CHAPTER II
LITERATURE REVIEW

2.1 Nanodelivery

The propose of drug delivery system is to control drug release and achieve drug target [31, 32]. The nanodelivery system has ability to cover these reasons. Due to its extremely small size, it can deliver the drug to reach the target easily and give high efficacy, on the contrary the reduction of side effect. The controlled release will ensure drug level in the target organ [33-35]. In addition to, nano-sized delivery systems offer other numerous advantages: small particle size, narrow size distribution, minimizes the irritant reactions at the injection site, protective insulation of drug molecules to enhance stability, surface features for target-specific localization, specific types of materials that respond to an applied stimulus, the ability to deliver multiple therapeutic payloads in a single formulation and temporal control over their release [36, 37].

Microemulsion could enhance the permeability of drugs such as lidocaine microemulsion expressed 17-fold greater permeability than solution formulation. Permeability of estradiol microemulsion and diltiazem HCl microemulsion can be increased 58-fold and 520-fold respectively compare with the solution formulations [38].

2.2 Microemulsion

2.2.1 Definitions

Microemulsion was first introduced by Hoar and Schulman more than 60 years ago. It is a thermodynamically stable dispersion of two immiscible liquids, usually water and oil, that can be merged with surfactants and/or cosurfactants [39]. Microemulsion can be considered as ideal liquid vehicles for drug delivery as they have most of requirements for this including the spontaneous formation, ease of formulation, low viscosity, high solubilization capacity and very small droplet size [40, 41]. The transparent property of microemulsion is due to the size of their inner phase of 10-100 nm diameters [42]. There are several mechanisms to explain the advantages of microemulsion for transdermal delivery of drug. First, a large amount of drug can be incorporated in the formulation due to the high solubilizing capacity. Second, the permeation rate of the drug from microemulsion may be increased by modification of the affinity of drug to the internal phase through flavor partitioning into stratum corneum, using different internal phase, changing its portion in microemulsion or adjusting its property. Third, the surfactant and cosurfactant in the microemulsions may reduce the diffusional barrier of the stratum corneum by acting as permeation enhancer [43, 44].

There are three main theories of microemulsion formation and stabilization reported by Schulman, Stoeckenius and Prince. In the mixed film theory, the interfacial film is considered as a duplex film having different properties on the water and oil side of the interface. The solubilization theory considers microemulsions as swollen micellar systems which is similar to micellar solubilization which internal phase was around with surfactant and continuous phase. The small size of droplets affected the microemulsions looks clear or tranlucent. The thermodynamic theory was

illustrated that a very low or slightly negative value of free energy (ΔG) affected to microemulsion formation [45].

There are two simple types of microemulsions. The oil-in-water (o/w) microemulsion represent oil droplet as internal phase when the volume fraction of oil is low. Conversely, water-in-oil (w/o) microemulsion express water droplet as internal phase when the volume fraction of water is low. If the volume of oil and water are equal, the bicontinuous microemulsion will be formed [46].

2.2.2 Components of microemulsion

2.2.2.1 Oil phase

The selection of oil is based on the property of the drug as well as the route of administration. The oil should have soluble capability for the drug. The oil influences the curvature and has the capability to swell the tail group of surfactant. The different of saturated and unsaturated fatty acids gave different penetration enhancing activity. The fatty acids increase the permeability of by disrupting densely packed lipids and filled up in extracellular spaces of stratum corneum. Amongst unsaturated fatty acids, oleic acid is an effective skin penetration enhancer. Also penetrating effect of fatty acids was depended on individual drug. The fatty acid which most used in the preparations were esters, isopropyl palmitate [47, 48].

Recent trend is towards use of semisynthetic oils that are more stable than their natural counterparts. The insoluble drugs need to have solubility in dispersed oil phase to form efficient o/w microemulsion system. Even with increase in oil content in o/w microemulsions leads to increase in droplet size. Substances that are commonly used as oil phase in transdermal formulations such as isopropyl palmitate,

isostearylic isostearate, ethyl oleate and alcohols such as octanol, decanol and benzyl alcohol [49].

