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Linker-based lecithin microemulsions for transdermal delivery of lidocaine

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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) 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).
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Keywords: Microemulsions; Transdermal drug delivery; Lecithin; Linker molecules; Skin permeation; Cytotoxicity; Lidocaine

1. 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, 2000) 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 skin irritation.

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). A microemulsion is a system which contains water and/or oil nano-domains coexisting in thermodynamic equilibrium due to the presence of a surfactant film at the oil/water interface. Depending on surfactant type and formulation conditions, several types of microemulsions can be formed, including oil-in-water (o/w, Winsor Type I), water-in-oil (w/o, Winsor Type II) and bicontinuous (Winsor Type III or IV) systems. Compared to conventional vehicles such as emulsions and hydrogels, microemulsions have shown larger drug solubility due to coexistence of hydrophilic and lipophilic solubilization sites, and larger

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oil/water interfacial area (Kreilgaard, 2002). It has been reported that the transdermal flux of lidocaine from a microemulsion is up to four times higher than that from an emulsion (Kreilgaard et al., 2000).

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 isopropyl myristate (IPM), have been formulated at several surfactant/cosurfactant mixing ratios (Aboofazeli and Lawrence, 1993; Corswant and Söderman, 1998; Saint Ruth et al., 1995). Recent studies suggested that lecithin microemulsions produce enhanced transdermal flux for oil-soluble drugs (Corswant et al., 1998; 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; Solans and Kunieda, 1997). One alternative to alcohol-based formulations is the use of polymeric additives such as polyethylene glycol (PEG) (Corswant and Söderman, 1998, 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. Linker molecules are amphiphiles that segregate near the oil/water interface (Sabatini et al., 2003). Lipophilic linkers such as long chain alcohols segregate near the surfactant tails to improve surfactant–oil interactions and oil solubilization capacity. Hydrophilic linkers are surfactant-like molecules with six to nine carbons per head group that coadsorb with the surfactant to increase surfactant–water interactions. The combination of both lipophilic and hydrophilic linkers leads to a special self-assembly which further increases the co-solubilization of oil and water. 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 safe TDD vehicles for poorly water-soluble drugs. To produce such formulations, we used the base linker-lecithin 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). Fig. 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 1.

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, 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 (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, 2003; Thomas and Finnin, 2004). In various instances, re-applying the dose represents a painful process in itself because of the mechanical occulation (massaging the skin) required to obtain the reported levels of lidocaine penetration with emulsion formulations (Lee, 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.

2. Materials and methods

2.1. Materials

2.1.1. 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 (98%), sodium chloride (99%+, Fluka brand), 1-pentanol (99%+), Dulbecco's phosphate

Table 1
Toxicological information for the materials used in microemulsions

Function	Material	Toxicity data	Irritation data
Surfactant	Lecithin	GRAS	Not available
	Sodium caprylate	Food additive status	Not available
Linkers	Caprylic acid	Food additive status, Oral, mouse, LD50 10080 mg/kg	Skin, rabbit, 500 mg/24 h, mild irritation effect
	Sorbitan monooleate	GRAS	Not available
Cosurfactant	Pentanol	Oral, mouse, LD50 200 mg/kg	Skin, rabbit, 20 mg/24 h, moderate irritation effect
Oil	Isopropyl myristate (IPM)	Oral, mouse, LD50 49700 mg/kg	Skin, rabbit, 426 mg/24 h, mild irritation effect
Drug	Lidocaine	Oral, mouse, LD50 220 mg/kg	Not available

Note: GRAS status according to 21 CFR part 184, toxicity and irritation data obtained from the MSDS of each component supplied by Sigma–Aldrich.

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.

2.1.2. 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 3 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 10 s 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.

2.2. Microemulsion preparation

Phase behavior 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 3 days, and left to equilibrate for 2 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.

