	24[c]	8[c]

[a]1:1 (w/w), [b]2:1 (w/w), . [c] 3:1 (w/w) Labrasol–Plurol Isostearique
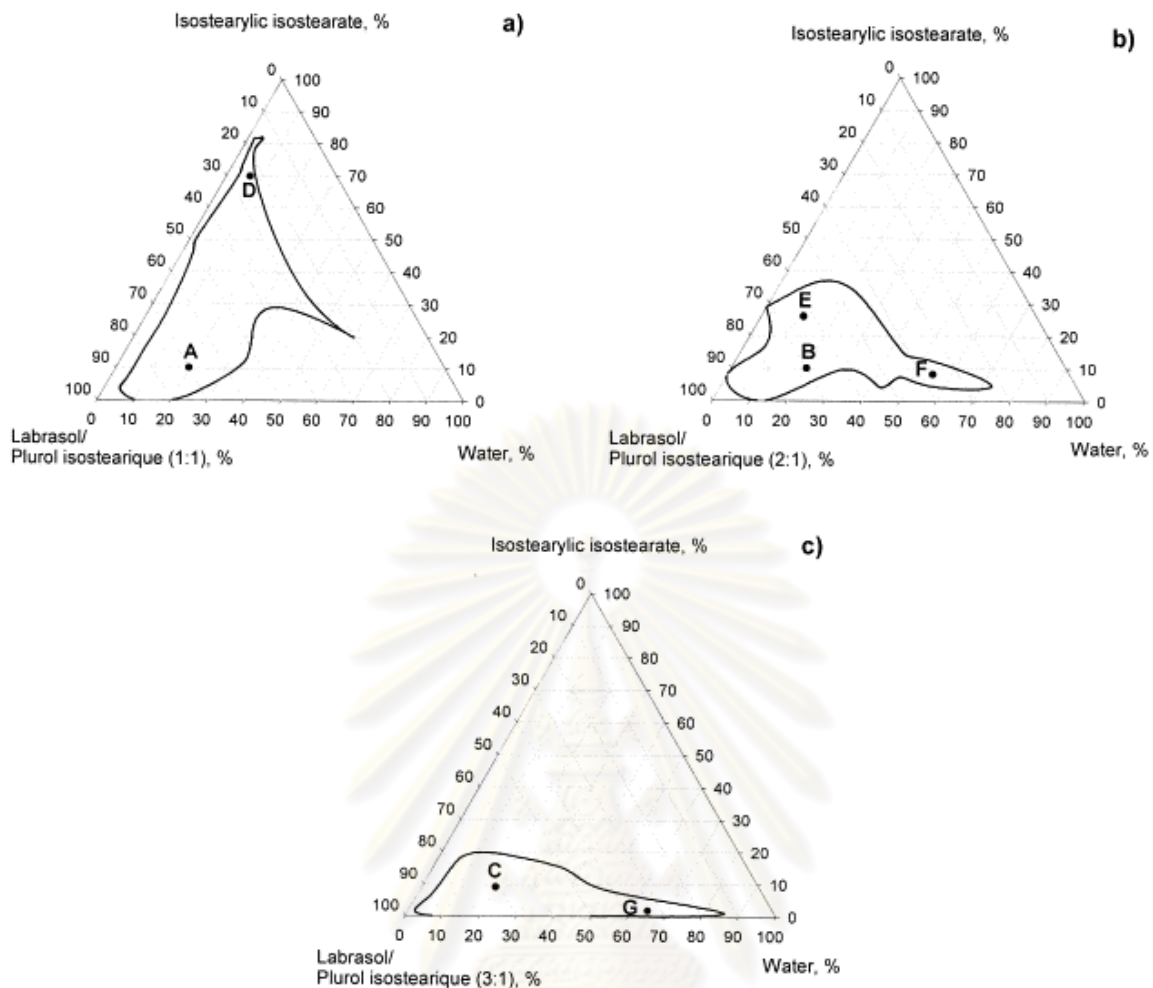
Figure 3.2 Pseudo ternary phase diagrams of microemulsion regions of existence (within the connected lines) with different surfactant/co-surfactant ratios ((a) 1:1; (b) 2:1; (c) 3:1) composed of Labrasol, Plurol Isostearique, isostearyle isostearate and water (% w/w) (Kreilgaard et al., 2000).

Not only surfactant/cosurfactant ratios, but also the chain length of oils phase affects to phase behavior. Lv et al. (2000) used Tween 80/Span 80 1:1 mol/mol with different chain length of oils; Isopropyl palmitate (IPP) and Isopropyl myristate (IPM), and Tween 20/ Span 20 1:1 mol/mol to study phase behaviors. The system are defined as; (a) Span 80/Tween 80 (1:1)/ n-butanol/ IPP/ H_2O; (b) Span 20 /Tween 20 (1:1)/ n-butanol/ IPP/ H_2O; (c) Span 80/Tween 80 (1:1)/ n-butanol (5% chloramphenicol)/ IPM/ H_2O; (d) Span 80/Tween 80 (1:1)/ n-butanol (5% chloramphenicol)/ IPP/ 0.05% sodium hyaluronate. From figure 3.3, the authors found that the area of (a) is a little larger microemulsion region than (b), and (c) is much larger than (a). These results may be understood in terms of the law of chain length

compatibility. Actually, chain length compatibility of surfactants and oil is a very important factor regarding the formation of microemulsions (Hou and Shah, 1987 and Garti et al., 1995). Bansal et al.(1980) showed that the maximum water solubilization by surfactants occurred when $la + lo = ls$ (la, lo, and ls are the lengths of hydrocarbon chains in alcohol, oil and surfactant, respectively) ,and it is also called BSO equation which reflects the requirement of chain length compatibility. As far as the present systems are concerned, according to the BSO equation, when $ls = 18$ for the mixed surfactants and $la = 4$ for n-butanol, the maximum solubilization of water should occur when lo is 14. So it is found that the system containing IPM (Fig. 3.3 c) has a larger area of microemulsion than that of the system containing IPP (Fig. 3.3 a). The same reason could explain the difference between Fig.3.3 (a) and (b). However, the chain length compatibility is an experimental rule and not necessarily fit to all systems (Lv et al., 2000).
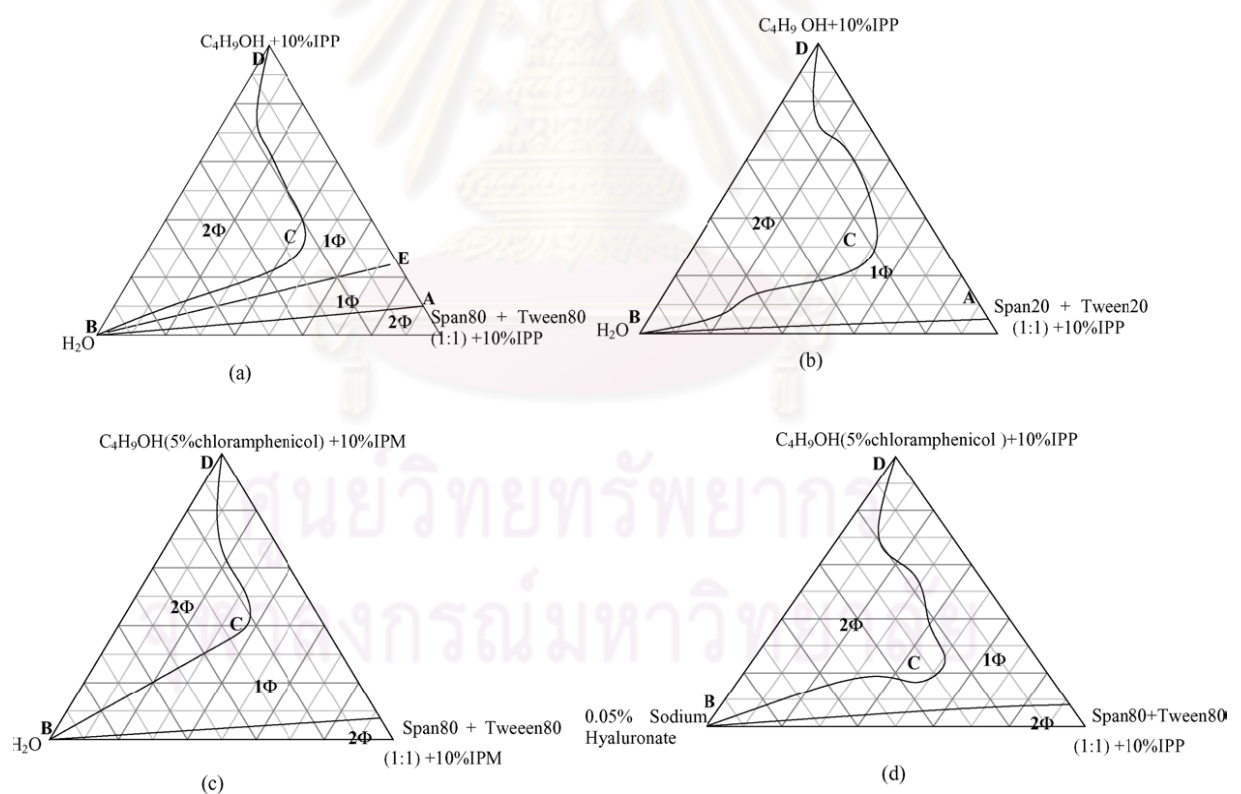
Figure 3.3 The pseudo ternary phase diagrams of various systems at 25 $^\circ$C in different ratios of Span 80 /Tween 80and Span 20/ Tween 20.

Additives may play an important role about modification of phase diagram. Zhu et al. (2006) found that acrylamide will be enlarge the w/o microemulsion zone in the pseudo ternary phase diagram of Span 80-Tween 85/isopar M (isoparaffinic mixture with a boiling range of 223–254 °C)/water system (Fig. 3.4). The reason is acrylamide favors hybridizing the advantages of macroemulsion and microemulsion for acrylamide polymerization.
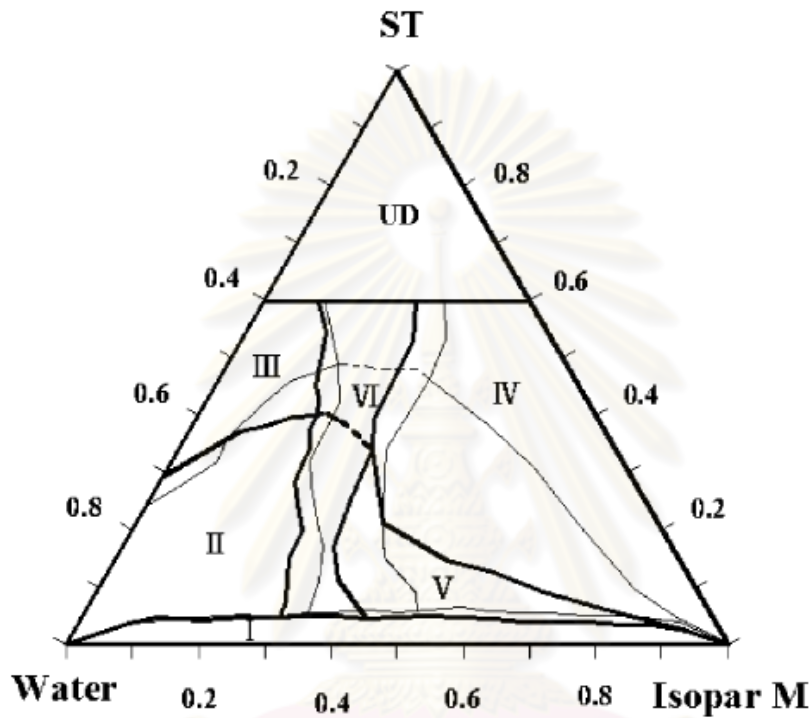
Figure 3.4 Influence of X_{AM} (weight fraction of acrylamide in its aqueous soltution) on phase diagrams of the pseudo ternary Span80-Tween85 /isoparM/water system, slime line represents $X_{AM} = 0$, and bold line is $X_{AM} = 0.50$. (I) Non-emulsion region; (II) o/w emulsion region; (III) o/w microemulsion region; (IV) w/o microemulsion region; (V) w/o emulsion region; (VI) phase inversion region (Zhu et al., 2006).

From phase diagram found that the total area of w/o region (IV and V) increased with the increase in X_{AM}. This would be the result of acrylamide locates at the isopar M/water interface to act as a surface-active agent by filling up the interspace left after the adsorption of emulsifier, decreasing the interfacial tension.

Furthermore, there is a report about using additives such as cholesterol and oleic acids (Schmalfuβ et al. 1996). The systems compose of ME 1: Tween 80/Span

20 (2:3)/ IPM/ DPH (diphenhydramine hydrochloride) dissolved in water, ME 2: Tween 80/Span 20 (2:3)-cholesterol dissolved in IPM- DPH dissolved in water, and ME 3: Tween 80/Span 20 (2:3) – Oleic acid – IPM – DPH dissolved in water. Form Figure 3.5, it is revealed that ME 1 have larger region of microemulsions, while adding cholesterol and oleic acid reduces the one-phase isotropic zone.
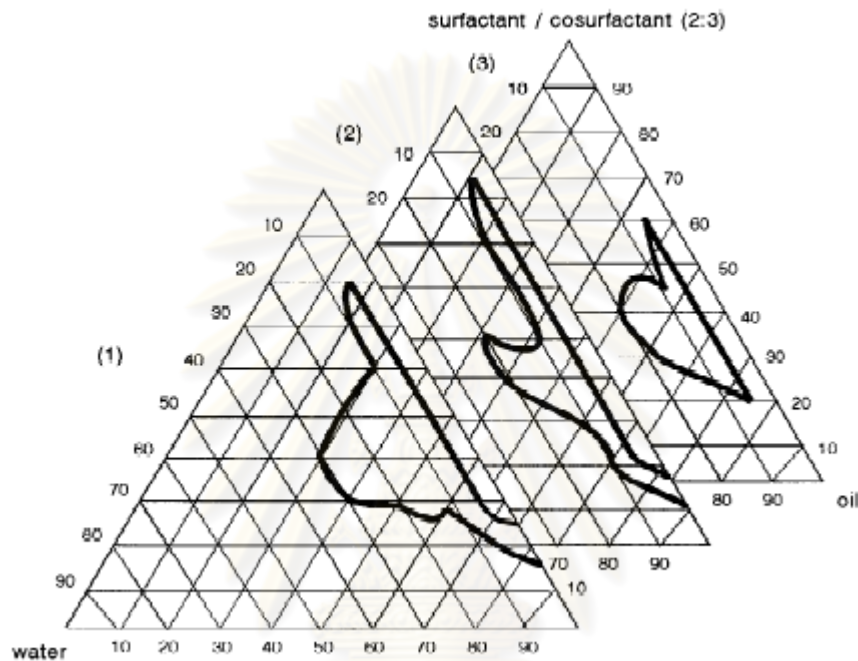
Figure 3.5 Phase diagrams: (1) standard microemulsion, (2) addition of cholesterol, (3) addition of oleic acid.

3.1.2   Influences of surfactants HLB

Griffin (1954) proposed to calculate the hydrophile-lipophile balance (HLB) number of a surfactant from its chemical structure. The HLB value describes the relative polarity of the surfactant, and its value may also indicate the extent of preference for surfactant migrating into the interface between water and oil phases. It seems to be important for surfactant being able to migrate into the interface to depress interfacial tension to a lower level to form microemulsion (Ho et al., 1996). HLB 8-18 from hydrophilic surfactants possess high water solubilizers and generally act as good aqueous solubilizing agents, detergents, and stabilizers for o/w microemulsions. On the other hands, HLB 3-6 from lipophilic surfactants are good for w/o microemulsions

(Myers, 1999). Nonionic surfactant such as Tween provides high HLBs value, while Span supplies low HLBs value as shown in Table 3.2.

