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# Effect of surfactant concentration on transdermal lidocaine delivery with linker microemulsions

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ABSTRACT

A limited number of studies have been conducted to investigate the effect of surfactant concentration on microemulsion-mediated transdermal transport. Some studies suggest that increasing surfactant concentration reduces the partition of the active in the skin, and the overall transport. Other studies suggest that increasing surfactant concentration improves mass transport across membranes by increasing the number of "carriers" available for transport. To decouple these partition and mass transport effects, a three-compartment (donor, skin, receiver) mass balance model was introduced. The model has three permeation parameters, the skin-donor partition coefficient ($K_{sd}$), the donor-skin mass transfer coefficient ($k_{ds}$) and the skin-receiver mass transfer coefficient ($k_{sr}$), also known as skin permeability. The model was used to fit the permeation profile of lidocaine formulated in oil-in-water (Type I) and water-in-oil (Type II) lecithin–linker microemulsions. The results show that surfactant concentration has a relatively minor effect on the mass transfer coefficients, suggesting that permeation enhancement via disruption of the structure of the skin is not a relevant mechanism in these lecithin–linker microemulsions. The most significant effect was the increase in the concentration of lidocaine in the skin with increasing surfactant concentration. For Type I systems such increase in lidocaine concentration in the skin was linked to the increase in lidocaine solubilization in the microemulsion with increasing surfactant concentration. For Type II systems, the increase in lidocaine concentration in the skin was linked to the increase in skin-donor partition. A surfactant-mediated absorption/permeation mechanism was proposed to explain the increase in lidocaine concentration in skin with increasing surfactant concentration. The penetration profiles of hydrophobic and amphiphilic fluorescence probes are consistent with the proposed mechanism.

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## 1. Introduction

A microemulsion is a system that contains water and/or oil nano-domains coexisting in thermodynamic equilibrium due to the presence of a surfactant film adsorbed at the oil/water interface. Oil-in-water (o/w) microemulsions are called Winsor Type I microemulsions, water-in-oil (w/o) are Winsor Type II, and microemulsions bicontinuous in oil and water are called Winsor Type III or Type IV if there are no excess phases. Microemulsions offer several advantages for pharmaceutical use including ease of preparation, thermodynamic stability, high solubilization capacity for lipophilic and hydrophilic drugs, and their ability to facilitate the transport of drugs through biological membranes (Hadgraft, 2004; Bagwe et al., 2001; Lawrence and Rees, 2000; Tenjarla, 1999). Several studies have shown that microemulsions are better transdermal drug delivery (TDD) systems than conventional vehicles such as hydrogels, emulsions and liposomes (Kreilgaard, 2002; Baroli et al., 2000; Delgado-Charro et al., 1997; Williams and Barry, 1992). For example, it has been reported that the transdermal flux of lidocaine base (a common anesthetic) from a microemulsion is higher than that from an emulsion (Kreilgaard et al., 2000). Lidocaine-containing products developed with microemulsion formulations are now available on the market (Date and Patravale, 2007).

Lidocaine has been used as model lipophilic drug in transdermal studies with microemulsions (Kreilgaard et al., 2000; Sintov and Shapiro, 2004). The transdermal delivery of lidocaine base is significantly limited by its relatively low water solubility (4 mg/ml). It has been shown that microemulsion-based gels for lidocaine delivery tend to have a longer lasting effect than emulsion-based systems (Lee, 2003), and produce nearly 50–100% larger fluxes of lidocaine than the emulsion-based EMLA® cream (Kreilgaard, 2002; Sintov and Shapiro, 2004). However, in most cases, the benefits of potentially larger fluxes obtained with microemulsions do

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not compensate for the increase in cytotoxic side effects related to the use of alcohols as cosurfactants (Kreilgaard, 2002; Changez et al., 2006).

To minimize the cytotoxicity of microemulsions, lecithin–linker formulations were introduced as a way to generate the desired microemulsion morphology without the need for medium or short chain alcohols that tend to produce the undesirable side effects (Acosta et al., 2005). The term linker is used to characterize asymmetric amphiphilic additives that are either too hydrophilic or too lipophilic and that tend to segregate near the oil–water interface (Sabatini et al., 2003). Lecithin–linker systems have been formulated using sorbitan monooleate (Span 80) as lipophilic linker and hexylpolyglucoside as hydrophilic linker (Acosta et al., 2005). Lecithin–linker formulations for transdermal delivery of lidocaine have been produced with mixtures of caprylic acid (CA) and sodium caprylate (SC) as hydrophilic linkers (Yuan et al., 2008; Yuan and Acosta, 2009). In these linker formulations a Type II – Type III or IV – Type I microemulsion transition is accomplished by increasing the concentration of hydrophilic linker in the system. Furthermore, it has been shown that the drop size of micelles or reverse micelles in lecithin–linker systems is dependent on the ratio between the concentration of the hydrophilic linker and the concentration lecithin in the formulation (Acosta et al., 2005). Using tissue cultures we have confirmed that lecithin–linker microemulsions are less cytotoxic than lecithin formulations where the hydrophilic linker is replaced by pentanol (a medium-chain alcohol), and that lecithin–linker systems produce higher transdermal flux (Yuan et al., 2008). The interpretation of this permeation behavior could not be explained by simple permeation constants used in donor–receiver (two-compartment) models that neglect the accumulation of drug in the skin. A new "skin permeability" term was introduced to account for the concentration of the drug in the skin at steady state in the calculation of the skin-receiver permeation constant (Yuan et al., 2008). Using this concept we learned that in lecithin–linker microemulsions, the increase in transdermal flux was not due to a permeation enhancing effect, but simply due to the increase in the concentration of the drug in the skin, a conclusion that is consistent with the observations of other authors (see the review of Heuschkel et al., 2008). This effect was later used to produce "in-situ" patches of topically absorbed microemulsions (Yuan and Acosta, 2009). These previous studies, however, did not elucidate the effect of surfactant concentration on drug transport.

One of the disadvantages of microemulsion formulations is that they typically require high surfactant concentrations (>20 wt.%). For example, Delgado-Charro et al. (1997) produced microemulsion systems containing 25–44% of a surfactant mixture (capryl-caproyl polyoxylglycerides and polyglyceryl fatty acid ester). The surfactant concentration can be as high as 70% in some microemulsion systems (Kreilgaard, 2002). Unfortunately, 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 to the skin (Attwood, 1994). Therefore, for a transdermal drug delivery (TDD) formulation, the surfactant/cosurfactant concentration should be minimized.

The current understanding of the influence of surfactant concentration on transdermal delivery with microemulsions is limited. There are only few articles (Sintov and Shapiro, 2004; Rhee et al., 2001; Chen and Xhou, 2006) that have discussed the influence of surfactant concentration on transdermal flux in microemulsions, but they have not studied this effect in a systematic way. In those articles, the authors suggest that an increase in surfactant concentration should reduce the transdermal flux of drugs. It has been argued that increasing surfactant concentration reduces the thermodynamic activity of the drug in the vehicle, thus decreasing the partition into the skin and the permeation of the drug (Heuschkel et al., 2008; Date and Patravale, 2007; Chen et al., 2004).