2.2.2.2 Surfactant

Surfactants are the molecules which when present in low concentration will adsorb to the surface of interfaces of a system and alter the interfacial energies of the system. The interfacial energy is the work required to create unit area of an interface. The actual purpose of surfactant is to lower the interfacial tension to negligible value facilitating the process of dispersion during preparation of microemulsion. Surfactant screening can be done with of hydrophilic lipophilic balance (HLB) value. The HLB provides a numerical value that indicates whether o/w or w/o emulsion will form. It relates molecular structure to interfacial packing and film curvature [50]. The HLB concept was introduced by GRIFFIN. The accepted fact is that generally low HLB surfactants are favorable for w/o microemulsion and high HLB surfactants are suited best for o/w microemulsions. Surfactants with HLB greater than 20 might require co-surfactants in order to reduce the HLB value within the range entailed by microemulsion to form.

Skin permeation enhancement is magnificent function of surfactants and the magnitude of permeation enhancement is largely dependent on physicochemical properties and nature of vehicle [51]. Nonionic surfactants are good replacement for naturally occurring surfactants. Tweens have been investigated for their minimal toxicity. Surfactants such as sorbitan fatty acid esters, polysorbates, pegylated fatty alcohols and poloxamers are recurrently used. Microemusions are highly dynamic structures, it is plausible that monomer surfactants can diffuse to the

skin surface and act as enhancers facilitating diffusion through barrier phase or by increasing the solubility of drug in the skin [52, 53].

The surfactants that used to form the microemulsion system are devided in 4 groups: (i) non-ionic, (ii) zwitterionic, (iii) cationic or (iv) anionic surfactants. Combination of ionic and non-ionic surfactants was reported to increase of microemulsion region [54]. Cosurfactant was reported to reduce the HLBs of ionic surfactants having HLBs greater than 20 for microemulsion formation [55].

Non-ionic surfactants are widely using in topical formulations. They are safe and have low irritation for skin [56]. Tween and Span are the non-ionic surfactant which usually include for microemulsion formulation [57]. It was found that Span 20 increase more fluidity of the stratum corneum and enhanced the flux of lipophilic drug through the skin [58]. Tween 20 enhances lipophilic drug penetration by allowing the polar molecule to partition across the barrier more easily [59].

2.2.2.3 cosurfactant

The cosurfactant is the additional component for microemulsion formation. It is also amphiphilic with an affinity for both the oil and aqueous phases. The cosurfactant partitions to an appreciable extent into the surfactant interfacial monolayer present at the oil-water interface. The medium-chain alcohols will always composed in the microemulsion formula [60, 61]. They have the effect of further reducing the interfacial tension, whilst increasing the fluidity of the interface thereby increasing the entropy of the system [62]. Medium chain length alcohols also increase the mobility of the hydrocarbon tail and also allow greater penetration of the oil into this region. Furthermore, any alcohol present may also influence the solubility properties of the aqueous and oily phases due to its

partitioning between these phases. Furthermore, other non-ionic surfactants [63, 64], alkanoic acids, alkanediols and alkyl amines [65] can be used as cosurfactants of microemulsion.

The effects of cosurfactants on the transdermal delivery of hydrocortisone from eucalyptus oil microemulsion were reported by Aboofazeli et al [66]. Eucalyptus oil which was successfully employed for steroidal drugs was used as the oil. Ethanol, isopropanol and propylene glycol which are relatively tolerable by the skin were employed as cosurfactants. Pseudo-ternary phase diagrams were constructed in the presence and absence of cosurfactants. Microemulsion formulations containing 20% eucalyptus oil, 20% water and 60% of either Tween 80 or 1:1 surfactant/cosurfactant mixture were compared. The results showed the microemulsion zone was expanded by the effect of cosurfactants. The cosurfactant free microemulsion was viscous showing pseudo-plastic flow. The cosurfactant containing preparations were less viscous with Newtonian flow. The drug loading and release rate were increased in the presence of cosurfactants with the release depending on the viscosity. Incorporation of hydrocortisone in microemulsion increased the transdermal flux compared to saturated aqueous solution. The presence of cosurfactants increased the transdermal drug flux compared to the cosurfactant free formulation. Ethanol produced the greatest effect followed by propylene glycol and isopropanol. The presence of cosurfactant and its type can thus affect both the phase behavior and the transdermal delivery potential of microemulsion.