Table 3.2   HLBs value of Tween and Span (http://pharmcal.tripod.com/ch17.htm)

Surfactant	HLB
Sorbitan trioleate (Span 85)	1.8
Sorbitan tristearate (Span 65)	2.1
Sorbitan monooleate, NF, (Span 80)	4.3
Sorbitan monostearate, NF, (Span 60)	4.7
Sorbitan monopalmitate, NF, (Span 40)	6.7
Sorbitan monolaurate, NF, (Span 20)	8.6
Polyoxyethylene sorbitan tristearate, (Tween 65)	10.5
Polyoxyethylene sorbitan trioleate, (Tween 85)	11.0
Polysorbate 80, NF, (Tween 80)	15.0
Polysorbate 60, NF, (Tween 60)	14.9
Polysorbate 40, NF, (Tween 40)	15.6
Polysorbate 20, NF, (Tween 20)	16.7

The effect of HLB of Tween 80/Span 20 on microemulsion system is studied. Huang et al. (2008) used different HLBs, in the other words, different ratios of Tween 80 /Span 20 at 1/4, 1/1 and 3/2 weight ratios. Aqueous phase and cosurfactant is 40% ethanol at the same time, whereas Isopropyl myristate (IPM) is oil phase. They found that the area of microemulsion isotropic region (Fig. 3.6) slightly increased in size with the increasing ratio of Tween 80/Span 20. A similar result was reported by Wu et al. (2001) which the isotropic regions tended to narrow down with increasing Span 80/Tween 80 ratios in the oil-poor part of the pseudo ternary diagram.
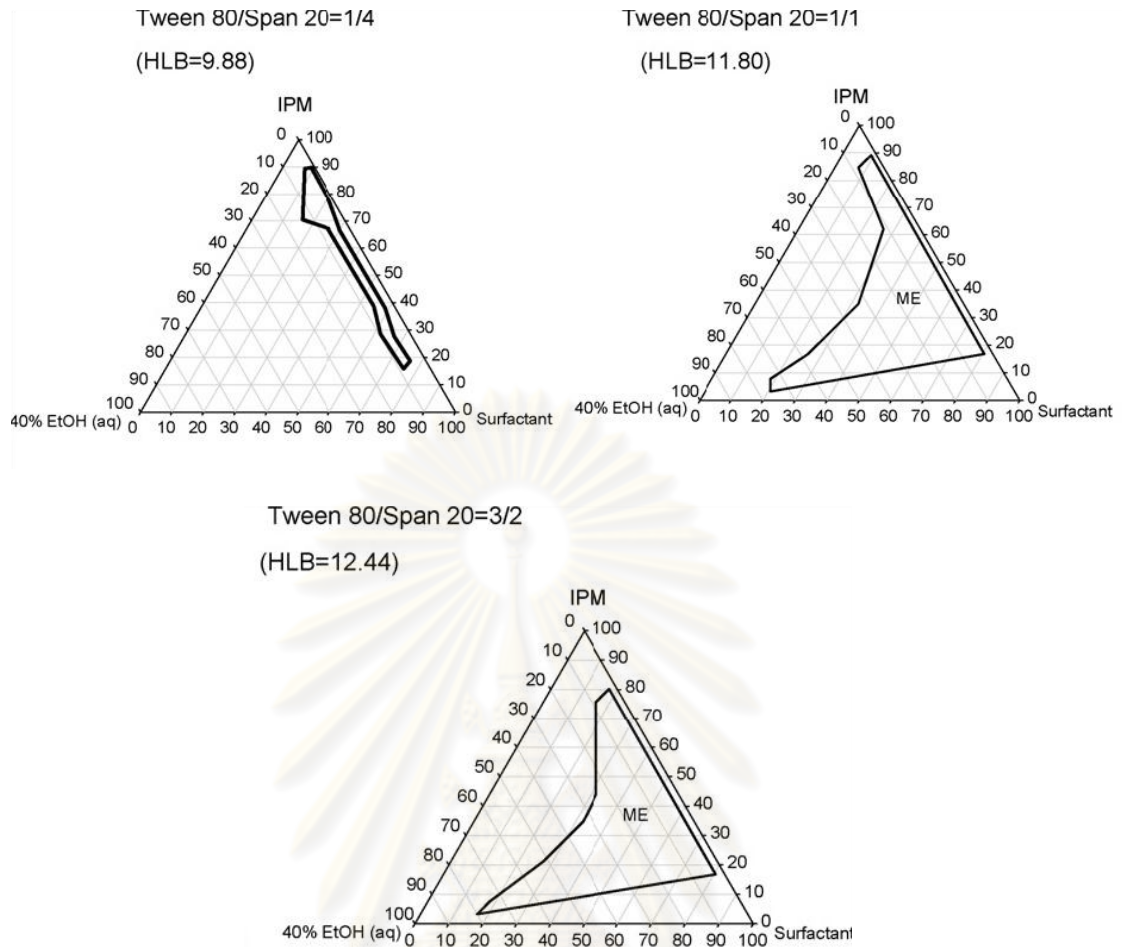
Figure 3.6 Pseudo ternary phase diagrams of microemulsion (ME) composed of
IPM, mixed surfactant (Tween 80/Span 20), cosurfactant (ethanol) and
aqueous solution (Huang et al., 2007).

In addition, different HLBs between 8 and 13 of polyglycerol fatty acid esters;
nonionic surfactants, such as tetraglycerol monolaurate (ML 310, HLB = 10),
hexaglycerol monooleate (MO 500, HLB = 11), decaglycerol monooleate (MO 750,
HLB = 13) and decaglycerol sesquioleate (SO 750, HLB = 11) are studied by Ho et
al. (1996) in the present of Captex 300 (Glyceryl tricaprylate/caprate) as oil phase and
water. They found that those surfactants necessary to add ethanol, 1-propanol, or 1-
butanol as cosurfactant to produce microemulsion, because of adding short chain
alcohols is able to reduce interfacial free energy and tension by their incorporation
into the interfacial layer. It is also reveal that 1-butanol is a good cosurfactant in
conjunction with polyglycerol fatty acid ester for the formation of microemulsions
(Fig 3.7).
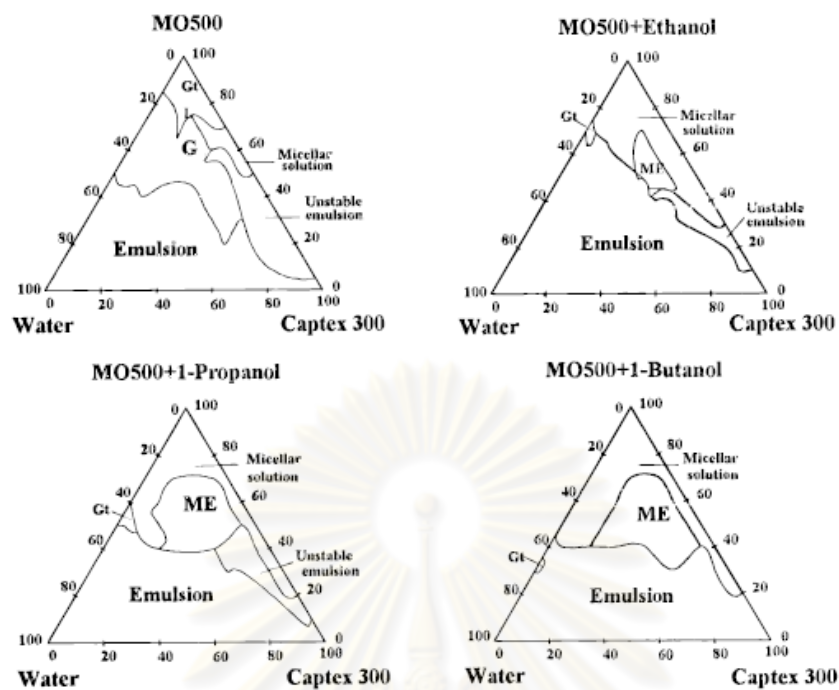
Figure 3.7 Phase diagrams of MO 500/Captex 300/water/alcohol systems (Ho et al.,1996)
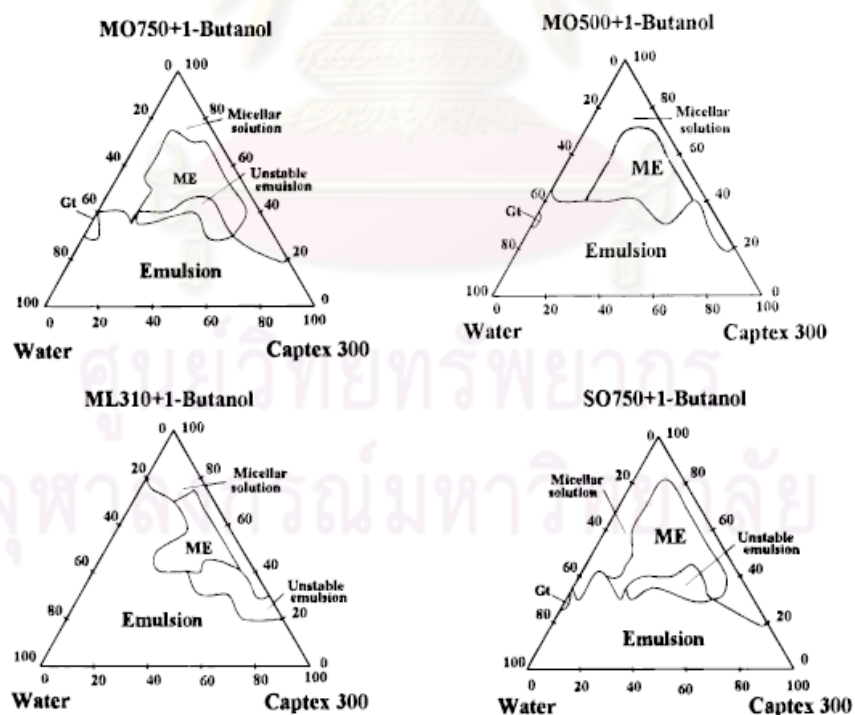
Figure 3.8 Phase diagrams of polyglycerol fatty acid esters/Captex 300/water/ 1-butanol systems (Ho et al.,1996)

From Figure 3.8, apparently, increasing of HLB value from ML 310 (HLB = 10) to MO 750 (HLB = 13) means increasing in microemulsion regions. This is similar results comparing to Wu et al.(2001) and Huang et al.(2007). However, all of them did not determine that the increasing microemulsion regions being w/o or o/w microemulsion. Looking at table 3.3, summary of HLB ranges and their application is shown below (Benita, 2005).

Table 3.3 Summary of HLB ranges and their application (Benita, 1996)

HLB range	Application
3–6	w/o microemulsion, emulsion
7–9	Wetting agents
8–18	o/w microemulsion, emulsion
13–15	Detergent
15–18	Solubilizer

3.2 Microemulsions for transdermal drug delivery

Microemulsions were found as an effective vehicle of the solubilization of certain drugs and as protecting medium for the entrapped of drugs from degradation, hydrolysis, and oxidation (Kogan and Garti, 2006) They are recognized as appropriate vehicles for the administration of drugs (Osborne et al., 1991; Swarbrink and Boylan, 1994; Thacharodi and Rao, 1994) since they provide thermodynamic stability (long shelf life), easy formation (zero interfacial tension and almost spontaneous formation), low viscosity with Newtonian behavior, high surface area (high solubilization capacity), and very small droplet size (Kogan and Garti, 2006). The advantage of using microemulsions as tranndermal vehicle is not only avoidance of hepatic metabolism, but also eases of administration (Krielgaard, 2002). The use microemulsions may enhance transdermal penetration by various mechanisms. Many molecules are solubilized in microemulsions. In addition, microemulsions induce a change in the thermodynamic activity of the drugs they contain, modifying their partition coefficient, and thus favor penetration of the stratum corneum (Delgado-Charro et al., 1997 ; Alvarez-Figueroa and Blanco-Mendez, 2000).

Lipophilic drug (lidocane) and hydrophilic drug (prilocaine hydrochloride) are studied as drugs model for microemulsions transdermal delivery and compared the drug delivery potential to conventional vehicles (emulsions and hydrogels) (Kreilgaard et al., 2000). The researchers used self-diffusion coefficients as determined by the pulsed-gradient spin-echo NMR (PGSE-NMR) to study the transdermal flux using Franz-type diffusion cells. They prepared phased diagrams where w/o and o/w microemulsions of Labrasol/Plurol isostearique/isostearyl isostearate/water (Fig. 3.2) and found that microemulsions increased the trandermal flux of lidocaine up to four times compared to a conventional o/w emulsions and the prilocaine hydrochloride almost 10-times compared to hydrogels. Focus on hydrophilic drug, Schmalfuβ et al.(1997), studied the penetration of diphenhydramine hydrochloride (DPH) from w/o microemulsion into human skin under *ex vivo* conditions. They studied the mechanism of drug penetration into the skin from microemulsion (ME) systems, particularly into the stratum corneum and the influence of the constituents of the ME on this penetration process. The w/o ME consisted of 5 wt% water and 15-20 % Tween 80/Span 20 (2/3 ratio) and approximately 70% IPM as oil phase in oil phase in the presence of 2% to 5% oleic acid or cholesterol and 1% of the DPH as the drug model. A standard microemulsion showed an accumulation of penetrated drug in the dermis, indicating a potentially high absorption rate. Incorporation of cholesterol into the system leads to an even higher penetration rate and shifting of the concentration profile further toward the epidermis. Addition of oleic acid had no effect.

Since this project will use Tween 80/ Span 80/1,2-octanediol/IPM/aqueous pH 5.5 to formulate microemulsion system for transdermal drug delivery, literature about using similar compositions to the project should be reviewed. Baroli et al. (2000) studied Tween 80:Span 80:1,2-octanediol (3:1:1.2 w/w)/IPM/water microemulsion system with purposed to delivery 8-methoxsalen (8 – MOP); drug for the treatment of hyper-proliferative skin diseases. The in vitro permeation data revealed that the novel microemulsions increased total penetration through the skin of 8-MOP 1.9–4.5 and and 3.5–8 fold higher than IPM and aqueous solution, respectively. Alvarez-Figueroa and Blanco-Mendez (2000), studied microemulsion system which consist of Tween 80:Span 80:1,2-octanediol (3:1:1.2 v/v/v) /IPM/water for Methotrexate transdermal

delivery. Similarly to Baroli et al (2000), the microemulsions were more effective than passive delivery from aqueous solutions of the drug.

Table 3.4 Overview of cutaneous drug delivery studies with microemulsion in vitro (Krielgaard, 2002).

