CHAPTER 2

LITERATURE REVIEW

2.1 Alzheimer's disease

AD is a chronic and progressive neurodegenerative disorder that begins with cognitive and memory impairments, accompanied with behavioral disturbance such as aggression, depression, hallucination, delusion, anger and agitation and eventually progresses to dementia, physical impairment and death (4). The definite diagnosis of AD is made upon histological verification, established by biopsy or at autopsy, of two main hallmarks: extracellular amyloid plaques and intracellular neurofibrillary tangles, which enclose hyperphosphorylated tau protein (1).

2.1.1 Epidemiology of Alzheimer's disease

AD is one of the most devastating diseases of the century, and the risk of this disease increases drastically along with advancing age (2). Approximately 10% of people over 65 years old have dementia, and about 70% of these individuals have AD. The rates of AD in those aged 85 and older maybe as high as 50% (1). A meta-analysis of studies on the incidence of AD indicated that the rates approximately double for every five-year age group over 60, with rates starting at 0.06% in 60-65 year olds and increasing to 6.69% in those over 95 years old (1).

2.1.2 Etiology of Alzheimer's disease

The actual cause of AD is still unknown but the most important risk factors appear to be age, genetics, and perhaps, vascular risk factors such as hypertension,

hypercholesterolemia, obesity, and diabetes (4). There are several hypothesis of AD development such as cholinergic hypothesis, amyloid cascade hypothesis, and glutamate hypothesis (4, 6). However, the most treatment strategies have been based on the cholinergic hypothesis.

2.1.2.1 Cholinergic hypothesis

The cholinergic hypothesis was the basis for the development of presynaptic, synaptic and postsynaptic treatment approaches designed to maintain and facilitate the activity of the surviving cholinergic system (17). This hypothesis postulates that memory impairments in patients suffering from AD results from a deficit of cholinergic function in the brain (5). Progressive deterioration of the cholinergic innervations of the human brain associated with decreasing levels of the neurotransmitter, ACh, choline acetyltransferase - the rate limiting enzyme for ACh synthesis, and AChE - the Ach hydrolysing enzyme and contributes to the remarkable cognitive and behavioral disturbances in AD (17).

The level of ACh, a neurotransmitter associated with memories, learning and behavior was lower in cases with Alzheimer's disease (18). Principal role of AChE is the termination of nerve impulse transmission at the cholinergic synapses by rapid hydrolysis of ACh (7). In the presence of the AChEIs, ACh hydrolysis is inhibited and leads to elevation of ACh levels in brain synapses. This inhibitory action has been reported to have additional clinical benefits (11). In the healthy brain, AChE predominates (80%) and butyrylcholinesterase (BChE) is considered to play a minor role in regulating brain ACh levels. In the AD brain, BChE activity rises while AChE activity remains unchanged or declines. Therefore, both enzymes are likely to have

involvement in regulating ACh levels and represent legitimate therapeutic targets to ameliorate the cholinergic deficit (19).

2.1.2.2 Amyloid cascade hypothesis

The amyloid cascade theory proposes β-amyloid protein (Aβ) as the central trigger of the pathological changes observed in the brains of AD patients, such as synapse loss, activation of inflammatory processes, the induction of neurofibrillary changes leading to the formation of paired helical filaments and, ultimately, neuronal death (20). To generate Aβ, amyloid protein precursor (APP) is first cleaved by β-secretase to produce a β-APPs ectodomain and a 99-residue carboxy-terminal fragment (C99). Proteolysis of C99 by γ-secretase produces the 40/42-amino acid Aβ-peptide (Aβ42) which is the principal Aβ species found in amyloid plaques (21). Moreover, accumulated toxic forms of Aβ (Aβ42) disrupt neuronal homeostasis, and destroy brain architecture (22).

2.1.3 Treatment of Alzheimer's disease

Because AD is a chronic disease with an insidious onset, there may be several opportunities for prevention and symptomatic treatment including primary, secondary and tertiary prevention or treatment strategies (1).

2.1.3.1 Primary and secondary prevention strategies

Primary prevention strategies attempt to reduce the incidence of disease before symptoms become evident. Thus, they attempt to prevent individuals who are cognitively normal, but perhaps at high risk of mild cognitive impairment, from

developing dementia. While secondary preventions attempt to retard the rate of progression of mild cognitive impairment to dementia (1). Unfortunately, there are no proven strategies for both preventions. Although epidemiologic studies have suggested that the consumption of several medications; includes nonsteroidal anti-inflammatory drugs (NSAIDs), vitamin supplements, and estrogens in women, is associated with a reduced risk of dementia (23-24). There is no delay or reduction in the onset of dementia observed (23-24). Therefore, there is no justification for recommending the use of NSAIDs, antioxidant vitamins C and E, and estrogen hormone replacement therapy for the primary or secondary prevention of AD (23).