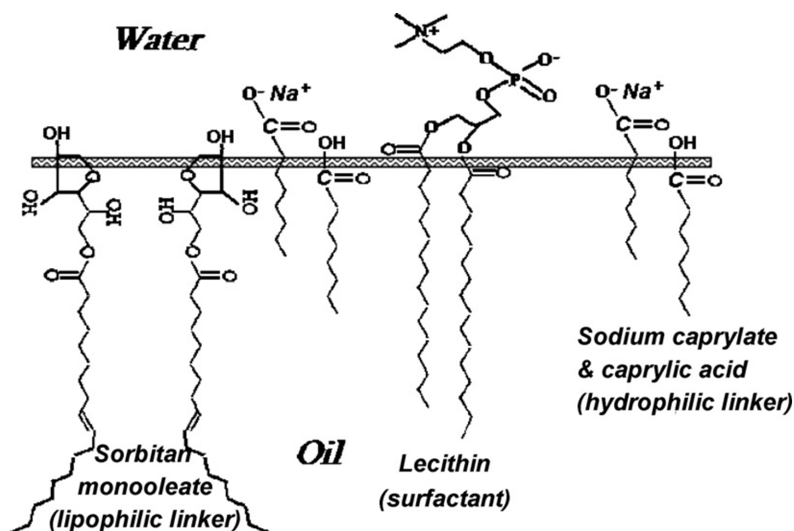

Fig. 1. Schematic of the linker effect using surfactant lecithin, hydrophilic linker sodium caprylate and caprylic acid, and lipophilic linker sorbitan monooleate.

Table 2
Summary of microemulsion formulations

(A) Microemulsions series used in phase behavior studies (%, w/w)

Series	%LE	%SM	%CA	%SC	%NaCl	%Water	%IPM
A	4.0	8.0	–	0.5 ~ 7.0	0.9	45.1 – %SC	42.0
B	4.0	12.0	–	0.5 ~ 7.0	0.9	45.1 – %SC	38.0
L	0.4	1.2	0.3	0.5 ~ 7.0	0.9	48.4 – %SC	48.8
	0.8	2.4	0.6	0.5 ~ 7.0	0.9	47.7 – %SC	47.6
	1.2	3.6	0.9	0.5 ~ 7.0	0.9	47.0 – %SC	46.4
	1.6	4.8	1.2	0.5 ~ 7.0	0.9	46.3 – %SC	45.2
	2.0	6.0	1.5	0.5 ~ 7.0	0.9	45.6 – %SC	44.0
	2.4	7.2	1.8	0.5 ~ 7.0	0.9	44.9 – %SC	42.8
	2.8	8.4	2.1	0.5 ~ 7.0	0.9	44.2 – %SC	41.6
	3.2	9.8	2.4	0.5 ~ 7.0	0.9	43.5 – %SC	40.2
	3.6	10.8	2.7	0.5 ~ 7.0	0.9	42.8 – %SC	39.2
	4.0	12.0	3.0	0.5 ~ 7.0	0.9	42.1 – %SC	38.0

(B) Microemulsion formulations used in transdermal delivery studies (%, w/w)

Formulation	%LE	%SM	%CA	%SC	%NaCl	%Water	%IPM
L-Type II	4.0	12.0	3.0	1.0	0.9	41.1	38.0
L-Type IV	4.0	12.0	3.0	4.0	0.9	38.1	38.0
L-Type I	4.0	12.0	3.0	7.0	0.9	35.1	38.0
			%Pentanol				
P-Type II	4.0	12.0	8.0		0.9	37.1	38.0

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 2(A) 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 behavior 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%. 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.

2.3. *Physicochemical characterization*

Dynamic light scattering measurements of the hydrodynamic radius and polydispersity of the microemulsion aggregates 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 min before measurement. Refractive indexes for the continuous phase were obtained from the literature (Lide, 2006). The viscosity of the samples was measured (in triplicate) at room temperature using a CV-2200 falling ball viscometer (Gilmont Instruments, Barrington, IL, USA).

2.4. *Lidocaine partitioning studies*

After equilibration, samples of the microemulsion and the excess phases were taken and the lidocaine concentration in each phase was measured using the HPLC method described in Section 2.7.

2.5. *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, 0.137 M 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/cm^2) was calculated by dividing the slope of the linear part of the curve ($\mathrm{d}m/\mathrm{d}t$) by the area of the exposed skin surface (A). The apparent lidocaine permeability (Kp, cm/h) is calculated as $Kp = J/\Delta Cv$, where ΔCv 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 ΔCv 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%. Eq. (1) reflects the flux and permeability described above.

$$J = \frac{1}{A} \times \frac{\mathrm{d}m}{\mathrm{d}t} = Kp \times \Delta Cv \quad (1)$$

2.6. *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.

2.7. *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, C_{18}, 4 μm, 150 mm × 4.6 mm). The mobile phase consisted of acetonitrile–0.05 M $NaH_2PO_4{\cdot}H_2O$ (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 min. 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%.

