LINKER-BASED LECITHIN MICROEMULSIONS AS TRANSDERMAL DRUG DELIVERY SYSTEMS

By

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ABSTRACT

The interest in microemulsions as transdermal delivery systems have been motivated by their large surface area for mass transfer, their high solubilization capacity of hydrophobic actives, and their ability to improve skin penetration. Lecithins (mixtures of phospholipids similar to those find in the skin) have been proposed as ideal surfactants in microemulsions due to their skin compatibility. Unfortunately, their incorporation into microemulsions used to require toxic medium-chain alcohols or viscous polymeric co-surfactants. Recently, microemulsion-base “green solvents” were formulated with lecithin and linker molecules. The main objective of this dissertation was to test this concept of linker-based lecithin microemulsions in transdermal delivery.

In the first part of this study, linker-based lecithin formulations were developed using soybean lecithin as main surfactant, sorbitol monooleate as lipophilic linker, and caprylic acid/sodium caprylate as hydrophilic linkers. These additives, at the suggested concentration, are safe for cosmetic and pharmaceutical applications. The low toxicity of these formulations was confirmed in cultured human skin tissues. The solubilization and permeation of a common anaesthetic, lidocaine, was evaluated. The concept of “skin” permeability was introduced to account for the differences in solvent-skin partition when comparing different delivery systems. The linker-
based lecithin microemulsion produced a substantial absorption of lidocaine into the skin, when compared to a conventional pentanol-lecithin microemulsion. The second part of this study takes advantage of the lidocaine adsorbed in the skin with the linker-based lecithin microemulsion as reservoir for in situ skin patches. The in situ patches were able to release 90% of the lidocaine over 24 hours, which is comparable to the release profile obtained from conventional polymer or gel-based patches. In the third part of this work, the role of surfactant droplets on the transport of lidocaine was studied. A mass balance model that accounted for mass transfer and partition coefficients was introduced. The parameters generated from the model confirm that in most cases the transport through the skin limits the overall penetration of lidocaine. Besides the conventional diffusion mechanism, the results suggest that surfactant droplets, carrying lidocaine, also penetrate into the skin and contribute to the accumulation of the lidocaine in the skin.
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1.1 OVERVIEW

In recent years, microemulsions have attracted considerable interest as promising colloidal drug delivery vehicles (Bagwe et al., 2001; Lawrence and Rees, 2000; Tenjarla, 1999). There are many advantages for microemulsions in pharmaceutical applications over other systems, including high solubilization capacity for both water-soluble and oil-soluble drugs, thermodynamic stability, transparency, and ease of preparation. Searching the database SciFinder® with “microemulsion” and “drug delivery” as key words retrieved over fifteen hundred references. Figure 1.1 presents the number of publications on the topic of microemulsions and drug delivery for each year between 1985 and 2007, making it clear that the level of interest in microemulsions as drug carriers continues to grow. Moreover, there are already commercial products based on microemulsions on the market like Neoral® for oral delivery of cyclosporine and Topicaine® for transdermal delivery of lidocaine.

![Figure 1.1](image-url)

**Figure 1.1** Number of publications per year containing the key words “microemulsion” and “drug delivery”.

Transdermal drug delivery (TDD) is a method of drug delivery in which a drug is administered by skin absorption, such as a patch. A well designed TDD system reduces drug degradation, side effects and patient discomfort (Amann and Osborne, 1990; Guy and Hadgraft, 2003). Because of the barrier properties of the skin, particularly stratum corneum (Michaels et al., 1975), only potent drugs with low molecular weight and adequate solubility can be easily delivered through the skin. In the development of a TDD vehicle, skin penetration of the active is a key factor, as well as low skin irritation.

Compared to conventional colloidal carriers like emulsions, hydrogels and liposomes, microemulsions have been shown to be superior for transdermal delivery of both oil-soluble and water-soluble drugs (Heuschkel et al., 2008; Date and Patravale, 2007; Kreilgaard, 2002). Microemulsions are thermodynamically stable systems comprised of oil, water and surfactants, which solubilize oil and/or water in nano-domains (1-100 nm) (Danielsson and Lindman 1981). Depending on the surfactant used in formulations, three types of microemulsions can be formed: oil-in-water (o/w, Winsor Type I), water-in-oil (w/o, Winsor Type II) and bicontinuous (middle-phase, Winsor Type III or IV) systems. The most important property of microemulsions for drug delivery is their excellent drug solubilization capacity due to the coexistence of hydrophilic and lipophilic solubilization sites (Kreilgaard, 2002).

The crucial point for microemulsions in pharmaceutical applications is the type and amount of surfactants selected in the systems. Generally, surfactants of natural origins are preferred over synthetic surfactants (Tenjarla, 1999). Among synthetic surfactants, nonionic surfactants are preferred over cationic and anionic surfactants. Microemulsions usually require large concentration of surfactants in formulations. For example, Delgado-Charro et al. formulated microemulsion systems with about 25-44% surfactant mixture to deliver drugs through the skin (Delgado-charro et al., 1997). In one case, the surfactant concentration was as high as 70% in
microemulsions (Kreilgaard et al., 2000). Unfortunately, skin irritation has been linked to high surfactant concentrations and therefore the surfactant concentration should be kept to a minimum in microemulsion formulations.

Aboofazeli and Lawrence showed that phospholipids, particularly lecithin, are good surfactants for microemulsion formulations because they are non toxic even at high concentrations (Aboofazeli and Lawrence, 1993). Lecithin, a mixture of phospholipids, is a naturally-occurring biological surfactant and can be found in all plants and animals. However, the problem in using lecithin as a surfactant for microemulsions is that phospholipids are too hydrophobic to spontaneously form zero curvature lipid layers as required for the formation of bicontinuous microemulsions (Bagwe, 2001; Tenjarla, 1999). Typical lecithin microemulsions have to use a large amount of medium-chain alcohols as cosurfactants. These alcohols can cause drying and skin irritation making them less attractive for clinical applications (Bommannan et al., 1991).

Alcohol-free lecithin microemulsions have been formulated using linker molecules to replace alcohols. Linker molecules are amphiphiles that segregate near the oil/water interface to enhance the surfactant-oil (lipophilic linkers) or surfactant-water (hydrophilic linkers) interactions (Sabatini et al., 2003). Acosta et al. found that linker-based lecithin microemulsions have a large solubilization capacity for a wide range of oils, when using hexyl polyglucoside as the hydrophilic linker and sorbitan monooleate as the lipophilic linker (Acosta et al., 2005). The authors proposed the lecithin-linker systems as “green solvents” in dry-cleaning applications and solvent delivery systems for pharmaceutical applications. However, before this project, there had been no report regarding the use of linker-based lecithin microemulsions in drug delivery, or any toxicity studies on these systems. Besides, hexyl polyglucoside is not registered in many countries, including Canada, as cosmetic, food or pharmaceutical ingredient and therefore cannot be used in these applications. Since the toxicity caused by surfactants is a major concern, it is
also necessary to investigate the relationship between the surfactant concentrations and the transdermal performance of linker-based microemulsion vehicles.

In addition, traditional form for topical treatment of skin diseases is drug-in-adhesive patches, in which polymers are commonly used as contact membranes to control drug release. Since patches can not be used over burns, cuts, and irritated skin, there is a need for developing sprayable formulations for extended release through the skin. Microemulsions are liquid and sprayable. They have been reported to provide high drug absorption in the skin (Baroli et al., 2000; Delgado-Charro et al. 1997; Willimann et al., 1992). This skin absorption portion could act as an “in-situ” patch and provide extended release of drugs. However, all microemulsion studies as TDD vehicles in the literature are for immediate release style (Kreilgaard, 2002; Lawrence and Rees, 2000), no work has been done to test them for extended release purposes.

1.2 HYPOTHESIS

Lecithin microemulsions can be formulated using another hydrophilic linker with Generally Recognized as Safe (GRAS) or food additive status, and the systems can be used as potentially safe and effective TDD vehicles for immediate and extended release. Moreover, the surfactant concentrations in the linker-based lecithin microemulsions can be optimized for transdermal delivery by understanding the drug release mechanism from microemulsions to the skin.

1.3 SPECIFIC OBJECTIVES

The overall objective of this study is to formulate and optimize the formulations of linker-based lecithin microemulsions and to investigate their potential on the transdermal immediate and
extended delivery of drugs through better understanding the mechanism of drug release from microemulsions. The specific aims of this work are three-fold:

1. To formulate lecithin microemulsions with another hydrophilic linker (sodium caprylate) and to investigate their effectiveness as TDD vehicles using in vitro permeation and cytotoxicity tests;

2. To investigate the possibility of in situ patches for extended drug release by applying the newly formulated lecithin-linker microemulsions;

3. To investigate the effect of surfactant concentration in the developed microemulsions on their transdermal potential and to understand the role of surfactants on drug release mechanism from microemulsions to the skin.

1.4 THESIS OUTLINE

This dissertation is organized in six chapters. A brief introduction of the research topic, hypothesis, and objectives is presented in Chapter 1. An overview of literature is given in Chapter 2, including a basic background of TDD, microemulsions, linker molecules and the scope of this work. The main contributions of this study are included in Chapter 3, 4, and 5. Chapter 3 reports the formulation and performance of linker-based lecithin microemulsions as transdermal systems for lidocaine. Chapter 4 takes advantage of the increased drug absorption found in Chapter 3 to produce an extended release profile where drug-loaded skin is used as an in situ patch. Chapter 5 describes the impact of surfactant concentrations on transdermal performance of the studied lecithin-linker systems. Finally, overall conclusions drawn from this work and an outlook on the potential impact of the findings of this work are presented in Chapter 6.
In addition, there are two appendices that present some experimental results related to this work. Appendix 1 attempts to investigate the effect of oils on the transdermal performance of the studied lecithin-linker microemulsions. Appendix 2 shows the effect of drugs on the incorporation of the developed lecithin-linker systems.

Indeed, this dissertation mainly consists of three publications/preparations submitted to refereed journals. Two papers based on Chapter 3 and 4 have been published or accepted in the International Journal of Pharmaceutics. Another paper based on Chapter 5 has been submitted to Journal of Controlled Release. Additionally, two conference papers derived from the thesis have been published in the Journal of Cosmetic Science. More than 11 presentations have been given at peer-reviewed or international conferences. A patent application (Pub. No. 2008/0139392) has been filed with the US patent office and we were awarded $50,000 Ontario Research and Commercialization Program grant from Province of Ontario.

1.5 INDUSTRIAL SIGNIFICANCE

The results of this work will enable us to formulate efficient and less toxic surfactant-based products to deliver a wide range of active ingredients in the pharmaceutical and cosmeceutical industries. The market for TDD in the U.S. alone was at $3 billion dollars in 2006 and is expected to reach $4.4 billion by 2008 (Shahani, 2003). Among the targeted nano-scale delivery vehicles, microemulsions are the only systems that can cosolubilize high concentrations of both water-soluble and oil-soluble active ingredients. However, there are two major challenges for microemulsion delivery systems: first the toxicity associated with synthetic surfactants commonly used in these formulations and second the lack of understanding of the mechanism of drug delivery in microemulsions which could lead to an optimization on the selection and
concentration of ingredients used to formulate the microemulsions. The results of this research work not only provide potentially safe and effective formulations as transdermal delivery vehicles, but also offer a drug release model for tailoring and optimizing the delivery systems.

1.6 REFERENCES


CHAPTER 2

BACKGROUND
Microemulsions have many advantages as transdermal drug delivery (TDD) vehicles, including high solubilization capacity for hydrophilic and lipophilic active ingredients, thermodynamic stability, high surface area to volume ratio (desirable for accelerated mass transfer); transparency and self-emulsifying properties. It has been reported that the transdermal flux of lidocaine from a microemulsion is up to four times than that from an emulsion (Kreilgaard et al., 2000). However, the major challenge for microemulsion delivery systems is the toxicity associated with surfactants and cosurfactants commonly used in these formulations. This chapter presents a detailed review of microemulsions used for transdermal delivery. To provide the rationale behind the approaches in this dissertation and address the questions present in the field, the background will focus on barrier properties of the skin, formulation constraints of microemulsions, and the approach of using linker molecules in microemulsions.

2.1 TRANSDERMAL DRUG DELIVERY

TDD is a method of drug delivery through the skin to the systemic circulation (Guy and Hadgraft, 2003). It is a convenient mode of administration enabling physicians to provide controlled and continuous drug delivery to patients without causing distress. Compared to oral and parenteral routes, the transdermal route of drug administration has the advantages of reducing gastrointestinal side effects and reducing drug degradation (Bronaugh and Maibach; 2002; Amann and Osborne, 1990). However, the skin itself is the major barrier preventing drugs into the body. Chemical approaches have been used to overcome this barrier and the dosage forms could be patches, creams, gels, ointments, or sprays (Williams and Barry, 2004). This review does not include physical approaches, such as the use of micro-needles, ultrasound, radio waves, etc.(Asbill, 2000).
2.1.1 The skin barrier

Human skin is the largest organ of the human body. It is accessible and provides a painless and compliant interface for systemic drug administration. However, one of the basic functions of the skin is to serve as a protective barrier impeding the penetration of external pathogens and toxins into the body. It also aids in balancing the water content of the body. Consequently, the entry of therapeutic agents (drugs) also becomes difficult due to the relatively impermeable nature of the skin (Guy and Hadgraft, 2003). The skin consists of two main layers: the epidermis and the dermis. The epidermis is the outermost layer of the skin, approximately 100 µm thick. The impermeability of the skin is mainly provided by the uppermost layer of the epidermis, the stratum corneum, whose thickness is approximately 15-20 µm (Hadgraft, 2001).

The stratum corneum is composed of dead, flattened, and interlocked keratin-rich cells (corneocytes). These dense cells are embedded in a lipid matrix. The structure of the stratum corneum is similar to a brick and mortar wall (Michaels et al., 1975), where the corneocytes of hydrated keratin comprise the bricks, and the epidermal lipids fill the space between the dead cells like mortar (Figure 2.1). The major lipids found in the stratum corneum are ceramides, fatty acids and their esters, and cholesterol and its sulfate (Wertz and Downing, 1991). They are arranged into ordered bilayers, and those structured bilayers confer a high degree of the impermeability of the skin to water and other penetrants (Hadgraft, 2001). Therefore, the stratum corneum has been organized as the rate controlling membrane that acts predominantly as a hydrophobic barrier (Langer, 2004; Prausnitz, 2004).
2.1.2 Routes of skin penetration

There are potentially three possible routes to penetrate the skin (Figure 2.2): transcellular, intercellular, and appendageal (through eccrine/sweat glands and hair follicles) (Hadgraft, 2001). Under normal conditions the appendageal (shunt) route is not considered to be important since the appendages occupy a relatively low fraction of skin area (~0.1%). Nevertheless, this route is more important for ions and large polar molecules which hardly permeate through the intact horny layer (Williams and Barry, 1992).
It is difficult to determine the differences between the transcellular and intercellular route. Daniels proposes that the penetration pathway is decided mainly by the partition coefficient (octanol/water Log P) (Daniels, 2004). Hydrophilic drugs partition preferentially into the intracellular domains, whereas lipophilic permeants (Log P >2) traverse the stratum corneum via the intercellular route. Most molecules pass the stratum corneum by both routes. However other researchers believe that the intercellular route is more important for the permeation of most drugs, whereas the nature of the intercellular channels is unclear (Hadgraft, 2001; Fartasch et al., 1993).

Even though the skin is such a heterogeneous membrane in most cases, the underlying transport process through the skin is controlled by simple passive diffusion (Hadgraft, 2001). Fick’s first law of diffusion can be used to describe these processes.

\[ J = \frac{KD\Delta C}{h} \]  
(Equation 2.1)

Where \( J \) is the flux per unit area, \( K \) is the skin-vehicle partition coefficient, and \( D \) is the diffusion coefficient in the skin; \( \Delta C \) is the concentration difference across the skin and \( h \) is the diffusional path length. This equation is often simplified to

\[ J = k_p \times \Delta C \]  
(Equation 2.2)

Where \( k_p \) (=\( KD/h \)) is a permeability coefficient, which has units of velocity (often quoted as cm/h), i.e., it is a heterogeneous rate constant and encodes both partition and diffusional characteristics.

### 2.1.3 Drugs

Only a small number of drug products are currently available via transdermal delivery. In many cases, the physical properties of a drug, including molecular size and polarity, limit its capacity
to be delivered transdermally. It is generally accepted that the best drug candidates for passive adhesive transdermal patches must be nonionic, of low molecular weight (less than 500 Daltons), have adequate solubility in oil and water (log P in the range of 1 to 3), a low melting point (less than 200°C), and are potent (dose is less than 50 mg per day, and ideally less than 10 mg per day) (Guy, 1996; Finnin and Morgan, 1999). On the other hand, the biological properties of drug molecules, including dermal irritation and insufficient bioavailability, have been problematic. Nevertheless, the use of gels, creams, and sprays makes a wider range of drug compounds possible to be delivered transdermally.

Currently, there are a number of notable examples of transdermal products on the market based on 13 drug molecules: fentanyl, nitroglycerin, estradiol, ethinyl estradiol, norethindrone acetate, testosterone, clonidine, nicotine, lidocaine, prilocaine, scopolamine, norelgestromin and oxybutynin (www.fda.gov/cder/ob).

2.1.4 Formulation approaches

To increase drug permeation across the skin, different classes of chemical penetration enhancers have been formulated using surfactants (e.g. Tween), fatty acid/esters (e.g. oleic acid), terpenes (e.g. limonene), and solvents (e.g. dimethylsulfoxide and ethanol) (Williams and Barry, 2004). In recent years, colloidal carriers such as emulsions, micelles, liposomes and deformable vesicles have been used extensively in the topical delivery of cosmetic and dermatological agents (Cevc, 2004). An alternative, perhaps more versatile approach, is the use of microemulsions because they can cosolubilize high concentrations of both water-soluble and oil-soluble drug molecules.

However, potent chemical enhancers are usually potent irritants to the skin at the concentrations necessary to achieve reasonable levels of penetration enhancement, making them physiologically...
incompatible (Lashmar, 1989). These chemicals may irritate the skin by causing an immune response, disrupting the epidermal barrier, being cytotoxic to any of the 30 types of skin cells, causing oxidative stress at a cellular level or any combination of these effects. When chronically irritated, the skin may become red, scaly, chapped, itchy and sensitive to touch. The skin immune response involves the coordinated actions of epidermal and dermal cells along with the intricate network of cytokines (Bos and Kapsenberg, 1993). First, many cytokines produced by activated keratinocytes in the epidermis are released. This local release, which serves as an early signal to the host immune system, then initiates the inflammatory cascade. Together, cytokines and inflammatory mediators allow the skin to recruit leukocytes and monocytes from the blood vessel in the dermis to the site of inflammation. This reaction is characterized by an increase in blood flow at the site inflammation (Rougier et al., 1994).

It is important to select chemical penetration enhancers free of irritancy potential. For example, dimethylsulfoxide may promote drug permeation, but this solvent can dissolve the skin cells and extract stratum corneum lipids. Short- (C\textsubscript{1}-C\textsubscript{3}) and medium-chain (C\textsubscript{4}-C\textsubscript{8}) alcohols are also common enhancers used in TDD with skin irritation as a consequence. Therefore, the researchers are challenged to come up with formulations that increase the permeability of the drug through the skin without irreversibly changing the skin barrier function (Wolff, 2000).

### 2.2 MICROEMULSIONS

The term “microemulsion” itself is sometimes used in a misleading way. Hoar and Schulman first introduced the word to define a clear solution obtained by titrating a milky emulsion with a medium-chain alcohol (Hoar and Schulman, 1943). The term was also used to describe various homogeneous surfactant-containing solutions in the past and the expression itself implies
emulsion-like properties with droplet sizes in submicron-range. To avoid the confusion in the terminology, Danielsson and Lindman proposed a definition for microemulsion: “a system of water, oil and amphiphile which is a single optically isotropic and thermodynamically stable liquid solution” (Danielsson and Lindman, 1981). This concept does not cover aqueous solutions of surfactant (micelles and reverse micelles), coarse emulsions (macroemulsions), liquid crystals, and systems that are surfactant-free. Recognizing the differences between a normal coarse emulsion and a microemulsion is very important. Table 2.1 lists the major differences between micro- and “macro” emulsions.

Table 2.1 Comparison of emulsions and microemulsions.

<table>
<thead>
<tr>
<th>Property</th>
<th>Emulsion</th>
<th>Microemulsion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Droplet size of dispersed phase</td>
<td>&gt; 500 nm (nanoemulsions: 1-100 nm)</td>
<td>1-100 nm</td>
</tr>
<tr>
<td>Appearance</td>
<td>Milky</td>
<td>Transparent/translucent</td>
</tr>
<tr>
<td>Formation</td>
<td>Input of external; External energy required</td>
<td>Spontaneous; Minimal energy required</td>
</tr>
<tr>
<td>Stability</td>
<td>Thermodynamically unstable; Two-phase system</td>
<td>Thermodynamically stable; One-phase system</td>
</tr>
<tr>
<td>Interfacial tension</td>
<td>(~ 50 \text{ mN m}^{-1})</td>
<td>(10^{-3} \sim 10^{-13} \text{ mN m}^{-1})</td>
</tr>
</tbody>
</table>

2.2.1 Structures

There are three basic types of microemulsions (Figure 2.3): (1) oil-in-water (o/w, Winsor Type I), in which water is the continuous phase; (2) bicontinuous (Winsor Type III or IV), in which approximately equal volumes of water and oil exist; and (3) water-in-oil (w/o, Winsor Type II), in which oil is the continuous phase (Kumar and Mittal, 1999). The presence of Type I microemulsion droplets is likely to be a feature in microemulsions where the volume fraction of oil is low. Conversely, Type II droplets are likely when the volume fraction of water is low.
When similar amounts of water and oil are present, a bicontinuous microemulsion may occur: both oil and water exist as a continuous phase in the presence of a continuously fluctuating surfactant-stabilized interface with a net curvature of zero. In the case of a Type III system, besides the microemulsion, there is an excess phase of oil and an excess phase of water, but other than that, it retains the same characteristics of a Type IV system (Bourrel and Schecter 1988). In addition, the microemulsion structures are greatly influenced by the physico-chemical properties of the components used, the ratios between the components, the salinity in the formulations and the surrounding temperature.

![Figure 2.3](image)

**Figure 2.3** Three basic microemulsion structures formed by oil phase (grey), aqueous phase (white) and surfactant/cosurfactant interfacial film: (1) o/w (Type I), (2) bicontinuous (Type III or IV), and (3) w/o (Type II) microemulsions (adapted from Kreilgaard, 2002).

### 2.2.2 Formation

When aqueous, oil and amphiphilic (surfactant and cosurfactant) solutions are mixed, a variety of complexes may be obtained including microemulsions and other colloidal systems such as micelles, emulsions, and liquid crystals (Kumar and Mittal, 1999). The region of microemulsions existence can be captured with the aid of pseudo-ternary phase diagrams, as the relationship between the phase behaviour of a mixture and its composition (Figure 2.4). For simplicity, microemulsions are often assumed to comprise of three components (oil, water, and surfactant)
in a phase diagram. With given oil-water-surfactant components, microemulsions are usually formed in narrow specific concentration ranges as shown in Figure 2.4 (Chen, 1990; Blum et al., 1985).

![Phase Diagram of Microemulsions](image)

**Figure 2.4** Regions where microemulsions are found (adapted from Kumar and Mittal, 1999).

The range of microemulsion formation is mainly determined by the physico-chemical properties of the aqueous phase, oil phase and surfactants. The mechanism of microemulsion formation is complex. The following are a few essential conditions (Kreilgaard, 2002): (1) the production of a very low interfacial tension at the water-oil interface; (2) the formation of a highly fluid interfacial surfactant film; (3) the effective interaction between the alkyl group of the surfactant and the oil molecules.

### 2.2.3 Dynamic behaviour

Dynamic behaviour of microemulsions is important to help one understand the thermodynamic equilibrium of the system. Microemulsions are dynamic, self-organizing systems in which
aggregation/disintegration processes operate simultaneously. In this process, dynamic exchange of matter (water, counter ions, cosurfactant, and surfactant) between dispersed phases occurs continuously, resulting in an overall equilibrium (Moulik and Paul, 1998). There are continuous exchanges of components between droplets by two types of processes (Figure 2.5): (1) droplet collisions accompanied by temporary merging of the droplets into a larger droplet (fusion) followed by breakdown of this larger droplet (fission); (2) partial breakdown (or fragmentation) of droplets with loss of droplet fragments, which can later associate with other droplets (coagulation). The time between interdroplet exchanges is in the range of 1 to 20 milliseconds (Bagwe et al., 2001).

Figure 2.5 Dynamic behaviour of microemulsions (adapted from Bagwe et al., 2001).

2.2.4 Advantages and disadvantages

Microemulsions have several advantages as TDD vehicles as mentioned below (Heuschkel et al., 2008; Date et al., 2007; Kogan and Garti, 2006; Kreilgaard, 2002).
1. High solubilization capacity of both hydrophobic and hydrophilic drugs: the various structures of microemulsions enable the entrapment of both oil-soluble and water-soluble drugs, either alone or in combination, making them versatile carriers.

2. Thermodynamic stability: the thermodynamic stability of microemulsions helps in improving the shelf-life of the product making them carriers of choice.

3. Ease of manufacture and scale-up: the spontaneous formation of microemulsions makes their manufacturing and scale-up very easy as compared to other colloidal carriers, which require specialized instruments such as high-pressure homogenizers for their manufacturing.

4. Improved stability of active pharmaceutical ingredients: encapsulation of the drugs in the microemulsion structures can improve the chemical, photochemical, and enzymatic stability of therapeutic agents (Date et al., 200).

Other advantages of microemulsions include clarity, their ability to be filtered, and low viscosity.

In recent years, microemulsions have been extensively studied to improve the drug permeability across the stratum corneum (Peltola et al., 2003; Lee et al., 2003; Baroli et al., 2000; Delgado-Charro et al., 1997; Kriwet et al., 1995; Trotta et al., 1994; Boltri et al., 1994). These studies demonstrated that microemulsions significantly increased transdermal flux and the permeation coefficient through the skin, when compared to other colloidal systems and aqueous solutions (Kreilgaard et al., 2000; Bhatnagar et al., 1994; Willimann et al., 1992). Furthermore, it has been suggested that the surfactants and the oil from the microemulsion interact with the rigid lipid bilayer structure and act as a permeation enhancer (Schmalfu et al., 1997).

The disadvantages of microemulsions stem from the use of a large concentration of surfactant and cosurfactant necessary for stabilizing the nanodroplets. As mentioned earlier, high level of
surfactants and cosurfactants are always a hazard due to irritations to the skin. Moreover, microemulsion stability is influenced by environmental parameters such as temperature, pH. These parameters change upon microemulsion delivery to patients. In addition, the structure of a microemulsion may change if diluted with water after it is introduced into a physiological environment (Podlogar et al., 2005). Thus, the effect of such changes on microemulsion stability must be evaluated in the formation development process.