2.1.3.2 Tertiary prevention or treatment strategies

Tertiary preventions or treatments may reduce the progression of dementia severity once it has been diagnosed or to improve cognitive, emotional, or behavioral symptoms in patients with dementia. Several pharmaceutical agents have been approved for the treatment of clinically established AD.

2.1.3.2.1 AChE inhibitors

There are four AChE inhibitors: tacrine (Cognex®), donepezil (Aricept®), rivastigmine (Exelon®), and galantamine (Reminyl®) (1). Tacrine was the first drug of this kind approved by the US Food and Drug Administration (FDA) for the treatment of AD; however, this drug was withdrawn later from the market due to its hepatotoxicity. The second generation of AChEI drugs, donepezil, galantamine and rivastigmine, demonstrated statistically significant benefits on cognitive measures relevant to dementia with improved safety profile compared to tacrine (4).

All along, there are many studies attempting to find out new AChEIs especially from plants. A variety of plants has been reported to show AChE inhibitory activity as shown in **Table 1**. Nowadays, products for improving AD symptoms from plant extract are available in the market. For example, *Ginkgo biloba* extract has been marketed as a supplement to improve mental alertness and to treat peripheral vascular disease. Furthermore, *Huperzia saururus* has been marketed in China as a new drug for AD treatment.

Table 1 Plants with AChE inhibitory activity

Plants	Parts used	Type of extract	Activity % inhibition (1 mg/mL)	Ref
Apocynaceae				
Tabernaemontana divaricata	Roots	Methanolic extract	99.72±0.26	(8)
Tabernaemontana divaricata	Stem	Methanolic extract	94.72±2.09	(8)
Coniferae				
Ginkgo biloba	Whole	Ethanolic extract	0.27 mg/mL*	(25)
Lycopodiaceae				
Huperzia saururus	Whole	alkaloidal extract	0.58 mg/mL*	(26)
Fumariaceae				
Fumaria vaillantii	Whole	$CHCl_3$:MeOH (1:1)	94.23±7.47	(27)
Fumaria capreolata	Whole	$CHCl_3$:MeOH (1:1)	96.89±7.17	(27)
Fumaria asepala	Whole	$CHCl_3$:MeOH (1:1)	91.99±7.70	(27)

***: IC_{50}, $CHCl_3$: Chloroform, MeOH : Methanol**

Table 1 (Cont.)

Plants	Parts used	Type of extract	Activity % inhibition (1 mg/mL)	Ref
Fumariaceae				
Fumaria densiflora	Whole	$CHCl_3$:MeOH (1:1)	93.42±7.92	(27)
Fumaria flabellate	Whole	$CHCl_3$:MeOH (1:1)	92.14±7.01	(27)
Fumaria macrocarpa	Whole	$CHCl_3$:MeOH (1:1)	93.43±7.64	(27)
Fumaria judaica	Whole	$CHCl_3$:MeOH (1:1)	96.47±7.63	(27)
Ericaceae				
Rhododendron ponticum	Whole	$CHCl_3$:MeOH (1:1)	93.03±7.12	(27)

***: IC_{50}, $CHCl_3$: Chloroform, MeOH : Methanol**

2.1.3.2.2 N-methyl-D-aspartate receptor antagonist

Nowadays, there is only one *N*-methyl-D-aspartate receptor antagonist (NMDA): memantine (Namenda®) available in the market (1). It is believed that memantine modulates the pathologic activation of NMDA receptors, hypothesized to occur in AD, while allowing normal physiologic activity important for learning and memory (28).

2.2 *Tabernaemontana divaricata* (L.) R. Br. Ex Roem. & Schult

TD is a common garden plant in Southeast Asia and other tropical countries. It has been used in Thai traditional rejuvenation remedies for improving memory while

native people in America, Africa and Continental Asia have used this plant as a central nervous system stimulant (9).

2.2.1 Description and taxonomy

TD belongs to the *Apocynaceae* family, *Plumeroidae* subfamily, *Tabermontanae* tribe and *Tabernaemontana* genus. The genus was named after the birthplace of its discoverer, J. Th. Mueller, Bergzabern, and Bergzabern was latinized into *Tabernaemontana* (29). TD has four typical characteristics as shown in **Figure 1**.