2.8. *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 Kp, 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. Results and discussion

3.1. *Phase behavior of linker-based microemulsions*

3.1.1. *Sodium caprylate scans*

The composition of linker-based microemulsion systems is shown in Table 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.

3.1.2. Sodium caprylate plus caprylic acid

Series L (Table 2(A)) incorporates a mixture of caprylic acid and sodium caprylate as hydrophilic linkers. With the addition of caprylic acid, the phase behavior 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. Fig. 1 shows a hypothetical schematic of the adsorption/segregation of sodium caprylate and caprylic acid at the oil/water interface.

Fig. 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.

Linker-based lecithin microemulsions produce a wide range of formulations (Fig. 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 (Fig. 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 Fig. 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, Fig. 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 Fig. 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 Fig. 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 and Söderman, 1998), and avoids the need for alcohol as cosurfactant.

Another important feature of Fig. 2C is that it can be used to predict the effect of dilution on the potential for phase transition. The dotted dilution line in Fig. 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.

3.1.3. Effect of lidocaine, temperature, and electrolyte

Fig. 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. 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/hydrophibicity of the oil phase. Other researchers have also found that, depending on the physicochemical 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).

Similar phase behavior studies to the one presented in Fig. 3 were carried out to evaluate the effect of electrolyte and temperature. The L-Series with 4% lecithin (Fig. 2B) were prepared in the absence and presence of electrolyte (0.9% NaCl); however, no significant difference in the phase volumes (compared to Fig. 2B) was observed. Similarly, when these formulations were produced at 37 °C, there were no significant changes in phase volumes. This insensitivity to electrolyte