### 2.2.5 Formulation constraints

In terms of the pharmaceutical applications, the most significant problem associated with formulating microemulsions is the selection of a surfactant, cosurfactant and oil. The components, which are able to form microemulsions should be nontoxic and fulfill the requirements of a good TDD vehicle, i.e., high solubility and high thermodynamic activity of the drug.

#### 2.2.5.1 Oil phase

The choice of an oil used in microemulsion formulations is often a balance between its ability to solubilize the drug candidate and its ability to facilitate formation of microemulsions (structures and stability). In general, oil molecules with high molecular weight are able to dissolve lipophilic compounds of multiple sizes. However, oil molecules with small molecular weight produce microemulsions with high solubilization capacity (Warisnoicharoen et al., 2000; Malcolmson et al., 1998). A variety of fatty acid and their esters, medium-chain triglycerides, and propylene glycol esters of fatty acids have been employed as oil phases to facilitate microemulsions (Kogan and Garti, 2006).
In this study, isopropyl myristate (IPM) is used as the oil phase. IPM is a well known permeation enhancer in transdermal formulations (Kreilgaard, 2002), but the mechanism of its action is poorly understood (Kogan and Garti, 2006). For example, in some cases, IPM does not show a significant effect (Morimoto et al., 1993). Some preliminary consideration of different oils is included in Appendix 1.

2.2.5.2 Surfactants

A crucial point for pharmaceutical microemulsions is the choice of surfactants. In certain cases, surfactants, at higher concentrations, may cause skin irritation. Generally, surfactants derived from natural sources are preferred over synthetic surfactants - for example, phospholipids are preferred whenever possible because they are naturally present in cell membrane. Among synthetic surfactants, nonionic surfactants are preferred over cationic and anionic surfactants, as anionic and cationic surfactants result in membrane perturbation and skin irritation when compared to nonionic surfactants (Tenjarla, 1999). In general, the surfactant concentration should be kept to a minimum. Lecithin (phospholipids), nonionic surfactants, and polyglycerol esters of fatty acid are commonly used in transdermal formulations.

2.2.5.3 Cosurfactants

Most of the time, surfactant alone can not lower the oil/water interfacial tension sufficiently to yield a microemulsion. Liquid crystalline phases are often formed when the surfactant film is too rigid (Bagwe et al., 2001; Tenjarla, 1999). It is necessary to add cosolvents (amphiphilic short-chain molecules) or cosurfactants to bring the interfacial tension close to zero. The amphiphilic nature of these additives (with the length of the carbon chain ranging from C₂ to C₉) enables them to interact with surfactant molecules at the interface, thereby affecting their packing (Vandamme, 2002; Lawrence and Rees, 2000). Cosurfactants penetrate the surfactant monolayer
providing additional fluidity to the interfacial film by disrupting liquid crystalline phases. Commonly used cosurfactants include alcohols, derivatives of glycols, polyglycerols or propylene glycols.

2.2.5.4 Aqueous phase

Water serves as the aqueous phase in most of microemulsions for TDD. In some of the cases, phosphate buffer of pH 7.4 is used (Trotta et al., 2003; Delgado-Charro et al., 1997). Isotonic solution (0.9% NaCl) is normally used in microemulsion formulations for drug delivery purpose.

2.2.6 Lecithin microemulsions

Lecithin microemulsions are a good choice for drug delivery because lecithin (phospholipids) is a naturally occurring biological, nontoxic surfactant. Phospholipids are present in the cell membrane and can be found in all plants and animals. When administrated in optimum amounts even in high concentrations, they do not have the toxic and sensitivity problems associated with other surfactants. In general, phospholipids are recognized as penetration enhancers because the absorption of phospholipids on the skin can increase tissue hydration, consequently increasing drug permeation (Williams and Barry, 2004). When phospholipids are applied to the skin as delivery vehicles, they can fuse with stratum corneum lipids, perturb its structure and facilitate drug delivery (Williams and Barry, 2004; Kirjavainen et al., 1999). Therefore, lecithin is the ideal surfactant for preparing pharmaceutically acceptable microemulsions.

In this work, the term lecithin refers to a naturally occurring mixture of phospholipids, including phosphatidylcholine, phosphatidylethanolamine, and phosphotidylinositol. Soybeans are the most important source of commercial lecithin and crude soy oil contains an average of 1.2-3.2%
(Du Bois and Christine, 2008; Shurtleff, 2006). Unrefined/natural lecithin is obtained in the process of degumming vegetable oil and contains 65-70% phospholipids and 30-35% crude soy oil. The oil in unrefined lecithin can be removed by extraction with acetone to give a dry granular product called “refined lecithin”. The fatty acid composition of soy lecithin is in roughly the same proportion as in soy oil: saturated and unsaturated. The profile is approximately as follow: 11% palmitic acid (C16:0-the first number means the length of carbon chain and the second number means the number of unsaturated bonds), 4% stearic acid (C18:0), 22% oleic acid (C18:1), 54% linoleic (C18:2) and 7% linolenic (C18:3). For pharmaceutical applications and research purposes, refined grade lecithin may contain 60-99.7% phosphatidylcholine (Dyson, 1978).

However, it is difficult to produce microemulsions with lecithin alone. Chemically, lecithin is phosphatidylcholine. As shown in Figure 2.6, phosphatidylcholine molecules consist of a hydrophilic polar head containing one or more phosphate groups and a hydrophobic tail from two fatty acyl chains. They are too hydrophobic to spontaneously form zero curvature lipid layers required for the formation of balanced microemulsions (Tenjarla, 1999). When many phosphatidylcholine molecules are placed in water, they tend to self-assemble in rigid bilayers (Bagwe, 2001). As a result, it is easy to form gels or liquid crystals when formulating lecithin microemulsions.

To form lecithin-based microemulsions, it is necessary to adjust the HLB (hydrophilic lipophilic balance) of lecithin and to inhibit its tendency to form lamellar liquid crystalline phases. The HLB is an empirical scale of hydrophobicity. HLB values typically range from 0 to 40. The value of 40 is indicative of highly hydrophilic surfactant such as sodium dodecyl sulphate (Griffin, 1949). The HLB can be modified by adding short- or medium-chain alcohols (cosurfactants) that increase interfacial area per lipid polar group. The penetration of the
cosurfactants into the surfactant film produces a more fluid interface by allowing the long hydrophobic tails of lecithin to move freely at the oil/water interface in a microemulsion (Moreno et al., 2003; Aboofazeli et al., 1995 and 1993). Hence, short or medium-chain alcohols are commonly used in lecithin microemulsions to “soften” the surfactant membrane.

Figure 2.6 Chemical structure of phosphatidylcholine.

Several groups have reported the preparation of alcohol-based lecithin microemulsions and their phase properties. It was found that the existence of the microemulsion region depends on the lecithin-alcohol ratios and the type of alcohols used. Attwood and coworkers prepared o/w microemulsions using lecithin, butanol or ethanol as cosurfactant, IPM as oil, and water (Attwood et al., 1992; Friberg, 1990). The microemulsion regions for the butanol system were detected at a surfactant/cosurfactant weight ratio between 1:0.33 and 1:0.6. In comparison to the butanol systems it was found that at the same amount of IPM more ethanol was required in the ethanol systems. Larger amounts of ethanol are needed to reduce the rigidity of the lecithin condensed film, creating the curvature that is needed for droplet formation. Aboofazeli and Lawrence extended the studies of lecithin-alcohol microemulsions (Aboofazeli et al., 1993, 1994
They have reported the phase properties of lecithin/alcohols/water/IPM systems in which the alcohols were $n$-propanol, isopropanol, $n$-butanol, $s$-butanol, $t$-butanol, and $n$-pentanol.

The lecithin-alcohol microemulsions have been extensively studied as transdermal formulations for enhancing the penetration of a range of drugs (Changez et al., 2006; Paolino et al., 2002; Brime et al., 2002; Rhee et al., 2001; Kreilgaard et al., 2000; Trotta et al., 1994 and 1998; Dreher et al., 1997; Bonina et al., 1995; Aboofazeli et al., 1994). Microemulsions were compared to creams, gels, lamellar liquid crystals and liposomes, where all formulations contained phospholipids. Kriwet et al. found that microemulsions enhance drug permeability compared to liposomes and lamellar liquid crystals across the skin (Kriwet et al., 1995). Bonina et al., also showed that lecithin microemulsions led a better skin partitioning of drugs than conventional formulations like creams and gels (Bonina et al., 1995). Furthermore, good human skin tolerability of a lecithin-based o/w microemulsions compared to conventional vehicles (o/w and w/o cream and gel) was observed by Panolino et al. (Paolino et al., 2002). In addition, Dreher et al. obtained higher drug flux by lecithin microemulsion gels compared to the drug solution in the pure oil (Dreher et al., 1997). Changez et al. studied the effect of the composition of microemulsions on barrier properties of the skin. They suggested the lecithin microemulsions generate a hydration gradient across the skin accompanied by an increased intercellular space in the epidermis and dermis facilitating drug uptake (Changez et al., 2006).

Unfortunately, the applications of lecithin microemulsions in TDD have been limited due to the alcohols used in the formulations. On one hand, short- or medium-chain alcohols are well-known penetration enhancers that help the drug partition into the skin. On the other hand, these alcohols also penetrate the skin, denaturing the membrane and triggering immunological responses (irritation) (Higgins and Du, 1992). For example, alcohols can extract the skin lipids and their presence in the skin will influence the solubility properties of the intercellular channels of the
stratum corneum (Bommannan et al., 1991). These changes in barrier function might make skin more susceptible to damage.

2.3 LINKER MOLECULES IN MICROEMULSIONS

Linker molecules are additives that are used in microemulsion systems to enhance the interaction between the surfactant and oil or water phases. They are defined as amphiphiles that segregate near the oil/water interface either near the surfactant tail (lipophilic linkers) or the surfactant head group (hydrophilic linkers) (Sabatini et al, 2003).

2.3.1 Lipophilic linker

The concept of lipophilic linkers was introduced in the early 90’s, as additives that increase the surfactant-oil interaction and oil solubilization in microemulsions (Graciaa et al., 1993a). Lipophilic linker molecules are believed to orientate along the surfactant tails and promote orientation of oil molecules further into the oil phase (Graciaa et al., 1993a). Thus they serve as a link between oil molecules and the surfactant tails. Figure 2.7 shows a schematic of the lipophilic linker effect originally proposed by Graciaa et al., illustrating the proposed orientational effect caused by lipophilic linkers. Later it was found that the solubilization enhancement plateaus (saturates) after a certain lipophilic linker concentration (Salager et al., 1998).

Long-chain (＞9 carbons in the alkyl group) alcohols are often used as lipophilic linkers (Graciaa et al., 1993b; Acosta et al., 2003a and 2003b). Alternative lipophilic linkers include fatty acids or amines, nonionic surfactants with a low degree of ethoxylation and non-ethoxylated sorbitol esters (Acosta et al., 2005; Acosta et al., 2002a and 2002b; Uchiyama et al., 2000; Salager et al., 1998; Graciaa et al., 1993a and 1993b).
2.3.2 Hydrophilic linker

Since lipophilic linkers become saturated at high concentrations, hydrophilic linkers have been recently introduced to compensate for the saturation effect for lipophilic linkers (Uchiyama et al., 2000). Hydrophilic linkers are defined as surfactant-like molecules with six to nine carbons in their tail per head group, which usually have short hydrophobe and strong hydrophile (Acosta et al., 2002a). Figure 2.8 shows a schematic of the hydrophilic linker coadsorbing along with surfactant molecules at the oil/water interface. Hydrophilic linkers modify the oil/water interface by adsorbing at it. They increase the surfactant-water interaction, but have a poor interaction with the oil phase due to their short tail. It has been proposed that the role of the hydrophilic linkers is to open holes in the interface. Hydrophilic linkers are found to increase the interfacial tension, decrease the thickness of the interface, and reduce the rigidity of the surfactant film (Acosta et al., 2003b; Acosta et al., 2002a).
Currently there is a very short list of molecules that can be considered as hydrophilic linkers. Hydrophilic linkers reported in the literature include sodium mono- and dimethyl-naphthalene sulfonate (SMDNS) (Acosta et al., 2003a and 2003b; Uchiyama et al., 2000), sodium caprylate (Acosta et al., 2002a), and hexyl polyglucoside (Komesvarakul et al., 2006; Acosta et al., 2005).

2.3.3 Self-assembly between hydrophilic and lipophilic linkers

When combining lipophilic and hydrophilic linkers together, they behave as a self-assembled surfactant at the oil/water interface (Acosta et al., 2004). A special synergism emerges which further increases the oil solubilization enhancement over lipophilic linkers alone. According to the conceptual model (Figure 2.7), the hydrophilic linker opens up a space on the oil side available for the lipophilic linker to segregate near the interface. It has been reported that the hydrophilic linker alone does not increase the oil solubilization capacity (Uchiyama et al., 2000).
Upon combination with the lipophilic linker, the solubilization capacity of oil and water increases proportionally to the combined linker concentration.

Linker-based microemulsion systems have shown great potential for different applications mainly because of their flexibility in tailoring the surfactant and linkers according to the needs of specific applications. Thanks to their self-assembly, the combined hydrophilic and lipophilic linkers can replace the main surfactant to a certain degree (Acosta et al., 2004). Since linker molecules are normally less expensive than surfactants, the combined linker approach in microemulsions is more appropriate for various applications. Furthermore, this technique allows the use of more environmentally friendly additives (linkers). In fact, microemulsions of a variety of chlorinated hydrocarbons have been formulated using linker molecules for environmental remediation purposes (Acosta et al., 2002a and 2002b; Uchiyama et al., 2000). Similarly, microemulsions of long-chain triglyceride-based oils are also formulated using linker molecules in cosmetic formulations (Komesvarakul et al., 2006).

2.3.4 Difference with other additives

Microemulsion formulations often use additives such as hydrotropes, cosurfactants, cosolvents, and electrolytes to affect the HLB of the amphiphile at the interface. The spectrum of additives can range from very hydrophobic to very hydrophilic. Figure 2.9 illustrates the relative hydrophilic/lipophilic character of different types of additives relative to their location at the oil/water interface. The main difference between a linker and a cosurfactant is that the cosurfactant has substantial interactions with both oil and water molecules; while a linker segregate near the oil/water interface but only from one side of the interface (Sabatini et al., 2003).
Graciaa et al. demonstrated the role of alcohol as lipophilic linkers from cosolvents or cosurfactants (Graciaa et al., 1993b). The author correlated the impact of alcohol molecules at the microemulsion interface with the length (number of carbons) of the alcohol. Short-chain alcohols (C$_2$-C$_4$) show a cosolvent effect that helps decrease the surfactant-surfactant interaction. Medium-chain alcohols (C$_3$-C$_9$) are cosurfactants because they interact strongly with the oil but retain their absorption at the oil/water interface. In contrast, long-chain alcohols ($\geq$C$_{10}$) have an interaction with the oil molecules but don’t adsorb at the interface.

Moreover, hydrophilic linkers were found to have interfacial properties between hydrotropes and cosurfactants (Acosta et al., 2002a). The authors used three similar additives in the family of naphthalene sulfonates to form microemulsion systems, and the additives represented a hydrotrope, a lipophilic linker and a cosurfactant. The results show that the hydrotrope has poor surface activity and no impact on the oil solubilization capacity of the system. The hydrophilic linker demonstrates substantial surface activity and makes the middle phase membrane more hydrophilic. While alone, it does not increase the oil solubilization capacity; when combined

**Figure 2.9** Relative hydrophilic/lipophilic character of microemulsion additives (adapted from Acosta et al., 2002a).
with a lipophilic linker (dodecanol), they show a synergistic effect and increase the oil solubilization. The cosurfactant shows increased surface activity over the hydrophilic linker and increases the oil solubilization alone. Moreover, it does not show a synergistic effect with the lipophilic linker.

### 2.3.5 Linker-based lecithin microemulsions

Alcohol-free lecithin microemulsions as “green solvents” have been reported using biocompatible linker molecules (Acosta et al., 2005). Hexyl polyglucoside was used as the hydrophilic linker and sorbitan monooleate served as the lipophilic linker in the microemulsion systems (Figure 2.10). It was found that the linker-based lecithin formulations were able to form microemulsions without the use of alcohols while achieving high solubilization capacity with a wide range of oils. These systems have been proposed as alternatives to chlorinated solvents in dry-cleaning applications and solvent delivery systems for pharmaceutical applications. However, there have been no direct experiments studying their use in drug delivery. Furthermore, the hexyl polyglucoside is reported to be a laxative and is not an FDA-approved ingredient.

### 2.4 CONCLUSIONS

The use of alcohols in conventional lecithin microemulsions is a shortcoming to transdermal delivery vehicles. Linker-based lecithin formulations reported in literature provide a competitive alternative to conventional alcohol-based lecithin microemulsions. The main objective of this dissertation is to evaluate the use of linker-based lecithin microemulsions as TDD vehicles.
To advance the field, we formulated new lecithin microemulsions using linker molecules with Generally Recognized as Safe (GRAS) or food additive status (21CFR172.860, 21CFR172.863). By demonstrating the capability of the developed systems to deliver a lipophilic drug (lidocaine), we provide and evidence the opportunity of using alcohol-free microemulsions designed for transdermal applications with non-toxic surfactant, lecithin.

Figure 2.10 Schematic of the linker effect using surfactant lecithin (adapted from Acosta et al., 2005). Hexyl polyglucoside is shown as hydrophilic linker and sorbitan monooleate as lipophilic linker.
2.5 SCOPE

To develop new linker-based lecithin microemulsions and demonstrate their capacity to deliver an oil-soluble drug, lidocaine, four parts of research were completed in this dissertation:

1. Chapter 3 reports alcohol-free lecithin microemulsions formulated with linker molecules to act as transdermal delivery vehicles. The food-grade linker system consists of a combination of sodium caprylate and caprylic acid (hydrophilic linkers), and sorbitan monooleate (lipophilic linker). IPM was used a “carrier” oil for a lipophilic drug, lidocaine. By varying the sodium caprylate concentration in the formulations, Type II (w/o), Type IV (bicontinuous), and Type I (o/w) lecithin microemulsions were obtained. The newly formulated lecithin-linker systems have low viscosity (10-40 cP) and small particle size (~10 nm). The in vitro transdermal permeation studies show that, compared to conventional lecithin-alcohol microemulsions, the newly formulated lecithin-linker microemulsions provide higher drug flux across the skin, higher drug absorption in the skin, and higher “skin” permeability. Furthermore, the in vitro cytotoxicity studies show that the linker-based microemulsions are significantly less toxic than the alcohol-based systems.

2. Chapter 4 takes advantage of the increased drug absorption found in Chapter 3 to produce an extended release profile where drug-loaded skin is used as in situ patches. The in situ patches after applying the lecithin-linker microemulsions are able to release 90% of their content over a 24 h period which rivals the performance of some polymer-based patches. Fluorescence micrographs of transversal cuts of skin loaded with Nile red are consistent with the observed release profiles.

3. Chapter 5 describes the impact of surfactant concentrations on transdermal performance of the studied lecithin-linker systems. As mentioned earlier, the use of microemulsions
has been limited in drug delivery due to large amounts of surfactants required in formulations. This chapter includes data on the *in vitro* permeation and absorption of lidocaine from the linker-based microemulsions as a function of surfactant concentrations. The results have shown that increasing surfactant concentration improves the drug absorption in the skin. Overall transdermal flux is found to increase as the surfactant concentration increases up to a point where a plateau is reached (saturation). In general, an optimal surfactant concentration exists in the linker-based lecithin microemulsions to provide high drug absorption in the skin and a maximum transdermal flux.

4. A differential mass balance model is proposed in Chapter 4 and 5 to describe the drug release profile from microemulsions to the skin. Chapter 4 presents the drug transfer from the skin (*in situ* patch) to the receiver follows a first order release that depends on the mass of drug initially loaded in the skin and a mass transfer coefficient. Chapter 5 extends the mass balance model from the donor (microemulsions) to the receiver by understanding the role of surfactants in the transport of drugs through the skin. The interdependence among different permeation parameters is illustrated.

Overall, the capacity of the new linker-based lecithin microemulsions as potentially safe and effective TDD vehicles is demonstrated in this dissertation. The lecithin-linker systems are proven to be less toxic and provide higher flux than the conventional lecithin-alcohol microemulsions. Moreover, *in situ* patches can be formed after applying linker-based microemulsions to provide extended release up to 24 h. There is an optimum surfactant amount in the linker-based lecithin microemulsions to achieve high enough permeation flux but with minimal cytotoxicity.
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CHAPTER 3

LINKER-BASED LECITHIN MICROEMULSIONS

FOR TRANSDERMAL DELIVERY OF LIDOCAINE

This chapter is derived from the following manuscript:

3.1 ABSTRACT

In this work, we introduce alcohol-free lecithin microemulsions formulated with linkers to produce transdermal delivery vehicles. The food-grade linker system consists of a combination of sodium caprylate and caprylic acid (hydrophilic linkers), and sorbitan monooleate (lipophilic linker). A “carrier” oil, isopropyl myristate (IPM), was used to predissolve a model lipophilic drug, lidocaine. The first part of the article describes the phase behavior and physical properties of these linker-based lecithin microemulsions. In the second part of the article, we evaluate the transdermal permeation and cytotoxicity of lidocaine formulated in oil-in-water (Type I), water-in-oil (Type II), and bicontinuous (Type IV) linker microemulsions. The transdermal permeation studies show that, compared to a conventional Type II alcohol-based lecithin microemulsion, Type II linker-based microemulsions provide twice the absorption and penetration of lidocaine through skin. The larger flux obtained with linker systems is due to the presence of sodium caprylate and caprylic acid. These hydrophilic linkers accelerate the microemulsion-skin mass transfer by reducing the interfacial rigidity of the systems. Furthermore, the cytotoxicity studies show that these linker microemulsions are significantly less toxic than the alcohol-based system. The Type II linker microemulsion (containing approximately 4% lidocaine) has a comparable cytotoxicity to water saturated with lidocaine (0.4% lidocaine).

3.2 INTRODUCTION

Transdermal drug delivery (TDD) is a convenient method of drug administration enabling physicians to provide controlled delivery of drugs to patients with minimum discomfort. Compared to oral and parenteral routes, the transdermal route of drug administration has the
advantages of reducing gastrointestinal side effects and reducing drug degradation (Amann and Osborne, 1990; Bronaugh and Maibach, 2002; Guy and Hadgraft, 2003). However, the poor permeability of the stratum corneum often limits the topical administration of novel drug formulations (Walters, 2002; Michaels et al., 1975). There are various chemical and physical methods to promote transdermal drug permeation through the disruption of the skin barrier. One such method is the use of permeation enhancers (Asbill, 2000a) such as solvents or surfactants that, although effective, tend to produce allergic reactions, skin irritation, and sensitization (Welss et al., 2004). The main challenge for many transdermal formulations is to effectively increase the permeability of the active ingredient through the stratum corneum while avoiding allergic reactions, skin irritation, or skin sensitization.

In recent years, microemulsions have emerged as potential TDD vehicles for delivering drugs through the skin (Bagwe et al., 2001; Kreilgaard, 2002; Lawrence and Rees, 2000; Tenjarla, 1999). Lecithin microemulsions are especially desirable because lecithin is a surfactant with Generally Recognized as Safe (GRAS) status. However, because of the tendency of lecithin to form lamellar and other liquid-crystal phases, it is necessary to use medium-chain alcohols as cosurfactants to prevent the formation of those undesirable phases and promote the formation of microemulsions (Tenjarla, 1999). Lecithin microemulsions containing pharmaceutically-acceptable oils, such as IPM, have been formulated at several surfactant/cosurfactant mixing ratios (Aboofazeli and Lawrence, 1993; Corswant and Söderman, 1998a; Saint Ruth et al., 1995). Recent studies suggested that lecithin microemulsions produce enhanced transdermal flux for oil-soluble drugs (Corswant et al., 1998b; Dreher et al., 1997; Paolino et al. 2002; Peltola et al., 2003). Unfortunately, medium-chain alcohols such as butanol and pentanol used in these formulations tend to dissolve cell membranes (McKarns et al, 1997) rendering the formulations cytotoxic, which is a common shortcoming of microemulsion formulas (Bommannan et al., 1991;
One alternative to alcohol-based formulations is the use of polymeric additives such as polyethylene glycol (PEG) (Corswant and Söderman, 1998a, US patent 6,638,537 B2). While more effective and less toxic than alcohol-based formulas, polymer-based formulations have a relatively high viscosity (this is desired in some, but not all formulations).

With the introduction of linker molecules, there is now a second alternative to produce alcohol-free microemulsion systems. It has been found that linker-based microemulsions with lecithin have an exceptional solubilization capacity for a wide range of oils (Acosta et al., 2005). In those linker-based lecithin microemulsions, sorbitan monooleate was used as lipophilic linker and hexyl polyglucoside as hydrophilic linker. It was proposed that this linker system should mitigate the toxicity concerns of lecithin formulations and act as a potential delivery vehicle. However, there have been no previous reports on the use of linker-based lecithin microemulsions as TDD vehicles, or any previous cytotoxicity studies of these systems.

In this work, we hypothesized that linker-based lecithin microemulsions can be formulated as effective and potentially safe TDD vehicles for poorly water-soluble drugs. To produce such formulations, we used the base of lecithin-linker formulation in the previous study (Acosta et al., 2005), but we replaced the hydrophilic linker hexyl polyglucoside (not FDA-approved) by a mixture of sodium caprylate and caprylic acid that have food additive status (21CFR172.860, 21CFR172.863). Figure 3.1 shows a schematic of surfactant and linker-self assembly at the oil/water interface. The toxicological information of the substances used in this work is shown in Table 3.1.
Figure 3.1 Schematic of the linker effect using surfactant lecithin, hydrophilic linker sodium caprylate and caprylic acid, and lipophilic linker sorbitan monooleate.