In contrast, a kinetic study of ion transport across microemulsions used as liquid membranes showed that the flux of ions increases with increasing surfactant concentration until the flux reaches a plateau value (Steytler et al., 2001). These researchers propose that micelles or reverse micelles act as carriers of the solute, 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. (1997).

In this work we developed a phase behavior diagram for lecithin-base linker microemulsions with the goal of selecting Type I and Type II microemulsions with similar domain (drop) size but containing six different surfactant concentrations. The solubilization of lidocaine base in these formulations as well as the transdermal permeation of lidocaine was evaluated as a function of the surfactant concentration. In order to quantify the partition and the mass transfer coefficients we introduced a three-compartment (donor, skin, receiver) mass balance model. This model was used to fit the transdermal delivery of lidocaine. This fit yielded the skin-donor partition ($K_{sd}$) coefficient for lidocaine and the mass transfer ($k_{ds}$, $k_{sr}$) coefficients for each formulation. A mechanism for lidocaine transport in lecithin–linker microemulsion is proposed in light of the effect of surfactant concentration on the transport parameters and the penetration of fluorescence probes (incorporated into lecithin–linker formulations) through the skin.

## 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 (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-$C_{12}$-$C_{16}$-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 article.

#### 2.1.2. Skin

Pig ears were obtained from the local market and frozen overnight. The pig ears were inspected for any signs of scalding (browning, stiffness) or other skin irregularities. Prior to use, they were thawed by rinsing with running water for 10 s at room temperature. The skin samples were dermatomed from the external side of the ear to a thickness that ranged from 700 to 900 μm (Yuan et al., 2008; Bronaugh and Maibach, 2002). After that, the dermatomed skin sample was cut in circles of 11.4 mm diameter (to fit the membrane housing section of the permeation device)

ready for use. Prior assembly into the permeation device the skin samples were visually inspected for defects such as open pores or cuts in the skin.

### 2.2. Microemulsion phase behavior and selection of formulations

#### 2.2.1. Phase behavior

The phase behavior (Type I–III–II phase boundaries) of linker-based lecithin microemulsions was obtained for systems containing 0–4% lecithin (evaluated at intervals of 0.4%). To carry out the phase behavior studies, the ratio of the aqueous phase to the oil phase was kept at 1:1. Furthermore, the ratio of lipophilic linker, sorbitan monooleate to lecithin was kept constant at 3:1, and the ratio of caprylic acid to lecithin was also kept constant at 0.75:1. For each lecithin concentration, the ratio of hydrophilic linker sodium caprylate to lecithin (SC/LE) was gradually increased from 0 to 3 (and up to 10 for 0.4% LE). Increasing SC/LE produces a Type II–Type III/IV–Type I transition (Yuan et al., 2008). The phase behavior was obtained at room temperature (23 ± 1 °C). The vials were kept in an enclosed cabinet away from sunlight and air currents that could produce changes in temperature. The electrolyte concentration in the aqueous phase was kept constant at 0.9% w/w NaCl. The pH of the formulations was approximately 5. It is relevant to note that lecithin linker formulations are not highly sensitive to changes in temperature and electrolyte concentration (Acosta et al., 2005).

In this study, 10 wt% lidocaine was pre-dissolved in isopropylmyristate (IPM), producing microemulsions with lidocaine content similar to that of commercial products (Yuan et al., 2008). Using this approach the concentration of lidocaine depends on the volume fraction of IPM in the microemulsion. The lidocaine concentration in the resulting microemulsions (donor solution) was measured and used to calculate the donor-skin partition coefficient. Fortunately, it was determined that the donor-skin partition of lidocaine obtained with microemulsions formulated with 10% and 20% lidocaine pre-dissolved in IPM were roughly the same (Yuan and Acosta, 2009). In other words, the microemulsion–skin partition coefficient is not highly dependent on the concentration of lidocaine pre-dissolved in the IPM.

#### 2.2.2. Selection of formulations

To study the effect of surfactant concentration on transdermal delivery, six samples of Type I and Type II microemulsions were selected containing 0.4%, 1.2%, 2.4%, 2.8%, 3.2% and 4% lecithin. Based on the studies of Steytler et al. (2001) the transport through microemulsions depends on the size of the droplet of the internal phase. The SC/LE ratio for Type I and Type II microemulsions at different lecithin concentrations were selected along paths of constant droplet size, as shown in Fig. 1. In these paths the difference between the ratio of SC/LE of the Type I or II microemulsion and the SC/LE ratio of net zero curvature bicontinuous systems (containing equal volumes of water an oil) is constant (Acosta, 2004). The composition of the entire preparation (including the excess phase) of selected Type I and Type II microemulsion systems are presented in Tables 1 and 2, respectively.

#### 2.2.3. Particle size

The hydrodynamic radius of microemulsion droplets was measured to confirm that the size of the droplets of the selected microemulsions was the same. The measurements were performed at room temperature (23 ± 1 °C) using a BI-200SM Brookhaven instrument equipped with a 35 mW green laser (λ = 514 nm) and a photomultiplier detector located at a fixed angle of 90°. Microemulsion samples were poured into standard glass cuvettes that were placed in the BI-200SM sample holder at least 10 min before measurement. The refractive indexes for the continuous phases (water and IPM) were obtained from the literature (Lide, 2006).

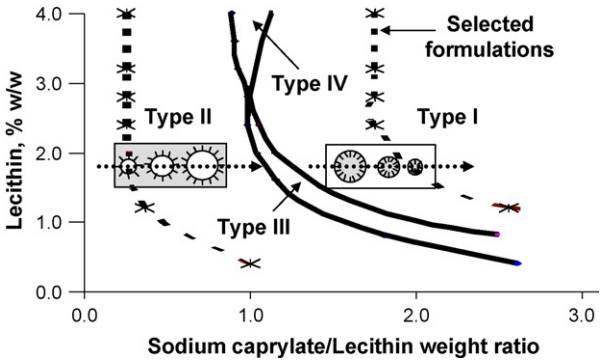


**Fig. 1.** Phase map of lecithin–linker microemulsions formulated with sorbitol monooleate/lecithin ratio of 3/1, caprylic acid/lecithin ratio = 0.75, oil (isopropylmyristate, IPM) to aqueous phase ratio of 1:1 at room temperature (23 ± 1 °C). Increasing the sodium caprylate to lecithin ratio produces a phase transition water-in-oil (Type II) to bicontinuous (Types III, IV) to oil-in-water (Type I) microemulsions. The solid lines represent the phase boundaries. The dashed lines represent the path of constant drop size.