Drug	Microemulsion			Reference
	Oil phase	Surfactants/cosurfactants	Aqueous phase	
5-Fluorouracil	isopropyl myristate	AOT	Water	Gupta, 2005
8-Methoxsalen	isopropyl myristate	Tween 80, Span 80, 1,2-octanediol	Water	Baroli, 2000
Apomorphine hydrochloride	isopropyl myristate, decanol	Epikuron 200,1,2-propanediol benzyl alcohol	water, Aerosil 200	Peira, 2001
Ascorbic acid	isopropyl myristate, cetearyl octanoate	dodecylglucoside cocoamide propylbetaine, phosphatidyl choline/2-ethyl-1,3-hexanediol	Water	Gallarate, 1999
Diclofenac	isopropyl myristate	Lecithin	Water	Dreher, 1997
	Diclofenac diethylamine	Lecithin	Water	Kriwet, 1995
	isopropyl myristate	Labrasol/Plurol Oleique	Water	Djordjevic, 2004
Lidocaine	iostearylic isostearate	Labrasol/Plurol Oleique	Water	Kreilgaard, 2000
Piroxicam	isopropyl myristate	hexadecyltrimethylammonium bromide	Aqueous buffer (pH 5.5)	Dalmora, 2001
Retinoic acid	isopropyl myristate	Epikuron 200, Oramix NS10/ethanol, 1,2 hexanediol	phosphate buffer	Trotta, 2003
Methotrexate	decanol	lecithin, benzyl alcohol	water, PG	Trotta, 1996
	ethyl oleate	Labrasol/Plurol Oleique	Aq. 154 mM NaCl (pH 7.4)	Alvarez-Figueroa, 2001
	isopropyl myristate	Tween 80, Span 80, 1,2-octanediol	Water	Alvarez-Figueroa, 2001

3.3 Methotrexate transdermal delivery via microemulsions

Methotrexate (MTX) is effective for the treatment of psoriasis when administered by the oral or parenteral. The systemic use of this drug, nevertheless, may provoke any of the numerous side effects, eapecially hepatotoxic effects and central nervous system (CNS) effects (Bookbinder et al., 1984; Van Dooren-Greebe et al., 1994). MTX enhances adenosine delivery in the CNS and its activation of A1 receptors in the brain can be responsible for induction of fatigue, lethargy, and headache (Stagni and shukla, 2003 ; Grim et al.,2003). To reduce and avoid such effects, it would clearly be preferable to administer MTX topically (Hwang et al., 1995). Consequently, techniques such as iontophoresis, electroporation or the use of appropriate vehicles such as hydrogels or microemulsions may enhance transdermal delivery of this drug (Alvarez-Figueroa and Blanco-Mendez, 2000; Singh and Singh, 1995). However, this research focuses on microemulsions only.

Trotta et al, (1996) studied the components of microemulsions which consisted of lecithin-benzyl alcohol/decanol/water-propylene glycol. They found that at pH 4.0 using counter ions such as dodecyl sulfate (SDS) and dioctyl sulfosuccinate (AOT) enhanced the transport of methotrexate across intact hairless mouse skin by increase the flux of the drug. The others microemulsion systems are investigated by Alvarez-Figueroa and Blanco-Mendez (2000). Two systems used were: I, Surfactant /cosurfactant 3:1 v/v Labrasol/(Plurol Isostearique); oil phase ethyl oleate; aqueous phase 154 mM NaCl pH 7.4 and II, Surfactant/cosurfactant 3:1:1.2 v/v/v (Tween 80):(Span 80):(1,2-octanediol); oil phase myristic isopropyl ester; aqueous phase water. System I was suggested as o/w microemulsion, where as system (2) is w/o microemulsion. The solubility of MTX was higher in system I than in system II. Since MTX is hydrosoluble, and thus has higher affinity for the aqueous phase: the external phase in the system I microemulsions, a greater amount of drug is solubilized. The assays of passive delivery of MTX from microemulsions are observed after 24 h at 37 ˚C. System I which consisted of 20% oil, 25% aqueous and 55% S/C, as well as, system II consisted of 10% oil, 15% water and 75% S/C at 50% saturation MTX concentration was detected the highest remaining MTX in the skin; 2.19 ±0.44 µg cm^{-2} and 2.91±0.39 µg cm^{-2} of MTX in the skin, respectively.

3.4 *In vitro* drug release studies

Franz diffusion cell are generally used to assess skin permeability of the drug in terms of design and development of novel formulations (Franz, 1978). Moreover, toxicity screening (Zorin et al., 1999) and quality control is evaluated using Franz diffusion cell (Siewert et al., 2003; Shah et al., 1999 and Ueda et al., 2009). Excised human or animal skins are normally used in the cell. However, synthetic membranes are employed when biological skin is not readily available. Two performances of the synthetic membranes applied in Franz cell drug diffusion studies are: simulation of the skin (Twist and Zatz , 1988) and quality control (Twist and Zatz, 1986 and Corbo et al., 1993). Transport of the drug across a membrane does control the drug release in the diffusion controlled membrane systems. The transport is dependent on the drug diffusivity through the membrane and the thickness of the membrane, according to Fick‟s law (Dimitrios et al., 2007).

Selection of membrane

Hydrophobic and possesses rate-limiting properties like skin has brought to Polymethylsiloxane (PDMS); synthetic membrane, often employed to simulate the skin (Twist and Zatz, 1986 and Pellett et al., 1997). On the other hand, drugs minimum diffusion resistance and only take action as supporting to separate the formulation from receptor medium is an important role of synthetic membranes for quality control (Siewert et al., 2003; Shah et al., 1999 and Ueda et al., 2009). The synthetic membrane should be a „continuous‟ medium of the receptor media, so the membrane should have pores called porous membrane.

Porous synthetic membranes with a large variety of materials, pores size and thicknesses have been used for a decades to asses topical drug diffusion as shown in Table 3.5. From the table, cellulose and cellulose acetate may be suitable for the study of release rates of w/o microemulsion containing methotrexate.

Table 3.5 Some artificial membranes that used to study in *vitro drug* release.

Drug	Membrane	Pore size (µm)	Thickness (µm)	MWCO (kDa)	Reference
Hydrocortisones (cream)	Cellulose acetate	0.50	150	-	Shah et al. (1989)
	Triton-free cellulose acetate	0.45	150	-	
	Glass fiber filter	0.45	450	-	
	Polysulfone	0.45	165	-	
Caffeine (concentrated w/o emulsion)	Polysulfone	0.45	165	-	Clément et al. (2000)
	Silicone	-	125	-	
	Cellulose acetate	NA	25	<5	
Diclofenac sodium (w/o microemulsion)	Cellulose	NA	NA	<12	Kantarci et al., 2007
Ibuprofen (saturated solution)	Cellulose nitrate	0.45	125	NA	Ng et al. (2010)
	Polysulfone	0.45	145	NA	
	Polyethersulfone	0.45	145	NA	
	Polycarbonate	0.10	10	NA	
	Polypropylene	0.05	20	NA	
Piroxicam (gels)	Cellulose nitrate	0.20	-	-	Santoyo et al. (1996)
Diclofenac sodium (carbopol gel)	Cellulose nitrate	0.20	-	-	Arellano et al. (1999)

CHARTER 4

MATERIALS AND METHODS

4.1 Equipments and chemicals

4.1.1 Equipments

Vortex mixer (G-560E, Scientific Industries, Inc., U.S.A.)

Magnetic Stirrer (IKA RCT, Germany)

Polarized light microscope (Olympus, model BHSP)

pH meter (MP220, Mettler Toledo, Switzerland)

Conductivity meter (Weilheim, Germany)

Dynamic Light Scattering (DLS) Spectrophotometer

Franz diffusion cells

4.1.2 Chemicals

Distilled water

Polyoxyethylene sorbitan monooleate (Tween80) 98% (Fluka, Germany)

Sorbitan monooleate (Span 80) 98% (Fluka, Switzerland)

1,2-octainediol 98% (Sigma-Aldrich, U.S.A.)

Isopropyl myristate (IPM) 98% (Fluka, Switzerland)

Acetic acid, AR grade (J.T. Baker, U.S.A.)

Sodium acetate, AR grade (J.T. Baker, U.S.A.)

Potassium phosphate monobasic dihydrate and dibasic, AR grade (E. Merck, Germany)

Methotrexate hydrate 98% (Sigma-Aldrich, U.S.A.)

Cellulose acetate membrane, pore size 0.45 μm, thickness 150 μm (Whatman, England)

4.2 Experimental procedures

4.2.1 Construction of phase diagrams

Pseudo-ternary phase diagrams will be constructed using the aqueous titration method at 37 ˚C in water bath until reaching equilibrium. The compositions between surfactants (Tween 80/ Span 80) and 1,2-octanediol are fixed to 4:1.2 w/w. In order to reach desired HLB of mixed surfactant, changing composition of those surfactants is required (table 4.1). Phase studies will be carried out, at first, mix surfactants and cosurfactant with above ratio. Afterwards, mix IPM in a required ratio with surfactants, cosurfactant using Vortex mixer and then slowly titrate the solution with 0.1 molar acetate buffer pH 5.5. After mixing, the systems are checked for visual clarity and fluidity. The fluid samples are further examined by polarized light microscopy (Olympus, model BHSP). The systems obtained will be classified into microemulsion or thermodynamically unstable system categories according to the transparency criterion. Those which do not show a change in the menicus after tilting to an angle of 90° will be classified as gels. The physical states of these systems will be marked on a pseudo-three component phase diagram with one axis representing acetate buffer pH 5.5, one representing IPM, and the third representing a mixture of surfactants and cosurfactant at a fixed volume ratio.

Table 4.1 Tween 80 and Span 80 compositions for required HLB with total weight 12 grams.

HLB	Tween 80		Span 80	
	weight (g)	volume[a] (ml)	weight (g)	volume[b] (ml)
6	1.907	1.765	10.093	10.195
7	3.028	2.804	8.972	9.063
8	4.150	3.842	7.850	7.930
9	5.271	4.881	6.729	6.797
10	6.393	5.919	5.607	5.664
11	7.514	6.957	4.486	4.531
12	8.636	7.996	3.364	3.398
13	9.757	9.034	2.243	2.266
14	10.879	10.073	1.121	1.133

Note: a is density of Tween 80, 1.080 g/ml

b is density of Span 80, 0.990 g/ml

4.2.2 Microemulsion characterization

4.2.2.1 Type of microemulsions

Dye solubility tests and dilution tests are used to determine the type of microemulsions. The dye solubility tests involve the addition of a water- (methyl orange) or oil- (Sudan III) soluble dye to the microemulsion. Thus, intense staining of the external phase after addition of a water-soluble dye indicated an o/w microemulsion. The addition of an oil-soluble dye to the same microemulsion would result in the staining of the droplets of the internal phase (Ho et al., 1996). The dilution tests involve observation of the microemulsion following its dilution with light mineral oil or water to see whether separation has been effected. If water is easily dispersed in the continuous phase, the microemulsion will be termed oil-in-water (o/w). On the other hands, the microemulsion will be water-in-oil (w/o) if light mineral oil is dispersible in the external phase.

4.2.2.2 Polarized light microscopy

To verify the isotropic nature of microemulsions, samples were examined using cross-polarized light microscopy (Olympus BHSP, Tokyo, Japan). A drop of sample was placed between a coverslip and a glass slide and observed under cross-polarized light. Isotropic material, such as a microemulsion, in contrast to anisotropic liquid crystals, will not interfere with the polarized light (Friberg, 1990) and the field of view remains dark.

4.2.2.3 Conductivity measurements

The electric conductivity (σ) was measured by means of an inoLab conductivity meter (Weilheim, Germany) operating at 50 Hz and room temperature. Conductivity measurements were carried out by titration of oil and surfactant/ cosurfactant mixture with buffer (along a dilution line).

4.2.2.4 Dynamic Light Scattering measurements

The particle size, the polydispersity and Zeta potential of the microemulsions are determined using dynamic light scattering (Malvern Zetasizer, Malvern, Worcestershire, UK) at a 90° angle by inserting the sample cell in the temperature-controlled chamber of the goniometer. The autocorrelation function of the scattered intensity is obtained from a 1024-channel photon correlator. The results of each sample are processed in a computer and the hydrodynamic diameter (r_h) of the droplets will be obtained based on the estimated diffusion coefficient (D) and the Stokes–Einstein equation,

$$r_h = \frac{k_B T}{6\pi\eta D} \qquad (4.1)$$

where T is absolute temperature in K, η viscosity of the continuous medium in Pa s, k_B Boltzmann constant in J/K, and D diffusion coefficient in m^2/s.

4.2.3 Construction of phase diagrams containing MTX

In this section, at first, MTX solubility in ABS pH 5.5 was determined using HPLC technique. Excess MTX was added to ABS pH 5.5 and stirred at 37 ºC for 24 hours with aluminum foil wrapped in order to protect MTX from light. After that, the suspension of MTX was centrifuge at 3000 g for 15 minutes, and an aliquot was suitable diluted before inject to HPLC using ascentis-C18 column (150x4.6), flow rate 1 ml/min, mobile phase was methanol: buffer solution (10: 90), UV-detector at 303 nm was employed. After that, the experiment was conducted in the same procedure to the section 4.2.1 except the change of aqueous phase compositions from ABS pH 5.5 to saturated MTX solution.

4.2.3 Determination of MTX amount released from w/o microemulsion

Approximately 0.5 ml of sampling aliquot was collected simultaneously from the lower region of the receptor chamber of Franz diffusion cell and replace with the same withdrawn volume of fresh receptor fluid. The samples were stored in capping vial 2 ml and kept at room temperature avoiding light. Without any dilution, 20 μl of sample solution was directly injected through HPLC.

Cumulative amount released (Q_t, μg) of MTX per contact surface area of membrane was as following:

$$Q_t = C_t \text{ x } V$$

Where,

Q_t is a cumulative amount of MTX released at anytime t (μg)

C_t is a concentration of MTX determined at anytime t (μg/ml)

V is a volume of individual Franz cell (ml), which was 14.00 ml in this study.

According to variation of initial of drug loading, the cumulative amount of MTX released should be divided by the amount of MTX presented initially in loading dose to correct the values of all data.

Cumulative amount released (Q, %) of MTX per unit initial drug loading dose was as following:

$$Q, \% = \frac{Q_t}{Q_0} \times 100 = (C_t \times V) \times \frac{100}{Q_0}$$

Where,

Q_0 is an amount of MTX presented initially in the system (μg).

Q_t is a cumulative amount of MTX released at anytime t (μg)

C_t is a concentration of MTX determined at anytime t (μg)

V is a volume of individual Franz cell (ml), which was 14.00 ml in this study.

100 is unit transformation (%).

4.2.4 Kinetics of release

Various kinetic release models such as the first order model, Baker – Lonsdale model, Higuchi model, Hixon – Crowell model, and Korsmeyer – Peppas model were used to fit the data. Correlation coefficients (R_c) from each models would be compared, and then the best R_c from any models would be selected to find rate constant.

CHAPTER 5

RESULTS AND DISSCUSSION

The study of Tween 80/Span 80 HLB effects on microemulsion systems for Methotrexate (MTX) delivery composes of three parts. Firstly, construction of phase diagrams of varied HLB values in order to determine microemulsion areas. Next, phase diagrams were investigated for systems with MTX. Finally, *in vitro* MTX drug release from microemulsions was studied using Franz diffusion cells for determination of MTX delivery.

5.1 Effects of HLB on microemulsion systems

5.1.1 Factors affecting HLB values

The hydrophile – lipophile balance (HLB) takes into account the relative contribution of hydrophilic and hydrophobic fragments of the surfactant molecules. Griffin (1954) proposed to calculate the HLB number of a surfactant from its chemical structure and match that number with the HLB of the oil phase which was to be dispersed. In this research, cosurfactant; 1,2-octanediol, might affect to HLB value of the system, but this effect was ignored in this research since the cosurfactant was added at a fixed weight in all the experiments.