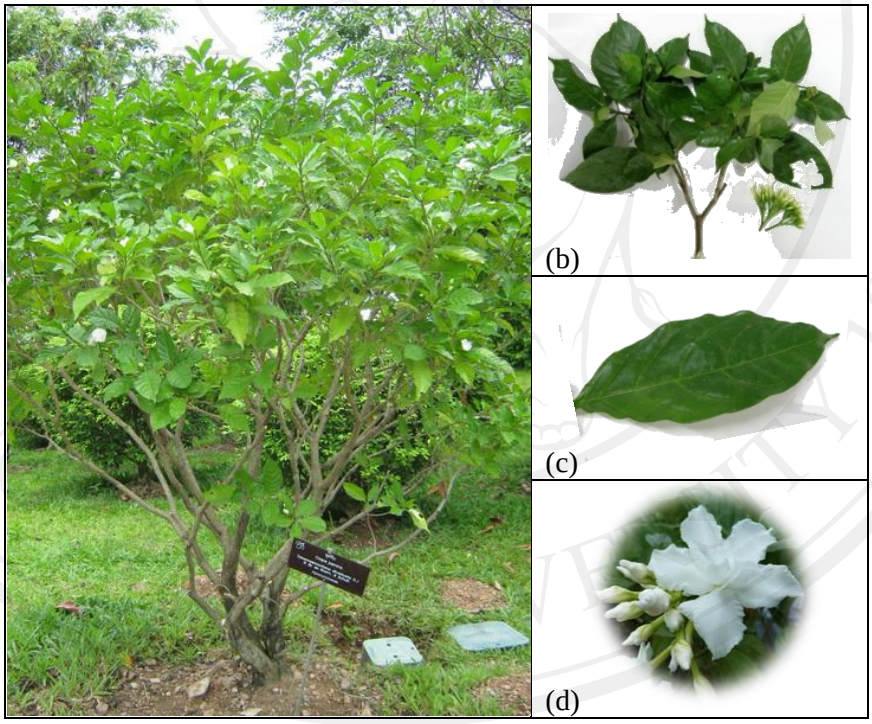
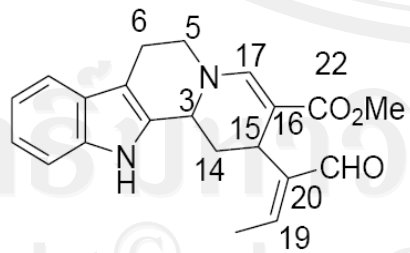


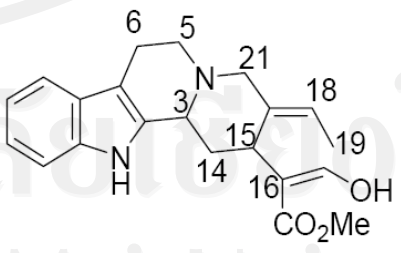
Figure 1. Four typical characteristics of TD including evergreen shrub forms shaped like symmetrical mounds 6-feet high (a), horizontal branches having the appearance of an attractive, almost horizontal shrub (the species name, *divaricata*, means an obtuse angle) (b), large, shiny, deep green leaves, 6 or more inches in length and 2 inches wide (c) and waxy blossoms with white, five-petal pinwheels, gathered in small clusters on the stem tips (d).

2.2.2 Phytochemistry

The phytochemistry and a number of chemical constituents from the leaves, stems and roots have been previously reported. TD is a rich source of various alkaloids because the major constituents of TD are alkaloid (29). Since 1974, 66 different alkaloids of TD have been identified (29). However, indole alkaloids were recognized as the important group of alkaloid from TD. According to van Beek *et al.* (1984), alkaloids of TD are arranged in 11 main classes: vallesiachotaman, corynanthean, strychnan, aspidospermatan, plumeran, ibogan, eburnan, tacaman, vincosan, bis-indole and miscellaneous (30). The structures are shown in **Figure 2**. Most of the phytochemical work on TD has been concerned with the alkaloidal constituents and most of the ethnomedical uses are related to the pharmacological activity of these substances (29). Two bisindole alkaloids (19,20-dihydrotabernamine and 19,20-dihydroervahanine A), isolated from root of TD, was found to have higher inhibitory activity on AChE in comparison with galantamine, a well-known AChE inhibitor (5). Beside alkaloids, there are non-alkaloid constituents in TD such as terpenoids, steroids, flavonoids, phenyl propanoids, phenolic acids and enzymes (29).



vallesiachotoman type



corynanthean type

Figure 2. Type of indole alkaloids found in *Tabernaemontana* species (30).