2.2.2.4 Aqueous phase

In general, water is used as an aqueous phase in a microemulsion. The suitable condition of aqueous phase such as pH or ionic strength

should be adjusted due to its considerable impact on phase behavior of microemulsions [50, 67]. For parenteral administration, aqueous phase should be adjusted by sodium chloride, glycerol, dextrose and sorbitol to isosmotic like blood [68].

2.3 Phase behavior

The microemulsion region is usually characterized by constructing ternary-phase diagrams as the triangular graph. Three components are the basic requirement to form a microemulsion: an oil phase, an aqueous phase and a surfactant. If a cosurfactant is used, it may sometimes be represented at a fixed ratio to surfactant as a single component, and treated as a single "pseudo-component". The relative amounts of these three components can be represented in a pseudo-ternary phase diagram (Gibbs' phase diagrams) as shown in Figure 2.1. It can be used to show the influence of changes in the volume fractions of the different phases on the phase behavior of the system [69, 70]. Generally, at low oil concentration (<30%) microemulsions are in the oil-in-water (o/w) form. On the contrary, at low aqueous concentration (<30%), microemulsions are in the water-in-oil (w/o) form [71].

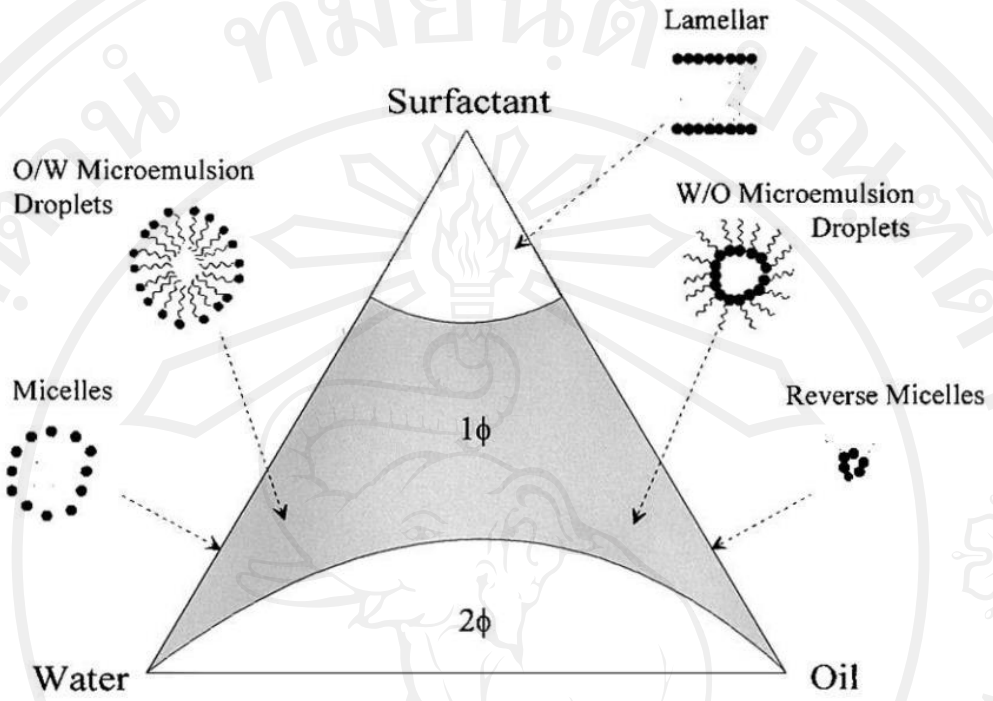

Figure 2.1 A hypothetical pseudo-ternary phase diagram of an oil/surfactant/water system [55].