Table 3.1 Toxicological information for the materials used in microemulsions

<table>
<thead>
<tr>
<th>Function</th>
<th>Material</th>
<th>Toxicity Data</th>
<th>Irritation Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surfactant</td>
<td>Lecithin</td>
<td>GRAS</td>
<td>Not available</td>
</tr>
<tr>
<td>Linkers</td>
<td>Sodium caprylate</td>
<td>Food additive status</td>
<td>Not available</td>
</tr>
<tr>
<td></td>
<td>Caprylic acid</td>
<td>Food additive status, Oral, mouse, LD50</td>
<td>Skin, rabbit, 500 mg/24h, mild irritation effect</td>
</tr>
<tr>
<td></td>
<td>Sorbitan monooleate</td>
<td>GRAS</td>
<td>Not available</td>
</tr>
<tr>
<td>Cosurfactant</td>
<td>Pentanol</td>
<td>Oral, mouse, LD50 200 mg/kg</td>
<td>Skin, rabbit, 20 mg/24h, moderate irritation effect</td>
</tr>
<tr>
<td>Oil</td>
<td>IPM</td>
<td>Oral, mouse, LD50 49700 mg/kg</td>
<td>Skin, rabbit, 426 mg/24h, mild irritation effect</td>
</tr>
<tr>
<td>Drug</td>
<td>Lidocaine</td>
<td>Oral, mouse, LD50 220 mg/kg</td>
<td>Not available</td>
</tr>
</tbody>
</table>

Notes: GRAS status according to 21 CFR part 184, toxicity and irritation data obtained from the MSDS of each component supplied by Sigma-Aldrich.
To evaluate the performance of linker-based lecithin microemulsions as TDD vehicles, a lipophilic drug, lidocaine, was chosen as a model drug in this work. Lidocaine is an anesthetic that has been used in topical formulations as a pain reliever in the treatment of minor burns, sunburn, insect bites and after various laser skin surgeries (Tetzlaff, 2000; Jesitus, 2001). The transdermal delivery of lidocaine is significantly limited by its poor water solubility (4 mg/ml). Most of the existing lidocaine delivery systems in the marketplace are emulsion-based, such as EMLA® cream. EMLA® cream and similar emulsions remain the treatment of choice because of their low toxicity (Friedman et al, 2001). It has been shown that microemulsion-based gels for lidocaine delivery tend to have a slightly longer lasting effect than emulsion-based systems (Lee, M-W 2003), and produce nearly 50% -100% larger fluxes of lidocaine compared to EMLA® cream (Kreilgaard, M., 2002; Syntov and Shapiro, 2004). However the benefits of potentially larger fluxes obtained with microemulsions do not compensate for the increase in cytotoxic side effects (Changez et al, 2006; Kreilgaard, 2002). Because of the limited capacity to deliver lidocaine and analgesics to the site of action, repeated dosing needs to be applied (Lee, M-W 2003; Thomas and Finnin, 2004). In various instances, re-applying the dose represents a painful process in itself because of the mechanical occlusion (massaging the skin) required to obtain the reported levels of lidocaine penetration with emulsion formulations (Lee, M-W 2003).

The linker-based lecithin microemulsion systems described in this work are designed to minimize the aforementioned cytotoxic side effects and yield comparable or higher lidocaine transdermal fluxes and lidocaine skin absorption than conventional microemulsions formulated with medium-chain alcohols.

Three specific objectives were addressed in this work. First, we studied the phase behavior of linker-based lecithin microemulsions and the properties of the Type I, IV, and II microemulsions obtained. Second, the effectiveness of selected Type I, II, and IV linker-based lecithin
microemulsions, and a Type II pentanol-based lecithin microemulsion as TDD vehicles was investigated using in vitro permeation tests. Finally, the in vitro cell viability of skin tissue cultures exposed to the microemulsion vehicles was evaluated using the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay.

3.3 MATERIALS AND METHODS

3.3.1 Materials

3.3.1.1 Chemicals

The following chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) at the concentrations shown in parentheses, and were used as received: sorbitan monooleate (Span® 80, 99%+), sodium caprylate (99%+), caprylic acid (99%+), isopropyl myristate (IPM, 98%), sodium chloride (99%+, Fluka brand), 1-pentanol (99%+), Dulbecco’s phosphate buffered saline (PBS) and lidocaine powder (base form, 98%+). Laboratory grade soybean lecithin (99%+) was purchased from Fisher Scientific (Fairlawn, NJ, USA). Soybean lecithin is a mixture of phospholipids (mainly phosphatidyl cholines) produced by acetone purification of soybean gum residues. Sodium phosphate monobasic, monohydrate (ACS grade) and acetonitrile (HPLC grade) were purchased from EMD Chemicals Inc. (Darmstadt, Germany), and they were used as received. Anhydrous ethyl alcohol was purchased from Commercial Alcohols Inc. (Brampton, ON, Canada). Unless otherwise stated, the composition is expressed on a mass basis (i.e. % wt.) throughout this paper.
3.3.1.2 Skin

Two types of skin models were used. Reconstructed human skin models, EpiDerm™ EPI-200 were purchased from MatTek Corporation (Ashland, MA, USA). The skin tissue was artificially derived from normal human epidermal keratinocytes (Faller et al., 2002). The tissues were stored in a refrigerator at 4°C and used within three days after being received. Pig ear skin from adult domestic pigs (approximately 6 months old) was studied as a surrogate for human epidermis. Pig ears were obtained from the local market and frozen overnight. Prior to use, they were thawed by rinsing with running water for ten seconds at room temperature. The skin of the external side of the ear was then dermatomed to a thickness that ranged from 700 to 900 µm (Bronaugh and Maibach, 1991). After that, the thin skin layer was cut in circles of 7.4 mm diameter ready for use.

3.3.2 Microemulsion preparation

Phase behaviour studies were performed using equal volumes of aqueous solution and oil (5 ml of each) in flat bottom test tubes. To obtain a phase transition of Winsor Type II - Type III or IV - Type I, the concentration of sodium caprylate was gradually increased (using a separate test tube for each concentration increment) while maintaining constant temperature (23±1°C – room temperature, unless stated otherwise), electrolyte concentration (0.9% NaCl in the aqueous solution) and pressure (1 atm). This procedure will be referred to as a hydrophilic linker (sodium caprylate) scan (Acosta et al., 2005). After introducing all the ingredients, the test tubes were thoroughly vortexed, then vortexed once a day for three days, and left to equilibrate for two weeks. After these systems reached equilibrium, the microemulsion volume fraction was calculated by measuring the volume of the microemulsion and excess phase(s) (if any) in the test
tube (Acosta et al., 2005). Selected systems were left to equilibrate in a water bath at 37°C with the objective of determining the phase behavior at body temperature. The pH of all microemulsions systems was 5.5±0.5.

Table 3.2 (a) The compositions of the linker microemulsions with different sorbitan monooleate to lecithin ratios (%w/w).

<table>
<thead>
<tr>
<th>Series</th>
<th>% LE</th>
<th>% SM</th>
<th>% CA</th>
<th>% SC</th>
<th>%NaCl</th>
<th>% water</th>
<th>% IPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>4.0</td>
<td>8.0</td>
<td>n/a</td>
<td>0.4</td>
<td>0.8</td>
<td>1.2</td>
<td>0.9</td>
</tr>
<tr>
<td>B</td>
<td>4.0</td>
<td>12.0</td>
<td>0.3</td>
<td>0.8</td>
<td>2.4</td>
<td>0.6</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>1.2</td>
<td>0.3</td>
<td>1.2</td>
<td>3.6</td>
<td>0.9</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>1.6</td>
<td>4.8</td>
<td>1.2</td>
<td>2.0</td>
<td>6.0</td>
<td>1.5</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>2.4</td>
<td>7.2</td>
<td>1.8</td>
<td>2.8</td>
<td>8.4</td>
<td>2.1</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td>9.8</td>
<td>2.4</td>
<td>3.6</td>
<td>10.8</td>
<td>2.7</td>
<td>0.9</td>
</tr>
<tr>
<td></td>
<td>4.0</td>
<td>12.0</td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
<td>0.5 ~ 7.0</td>
</tr>
</tbody>
</table>

(b) The compositions of the selected microemulsion formulations (%w/w).

<table>
<thead>
<tr>
<th>Formulation</th>
<th>% LE</th>
<th>% SM</th>
<th>% CA</th>
<th>% SC</th>
<th>%NaCl</th>
<th>% water</th>
<th>% IPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Type II</td>
<td>4.0</td>
<td>12.0</td>
<td>3.0</td>
<td>1.0</td>
<td>0.9</td>
<td>41.1</td>
<td>38</td>
</tr>
<tr>
<td>L-Type IV</td>
<td>4.0</td>
<td>12.0</td>
<td>3.0</td>
<td>4.0</td>
<td>0.9</td>
<td>38.1</td>
<td>38</td>
</tr>
<tr>
<td>L-Type I</td>
<td>4.0</td>
<td>12.0</td>
<td>3.0</td>
<td>7.0</td>
<td>0.9</td>
<td>35.1</td>
<td>38</td>
</tr>
</tbody>
</table>

% Pentanol

<table>
<thead>
<tr>
<th>Formulation</th>
<th>% LE</th>
<th>% SM</th>
<th>% CA</th>
<th>% SC</th>
<th>%NaCl</th>
<th>% water</th>
<th>% IPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-Type II</td>
<td>4.0</td>
<td>12.0</td>
<td>8.0</td>
<td>0.9</td>
<td>37.1</td>
<td>38</td>
<td></td>
</tr>
</tbody>
</table>

LE – lecithin  SM - sorbitan monooleate  CA - caprylic acid  SC - sodium caprylate
Sorbitan monooleate to lecithin weight ratios of 1:1, 2:1 and 3:1 were investigated. Using a sorbitan monooleate to lecithin ratio of 1:1, microemulsions could not be formed; instead, highly viscous liquid crystalline phases were found. Table 3.2a presents a summary of the formulations considered in this study. In series A, the sorbitan monooleate to lecithin ratio was 2:1. In series B, a sorbitan monooleate to lecithin mass ratio of 3:1 was used. In series L, the 3:1 sorbitan monooleate to lecithin mass ratio was kept, but caprylic acid was introduced to facilitate the formation of Type III/IV microemulsions. For each series, sodium caprylate scans were performed for phase behaviour studies. A lecithin concentration of 4% was used in Series A and B, while different lecithin concentration from 0.4% to 4% were investigated in Series L.

A pentanol-based lecithin microemulsion was formulated using 4% lecithin, 12% sorbitan monooleate, and 0.9% NaCl. The idea of keeping the base lecithin formulation the same as in one of Series L, but replacing the hydrophilic linker mixture (sodium caprylate plus caprylic acid) by pentanol was to evaluate the difference between a fully formulated linker system and a system that resembles the more conventional formulation approach of using medium-chain alcohols as cosurfactants. Unfortunately, this strategy did not yield a microemulsion phase transition (II-IV-I) for the pentanol series. A value of 8% pentanol in the mixture (which produces a Type II microemulsion) was fixed for comparative studies with linker-based lecithin microemulsions.

Lidocaine was introduced in the formulations by predissolving this drug in IPM to a concentration of 10% (10% lido-IPM). In this study, all microemulsions were loaded with lidocaine, unless otherwise stated. After the microemulsion formulated with the lidocaine-IPM mixture reached equilibrium (for Type I and Type II systems), the lidocaine that partitioned into the microemulsion and the excess phase was measured using high performance liquid chromatography as explained below.
3.3.3 Physicochemical characterization

Dynamic light scattering measurements of the hydrodynamic radius and polydispersity of the microemulsion droplets were performed at room temperature (23±1°C) using a BI-200SM Brookhaven instrument equipped with a 35 mW green laser (wavelength ~514 nm) and a photomultiplier detector located at a fixed angle of 90°. Microemulsion samples were poured into standard glass cells that were placed in the BI-200SM sample holder at least 10 minutes before measurement. Refractive indexes for the continuous phase were obtained from the literature (CRC Handbook, 1988). The viscosity of the samples was measured (in triplicate) at room temperature using a CV-2200 falling ball viscometer (Gilmont Instruments, Barrington, IL, USA).

3.3.4 Lidocaine partitioning studies

After equilibration, the microemulsion and the excess phases in a test tube were sampled using a gas-tight syringe. To collect the sample of the lower phase, a gap of air was left in the syringe as the needle penetrated the upper phase. Once in the lower phase the air bubble was pushed out and the sample was taken. The lidocaine concentration in each phase was measured using the HPLC method described in section 3.3.7.

3.3.5 In vitro permeation studies

The transdermal flux and drug permeability from test formulations were measured in vitro using reconstructed human skin EpiDerm™ EPI-200 and pig ear skin. All permeation experiments were performed according to the MatTek standard percutaneous absorption protocol. Briefly, the
model skin was placed in a MatTek Permeation Device (MPD), with the epidermis facing up. The microemulsion formulation (0.4 ml) was applied in the donor compartment. The receptor compartment was filled with 5 ml of PBS (0.01M phosphate, 0.137M NaCl, pH 7.4). At predetermined times (0.5 h, 1.5 h, 2.5 h, 3.5 h, 4.5 h and 5.5 h), the receiver solution was withdrawn completely from the receptor compartment and was immediately replaced by fresh PBS solution. At 5.5 h, the experiment was terminated. All permeation experiments were conducted in triplicate at room temperature. The MatTek human skin remaining after in vitro permeation study was used for in vitro cytotoxicity study, while the pig skin remaining after the permeation study was used to test the lidocaine absorbed into the skin.

Prior to measuring the absorbed lidocaine, the pig skin was rinsed with a PBS solution and placed into 2 ml methanol for overnight extraction of lidocaine. The equivalent lidocaine concentration absorbed in skin was calculated as the mass of lidocaine extracted from the skin divided by the volume of the skin (exposed area×thickness), and is expressed in mg/ml. We have determined, using a mass balance closure in selected samples, that the efficiency of this methanol extraction procedure is more than 95%.

The cumulative mass of lidocaine (µg) permeated across the skin was plotted as a function of time (h), and the average steady-state flux (J, µg/h/cm²) was calculated by dividing the slope of the linear part of the curve (dm/dt) by the area of the exposed skin surface (A). The apparent lidocaine permeability (k_p, cm/h) is calculated as \( k_p = \frac{J}{\Delta C_v} \), where \( \Delta C_v \) is the lidocaine concentration difference between the donor and receptor. While the concentration of lidocaine in donor and receptor varies as a function of time, we calculated \( \Delta C_v \) as the difference between the initial concentration of the drug in the donor minus the final concentration of the drug in the receiver after each time interval. This approximation represents an error of less than 5% for most cases. Equation 3.1 reflects the flux and permeability described above.
In vitro cytotoxicity studies

The MTT cell viability assay was performed on the reconstructed human skin EpiDerm™ EPI-200 as described in the standard MTT-ET-50 protocol provided by MatTek (Faller et al., 2002). In this method, the yellowish MTT indicator is transformed into an insoluble purple formazan by mitochondrial dehydrogenases of living cells. At the end of the exposure period during the permeation studies (5.5 h), the EpiDerm skin model was removed from the MPD, and incubated with 1 mg/ml MTT for 3 h to form formazan. The water-insoluble formazan was then extracted and analyzed spectrophotometrically. In this assay, 1% (w/v) Triton X-100 was used as the positive control and PBS was used as a negative (non-toxic) control. The cell viability is calculated as the ratio of optical density of the sample divided by the optical density of the negative control.

3.3.7 Lidocaine quantification

The concentration of lidocaine in the microemulsion, the excess phase, donor solutions and skin was quantified using high performance liquid chromatography (Shimadzu HPLC equipped with a Perkin Elmer LC235C Diode Array Detector, SIL-10AP autosampler, a 20 μl loop, and a 200LC pump) with a reverse phase column (Genesis, C₁₈, 4 μm, 150×4.6 mm). The mobile phase consisted of acetonitrile-0.05 M NaH₂PO₄·H₂O (pH 2.0) (30:70, v/v) and the measurement was conducted under isocratic conditions (1.0 ml/min). The UV detector was set to 230 nm. The retention time of lidocaine under these conditions was approximately 2.5 minutes. The peak area
correlated linearly with the concentration of lidocaine \((R^2=0.9998)\) in the range of 0.05-600 μg/ml. Limit of quantitation was 0.05 μg/ml; coefficient of variation (CV) was 1.0% at 2 μg/ml.

Lidocaine in receiver solutions was assayed by a UV spectrophotometer (Ultrospec Plus, Amersham Pharmacia Biotech, USA). Receiver solutions were diluted with methanol and the absorbance at 230 nm was measured. A linear calibration curve for lidocaine was obtained at 230 nm in the range of 0.01-100 μg/ml with a correlation \((R^2)\) of 0.999. Randomly selected samples of the receiver were also measured using the HPLC method. The difference between these two methods was <10%.

### 3.3.8 Statistical data analysis

All transdermal permeation values were calculated from three independent experiments, and data are expressed as the mean value ± S.D. Statistical analysis of \(J\) and \(k_p\), as well as cytotoxicity values, was performed using a one-way analysis of variance (ANOVA) to test the difference between the means of two or more delivery systems. Data with \(P<0.05\) are considered statistically significant.

### 3.4 RESULTS AND DISCUSSION

#### 3.4.1 Phase behaviour

3.4.1.1 Sodium caprylate scans

The composition of linker-based microemulsion systems is shown in Table 3.2. Series A and B were formulated with 4% lecithin using a sorbitan monooleate to lecithin ratio of 2:1 and 3:1,
respectively. For Series A, the transition from Type II to Type I occurs when the sodium caprylate concentration increases from 3.5% to 4%. For Series B, the Type II–I transition occurs when the sodium caprylate concentration increases from 4% to 4.5%.

The observations discussed above lead us to infer that scanning the sodium caprylate concentration is an effective method to formulate linker-based lecithin microemulsions. One could fix the desired lecithin and lipophilic linker concentration, electrolyte composition, drug and oil and simply adjust the sodium caprylate concentration to obtain the Type II-I transition. However, the drawback of sodium caprylate formulations is that, when approaching the phase inversion (II-I), stable gel-like emulsions were found and there were no Winsor Type III or IV microemulsions formed. The appearance of these metastable gel phases was more severe for systems enriched in lecithin (series A).

One of the roles of hydrophilic linkers is to prevent the formation of gel and liquid crystal phases. These results suggest that sodium caprylate alone is not an effective hydrophilic linker. The initial selection of sodium caprylate as an alternative to hexyl polyglucoside (Acosta et al., 2005) was based on a previous study that suggested that sodium caprylate could be used as hydrophilic linker (Acosta et al., 2002). However, the same study proposes that the effectiveness of a hydrophilic linker depends on the degree to which this linker participates at the oil/water interface. Unfortunately, sodium caprylate is highly hydrophilic and tends to be more water soluble (i.e. higher critical micelle concentration, CMC) than other hydrophilic linkers, suggesting that caprylate does not effectively coadsorb with lecithin at the oil/water interface. The simplest way to reduce the hydrophilicity of sodium caprylate is by reducing its degree of saponification. One way to simulate partially saponified caprylic acid is by using a mixture of sodium caprylate and caprylic acid as hydrophilic linkers. This is evaluated in the next section.
3.4.1.2 Sodium caprylate plus caprylic acid

Series L (Table 3.2a) incorporates a mixture of caprylic acid and sodium caprylate as hydrophilic linkers. With the addition of caprylic acid, the phase behaviour of the Series L formulations with same lecithin concentration (i.e. 4%) was significantly improved when compared to Series A and B. When conducting the sodium caprylate scan, a clear Type II – Type IV – Type I phase transition was achieved in the L-Series with 4% lecithin. The presence of caprylic acid helps to balance the hydrophilicity of the formulation. Figure 3.1 shows a hypothetical schematic of surfactant and linker-self assembly at the oil/water interface. According to this schematic, the surfactant molecules (lecithin, shown as phosphatidylcholine) are present at the interface with the hydrophilic group (surfactant head) in the water and the hydrophobic group (surfactant tail) in the oil. The lipophilic linker molecules (sorbitan monooleate) orientate along the surfactant tail to increase oil solubilization. The hydrophilic linker molecules (the combination of sodium caprylate and caprylic acid) coadsorb along with the surfactant head, increasing the interfacial area. It should be noted, however, that this schematic proposed by Acosta et al. (Acosta et al., 2002) has not been fully confirmed experimentally.

Figure 3.2b illustrates the phase behavior for the L-Series formulated with 4% lecithin. As the concentration of sodium caprylate increases from 0.5% to 3%, the volume fraction of the excess water reduces due to the increased solubilization of water in reverse micelles. At 3.5% and 4% sodium caprylate, clear Type IV bicontinuous phases are formed with no excess phases. At concentrations of sodium caprylate of 4.5% and higher, Type I microemulsions are produced.
Figure 3.2 Phase behaviour of Series L (linker) formulations containing lidocaine at room temperature and 0.9% NaCl. (a) Ternary phase diagrams of the L-Type II, L-Type IV, and L-Type I formulations, indicating the presence of one phase (1p), two phases (2p\textsubscript{m,o} and 2p\textsubscript{m,w}), and three coexisting phases (3p\textsubscript{m,o,w}). The subscript m, o, and w stand for microemulsion phase enriched with surfactant, excess oil phase and excess aqueous phase, respectively. The point “Δ” indicates the exact composition in the corresponding test tube. (b) The Type II-IV-I phase transition for the L-Series with 4% lecithin. (c) Phase map of Series L with different lecithin concentrations. The dashed line indicates the optimum formulation, and the dotted line indicates a dilution path with water.
Linker-based lecithin microemulsions produce a wide range of formulations (Figure 3.2b alone shows 14 different formulations to choose from), however, only three representative samples of the L-Series with 4% lecithin were selected to generate ternary phase diagrams (Figure 3.2a). The same samples are later used for in vitro permeation studies. These samples were L-Type II, L-Type IV and L-Type I containing 1%, 4%, and 7% sodium caprylate, respectively.

The ternary phase diagrams presented in Figure 3.2a show that the surfactant mixture is not completely soluble in either water or IPM. This is explained on the basis that the lipophilic linker sorbitan monooleate is soluble in oil but is not soluble in water; conversely, sodium caprylate is soluble in water but not in IPM. Furthermore, Figure 3.2a shows that the formulations L-Type II and L-Type I are saturated with oil and water respectively, however, the L-Type IV formulation is located above the saturation line (no excess phases). These observations reflect the fact that the L-series formulation is optimized for the production of Type IV microemulsions.

To investigate the effect of lecithin concentration on these phase transitions, a “phase map” of Series L is presented in Figure 3.2c. The amount of sodium caprylate that produces the phase transitions is plotted for formulations with lecithin concentrations ranging from 0.4% to 4%. Increasing the sodium caprylate concentration (sodium caprylate scan) produces a Type II - Type III or IV - Type I phase transition in all Series L formulations. As the lecithin concentration increases, more sodium caprylate is required to reach this phase transition. For example, the Type II - III and Type III - I transitions occur at 1.6 and 2.0 % sodium caprylate, respectively, for a 1.2% lecithin formulation. Furthermore, according to Figure 3.2c the saturated Type IV microemulsion contains 2.8% lecithin, 3% sodium caprylate and 8.4% sorbitan monooleate. This system requires less than half the lecithin that standard lecithin-polyethyleneglycol-ethanol systems do (Corswant et al., 1998a), and avoids the need for alcohol as cosurfactant.
Another important feature of Figure 3.2c is that it can be used to predict the effect of dilution on the potential for phase transition. The dotted dilution line in Figure 3.2c indicates that a Type I microemulsion will, upon dilution with water, undergo a Type I - III - II phase transition; while a Type II microemulsion phase will not experience any significant change upon dilution. Dilution is an important factor in parenteral and oral drug delivery, where dilution factors as large as 500 are common. However, in transdermal delivery, dilution plays less of a role since the volume of “free” water in the skin is relatively small.

3.4.1.3 Effect of lidocaine, temperature, and electrolyte

Figure 3.3 presents the phase volumes for the L-Series with 4% lecithin as a function of the sodium caprylate concentration, in the presence and absence of lidocaine. The drug was dissolved in the Type II and I systems formulated with 10% lido-IPM, and the actual drug concentration in each system can be found in Figure 3.5. From Figure 3.3, in the presence of lidocaine, the Type II - IV - I transition occurs at lower sodium caprylate concentrations.

This observation can be explained by the fact that lidocaine itself is polar (Attwood, 1983). Because of its polarity, lidocaine may interact with the surfactant and linkers near the oil/water interface and increase the hydrophilicity of the oil phase, such that less hydrophilic linker (sodium caprylate) is required to match the hydrophilicity/hydrophobicity of the oil phase. Other researchers have also found that, depending on the physico-chemical properties of the constituents and composition, the phase behavior of microemulsions may be affected by the addition of drugs (Carlfors et al., 1991; Malcolmson et al., 2002).
Figure 3.3 The Type II-IV-I phase transition for the L-Series with 4% lecithin in the presence and absence of lidocaine at room temperature. The Type IV phases are formed between Type II and I for both cases. In the presence of lidocaine, Type IV systems are obtained when the concentration of sodium caprylate is 3.5-4.5%; In the absence of lidocaine, Type IV are formed when the concentration of sodium caprylate is 4.5-5.5%.

Similar phase behavior studies to the one presented in Figure 3.3 were carried out to evaluate the effect of electrolyte and temperature. The L-Series with 4% lecithin (Figure 3.2b) were prepared in the absence and presence of electrolyte (0.9% NaCl); however, no significant difference in the phase volumes (compared to Figure 3.2b) was observed. Similarly, when these formulations were produced at 37°C, there were no significant changes in phase volumes. This electrolyte and temperature insensitivity is a desirable feature in drug delivery vehicles. In the literature, it has been suggested that lecithin, being a zwitterionic surfactant, is responsible for the insensitivity to electrolyte concentration and temperature (Acosta et al., 2005).
3.4.2 Physicochemical characterization

3.4.2.1 Viscosity

Figure 3.4a presents the viscosity of the microemulsions in the L-Series with 4% lecithin at room temperature as a function of the sodium caprylate concentration. For Winsor Type II microemulsions viscosity values are close to 2-5 times the viscosity of pure IPM (~5 cP), while in the case of Winsor Type I microemulsions viscosity values are between 30 to 40 cP. An increase in viscosity occurs upon approaching the Winsor Type IV phase in either Type II or I phase, but the viscosity is slightly lower for Type IV systems. The viscosity peak that appears at the Type IV-I transition has been attributed to a clustering of oil-swollen micelles (Kumar and Mittal, 1999). The low viscosity (10 to 40 cP) of these linker formulations has been reported for other microemulsion systems (Kumar and Mittal, 1999; Haße and Keipert, 1997) and makes them suitable for spray, roll-on, and gel products for topical applications. However, lecithin microemulsions formulated with medium-chain alcohols could reach viscosities as high as 1000 cP (Luisi et al., 1990).

It is somewhat difficult to compare the viscosity of different formulations because viscosity is a function of surfactant, water, and oil type and concentration. Perhaps the closest points of comparison are the formulations of Moreno et al. (Moreno et al., 2003) who produced Type I microemulsions using lecithin, IPM and polyethyleneglycol-PEG-(20) sorbitan monooleate. Using a total surfactant concentration between 20% and 25% they obtained a viscosity that ranged between 15 to 50 cP, which is comparable to the viscosities obtained in our Type I systems (Figure 3.4a). However, the formulation of Moreno et al. only contained 10% IPM, whereas our Type I systems near the Type I - IV transition contained almost 30% oil phase (a mixture of IPM and lidocaine). When Moreno et al. increased the oil content to 15% (25% total
surfactant concentration) the viscosity of the formulation approached 180 cP. The larger viscosity of the polymer-based microemulsion of Moreno et al. is, in part, due to the viscous nature of the lecithin and the polymer, but also due to the larger drop size as discussed below. It should be clarified that the viscosity of the linker-based microemulsion systems may depend on the shear conditions (i.e. non-newtonian effects).