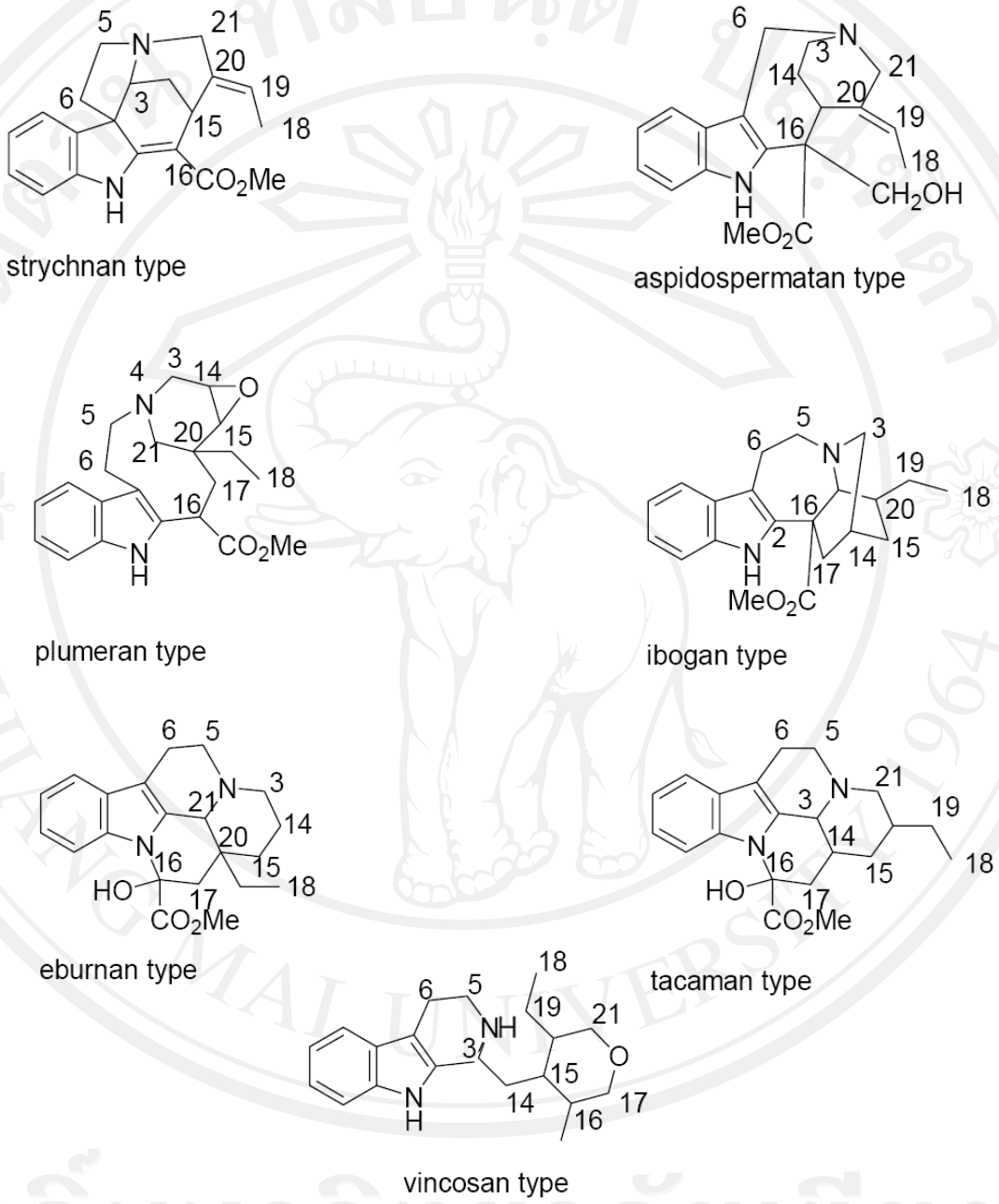


Figure 2. (Cont.)

2.2.3 Neuropharmacological properties

Recently, Ingkaninan *et al*. (2003) demonstrated that methanolic extracts of TD (0.1 mg/ml) inhibited more than 90% of AChE activity in their *in vitro* study (8).