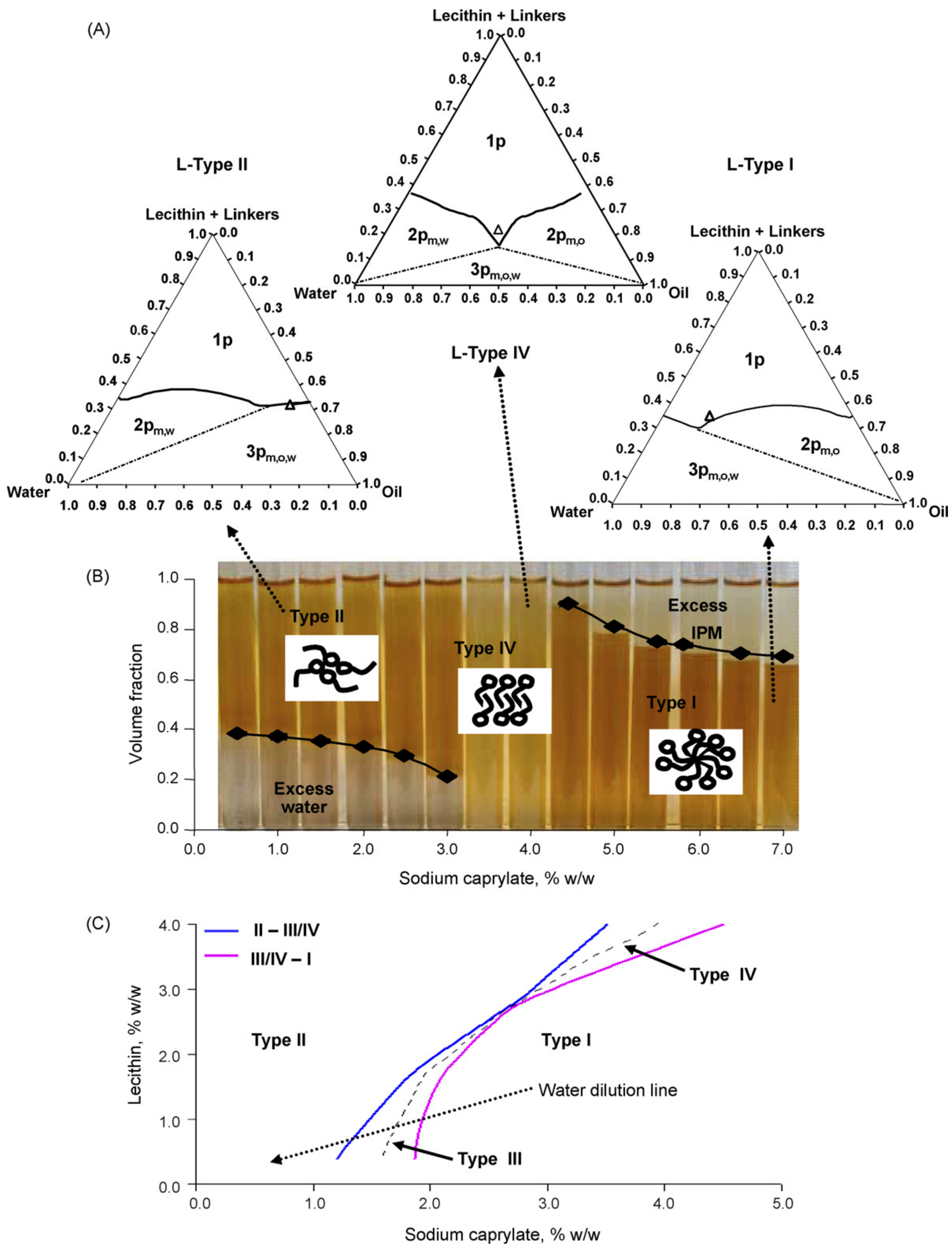

Fig. 2. Phase behavior of Series L (linker) formulations containing lidocaine at room temperature and 0.9% NaCl. (A) Ternary phase diagrams of the L-Type II, L-Type IV, and L-Type I formulations, indicating the presence of one phase (1p), two phases ($2p_{m,o}$ and $2p_{m,w}$), and three coexisting phases ($3p_{m,o,w}$). The subscripts 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.

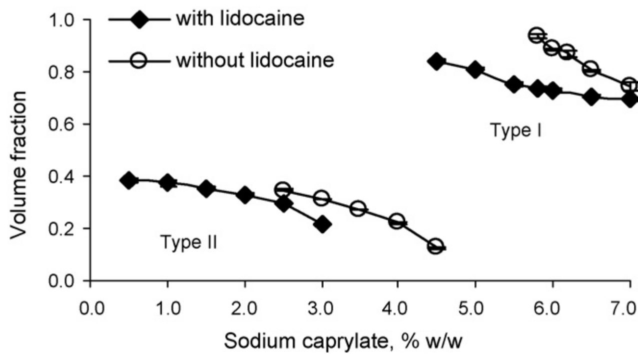

Fig. 3. The Type II–IV–I phase transition for the L-Series with 4% lecithin in the presence and absence of lidocaine at room temperature.

concentration and temperature is explained by the fact that the main surfactant, lecithin, is a zwitterionic surfactant which is relatively insensitive to these factors. Electrolyte and temperature insensitivity is a desirable feature in drug delivery vehicles.

3.2. Physicochemical characterization of linker-based microemulsions

3.2.1. Viscosity

Fig. 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 mPa s), while in the case of Winsor Type I microemulsions viscosity values are between 30 and 40 mPa s. 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 (10–40 mPa s) 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 mPa s (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. (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 and 50 mPa s, which is comparable to the viscosities obtained in our Type I systems (Fig. 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 mPa s. 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).

3.2.2. Particle size

Fig. 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 aggregate 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 aggregate 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).

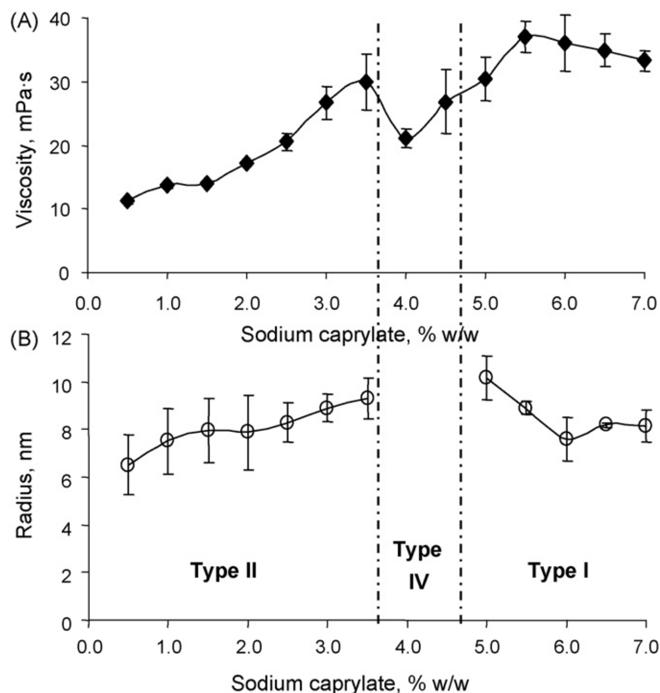

Fig. 4. (A) Viscosity and (B) droplet hydrodynamic radius of the microemulsions in the L-Series with 4% lecithin containing lidocaine at room temperature.