**Table 1**
Overall composition of selected lecithin–linker Type I microemulsion formulations (composition given on a weight basis).

| % Lecithin | % NaCl | % CA | % SC | % Water | % SM | % Lidocaine | % IPM |
|---|---|---|---|---|---|---|---|
| 0.4 | 0.45 | 0.3 | 0.4 | 48.5 | 1.2 | 4.88 | 43.92 |
| 1.2 | 0.45 | 0.9 | 0.5 | 47.0 | 3.6 | 4.64 | 41.76 |
| 2.4 | 0.45 | 1.8 | 0.6 | 44.8 | 7.2 | 4.28 | 38.52 |
| 2.8 | 0.45 | 2.1 | 0.7 | 44.0 | 8.4 | 4.16 | 37.44 |
| 3.2 | 0.45 | 2.4 | 0.8 | 43.2 | 9.6 | 4.04 | 36.36 |
| 4.0 | 0.45 | 3 | 1 | 41.6 | 12 | 3.8 | 34.2 |

CA: capryilic acid, SC: sodium caprylate, SM: sorbitan monooleate, IPM: isopropyl myristate.

### 2.3. In vitro permeation studies

The permeation experiments were performed using a modified version of the MatTek standard percutaneous absorption protocol (Yuan et al., 2008). Briefly, the skin sample was placed in a MatTek Permeation Device (MPD), with the epidermis facing towards the donor compartment. The microemulsion formulation (0.4 ml) was applied in the donor compartment by gently pipetting the solution into the compartment. The skin was not occluded. The receptor compartment was filled with 5 ml of PBS (0.01 M phosphate, 0.137 M NaCl, pH 7.4). At predetermined times (0.5, 1.5, 2.5, 3.5, 4.5 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. In all cases we observed steady state flux of lidocaine starting at 2.5 h or sooner, leaving at least four data points to calculate the steady state flux. All permeation experiments were conducted in triplicate at room temperature. The pig skin remaining after the permeation study was used to measure the steady state lidocaine concentration in the skin.

**Table 2**
Overall composition of selected lecithin–linker Type II microemulsion formulations (composition given on a weight basis).

| % Lecithin | % NaCl | % CA | % SC | % Water | % SM | % Lidocaine | % IPM |
|---|---|---|---|---|---|---|---|
| 0.4 | 0.45 | 0.3 | 2.8 | 46.1 | 1.2 | 4.88 | 43.92 |
| 1.2 | 0.45 | 0.9 | 3.1 | 44.4 | 3.6 | 4.64 | 41.76 |
| 2.4 | 0.45 | 1.8 | 4.2 | 41.2 | 7.2 | 4.28 | 38.52 |
| 2.8 | 0.45 | 2.1 | 4.9 | 39.8 | 8.4 | 4.16 | 37.44 |
| 3.2 | 0.45 | 2.4 | 5.6 | 38.4 | 9.6 | 4.04 | 36.36 |
| 4.0 | 0.45 | 3 | 7 | 35.6 | 12 | 3.8 | 34.2 |

The cumulative mass of lidocaine (mg) permeated across the skin was plotted as a function of time (h), and the average steady-state flux ($F_{ss}$, mg/h/cm$^2$) was calculated by dividing the slope of the linear part of the curve ($dm/dt$) by the exposed skin area ($A$). This exposed area of the skin placed in the MPD was 0.256 cm$^2$.

$$F_{ss} = \frac{1}{A} \times \frac{dm}{dt} \quad (1)$$

*2.3.1. Lidocaine absorption in the skin*

At the end of the *in vitro* permeation studies (5.5 h), the MPD was disassembled, and the skin sample was removed and rinsed with phosphate buffer solution (PBS). The skin was then blotted with Kimwipes and then placed into 2 ml methanol for overnight extraction of lidocaine (Yuan and Acosta, 2009). The concentration of lidocaine in the extract solution was measured via HPLC. The equivalent lidocaine concentration absorbed in skin ($C_{s\text{-}ss}$) 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% (Yuan and Acosta, 2009). This equivalent concentration is not physically correct because skin is not a homogenous phase, and because the concentration of lidocaine in skin is different at different skin depths. This is, however, a necessary simplification for the three-compartment model.

*2.3.2. 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. Lidocaine was separated using a reverse phase column (Genesis, $C_{18}$, 4 μm, 150 mm × 4.6 mm) and detected using an UV–vis absorbance (AD25) set at 230 nm. A mixture of acetonitrile and 0.05 M $NaH_2PO_4 \cdot H_2O$ (pH 2.0) solution (30:70, v/v) was used as the mobile phase with a flow rate of 1.0 ml/min. The column temperature and the injection volume were 25 °C and 10 μl, respectively. The retention time of lidocaine under these conditions was approximately 2.7 min. The peak area correlated linearly with the concentration of lidocaine ($R^2$ = 0.999) in the range of 1–100 μg/ml. Limit of quantitation was 1 μg/ml; coefficient of variation (CV) was 1.5% at 2 μg/ml. Details about the development and validation of this method were described by Yuan et al. (2008).

To measure the concentration of lidocaine in the selected microemulsion, a sample of the microemulsion was carefully withdrawn with a syringe and diluted using methanol as solvent. The diluted sample was analyzed using the method described in the previous paragraph. The run time was extended to 15 min to make sure that all the components of the microemulsions eluted the system before the following injection.

*2.3.3. 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 containing 4% lecithin and 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 h (enough time to reach steady state), 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 63× objectives and exposure time 2.0 s. A solution of 0.001% Nile red in IPM was used as a reference for "hydrophobic" delivery using hydrophobic solvents. The penetration of each dye into the skin was assessed using image analysis (histogram tool of Corel Paint Shop Pro™ version 9.02) to determine the grey level 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 $\{\text{Intensity} - \text{Intensity}_{\min}\}/\{\text{Inensity}_{\max} - \text{Intensity}_{\min}\}$, which assumes that the minimum intensity corresponds to the background fluorescence from the skin (if any).

*2.3.4. Statistical data analysis*

All permeation values were calculated from three independent experiments, and data are expressed as the mean value ± S.D. Statistical analyses were performed using a one-way analysis of variance (ANOVA) to test the significance of the difference between the value of a transport parameter (Figs. 2–5, 7 and 8) obtained at a given lecithin concentration (for Type I or Type II systems) and the value of that parameter at 2.4% lecithin (used as reference concentration). Data with $P < 0.05$ are considered statistically significant.

## 3. Results and discussion

*3.1. Phase behavior and properties of the selected microemulsions*

Fig. 1 presents the phase boundaries for Type II–III or IV–I transitions in a space map of lecithin concentration vs. SC/LE ratio. This phase map is consistent with that of Acosta et al. (2005). In theory, this phase map should have an "X" shape if all surfactants and cosurfactants participate at the interface. Deviations from this theoretical shape suggest that some of the surface active species partition in the bulk phases (Bourrel and Schecter, 1988). In this case, the deviation towards higher SC/LE ratios at low surfactant concentrations is likely due to the partition of the hydrophilic linker (sodium caprylate) into the aqueous phase (Acosta et al., 2005).

The intersection point of the "X" in Fig. 1 occurs at approximately 2.5% lecithin, and at a SC/LE = 1. This implies that a single phase bicontinuous microemulsions can be obtained using approximately 2.5% LE. The Type I and Type II obtained at higher surfactant concentrations are, from the solubilization point of view, underoptimized because the surfactant itself has the capacity to dissolve more oil and surfactant. However the selection of Type I and Type II microemulsions was not based on solubilization capacity, but on maintaining a constant drop size for different lecithin concentrations.