The further investigation on each part of the plant found that the ethanolic extract of both root and stem also exhibited very high inhibitory activity (>90%) against AChE (5). Two vobasinyl-iboga bisindole alkaloids (19,20-dihydrotabernamine and 19,20 dihydroervahanine A) were isolated and AChE inhibitory activity of them were higher than that of the standard inhibitor, galantamine (5). The preclinical analyses using *in vivo* enzymatic techniques in the study of Chattipakorn *et al*. (2007) demonstrated that ethanolic extract of TD is a reversibly selective AChEI and can enhance neuronal activity (9). Moreover, subchronic administration of TD extract via oral route markly improves cognitive deficits induced by Aβ25-35 peptides which is presumed to be one of the causes of AD (10). Therefore, TD could potentially be developed to become one of AChEIs for those elderly people suffering from dementia such as the AD patients (10).

2.2.4 Toxicity

Since TD has a number of pharmacological activities (neurotonic, analgesic, cell proliferation inhibition, and anti-inflammatory), further toxicological studies are necessary. Henriques *et al.* (1996) investigated the acute toxicity of TD using the behavior screening test in mice treated with alcoholic or aqueous extracts of TD stem at doses of 150-200 mg/kg body weight via oral route. They reported that the results were indistinguishable from control animals, indicating that no toxicity was found at these concentrations (31). Moreover, no toxicity was found in the study of Nakdook *et al*. (2010) that pretreated the mice with TD root extract (250, 500 and 1000 mg/kg body weight) for 28 days via oral route (10).

2.3 Transdermal drug delivery system

Transdermal drug delivery system represents the most successful non-oral systemic drug delivery since it offers several advantages compared to the oral and parental route in terms of decreased drug degradation before entrance into the systemic circulation and higher patient comfort and compliance, respectively (32, 33). However, the skin often limits the permeability of those drugs.

2.3.1 Skin

Skin is the largest human organ and behaves like an excellent biological barrier (34). Despite being normally less than 2 mm thin, the skin contributes around 4% to a body weight and is 102-104 times less permeable than a blood capillary wall (34). 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 (35). There is a fat layer underneath the dermis which is hypodermis or so called subcutaneous. The cross section of the skin is illustrated in **Figure 3.**

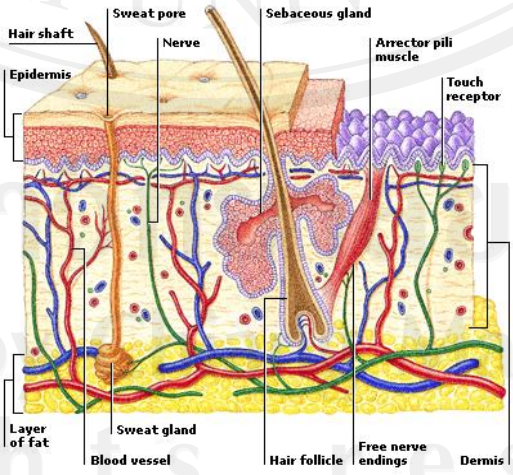


Figure 3. The cross section of skin presents epidermis, dermis and hypodermis (36).

2.3.1.1 Epidermis

Epidermis is the outer skin part in humans which is typically 50-150 μm thin (34). Stratum corneum or horny layer is the uppermost part that controls the penetration of drugs and provides a very effective barrier to penetration, despite its thickness of only 15-20 μm (35). Moreover, this layer is the primary skin barrier to the entry of drug molecules into the organism since its lipophilic character and intrinsic tortuosity (34, 37).

2.3.1.2 Dermis

Dermis is the region below epidermis. Many different structures exist in the dermis, including blood vessels, nerves, hair follicles, and sweat glands which taken together usually 2 mm in thickness (5-20 times thicker than epidermis) (34, 36). The segmental area of cutaneous blood vessels peaks approximately 100-150 μm below the surface (35).

2.3.2 Transdermal process

The permeation of the drug through the skin has several routes include transcellular, intercellular and appendageal route. The intercellular spaces consist of a mixture of lipids-ceramides, free fatty acids and their esters, and cholesterol and its sulphates that are structured in bilayers. The appendageal route includes penetration through eccrine (sweat) glands or hair follicles (37). All the permeation routes are shown in **Figure 4**.

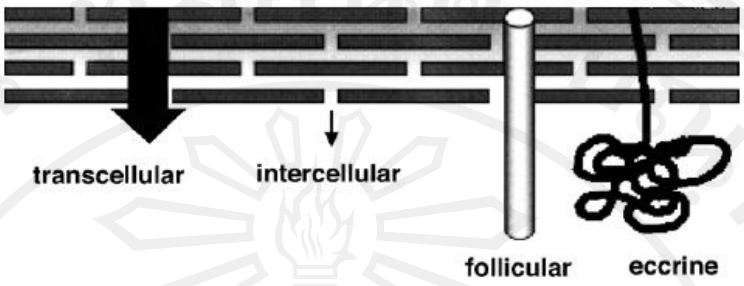


Figure 4. Schematic representation of the different possible routes of penetration through the skin (35).