![Graph showing viscosity and droplet hydrodynamic radius](image)

**Figure 3.4** (a) Viscosity and, (b) droplet hydrodynamic radius of the microemulsions in the L-Series with 4% lecithin containing lidocaine at room temperature.
3.4.2.2 Particle Size

Figure 3.4b shows the hydrodynamic radius of the microemulsions in the L-Series with 4% lecithin obtained from dynamic light scattering measurements as a function of sodium caprylate concentration. Microemulsion droplets increase in size as the formulation approaches the Winsor Type IV bicontinuous system. This observation correlates with the viscosity measurements and is consistent with existing microemulsion models (Acosta et al., 2003a). The droplet size (oil-swollen micelles or water-swollen reverse micelles) of the systems is small with all microemulsions having mean hydrodynamic radii between 6 and 11 nm, which makes these systems optically transparent, and of high interfacial area. The droplet sizes of the linker-based systems measured here are five times smaller than those of lecithin-IPM microemulsions reported in the literature (Moreno et al., 2003; Saint Ruth et al., 1995).

The smaller drop size of linker-based microemulsions can be explained by the fact that, as the hydrophilic linker (sodium caprylate+caprylic acid) adsorbs at the oil/water interface, the interfacial area increases, thus reducing the sizes of oil-swollen micelles and/or water-swollen reverse micelles (Acosta et al., 2002; Acosta et al., 2004).

In this study, the particle size was measured by DLS, which is a common method for determining the size of sub-micron particles (Merkus, 2009). However, DLS has its limitations. The measurement of hydrodynamic particle size by DLS is somewhat larger than the actual particle size (Merkus, 2009; Acosta, 2004). There are other methods for determining particle size, such as Transmission Electron Microscopy at cryogenic temperature (Cryo-TEM), or small-angle X-ray scattering (SAXS) or small-angle neutrons scattering (SANS). Further work can be done using other methods to double check the particle sizes of linker-based lecithin microemulsions.
3.4.3 Lidocaine partitioning between microemulsion and excess phases

The lidocaine that partitioned into the microemulsion and the excess phase was studied in the L-Series with 4% lecithin. Figure 3.5 shows that lidocaine concentrations in Winsor Type II linker microemulsions are higher than those in their Type I counterparts. The high lidocaine concentration in Type II systems is due to the hydrophobic nature of lidocaine and its tendency to concentrate in the continuous oil phase of Type II systems. This suggests that the continuous oil phase of Type II microemulsions acts as drug reservoir for lipophilic drugs such as lidocaine as concluded by previous reports (Kreilgaard et al., 2000; Lee et al., 2003). The concentration of lidocaine in the excess water phase is almost constant and close to the solubility limit of lidocaine in water (4.0 mg/ml). In Type IV microemulsions the concentration of lidocaine reaches 40 mg/ml (as expected, considering that there are no excess phases).

Figure 3.5 Lidocaine concentrations in the microemulsion and excess phases for the L-Series with 4% lecithin.
It is interesting to note that the concentration of lidocaine in Type I microemulsions is almost equal to the concentration of lidocaine in the excess oil phase (IPM). According to Figure 3.5, the lidocaine concentration in the Type I microemulsions is close to 40 mg/ml which is 10 times higher than the lidocaine solubility in water. This difference reveals that IPM-swollen micelles in Type I microemulsions significantly improve lidocaine solubilization when compared to pure water. However, this increase in solubilization cannot be explained by the solubilization of IPM and lidocaine in the core of oil-swollen micelles. To understand the magnitude of this solubilization enhancement, we need to remember that lidocaine is a hydrophobic but polar drug. For this kind of molecule, the solubilization sites include the hydrophobic core of the micelle as well as the palisade layer of the micelle (i.e. between the surfactant and linkers, on the oil side of the interface) (Rosen, 1989). The large interfacial area exhibited by microemulsions leads to additional solubilization sites (due to larger palisade layer) for lidocaine (Kreilgaard, 2002; Lawrence and Rees, 2000). This finding is consistent with the previous discussion regarding the changes in sodium caprylate concentration to produce II-IV-I transitions induced by lidocaine.

It should be clarified that the concentration of lidocaine in the microemulsions can be increased, at least, by two fold in these linker formulations (this is limited by the saturation of lidocaine in IPM which is approximately 20%). This is consistent with lidocaine solubilization capacity reported for other lidocaine microemulsion systems (Sintov and Shapiro, 2004).

3.4.4 Permeation flux and permeability

The transdermal permeation profiles (Figure 3.6a and 3.6b) show the cumulative mass of lidocaine permeated across the skin as a function of time. In general, lidocaine in the linker microemulsions produced higher lidocaine permeation than the P-Type II microemulsion through
the reconstructed human skin and pig skin models. In particular, the Type II linker microemulsion provided exceptionally higher lidocaine permeation through pig skin. Table 3.3 summarizes the transdermal flux calculated from the drug permeation profiles for the systems of Figure 3.6. Among different types of linker microemulsions, L-Type II provides highest flux, followed by L-Type IV and then L-Type I. One way ANOVA analysis provided $P << 0.001$ for various delivery systems. The higher transdermal flux for L-Type II versus L-Type I maybe related to the mechanisms of the drug penetrated into the skin. One mechanism is that the skin barrier resembles a lipid layer along which individual molecules (free drug) migrate via diffusion (Cussler, 1997). In this case, the Type II system should have higher permeability (i.e. higher permeation and diffusion coefficients) than the Type I system. The other mechanism is that the skin behaves as a mechanical nano-porous barrier for droplet transport (drug associated with colloids) through the skin (Maghraby et al., 2008; Cevc, 2004). The Darcy or Washburn penetration equations apply in this case, then the flux is proportional to $1/\text{viscosity}$ (Washburn, 1921). Therefore, the higher the viscosity, the lower the flux of the system.

These findings conflict with those of Lee et al. who reported that, in their formulations, Type I microemulsions produce larger fluxes than Type II systems (Lee et al., 2003). Since the concentrations of lidocaine used in our studies are similar to that used by Lee et al., the difference could only be attributed to the differences in formulations. Lee et al. used polymer-alcohol systems containing 40% PEG(20) sorbitan monooleate. As discussed above, the linker microemulsions have higher oil solubilization capacity than polymer-based systems; additionally, the lidocaine partition studies (Figure 3.5) show that lidocaine is highly soluble in Type I linker microemulsions. The high solubility of lidocaine in Type I linker systems may represent a problem in terms of improving the permeation of lidocaine through skin. It is well known that
higher drug solubility typically results in lower drug permeability (thus less drug permeation) (Santi et al., 1991).

Figure 3.6 Permeation profile of lidocaine through (a) MatTek human skin, and (b) pig ear skin from linker microemulsions (L-Type II, L-Type IV, and L-Type I), pentanol microemulsion (P-Type II), water and IPM. The formulations of these microemulsions are indicated in Table 3.2b.

Table 3.3 The steady-state flux ($J$, µg/h/cm²) of lidocaine, through MatTek human skin and pig ear skin from different delivery systems

<table>
<thead>
<tr>
<th>Delivery system</th>
<th>MatTek human skin</th>
<th>Pig ear skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Type II</td>
<td>559.4±30.4</td>
<td>479.1±70.3</td>
</tr>
<tr>
<td>L-Type IV</td>
<td>467.9±16.2</td>
<td>342.3±36.2</td>
</tr>
<tr>
<td>L-Type I</td>
<td>308.0±53.4</td>
<td>180.6±32.9</td>
</tr>
<tr>
<td>P-Type II</td>
<td>151.8±20.4</td>
<td>127.0±21.6</td>
</tr>
<tr>
<td>H₂O</td>
<td>325.4±16.5</td>
<td>140.6±10.0</td>
</tr>
<tr>
<td>IPM</td>
<td>315.1±22.1</td>
<td>130.1±24.0</td>
</tr>
</tbody>
</table>
The data in Figure 3.6 reveal that the L-Type II microemulsion produces a 4-fold ($P=0.00004$ and 0.003 for MatTek human skin and pig ear skin, respectively) increase in lidocaine permeation compared to the P-Type II system. Considering that the only difference between the two formulations is the substitution of the hydrophilic linkers by pentanol, one can attribute such a significant difference to the role that hydrophilic linkers play in such systems. It has been shown that the addition of hydrophilic linkers accelerates the rate of coalescence and solubilization in microemulsions (Acosta et al., 2003b). It has been proposed that hydrophilic linkers coadsorb between surfactants (Figure 3.1), increasing the surfactant-surfactant spacing, weakening the surfactant-surfactant interactions, and producing a more fluid (less rigid) interface.

Our preliminary estimations of the interfacial rigidity of the L-Type II and P-Type II systems (see Acosta et al., 2003a,b for details on interfacial rigidity calculations) indicate that interfacial rigidity of the pentanol Type II microemulsion is twice that of the linker Type II system. The interfacial rigidity has been found to be proportional to the activation energy of coalescence (Acosta et al., 2003b), making the kinetic coalescence constant an exponential function of the interfacial rigidity. We speculate that this interfacial rigidity may also be linked to the activation energy for the lidocaine transfer from the oil continuous phase to the skin. This hypothesis is supported by microemulsion mass transfer studies that show that the solute transfer flux is proportional to the coalescence kinetic constant (Steytler et al., 2001). Therefore, the lower rigidity of the L-Type II microemulsion could be responsible for the higher flux obtained with this microemulsion.

Compared to the IPM-only vehicle, the L-Type II microemulsion yields twice ($P=0.02$) the lidocaine flux through the reconstructed human skin and four ($P=0.0005$) times the flux through pig skin. Considering that the IPM-only vehicle contains more lidocaine (10%) than the L-Type II microemulsion (close to 6%), and that in both cases lidocaine is dissolved in an IPM-
continuous phase, one can conclude that the presence of the surfactant and linkers facilitates the mass transfer of lidocaine as discussed above. Similar permeation enhancement of Type II polymer-based formulations over IPM-only vehicles have been reported (Lee et al., 2003).

As indicated by Equation 3.1, the transdermal flux of lidocaine is associated with the difference in concentration ($\Delta C$) between the donor and the receiver solution. Certainly, higher lidocaine concentration in the donor solution produces a larger concentration gradient across the skin that should result in higher flux (Kreilgaard, 2002; Lawrence and Rees, 2000). The value of permeability ($k_p$) calculated by Equation 3.1 has been used to determine the effectiveness of delivery systems. Table 3.4 summarizes the values of permeability for the delivery systems considered in Figure 3.6. According to Table 3.4, water is, by far, the most effective vehicle for lidocaine. The same observation has been made in the literature (Lee et al., 2003). This result can be explained based on the principle of solubility versus permeability explained above: the lower the solubility (such as the case of lidocaine in water), the larger the permeability. If we consider this issue of solubility, then Equation 3.1 is not an appropriate expression to evaluate the effectiveness of microemulsion-based drug delivery vehicles because it should be based on the difference of chemical potential, and not on a simple difference of concentration. Equation 3.1 is theoretically more suitable for a drug transfer in a simple solvent, but the mechanism of drug release from microemulsions is more complex and not well established yet (Washington, 1990).

The only way to compare the permeability of the delivery systems considered here is if we express the concentrations on the basis of a common phase, in this case the skin. We measured the equivalent lidocaine concentration remaining in the skin after the permeation test to calculate the lidocaine “skin” permeability ($k_{sr}$, cm/h) for the vehicles considered in Figure 3.6. Figure 3.7 shows the equivalent lidocaine concentration remaining in the skin after treatment by these vehicles. All the microemulsion systems significantly improved the absorption of lidocaine into
the skin (i.e. larger equivalent skin concentrations) which suggests that these systems could also be used as topical delivery vehicles. Table 3.4 presents the calculated lidocaine “skin” permeability ($k_{sr}$) for these vehicles, where the lidocaine concentration difference, $\Delta C_v$, in Equation 3.1 is calculated based on the lidocaine concentration in the skin and in the receiver solution. The drug “skin” permeability from L-Type II is higher than those from P-Type II, L-Type I, IPM and water. These “skin” permeability values capture the permeation enhancing abilities of surfactants. Based on these results, it is highly recommended that future microemulsion-based drug delivery studies revisit Equation 3.1 by either using a difference in chemical potential (as opposed to absolute concentrations) or the concept of “skin” permeability.

![Figure 3.7](image)

**Figure 3.7** Lidocaine concentration remaining in the skin after treatment by different delivery systems. The formulations of these microemulsions are indicated in Table 3.2b.

It has to be mentioned that the values of the transdermal flux and permeability of lidocaine presented in this work are one order of magnitude higher than those reported in the microemulsion literature (Kreilgaard et al., 2000; Lee et al., 2003; Sintov and Shapiro, 2004). For example, Lee et al. reported the permeability of lidocaine in water to be 0.00133 cm/h through human cadaver skin whereas we obtained a value of 0.036 cm/h through pig skin. Depending on
the way pig ear skin is treated there could be significant changes in permeability (Sekkat et al., 2004). In the literature, gel lidocaine microemulsions (Shin et al., 2004) produce fluxes of the order of magnitude reported in Table 3.3. Furthermore, Kushla and Zatz (Kushla and Zats, 1991) have reported lidocaine permeabilities of 0.018 cm/h through human skin, which is of the same order of magnitude reported in this work for pig skin. While we obtained consistent results with our skin sources, the variability between different skin sources/treatments suggest that one to one comparisons of absolute flux values or permeability values is not advisable. The analysis of drug delivery studies should be based on baseline systems such as the water and IPM included in our studies and the studies of Lee et al.

Table 3.4 The normal permeability ($k_p, \times 10^3$ cm/h) and “skin” permeability ($k_{sr}, \times 10^3$ cm/h) of lidocaine calculated for two types of skin sources.

<table>
<thead>
<tr>
<th>Delivery system</th>
<th>MatTek human skin</th>
<th>Pig ear skin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$k_p$</td>
<td>$k_{sr}$</td>
</tr>
<tr>
<td>L-Type II</td>
<td>10.1±0.6</td>
<td>9.0±1.0</td>
</tr>
<tr>
<td>L-Type IV</td>
<td>11.2±0.9</td>
<td>8.2±0.8</td>
</tr>
<tr>
<td>L-Type I</td>
<td>7.9±1.1</td>
<td>4.6±0.6</td>
</tr>
<tr>
<td>P-Type II</td>
<td>2.4±0.1</td>
<td>1.9±0.1</td>
</tr>
<tr>
<td>$H_2O$</td>
<td>82.8±4.6</td>
<td>35.8±1.8</td>
</tr>
<tr>
<td>IPM</td>
<td>2.9±0.2</td>
<td>1.3±0.2</td>
</tr>
</tbody>
</table>

The difference in lidocaine flux between skin sources is evident in Table 3.3. Compared to pig ear skin, MatTek reconstructed human skin produced higher lidocaine transdermal flux and permeability. However, it has to be mentioned that , unlike human skin, the reconstructed human skin has a more permeable stratum corneum (Asbill et al., 2000b; Ponec et al., 2000). On the other hand, pig ear skin yields similar flux and permeability to human cadaver skin (Songkro et al., 2003). As a result, pig ear skin is a reliable model for in vitro permeation studies.
3.4.5 Cytotoxicity

Figure 3.8 summarizes the cell (keratocyte) viability after 5.5 hours exposure of MatTek EPI-200 tissues to different lidocaine vehicles. In these viability tests, PBS and 1% Triton X-100 were used as a negative (non-toxic) and positive control, respectively. A solution of 1% Triton X-100 is usually used as a reference for a mild formulation, and its cell viability is expected to represent mild in vivo skin irritancy (Faller et al., 2002). According to recent European regulations, a test vehicle should be considered to be corrosive to skin if the viability of keratocytes after 1 h of exposure is less than 15% (Liebsch et al., 2000). The test conditions of Figure 3.8 are more restrictive than such regulations (exposure time of 5.5 h versus 1 h for the European regulations), yet all the delivery vehicles show viabilities larger than 15%, especially for the case of the water-lidocaine system and L-Type II microemulsion. These results are in agreement with the low toxicity of lecithin o/w microemulsion delivery systems evaluated using animal studies (Brime et al., 2002; Moreno et al., 2003).

To interpret the results of Figure 3.8, it is necessary to clarify that the water only system contains 0.4% lidocaine and no surfactant, whereas the L-Type II formulation contains more than 20% surfactant + linkers, and 6% lidocaine. Despite this large difference in composition the L-Type II microemulsion has the second-best (P=0.01) viability of all these systems. This result confirms our initial hypothesis that it is possible to produce mild microemulsions using lecithin and avoiding the use of short or medium-chain alcohols (Kumar and Mittal, 1999). The low toxicity of the linker formulation comes as a result of selecting formulation ingredients with chemical structure similar to the lipid composition of the skin (lecithin, caprylic acid, and the oleic moiety in sorbitan monooleate). It is quite possible that the lower viability of keratocytes exposed to the L-Type II microemulsion, compared to water only, comes from the larger lidocaine skin absorption, and the cytotoxicity of lidocaine itself (Table 3.1).
One problem with using MTT tests with MatTek EPI-200 skin models is that this test is most sensitive to toxic vehicles but can not differentiate well between two mild systems (Faller et al., 2002). Future studies should consider cytokine release (e.g. using IL1-α EIA test kits), which are best suited to evaluate mild irritation effects (Faller et al., 2002).

Figure 3.8 Cytotoxicity of different delivery systems according to the MTT assay. The formulations of these microemulsions are indicated in Table 3.2b.

Compared to the P-Type II microemulsion, the L-Type II system yields higher ($P=0.002$) cell viability. As discussed, the only difference between the two formulations is that the L-Type II uses sodium caprylate and caprylic acid as hydrophilic linkers while P-Type II uses pentanol as cosurfactant. Thus, the difference in cytotoxicity is explained by the fact that a medium-chain alcohol (such as pentanol) tends to disrupt the cell membrane, triggering allergic reactions (McKarns et al., 1997). Unlike pentanol, caprylic acid has direct food additive status (21CFR172.860) and has been found to be a non-irritant of human skin (Whittle et al., 1996). Furthermore, according to the irritation data in Table 3.1, the onset of irritation on rabbit skin with caprylic acid occurs at a dosage 25 times larger than that required to trigger an irritation using pentanol.
It is still unclear why the L-Type II system generates a larger ($P=0.0008$) cell viability than L-Type I and IPM-lidocaine systems. To our knowledge, this is the first time that the toxicity of Type I and Type II microemulsions have been compared side by side.

### 3.5 CONCLUSIONS

In this study alcohol-free lecithin microemulsions have been formulated using sorbitan monooleate as lipophilic linker and a combination of sodium caprylate and caprylic acid as hydrophilic linkers. Increasing the concentration of sodium caprylate in the systems increases the hydrophilicity of the formulations, and induces a Type II-IV-I phase transition. The presence of lipophilic and hydrophilic linkers makes the surfactant+linker system partially insoluble in pure oil or pure water, but is optimized for co-solubilizing oil and water in equal proportions using as little as 2.8% lecithin. These linker microemulsions produced relatively small droplets (less than 10 nm in diameter) which suggest the existence of a large surface area for mass transfer and explain the relatively low viscosity of these formulations.

Furthermore, the model drug studied in this work, lidocaine, was highly soluble in these linker microemulsions. The lidocaine partition and phase behavior studies suggest that this hydrophobic but polar drug solubilizes in the core and the palisade layer of the micelles.

It was also found that although Type I and IV microemulsions may experience a phase transition upon dilution with water, Type II microemulsions will not undergo this transition. Other formulation conditions such as temperature and electrolyte concentration do not affect the phase behavior of these systems significantly due to the zwitterionic nature of lecithin, which is the main surfactant used in the formulation.
Linker-based lecithin microemulsions were also examined as potential vehicles for TDD of lidocaine. Type II and IV linker-based vehicles produce larger transdermal lidocaine flux, larger lidocaine skin absorption and larger lidocaine “skin” permeability than water, pentanol-based Type II microemulsion, linker Type I microemulsion, and IPM. The superior flux obtained with linker microemulsions is due to the use of hydrophilic linkers that accelerate the interfacial mass transfer. The concept of “skin” permeability was also introduced and recommended as a method to compare the permeability obtained from different drug delivery vehicles.

Furthermore, the cytotoxicity studies indicate the linker-based Type II systems are mild, and their cytotoxicity compares to that of dilute lidocaine solutions in water. These linker-based systems are also less toxic than the alcohol-based lecithin microemulsion. The difference between hydrophilic linkers and pentanol is that the former concentrates on fluidizing the oil/water interface and probably the interstitial spaces between cells whereas the latter is more efficient at fluidizing the membranes of living cells, inducing cell lysis.

3.6 REFERENCES


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CHAPTER 4

EXTENDED RELEASE OF LIDOCAINE

FROM LINKER-BASED LECITHIN MICROEMULSIONS

This chapter is derived from the following manuscript:

4.1 ABSTRACT

In Chapter 3, we reported on the use of linker-based lecithin microemulsions as effective transdermal delivery vehicles for lidocaine. It was determined at that time that the performance of these vehicles was in part due to a permeability enhancement effect, but also due to the amount of lidocaine absorbed in the skin. In this chapter, we take advantage of this drug absorbed in the skin to produce an extended release profile where the lidocaine-loaded skin is used as an *in situ* patch. The release of lidocaine from the skin is modeled using a differential mass balance that yields a first order release profile. This profile depends on the mass of drug initially loaded in the skin and a mass transfer coefficient. When the release profile of lidocaine was evaluated as a function of the concentration of lidocaine in the microemulsion, application time, and microemulsion dosage; we observed that all these different conditions only change the mass of lidocaine initially loaded in the skin. However, these parameters do not change the mass transfer coefficient. When the release profile of Type I and Type II microemulsions was compared, it was determined that the mass transfer coefficient of Type II systems was larger than that of Type I. This suggests that the morphology of the microemulsion plays an important role on the release kinetics. These linker microemulsions are able to release 90% of their content over a 24 h period which rivals the performance of some polymer-based patches. Fluorescence micrographs of transversal cuts of skin loaded with Nile red are consistent with the observed release profiles.

4.2 INTRODUCTION

The goal of transdermal drug delivery (TDD) is to achieve better percutaneous absorption and permeation of active ingredients for local treatment (Bronaugh and Maibach, 2005).
Microemulsions have been reported to improve the absorption of drugs in the skin significantly compared to aqueous solutions, gels, or cream formulations (Baroli et al., 2000; Delgado-Charro et al., 1997; Williams and Barry, 1992). They provide many advantages as topical TDD systems, including high solubilization for both oil-soluble and water-soluble drugs, being thermodynamically stable, transparent, and spontaneous formation. The enhanced absorption is typically associated with the high surface area of microemulsion droplets and the presence of surfactant that act as permeation enhancers. However, the use of microemulsions has been limited by the toxicity of ionic surfactants and alcohols used in most formulations.

Mild alcohol-free lecithin microemulsions formulated with linker molecules with Generally Recognized as Safe (GRAS) or food additive status were reported in Chapter 3. In this work, we hypothesize that by topically administering an active ingredient using the linker microemulsions formulated in Chapter 3, the drug will be safely absorbed into the skin, thus producing an in situ delivery patch. Potential advantages of this in situ patch include its application on uneven and exposed parts, its low cost, and customizable dose.

The typical controlled-release form for topical treatment of skin diseases is drug-in-adhesive patches. Since the transdermal absorption occurs through a slow process of diffusion, driven by a concentration gradient, the patches must be kept in continuous contact with the skin for a considerable time (hours and days) (Scheindlin, 2004). This often causes skin irritation at the site of administration (Shah, 1987; Kurihara-Bergstrom et al., 1991). For extreme situations such as patients with painful skin irritations, blisters, burns, or other skin wounds, the use of sprayable therapeutic microemulsion formulations may represent a significant advance over the patches that require direct contact with the skin. Since these microemulsions are transparent, the formulation could be used to treat various skin conditions without affecting the appearance of the patient.
Another advantage of *in situ* patches over conventional ones is that a large excess of drug has to be placed in the conventional patches to maintain the concentration gradient. For example, the label of lidocaine patch (Lidoderm®) states that only 3% of the applied dose is absorbed (Scheindlin, 2004). The cost of excess drug in the patches may be significant for numerous drugs.

To evaluate the performance of the linker-based lecithin microemulsions as *in situ* patches, a lipophilic drug, lidocaine, was chosen as a model drug in this work. Lidocaine is an anesthetic that has been used in topical formulations as a pain reliever in the treatment of minor burns, sunburn, insect bites and after various laser skin surgeries (Tetzlaff, 2000; Jesitus, 2001). The problem with the existing lidocaine delivery systems on the market is the short half-life. For instance, the pain relieving effects of EMLA cream (a commercial macroemulsion containing 1:1 eutectic mixture of 2.5 wt.% lidocaine and 2.5 wt.% prilocaine; Astra, Lakemedel, Sweden) only lasts between two to four hours after application (Pasero et al., 1995; Skaaret, 2006). In the case of wound dressings, adequate pain relief results in better compliance with treatment and a better quality of life (Heinen et al., 2004). Since these dressings are unlikely to be replaced as often as every 2 to 4 hours, there is a need to find formulations for prolonged release of lidocaine.

The delivery of lidocaine by microemulsions has been compared to the currently available lidocaine formulations (Sintov and Shapiro 2004; Kreilgaard et al., 2000). Kreilgaard et al. compared the transdermal delivery of lidocaine from microemulsions and from conventional formulations, such as 5% lidocaine cream (Xylocaine®, AstraZeneca), 2% lidocaine hydrochloride gel (Xylocaine® gel, AstraZeneca) and EMLA cream (Kreilgaard et al., 2000). The results show that the optimized microemulsions increased transdermal flux of lidocaine up to four times compared to a conventional o/w emulsion, and approximately two times compared to EMLA. Furthermore, Sintov and Shapiro demonstrated that microemulsions as transdermal
delivery vehicles showed a significant increase in the skin absorption of lidocaine compared to application of EMLA cream (Sintov and Shapiro 2004).

However, there is no previous report that studied extended transdermal release of lidocaine from microemulsions. In order to reduce patient discomfort, a long release time of 12- or 24-h would be desirable when compared to the 3-5 h of existing formulations. In this work, the release of more than 12 h is defined as extended release in this study. The aim of the study is to test the in situ delivery patch hypothesis by evaluating the skin absorption and the release profile of lidocaine via linker microemulsions. Lidocaine-laden linker microemulsions were applied on pieces of excised pig ear skin and in vitro extended release studies were conducted. The release profile from these studies were analyzed by a controlled-release model (described in the next section) and compared to the release profile of traditional transdermal patches, gels and polymer films reported in the literature. Furthermore, fluorescence microscopy was used to study the permeation and location of a lipophilic fluorescent dye (Nile red) within the skin.

4.3 MATERIALS AND METHODS

4.3.1 Materials

4.3.1.1 Chemicals

The following chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) at the concentrations shown in parentheses, and were used as received: sorbitan monooleate (Span® 80, 99%), sodium caprylate (99%), caprylic acid (99%), isopropyl myristate (IPM, 98%), sodium chloride (99%, Fluka brand), Dulbecco’s phosphate buffered saline (PBS), Nile red and lidocaine powder (base form, 98%). Laboratory grade soybean lecithin (99%) was purchased
from Fisher Scientific (Fairlawn, NJ, USA). Soybean lecithin is a mixture of phospholipids (mainly phosphatidyl cholines) produced by acetone purification of soybean gum residues. Sodium phosphate monobasic, monohydrate (ACS grade) and acetonitrile (HPLC grade) were purchased from EMD Chemicals Inc. (Darmstadt, Germany), and they were used as received. Anhydrous ethyl alcohol and methanol were purchased from Commercial Alcohols Inc. (Brampton, ON, Canada). Unless otherwise stated, the composition is expressed on weight basis (i.e. wt. %) throughout this Chapter.