Fig. 2 presents the radius of the selected Type I and II microemulsion samples obtained from dynamic light scattering measurements. The droplet radius remains essentially constant for all samples with increasing surfactant concentration, which validates the procedure used to select the microemulsion samples. The droplet sizes of the selected samples are approximately 6 nm, consistent with values reported for lecithin–linker systems (Yuan et al., 2008; Acosta, 2004).

The lidocaine concentration in the selected microemulsions ($C_d$), used as donor solutions, is shown in Fig. 3. For Type I systems, increasing the surfactant concentration from 0 to 2.8% lecithin increases the number of the micelles per unit of volume, and therefore the overall solubilization capacity of lidocaine. After 2.8% lecithin there are no substantial gains in the solubilization capac-

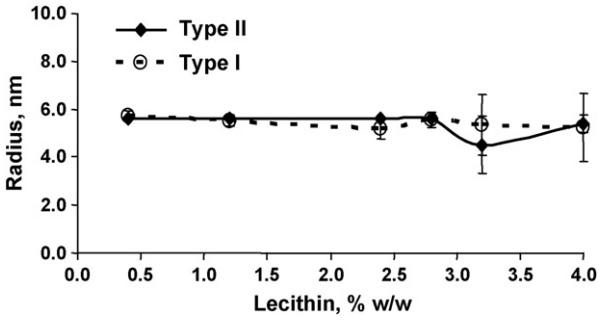


**Fig. 2.** Droplet radius, obtained via dynamic light scattering, for Type I and Type II microemulsions selected from the dashed paths shown in Fig. 1. There was no significant difference among the measured drop radii ($P < 0.05$).

ity of lidocaine since most of the oil is solubilized in micelles. The high drug solubilization capacity is an important feature of microemulsions when delivering oil-soluble drugs using aqueous surfactant solutions (Lawrence and Rees, 2000; Kreilgaard, 2002). On the other hand, the lidocaine concentration in Type II systems

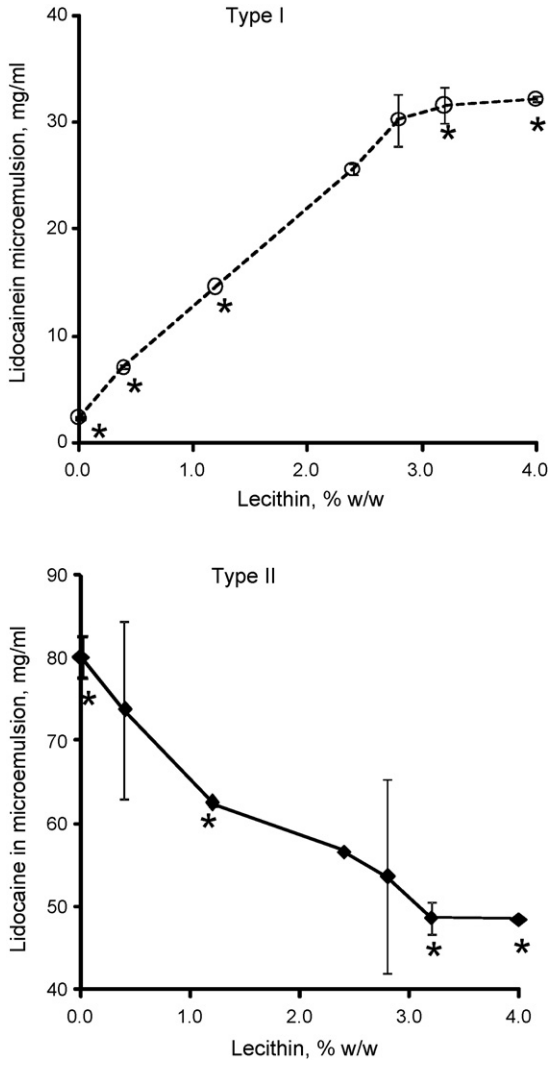


**Fig. 3.** Lidocaine concentration in Type I and Type II microemulsions ($C_d$) as a function of lecithin concentration. The composition of these formulations is indicated by the dotted line (selected formulations) in Fig. 1. The asterisk (*) indicates that the lidocaine concentration in the microemulsion at a given lecithin concentration is statistically different ($P < 0.05$) than the concentration at 2.4% lecithin for the corresponding Type I or Type II system.

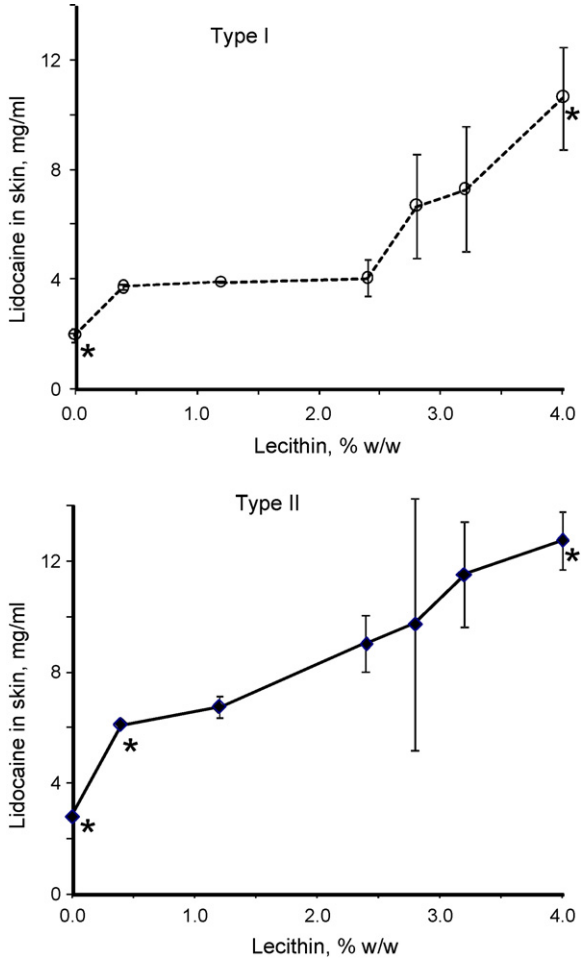


**Fig. 4.** Equivalent lidocaine concentration in skin ($C_{s-ss}$) at steady state absorbed from Type I and Type II microemulsions as a function of lecithin concentration. The asterisk (*) indicates that the lidocaine concentration in skin at a given lecithin concentration is statistically different ($P < 0.05$) than the concentration at 2.4% lecithin for the corresponding Type I or Type II system.

decreases with increasing surfactant concentration. This "dilution" effect in Type II systems is due to the fact that increasing surfactant concentration more water is solubilized in the core of the reverse micelles, increasing the volume of Type II system. This dilution effect levels off at 2.8% LE when most of the water is solubilized.