From equation 2.2 , the HLB value of surfactants can be calculated as the ratio between relative molecular mass of hydrophile group and relative molecular mass of surfactant multiply by 20 (Griffin, 1954). Tween 80 is of HLB 15 which indicates its high water solubility and favors to form o/w microemulsion (Griffin, 1954). Figure 5.1 demonstrates that Tween 80 contains many polyoxyethylene groups that can form hydrogen bonding with water molecules resulting in a stable solution. However, a long single chain hydrocarbon (monooleate) in Tween 80 makes it also slightly oil soluble. Considering Span 80, its HLB value is 4.3 which means Span 80 is only slightly water soluble and favors to form w/o microemulsion (Griffin, 1954). Figure 5.2, confirms this statement since Span 80 composes of only a small hydrophilic group in comparison to hydrophobic one.
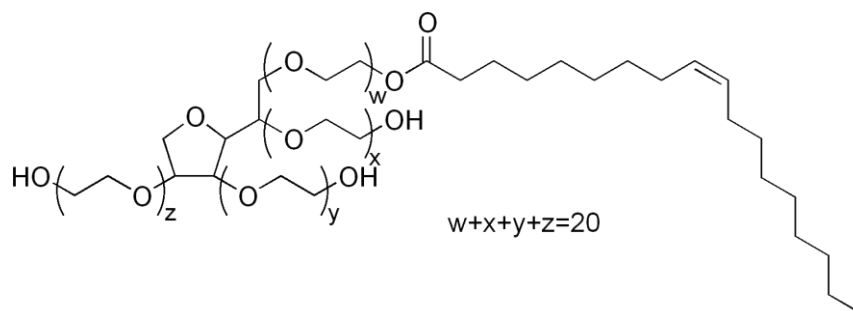
Figure 5.1 Chemical structure of Tween 80, molar mass 1310 g/mol.
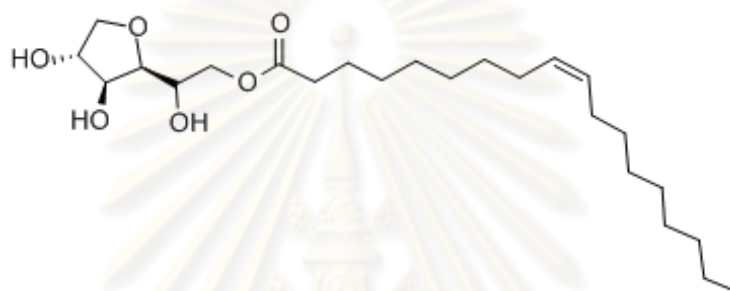
Figure 5.2 Chemical structure of Span 80, molar mass 428.6 g/mol.

Both Tween 80 and Span 80 are single long chain monooleate sorbitan surfactants, so their molecules adsorb each other simultaneously. Therefore, different mixing ratios between Tween 80 /Span 80 result in different HLB values.

According to equation of packing parameter (p) which relates the ability of surfactants to form particular aggregates to the geometry of the molecule itself, can be calculated by using the following equation.

$$p = \frac{V}{a_0 l_c} \qquad (5.1)$$

Increasing the ratio of Tween 80 for increasing HLB of the system directly decreases volume of the hydrocarbon per amphiphile (V) since an amphipile group increases with Tween 80 ratio whereas hydrocarbon volume remains constant. Optimal area per polar head (a_0) also increases with Tween 80 proportion. And the length of extended alkyl chain (l_c) maintained the same due to same hydrophobic part of both surfactants. As the result, increasing Tween 80/ Span 80 weight ratio in order

to increase an HLB value will reduce the packing parameter which influence to the type of aggregation in aqueous (o/w, lamellar or w/o) of the surfactants. Generally, if p is less than 1/3, o/w microemulsion will favor to form (Lawrence and Rees, 2000). However, it should be emphasized that since predictions based on p consider only geometrical arguments (ignoring, for instances, chain elasticity and entropy), they should be treated with caution (Chevalier and Zemp, 1990). Furthermore, the calculation of p for multicomponent systems is not simple: oil may penetrate into the hydrophobic core and increase V; chain folding may shorten the alkyl chain length l_c (Kumar and Mittal, 1999).
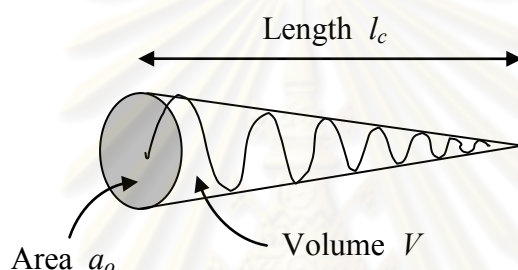
Figure 5.3 A surfactant molecule with head group area a_o, hydrocarbon critical chain l_c, and hydrocarbon volume V. (Holmberg, 1998)

Many studies revealed that single-chain surfactants alone are unable to reduce adequate oil/water interfacial tension to enable forming microemulsion (Lawrence and Rees, 2000). As a result, medium chain length alcohols are commonly used as cosurfactants not only to reduce the interfacial tension, but also to increase the fluidity of the interface. Thereby entropy of the system increases (Attwood, 1994; Eccleston, 1994; and Tenjara, 1999). Furthermore, alcohols may influence the solubility properties of aqueous and oil phases due to its partitioning on both phases (Lawrence and Rees, 2000). In this study, 1,2-octanediol (Fig. 5.4) was used as a cosurfactant.
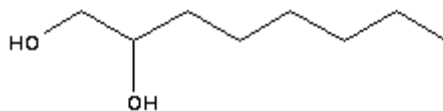
Figure 5.4 Chemical structure of 1,2-octanediol.

Mixed surfactant systems co-operated with 1,2-octanediol as a cosurfactant were fixed at 4:1.2 w/w ratios of surfactants/cosurfactant in all the experiments, so effects from cosurfactant HLBs could be ignored in this research even. Consequently, the only factor considered HLB values was the weight fraction of blended surfactants which can be calculated according to equation 2.3 as shown below,

$$HLB = \Sigma \, (HLB_i \, x \, f_i) \tag{2.3}$$

where HLB_i is HLB value of surfactant i

   f_i is mass or weight fraction of surfactant i

5.1.2 HLB effects on phase diagrams

5.1.2.1 Phase diagram without MTX

Phase diagrams were constructed using an aqueous titration method at 37 ˚C in a water bath where the solution was left incubated for 24 h before the equilibrium was reached. The compositions between surfactants (Tween 80/ Span 80) and 1,2-octanediol were fixed at 4:1.2 w/w. Phase preparation was carried out, at first, mixed surfactants and cosurfactant at the specified ratios. Afterwards, IPM was added into surfactants/cosurfactant (S/C) mixture at a required ratio, and vortex mixer was used to mix the system. Acetate buffer solution (ABS) pH 5.5 was slowly titrated to the prepared solution and mixed again by a vortex mixer. Each time of titration, the system was observed for its viscosity and transparency. Microemulsion systems were visually determined by its low viscous transparent solution. In addition, different methods were carried out to confirm the formation and types of microemulsion solutions. According to phase characterization, single isotropic region (1Φ) and non-isotropic regions such as lamellar-liquid crystals, macroemulsions, two phases and three phases were found. After that, phase diagrams were constructed for systems with HLB 6, 8, 10, 12 and 14 as shown in Figures 5.5 – 5.9.
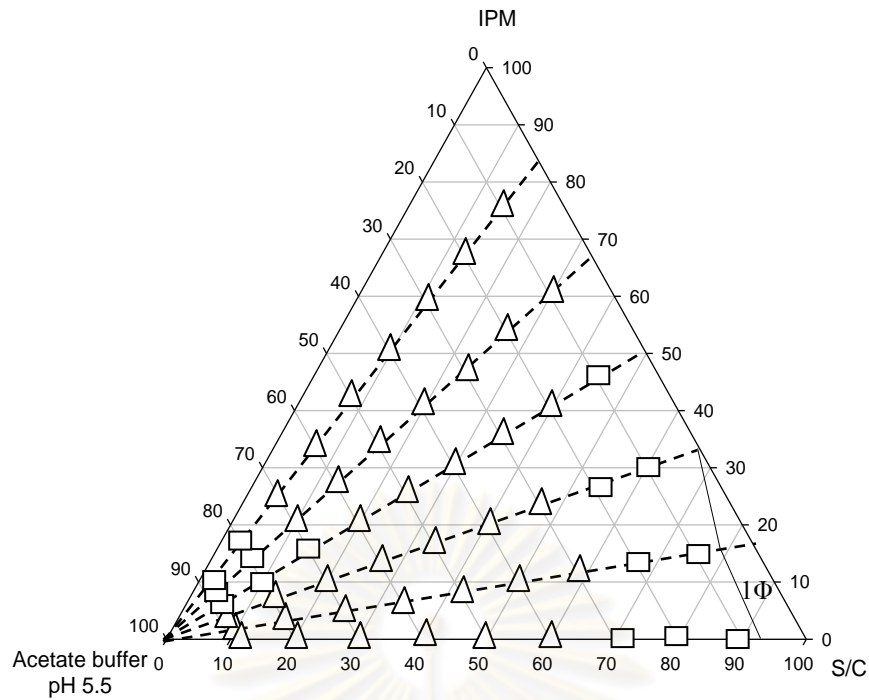
Figure 5.5 Pseudo-ternary phase diagram of Tween 80/Span 80/1,2-octanediol, IPM
and acetate buffer pH 5.5 system for HLB 6 at temperature of 37° C.
(□ two phases, and △ three phases)

Figure 5.6 Pseudo-ternary phase diagram of Tween 80/Span 80/1,2-octanediol, IPM
and acetate buffer pH 5.5 system for HLB 8 at 37° C. (□ two phases,
△ three phases, and ○ macroemulsions)
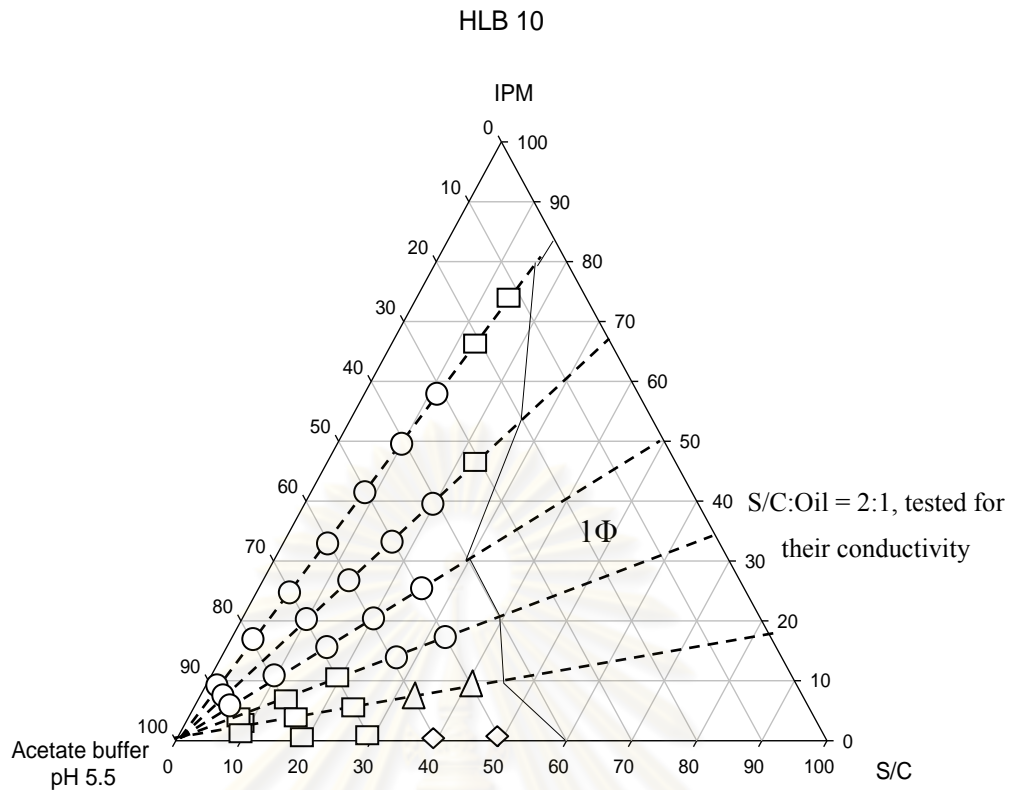
HLB 10

Figure 5.7 Pseudo-ternary phase diagram of HLB 10 system at 37° C. (□ two phases,
△ three phases, ○ macroemulsions, and ◇ lamellar-liquid crystals)
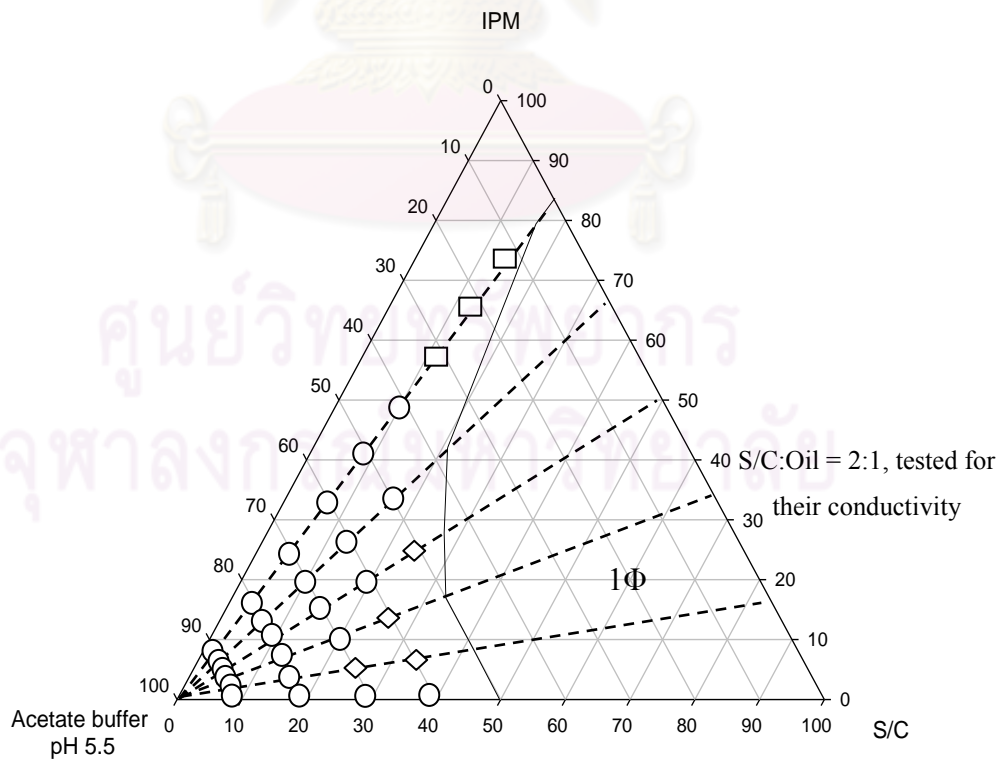
Figure 5.8 Pseudo-ternary phase diagram of HLB 12 system at 37° C**.** (□ two phases,
○ macroemulsions, and ◇ lamellar-liquid crystals)
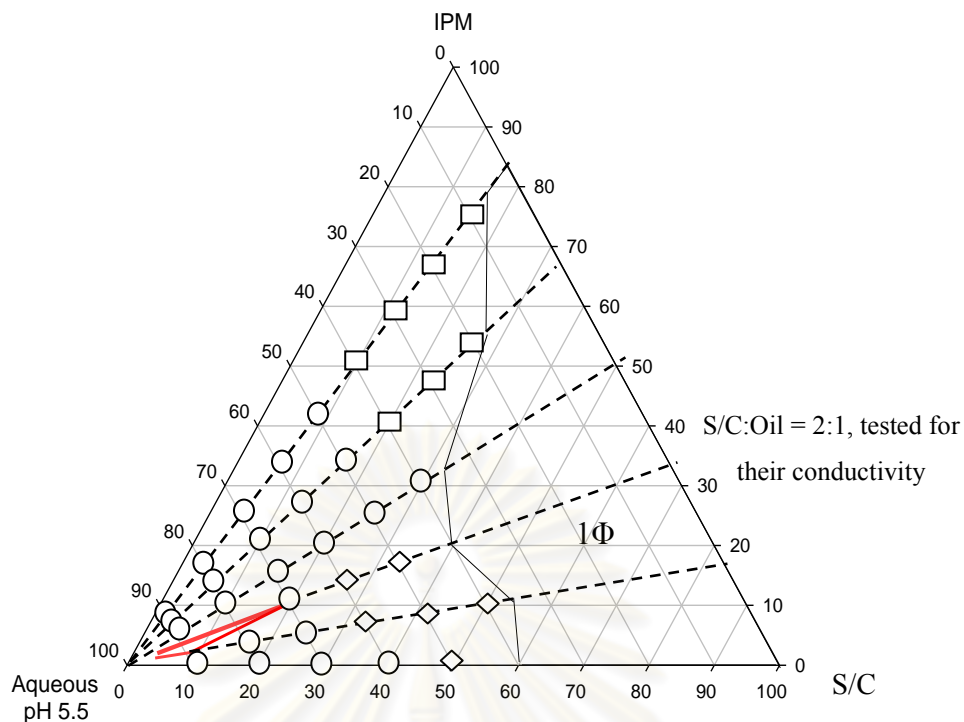
Figure 5.9 Pseudo-ternary phase diagram of HLB 14 system at 37° C. (□ two phases, ○ macroemulsions, ◇ lamellar-liquid crystals)

Considering Figure 5.5 – 5.9, it was found that HLB values affected phase behavior. Three phases was the largest region found in HLB 6, and then decreased in HLB 8 and HLB 10, while HLB 12 and 14 were not found. In addition, macroemulsions was not found in HLB 6, whereas they appeared in HLB 8 – 14. Lamellar-liquid crystals were found along the S/C line in HLB 10 which also found in Baroli et al. (2000) experiment using 3:1:1.2 w/w/w Tween 80:Span 80:1,2 – octanediol system. Moreover, lamellar-liquid crystals were also found in HLB 12 and 14 systems. Surprisingly, o/w microemulsion was only found in HLB 14.