Transdermal drug permeability is influenced mainly by three factors including the mobility of the drug in the vehicle, the release of the drug from the vehicle, and drug permeation through skin (35). The release of a therapeutic agent from a formulation applied to the skin surface and its transport to the systemic circulation is a multistep process which involves:

2.3.2.1 Dissolution within and release from the formulation.

2.3.2.2 Partitioning into the skin's outermost layer, the stratum corneum.

2.3.2.3 Diffusion through the stratum corneum (the rate-limiting step for most compounds).

2.3.2.4 Partitioning from the stratum corneum into the aqueous viable epidermis.

2.3.2.5 Diffusion through the viable epidermis and into the upper dermis.

2.3.2.6 Uptake into the local capillary network and eventually the systemic circulation

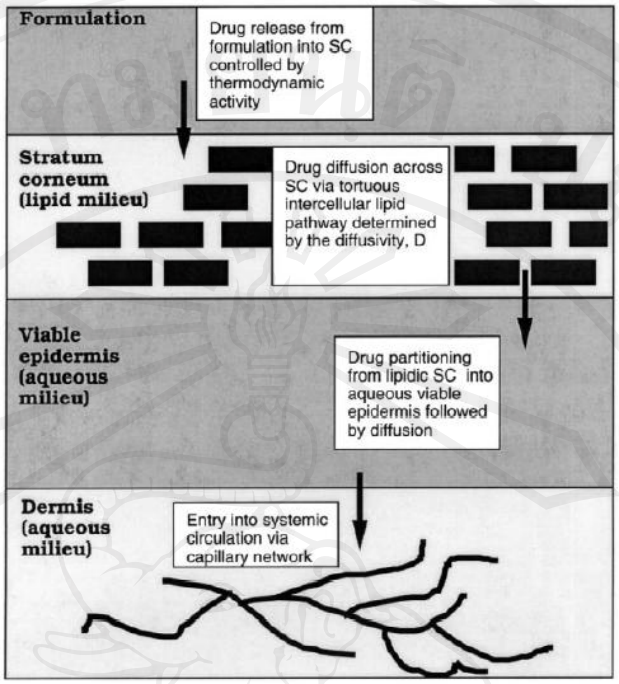


Figure 5. Schematic representation of the transport processes involved from the release of the drug from the formulation to its eventual uptake by the dermal capillaries (37).

2.3.3 Advantages of transdermal drug delivery system

Transdermal administration is able to provide continuous delivery of drug with reduced fluctuations in plasma levels (i.e., lessening the rapid rise and fall of drug concentration), prolong t_{max}, and achieve a lower C_{max} (11). The transdermal application is expected to reduce side effects and offer additional therapeutic advantages over oral administration, such as access to higher doses, with the potential to improve compliance and treatment effects (11). Moreover, transdermal drug delivery has many advantages over the oral route of administration: it avoids hepatic metabolism, the administration is easier and more convenient for the patient, and there is the possibility of immediate withdrawal of the treatment if necessary (35).

2.3.4 Transdermal drug delivery system of anticholinesterase

As people grow old, their need for medications increases dramatically because of the higher incidence of chronic pain, diabetes mellitus, cardiovascular and neurological diseases in the elderly population. Furthermore, the elderly requires special consideration with respect to drug delivery, drug interactions and adherence. In particular, patients with chronic neurological diseases often require multiple administrations of drugs during the day to maintain constant plasma medication levels, which in turn increases the likelihood of poor adherence (38). Consequently, several attempts have been made to develop pharmacological preparations that can achieve a constant rate of drug delivery. Transdermal administration is the ideal therapeutic approach for chronic neurological disorders in elderly people because it provides sustained therapeutic plasma levels of drugs, is simple to use, and may reduce systemic adverse effects. Several transdermal delivery systems are currently under investigation for the treatment of AD. Because of the favourable pharmacological profile and bioavailability of transdermal treatment, the cholinestersae inhibitors (ChEIs) tacrine and rivastigmine are expected to show at least the same benefits as oral formulations of these drugs, but with fewer severe adverse effects (39, 40). Although most transdermal delivery systems treatments cannot be considered as first-line therapy at present, some of them provide clear advantages compared with other routes of administration and may become the preferred treatment in selected patients. In general, however, most transdermal treatments still require long-term evaluation in large patient groups in order to optimise dosages and evaluate the actual incidence of local and systemic adverse effects (38).