### 3.2. Effect of surfactant concentration on topical and transdermal delivery of lidocaine

Fig. 4 shows the equivalent lidocaine concentration in the skin at steady state ($C_{s-ss}$) as a function of lecithin (surfactant) concentration. This concentration of lidocaine in the skin is indicative of the topical delivery performance of the formulation. Increasing the concentration of lecithin produced an increase in the equivalent lidocaine skin concentration ($C_{s-ss}$) in both Type I and II microemulsions (statistical significance illustrated by "*" in Fig. 4). For Type I microemulsions the addition of 0.4% LE produces a substantial increase in lidocaine absorption in the skin, but no substantial increase was seen between 0.4% LE and 2.4% LE. The concentration of lidocaine in the skin continues to increase for systems containing more than 2.4% LE. For Type II systems there is a gradual increase in lidocaine concentration in the skin with increase in lecithin concentration. This concentration of lidocaine in the skin is the result of a combination of factors, particularly the rate of lidocaine pen-

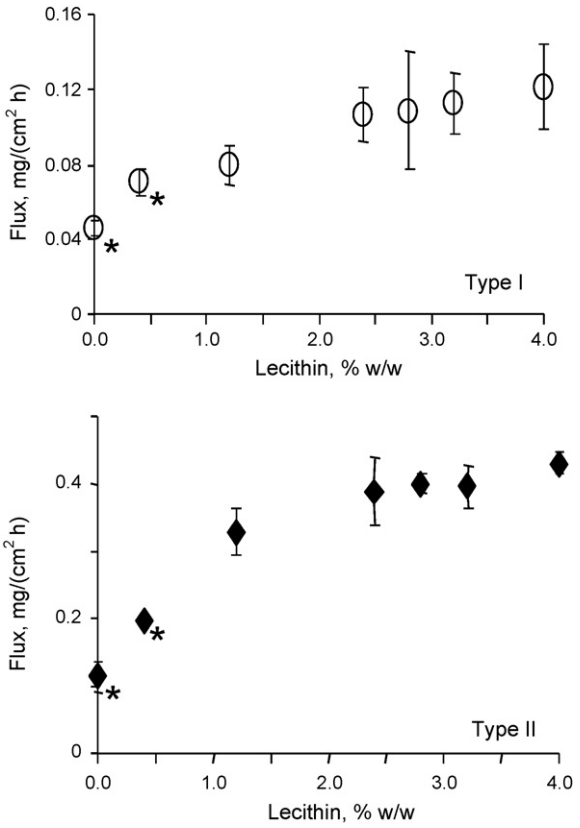


**Fig. 5.** Steady state flux of lidocaine permeated from the skin into the receiver compartment ($F_{ss}$) for Type I and Type II microemulsion formulations, as a function of lecithin concentration. The asterisk (*) indicates that the lidocaine flux at a given lecithin concentration is statistically different ($P < 0.05$) than the flux at 2.4% lecithin for the corresponding Type I or Type II system.

etration into the skin, and the rate of lidocaine release from the skin into the receiver. If the penetration of lidocaine into the skin is faster than its rate of release from the skin, then the concentration of lidocaine in the skin is determined by the concentration of lidocaine in the microemulsion (the donor solution) and its partition into the skin.

Fig. 5 shows the effect of surfactant concentration on the transdermal flux of lidocaine. This transdermal flux increases with surfactant concentration in both Type I and Type II systems within the range of 0–2.4% LE (statistical significance illustrated by "*" in Fig. 5). For lecithin concentrations larger than 2.4% there is only a marginal (not statistically significant) increase in the transdermal flux. The increase in lidocaine concentration in the skin observed in Fig. 4 helps explain the increase in transdermal flux observed in Fig. 5 because the larger lidocaine concentration difference between the skin and the receiver driving the skin-receiver transport. However, this explanation does not apply for systems with 2.8% LE or more, suggesting that there might be dynamic (mass transfer effects) that start to play a role.

### 3.3. In vitro permeation parameters

Various models have been proposed to understand the transdermal transport of drugs in microemulsion systems. Trotta et al. (1989) proposed a simple two-compartment (donor, receiver) membrane permeation model, however the donor–receiver permeation parameter is not suitable to assess the effective permeation of linker–lecithin systems (Yuan et al., 2008). Another donor–receiver model with built-in lag time has also been introduced (Kreilgaard, 2001; Kreilgaard et al., 2001), but this model also neglects the skin compartment. Grassi et al. (2000) introduced a more complete description of the transport by introducing a three-compartment model that assumed a linear concentration profile of the drug in the skin. Unfortunately this model does not fit permeation profiles with an induction (lag) period, typical of systems with moderate to high surfactant concentration, and thick skin membranes (Sirotti et al., 2002). As will be discussed later, the transport through skin does not produce linear concentration profiles, the skin is not homogeneous, and these models neglect the possibility that some of the drug could penetrate with the microemulsion.

To separate the dynamic (mass transfer coefficients) and equilibrium (partition constants) considerations that explain the topical and transdermal delivery of lidocaine, here we introduce a three-compartment (donor, skin, receiver) mass transfer model to fit the permeation profile of lidocaine formulated in lecithin–linker microemulsions. In this three-compartment model it is 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: the flux $F_{ds}$ from the donor solution to the skin, and the flux $F_{sr}$ from the skin to the receiver solution. The differential mass balance of the drug in the skin is:

$$\frac{dC_s}{dt} \times V_s = (F_{ds} - F_{sr})A \tag{2}$$

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

Mass transfer coefficients can be used to calculate the flux of lidocaine transferred from the donor to the skin ($F_{ds}$) and the lidocaine transferred from the skin to the receiver ($F_{sr}$),

$$F_{ds} = k_{ds}(K_{sd}C_d - C_s) \tag{3}$$

$$F_{sr} = k_{sr}(C_s - K_{sr}C_r) \tag{4}$$

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. This assumption is relatively accurate in our experiments because there is less than 5% between the concentration of lidocaine in the microemulsion before and after completing the permeation experiment. Furthermore, 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, $C_r \sim 0$.

Introducing Eqs. (3) and (4) in Eq. (2), considering $C_r = 0$, then:

$$\frac{dC_s}{dt} \times \frac{V_s}{A} = k_{ds}(K_{sd}C_d - C_s) - k_{sr}C_s \tag{5}$$

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 a steady state before the end of the experiment, then this skin concentration is the steady state skin concentration, or $C_{s\text{-}ss}$. The steady state assumption also helps in calculating the skin-receiver mass transfer coefficient (or skin permeability) $k_{sr}$, since at steady state Flux = $F_{ds} = F_{sr}$. Using Eq. (4), and considering that $C_r \sim 0$, then $F_{sr} = k_{sr}\,C_{s\text{-}ss} = F_{ss}$ (flux at steady state). Also using the steady state assumption, the accumulation