From Figure 5.5 – 5.9, the areas of microemulsions were calculated as shown in Table 5.1. It was found that percent area of w/o microemulsion isotropic region significantly increased with the increased ratio of Tween 80 and Span 80; an increasing of HLB. Ho et al. (1996), Wu et al. (2001), Huang et al. (2007) and Chaiyana et al.(2010) also found the same phenomenon in their researches using Tween 80/Span 80/Olive oil/water systems, Tween 80/Span 20/ethanol/IPM/water systems, and Brij 92, Triton X-114, Tween 20 and Tween 85/ethanol/ *C. Citratus* oil/water systems respectively. Figure 5.10 showed the similar trend to this research that microemulsion area increased when increasing HLB values.

Table 5.1 Weight fractions of Tween 80/ Span 80 at specified HLB value and corresponding percent area of microemulsions.

HLB	Surfactant weight fraction		% area of w/o microemulsion	% area of o/w microemulsion
	Span 80	Tween 80		
6	0.84	0.16	2.82	ND
8	0.65	0.35	6.28	ND
10	0.47	0.53	46.89	ND
12	0.28	0.72	55.64	ND
14	0.094	0.906	41.19	0.59
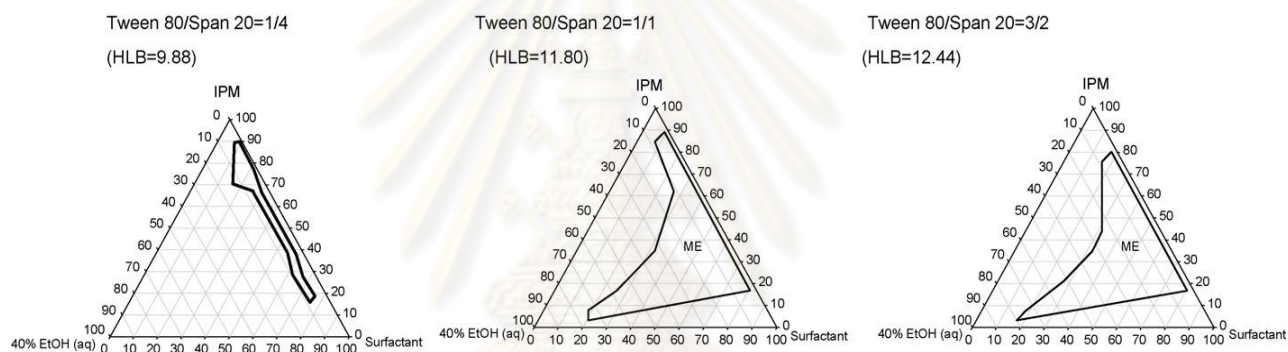
Figure 5.10 Pseudo-ternary phase diagrams of microemulsion (ME) composed of IPM, mixed surfactant (Tween 80/Span 20), cosurfactant (ethanol) and aqueous solution (Huang et al., 2008).

However, increasing HLB value from 12 to 14 resulted in a decrease of w/o microemulsion area and an appearance of o/w microemulsion. Baroli et al. (2000) found that 3:1 w/w ratio of Tween80/Span80 as an optimum ratio for microemulsion formulation for topical delivery of 8-Methoxalen. Considering HLB 12 system which contained the largest area of w/o microemulsion, was found in which weight ratio of Tween 80 to Span 80 was ~ 3:1 weight ratio.

From phase diagrams demonstrated above, HLB 10, 12, and 14 were selected to study the effects of drug loaded due to their large regions of w/o microemulsion. In addition, all microemulsion systems were stable at 37 ºC for at least 1 month that neither flocculation nor phase separation was noticed.

5.1.2.2 Phase diagram with loaded MTX

Loading drug into microemulsion system may influence phase behavior (Attwood and Florence, 1983). Therefore, phase diagrams were re-constructed when MTX was loaded into the system using the same aqueous titration method at 37 ˚C in the water bath for 24 hours in order to reach equilibrium. The experiment was conducted using the same procedure as the previous ones except the change of aqueous phase compositions. The aqueous acetate buffer solution (ABS) pH 5.5 was primarily saturated with MTX. MTX structure is shown in Figure 5.11 and phase diagrams with MTX loading are presented in Figures 5.12 – 5.14. It should be noted that two COOH groups of MTX ionized to negative charges; COO ‾, when it dissolved in solution pH 5.5 since its pKa were 3.8, 4.8 and 5.6 (Lund, 1994). Therefore, ionic strength of solution increased and might affect to microemulsion formulation especially hydrodynamic diameter (http://www.malvern.com/common/ downloads/campaign /MRK 656-01.pdf). It would be said that microemulsion region might change when drug was loaded.
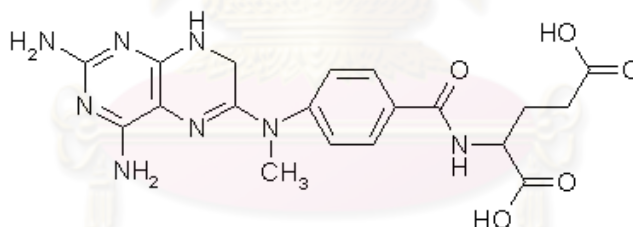
Figure 5.11 Chemical structure of methotrexate (www.steve.gb.com).

From the experiments, it was found that concentration of saturated solution of MTX in ABS pH 5.5 was 1160 μg/ml at 37 ºC. Adding saturated solution of MTX, which used ABS pH 5.5 as a solvent, resulted in the change of phase diagrams from the unloaded one. Furthermore, the microemulsion systems from HLB 10, 12 and 14 were stable at 37 ºC for at least 1 month.
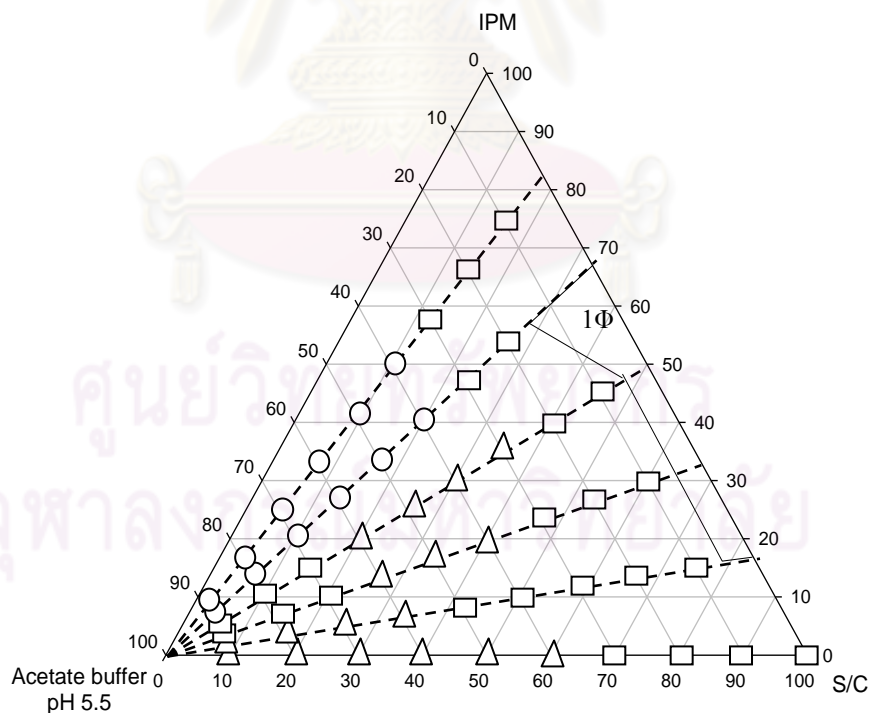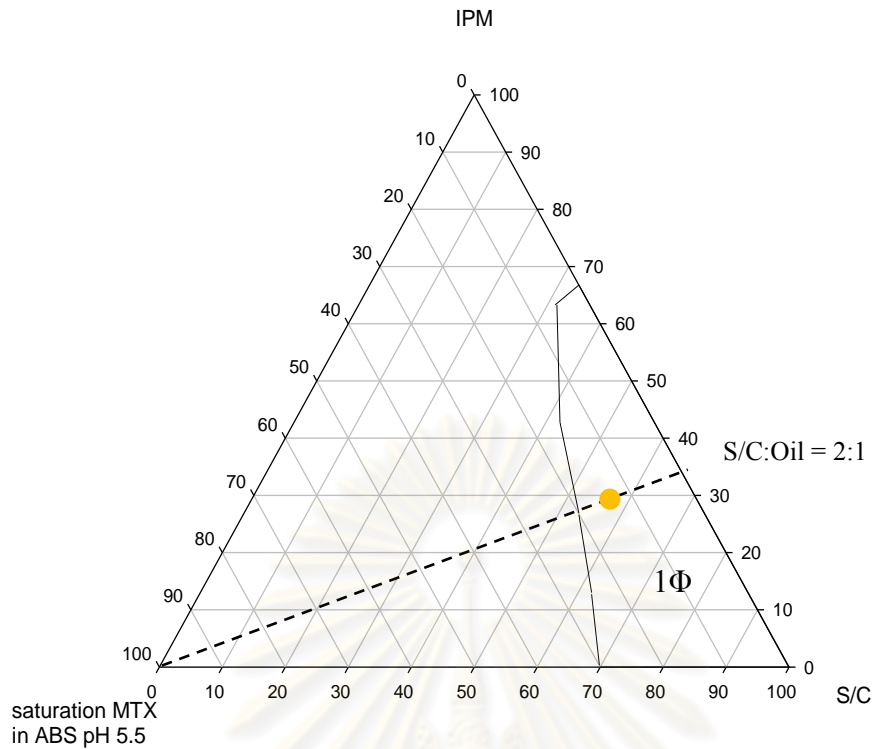
Figure 5.12 Pseudo-ternary phase diagram of Tween 80/Span 80/1,2-octanediol, IPM
and saturation MTX in ABS pH 5.5 system for HLB 10 at 37° C
(● selected compositions for droplet characterization).
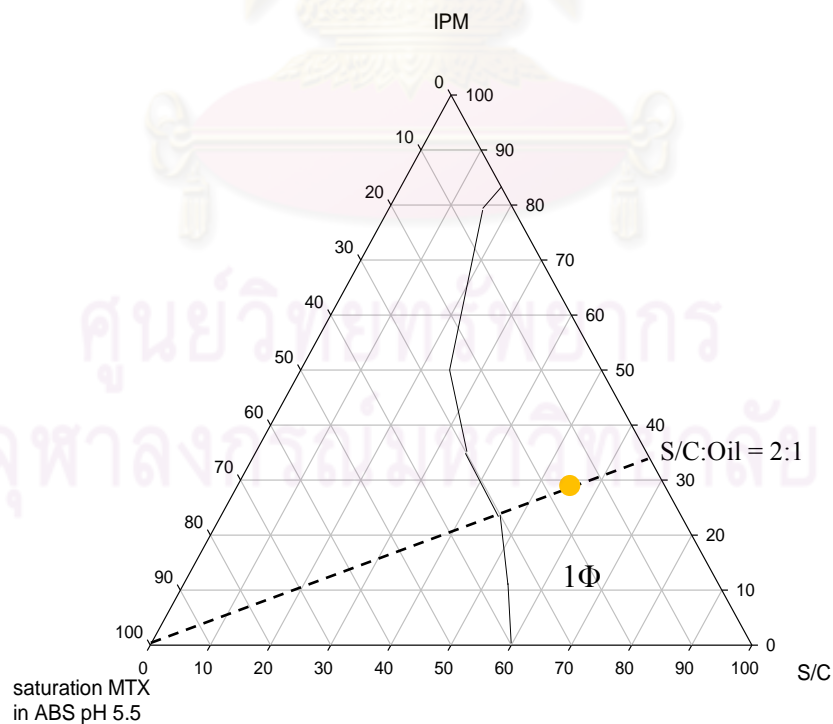
Figure 5.13 Pseudo-ternary phase diagram of Tween 80/Span 80/1,2-octanediol, IPM
and saturation MTX in ABS pH 5.5 system for HLB 12 at 37° C**.**
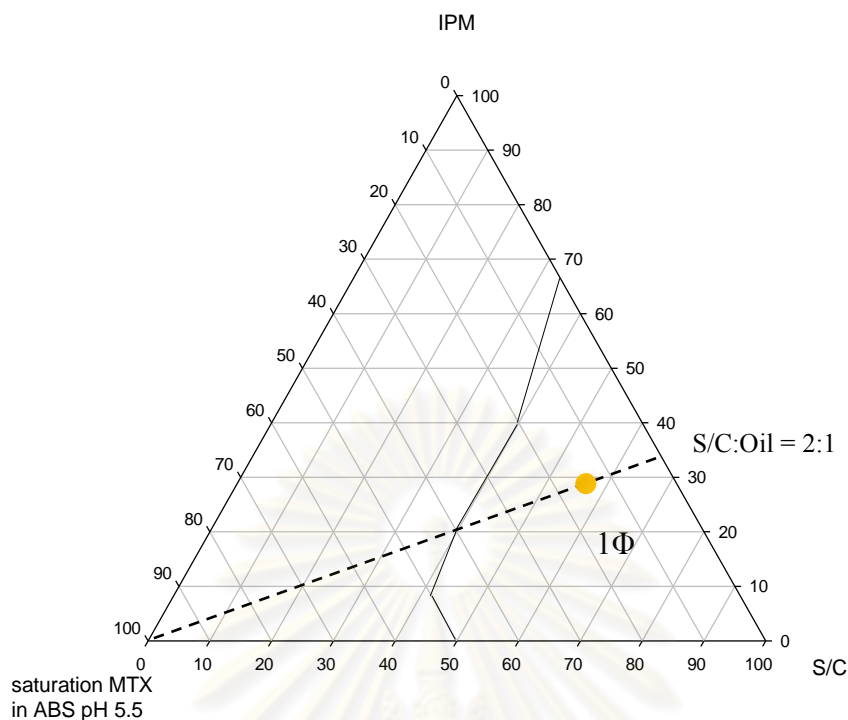
Figure 5.14 Pseudo-ternary phase diagram of Tween 80/Span 80/1,2-octanediol, IPM and saturation MTX in ABS pH 5.5 system for HLB 14 at 37° C.