4.3.1.2 Skin

Pig ears were purchased from the local market and frozen overnight. Prior to use, they were thawed by rinsing with running water for ten seconds at room temperature. The skin of the external side of the ear was then dermatomed to a thickness that ranged from 700 to 900 μm (Bronaugh and Maibach, 1991). After that, the thin skin layer was cut in circles of 11.4 mm diameter ready for use.

4.3.2 Microemulsion preparation

Microemulsion formulations were prepared using equal volumes of aqueous solution and oil (5 ml of each) in flat bottom test tubes at constant temperature (25±1°C), electrolyte concentration (0.9% NaCl in the aqueous solution), and pressure (1 atm) (Yuan et al., 2008). The oil used in this study is IPM. Lidocaine was loaded in the microemulsion formulations by predissolving this drug in IPM to a concentration of 10 % (10% lido-IPM) and 20% (20% lido-IPM, near the solubility limit of lidocaine in IPM). After introducing all the ingredients, the test tubes were thoroughly vortexed, then vortexed once a day for three days, and left to equilibrate for two weeks. The composition of the linker-based lecithin microemulsions is shown in Table 4.1.
Additional information regarding the phase diagram, viscosity, and particle size for these linker formulations have been previously reported (Yuan et al, 2008).

Table 4.1 Compositions of Type II and Type I linker-based lecithin microemulsions (% w/w)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>LE</th>
<th>SM</th>
<th>SC</th>
<th>CA</th>
<th>NaCl</th>
<th>Water</th>
<th>10% or 20% Lido-IPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type II</td>
<td>4.0</td>
<td>12.0</td>
<td>1.0</td>
<td>3.0</td>
<td>0.9</td>
<td>41.1</td>
<td>38</td>
</tr>
<tr>
<td>Type I</td>
<td>4.0</td>
<td>12.0</td>
<td>7.0</td>
<td>3.0</td>
<td>0.9</td>
<td>35.1</td>
<td>38</td>
</tr>
</tbody>
</table>

LE – lecithin  SM - sorbitan monooleate  CA - caprylic acid  SC - sodium caprylate

4.3.3 In vitro extended release studies

The in vitro extended release experiments were conducted in MatTek Permeation Devices (MPD) supplied by MatTek Corporation (Ashland, MA, USA). The exposed area of tissue for the MPD is 0.256 cm². The pig ear skin was placed in the MPD, with the epidermis facing up. A test microemulsion formulation (400 μl) was applied in the donor compartment. The receptor compartment was filled with 5 ml of PBS (0.01M phosphate, 0.137M NaCl, pH 7.4). After 30 min (unless stated otherwise), the donor microemulsion was withdrawn and the skin surface was blotted dry with Kimwipes and then used for extended release. This loading time of 30 minutes was selected based on a previous study that shows that the continuous release reaches steady state after 30 minutes (Yuan et al, 2007). At predetermined times (1 h, 3 h, 6 h, 12 h, 24 h and 48 h), the receiver solution was withdrawn completely from the receptor compartment and was immediately replaced by fresh PBS solution. At 48 h, the experiment was terminated. All permeation experiments were conducted in triplicate at room temperature.
4.3.3.1 *In vitro* skin absorption

The pig ear skin samples at the end of the *in vitro* extended release studies were used to test the final concentration of the drug absorbed in the skin \((C_f)\). Prior to measuring the absorbed lidocaine, the pig skin was rinsed with a PBS solution and placed into 2 ml methanol for overnight extraction of lidocaine (Yuan et al., 2008). The equivalent lidocaine concentration absorbed in skin was calculated as the mass of lidocaine extracted from the skin divided by the volume of the skin \((\text{exposed area} \times \text{thickness})\), and is expressed in mg/ml. We have determined, using a mass balance closure in selected samples, that the efficiency of this methanol extraction procedure is more than 95%. The initial lidocaine concentration \((C_s^0)\) absorbed into skin after 30 min application of a microemulsion was then calculated by adding the cumulative amount of drug permeated to the receiver and the final amount of drug extracted from the skin and dividing this value by the volume of the skin.

4.3.3.2 Lidocaine quantification

The concentration of lidocaine in the microemulsions, receiver solutions and skin was analyzed using a Dionex ICS-3000 (Sunnyvale, CA, USA) liquid chromatography system consisting of single pump, detector, AS40 automated sampler, AD25 absorbance detector and Chromelon chromatography software (Dionex). Lidocaine was separated by a reverse phase column (Genesis, C18, 4 μm, 150×4.6 mm) and detected through its absorbance at 230 nm (AD25 detector). A mixture of acetonitrile and 0.05 M NaH2PO4·H2O (pH 2.0) solution (30:70, v/v) was used as a mobile phase with a flow rate at 1.0 ml/min. The column temperature and the injection volume were 25°C and 10μl, respectively.
4.3.3.3 Statistical data analysis

All extended release values were calculated from three independent experiments, and data are expressed as the mean value ± S.D. The statistical analysis of $k_{sr}$, and $t_{50\%}$, was performed using a one-way analysis of variance (ANOVA) to test the difference between the means of two or more delivery systems. Data with $P<0.05$ are considered statistically significant.

4.3.4 Fluorescence Microscopy

To visualize the permeation of the microemulsions into the skin tissues, the linker-based lecithin microemulsions containing a fluorescent dye, Nile red (0.001%), were prepared. The dye was simply dissolved into the Type II and I systems with 10% lido-IPM (Table 4.1). The systems were vortexed and left to equilibrate overnight. The microemulsions containing Nile red were then used to conduct in vitro extended release studies. After 1 hour, the skin samples were taken off the permeation device, blotted dry with Kimwipes, and then rinsed twice with PBS. The clean skin samples were snap frozen using dry ice and were cross sectioned to 30 µm thick slices by a cryostat microtome (Leica Jung CM3000, Bensheim, Germany). The skin slices were observed and photographed with a Leica MZFIII fluorescence stereomicroscope (Leica, Heerbrugg, Switzerland) equipped with a Leica DFC 320 Digital Camera (Leica, Heerbrugg, Switzerland). Sections were photographed using a red filter ($\lambda_{exc}= 450-500$ nm) to visualize Nile red. All photographs were taken with 63x objectives and exposure time 2.0 sec. Solutions of 0.001% Nile red in IPM was considered as the control.
4.4 RESULTS AND DISCUSSION

4.4.1 In vitro extended release

To investigate the potential of linker-based lecithin microemulsions absorbed in skin as in situ patches for extended delivery, we examined the release profile of these systems with different formulation and application conditions. In terms of formulation conditions, we evaluated the effect of drug loading in the microemulsion (10% lidocaine in IPM and 20% lidocaine in IPM) and the effect of microemulsion morphology (Type I-o/w- vs. Type II-w/o- microemulsions). These effects are described in section 4.4.4.1, and the first order release kinetics (Equation 4.7) is applied to these results. To evaluate the effect of application conditions (section 4.4.4.3), the release profile was examined as a function of dosage (mg of microemulsion/cm² skin) and application time.

4.4.4.1 Effect of formulation conditions: drug loading and microemulsion morphology

Figure 4.1a and 4.1b report the drug release profiles obtained by plotting the cumulative amount of lidocaine released as a function of time. For all of the in situ patches, the cumulative release profiles typically show a large increase in the first 24 h. When increasing the drug loading from 10% lido-IPM to 20% lido-IPM in either the Type II or Type I formulation, it shows that the lidocaine release also doubled. The increase in drug released from the in situ patches with increasing lidocaine loading in the microemulsion is attributed to the larger lidocaine concentration absorbed in the skin. For the Type II formulation with 10% lido-IPM, the lidocaine absorption in the skin was 24.2±3.3 mg/ml, while that for the Type II formulation with 20% lido-IPM doubled to 50.2±4.6 mg/ml (Table 4.2). Similarly, the data in Table 4.2 also shows that for Type I systems, increasing the concentration of lidocaine from 10% to 20% in IPM, the concentration of lidocaine absorbed in the skin increased from 21.0±1.9 mg/ml to 46.6±5.4...
mg/ml. These observations are in agreement with the results obtained by Kreilgaard et al., where they determined that transdermal delivery increases as the drug loading in the microemulsions increases (Kreilgaard et al., 2000).

Table 4.2 Formulation and release kinetic parameters: lidocaine loading in microemulsions, initial drug absorption in the skin ($C_s^0$, mg/ml), release rate constants of lidocaine ($k_{sr}$, ×10³ cm/h) and the time required to reach 50% release ($t_{50\%}$, h) calculated by the first-order model fitted to the 24 h release data.

<table>
<thead>
<tr>
<th>Microemulsion systems</th>
<th>Drug loading (mg/ml)</th>
<th>Drug absorption $C_s^0$ (mg/ml)</th>
<th>$k_{sr}$, x10³ (cm/h)</th>
<th>$t_{50%}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% lido-IPM</td>
<td>55.8±0.8</td>
<td>24.2±3.3</td>
<td>8.4±0.6</td>
<td>6.2±1.1</td>
</tr>
<tr>
<td>20% lido-IPM</td>
<td>104.0±0.0</td>
<td>50.2±4.6</td>
<td>7.2±1.3</td>
<td>7.7±0.4</td>
</tr>
<tr>
<td>Type I</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10% lido-IPM</td>
<td>40.8±1.6</td>
<td>21.0±1.9</td>
<td>6.0±0.6</td>
<td>9.5±0.3</td>
</tr>
<tr>
<td>20% lido-IPM</td>
<td>71.0±0.1</td>
<td>46.6±5.4</td>
<td>6.0±0.7</td>
<td>9.2±0.4</td>
</tr>
</tbody>
</table>

Figure 4.1 In vitro release profiles of lidocaine from the in situ patches produced with (a) Type II, and (b) Type I microemulsions formulated with 10% and 20% of lidocaine in IPM.
During the extended release experiments, most of the drug absorbed in the skin was released. Figure 4.2a and 4.2b help illustrate this observation. In Figure 4.2a and b, the fraction of lidocaine released is plotted as a function of time for the *in situ* patches of Figure 4.1. The fraction release curves show that approximately 80-90% of the drug was released by 24 h and nearly 100% released by 48 h. According to Figure 4.2a and b, increasing the drug loading from 10% lido-IPM to 20% lido-IPM had no impact on the fraction of lidocaine released (ANOVA test - *P* ranges from 0.08 to 0.74 for Type I systems, and from 0.11 to 0.57 for Type II systems). These observations are consistent with Equation 4.7 in that the fraction of drug release is not a function of the mass of drug loaded in the skin (*M*<sub>∞</sub>).

![Figure 4.2 Fraction of lidocaine released from *in situ* patches produced with (a) Type II, and (b) Type I microemulsions formulated with 10% and 20% of lidocaine in IPM. The solid and dashed lines represent the fits (24-h data) to the first-order model (Equation 4.7).](image-url)
It is also important to highlight that the shapes of the fractional release curves of Figure 4.2a and b are different for Type I and Type II microemulsions. The release from Type I microemulsions (Figure 4.2b) is slower than Type II microemulsions (Figure 4.2a) either for 10% or 20% lidocaine-IPM. After 12 hrs of release, the Type I microemulsion has released almost 60% of its load whereas the Type II systems has released approximately 75%. Furthermore, the Type I system seems to approach a linear release profile during the first 12 hrs of release. These observations are consistent with those of Yuan et al (2008). At this point there is no clear explanation for the lower rate of penetration for Type I systems, but this may be related to the different mechanisms of the drug penetrated into the skin. For free drug molecules, the skin works as diffusion barrier and Type I has less free drug in the continuous phase (Cussler, 1997). For drug associated with colloids, the droplets penetrated the skin as a fluid that moves through a porous media (Maghraby et al., 2008; Cevc, 2004). According to the Darcy or Washburn equation (Washburn, 1921), the flux is inversely proportional to viscosity and Type I has higher viscosity.

4.4.4.2 Drug release kinetics

For many controlled-release systems, three main diffusion models are commonly used to describe the drug release kinetics (Cussler, 1997): the zero-order model, the first-order model, and the Higuchi square root time model. The zero-order kinetics was not applicable for the in situ patches in this study, as the fraction released vs. time curves obtained for Type I and Type II microemulsions were non-linear within the 48 hour timeframe. The Higuchi square root time model is a simplified form of the first-order model and only applicable for low to intermediate release fractions (Higuchi, 1962).

In this study, drug release data obtained was analyzed in terms of the fraction of lidocaine released as a function of time. To this end, we propose a mass transfer mechanism illustrated in
Figure 4.3. As shown in Figure 4.3, the drug transport in the experiment can be divided into two parts: (1) the flux $F_{ds}$ from the donor solution to the skin, and (2) the flux $F_{sr}$ from the skin to the receiver solution. The differential mass balance of lidocaine yields the following equation:

$$\frac{dC_s}{dt} \times V = (F_{ds} - F_{sr}) A$$  \hspace{1cm} (Equation 4.1)

where $C_s$ is the drug (lidocaine) concentration in the skin at time $t$, $V_s$ is the volume of the skin, $A$ is the area of the skin, and $V_s/A = l$, which is the thickness of the skin.

![Schematic of the mechanism of drug transport for in vitro extended release studies.](image)

**Figure 4.3** Schematic of the mechanism of drug transport for *in vitro* extended release studies.

In the *in vitro* extended release studies, the applied microemulsion was withdrawn from the donor compartment before the extended release. In this case, $F_{ds} = 0$, and Equation 4.1 is then can be written as,

$$\frac{dC_s}{dt} \times l = -F_{sr}$$  \hspace{1cm} (Equation 4.2)

We can now introduce a mass transfer coefficient to calculate the flux of lidocaine transferred from the skin to the receiver solution $F_{sr}$ is given by,

$$F_{sr} = k_{sr} (C_s - K_{sr} C_r)$$  \hspace{1cm} (Equation 4.3)
where \( k_{sr} \) is the mass transfer coefficient between the skin and the receiver solution, \( K_{sr}=C_s^*/C_r \) is the partition constant between the drug concentration the skin \((C_s^*, \text{ in equilibrium with } C_r)\) and the drug concentration in the receiver solution \( C_r \) at time \( t \).

Substituting \( F_{sr} \) in Equation 4.2 by Equation 4.3 we obtain that:

\[
\frac{dC_s}{k_{sr}(K_{sr}C_r - C_s)} = l \times \frac{dt}{t}
\]

(Equation 4.4)

Integrating between \( t=0 \) at the beginning of the extended release and any “\( t \)”: \[
\int_0^t dt = \int_{C_s^0}^{C_s} \frac{dC_s}{k_{sr}(K_{sr}C_r - C_s)} \times l
\]

(Equation 4.5)

where \( C_s^0 \) is the drug concentration in the skin at time \( t=0 \), i.e., the initial lidocaine concentration absorbed into skin. In our extended release experiments, at predetermined times, the receiver solution was withdrawn completely from the receptor compartment and was immediately replaced by fresh PBS solution. In this way, we can assume that the drug concentration in the receiver is negligible as each time the receiver the receiver medium is being replaced with fresh medium, that is, \( C_r \sim 0 \). From Equation 4.5, the drug concentration in the skin at time \( t \) can be written as,

\[
C_s = C_s^0 \exp\left(-\frac{1}{l}k_{sr}t\right)
\]

(Equation 4.6)

An alternate solution useful for interpretation of the fraction of drug release is given as,

\[
\frac{M_t}{M_s} = 1 - \exp\left(-\frac{1}{l}k_{sr}t\right)
\]

(Equation 4.7)

where \( M_t \) is defined as the mass of drug released at time \( t \), and \( M_s \) is the mass of drug released at time approaches infinity (this is also the mass of drug loaded in the skin). This equation follows the classic first-order drug release model \( M_t/M_s=1-\exp(-kt) \) (Cussler, 1997).
We proceeded to fit the fractional release profiles of Figure 4.2a and 4.2b to the first-order release model (Equation 4.7). Figure 4.4a and b presents the value \( \log(1-M_t/M_{\infty}) \) against time \( t \) for the 24-h and 48-h release data. The high correlation factor \( R^2>0.95 \) of all the fits in Figure 4.4a and b is consistent with the hypothesis that the mechanism of drug release from all of the \textit{in situ} patches applying either Type II or I microemulsions obey the first-order release kinetics. However, the 48-h fit (Figure 4.4a) of Equation 4.7 show substantial deviations from the release data for Type I microemulsions. The 24-h fit (Figure 4.4b), on the other hand, show good agreement with the data. Even when the 24-h fit is extended to 48-h release, shown as solid and dashed lines in Figure 4.4a and b, the error obtained at 48-h is not substantial.

Table 4.2 presents the release rate constants \( k_{sr} \) obtained after fitting the 24-h fractional release data with Equation 4.7. The time to release 50% of the load \( t_{50\%} \) was determined by Equation 4.7 and is also presented in Table 4.2. We were able to confirm that the values of \( k_{sr} \) do not
depend on the loading of lidocaine in Type I ($P=0.98$) or Type II ($P=0.31$) microemulsions. Furthermore, we were able to determine that the value of $k_{sr}$ was different for Type I and Type II microemulsions formulated with 10% lidocaine in IPM ($P=0.045$), but not distinctly different for formulations produced with 20% lidocaine in IPM ($P=0.21$). The analysis of these fits suggests that, consistent with the earlier observations, the loading of lidocaine in the microemulsion does not affect the fractional release. However, the morphology of the microemulsion (Type I vs. Type II) does have an impact on the release kinetics. The slower lidocaine release from Type I microemulsion is consistent with previous observations in continuous delivery studies (Yuan et al, 2008). In that work, it was shown that the “skin” permeability of Type II microemulsions loaded with lidocaine was almost twice that of Type I microemulsions. This “skin” permeability can be interpreted as a mass transfer coefficient for continuous delivery when the donor is not removed from the permeation device. The ratio between $k_{sr}$ for Type II and $k_{sr}$ for Type I systems formulated with 10% of lidocaine in IPM (Table 4.2) is close to 1.4, which approaches the ratio observed for the “skin” permeability by Yuan et al (2008). Using the argument of viscosity (35cP for Type I and 15cP for Type II), and Darcy’s law or Washburn equation (flux~1/viscosity) then the ratio of “skin” permeabilities should be close to 2. Further studies to explore this phenomenon and to explain the effect of microemulsion morphology are still needed.

In order to compare the extended release performance of Type I and II formulations with other systems, we plotted the release profile of the microemulsions, and the release profile of other lidocaine patches reported in the literature (Repeka et al., 2005; Glasvas-Dodov et al., 2002; Burgalassi et al., 1996; Gref et al., 1994). These release profiles are presented in Figure 4.5 for Type I and Type II microemulsions formulated with 10% lido-IPM. The lidocaine release from the in situ patch is slower than that from lidocaine embedded in a polymer (HPC:HPMC) film (Repeka et al., 2005) and in polymeric nanospheres (Gref et al., 1994), however, it has similar
performance to buccal patches produced with chitosan gels (Burgalassi et al., 1996). It is necessary to clarify that the *in situ* patches rely, for the most part, on the properties of the skin and the interactions between the drug and the surfactants/oil in the microemulsion to regulate the transport of the active. In the polymer film of Repka et al or in the buccal patch, the mechanism of controlling the rate of release is different because there is a physical film/membrane to regulate the release of the drug. While the mechanisms of delivery are different, we confirmed the possibility of using linker-based lecithin microemulsions absorbed in skin as *in situ* patches for extended delivery.

**Figure 4.5** Comparison of lidocaine release from *in situ* patches produced with Type II and Type I linker microemulsion (10% lido-IPM) to other delivery systems in the literature: a polymer (HPC:HPMC) film (Repka et al., 2005), polymeric nanosphere (Gref et al., 1994), a buccal patch (Burgalassi et al., 1996) and a hydrogel (Glasvas-Dodov et al., 2002).
On the other hand, the drug release from the *in situ* patch is relatively fast when compared to the released profile of lidocaine from a crosslinked hydrogel (Glasvas-Dodov et al., 2002). One possible reason is because the microemulsion does not have the high viscosity (Yuan et al., 2008) or the fixed 3-D pore network structure of the polymer that help regulate the fraction of lidocaine release.

The comparisons discussed above should be interpreted in light of the fact that the release profile not only depends on the formulation and properties of the polymer, but also on the flow dynamics and concentration profiles around the film reservoir. With the exception of the work of Glasvas-Dovos (2002), all the other profiles were obtained using USP dissolution tests. In those tests, the higher shear provided by the agitation of the paddles may have increased the rate of release. However, it is important to remember that we also maintained “sink” conditions by periodically replacing the receiver solution with fresh buffer solution.

The relative short lasting effect of commercial topical lidocaine formulations (2 to 4 hrs of effective action), could be improved with the formulations presented in this work. For example, the release profile obtained with Type I systems is almost linear during the first 12 hrs, and still significant release occur over the next 12 hrs. *In vivo* studies to confirm the long lasting effects of these linker formulations are still needed.

4.4.4.3 Effect of application conditions: dosage and application time

In order to investigate the influence that microemulsion dosage has on extended release, the release profiles of Type II microemulsions formulated with 10% lidocaine in IPM were obtained for dosages of 40, 80 and 120 µl of microemulsion per cm² of skin, and placed on the top of the skin for 30 min. We concentrated on Type II microemulsions because they are less toxic than Type I systems (Yuan et al, 2008), and therefore more likely to be used as delivery systems. The
results were compared with that for a dosage of 1600 µl/cm² (400 µl applied on the MPD mentioned in Section 4.3.3). The release profiles for different dosages are given in Figure 4.6a. According to these cumulative release profiles, the larger the microemulsion dosage, the larger the amount of lidocaine released.

Figure 4.6 (a) In vitro release profiles of lidocaine and (b) Log (1-\(M_t/M_\infty\)) against time for the 24-h data from the Type II microemulsion formulated with 10% lido-IPM. Different curves present different dosages.

To understand the data in Figure 4.6a, it is important to consider the initial lidocaine concentration absorbed in the skin after different dosages. The initial lidocaine concentration in the skin was 11.1±3.2, 16.0±4.0, and 21.4±6.5 mg/ml for the dosage of 40, 80 and 120 µl/cm² respectively. Based on this information, the larger the microemulsion dosage, the larger the initial lidocaine concentration in the skin, and the larger the cumulative amount of drug released. Figure 4.6b presents the value log(1-\(M_t/M_\infty\)) against time (t) for the 24-h data of different dosage.
The variation of the slopes (-k\textsubscript{sr}/l) for different curves is small, which indicates that the change of application dosage do not change the mass transfer coefficient (k\textsubscript{sr}).

To explain the effect of microemulsion dosage, one can consider that transdermal delivery with microemulsions resembles the general phenomena of imbibition of liquids into porous media, which is explained by the Washburn equation (Washburn, 1921). According to this equation, a wetting fluid (such as microemulsions) will penetrate a capillary tube as long as there is liquid in the mouth of the tube. Larger doses provide a larger reservoir of fluid on the mouth of the pores, thus imbibing more microemulsion into the skin. In reality, dosages of 10 - 40 µl/cm\textsuperscript{2} can be used for topical applications. However, a dosage of 1600 µl/cm\textsuperscript{2} is unrealistic because the microemulsion, having the relatively low viscosity of 15 cP and a wetting contact angle on the skin (less than 10°), would quickly spread over the skin. In this work most of the studies were conducted at 1600 µl/cm\textsuperscript{2} for the purpose of evaluating effects other than dosage in order to ensure that the pores are filled during the time that the microemulsion is in contact with the skin.

Besides dosage, application time is also a factor that may affect the extended release of lidocaine. To evaluate this effect, the released profile was obtained for skins dosed with 1600 µl/cm\textsuperscript{2} of Type II systems (10% lidocaine in IPM) and exposed for 5 min and 15 min. Figure 4.7a shows the cumulative lidocaine release profiles for 5, 15 and 30 minutes of application time. As expected, longer application times resulted in more drug permeated through the skin. The drug initially absorbed in the skin also increases from 21.2±4.2 to 24.2±3.3 mg/ml as the application time increases from 5 to 30 minutes. This observation is consistent with the full differential mass balance equation (Equation 4.1), where the concentration in skin is expected to increase from zero, when the donor is placed on top of the skin, to a steady state value when the flux of lidocaine received from the donor equals the flux of lidocaine released from the skin. In Chapter 3, it was found that the time for linker-based lecithin microemulsions to reach steady state during
continuous permeation is close to 30 minutes. For application times shorter than 30 minutes, it is expected that the concentration of the drug in the skin to be lower than the steady state value, which is consistent with the experimental observation. However, even for an application time of 5 minutes, the amount of lidocaine initially absorbed and later released from the skin is comparable to that obtained with a 30 minutes application time.

Figure 4.7b presents the value $\log(1 - M_t/M_\infty)$ against time ($t$) for the 24-h data of different application times. According to this figure, the application time has no effect on the mass transfer coefficient ($k_{sr}$), suggesting that the differences observed in Figure 4.7a (absolute release) are only due to the differences in the mass of lidocaine loaded into the skin ($M_\infty$), as would be predicted by Equation 4.7.

**Figure 4.7** (a) *In vitro* release profiles lidocaine, and (b) $\log (1 - M_t/M_\infty)$ against time for the 24-h data from the Type II microemulsion formulated with 10% lido-IPM. Different curves present different application times.
4.4.2 Microscopic observations

A fluorescent lipophilic dye, Nile red, was used to visualize the absorption and permeation of linker microemulsions into the skin. The Type II and I linker microemulsions formulated with 10% lidocaine in IPM were applied on the skin surface for 30 min and then withdrawn afterwards. For comparison, 10% lidocaine dissolved in IPM only (no microemulsion) was also included. The skin samples were cross-sectioned and viewed by fluorescence microscopy. The micrographs showing the presence of Nile red (red fluorescent dye) in the skin are presented in Figure 4.8.

![Microscopic observations](Image)

**Figure 4.8** Penetration of Nile red into pig ear skin from (a) Nile red in IPM after 1h, (b) Type II linker microemulsion after 1h, and (c) Type I linker microemulsion after 1h.

Figure 4.8a show the location of Nile red formulated in IPM after 1h of release. Figure 4.8b and 8c show the location of Nile red formulated in the Type II and I microemulsions remaining in the pig skin after 1h. After 1h of extended release, all formulations showed Nile red absorption in the superficial level of stratum corneum (SC). In comparison to the IPM system (Figure 4.8a), both Type II (Figure 4.8b) and Type I (Figure 4.8c) linker formulations absorbed more of the hydrophobic fluorescent compound in the superficial layer and more of the dye permeated deeper into skin. This observation is consistent with the larger absorption values (lidocaine skin concentrations) of Table 4.2 and those obtained in Chapter 3. The Type II microemulsion had the
highest deposition of Nile red on the uppermost skin layer because its external phase is oil which contains more hydrophobic Nile red.