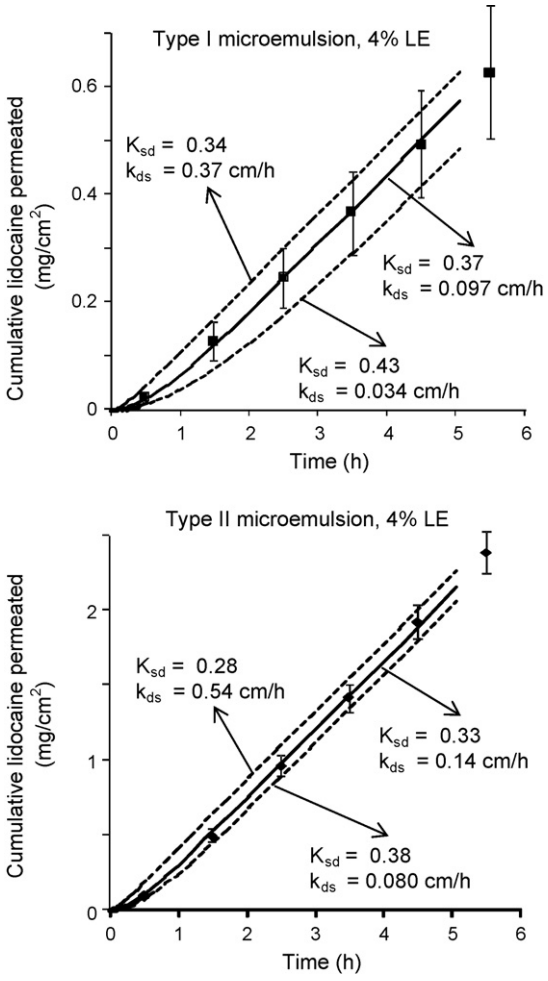


**Fig. 6.** Cumulative mass of lidocaine released into the receiver solution from Type I and Type II microemulsions formulated with 4% lecithin as a function of time (release profile). The solid line represents the theoretical release profile fitted using the value of $K_{sd}$ presented in each figure. The dashed lines show the theoretical profiles using $K_{sd}$ values larger and smaller than the fitted $K_{sd}$ value to illustrate the sensitivity of the fit.

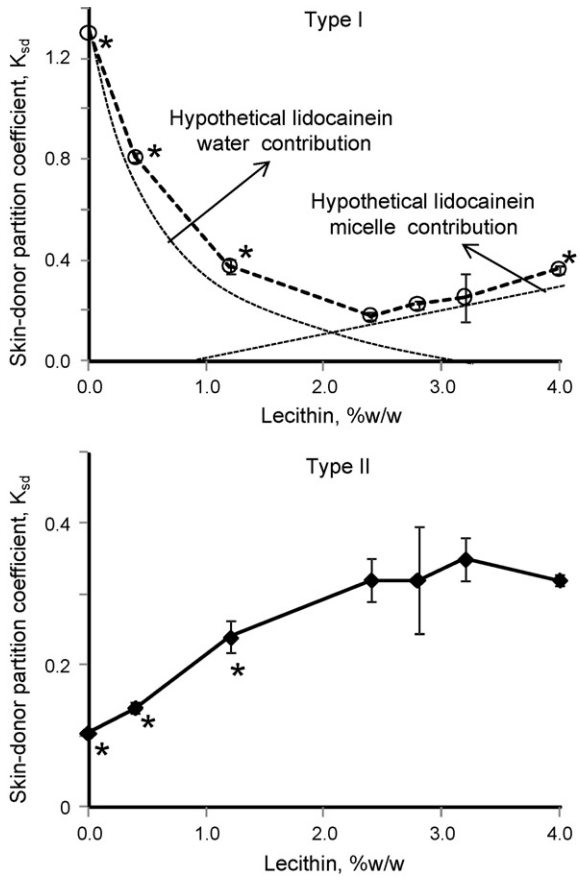


**Fig. 7.** Fitted values of skin-donor partition coefficient ($K_{sd}$) for Type I and Type II microemulsions as a function of lecithin concentration. The asterisk (*) indicates that the lidocaine skin-donor partition at a given lecithin concentration is statistically different ($P < 0.05$) than the partition at 2.4% lecithin for the corresponding Type I or Type II system.

term of Eq. (5) ($dC_s/dt \times V_s/A$) is zero, and solving for $k_{ds}$, we obtain:

$$k_{ds} = \frac{k_{sr} C_{s\_ss}}{K_{sd} C_d - C_{s\_ss}} \quad (6)$$

To find the values of $k_{ds}$ and $K_{sd}$, the theoretical release profiles were fitted the experimental release profiles. To obtain the theoretical release profile, first we assumed a value of $K_{sd}$ (the lower boundary of the initial assumption being $K_{sd} = C_{s\_ss}/C_d$) and using Eq. (6), then calculate $k_{ds}$. With $K_{sd}$, $k_{ds}$, $k_{sr}$ and $C_d$ (the last two measured experimentally) then Eq. (5) can be solved for $C_s$ at any given time. Then with $C_s$ for any given time, Eq. (4) 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 accumulated release profile is obtained. Using the Solver tool of Microsoft Office (2007) Excel, the total error (sum of the absolute differences between the mass of lidocaine released and the predicted value) was minimized by optimizing the value of $K_{sd}$. The Solver tool used a maximum of 100 iterations, a convergence of 0.01%, and a quasi-Newtonian numerical method.

Fig. 6 shows two examples of experimental permeation profiles for selected Type I and II microemulsions formulated with 4% LE. The fitted theoretical profiles are also presented as solid lines in Fig. 6. It is observed that the theoretical profiles fit the experimental data well for both the Type II and I systems. To give an idea of the sensitivity of the predicted profile to the value of $K_{sd}$, the dashed lines present predicted release profiles with different values of $K_{sd}$. According to those lines, even a deviation of 10% from the fitted $K_{sd}$ value produces a significant deviation from the permeation profile. Furthermore the calculated values of $k_{ds}$ are highly sensitive to changes in the value of $K_{sd}$. Although the examples of Fig. 6 presents the average permeation profile of three different repetitions, the reported permeation parameters were calculated (or fitted) for each individual repetition, and then averaged.

### 3.3.1. Skin-donor partition coefficient

Fig. 7 shows the skin-donor partition coefficient ($K_{sd}$) obtained for Type I and Type II formulations as a function of lecithin concentration. For Type I systems, the partition coefficient ($K_{sd}$) gradually decreases with increasing surfactant concentration until the concentration of lecithin is approximately 2.4% (statistical significance illustrated by "*" in Fig. 7). For surfactant concentrations larger than 2.4% LE the partition slightly increases with increasing surfactant concentration. To interpret these results it is important to consider that lidocaine is an amphiphilic molecule that can be partially dissolved in water (solubility 4 mg/ml), in IPM (solubility 220 mg/ml), and it also coadsorbs with the phospholipid (lecithin) at the oil–water interface (Fernandes-Fraceto et al., 2002). Such association between amphiphilic oil-soluble actives is also called palisade layer solubilization (Rosen, 2004). Therefore, increasing the lecithin concentration in Type I microemulsion increases the number of IPM-swollen micelles in the aqueous solution, therefore providing the IPM environment and the surfactant (lecithin

+ linkers) environment where the lidocaine can solubilize. The molecules of lidocaine prefer these environments to the aqueous continuous phase, therefore reducing the chemical potential of lidocaine in water. Having a lower chemical potential, the skin-donor partition of the lidocaine in water is expected to reduce with increasing surfactant concentration. This would explain the decrease in $K_{sd}$ for systems containing 2.4% LE or less. This interpretation is consistent with the observations of Heuschkel et al. (2008) and Date and Patravale (2007), Lee et al. (2003), Shah (1994), and Osborne et al. (1991). For systems containing more than 2.4% LE there seem to be a secondary contribution to lidocaine partition, most likely a partition phenomenon linked to the lidocaine solubilized in IPM-swollen micelles. The hypothetical contributions of lidocaine in water and lidocaine in micelle are illustrated in Fig. 7.