According to Figures 5.5 – 5.9 and 5.12 – 5.14, adding MTX to pseudo-ternary systems resulted in the change of phase diagram. It was found that the area of w/o microemulsion decreased when MTX was loaded into the system as shown in Table 5.2.

Table 5.2 Comparison of % area of w/o microemulsions of HLB 10, 12 and 14 systems with and without drug loaded.

HLB	% area of w/o microemulsions		% area difference of unloaded/ loaded MTX
	unloaded MTX	loaded MTX	
10	46.89	22.70	24.19
12	55.64	41.48	14.16
14	41.19	32.64	8.55

Attwood and Florence (1983) explained that drug would be expected to influence phase behavior in spite of a large amount of drugs molecules loaded into the system are themselves surface active. From Table 5.2, it was found that micro-emulsion regions decreased when MTX was loaded into the systems for all HLB formulations. Schmalfuβ et al. (1996) also found similar phenomenon to this research and reported that adding diphenhydramine hydrochloride reduced microemulsion area compared to unloaded drug system. In addition, similar trend to unloaded MTX, HLB 12 gave the highest percent area microemulsion when drug was loaded. It was notably observed that percent area difference of unloaded/loaded MTX decreased when increasing HLB value.

Considering Figure 5.14 of HLB 14 system, maximum aqueous phase content was 50%. Calculation from saturated solution of MTX found that 580 μg/ml was the maximum loading in this research which related to other researches as shown in Table 5.3.

Table 5.3 Comparison of amounts of MTX loading with other researches.

Phase compositions			Type of ME	MTX loading (μg/ml)	References
Oil	Surfactants/ cosurfactant	water			
Decanol	Lecithin/benzyl alcohol	Water, PG	w/o	2.5 – 2.8	Trotta (1996)
Ethyl Oleate	Labrasol/Plurol Oleique	154 mM NaCl pH 7.4	o/w	977 – 1358	Alvarez – Figueroa and Blanco – Mendez (2001)
Isopropyl myristate	Tween 80,Span80 /1,2-octanediol	water	w/o	596 – 670	
Soybean oil	Cremophore EL, Span 80/ Isopropyl alcohol	0.2 M NaOH	w/o	2500	Karasulu (2007)
Decanol	Rhamnolipid/ Butanol	0.1 M ABS pH 5.5	w/o	603 – 656	Patcharapa (2008)
		0.2 M PBS pH 7.4	w/o	301 – 329	
Isopropyl myristate	Tween 80,Span80 /1,2-octanediol	0.1 M ABS pH 5.5	w/o	580	This research

From Table 5.3, the amount of MTX loading in w/o microemulsion system from this research was near to Alvarez – Figueroa and Blanco – Mendez (2001) which used same surfactants/cosurfactant system. In addition, it should be noticed that loading saturated drug solution in this research gave the amount of drug in microemulsion closed to loading excess drug method. However, comparison with Karasulu (2007) and Trotta (1996), the amount of MTX loading from this study was extremely different which might be the effect of using different surfactants/ cosurfactants.

5.2 Characterization of microemulsions

Since microemulsions are optically isotropic and thermodynamic stable liquid solution (Danielsson and Lindman, 1981); hence, qualitative tests such as visual observation and isotropic test using polarized light were used to define microemulsion system in this research. Not only dye solubility tests, but also conductivity measurements were used to determine the type of microemulsions. Moreover, particle size distribution, polydispersity index and zeta potential were determined using Zeta sizer 3000 HS$_A$ (Malvern HPPS, UK).

5.2.1 Visual observation and isotropic test

After blending Tween 80/Span 80, 1,2-octanediol, isopropyl myristate (IPM), and aqueous phase under calculated fraction using vortex mixer and equilibrated in water bath at 37 ℃ for 24 hours, the system was optically check for its transparent isotropic solution. The system which was transparent and had low viscosity as low as water would be marked as microemulsion. The system which was gel liked would be classified as lamellar-liquid crystal, and the milky system would be classified as macroemulsion.

In order to ensure the formation of microemulsion solution after optical inspection, isotropic test by using polarized light was also employed to check the systems. Isotropic solution such as microemulsion does not scatter polarized light; hence, birefringence would be not observed. On the other hand, polarized light would be scattered under anisotropic phase such as liquid crystal and emulsion, birefringence would be observed (Fig. 5.15).

(a)	(b)

Figure 5.15 Light scattering under polarized light of liquid crystal (a) HLB 12 which contained 30 % S/C, 15 % oil and 55% aqueous phase. (b) HLB 14 which contained and 36.67 %S/C, 18.33% oil and 45% aqueous phase.

5.2.2 Dye solubility test for types of microemulsion

Types of microemulsion were determined using dye solubility tests. Adding water- (methyl orange) or oil- (Sudan III) soluble dye into the microemulsion systems enable the characterization of w/o or o/w microemulsion types. After adding methyl orange to the system, aqueous continuous of the o/w microemulsions would be homogeneously stained with the orange color. For w/o microemulsion, on the other hand, addition of methyl orange would result in the appearance of the lower orange phase. Addition of Sudan III to the system, the result was opposite to methyl orange. Nevertheless, determination of microemulsion type using Sudan III is better because of the more contrast color change.

(a)	(b)

Figure 5.16 Dye solubility test (a) A w/o microemulsion after adding methyl orange, opaque solution occurred. (b) A w/o microemulsion after adding Sudan III, dye dispersed homogenously.

5.2.3 Conductivity measurement

Electrical conductivity is often used to investigate structural changes oil/water/surfactant systems (Kumar and Mittal, 1999; Bauduin et al., 2005). In other words, conductivity is used to indicate which system was water or oil continuous phase. Continuous aqueous phase; o/w microemulsion, should give fairly high conductivity whereas continuous oil phase; w/o microemulsion, should give fairly low conductivity (Trados, 2005). Without addition of water, nonionic surfactants, cosurfactant and oil system had very small conductivity but not zero (Kantarci et al., 2007). Adding ABS pH 5.5 – an aqueous solution – to the system by titration method, electrical conductivity was found gradually increase, and then dramatically increased when structural change occurred in the phase separation region from water continuous to oil continuous phases (Lv et al., 2005). Chayana et al.(2010), Boonmee et al.(2006) and Baker et al.(1984) reported similar trend of o/w microemulsion conductivity.
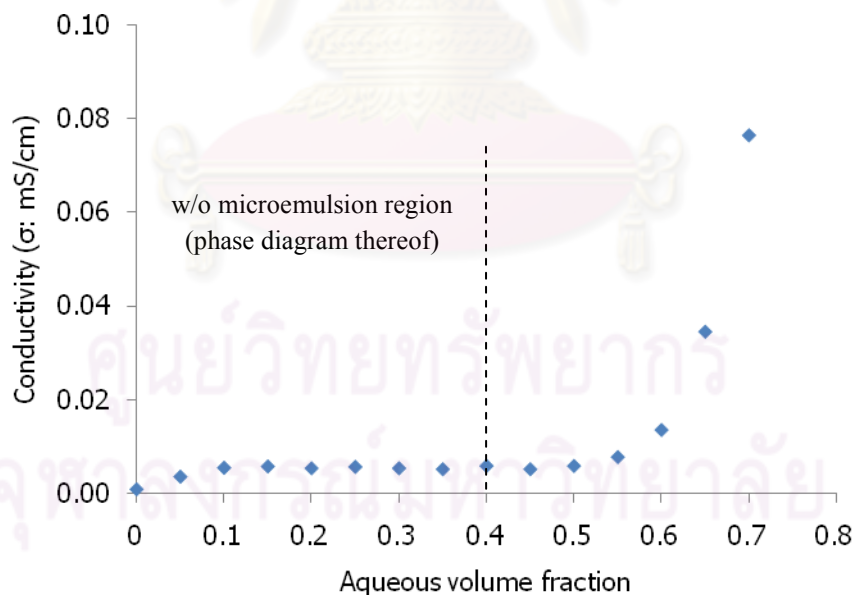
Figures 5.17 Electrical conductivity of 2:1 S/C:O of HLB 10 which titrated with ABS pH 5.5 (σ = 11.73 mS/cm at room temperature)

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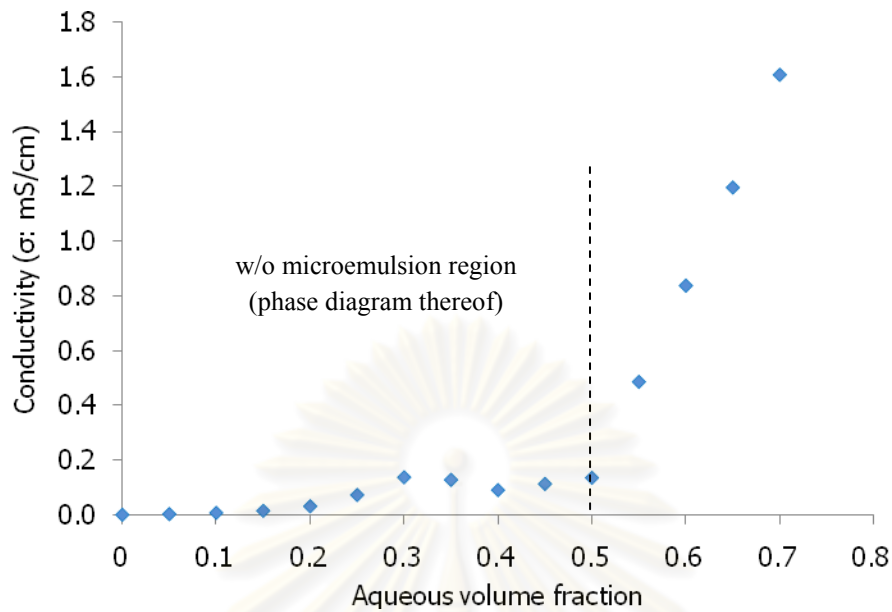
Figures 5.18 Electrical conductivity of 2:1 S/C:O of HLB 12 which titrated with ABS pH 5.5 ($\sigma = 11.70$ mS/cm at room temperature).
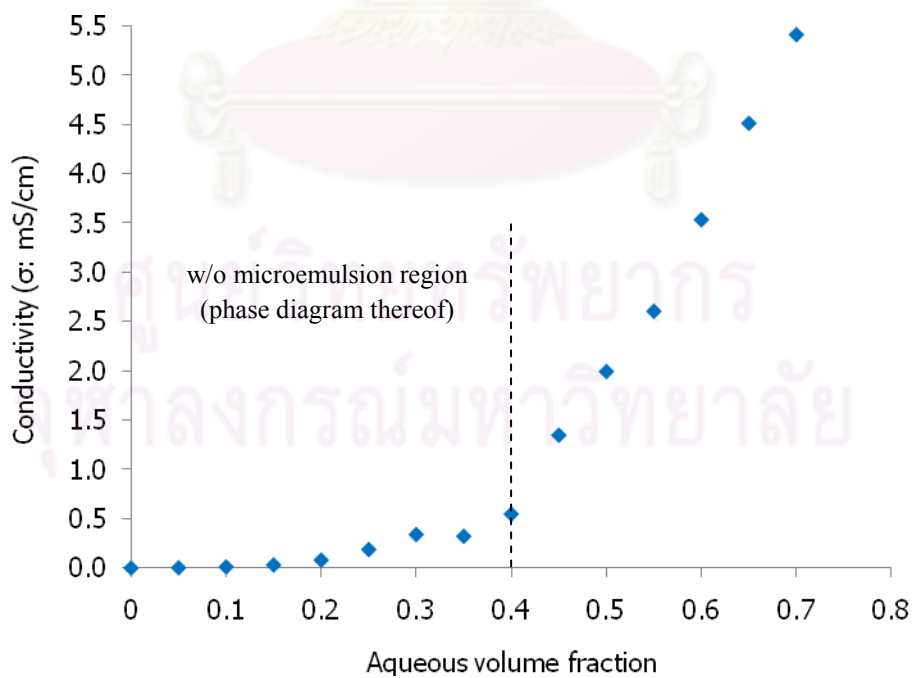
Figures 5.19 Electrical conductivity of 2:1 S/C:O of HLB 14 which titrated with ABS pH 5.5 ($\sigma = 11.79$ mS/cm at room temperature).

Considering Figure 5.19, electrical conductivity of HLB 10 with 2:1 ratio of surfactant/cosurfactant to oil was 0.8 µS/cm at room temperature. After titration ABS pH 5.5 into the system at room temperature, the conductivity increased slowly from 0 to 10% aqueous and then remained constant about 5.3 µS/cm from 10% to 50% aqueous. This could be said that 0 – 50% aqueous or may be until 55% could be oil continuous phase because of its low conductivity. This result related to other characterization methods that 0 – 40% aqueous of 2:1 S/C:O of HLB 10 system defined as w/o microemulsion (Fig. 5.7).

From figure 5.20 and 5.21, the HLB 12 and HLB 14 conductivity resulted similar to HLB 10 in terms of conductivity trend. For HLB 12, this result fitted to other characterization methods that 0 – 50% aqueous was oil continuous called w/o microemulsion. As same as HLB 14, the conductivity result fitted to visual check, polarized check and dye test check that 0 – 40% was w/o microemulsion which had oil continuous phase.

From these three conductivity graphs, it could be said that visual observation, isotropic test and dye solubility test were good methods to characterize microemulsion system and type of microemulsion and the phase diagrams displayed quite accurately the areas of w/o microemulsions.

5.2.3 Drop characteristics

The particle diameters of microemulsions, hydrodynamic diameters, poly-dispersity index (PDI) and Zeta potential (δ) of HLB 10, HLB 12 and HLB 14 which contained 15 %aqueous, 28.3 %oil and 56.67 %S/C were measured on both unloaded and drug-loaded microemulsions. Zeta sizer was used for the measurements. The sizing measurements were carried out at a fixed angle of 90º. The reported results are the mean and standard deviation (S.D.) of three measurements on the sample.

Table 5.4 Photon correlation spectroscopy characterization of w/o microemulsion with/without MTX (mean ± SD, *n*=3).

Microemulsions	Peak diameter[a] (nm) (% area)			Hydrodyna-mic diameter (nm)	Polydisper-sity index (PDI)	Zeta potential (mV)
HLB 10	72 (4.07)	582 (95.50)	4986 (0.43)	365 ± 5	0.383 ± 0.060	0.07 ± 0.02
HLB10 + MTX[b]		731 (98.10)	4850 (1.90)	400 ± 19	0.470 ± 0.025	-0.43 ±0.13
HLB 12	20 (5.60)	480 (68.87)	4532 (25.53)	499 ± 7	0.844 ± 0.010	-0.26 ± 0.14
HLB12 + MTX[b]	18 (9.63)	383 (86.72)	5389 (3.65)	366 ± 8	0.571 ± 0.026	-0.38 ± 0.14
HLB 14	11 (21.67)	226 (78.33)		449 ± 19	0.844 ± 0.137	-0.85 ± 0.02
HLB14 + MTX[b]	11 (19.87)	228 (80.13)		478 ± 4	0.661 ± 0.115	-0.16 ± 0.04

[a] The values higher than 100 nm correspond to the apparent diameter of the temporal aggregate domains.
[b] Saturated solution of MTX in ABS pH5.5 (1160 μg/ml) were added to the microemulsion systems to be a ratio of 15% w/w.

From Table 5.4, there were two or three ranges of peak diameter distribution as shown for an example in Figure 5.20. Typically, in this research, it was found that the distribution of droplets diameter were about 226 – 731 nm.