These fluorescence microscopy studies are consistent with the hypothesis that the active ingredient (the hydrophobic fluorescence dye Nile red, in this case) is carried by the linker microemulsions to the deeper layers of the skin.

4.5 CONCLUSIONS

We observed the extended release of lidocaine from the in situ skin patches produced with linker-based lecithin microemulsions. After applying the microemulsions for a period of time, lidocaine was absorbed in the skin. The microemulsion imbibed in the skin acted as drug reservoir and provided extended release for over 24 hours. The lidocaine release from the microemulsions in situ patches follows a first-order kinetics. Increasing the drug loading in the microemulsion, the microemulsion dosage, and the application time increases the drug uptake in the skin, and the cumulative amount of drug release. However, the mass transfer constant ($k_{sr}$) is not affected by these formulation/application conditions, and depends on the morphology of the microemulsion, and quite likely on the properties of the skin. Microscopic observations illustrated the uptake of a lipophilic dye by the upper layers of the skin.

In conclusion, linker microemulsions can act as in situ delivery patches for extended release of active ingredients. Potential advantages of this in situ patch include its application on uneven and exposed parts, its low cost, customizable dose and flexibility to formulate for a wide range of drugs.
4.6 REFERENCES


Skaaret, I., 2006. Extended release of lidocaine hydrochloride from a new formulation, Department of Pharmacy, Uppsala University (http://www.farmfak.uu.se/farm/galfarm-web/DiplomaWork/Ingrid_Skaaret.pdf)


CHAPTER 5

EFFECT OF SURFACTANT CONCENTRATION
ON TRANSDERMAL DELIVERY OF LIDOCAINE
WITH LINKER-BASED LECITHIN MICROEMULSIONS

This chapter is derived from the following manuscript:

5.1 ABSTRACT

In Chapter 3 and 4, linker-based lecithin microemulsions have been shown to produce substantial transdermal flux and skin absorption of lidocaine. From a practical prospective, it is desirable to reduce the surfactant and additive content while maintaining a desirable flux and absorption of the active. In this chapter, we investigate the effect of surfactant and additive (linker) content on transdermal flux and absorption of lidocaine. The literature on this issue suggests that high surfactant concentrations reduce the partition of the active into the skin, which is undesirable. However, other literature on microemulsions transport suggests that increasing surfactant concentration improve the mass transport by increasing the number of micelle or reverse micelle “carriers”. To resolve these issues, a differential mass balance model was introduced to analyze the lidocaine permeation profiles. The model contains three basic parameters, the skin-donor partition ($K_{sd}$), the donor mass transfer coefficient ($k_{ds}$) and the skin mass transfer coefficient (skin permeability, $k_{sr}$). For Type I (oil-swollen micelles) microemulsions, increasing surfactant concentration reduces $K_{sd}$ up to a point, but this was not observed for Type II (water-swollen reverse micelles) microemulsions. Increasing surfactant concentration also increases $k_{ds}$, but in most cases the permeability of the skin ($k_{sr}$) limits the overall transport. The “skin” permeability slightly increased with surfactant concentration up to a certain maximum. A surfactant-mediated absorption/permeation mechanism is proposed to explain the drug transport through the skin. Fluorescence studies for the permeation of hydrophilic, hydrophobic and amphiphilic probes are consistent with the proposed mechanism of transport.
5.2 INTRODUCTION

Over the last few decades, microemulsions have gained considerable interest as potential drug delivery systems (Bagwe et al., 2001; Lawrence and Rees, 2000; Tenjarla, 1999). Microemulsions offer many advantages for pharmaceutical use, such as ease of preparation, thermodynamic stability, and high solubilization capacity for both lipophilic and hydrophilic drugs. However, the main limitation in microemulsion formulations is that they typically require large amount of surfactants and cosurfactants (>20 wt.%) in formulations. For example, Delgado-Charro et al. formed microemulsion systems with about 25-44% surfactant mixture (caprylocaproyl polyoxylglycerides and polyglyceryl fatty acid ester) to delivery drugs through the skin (Delgado-charro et al., 1997). The surfactant concentration can be as extremely high as 70% in similar microemulsion systems (Kreilgaard et al., 2002). High surfactant concentrations may cause skin irritation (McKarns et al, 1997; Attwood, 1994). In addition, the cosurfactants employed in microemulsions are usually medium-chain alcohols which are potentially irritating the skin (Attwood, 1994). Therefore, for a TDD formulation, the surfactant/cosurfactant concentration should be as low as possible for a given drug delivery goal. The use of microemulsions as drug carriers in the future depends on the choice of well-tolerated surfactants and the optimization of their concentration and dosage.

The influence of surfactant concentration on the transdermal potential of microemulsions has not been studied thoroughly. In the literature, there are only fewer papers (Chen, et al., 2006; Sintov and Shapiro, 2004; Rhee et al., 2001) that have discussed the influence of surfactant concentration on the transdermal flux of drugs in microemulsion systems, but none of them has systematically investigated this effect thus far. In those articles, the authors observed that an increase in surfactant concentration reduces the flux of drugs delivered through the skin. It was suggested that the increase in the surfactant concentration lowers the thermodynamic activity of
the drug in the delivery vehicle, which ultimately leads to a decrease of the partition into the skin and a decrease in drug permeation (Heuschkel et al., 2007; Date and Patravale, 2007).

In contrast, a kinetic study of ion transport across the water-oil interface in microemulsions showed that the flux increases with increasing surfactant concentration (Steytler et al., 2001). The author observed that the solute flux increased with increasing the surfactant concentration in the microemulsion phase, up to a certain point where the flux reaches a plateau value. These researchers propose that micelles or reverse micelles act as carriers of the solute from the bulk to the interface, and that the more micelles the more carriers are available, but that there is a point where the interfacial area for mass transfer is saturated by carriers (a phenomenon that can be modeled using a Langmuir adsorption isotherm). Such dynamic model has been supported by the results of Nitsch et al. (Nitsch et al., 1990).

The objective of this work is to investigate the relevance of surfactant concentration on the transdermal flux and absorption in linker-based lecithin microemulsions, and elucidate the apparently contradictory observations introduced above. These linker-based lecithin microemulsions have been proven to be effective vehicles for transdermal delivery, featuring high drug permeation, high skin absorption, and minimal cytotoxicity in Chapter 3 and 4. The drug absorption/permeation parameters of interest in this Chapter include: drug solubilization capacity, drug absorption in the skin, partition of the drug between skin and the microemulsions, “skin” permeability, mass transfer constant between skin and the microemulsion, and overall transdermal flux. The value of these parameters will be evaluated as a function of surfactant concentration in Type I and Type II microemulsions.

Lidocaine, a lipophilic drug, was used as a model drug in this work. It is a common anesthetic used in topical formulations and has been used as drug models by many groups (Sintov and
Shapiro 2004; Kreilgaard et al., 2000). The transdermal delivery of lidocaine is significantly limited by its poor water solubility (4 mg/ml). Most of the existing lidocaine delivery systems in the market place are emulsion-based, such as EMLA® cream. It has been shown that microemulsion-based gels for lidocaine delivery tend to have a slightly longer lasting effect than emulsion-based systems (Lee, 2003), and produce nearly 50% -100% larger fluxes of lidocaine compared to EMLA® cream (Kreilgaard, 2002; Sintov and Shapiro, 2004). However the benefits of potentially larger fluxes obtained with microemulsions do not compensate for the increase in cytotoxic side effects related to the use of alcohols as cosurfactants (Changez et al, 2006; Kreilgaard, 2002). Our developed lecithin-linker systems minimize these cytotoxic side effects and yield higher lidocaine transdermal flux than a conventional alcohol-based microemulsion as discussed in Chapter 3.

5.3 MATERIALS AND METHODS

5.3.1 Materials

5.3.1.1 Chemicals

The following chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA) at the concentrations shown in parentheses, and were used as received: sorbitan monooleate (Span® 80, 99%+), sodium caprylate (99%+), caprylic acid (99%+), isopropyl myristate (IPM, 98%), Nile Red (98%+), sodium chloride (99%+, Fluka brand), Dulbecco’s phosphate buffered saline (PBS), lidocaine powder (base form, 98%+). Laboratory grade soybean lecithin (99%+) was purchased from Fisher Scientific (Fairlawn, NJ, USA). Soybean lecithin is a mixture of phospholipids (mainly phosphatidyl cholines) produced by acetone purification of soybean gum residues. The fluorescent lecithin, 2-(12-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino)dodecanoyl-1-
hexadecanoyl-sn-glycero-3-phosphocholine (NBD C_{12-16}-PC) was purchase from Invitrogen (Carlsbad, California). Sodium phosphate monobasic, monohydrate (ACS grade) and acetonitrile (HPLC grade) were purchased from EMD Chemicals Inc. (Darmstadt, Germany), and they were used as received. Anhydrous ethyl alcohol and methanol were purchased from Commercial Alcohols Inc. (Brampton, ON, Canada). Unless otherwise stated, the composition is expressed on weight basis (i.e. wt. %) throughout this Chapter.

5.3.1.2 Skin

Pig ears were obtained from the local market and frozen overnight. Prior to use, they were thawed by rinsing with running water for ten seconds at room temperature. The skin of the external side of the ear was then dermatomed to a thickness that ranged from 700 to 900 μm (Yuan et al., 2008; Bronaugh and Maibach, 1991). After that, the thin skin layer was cut in circles of 11.4 mm diameter ready for use.

5.3.2 Microemulsion preparation and selection

5.3.2.1 Preparation

Different series of linker-based lecithin microemulsions from A to L with increasing lecithin concentrations from 0% to 4% at intervals of 0.4%. Sorbitan monooleate to lecithin weight ratio was kept 3:1, consistent with Chapter 3 and 4. For a given lecithin concentration (and therefore sorbitan monooleate concentration), sodium caprylate scan was gradually increased from 0.5% to 7.0% to achieve a Type II-Type III/IV-Type I transition at constant temperature (22°C) and electrolyte concentration (0.9% w/w NaCl) (Acosta et al., 2005). Details of this procedure are described in Chapter 4. Lidocaine was loaded in the microemulsion formulations by
predissolving this drug in IPM to a concentration of 10%. The composition of the linker-based lecithin microemulsions is shown in Table 5.1.

Table 5.1 The compositions of the linker-based lecithin microemulsions from Series A to L with different surfactant and linker concentrations (% w/w).

<table>
<thead>
<tr>
<th>Systems</th>
<th>% LE</th>
<th>% SM</th>
<th>% CA</th>
<th>% SC</th>
<th>Selected Type II</th>
<th>Selected Type I</th>
<th>% NaCl</th>
<th>% water</th>
<th>% IPM/lidocaine</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>0.4</td>
<td>1.2</td>
<td>0.3</td>
<td>0.4~7.0</td>
<td>0.4</td>
<td>2.8</td>
<td>0.9</td>
<td>48.4-%SC</td>
<td>48.8</td>
</tr>
<tr>
<td>B</td>
<td>0.8</td>
<td>2.4</td>
<td>0.6</td>
<td>0.5~7.0</td>
<td>-</td>
<td>-</td>
<td>0.9</td>
<td>47.7-%SC</td>
<td>47.6</td>
</tr>
<tr>
<td>C</td>
<td>1.2</td>
<td>3.6</td>
<td>0.9</td>
<td>0.5~7.0</td>
<td>0.5</td>
<td>3.1</td>
<td>0.9</td>
<td>47.0-%SC</td>
<td>46.4</td>
</tr>
<tr>
<td>D</td>
<td>1.6</td>
<td>4.8</td>
<td>1.2</td>
<td>0.5~7.0</td>
<td>-</td>
<td>-</td>
<td>0.9</td>
<td>46.3-%SC</td>
<td>45.2</td>
</tr>
<tr>
<td>E</td>
<td>2.0</td>
<td>6.0</td>
<td>1.5</td>
<td>0.5~7.0</td>
<td>-</td>
<td>-</td>
<td>0.9</td>
<td>45.6-%SC</td>
<td>44.0</td>
</tr>
<tr>
<td>F</td>
<td>2.4</td>
<td>7.2</td>
<td>1.8</td>
<td>0.5~7.0</td>
<td>0.6</td>
<td>4.2</td>
<td>0.9</td>
<td>44.9-%SC</td>
<td>42.8</td>
</tr>
<tr>
<td>G</td>
<td>2.8</td>
<td>8.4</td>
<td>2.1</td>
<td>0.5~7.0</td>
<td>0.7</td>
<td>4.9</td>
<td>0.9</td>
<td>44.2-%SC</td>
<td>41.6</td>
</tr>
<tr>
<td>H</td>
<td>3.2</td>
<td>9.8</td>
<td>2.4</td>
<td>0.5~7.0</td>
<td>0.8</td>
<td>5.6</td>
<td>0.9</td>
<td>43.5-%SC</td>
<td>40.2</td>
</tr>
<tr>
<td>I</td>
<td>3.6</td>
<td>10.8</td>
<td>2.7</td>
<td>0.5~7.0</td>
<td>-</td>
<td>-</td>
<td>0.9</td>
<td>42.8-%SC</td>
<td>39.2</td>
</tr>
<tr>
<td>L</td>
<td>4.0</td>
<td>12.0</td>
<td>3.0</td>
<td>0.5~7.0</td>
<td>1.0</td>
<td>7.0</td>
<td>0.9</td>
<td>42.1-%SC</td>
<td>38.0</td>
</tr>
</tbody>
</table>

LE - lecithin  SM - sorbitan monooleate  CA - caprylic acid  SC-sodium caprylate

5.3.2.2 Selection

To study the effect of surfactant contents on transdermal delivery from the linker-based microemulsions, six samples of Winsor Type II microemulsions and another six samples of Winsor Type I microemulsions were selected (Table 5.1) from Series A, C, F, G, H, and L, respectively. According to a microemulsion critical scaling model, the radius of micelles or reverse micelles ($R_d$) in a similar linker-based microemulsions is a function of the ratio of the hydrophilic linker (sodium caprylate in this case) to lecithin ratio ($SC/LE$), with respect to the ratio that produce optimum Type III/IV system ($SC/LE$)* such that $R_d \propto \frac{(SC/LE) - (SC/LE)^*}{(SC/LE)^*}$
(Acosta et al., 2004). This can be easily described when using a classical two-dimensional “fish diagram” as shown in Figure 5.1. In this study, we keep the ratio \( \frac{(SC/LE) - (SC/LE)^*}{(SC/LE)^*} \) constant for all selected Type II and I samples to ensure that the particle sizes is approximately the same. This is confirmed later in the result section.

![Figure 5.1](image)

**Figure 5.1** Schematic of sample selection in a “fish” phase diagram. The dotted line indicates the optimum formulation (equal volume of oil and water solubilized in middle phase). The shaded area means no microemulsion formation.

5.3.2.3 Particle Size

Dynamic light scattering measurements of the hydrodynamic radius and polydispersity of the microemulsion droplets were performed at room temperature (23±1°C) using a BI-200SM Brookhaven instrument equipped with a 35 mW green laser (wavelength ~514 nm) and a photomultiplier detector located at a fixed angle of 90°. Microemulsion samples were poured
into standard glass curettes that were placed in the BI-200SM sample holder at least 10 minutes before measurement. Refractive indexes for the continuous phase were obtained from the literature (Lide, 2006).

5.3.3 *In vitro* permeation studies

The transdermal flux and “skin” permeability of the drug from test formulations were measured *in vitro* using pig ear skin (Yuan et al., 2008). All permeation experiments were performed according to the MatTek standard percutaneous absorption protocol. Briefly, the skin piece was placed in a MatTek Permeation Device (MPD), with the epidermis facing up. The microemulsion formulation (0.4 ml) was applied in the donor compartment. The receptor compartment was filled with 5 ml of PBS (0.01M phosphate, 0.137M NaCl, pH 7.4). At predetermined times (0.5 h, 1.5 h, 2.5 h, 3.5 h, 4.5 h and 5.5 h), the receiver solution was withdrawn completely and immediately replaced by fresh PBS solution. At 5.5 h, the experiment was terminated. All permeation experiments were conducted in triplicate at room temperature. The pig skin remaining after the permeation study was used to test the lidocaine absorbed into the skin.

The cumulative mass of lidocaine (μg) permeated across the skin was plotted as a function of time (h), and the average steady-state flux ($F_{ss}$, μg/h/cm²) was calculated by dividing the slope of the linear part of the curve ($dm/dt$) by the area of the exposed skin surface ($A$).

$$F_{ss} = \frac{1}{A} \times \frac{dm}{dt}$$  \hspace{1cm} (Equation 5.1)
5.3.3.1 Determination of skin absorption

The amount of lidocaine retained in the skin was determined at the end of the in vitro permeation studies (5.5 h). Prior to measuring the absorbed lidocaine, the pig skin was rinsed with PBS solution and placed into 2 ml methanol for overnight extraction of lidocaine (Yuan and Acosta, 2009). The resulting solution was analyzed by HPLC. The equivalent lidocaine concentration absorbed in skin \( (C_{ss}) \) was calculated as the mass of lidocaine extracted from the skin divided by the volume of the skin (exposed area \( \times \) thickness), and is expressed in mg/ml. We have determined, using a mass balance closure in selected samples, that the efficiency of this methanol extraction procedure is more than 95%.

5.3.3.2 Lidocaine quantification

The concentration of lidocaine in the microemulsions, receiver solutions and skin was analyzed using a Dionex ICS-3000 (Sunnyvale, CA, USA) liquid chromatography system consisting of single pump, detector, AS40 automated sampler, AD25 absorbance detector and Chromeleon chromatography software (Dionex) (Yuan and Acosta, 2009). Lidocaine was separated by a reverse phase column (Genesis, C\(_{18}\), 4 \( \mu \)m, 150\( \times \)4.6 mm) and detected through its absorbance at 230 nm (AD25 detector). A mixture of acetonitrile and 0.05 M NaH\(_2\)PO\(_4\)-H\(_2\)O (pH 2.0) solution (30:70, v/v) was used as the mobile phase with a flow rate at 1.0 ml/min. The column temperature and the injection volume were 25°C and 10\( \mu \)l, respectively.
5.3.3.3 Statistical data analysis

All permeation values were calculated from three independent experiments, and data are expressed as the mean value ± S.D. The statistical analysis of $C_d$, $C_s$, $K_{ds}$, $k_{sr}$, $k_{ds}$ and $J$ was performed using a one-way analysis of variance (ANOVA) to test the difference between the means of two or more delivery systems. Data with $P<0.05$ are considered statistically significant.

5.3.4 Fluorescence Microscopy

To visualize the penetration of hydrophobic and hydrophilic solutes as well as the penetration of lecithin into the skin in microemulsions, various fluorescence probes were dissolved in the Type II formulation of system L of Table 5.1 (4%LE, 1% SC). A red-fluorescing probe, Nile Red (0.001%) was used as a surrogate for hydrophobic actives. A green-fluorescing probe, NBD C$_{12}$-C$_{16}$-PC (0.01%) was used as a surrogate to trace the penetration of lecithin. The systems were vortexed and left to equilibrate overnight. The microemulsions containing each fluorescence probe were used to conduct in vitro extended release studies. After 1 hour, the skin samples were taken off the permeation device, blotted dry with Kimwipes, and then rinsed twice with PBS. The clean skin samples were snap frozen using dry ice and were cross sectioned to 30 μm thick slices by a cryostat microtome (Leica Jung CM3000, Bensheim, Germany). The skin slices were observed and photographed with a Leica MZFIII fluorescence stereomicroscope (Leica, Heerbrugg, Switzerland) equipped with a Leica DFC 320 Digital Camera (Leica, Heerbrugg, Switzerland). Sections were photographed using a red filter (λexc= 450-500 nm) suitable for these fluorescence probes. All photographs were taken with 63x objectives and exposure time 2.0 sec. Solutions of 0.001% Nile red in IPM was considered as the “hydrophobic” control. The penetration of each dye into the skin was assessed using image analysis (histogram tool of Corel
Paint Shop Pro™ version 9.02) to measure the intensity of the red (for Nile red) and green (for NBD-C_{12}-C_{16}-PC) hue for different skin penetrations. That intensity was normalized as \( \frac{{\text{Intensity} - \text{Intensity}_{\text{min}}}}{{\text{Intensity}_{\text{max}} - \text{Intensity}_{\text{min}}}} \), which assumes that the minimum intensity corresponds to the background fluorescence from the skin (if any).

5.4 RESULTS AND DISCUSSION

5.4.1 Phase behaviour

The composition of the linker-based lecithin microemulsions are shown in Table 5.1. The total surfactant concentrations (lecithin+linkers) in the formulations from Series A to L were gradually increased at regular intervals. The phase behaviour of the linker-based systems was obtained by scanning the concentration of the hydrophilic component of the surfactant mixture, sodium caprylate, from 0.5% to 7% at intervals of 0.5%. In this manner, the following phase transition occurred in all series with increasing sodium caprylate concentrations (Figure 5.2): Winsor Type II (water-swollen reverse micelles) – Winsor Type III or IV (bicontinuous phase) – Winsor Type I (oil-swollen micelles). This phase map is consistent with maps observed in Chapter 3 and a previous study (Acosta et al., 2005). In those previous studies, as in this case, the boundaries shift to higher SC/LE ratios at lower lecithin concentrations. This shift has been explained based on the partition of the hydrophilic linker (sodium caprylate) that tends to be preferentially dissolved in water at low surfactant concentration.

This phase map has been used for the selection of Type II and I microemulsion samples for in vitro transdermal study according to the sample selection method mentioned earlier. In Figure 5.2, the points “•” indicate the compositions of the selected Type II and I samples used in this
study. All selected samples have the approximately equal ratio \( \frac{(SC / LE) - (SC / LE)^*}{(SC / LE)^*} \) in hopes of obtaining with similar droplet size.

Figure 5.2 Phase map for the linker-based lecithin microemulsions. The dotted line indicates the optimum formulation (equal volume of oil and water solubilized in middle phase). The points “*” indicate the selected Type II and I samples with the exact compositions in the corresponding test tube.

5.4.2 Particle size

Figure 5.3 presents the radius of the selected Type II and I microemulsion samples obtained from dynamic light scattering measurements. The droplet radius remains essentially constant for all samples with increasing the surfactant concentration in the systems at 25°C, which confirms the
appropriateness of using the ratio \( \frac{(SC/LE) - (SC/LE)^*}{(SC/LE)^*} \) to select the samples. The droplet sizes of the selected samples are around 6 nm, which is consistent with values reported in Chapter 3 and a previous study (Acosta et al., 2005).

It is important to select samples with constant droplet size for the transdermal studies because some microemulsion mass transfer models (Steytler et al., 2001; Nitsch et al., 1990) suggest this is one of the factors that influence mass transport. From the results of Figure 5.3, we can conclude that with increasing surfactant concentration, we increase the number of droplets per volume in the system, but the droplet size remains constant.

Figure 5.3 Droplet radius of Type II and I linker-based microemulsions of different surfactant concentrations.
5.4.3 Effect of surfactant on drug partitioning into microemulsions

After the studied microemulsions were prepared and reached equilibrium, the drug, lidocaine, partitioned into the microemulsion and the excess phases. The lidocaine concentration in the microemulsion samples ($C_d$, these samples are used as donor solutions) is shown in Figure 5.4. Saturated lidocaine in water (4.0 mg/ml lidocaine) and 10% lidocaine in IPM (80.1 mg/ml lidocaine) were used as controls of surfactant-free formulations (0% lecithin) for the Type I and II samples, respectively. In general, the increasing surfactant concentration in microemulsions increases the load capacity of the dispersed phase in both Type II and I systems.

![Graph showing lidocaine concentration in Type II and I linker-based microemulsions of different surfactant concentrations.](image)

**Figure 5.4** Lidocaine concentration in Type II and I linker-based microemulsions of different surfactant concentrations.

For Type I systems, the concentration of lidocaine in the microemulsion increases with increasing surfactant concentration. Type I systems consist of oil-swollen micelles in a
continuous aqueous phase, and therefore with increasing surfactant concentration, the number of the micelles per volume increases, and also the amount of oil (and lidocaine) solubilized. Compared to saturated lidocaine in water (4.0 mg/ml), the addition of incremental amounts of surfactants from Series A to L gradually improves the solubilization of lidocaine in Type I systems. The high drug solubilization capacity is the most important feature for delivering oil-soluble drugs using aqueous surfactant solutions (Kreilgaard, 2002; Lawrence and Rees, 2000).

On the other hand, the lidocaine concentration in Type II systems decreases with increasing surfactant concentration. With the addition of more surfactants, more water is solubilized in the core of the reverse micelles, and thus increasing the volume of Type II system, which “dilute” the lidocaine in the Type II system.

5.4.4 Effect of surfactant on lidocaine absorption in the skin

Figure 5.5 shows the concentration of lidocaine absorbed in the skin ($C_{ss}$) as a function of surfactant concentration in the microemulsions. Same as in Figure 5.4, the first points of 0% lecithin are the controls of surfactant-free formulations. Lidocaine skin absorption was significantly affected by surfactant concentration. Increasing surfactant (lecithin) concentration in the microemulsion increased the lidocaine skin concentration ($C_{ss}$) in both Type II and I microemulsions. This indicates that the high surfactant concentrations are desirable if the goal of transdermal delivery is to accumulate drugs in the skin.
Figure 5.5 Drug absorption in the skin after treatment by Type II and I linker-based microemulsions of different surfactant concentrations.

The increase in skin absorption may be explained by a surfactant-mediated absorption/permeation mechanism. As we discussed earlier, more surfactant in either Type II or I microemulsion results in more surfactant droplets (micelles/reverse micelles) with constant size. We propose that these droplets carry the drug into the skin. In the case of lidocaine, this drug is located either in the core of the micelles or in the palisade layer of reverse micelles. When increasing the surfactant concentration in the formulations, more droplets can carry more drugs and increase the drug absorption in the skin. Figure 5.6 presents a schematic diagram of this hypothetical surfactant-mediated absorption/permeation mechanism. Consistent with this schematic, Kreilgaard and collaborators showed that transdermal delivery in microemulsions is not fully explained by the permeability enhancing effect of surfactants, and that surfactants play an alternative role in these systems (Kreilgaard et al., 2002).
**Figure 5.6** Schematic diagram of the surfactant-mediated absorption/permeation from the microemulsion to the skin. Partitioning of the incorporated drug can take place by: droplet partitions between the microemulsion to the skin (step a); and drug partitions between the droplet and the skin (step b).

### 5.4.5 *In vitro* permeation parameters

The *in vitro* drug permeation experiments were performed using the selected linker-based microemulsion samples for Type II and I systems. The experimental permeation profiles can be obtained by plotting the cumulative mass of lidocaine permeated per unit area of the skin as a function of time. In order to obtain the *in vitro* permeation parameters, we proposed a 3-compartment mass transfer model to produce a theoretical release profile that can be fitted to the experimental profile. Here we use the full differential mass balance equation (Equation 4.1) proposed in Chapter 4.