For Type II systems, the partition coefficient ($K_{sd}$) gradually increases as the surfactant concentration increases. In this case, increasing the concentration of the surfactant increases the number of water-swollen reverse micelles. The palisade layer of these reverse micelles provides an additional solubilization site for lidocaine. The increase in skin-donor partition coefficient is most likely linked to the lidocaine associated with the palisade layer of the reverse micelles. The presence of the phospholipid (lecithin) and linkers may facilitate the penetration of the associated lidocained into the skin.

Yuan (2009) measured the partition coefficient of lidocaine between donor and skin by submerging the skin in lidocaine solutions for 24 h, followed by rinse and extraction. Yuan (2009) obtained a skin-water partition of 1.4 and a skin-IPM partition of 0.085. These values are close to the $K_{sd}$ values of 1.35 and 0.1 in Fig. 7 obtained with water and IPM as donor solutions. For Type I and Type II systems with 4% LE, Yuan (2009) measured partition coefficients close to 0.25 that are slightly lower than the partition coefficients of Fig. 7 for 4% LE. Overall, the fitted $K_{sd}$ values are a reasonable estimation of the skin-donor partition.

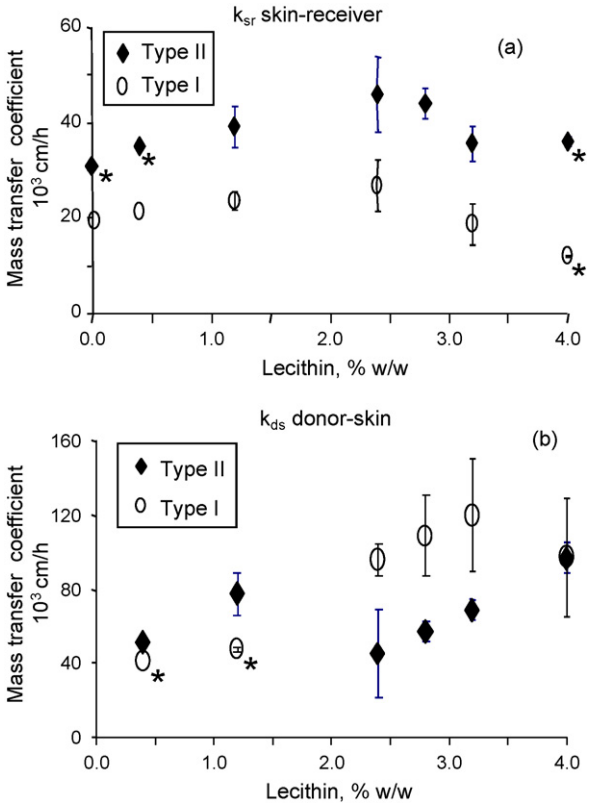


**Fig. 8.** Donor-skin ($k_{ds}$) and skin-receiver ($k_{sr}$) mass transfer coefficient for Type I and Type II microemulsions as a function of lecithin concentration. The asterisk (*) indicates that the mass transfer coefficient ($k_{ds}$ or $k_{sr}$) at a given lecithin concentration is statistically different ($P < 0.05$) than the coefficient at 2.4% lecithin for the corresponding Type I or Type II system.

### 3.3.2. Skin-receiver mass transfer coefficient ($k_{sr}$)

Fig. 8a presents the skin-receiver mass transfer coefficient (skin permeability, $k_{sr}$) of lidocaine in Type I and II systems as a function of lecithin concentration. For lecithin concentration less than 2.4%, 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 for Type I systems (statistical significance illustrated by "*" in Fig. 8a). This suggests that, as proposed in previous articles (Yuan et al., 2008; Yuan and Acosta, 2009) the lecithin and the linkers used in these formulations do not act as permeation enhancers. Sintov and Shapiro (2004) found that high surfactant concentration resulted in lower fluxes, suggesting that permeation enhancing effects are not dominant in microemulsion systems (Rhee et al., 2001). A review by Heuschkel et al. (2008) presents more evidence that supports the thesis that permeation enhancing effects are not significant for most microemulsions.

For Type I and II systems containing more than 2.4% lecithin, the value of $k_{sr}$ reduces with increasing surfactant concentration. One potential explanation for the reduction in $k_{sr}$ is that the viscosity of the formulation increases with increasing surfactant concentration. For Type I, the viscosity at 2.4% LE is 5 cP and at 4% LE is 34 cP. For Type II, the viscosity at 2.4% LE is 8 cP and at 4% LE is 14 cP. Slow transdermal permeation in lecithin–linker microemulsion has been linked to high viscosities (Yuan and Acosta, 2009). Alam et al. (2010) have also observed that increasing surfactant concentration increases the viscosity and reduces the transdermal flux of the formulation. Another possible explanation is the accumulation of surfactant and additives in the skin, or a change in the morphology of the microemulsion as it penetrates through the skin.

Another important observation from Fig. 8a is that the skin-receiver mass transfer coefficient of Type II systems is higher than that of Type I systems when compared at a given lecithin concentration ($P < 0.05$). This is consistent with previous findings for lecithin–linker systems (Yuan et al., 2008) and the results of Jurkovič et al. (2003) but contrasts with other studies that use alcohol as aqueous co-solvent in the microemulsion (Lee et al., 2003). Jurkovič et al. suggested that oil-in-water formulations accumulate the drug in stratum corneum and epidermis whereas the water-in-oil microemulsions delivered the drug into deeper skin regions. Drug accumulation in the skin is a common feature with our systems.

### 3.3.3. Donor-skin mass transfer coefficient ($k_{ds}$)

Fig. 8b presents the donor-skin mass transfer coefficient as a function of surfactant concentration. This parameter was calculated after fitting the theoretical permeation profile using the skin-donor partition $K_{sd}$. The fitting method introduced variability in the calculated value of the $k_{ds}$. Considering the variability of the calculated values presented in Fig. 8b, the only conclusion is that there is a slight increase in $k_{ds}$ with surfactant concentration (statistical significance illustrated by "*" in Fig. 8b). This is consistent with the microemulsion mass transfer model of Steytler et al. (2001).

In most cases, the donor-skin mass transfer coefficient ($k_{ds}$) of lidocaine is larger than the skin-receiver mass transfer coefficient (i.e. $k_{ds} > k_{sr}$). For Type II systems $k_{ds} \sim 1$–2.5 times $k_{sr}$, and for Type I $k_{ds} \sim 2$–8 times $k_{sr}$. This indicates that in most cases the rate limiting step for lidocaine permeation in lecithin–linker microemulsions is the transport through the skin.