2.4 Microemulsion

Microemulsions belong to the group of colloidal drug delivery systems (41). Microemulsions are optically transparent, low viscosity and thermodynamically stable dispersions of oil, water, and surfactant, frequently in combination with a co-surfactant (35, 40). The systems can be differentiated from a coarse emulsion by visual inspection (microemulsion = clear, coarse emulsion = white). The differentiation from a liquid crystal can be undertaken by PLM (microemulsion = isotropic/non birefringent, liquid crystal = usually anisotropic /birefringent) and by viscosity determination (microemulsion = low, Newtonian viscosity, liquid crystal = high, non-Newtonian viscosity) (41).

2.4.1 Type of microemulsion

Depending on their microstructure, microemulsions can be categorized into 3 types: droplet, bicontinuous or solution type (41).

2.4.1.1 Droplet microemulsions

In a droplet microemulsion, the dispersed oil or water is surrounded by surfactant molecules forming micelles or reverse micelles in the continuous component as shown in **Figure 6a** and **6c**, respectively (41).

2.4.1.1.1 Micellar droplet structures: oil-in-water (O/W) microemulsion : oil droplet disperse in continuous aqueous (42).

2.4.1.1.2 Inverted micellar droplet structures: water-in-oil (W/O) microemulsion : water droplet disperse in continuous oil (42).

2.4.1.2 Bicontinuous microemulsions

Bicontinuous microemulsions are characterized by a sponge-like microstructure, with comparatively large oil and water domains intertwining, separated by a surfactant layer as shown in **Figure 6c** (33).

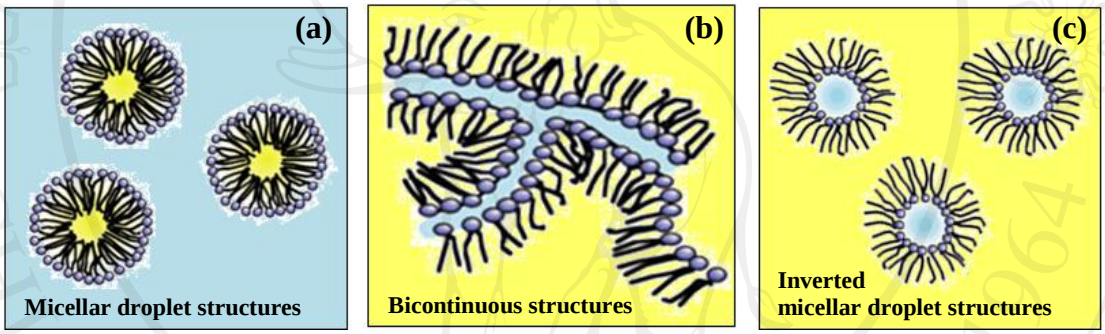


Figure 6. Basic microemulsion structures formed by oil phase (yellow), aqueous phase (blue) and surfactant/co-surfactant interfacial film (43).

2.4.1.3 Solution type microemulsions

Molecular dispersions of all components are termed solution type microemulsions (43). Despite the absence of droplets or swollen micelles in these microemulsions, it has been shown that nanoparticles can be prepared by interfacial polymerization of such systems (41).

2.4.2 Microemulsion development

Phase diagram is used to provide a means of characterizing emulsions. Generally, ternary diagrams are employed to depict mixtures of three components which are oil, water and surfactant. In case of microemulsion development, the diagrams are called pseudoternary phase diagrams as shown in **Figure 7**, since a microemulsion is a complex system of oil, water and emulsifiers (surfactant and co-surfactant). Surfactant and co-surfactant is prepared in defined mixtures and is presented on one axis in the ternary phase diagram. Contrary to submicron emulsions, microemulsions are formed spontaneously without the input of energy as soon as the required ratio of components has been reached. Microemulsions are optically transparent and isotropic liquids, which are in thermodynamic equilibrium. Apart from single-phase ranges, the ranges of the microemulsion, such mixtures also have two- and three-phase regions and are usually only formed in narrow specific concentration ranges of the ingredients. Pseudoternary phase diagrams enable the homogeneous or the heterogeneous regions to be depicted (44).