As illustrated in Figure 4.3, we assumed that lidocaine is first transferred from the donor to the skin and then from the skin to the receiver. Such transfer leads to two transfer fluxes: (1) the flux
$F_{ds}$ from the donor solution to the skin, and (2) the flux $F_{sr}$ from the skin to the receiver solution. The differential mass balance of the drug in the skin yields the following equation:

$$\frac{dC_s}{dt} \times V_s = (F_{ds} - F_{sr})A$$  \hspace{1cm} \text{(Equation 4.1)}$$

where $C_s$ is the drug concentration in the skin at time $t$, $V_s$ is the volume of the skin, $A$ is the area of the skin.

A mass transfer coefficient was introduced to calculate the flux of lidocaine transferred from the donor to the skin ($F_{ds}$) and that transferred from the skin to the receiver solution ($F_{sr}$),

$$F_{ds} = k_{ds} (K_{sd} C_d - C_s)$$  \hspace{1cm} \text{(Equation 5.2)}$$

$$F_{sr} = k_{sr} (C_s - K_{sr} C_r)$$  \hspace{1cm} \text{(Equation 4.3)}$$

where $k_{ds}$ and $k_{sr}$ are the mass transfer constant from the donor to the skin and from the skin to the receiver, respectively. $K_{sd}$ and $K_{sr}$ are the partition coefficient of the drug between the skin and the donor, and between the skin and the receiver, respectively. $C_d$ and $C_r$ are the drug concentration in the donor and receiver solution at time $t$. The drug concentration in the donor ($C_d$) is assumed to be constant during the experiments since we found it remained same before and after the permeation experiments. In our permeation experiments, at predetermined times, the receiver solution was withdrawn completely from the receptor compartment and was immediately replaced by fresh PBS solution. In this way, compared to the lidocaine concentration in the donor ($C_d$), we can assume that the drug concentration in the receiver ($C_r$) is negligible, that is, $C_r \sim 0$.

Substituting $F_{ds}$ and $F_{sr}$ in Equation 4.1 by Equation 5.2 and Equation 4.3, we obtain that:

$$\frac{dC_s}{dt} \times \frac{V_s}{A} = k_{ds} (K_{sd} C_d - C_s) - k_{sr} C_s$$  \hspace{1cm} \text{(Equation 5.3)}$$
Experimentally, we can measure the mass of drug permeated as a function of time, and therefore we can calculate the flux of drug at different time intervals. Furthermore, we can also extract the remaining drug in the skin and divide this mass of drug by the volume of the skin to obtain equivalent drug concentration in the skin. Assuming that these systems reach an steady state before the end of the experiment, then this skin concentration is the steady state skin concentration, or \( C_{s,ss} \). The steady state assumption also helps calculating the “skin” permeability \( k_{sr} \), since at steady state \( \text{Flux} = F_{ds} = F_{sr} \). Using Equation 5.2, and considering that \( C_r \sim 0 \) (ppm levels), then \( F_{sr} = k_{sr} C_{s,ss} = F_{ss} \) (flux at steady state). Also using the steady state assumption, the accumulation term of Equation 5.3 \( \int \frac{dC_s}{dt} \times \frac{V}{A} \) is zero, and solving for \( k_{ds} \), we obtain:

\[
 k_{ds} = \frac{k_{sr} C_{s,ss}}{K_{sd} C_d - C_{s,ss}} \tag{Equation 5.4}
\]

To find the values of \( k_{ds} \) and \( K_{sd} \), we fitted a theoretical release profile (cumulative mass of lidocaine released versus time) to the experimental release profile. To obtain the theoretical release profile, first we assumed a value of \( K_{sd} \) (the initial assumption being \( K_{sd} = C_{s,ss}/C_d \)) and using Equation 5.4, then calculate \( k_{ds} \). With \( K_{sd} \), \( k_{ds} \), \( k_{sr} \) and \( C_d \) (measured experimentally) then solve Equation 5.3 for \( C_s \) at any given time. Then with \( C_s \) for any given time, Equation 5.2 can be used to calculate the flux of drug released from the skin to the receiver at any given time \( F_{sr} \) and by integrating this flux over the course of the experiment, the theoretical release profile is obtained. The value of \( K_{sd} \) is then iterated until the theoretical release profile matches the actual profile.

Figure 5.7a and 5.7b shows two examples of experimental permeation profiles for selected Type II and I samples of the F-Series. The fitted theoretical profiles are also presented as solid lines in this figure. It is observed that the theoretical profiles fit the experimental data well for both the
Type II and I systems. The in vitro permeation parameters, $K_{sd}$, $k_{ds}$, and $k_{sr}$ are then calculated or iterated for all selected Type II and I samples.

**Figure 5.7** Permeation profile of lidocaine from the selected (a) Type II, and (b) Type I linker-based microemulsion of the F-Series. The solid line represents the mass transfer model of Equation 5.3.

5.4.5.1 Partitioning between the skin and the donor

Figure 5.8 shows the fitted values of partition coefficient ($K_{sd}$) as a function of the surfactant content in the studied microemulsions. These fitted $K_{sd}$ values are close to the $C_{s,ss}/C_d$ ratio used as initial estimated value for $K_{sd}$.

For Type II systems, the partition coefficient ($K_{sd}$) gradually increases as the surfactant concentration increases. This can be explained by the increased drug absorption in the skin due to the surfactant-mediated permeation/absorption. As mentioned earlier, the higher surfactant concentration results in more droplets and consequently, more lidocaine is carried by reverse
micelles into the skin. Lidocaine, like other polar hydrophobic molecules, is likely associated to the palisade layer of these reverse micelles (Rosen, 2004).

![Graph showing the fitted values of the partition coefficients of lidocaine between the skin and the donor ($K_{sd}$) for Type II and I linker-based microemulsions of different surfactant concentrations.](image)

**Figure 5.8** The fitted values of the partition coefficients of lidocaine between the skin and the donor ($K_{sd}$) for Type II and I linker-based microemulsions of different surfactant concentrations.

For Type I linker-based microemulsions, the partition coefficient ($K_{sd}$) gradually decreases with increasing surfactant concentration until the concentration of lecithin is about 2.5%; After that, the partition slightly increase with increasing surfactant concentration. In this case, the partition coefficient ($K_{sd}$) comes from two contributions: one from the lidocaine molecularly dissolved in water and the other, the lidocaine solubilized in micelles. At low surfactant concentration, most of the lidocaine is molecularly dissolved in the external aqueous phase where it has a higher tendency to partition into the skin, leading to high $K_{sd}$ (Lee et al., 2003; Osborne et al., 1991). As the surfactant concentration increases, the fraction of lidocaine molecularly dissolved in water reduces, and the partition of lidocaine between micelles and skin (substantially small $K_{sd}$)
dominates the overall partition. At certain point when the surfactant concentration is high enough, the contribution from micelles becomes dominant. Similar to Type II systems, more surfactant results in more droplets which carry more drugs into the skin (in this case, most of the drug is located in the core of micelles).

5.4.5.2 “Skin” permeability

The “skin” permeability ($k_{sr}$) of lidocaine in Type II and I systems is presented in Figure 5.9 as a function of surfactant concentrations. For surfactant concentration less than 2.4% lecithin, an increase in surfactant concentration slightly increases the value of $k_{sr}$ for both Type II and I microemulsions. However this increase in $k_{sr}$ is not significant ($P=0.16$ and 0.36 for Type II and I systems, respectively). The observation reinforces our hypothesis that the surfactants used in the linker-based formulations do not act as permeation enhancers; Otherwise, a significant increase in $k_{sr}$ would have been observed with increasing surfactant concentration.

For surfactant concentrations above 2.4% lecithin, the value of $k_{sr}$ actually reduces with increasing surfactant concentration for both Type II and I systems. This would be due to two combined effects. The first effect is that the viscosity of the formulation increases with increasing surfactant concentration. For Type II, the viscosity at 2.4% lecithin is approximately 8 cP and that for 4% lecithin is 14 cP. For Type I, the viscosity at 2.4% lecithin is about 5 cP and that for 5% lecithin is 34 cP. The second effect is the accumulation of surfactant and additives in the skin which, due to its affinity with lidocaine may increase the retention of the lidocaine in the skin. The idea that surfactant and additives accumulate in the skin and they are not released to the receiver is based on an earlier observation in Chapter 3 that lecithin and the linkers do not appear in the chromatograms of the receiver solution.
Figure 5.9 The “skin” permeability \( (k_{sr}) \) of lidocaine from Type II and I linker-based microemulsions of different surfactant concentrations.

The same observations has been made by other researchers that increasing surfactant contents partly resulted in lower permeation rates (Chen et al., 2006; Rhee et al., 2001). It has been proposed that increasing the surfactant concentration reduces the chemical potential of the drug (Shah, 1994), which in our case seems to be reflected in lower \( k_{sr} \) with increasing surfactant concentration above 2.4% lecithin. Sintov and Shapiro found similar observations the highest surfactant contents resulted in lower fluxes (Sintov and Shapiro, 2004), so that the general permeation enhancing effect of the pure surfactants was ruled out (Rhee et al., 2001). Our observation that excess surfactant inhibits the flux increase thus supports the surfactant-mediated absorption/permeation hypothesis on transdermal drug delivery.

From Figure 5.9, it is obvious that the “skin” permeability of Type II systems are higher than those of Type I systems. This observation is consistent with the data reported in Chapter 3. The
higher permeation rates for the Type II systems may be related to the better interaction of their external phase (oil in this case) with the skin, which is highly lipophilic and more amenable for lidocaine. Jurkovic et al. also reported that a w/o microemulsions showed a better release of a hydrophobic drug than an o/w systems (Jurkovic et al., 2003). The authors suggested that the microemulsions led a different partitioning behaviour of the drug within the skin. The o/w formulation was supposed to accumulate the drug in stratum corneum and epidermis whereas the w/o system delivered it primarily into deeper skin regions.

5.4.5.3 The mass transfer constants from the donor to the skin

The mass transfer constants ($k_{ds}$) from the donor to the skin are shown in Figure 5.10 as a function of surfactant concentration. This parameter is the only one that obtained based purely on the fitting procedure, therefore it is highly variable. Considering the variability of the data, Figure 5.10 only hints a slight increase in $k_{ds}$ with surfactant concentration. This is consistent with the microemulsion mass transfer model of Steytler et al. (Steytler et al., 2001).

In most cases, the mass transfer constants ($k_{ds}$) of lidocaine from the donor to the skin is larger than the “skin” permeability ($k_{sr}$) of lidocaine from the skin to the receiver (i.e. $k_{ds} > k_{sr}$). For Type II systems, this difference is about 1-2.5 times; whereas for Type I system, the difference is about 2-8 times. This indicates that in most cases, the rate limiting step for the drug transport from the linker-based microemulsions is the transport of the drug from the skin to the receiver, for both Type II and I linker-based systems. Most of researchers treat the whole drug transfer process as a whole and only study one parameter, i.e. the drug transport rate from the formulation (donor) to the receiver. Through the use of 3-compartment mass balance, we can decouple this parameter and gain a better understanding of transdermal permeation. Further studies to explore this phenomenon are still needed.
Figure 5.10 The lidocaine permeability ($k_{ds}$) from the donor to the skin from Type II and I linker-based microemulsions of different surfactant concentrations.

5.4.6 Effect of surfactant on transdermal flux

The transdermal flux of lidocaine was calculated from the experimental drug permeation profiles for different systems according to Equation 5.1. Figure 5.11a and 11b show the plot of flux against the surfactant concentration either for the selected Type II or I samples. For both Type II and I systems, the results show that the flux is dependent on the surfactant concentration and increases as the surfactant concentration increases at low surfactant levels, but it reaches a limiting value at high surfactant concentration. The minimum surfactant amount needed to achieve flux saturation is about 2.4% lecithin for both Type II and I samples. In addition, the flux values for the Type II systems are much higher than those for the Type I systems. This result is consistent with Chapter 3 and 4.
Figure 5.11 The transdermal flux of lidocaine from the (a) Type II and, (b) Type I linker-based microemulsions of different surfactant concentrations.

Table 5.2 Effect of increasing surfactant concentration on permeation parameters.

<table>
<thead>
<tr>
<th>Permeation parameters</th>
<th>Type II</th>
<th>Type I</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low LE</td>
<td>High LE</td>
</tr>
<tr>
<td>Drug concentration in microemulsions ($C_d$)</td>
<td>↓</td>
<td>~</td>
</tr>
<tr>
<td>Drug concentration in skin ($C_s$)</td>
<td>↑</td>
<td>~</td>
</tr>
<tr>
<td>Partition coefficient from the skin to the donor ($K_{sd}$)</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>“Skin” permeability from the skin to the receiver ($k_{sr}$)</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Mass transfer constants from the donor to the skin ($k_{ds}$)</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Tranadermal Flux ($J$)</td>
<td>↑</td>
<td>~</td>
</tr>
</tbody>
</table>

LE – lecithin

Table 5.2 summarizes the effect of increasing surfactant concentration on the values of the permeation parameters for Type II and I systems. This table reflects the complex interdependence of various factors in TDD.

For Type II systems, at low surfactant concentration, the drug flux increases significantly with increasing lecithin concentrations. This is not surprising since both $K_{sd}$ (Figure 5.8) and $k_{sr}$
(Figure 5.9) increases when surfactant concentration increases. From the most basic diffusion equation (Hadgraft, 2004), it is well known that the transdermal flux is dependent on the permeability coefficient \( k_{sr} \) (in this case) as well as on the partition of the drug between the skin and the applied formulation \( K_{sd} \) (in this case). At high surfactant concentration, \( K_{sd} \) keeps increasing, but \( k_{sr} \) reduces and hence the drug flux does not change significantly.

For Type I systems, the complex interdependence among various parameters is more complex. Table 5.2 summarizes these findings.

### 5.4.7 Fluorescence studies

To test some of the postulates of the mechanisms depicted in Figure 5.6, the skin penetration of the fluorescence probe NBD-C\(_{12}\)-C\(_{16}\)-PC, a surrogate for lecithin in Type II microemulsions, was evaluated. This penetration profile is shown in Figure 5.12a. According to Figure 5.12a, the NBD-C\(_{12}\)-C\(_{16}\)-PC probe penetrated almost 300 µm into the skin. To interpret this value, it is important to consider that the thickness of the epidermis of pig ears skin is approximately 50µm, and the thickness of the stratum corneum is approximately 10 µm (Monteiro-Riviere et al, 1990). The fact that there is a significant penetration into the dermis supports the idea that lecithin droplets (reverse micelles in this case) penetrate deeper into the formulation, carrying the lidocaine. It is also pertinent to mention that the NBD-C\(_{12}\)-C\(_{16}\)-PC probe is fluorescence only when associated in amphiphilic environments. This property is frequently used to track the phase of lipid droplets like vesicles, or in this case, reverse micelles (Chattopadhyay, 1990).

To evaluate the penetration of hydrophobic solutes, like lidocaine, the fluorescence probe, Nile Red was used in Type II lecithin microemulsions formulated with 4%. The penetration of Nile

- 135 -
red in Type II is presented in Figure 5.12b. According to that Figure, Nile red also penetrates nearly 250 microns into the skin, which is compatible with the proposition that lecithin droplets carry the active ingredients into the skin. As a control, the penetration of Nile Red dissolved in IPM (no microemulsion) is presented in Figure 5.12c. As shown in that Figure, in this formulation Nile Red penetrates less than 100 micron, suggesting that, by partition and diffusion alone, hydrophobic components can only penetrate the stratum corneum and the epidermis.

Figure 5.12. Skin penetration profiles for (a) NBD-C12-C16-PC formulated in 4% LE Type II microemulsion, (b) Nile Red formulated in 4% LE Type II microemulsion, and (c) Nile red formulated in IPM.
While these findings are not complete proof of the mechanism of Figure 5.6, they are consistent with that schematic. Furthermore, there is increasing evidence that small (< 100 nm) and “soft” lipid-base carriers penetrate into skin by a network of pores, similar to that depicted in Figure 5.6 (Maghraby et al, 2008; Cevc, 2004).

5.5 CONCLUSIONS

Based on above results and discussions, it is evident that increasing surfactant concentration in the linker-based lecithin microemulsions improves the drug absorption into the skin. However, the increase in surfactant concentration also affects the partition of the drug and the mass transfer coefficients. As indicated by previous studies, increasing the surfactant concentration does reduce the activity of the drug in the continuous solvent, but at the same time it seems to opens a secondary mechanism of mass transfer than involves the penetration of the carrier into the skin. Similar to the findings in other microemulsion-based mass transport, the increase of surfactant concentration seem to increase the mass transfer coefficient in the microemulsion phase. However, the effect of surfactant concentration on the “skin” permeability is more complex. Certainly the modest increase in skin permeability supports the idea that increase permeability is not the main mechanism of transport enhancement when using linker-based lecithin microemulsions. Overall the transdermal flux seems to plateau at approximately 2.5% of lecithin. This lecithin concentration also corresponds to the point where the “skin” permeability reaches a maximum value. The proposed mathematical model to describe the release curves only use one fitting parameter, and it provides a frame work that can be used for other microemulsion systems. The fluorescence microscopy studies support the idea of a surfactant-mediated adsorption-permeation mechanism for linker-based lecithin microemulsions.
5.6 REFERENCES


CHAPTER 6

CONCLUSIONS AND FUTURE RECOMMENDATIONS
6.1 CONCLUSIONS

In conclusion, linker-based lecithin microemulsions have been formulated with food-grade additives, and are shown be safe and effective TDD vehicles \textit{in vitro}. When compared to a conventional alcohol-based lecithin system, the new lecithin-linker systems not only produce higher transdermal flux and larger drug absorption in the skin, but are also less toxic. After applying the linker microemulsions on the skin, \textit{in situ} delivery patches are formed and provide extended release of active ingredients up to 24 h. The mass of drug initially absorbed in the skin has a significant impact on drug release profiles. It is found that increasing surfactant concentration in the linker-based lecithin microemulsions improves the drug absorption in the skin, but only increases drug flux up to a plateau value. These conclusions rely on the following results:

1. Alcohol-free lecithin microemulsions can be formulated using the combination of sodium caprylate and caprylic acid as hydrophilic linkers. From this, different types of microemulsions can be obtained including Type II (w/o), Type III or IV (bicontinuous), Type I (o/w). These linker microemulsions produced relatively small droplets (less than 10 nm in diameter) which suggest the existence of a large surface area for mass transfer and explain the relatively low viscosity of these formulations. The formulation conditions such as temperature and electrolyte concentration do not affect the phase behaviour of these systems.

2. The superior transdermal flux obtained with the linker microemulsions is likely to be due to the presence of sodium caprylate and caprylic acid, which accelerate the microemulsion-skin mass transfer by reducing the interfacial rigidity of the systems. It is difficult to formulate alcohol-free lecithin microemulsions since there is only a small pool
of molecules with six to nine carbons that can be considered as hydrophilic linkers. The approach of using the combination of sodium caprylate and caprylic acid as hydrophilic linkers in lecithin microemulsions offers many exciting opportunities in the broad field of drug delivery.

3. The concept of “skin” permeability, for the first time, is introduced and used to determine the effectiveness of microemulsions as transdermal vehicles, especially useful when comparing different delivery systems. We express the “skin” permeability using the concentration difference between a common phase (the skin) and the receiver. The permeability commonly used in the literature (Equation 2.2 in Chapter 2) simply considers the concentration difference between the donor and the receiver. It ignores the solubility and chemical potential of the drug in the vehicles. Hence, the concept of permeability is suitable for simple solutions, but is not appropriate to evaluate microemulsion-based drug delivery vehicles. The new concept of “skin” permeability captures the permeation enhancing abilities of surfactants, and allows one to compare the permeability obtained from different delivery systems.

4. For the first time, in situ patches are produced with the developed linker-based lecithin systems loaded with drugs. After applying the microemulsions for a short period of time, the drug can be absorbed in the skin and the drug-loaded skin can act as in situ patches for extended drug release. The in situ patches are able to release 90% of their drug content over a 24 h period which rivals the performance of some polymer-based patches. Indeed, we observed the in situ patches under the fluorescent microscopy.

5. The increase of surfactant concentration in the developed microemulsions is found to increase the drug absorption in the skin, but only increase the transdermal flux of lidocaine up to a point where a plateau value of transdermal flux is reached. Hence, an
optimal surfactant concentration exists in the linker-based lecithin systems to provide high enough transdermal flux with minimal side effects.

6. The mass balance model proposed in this work can be applied to the drug transport mechanism from microemulsions (the donors) to deep skin layers (the receivers). The underlying mechanisms of the drug release from microemulsions are complex and less understood. Before this project, there was only one attempt to establish a mathematical model for describing drug release kinetics from microemulsions (Grassi et al., 2000). The authors considered microemulsions as emulsions with extremely small drops and adopted a drug release model of emulsions for microemulsions. As discussed in Chapter 2, microemulsions are distinctly different from emulsions in that the former are thermodynamically stable single-phase systems whereas the latter are thermodynamically unstable two-phase dispersions. These differences make the model for emulsions inappropriate for microemulsions. Hence, we developed a mathematical model based on a mass transfer balance along three drug compartments (Figure 4.3). The model shows the interdependence among a number of permeation parameters, but only uses one fitting parameter and has a perfect match with the experimental data. The drug loading in the skin is found to be the key factor for TDD, which is easy to be obtained by experimental measurements. The model provides a framework that can be used for other microemulsion systems.
6.2 RECOMMENDATIONS FOR FUTURE WORK

In this dissertation, we have demonstrated the formulation and characterization of the linker-based lecithin microemulsions, their phase behaviour, drug solubilization capacity, the transdermal performance of delivery lidocaine in vitro, the formation of in situ patches and the effect of surfactant concentration. Although these promising results demonstrated that these newly formulated lecithin-linker microemulsions are efficient drug carriers in vitro, there are important questions that arise from these studies and need to be addressed in future studies. For example, how well the linker-based systems will performance transdermally under in vivo circulation? Is there more evidence to prove the proposed surfactant-mediated absorption/permeation mechanism for drug transport from microemulsions to the skin? Can the linker-based systems deliver other drugs in spite of lidocaine? In addition, given the low viscosity of the linker-based systems, is it possible to increase the viscosity for ease of application? Based on the present work, recommendations for future research needs are put forward, including:

1. Investigating the effect of oils on the linker-based delivery systems

One of important issue of microemulsions is the selection of oils. The oil currently used in the studied linker-based lecithin microemulsions is isopropyl myristate (IPM), which has a relatively large molecular weight and requires high surfactant concentration to be solubilized. Appendix 1 compares IPM and another oil (ethyl caprate) with a smaller molecular weight in the systems to delivery lidocaine. The results show that the hydrophobicity of the oil is not the only factor to be considered. It would be of interest to explore the effect of other oils on the linker-based lecithin microemulsions for
transdermal delivery, and to find out which factors related to oils have the greatest influence on drug delivery.

2. Investigating the effect of drugs on the linker-based delivery systems

The delivery of a lipophilic drug, lidocaine, has been demonstrated using the developed linker-based lecithin systems. As discussed in Chapter 2, the major advantage of microemulsions is that microemulsions are able to entrap both hydrophobic and hydrophilic active ingredients. It is necessary to investigate the effect of drugs on the linker-based lecithin microemulsions for transdermal delivery. Appendix 2 reports the incorporation of an extremely hydrophobic drug (α-tocopherol acetate) and a hydrophilic drug (sodium salicylate) in the developed microemulsion formulations. The results of phase behaviour of the systems are promising. Future work can continue in this direction, to examine the in vitro transdermal flux and “skin” permeability.

3. Further prove of the proposed surfactant-mediated absorption/permeation mechanism

This dissertation proposes that the drug transfer from the microemulsions to the skin rely on the surfactant absorption/permeation mechanism, in which surfactant droplets are considered as vehicles to carry drugs into the skin. Although the fluorescence studies were used to visualize the penetration of lecithin and drug into the skin, it’s better to find other methods (i.e. mass transfer) to further prove this mechanism.

In addition, Eq. 5.3 in Chapter 5 only looks for overall mass transfer of the drug. In reality, there are two kinds of mass transfer mechanisms that occur simultaneously. In one of them the drug is carried directly by droplets, and in the other the drug is
transferred from droplet to the continuous phase first, and then from the continuous phase diffusing into the skin (Grassi et al., 2000). Therefore, the new version of Eq.5.3 should account for both of them: surfactant-mediated mechanism and the indirect mass transfer from droplet-continuous phase-skin.

4. Improving the techniques of *in vitro* permeation studies and fluorescence microscopy

There are two experimental details needed to be improved in future. First, the receiver solution used for the *in vitro* permeation studies is pure PBS, which has a (very) low solubility towards extremely hydrophobic drugs (e.g. α-tocopherol acetate). The drug transported across the skin may not be able to dissolve completely in the receiver. Current research is underway by adding 1% albumin bovine serum in PBS in order to better dissolve the permeants. Secondly, water loss occurred during the freeze drying step for the fluorescence microscopy studies. There is a need for proper preparation of the skin before the freeze dry process to improve the effectiveness of fluorescence technique.

5. Studying the effect of viscosity

In terms of the low viscosity of the studied lecithin-linker systems, there are two sides to the coin. On one hand, the low viscosity is good for the dosage form of spray for topical use, and may be suitable for oral, parenteral, pulmonary or even ocular delivery. On the other side, for practical reasons, the systems need to be thickened for the topical form of creams or gels. The question remaining is whether the viscosity will affect the drug permeability. Current research is underway to study this phenomenon using xanthan gum or gelatin.
6. **Evaluation of *in vivo* efficacy of the linker-based systems**

In this work, we have demonstrated the capacity of the linker-based lecithin microemulsions as efficient TDD vehicles *in vitro*. Currently we are cooperating with a Canadian company and using a modified version of the developed lecithin-linker microemulsions for anti-cellulites delivery. *In vivo* Safety tests, such as Ames test for mutagenic potential and human repeat insult patch test (HRIPT), have been done on the modified formulations. The result show no carcinogenic, irritation or allergenic potential caused by the lecithin-linker microemulsions systems. Future *in vivo* studies are necessary to further prove the efficacy of the developed systems as transdermal drug carrier. For example, *in vivo* percutaneous absorption studies can be performed through topical applications on animal models. This will directly improve the possibility of the developed microemulsions to be a pharmaceutical product.

7. **Evaluating the phase transition of the linker-based systems in the skin**

When microemulsion droplets penetrate into the skin, they may change their shape or size or even microemulsion type due to temperature changes, dilution effects or the change in electrolyte concentration. In Section 3.4.1.2 and Section 3.4.1.3, these factors have been studied briefly, but not directly in the skin.

8. **Studying the effect of skin variability on the effectiveness of microemulsion systems**

When using pig skin for the *in vitro* permeation studies and *in vitro* cytotoxicity studies, we found that the results were variable and in most cases, the experiments had to be repeated more than three times to obtain reasonable statistics. The reason is because the skin properties are not consistent from different batches. Hence, a protocol should be
established to prescreen the skin can be used for transdermal studies. Otherwise, the impact of skin variability on the effectiveness of microemulsion systems should be investigated.