Size Distribution by Intensity
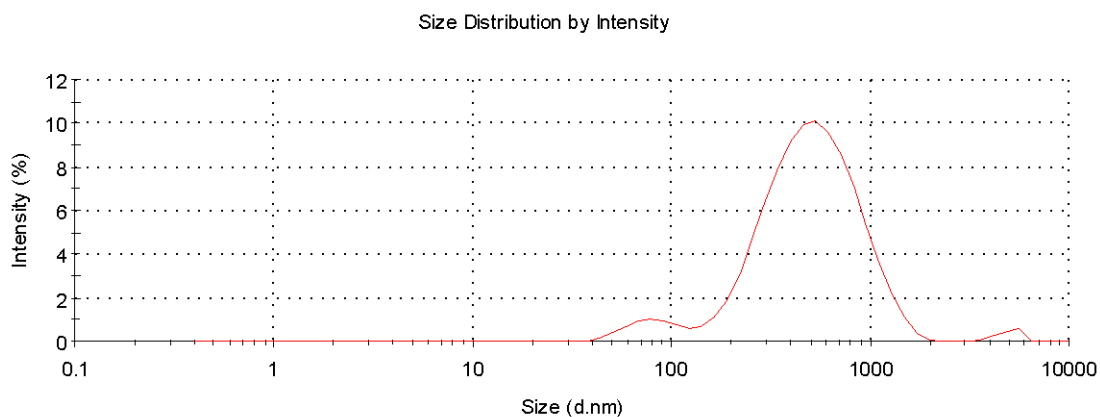
Figure 5.20 An example of size distribution curve (HLB 10, without MTX).

As seen in Table 5.4, it was investigated that a large number of droplets diameter in all HLBs were larger than 100 nm, which was above normal microemulsion range (Trados, 2005). This may be the effects of the temporal aggregation of droplets. Assuming that microemulsion droplets behave like rigid spheres, they will be forced via hydrodynamic interactions to interact more strongly with each other as their number increase (Lissant, 1974). Baroli et al.(2000) discovered that unloaded w/o microemulsion systems had particle average radius between 200 – 700 nm and 100 – 550 nm when drug (8-methoxalen) loading. They proposed that large droplet diameters formed due to the temporal aggregation of microemulsions. In addition, El Maghraby (2008) also found large w/o microemulsion droplet sizes about 170.5 ± 17.8 nm when drug (hydrocortisone) was loaded.

Moreover, adding saturated solution of MTX in ABS pH 5.5 into microemulsion system as an aqueous phase increased the hydrodynamic diameters of microemulsion in HLB 10 and HLB 14. Sintov and Shapiro (2004) found similar result that microemulsions contained lidocaine at a concentration of 2.5% were larger than unloaded microemulsion. However, it was found that the hydrodynamic diameter of microemulsion of HLB 12 decreased when saturated solution of MTX was applied to the system. Kantarci et al.(2007) found that three formulations of w/o microemulsions; contained 1%w/w of diclofenac sodium, decreased in size diameter compared with unloaded-drug formulations. Consequently, loading drug into microemulsion systems affected to microemulsion diameters which might be larger or smaller than unloaded systems.

Size distribution of microemulsions could be described by the polydispersity index (PDI) and the size distribution graph (shown in Appendix C). From Table 5.1, increasing in HLB value promoted the increase the PDI, especially in MTX loaded system. For HLB 10 system, it was found that loading drug into the system affected to an increased of PDI, increasing in size distribution. Nonetheless, microemulsions size distributions of HLB 12 and HLB 14 decreased when added drug into the system.

5.3 *In vitro* drug release

In vitro drug release study was used as a tool for determining the effect of HLB on the release of MTX from microemulsions. Therefore, all the systems investigated contained the same amount and concentration of MTX. Thus, saturated solution of MTX was dosed to all formulations at 15%w/w. The *in vitro* drug release study was performed by using modified Franz diffusion cells.

At the starting of the release experiment, the amount of MTX in the donor compartment of each formulation was determined to correct the release values since weight of microemulsions were not exactly the same when loaded to the donor chambers. The data is shown in Table 5.2 for all formulations.

Table 5.5 Actual amounts of MTX in applied doses (Q_0, µg) in all system (mean ± SD, *n*=6).

Formulations	HLB 10	HLB 12	HLB 14
Oil (%)	28.3	28.3	28.3
Aqueous (%)	15	15	15
Tween 80 (%)	23.2	31.4	39.5
Span 80 (%)	20.4	12.2	4.1
1,2-Octanediol (%)	13.1	13.1	13.1
Q_0 (µg)	**173.5 ± 5.4**	**177.0 ± 2.6**	**175.0 ± 3.5**

5.3.1 Release studies

Figure 5.21 depicted the released profile of MTX in micro-emulsions with time. HLB 10 microemulsion system was found to have least amount of MTX release within 24 h time; only 4.67 % of MTX was released cumulatively. Moreover, the low drug release was also found in HLB 12 system, about 6.17 % of MTX release was investigated within 24 h. On the other hand, HLB 14 system provided about 59.66 % of MTX release which much higher than HLB 12 and HLB 10 respectively. We suspected that this high release rate detected was likely due to the leak of microemulsions with HLB 14 to the receiving phase since its average was determined at 228 nm while the membrane pore size was 450 nm.
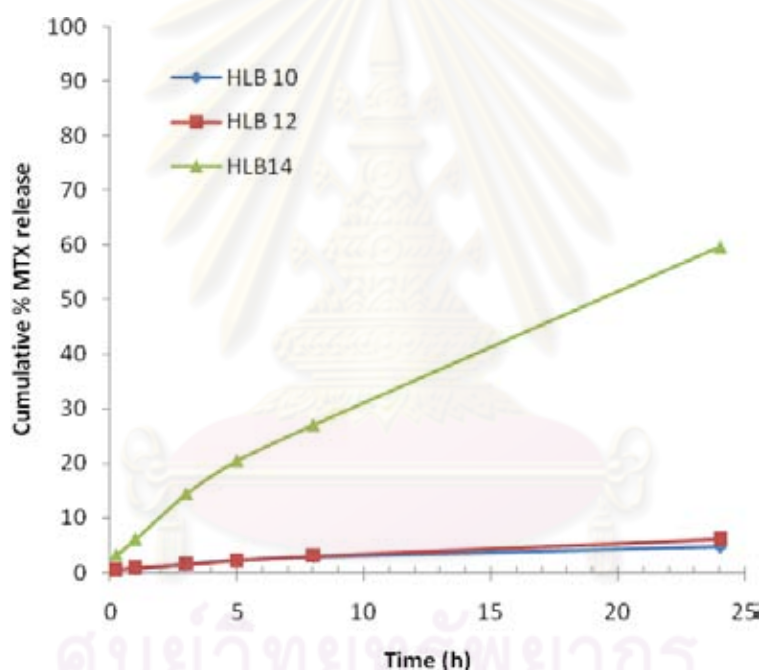
Figure 5.21 Cumulative amount released of MTX per initial amount of drug loading (%) as a function of time of HLB 10, 12 and 14 systems.

5.3.2 The kinetics of release

Drug release from microemulsion can be either diffusion controlled or dissolution controlled or both. Since the types of controlled are not know, various kinetic models were used to describe the release kinetics. Higuchi (1963) described the release of drugs from insoluble matrix as a square root of time dependent process based on Fickian diffusion (Eq. 5.2). Baker and Lonsdale (1974) developed the model

(Eq. 5.3) from Higuchi model and described the drug release from microcapsules or microspheres. And Korsmeyer – Peppas (Eq. 5.4) model was used to describe drug release from polymeric system with unknown mechanism (Korsmeyer et al., 1983).

$$\text{Higuchi model: } F = k\sqrt{t} \qquad\qquad (5.2)$$

$$\text{Baker – Lonsdale model: } \frac{3}{2}\left[1 - (1 - F)^{2/3}\right] - F = kt \qquad\qquad (5.3)$$

$$\text{Korsmeyer – Peppas model: } F = kt^{n} \qquad\qquad (5.4)$$

Where,

F is the fraction of drug release at time t, equal to Q_t/Q_0.

Q_t is initial amount of drug in microemulsion.

Q_0 is amount of drug release at time t.

k is the rate constant which varied on the model.

It should be noted that the zero order kinetic was ignored to fit the data since the released profile was non-linear. In addition, the surface area and porosity of the substrate were assumed to be constant during the release experiment. Moreover, cumulative fraction was sometimes used to fit the model instead of fraction at time t (Shoaib et al., 2006; and Singhvi and Singh, 2011). Thus, cumulative fraction was used in this research. According to Table 5.6, correlation coefficients, R_c, for drug release when applying each of the models to the data were listed. And then the appropriate model that fitted drug released would be determined by selecting the highest value of Rc, even though the Rc values obtained were quite similar (Mallick et al., 2002 ; Shoaib et al., 2006; Nagda et al., 2008; Radin et al., 2009; and Iliger and Demappa, 2011) . The highest R_c from any models of the systems, would be tested for its minimum sum of squares of residuals or Least Square (LS) using *Solver* in Microsoft Excel® and rate constant (k) would be also investigated which is shown in Table 5.7.

Table 5.6 Correlation coefficient R_c for MTX release from microemulsion expressed by each of the Eqs. 5.2 –5.4.

Systems	Higuchi	Baker – Lonsdale	Korsmeyer – Peppas
HLB 10	0.9947	**0.9985***	0.9865
HLB 12	0.9934	**0.9955***	0.9946

* The highest Rc for each systems

Table 5.7 Least Square (LS) of the best fitted model for drug released and estimation of rate constant (k) for each system using *Solver*.

Systems	Baker – Lonsdale	
	LS	k (h^{-1})
HLB 10	2.7×10^{-7}	1.56×10^{-5}
HLB 12	5.7×10^{-6}	2.60×10^{-5}

According to Table 5.6, fitting release data to the models was found that Baker – Lonsdale model was the most fitted for MTX released from HLB 10 (R_c = 0.9985) and HLB 12 (R_c = 0.9955) systems. As followed Higuchi model, MTX release of HLB 10 and HLB 12 would be diffusion controlled release. Table 5.7 revealed that LS were much smaller than rate constant (k); $2.7 \times 10^{-7}\,h^{-1}$ and $5.7 \times 10^{-6}\,h^{-1}$ for HLB 10 and HLB 12 systems respectively. It could be deduced that this model was suitable to use describing MTX release mechanism because of its small LS and good correlations on fitting data.

However, it should be noted that fitting model from HLB 14 MTX release was neglected because we suspected that there was a leak of microemulsion to the receiving phase.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

In this chapter, section 6.1 provides the conclusions obtained from the experimental results of characterization and phase construction of HLB Tween80/ Span 80 values of 6, 8, 10, 12 and 14. In addition, *in vitro* drug release is summarized in this section. Consequently, recommendations for further study are given in section 6.2.

6.1 Conclusions

1. HLB significant effects on phase behavior, maximum water content; 50 %w/w aqueous, of w/o microemulsion was found in HLB 12 system. Moreover, o/w microemulsion region was only found in HLB 14 system.

2. Loading MTX into these systems reduced microemulsion regions. The area of microemulsion decreased 8.55% for HLB 14, 14.16% for HLB 12, and 24.19% for HLB 10 when compared to unloaded MTX system.

3. HLB 12 provided maximum MTX loading that was 580 µg/ml.

4. HLB 14 system had the smallest droplet size diameter, came after with HLB 12 and HLB 10 respectively both unloaded and loaded MTX into the system.

5. Baker – Lonsdale model was the best fitted model for both HLB 10 and 12 systems for MTX controlled release from microemulsion.

6.2 Recommendations

1. It should be varied the percentage of MTX in microemulsion system at fix HLB to find the optimum percent of MTX for drug release.

2. Microemulsions prepared in this research aimed to study as drug vehicle for MTX delivery, *in vitro* skin permeation should be conducted in future and also *in vivo*.

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APPENDICES

APPENDIX A

CALCULATION FOR HLB FORMULATIONS

Calculation Tween 80/ Span 80ratios for required HLB

Preparation of mixed surfactants between Tween 80 (HLB 15.0) and Span 80 (HLB 4.3) can be calculated as equation below;

$$HLB = \Sigma \, (HLB_i \, x \, f_i)$$

Where HLB_i is HLB value of surfactant i

                 f_i is mass or weight fraction of surfactant i

Given: f_i is weight fraction of Span 80

                 $1 - f_i$ is weight fraction of Tween 80

For example HLB 6 : $6 = 4.3f_i + 15(1 - f_i)$

                 $f_i = 0.8411$

Hence, weight fraction of Span is 0.84 while Tween 80 is 0.16.

Table A.1 Tween 80 and Span 80 compositions for required HLB with total weight 12 grams.

HLB	Span 80		Tween 80	
	weight fraction	weight (g)	weight fraction	weight (g)
6	0.8411	10.093	0.1589	1.907
8	0.6542	7.850	0.3458	4.150
10	0.4673	5.607	0.5327	6.393
12	0.2804	3.364	0.7196	8.636
14	0.0935	1.121	0.9065	10.879

Calculation of 1,2 – octanediol add to mixed surfactant

Due to 4:1.2 w/w of surfactants to cosurfactant ratio is fixed, weight of 1,2-octaindiol can be calculated as followed.

Mixed surfactant   =   12 grams

1,2 – octanediol   = $12 \text{ g surfactants} \times \dfrac{1.2 \text{ g cosurfactant}}{4 \text{ g surfactants}}$

                            =   3.6 grams

Calculation of isopropyl myristate (IPM); an oil phase

IPM is added to mixed surfactant and cosurfactant at the S/C: Oil ratios of 1:5, 1:2, 1:1, 2:1 and 5:1 to act as a titration line in phase diagram. First, amount of S/C is weighed and calculated IPM is added at the fixed ratios.

Table A.2 Amount of IPM added to surfactants/cosurfactant.