Microemulsions may also co-exist in equilibrium with excess oil or excess aqueous phase. At low surfactant concentrations, there is a sequence of equilibria between phases, commonly referred to as Winsor phases. Lower phase microemulsion, or Winsor I expressed in equilibrium with excess oil, in the contrary upper phase microemulsion, or Winsor II reached the equilibrium with excess water. With both excess phases, the microemulsion was formed in the middle phase between oil and water (Winsor III). The Winsor phase IV was referred to isotropic micellar solution or single phase system [72].

2.4 Topical microemulsion and recent researches on microemulsion

In topical formulations, microemulsions have been proved to increase the cutaneous absorption of both lipophilic and hydrophilic drugs when compared to conventional vehicles (emulsions, pure oils, aqueous solutions, etc.). The vehicles may also act as penetration enhancers depending on the oil/surfactant constituents, which involve a risk of inducing local irritancy [73].

Microemulsion is used as the excellent drug carriers. The advantages of microemulsions are spontaneous formation, ease of manufacture, and incorporate both oil and aqueous phases into the preparations [74]. However the microemulsions required high concentration of surfactant which may be concerned for oral preparation [75].

The potential application of microemulsions as a dermal drug delivery was studied by Zhu et al [35]. In this study, penciclovir was loaded into the microemulsion of oleic acid, cremorphor EL and ethanol. The study revealed that the cumulative amount of penciclovir of the developed products penetrated through excised mouse skin was about 3.5 times higher than that of commercial cream.

Many researchers have reported on essential oil and fixed oil utilization to produce microemulsions. For example, soybean oil was used as oil phase for both o/w and w/o microemulsions of ethoxylated mono- and diglyceride (EMD), phospholipid and polyoxyethylene oleyl ether (POE) [76]. The coconut oil was combined with octadecyltrimethyl ammonium bromide, iso-pentanol and water for microemulsion forming system [77]. Microemulsion composed of olive oil, water, lecithin and propanol was also reported [78]. Furthermore, these studies found that short-chain alcohol such as ethanol, propanol and iso-pentanol can increase the area of

microemulsion of phase diagram. The essential oil such as eucalyptus oil [79] and clove oil [80] was used as the oil phase of microemulsion. The microemulsion zone of eucalyptus oil was found to be dependent upon the mixing ratios of surfactant and cosurfactant with 1:1 (w/w) AOT and Brij 35:butanol.

Most of microemulsions were aimed as topical or transdermal drug delivery system for reduced the unwanted activity of drug such as gastrointestinal side effect and hepatotoxic. Some microemulsions have shown the effect on drug pharmacodynamics through the skin. Quarcetin was encapsulated in clove oil/ Tween 20/water microemulsion and it was found that topical administration of this microemulsion reduced the side effect of quercetin [80]. Microemulsion formulations of oleic acid, Cremophor RH40/Labrasol (1:2) and water were used as transdermal delivery of theophylline. These studies showed that topical microemulsion increased drug bioavailability to 1.65-fold higher than that of oral solution administration [81]. Celecoxib (selective cyclooxygenase inhibitor NSAIDs) topical microemultion was evaluated and compared with oral preparation. The topical microemulsions composed of isopropyl myristate/medium-chain glyceride/polysorbate 80/water increased the permeation rate of celecoxib up to 5 and 11 times compared with gel and cream, respectively [82]. Transdermal enhancers such as n-methyl pyrrolidone (NMP) and oleyl alcohol were incorporated into the systems of lidocaine w/o and o/w microemulsion (omposed of water, isopropyl myristate (IPM) and Tween 80). These studies demonstrated that the o/w microemulsion provides significantly greater flux ($p < 0.025$) than w/o microemulsion [72]. Microemulsion-base hydrogel for topical delivery of ibuprofen were constructed for increasing skin permeation. The formulation consisted of 3% ibuprofen, 6% ethyl oleate, 30% Tween 80/PG (2:1) and

water, showed a high permeation rate of 38.06 μgcm^{-2} h^{-1} through porcine skins [83].

It was reported that the skin permeation of 0.375% meloxicam (COX-2 NSAIDs) was improved by IPM, Tween 85/ethanol (1:1) and water oil-in-water microemulsions [84]. Rifampicin, an antitubercular drug, microemulsion which composed of oleic acid, phosphate buffer, Tween 80, and ethanol promoted the drug through skin [79].