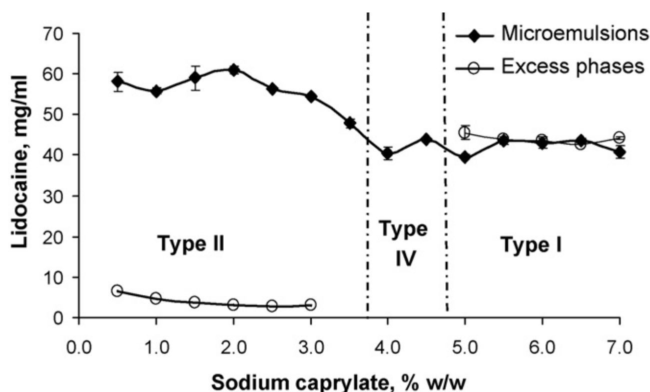

Fig. 5. Lidocaine concentrations in the microemulsion and excess phases for the L-Series with 4% lecithin.

3.3. 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. Fig. 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).

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 Fig. 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. In vitro permeation flux and permeability

The transdermal permeation profiles (Fig. 6A and B) 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

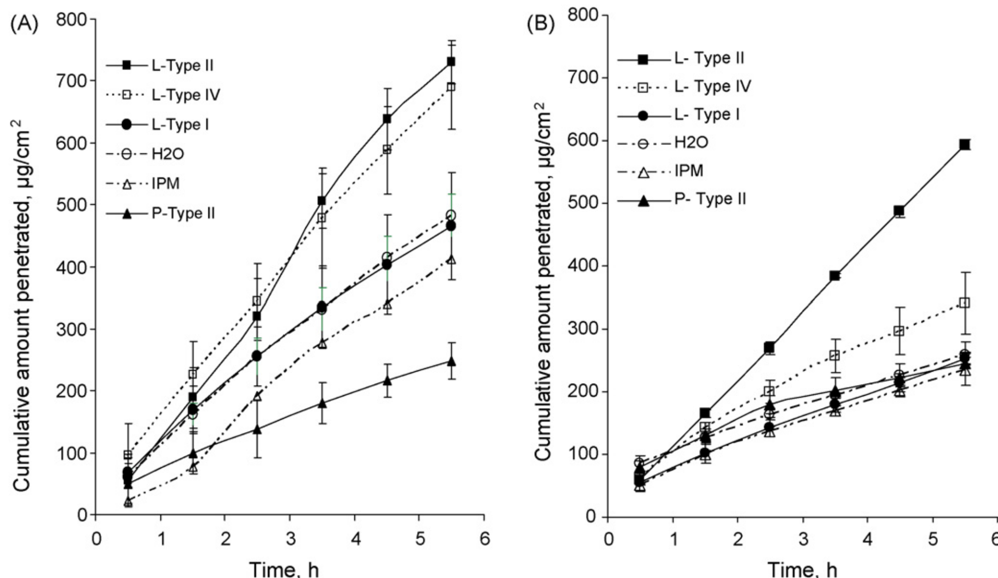

Fig. 6. Permeation profile of lidocaine through (A) MatTek human skin, and (B) pig ear skin from linker microemulsions (L-Type II, L-Type IV, and L-Type I), pentanol microemulsion (P-Type II), water and IPM. The formulations of these microemulsions are indicated in Table 2(B).

Table 3
The steady-state flux (J, μg/h/cm^2) of lidocaine, through MatTek human skin and pig ear skin from different delivery systems

Delivery system	MatTek human skin	Pig ear skin
L-Type II	559.4 ± 30.4	479.1 ± 70.3
L-Type IV	467.9 ± 16.2	342.3 ± 36.2
L-Type I	308.0 ± 53.4	180.6 ± 32.9
P-Type II	151.8 ± 20.4	127.0 ± 21.6
H_2O	325.4 ± 16.5	140.6 ± 10.0
IPM	315.1 ± 22.1	130.1 ± 24.0

skin models. In particular, the Type II linker microemulsion provided exceptionally higher lidocaine permeation through pig skin. Table 3 summarizes the transdermal flux calculated from the drug permeation profiles for the systems of Fig. 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.