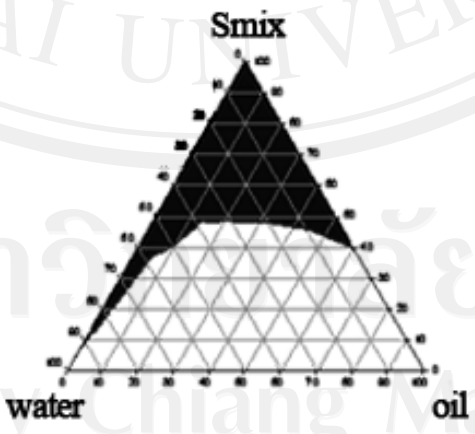


Figure 7. Pseudoternary phase diagrams of oil/water/Smix (surfactant and co-surfactant) (45).

2.4.3 Advantages of microemulsion

The use of microemulsions in pharmaceutics is advantageous not only due to the low cost and ease of preparation (zero interfacial tension and almost spontaneous formation), but also because of the improved bioavailability, stability (long shelf-life), high surface area (high solubilization capacity), very small droplet size (5-100 nm) and good appearance (35, 42). The small droplets have better chance to transport bioactive molecules in a more controlled fashion (35). Using the microemulsion vehicles, water-soluble and oil-soluble components from different plant extracts can be co-solubilized in order to attain synergistic effect for a specific therapeutic goal (35). Microemulsions were also found as protecting medium for the entrapped of drugs from degradation, hydrolysis and oxidation (35).

It was demonstrated that permeation rates from microemulsions were significantly higher than from conventional emulsions and other formulations. Both, increase in solute concentration and the tendency of the drug to favor partitioning into the stratum corneum make the microemulsion a useful vehicle to enhance transdermal drug permeability (46, 47). By far, it has been shown in many studies that microemulsion formulations possessed improved transdermal and dermal delivery properties. Recently many drugs such as lidocaine, diclofenac, 5-Fluorouracil and ascorbic acid using microemulsion for transdermal delivery had been reported (43, 46, 48, 49). Kreilgaard *et al*. (2002) have reported that the transdermal flux of lidocaine from a microemulsion is up to four times higher than that from a conventional O/W emulsion (43). In addition, Sintov *et al*. (2006) found that the transdermal administration of the microemulsion containing diclofenac to rats resulted in 8-fold higher drug plasma levels than those obtained after application of Voltaren Emulgel

(46). Moreover, Gupta *et al.* (2005) indicated that incorporation of 5-Fluorouracil into microemulsions increased the flux of the drug 2-6 fold in comparison to the aqueous solution of the drug (48).

Since microemulsions were also found as protecting medium from degradation (35), Gallarate *et al*. (1999) found that microemulsion systems provided protection against oxidation for ascorbic acid, and therefore its degradation rate was slower than in aqueous solutions (49).

Moreover, microemulsion can be used as a template for developing many formulations such as hydrogel and nanoparticles. Gasco and Trotta (1986) indicated that the use of a microemulsion instead of a coarse or submicron emulsion frequently used as a template for the preparation of nanoparticles offers advantages in terms of enhanced physical stability of the system, the minimal need of energy input to form the polymerization template, and their small and uniform droplet size that is reflected in the size of the nanoparticles produced (50). Moreover, Watnasirichaikul *et al*. (2000) developed a method for producing poly(alkylcyanoacrylate) (PACA) nanoparticles from biocompatible microemulsions in a simple one-step process thereby omitting the need of having to separate the resulting nanoparticles after polymerization and retaining a high yield of encapsulated peptide (51). In another hand, Chen *et al*. (2006) developed the microemulsion-based hydrogel formulation containing ibuprofen with a suitable viscosity for topical administration by swelling xanthan gum in microemulsion system (52). The advantages of microemulsion in a comparison with the conventional emulsion are shown in **Table 2**.

Table 2. Comparison of conventional emulsion and microemulsion.

Conventional emulsion	Microemulsion
The strong interactions between the surfactants that occur in the interfacial membrane film limit the mobility of the drug between the internal and external phases within the formulation.	In microemulsion, the co-surfactant assists in lowering the interfacial tension of the surfactant film.
Strong fluctuation in bioavailability was detected.	Less fluctuation in bioavailability was detected.
Emulsions are not stable formulations.	Microemulsions are thermodynamic stable.

2.4.4 Essential oil as an oil phase in microemulsion

Essential oil, also known as volatile oil, is multi-component system composed of volatile aromatic compounds from plants. It has a characteristic odor or flavor of the plant from which it is obtained. Essential oil has been used as an oil phase in microemulsions for transdermal applications (15). The advantages of using essential oil in microemulsion were not only a large microemulsion region investigated in the psuedoternary phase diagram but terpenes, the main constituents of essential oil, also acted as skin penetration enhancers (35).