Ratios of S/C:Oil	Surfactants/cosurfactant (g)	IPM (g)
1 : 5	1.00	5.00
1 : 2	2.00	4.00
1 : 1	3.00	3.00
2 : 1	4.00	2.00
5 : 1	5.00	1.00

APPENDIX B

CALIBRATION CURVE

This appendix showed the calibration curve of MTX concentration in PBS pH 7.4 and ABS pH 5.5 using HPLC Variance, USA , ascentis column- C18, flow rate 1 ml/min, methanol: buffer solution (10:90) as mobile phase, UV-detector at 303 nm, and 20 µl of injection volume.
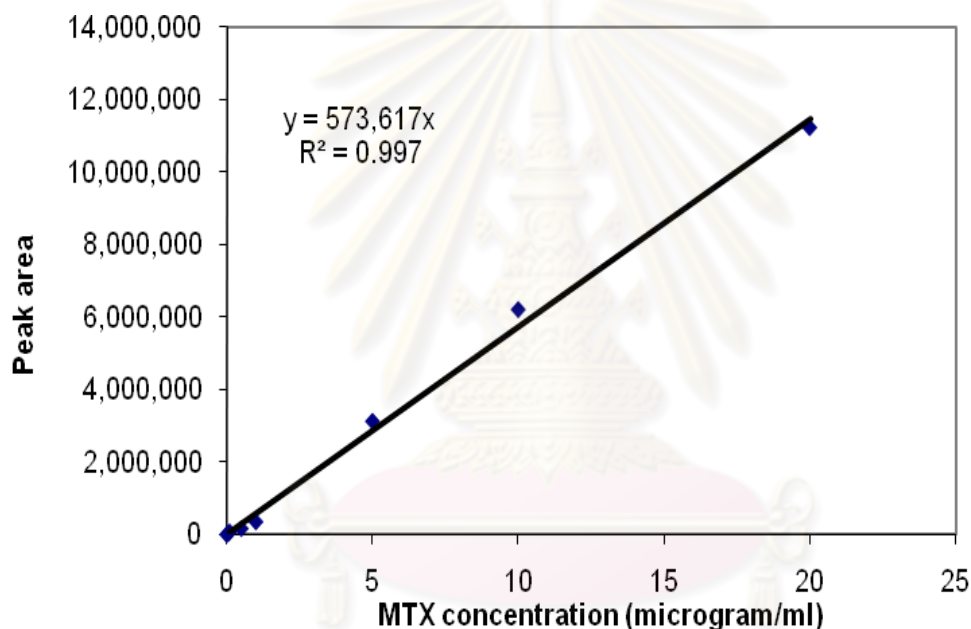

The equation shown on the graph: $y = 573{,}617x$, $R^2 = 0.997$

Figure B.1 Calibration curve of MTX in PBS pH 7.4 at MTX concentration of 0.05, 0.10, 0.50, 1.00, 5.00, 10.0 and 20.0 microgram/ml.
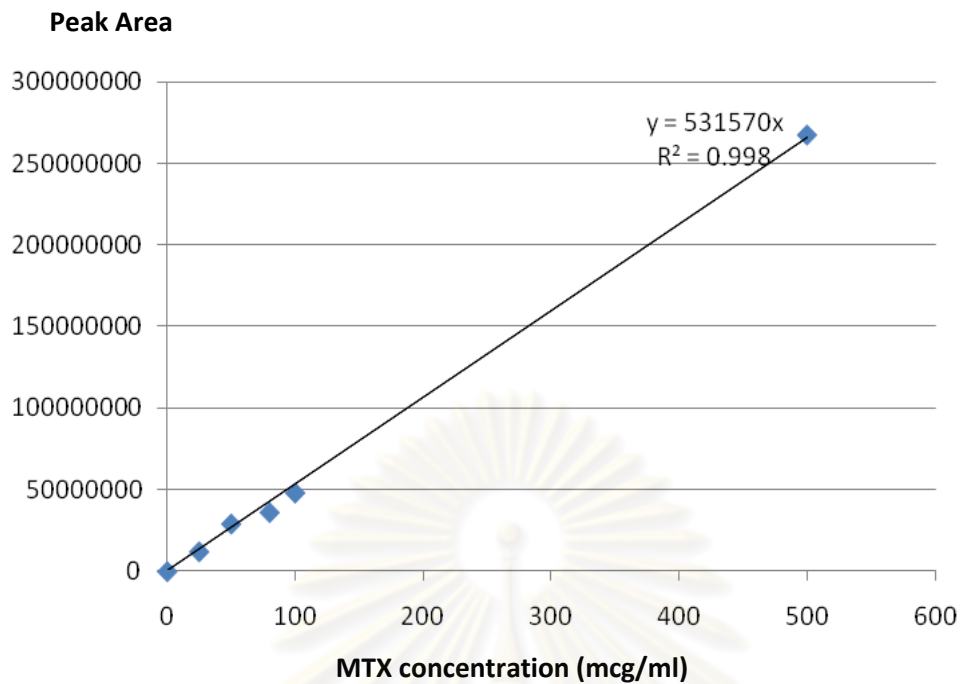
Peak Area

Figure B.2 Calibration curve of MTX in ABS pH 5.5 at MTX concentration of 25, 50, 80, 100, and 500 microgram/ml.

APPENDIX C

PARTICLE SIZE DISTRIBUTION CURVE

In this section showed the particle size distribution of microemulsions of HLB 10, HLB 12 and HLB 14 system with and without loading MTX using photon correlation scattering (PCS) technique (Malvern, UK).
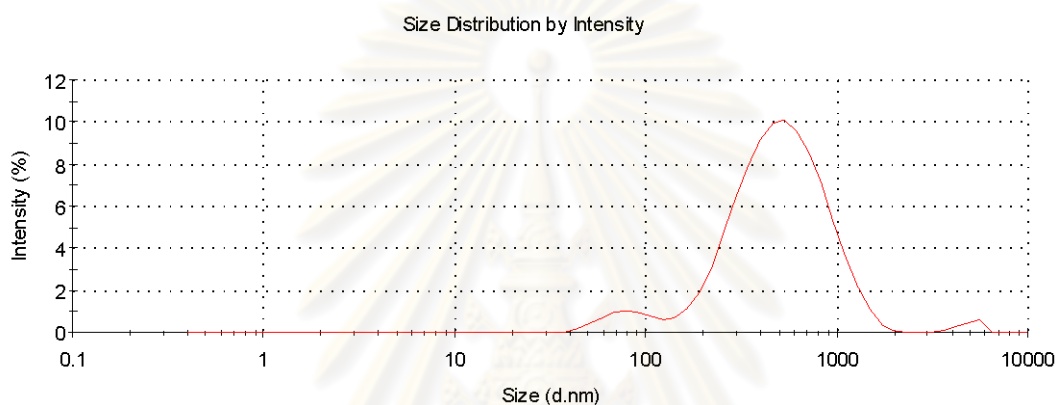

Figure C.1 Particle size distribution curve of HLB 10 micremulsion contained 15% aqueous phase of ABS pH 5.5.
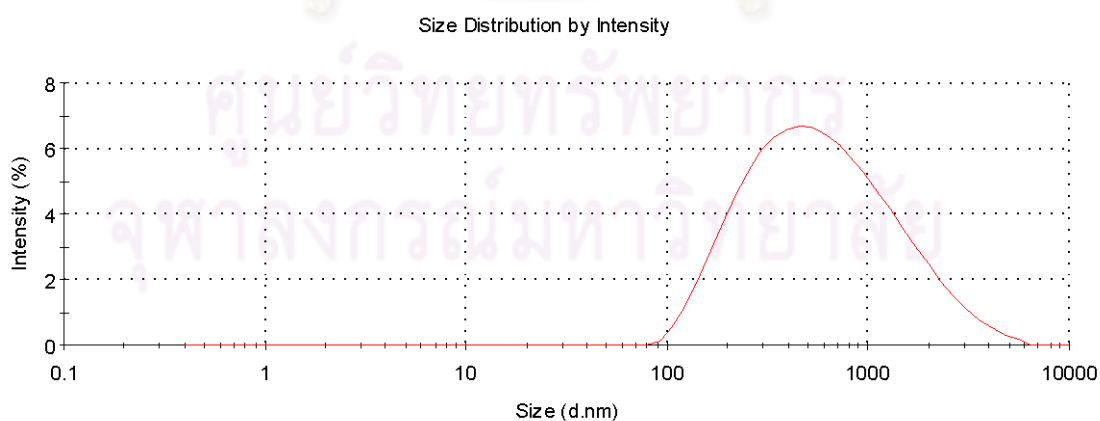

Figure C.2 Particle size distribution curve of HLB 10 micremulsion contained 15% aqueous phase of saturated solution of MTX in ABS pH 5.5.
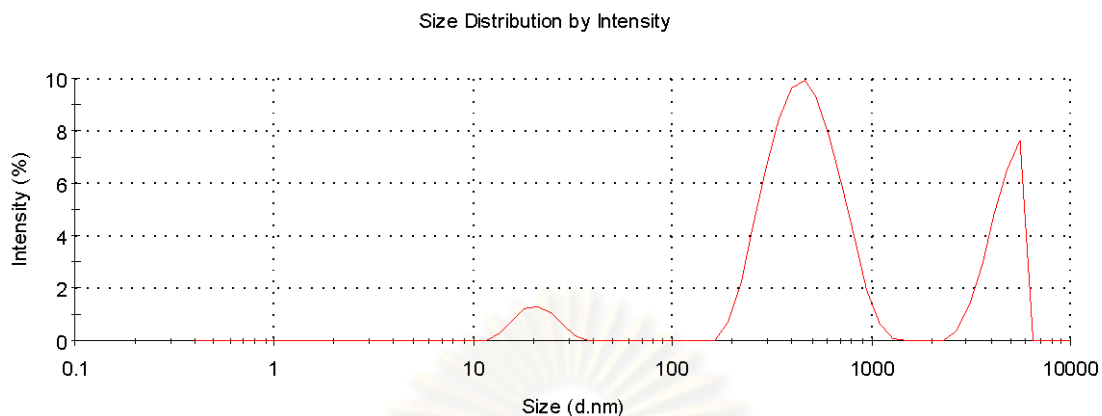
Size Distribution by Intensity

Figure C.3   Particle size distribution curve of HLB 12 micremulsion contained 15%
aqueous phase of ABS pH 5.5

Size Distribution by Intensity

Figure C.4   Particle size distribution curve of HLB 12 micremulsion contained 15%
aqueous phase of saturated solution of MTX in ABS pH 5.5.

Size Distribution by Intensity

Figure C.5 Particle size distribution curve of HLB 14 micremulsion contained 15% aqueous phase of ABS pH 5.5
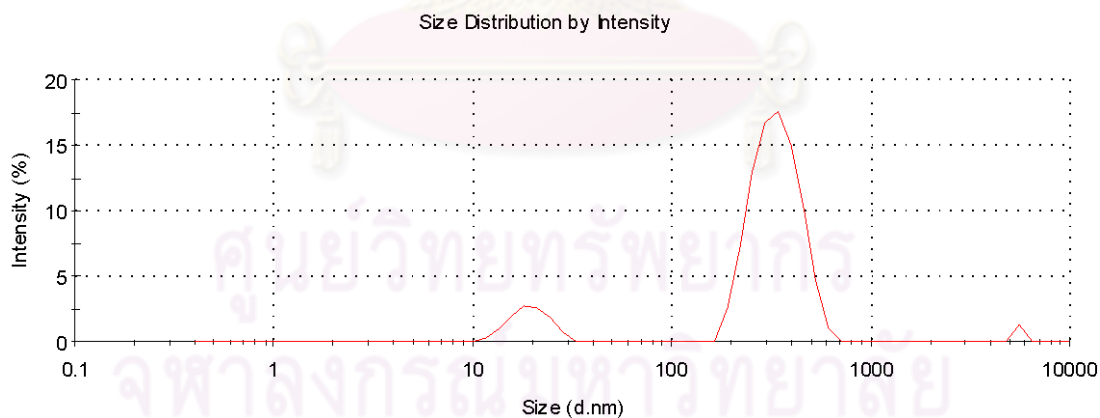
Size Distribution by Intensity

Figure C.6 Particle size distribution curve of HLB 12 micremulsion contained 15% aqueous phase of saturated solution of MTX in ABS pH 5.5.
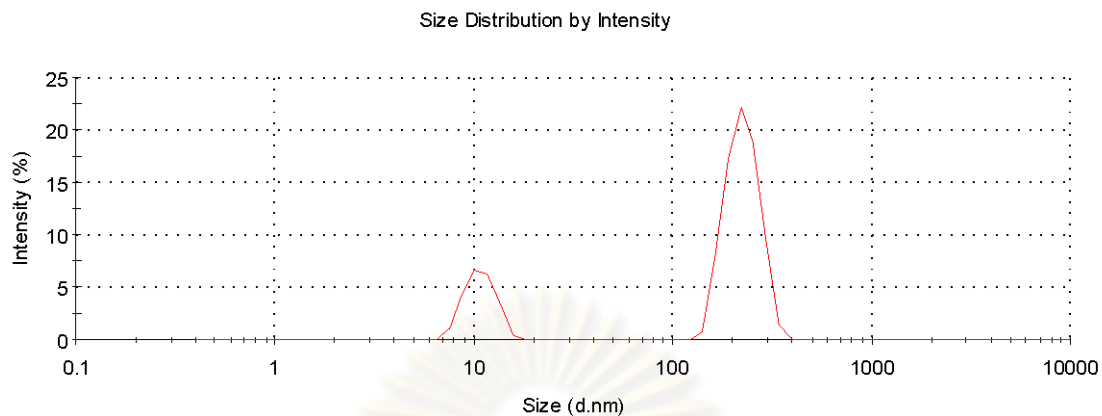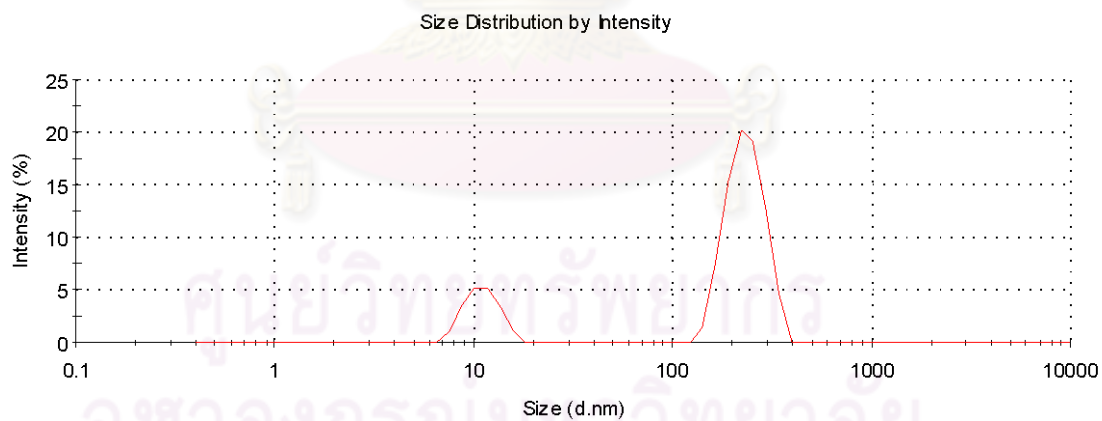
APPENDIX D

IN VITRO DRUG RELEASE EXPERIMENT

This section depicted the *in vitro* drug release experiment. The experiment was conducted using Franz diffusion cell and the samples were collected at 0.25, 1, 3, 5, 8, and 24 hours. Approximately 0.2 ml of sampling aliquot was withdrawn from 14 ml of receptor fluid. The cumulative amount of release was calculated as shown below,

$$Q_n = V_R C_n + \sum_{i=0}^{n-1} V_s C_i \qquad (D.1)$$

Fraction of cumulative release (F) = Q_n/Q_0

Where,

C_n is a concentration of MTX determined at time of sample n^{th}.

C_i is a concentration of MTX determined of i sample.

Q_n is the cumulative amount of MTX released at time of sample n^{th}.

Q_0 is the initial amount of MTX in the system.

V_R is the volume of the receptor solution (14 ml).

V_s is the volume of the sample (0.2 ml)

Table D.1 MTX concentration (C_n) and cumulative amount of MTX released (mean ± S.D., *n* = 6) of HLB 10.

Time (h)	C_n (µg/ml)	Q_n (µg)
0.25	ND	ND
1	0.076 (±0.010)	1.072
3	0.190 (±0.022)	2.669
5	0.273 (±0.031)	3.875
8	0.341 (±0.044)	4.882
24	0.566 (±0.045)	8.100

Table D.2 MTX concentration (C_n) and cumulative amount of MTX released (mean ± S.D., n = 6) of HLB 12.

Time (h)	C_n (µg/ml)	Q_n (µg)
0.25	0.067 (±0.017)	0.937
1	0.131(±0.027)	1.847
3	0.198 (±0.017)	2.811
5	0.288 (±0.019)	4.111
8	0.383 (±0.028)	5.499
24	0.765 (±0.056)	10.92

Table D.3 MTX concentration (C_n) and cumulative amount of MTX released (mean ± S.D., n = 6) of HLB 14.

Time (h)	C_n (µg/ml)	Q_n (µg)
0.25	0.412 (±0.052)	5.768
1	0.765 (±0.044)	10.79
3	1.793 (±0.108)	25.34
5	2.524 (±0.205)	35.93
8	3.307 (±0.217)	47.39
24	7.332 (±0.328)	104.41

VITA

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LIST OF PUBLICATION

Proceeding

Kantharakorn Macharoen, Sarada Kanokpanont and Seeroong Prichanont "Effect of HLB on the Formation of Tween 80/ Span 80 Microemulsion Systems" Proceeding of 19th TiChE conference, Kanchanaburi Thailand, October., 2009.