These findings conflict with those of Lee et al. (2003) who reported that, in their formulations, Type I microemulsions produce larger fluxes than Type II systems. 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 (Fig. 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).

The data in Fig. 6 reveal that the L-Type II microemulsion produces a four-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 (Fig. 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 has been reported (Lee et al., 2003).

As indicated by Eq. (1), the transdermal flux of lidocaine is associated with the difference in concentration (ΔC) 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 (Kp) calculated by Eq. (1) has been used to determine the effectiveness of delivery systems. Table 4 summarizes the values of permeability for the delivery systems considered in Fig. 6. According to Table 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:

Table 4
The permeability (Kp, × 10^3 cm/h) of lidocaine calculated using Eq. (1), where ΔC is different for pig ear skin

Delivery system	MatTek human skin	Pig ear skin	
		Normal permeability $\Delta C = C_{donor} - C_{receiver}$	"Skin" permeability $\Delta C = C_{skin} - C_{receiver}$
L-Type II	10.1 ± 0.6	9.0 ± 1.0	33.1 ± 2.4
L-Type IV	11.2 ± 0.9	8.2 ± 0.8	25.9 ± 4.2
L-Type I	7.9 ± 1.1	4.6 ± 0.6	14.9 ± 3.1
P-Type II	2.4 ± 0.1	1.9 ± 0.1	19.2 ± 2.3
H_2O	82.8 ± 4.6	35.8 ± 1.8	20.6 ± 5.4
IPM	2.9 ± 0.2	1.3 ± 0.2	16.3 ± 1.7

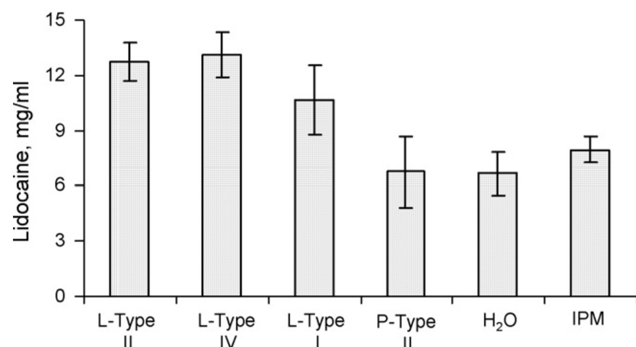

Fig. 7. Lidocaine concentration remaining in the skin after treatment by different delivery systems. The formulations of these microemulsions are indicated in Table 2(B).

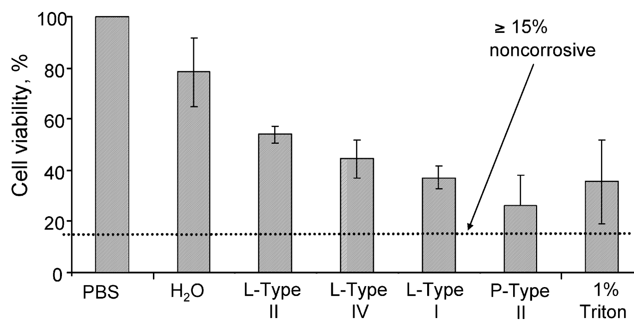

Fig. 8. Cytotoxicity of different delivery systems according to the MTT assay. The formulations of these microemulsions are indicated in Table 2(B).

the lower the solubility (such as the case of lidocaine in water), the larger the permeability. If we consider this issue of solubility, then Eq. (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. Eq. (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 for the vehicles considered in Fig. 6. Fig. 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 4 presents the calculated lidocaine "skin" permeability for these vehicles, where the lidocaine concentration difference, ΔCv, in Eq. (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 Eq. (1) by either using a difference in chemical potential (as opposed to absolute concentrations) or the concept of "skin" permeability.

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 microeoemulsions (Shin et al., 2004) produce fluxes of the order of magnitude reported in Table 3. Furthermore, Kushla and Zatz (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. (2003).

The difference in lidocaine flux between skin sources is evident in Table 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., 2000; 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.

3.5. Cytotoxicity of linker-based microemulsions

Fig. 8 summarizes the cell (keratocyte) viability after 5.5 h 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. The cell viability of 1% Triton X-100 is expected to represent mild *in vivo* skin irritancy. 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 Fig. 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 Fig. 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 biocompatible 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 1).

One problem with using MTT tests with MatTek EPI-200 skin models is that this test is most sensitive to toxic vehicles but cannot 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).

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 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.

4. 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 aggregates (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.

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