6.3 REFERENCES

APPENDIX 1

EFFECT OF OILS

ON LINKER-BASED LECITHIN MICROEMULSIONS

FOR TRANSDERMAL DELIVERY OF LIDOCAINE

Co-authors for this portion of the work include Micheline Samaan and Edgar Acosta.
1. INTRODUCTION

In Chapter 3, new alcohol-free lecithin microemulsions have been formulated with food-grade linker molecules and the linker-based system has been proved to be effective vehicles for transdermal drug delivery (TDD). However, the major challenge was found to be the use of an oil, particularly isopropyl myristate (IPM), which causes the lidocaine to partition preferentially into the oil phase rather than into skin. Another challenge is that the oil currently used, IPM, is a relatively long 17-carbon oil and thus requires high surfactant concentration in microemulsions to solubilize it.

The purpose of this study is to investigate the use of a pharmaceutically acceptable oil, ethyl caprate (EC), to overcome the above obstacle. EC (Log P = 4.3) is a 12-carbon oil and less hydrophobic than IPM (Log P = 7.4) and their physico-chemical parameters and molecular structure were shown in Table 1. We hypothesized that the use of EC in linker-based microemulsions would require less surfactant and in turn will increase the drug solubilization capacity. Most importantly, it would decrease the drug partition into the oil phase, resulting in a relatively higher flux and permeability of lidocaine through skin.

Table 1. Physico-chemical parameters and molecular structures of the drug and oils

<table>
<thead>
<tr>
<th>Compound</th>
<th>Molecular weight</th>
<th>Log P</th>
<th>Molecular structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lidocaine</td>
<td>234.34</td>
<td>2.50</td>
<td><img src="image1" alt="Lidocaine structure" /></td>
</tr>
<tr>
<td>EC</td>
<td>200.32</td>
<td>4.34</td>
<td><img src="image2" alt="EC structure" /></td>
</tr>
<tr>
<td>IPM</td>
<td>270.45</td>
<td>7.43</td>
<td><img src="image3" alt="IPM structure" /></td>
</tr>
</tbody>
</table>
In order to test this hypothesis, microemulsion formulations of Series L with 4% lecithin in Chapter 3 were prepared using EC as the carrier oil instead of IPM in this study. Sorbitan monooleate is still used as hydrophobic linker, a mixture of sodium caprylate and caprylic acid serves as hydrophilic linkers. Lidocaine is the active drug ingredient. The microemulsions with EC oil were then used to test *in vitro* drug permeability and partitioning of drug in the different microemulsions (Types I, II, and IV) and skin remaining to examine the effect of using EC oil, as opposed to a supposedly more hydrophobic oil such as IPM.

As will be shown in the results, using EC indeed enhances the drug solubilization capacity in microemulsions and has proved one component of the hypothesis correct. On the other hand, lidocaine was shown to have a stronger affinity to EC than IPM due to an increased drug solubility, Therefore, the use of EC oil instead of IPM as reflected by the differences in through the skin, rather than improves it.

1.1 Effect of oil on microemulsions

The type of oil used in microemulsion formulations is very important because it must be solubilized by the surfactant in order to form microemulsions. The selected oil affects not only the phase structures and stability of microemulsions, but also the level of drug solubilization. Malcolmson et al. examined the solubility of testosterone propionate (hydrophobic) in microemulsions with regards to different oil constituents (Malcolmson et al. 1998). The solubilization of the lipophilic drug depends on the solubility of the drug in the bulk oil and the influence of the oil on the nature of the microemulsion droplet. Warisnoicharoen et al. investigated the effect of oil type on phase behaviour of microemulsions using different nonionic surfactants (Warisnoicharoen et al., 2000). They found depending on types of surfactant used, in
some cases short single-chained oils were easier to solubilize than triglycerides, and vice versa. Another important finding was that within the same series of oil, the shortest alkyl chain was solubilized more than the longer chain oils. Kantaria et al. also studied the effect of the oil’s chain length on solubilization and found that optimal solubilization was obtained using oils of approximately 6-8 carbon chain length (Kantaria et al., 2003). Lawrence and Rees also claimed that small molecular volume oils penetrated the hydrophobic chain region of the surfactant and aided microemulsion formation (Lawrence and Rees, 2000).

Using an extended surfactant, which is a surfactant combined with a lipophilic or hydrophilic linker, improves the surfactant-oil or surfactant-water interactions. Miñana-Perez et al. have shown that at optimum formulation, a Type III formulation can be obtained with a variety of long-chained oils, particularly mono, and triglyceride esters, but solubilization is much higher for long single-chain polar oils, such as ethyl oleate (Miñana-Perez et al., 1995).

Therefore, using single-chain oils or triglycerides depends on the surfactant used along with the presence of cosurfactants and linkers. If the latter two molecules are present, microemulsion formation becomes easier because of the decrease in interfacial tension and rigidity. The different oil experiments performed by Warisnoicharoen and Miñana-Perez et al. did not use lecithin, but rather, they used nonionic surfactants such as polyoxyethylene, which have different structures and properties; and therefore, will exhibit different physicochemical characteristics when mixed with different oils. Thus, since no experiments have been performed using lecithin as surfactant in contact with a variety of oils, no solid conclusions can be made on the solubilization of the different types of oils in lecithin surfactant.
1.2 Effect of oil on drug release from microemulsions

Several studies have pointed out the influence of the oil phase composition used in microemulsions on transdermal administration of drugs (Montenegro et al., 2006; Trotta 1997). The drug release rate from microemulsions was found to be dependent on oil phase lipophilicity, drug partition coefficient, and type of surfactant used. Trotta et al. studied o/w microemulsions containing benzyl alcohol or different ratios of benzyl alcohol and IPM as oil phase for transdermal absorption of felodipine. They found the highest drug flux was from the microemulsion with the highest drug solubility. Montenegro et al. also investigated effect of oil on drug release from o/w microemulsions formulated with different oils IPM, isopropyl palmitate, and isopropyl stearate. They observed that the vehicles containing IPM as oil phase produced the highest release rate for a hydrophobic drug naproxen (Log P = 2.9). The authors concluded that the microemulsions containing the most lipophilic oil increase the drug solubility and reduce the drug thermodynamics activity, which lead to an unfavorable drug partition from the oil phase.

The drug release from microemulsions also dependent on the active compound incorporated and type of surfactant used. According to Montenegro’s study, the drug release rate was inversely related to drug lipophilicity. Previous studies on effect of oil drug release from microemulsions did not use lecithin as surfactant and lidocaine as model drug. Consequently, the effect of oil on drug release from linker-based lecithin microemulsions had to been found out.
2. MATERIAL AND METHODS

The materials and methodology used in this study are same as those described in Chapter 3. Ethyl caprate (99%+) was purchased from Sigma (St Louis, MO, USA) at the concentrations shown and were used as received. Only pig ear skin was used for in vitro permeation studies.

The saturation solubility of lidocaine was determined in EC and IPM. Excess drug was added to known volumes of the solvent, vortexed for 2 min, followed by hot water bathing to dissolve the drug and then equilibrated at room temperature (approximately 22°C) overnight. The contents were then centrifuged at 3,000 rps/min for 20 min (Beckman Avanti™ 30 centrifuge) and the aliquots of supernatant saturated solvent were diluted appropriately and analyzed by HPLC.

Partition coefficient studies between oil and water were done using different oils: EC and IPM. First, 10% lidocaine in oil was diluted to 5%, 2%, 1%, 0.2%, and 0.1% lidocaine in oil. Second, 5 ml of water was added into 5 ml of the oil with different drug concentration. The mixture was shaken, vortexed, and centrifuged consecutively several times per day. After equilibrium, the drug partitioning in the water and oil phases were analyzed by HPLC.

3. RESULTS AND DISCUSSION

3.1 Phase behaviour of linker-based EC microemulsions

The linker-based EC microemulsions were formulated with 4% w/w (unless stated otherwise) lecithin, 12% sorbitan monooleate, 1.5-7% sodium caprylate, 3% caprylic acid, 0.9% sodium chloride, and 38% lidocaine-EC solution (10% lidocaine in EC). With increasing sodium caprylate (hydrophilic linker) concentrations, the system becomes more hydrophilic and the
following phase transition occurred: Type II (water-swollen reverse micelles) – Type IV (bicontinuous phase) – Type I (oil-swollen micelles). The phase behaviour of the linker-based EC system is very similar with that of the linker-based IPM microemulsion system in Chapter 3. Figure 1 displays the phase behaviour photos for both systems and the numbers at the bottom of the picture represent the percent by weight of sodium caprylate in each vial. Compared to the IPM formulations, the EC formulations require more sodium caprylate to reach a phase inversion. This result is in agreement with that of linker-based lecithin microemulsions studied with a variety of oil (Acosta et al. 2005). According to an equation to model the phase behaviour of linker microemulsions, the hydrophilic linker-to-lecithin ratio decreases in a linear manner as the oil becomes more hydrophobic. In our case, EC is less hydrophobic than IPM and it requires higher amount of hydrophilic linker (sodium caprylate) given that the lecithin concentration is constant within the system.

**Figure 1.** The phase behaviour photos of linker-based EC (upper) and IPM (bottom) microemulsions.
Figures 2 below shows the phase behaviour of the EC and IPM microemulsions by plotting the volume fraction of microemulsions versus the sodium caprylate concentration in each formulation. The volume fraction was calculated by measuring the height of the microemulsion phase and dividing that by the total height of the formulation in the vial at room temperature. For the Type II (w/o) systems, the microemulsion phase volumes are similar when using either EC or IPM. Unlike, for the Type I (o/w) systems, the phase volumes of the EC microemulsions are significantly higher than those of the IPM microemulsions. Obviously, more EC oil was solubilized in the water-continuous microemulsions by same amount of surfactants in formulations. As we expected, since EC has a shorter carbon chain than IPM, it is easier to be solubilized and in turn requires less surfactant in formulations.

![Graph showing phase behaviour](image)

**Figure 2.** The Type II-IV-I phase transition for the linker-based EC and IPM microemulsions.

### 3.2 Characterization of linker-based EC microemulsions

The viscosity of the linker-based EC microemulsions was determined at room temperature and compared with that of the linker-based IPM microemulsions. Figure 3 presents the difference between using the two oils for formulating linker microemulsions. For the Type I and IV
systems, the viscosity of both oil systems is similar. However, for the Type II systems, the viscosity value using EC is lower than that using IPM. This may be because the Type II systems have oil as the continuous phase, and therefore, the difference in viscosity seen in the graph is due to the difference in density as well as the sizes of the two oils. The specific gravity of 10\% lidocaine in IPM is 0.852, while for 10\% lidocaine in EC it is 0.89. Since the density of lidocaine in IPM is lower, it should have a higher viscosity, and also because IPM is a larger molecule than EC, this should also impact the viscosity of Type II microemulsions.

3.3 Drug solubilization

3.3.1 Drug solubility in oil

Saturation solubility of lidocaine in EC was found to be significantly higher than that in IPM. The data for EC and IPM was 182K and 311K mg/l, respectively.

**Figure 3.** Viscosities of linker-based EC and IPM microemulsions.
3.3.2 Drug partitioning between microemulsions and excess phases

The solubilization capacity of the linker-based EC microemulsions for a poorly water-soluble drug, lidocaine, was measured and compared to that of the linker-based IPM microemulsions. The initial lidocaine concentration dosed in all formulations was the same. The drug concentration in microemulsions and excess phases was measured after the samples were equilibrated. Figure 4(a) and 4(b) below displays the plot of lidocaine concentration in microemulsions and excess phases versus the amount of sodium caprylate. The trend is similar for the EC and IPM systems: Type II > excess oil > Type I > excess water.

At low quantities of sodium caprylate, the systems are Type II and lidocaine is solubilized mostly in the microemulsion phase. This is because the oil phase is continuous in this system and lidocaine, being slightly hydrophobic, preferentially remains in the oil continuous phase. This inevitably leads to a high partition coefficient of lidocaine (K_{II/H2O}) between the Type II microemulsions and their excess aqueous phases.

At high quantities of sodium caprylate, Type I exists where the aqueous phase is continuous. The partition coefficient (K_{I/oil}) between the Type I microemulsions and their excess oil phases is relatively low and constant. For these systems, lidocaine is still solubilized more in the excess oil phase, but is also solubilized in the aqueous microemulsion phase. This indicates an important effect that microemulsions enhance the solubilization capacity of the drug; and this is very apparent from the fact that the Type I microemulsions have a much higher concentration of lidocaine in comparison with that in water.

It is interesting to note that lidocaine in the Type II EC microemulsions and the excess EC phases are significantly higher than those in the IPM case. Also, lidocaine concentration in the Type I EC microemulsions is also higher. The reveals that EC has a greater affinity for lidocaine
than IPM and enhance the solubilization capacity of lidocaine in microemulsions. The higher affinity of lidocaine in EC could be attributed to the increased drug solubility. For example, in the phase behaviour studies, we confirmed that more EC was solubilized in Type I microemulsions and thus less EC remained as the excess oil phase when compared to the IPM oil. From Figure 4, however, less EC oil volume as the excess phases provided higher lidocaine concentration. The higher transdermal flux and permeability was expected with the higher drug solubility in microemulsions using EC oil according to Trotta’s result (Trotta 1997).

![Figure 4](image_url)

**Figure 4.** Lidocaine concentrations in the (a) microemulsions, and (b) excess phases for linker-based EC and IPM microemulsions.
3.4 *In vitro* permeation flux and permeability

Three representative Type II (1.5% sodium caprylate), IV (4% sodium caprylate), and I (7% sodium caprylate) samples of the linker-based EC microemulsions were selected to test transdermal delivery of lidocaine across pig ear skin. The results were compared to those of linker-based IPM microemulsions, pure EC and IPM (10% lidocaine in oils), and water (saturated lidocaine in water). Figures 5a and 5b display the flux and permeability data for all vehicles. For both oil systems, the trend for transdermal flux and permeability is the same. The Type II microemulsions produce highest flux than other vehicles, which is obviously because the higher lidocaine concentration oil-continuous phases.

![Figure 5](image)

Figure 5. (a) Transdermal flux and, and (b) “skin” permeability of lidocaine from linker-based EC and IPM microemulsions through pig skin.

Comparing the flux and permeability of the EC and IPM series results, it is evident that both parameters are generally higher for the IPM systems than for the EC systems. From Figure 5, the flux of lidocaine in IPM itself is slightly higher than that of lidocaine in pure EC. Recall that EC has a greater affinity for lidocaine than IPM and therefore lidocaine tends to remain in EC more
than in IPM. This explains why the flux of all types EC microemulsions are lower than the IPM case although the EC microemulsions have higher lidocaine concentrations. This observation is contrary to the initial hypothesis that because EC is a supposedly less hydrophobic oil, it will increase the flux of lidocaine through skin. Several conclusions may be drawn from these results by examining the molecular structures of both oils. EC is a 12-carbon oil with an ester group at one end, while IPM is a 17-carbon oil. The shorter carbon chain of EC causes one to assume that it is less hydrophobic; however, the ester group and shorter chain of EC, may be the cause of this unexpected result. As seen in Table 1, IPM also contains an ester group; however, this ester group combined with a short carbon chain creates stronger dipole moment in EC than IPM, since for a given volume of oil, more dipole interactions will exist for a smaller molecule rather than a larger one. Thus, this leads to the conclusion EC has a greater affinity for lidocaine than IPM, since lidocaine is also a polar molecule. Consequently, the greater affinity could reduce the thermodynamic activity of lidocaine in the vehicles containing EC oil, which leads to an unfavorable drug partition from the oil phase and hence provides a lower drug flux and permeability across the skin.

3.5 Drug partition and transport

3.5.1 Lidocaine partitioning between oil and water

Lidocaine partitioning studies have been done to determine the partition coefficient of lidocaine between the oil (EC or IPM) and water phases. Figure 6 below is a plot of the partition coefficient of lidocaine in different oils versus the total concentration of lidocaine in the mixture. It is clear that the partition coefficient is higher for EC than IPM. This indicates that lidocaine
partitions more in EC compared to IPM, which confirms the observation made in previous section that EC has a greater affinity for lidocaine than IPM.

![Graph showing partition coefficient of lidocaine in oil and water](image)

**Figure 6.** Plot of the Partition Coefficient of Lidocaine in Oil and Water

### 3.5.2 Lidocaine partitioning between microemulsions and skin

The results for the partition coefficient of lidocaine between the donor solutions and skin are more interesting when comparing formulations using EC and IPM as carrier oils. Figure 7 below is a graph of both systems and their partition coefficients in pig skin. In general, for every type of microemulsions, the $K_{\text{donor/skin}}$ is higher when using the EC microemulsions as donors than using the IPM microemulsions. The reason for this is because lidocaine tends to remain more in EC relative to IPM. These results confirm the observation that lidocaine has a greater affinity for EC than IPM. In another word, lidocaine has more skin absorption from the IPM microemulsions than the EC microemulsions. This results in higher transdermal flux and permeability from the IPM systems, not the EC systems.
4. CONCLUSIONS

Based on the above results and discussions, it is evident that the original hypothesis of using EC as a less hydrophobic oil was partially incorrect. This was discovered by comparing flux, permeability, and partitioning in both oil and water, and in microemulsions and skin. In all these studies the same conclusions were drawn: lidocaine has more affinity for EC than IPM. This is based on obtaining lower flux and permeability of the EC formulations. As well, in studying the partitioning coefficient, the concentration of lidocaine in EC was higher than in IPM when the same oil-water system was examined.

Originally, EC was thought to be less hydrophobic than IPM because of the chemical formula of each oil. EC has a shorter carbon chain than IPM and may still be less hydrophobic; however, it is concluded that one cannot rely on solely their hydrophobicity of the oil to predict its impact on drug permeability. But rather, it is necessary to compare the entire molecular structure. Both oils have an ester group which creates an overall dipole moment in both molecules. Since lidocaine is also slightly hydrophobic and polar, it has an affinity for both oils. However, one
can now presume that would have a stronger affinity for EC due to the very fact that it is the smaller molecule of the two oils. This resulted in lower flux of lidocaine because it had a tendency to remain in EC more than in IPM. As a result, the hydrophobicity of the oil is not necessarily the only factor to be considered for drug delivery through the skin.

On the other hand, part of the hypothesis was found to be correct, because EC is easier to be solubilized in microemulsions and the drug solubility in EC is high, therefore enhance the drug solubilization capacity in linker-based microemulsions. In another words, lower surfactant concentration can be used to create microemulsions using EC. This will ensure to reduce the formulation cost and further reduce the surfactant toxicity probably for delivering another drug, not lidocaine.

Overall, this project is successful in further proving that microemulsions are excellent drug delivery vehicles, and that in choosing an oil in formulations, one must consider all of its chemical and physical properties rather than only its hydrophobicity; as this does not prove to be the main factor in affecting the permeability of the drug.

5. RECOMMENDATIONS

To further investigate the effect of oil on linker-based microemulsions as TDD vehicles, we need to select study more oils and select one which is less hydrophobic than IPM but with lower affinity for lidocaine.
6. REFERENCES


Warisnoicharoen, W., Lansley, A.B., Lawrence, M.J. 2000, Nonionic oil-in-water microemulsions: the effect of oil type on phase behaviour, Int. J. Pharm.198, 7-27.

APPENDIX 2

EFFECT OF DRUGS

ON LINKER-BASED LECITHIN MICROEMULSIONS

FOR TRANSDERMAL DELIVERY
1. INTRODUCTION

In Chapter 3, alcohol-free lecithin microemulsions have been formulated with food-grade linker molecules and the linker-based system has been proved to be effective vehicles for transdermal drug delivery (TDD). The newly developed systems should be able to deliver both oil-soluble and water-soluble active ingredients. The purpose of this work is to investigate the influence of drugs on the incorporation of linker-based lecithin microemulsions. The newly formulated lecithin-linker microemulsions in Chapter 3 are used as vehicles for transdermal delivery of a hydrophobic drug (α-tocopherol acetate), a less hydrophobic drug (lidocaine) as well as a hydrophilic drug (sodium salicylate). Figure 1 shows the chemical structures of the drugs. Table 1 presents the physico-chemical properties and the toxicological information of the model drugs.

Figure 1. Chemical structures of the model drugs: (1) α-tocopherol acetate, (2) lidocaine, and (3) sodium salicylate.

Table 1. Physico-chemical parameters and molecular structures of drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Molecular weight</th>
<th>Log $K_{ow}$</th>
<th>Toxicity Data</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-TAc</td>
<td>472.76</td>
<td>8.7</td>
<td>Not available</td>
</tr>
<tr>
<td>Lidocaine</td>
<td>234.34</td>
<td>2.26</td>
<td>Oral, mouse, LD50 220 mg/kg</td>
</tr>
<tr>
<td>Sodium salicylate</td>
<td>160.11</td>
<td>1.5</td>
<td>Oral, mouse, LD50 540 mg/kg</td>
</tr>
</tbody>
</table>
DL-α-tocopherol acetate (α-TAc) is a typical hydrophobic component (its partition coefficient between octanol and water log K_{ow}=8.7) because of its longer carbon tail. It is a lipid-soluble version of vitamin E and usually used in skin care products as emollient. It accumulates in the epidermis and forms a barrier against moisture evaporation from the skin. α-TAc is also used as a powerful antioxidant and free radical scavenger and helps prevent free radical damage in the skin tissues. This drug has been incorporated in microemulsions containing mixed phosphatidylcholines (similar to phospholipids) (Darfler, 1990). A recent publication also mentions microemulsion systems containing α-TAc (Kogan et al., 2008).

Sodium salicylate is a hydrophilic solute (log K_{ow}=1.5). It is salt form of salicylic acid (BHA) and is usually used for anti-aging purpose in skin care products. The phase behaviour of microemulsions containing isopropyl myristate (IPM), lecithin, medium-chain alcohols and sodium salicylate solution was reported by Aboofazeli et al. (2000). The authors only obtained microemulsion systems in the oil rich part of the phase diagram, i.e. w/o microemulsions.

Lidocaine is less hydrophobic than α-TAc (log K_{ow}=2.26), but it is still considered a hydrophobic drug. The details of lidocaine incorporated in microemulsions have been discussed in the dissertation.

2. MATERIAL AND METHODS

2.1 Chemicals

The materials and methodology used in this study are the same as those described in Chapter 3. DL-α-Tocopherol acetate (97%+, Fluka brand) and sodium salicylate (99.5%+, Fluka brand)
were purchased from Sigma (St Louis, MO, USA) at the concentrations shown and were used as received. Only pig ear skin was used for in vitro permeation studies.

2.2 Microemulsion preparation

The preparation of microemulsions was described in Chapter 4. Lidocaine or α-TAc was loaded in the microemulsions by predissolving the drug in IPM to a concentration of 10%. Sodium salicylate was loaded in the microemulsions by predissolving the drug in certain amount of water to a concentration of 38%.

2.3 Drug quantification

Lidocaine quantification is described in Chapter 4. The concentration of α-TAc in the microemulsions and excess phases was analyzed using a Dionex ICS-3000 (Sunnyvale, CA, USA) liquid chromatography system consisting of single pump, detector, AS40 automated sampler, AD25 absorbance detector and Chromeleon chromatography software (Dionex). α-TAc was separated by a reverse phase column (Genesis, C\text{18}, 4 \text{ µm}, 150×4.6 mm) and detected through its absorbance at 284 nm (AD25 detector). A mixture of methanol and water (98:2, v/v) was used as a mobile phase with a flow rate at 1.0 ml/min. The column temperature and the injection volume were 25°C and 25µl, respectively.
3. RESULTS AND DISCUSSION

3.1 Phase behaviour of linker-based EC microemulsions

The linker-based microemulsions were formulated with 4% w/w (unless stated otherwise) lecithin, 12% sorbitan monooleate, 1.5-7% sodium octanoate, 3% octanoic acid, 0.9% sodium chloride, and 38% IPM solution. Individual drug was incorporated into the formulation by pre-dissolving the drug into oil (for lidocaine or α-TAc) or water (for sodium salicylate). With increasing sodium caprylate (hydrophilic linker) concentrations, the system becomes more hydrophilic and the following phase transition occurred: Type II (water-swollen reverse micelles) – phase transition – Type I (oil-swollen micelles). Figure 2 displays the phase behaviour photos for both systems and the numbers at the bottom of the picture represent the percent by weight of sodium caprylate in each vial. From Figure 2, we can see that the phase behaviour of the linker-based systems loaded with either α-TAc or sodium salicylate is very similar with that of the systems loaded with lidocaine. Compared to the lidocaine-loaded formulations, the α-TAc-loaded formulations require more sodium caprylate to reach a phase inversion. This may because α-TAc is more hydrophobic and thus more hydrophilic linkers are needed to balance the hydrophobicity of the systems. On the other hand, similar percentages of hydrophilic linker were used for sodium salicylate-loaded formulations to reach the phase transition when compared to lidocaine-loaded formulations. This fact is rather surprising because sodium salicylate is much hydrophilic than lidocaine. In addition, there is no bicontinuous microemulsion formed in sodium salicylate-loaded formulations.
Figure 2. The phase behaviour photos of lecithin-linker microemulsions loaded with sodium salicylate, lidocaine or α-TAc. “T” means the transition phase from Type II to Type I.

Figures 3 below shows the phase behaviour of the linker-based microemulsions loaded with α-TAc or lidocaine by plotting the volume fraction of microemulsions versus the sodium caprylate concentration in each formulation. The volume fraction was calculated by measuring the height of the microemulsion phase and dividing that by the total height of the formulation in the vial at room temperature. From Figure 3, the phase volumes of the α-TAc microemulsions are slightly higher than those of the lidocaine microemulsions. Obviously, the hydrophobicity of the drug changes the phase behaviour of the microemulsions.
Figure 3. The Type II-IV-I phase transition for the linker-based microemulsions loaded with lidocaine or α-TAc.

3.2 Drug partitioning between microemulsions and excess phases

The drug solubilization capacity of the linker-based microemulsions incorporated with α-TAc or lidocaine were compared. The initial drug concentration dosed in all formulations was the same, i.e. 10% drug in IPM. The drug concentration in microemulsions and excess phases was measured after the samples were equilibrated. Figure 4a and 4b below display the plot of drug concentration in microemulsions and excess phases versus the amount of the hydrophilic linker (sodium caprylate). For the microemulsions, the drug solubilization either in Type II or in Type I is similar for the two systems loaded with different drugs. For excess phase, both of the drugs are poorly solubilized in aqueous phase. However, it is obvious that α-TAc is solubilized much more than lidocaine in excess oil phase.
Figure 4. Drug concentrations in the (a) microemulsions, and (b) excess phases for linker-based lecithin microemulsions.

4. CONCLUSIONS

The observations in this work confirm that the linker-based lecithin microemulsions can be potential transdermal vehicles for both oil-soluble and water-soluble drugs. They can incorporate not only hydrophobic lidocaine, but also extremely hydrophobic α-TAc or hydrophilic sodium caprylate.
salicylate. Different types of microemulsions can be formed included Type II, bicontinuous phases, and Type I.

5. RECOMMENDATIONS

To further investigate the effect of drugs, the *in vitro* permeation studies should be conducted to test the drug release from the studied linker-based lecithin microemulsions.

6. REFERENCES