Even there are a lot of reports on microemulsion, but the systems composed of Thai medicinal oil are still less. The essential oils of *Ocimum basilicum* L. (sweet basil), *Ocimum sanctum* L. (holy basil) and *Ocimum americanum* L. (hoary basil) were formulated as microemulsions against *P. acnes*. This study indicated that, the formulations containing sweet basil oil exhibited higher activity against *P. acnes* than those containing holy basil oil, and the stable o/w micro-emulsion system for basil oil consisted of 55.0% v/v water phase, 10.0% v/v oil phase (2.0 or 3.0% v/v sweet basil or 3.0% v/v holy basil oil plus 7.0% v/v isopropyl myristate), 29.2% v/v polysorbate 80 and 5.8% v/v 1,2-propylene glycol [85].

2.5 Antityrosinase activity

Tyrosinase (EC 1.14.18.1) is an enzyme capable of catalyzing melanin synthesis [86] which a pigment produced by organisms in all plants and animals [87]. In mammalian, melanin is responsible for protecting the skin against ultraviolet (UV)-induced damage and it is involved in dermatological disorders such as hyperpigmentation [88] and Parkinson's disease [89, 90]. Tyrosinase is responsible for enzymatic browning of fruits and plants products [91]. In insects, tyrosinase is involved in sclerotization of cuticle, encapsulation and melanization of foreign organisms and wound healing [92]. On the other hand, melanin reduces the

susceptibility of melanized microbes to host defence [93]. Tyrosinase is a multifunctional, glycosylated, copper containing oxidase and found exclusively in melanocytes [94]. The biosynthetic pathway is initiated with the hydroxylation of L-tyrosine to 3-4-dihydroxyphenylalanine (L-DOPA) and the oxidation of L-DOPA to o-dopaquinone. This oquinone is a highly reactive compound and can polymerize spontaneously to form the pigment melanin; this presents a serious aesthetic problem in human beings [95]. The pathway of melanin biosynthesis is shown in Figure 2.2

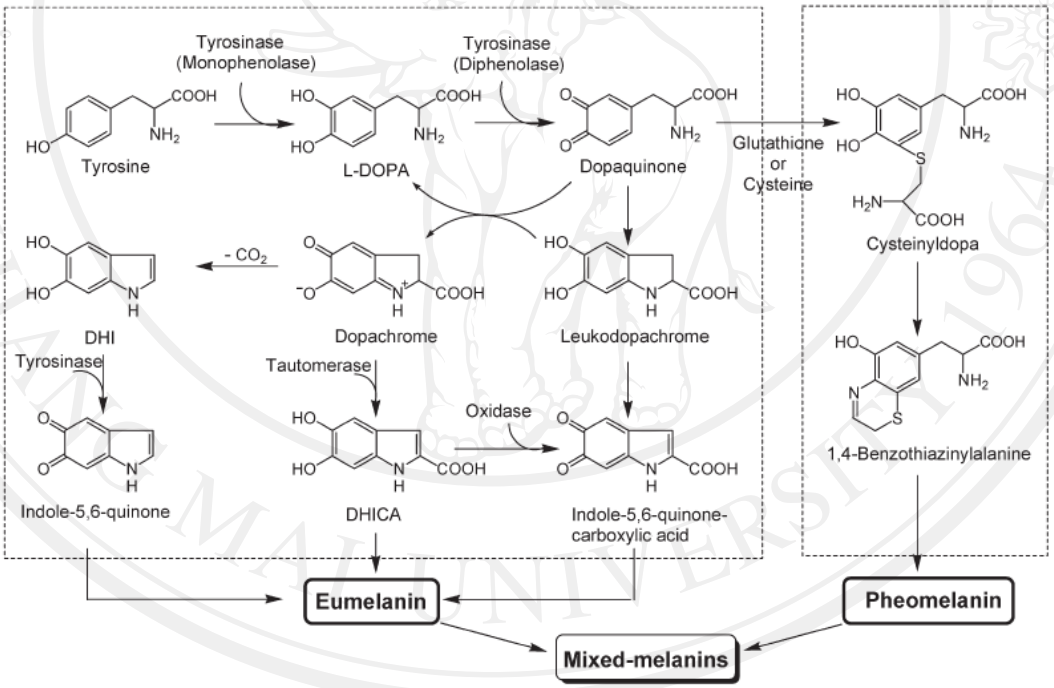

Figure 2.2 The pathway of melanin biosynthesis [96, 97]

Hyperpigmentation is the most common pigmentary disorder on face and skin. It has been observed that a local increase in melanin synthesis or uneven distribution of melanin can cause local hyperpigmentation or spots. Hence, tyrosinase inhibitors result in the best strategy for providing therapeutic or cosmetic agents [98].

Tyrosinase inhibitors or whitening agents such as hydroquinone [99], arbutin [100, 101], kojic acid [102] and azelaic acid [103] are widely used in cosmetic products as active substances), however many of them show side effects [104, 105]. Plants are the source of compounds with different activities including tyrosinase inhibitors [106, 107].

Plants have a good melanin formation inhibitory effect. There are many reports about the whitening facial skin and protection against skin darkening [98, 108]. The plant extracts and oil were studied as antityrosinase for whitening agents [109, 110].

2.6 Antioxidant activity

The main characteristics of antioxidants are their ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. A free radical may be defined as a molecule or molecular fragment containing one or more unpaired electrons (superoxide, hydroxyl, peroxyl) in its outermost atomic or molecular orbital. These when formed can be highly reactive and can start a chain reaction [111]. The sources of free radicals can be endogenous and exogenous in nature. Endogenous sources of free radicals are intracellular and generated from auto-oxidation or inactivation of small molecules. Exogenous sources of free radicals are certain pollutants, tobacco smoke, organic solvents, anesthetics and pesticides. The sites of free radical generation surround all cellular constituents including mitochondria, peroxisomes, endoplasmic reticulum, lysosomes, plasma membrane and sites within the cytosol [112]. Furthermore, certain medications metabolized to free radical intermediate products also cause oxidative

damage within the target tissues. Exposure to radiation results in the formation of free radicals within the target tissues.

Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species. Free radicals or reactive oxygen species (ROS) are produced in vivo from various biochemical reactions and also from the respiratory chain as a result occasional challenges. These free radicals are the main unwanted reaction in lipid peroxidation. Plants congaing bioactive compounds have been reported to occupy strong antioxidant properties. In many inflammatory disorders there is excessive activation of phagocytes, production of 0_2^-, OH radicals as well as non-free radicals species (H_20_2) [113, 114], which can harm severely tissues either by powerful direct oxidizing action or indirect with hydrogen peroxide and –OH radical formed from O_2^- which initiates lipid peroxidation resulting in membrane destruction. Tissue damage then provokes inflammatory response by production of mediators and chemotactic factors [115].

The body has several mechanisms to counteract oxidative stress by producing antioxidants, either naturally generated endogenous, or externally supplied through foods (exogenous). The role of antioxidants is to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention [116]. Antioxidants from our diet play an important role in helping endogenous antioxidants for the neutralization of oxidative stress. Each nutrient is unique in terms of its structure and anti-oxidant function [117].

A lot of research is being undertaken to identify new plant resources which have no or low side effects and potent antioxidant activity.

Hamsar et al reported that the screening of antioxidant, content of total phenolics, total flavonoid and glutathione-S-transferase inhibitory activities was performed on various extracts of *A. catechu* [118]. Tenore et al indicated that *S. lanigera* essential oil possessed a good antioxidant activity and also showed a broad spectrum of antimicrobial activity against referenced strains, especially the Gram-positive bacteria [119]. Gulcin et al revealed that *E.aromatica* (clove oil) could be used for minimising or preventing lipid oxidation in food and pharmaceutical products, retarding the formation of toxic oxidation products, maintaining nutritional quality and prolonging the shelf life of food and pharmaceuticals [120].