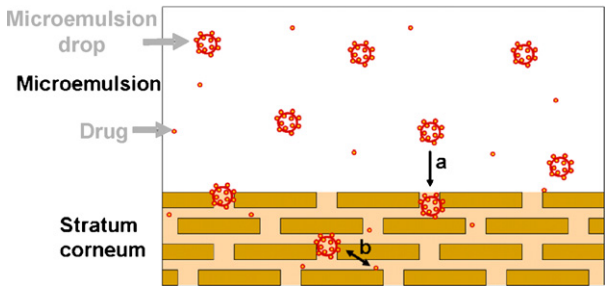


**Fig. 9.** Schematic of the mechanism of microemulsion-mediated transport. In step (a) the microemulsion drop (oil-swollen micelles or water-swollen reverse micelles) penetrates through the porous structure of the skin carrying the drug associated with it. In step (b) the drug is released from the microemulsion droplet.

### 3.4. Mechanism of drug transport in lecitihin–linker microemulsions

The *in vitro* permeation parameters suggest that in lecithin–linker microemulsions the surfactant mixture (lecithin + linkers) does not produce the permeation enhancing effect that has been reported for other microemulsions (Lee et al., 2003). Instead, the accumulation of lidocaine in the skin, which increases the driving force for skin-receiver transport, explains the increase in transdermal flux with increasing surfactant concentration. In Type II systems the increase in $K_{sd}$ with increasing surfactant concentration suggests that the lidocaine associated with reverse micelles is capable of penetrating the skin, but not the lidocaine dissolved in the IPM. For Type I systems, the increase in $K_{sd}$ with increasing surfactant concentration for systems containing more than 2.4% LE (the micelle contribution in Fig. 7) also suggests that the presence of microemulsion droplets (micelles) contributes to the penetration of lidocaine into the skin.

The hypothesis that microemulsion droplets mediate the transport of lidocaine into the skin is illustrated in Fig. 9. According to that schematic the microemulsion droplet carries with it the drug (lidocaine) into the skin (step a). Once in the skin, the microemulsion droplet releases the lidocaine into the surrounding media and eventually the drug permeates into the receiver compartment (step b).

Besides the proposed microemulsion-mediated transport, there is another potential route of transport. Lidocaine can partition as a single molecule directly from the continuous phase of the microemulsion and into the skin. Such mechanism has been used by Grassi et al. (2000) to model transdermal drug delivery from microemulsions. The transport of lidocaine across the skin is most likely a combination of both mechanisms of transport.

The mechanism of Grassi et al. (2000) is consistent with the conventional transport mechanism across the stratum corneum where only individual molecules move through the mortar-brick structure of the stratum corneum. According to that view, surfactants partition into the stratum corneum as free monomers, not in micelles, and that those free surfactant monomers disrupt the mortar-brick structure, facilitating the permeation of the drug. Such view has been challenged by Moore et al. (2003) who have been able to identify micellar structures of sodium dodecyl sulfate penetrating into the skin. According to these authors the key is to use micelle drop sizes similar to that of the pore size of the stratum corneum, which is in the order of 1–10 nm (Li et al., 1998). Furthermore, there is increasing evidence that small (<100 nm) and "soft" lipid-based carriers penetrate into skin by a network of pores (Maghraby et al., 2008; Cevc, 2004). Since the lecithin–linker microemulsion droplets have a droplet size close to 6 nm, and considering that these droplets are soft structures, it is conceivable that, indeed the microemulsion droplets can penetrate through the porous structure of the skin.

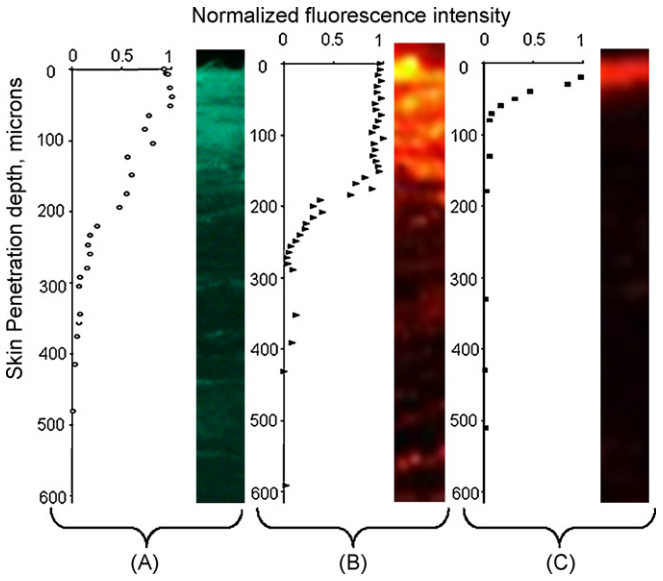


**Fig. 10.** Skin penetration profiles for (A) NBD-$C_{12}$-$C_{16}$-PC formulated in 4% LE Type II, (B) Nile red formulated in 4% LE Type II, and (C) Nile red formulated in IPM.

### 3.5. Fluorescence studies

To test the proposed microemulsion-mediated transport mechanism illustrated in Fig. 9, the skin penetration of the fluorescence probe NBD-$C_{12}$-$C_{16}$-PC (used as a surrogate for lecithin in 4% LE Type II microemulsions) was evaluated. This penetration profile is shown in Fig. 10a. According to Fig. 10(A), 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 50 μm, 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 aggregates (reverse micelles in this case) penetrate deeper into the formulation, carrying lidocaine with them. It is also pertinent to mention that the NBD-$C_{12}$-$C_{16}$-PC probe fluoresce when is associated in amphiphilic environments, a property which is commonly used to track lipid aggregates like vesicles, or in this case, reverse micelles (Chattopadhyay, 1990). Because the concentration of phospholipid in the formulation is 4 wt%, and the fact that the critical micelle concentration (CMC) of di-C12 or longer chain phospholipids is in the order $10^{-6}$ wt% or even lower (extrapolated from Tausk et al., 1974), it is unlikely to observe any fluorescence signal from single phospholipid monomers.

To evaluate the penetration of hydrophobic solutes, like lidocaine, the fluorescence probe, Nile Red was used in Type II microemulsions formulated with 4% lecithin. The penetration of Nile red in Type II is presented in Fig. 10(B). According to that Figure, Nile red also penetrates nearly 250 microns into the skin, which is compatible with the proposition that lecithin aggregates carry the drug into the skin. As a control, the penetration of Nile Red dissolved in IPM (solvent) is presented in Fig. 10(C). As shown in that Figure, in this formulation Nile Red penetrates less than 100 microns, suggesting that, by partition and diffusion of single molecules alone, hydrophobic components only penetrate the stratum corneum and part of the epidermis.

## 4. Conclusions

In lecithin–linker microemulsions, increasing surfactant concentration leads to an increase in the concentration of lidocaine absorbed in the skin. This increase in lidocaine concentration in the skin leads to a larger transdermal flux of lidocaine. In Type I systems the increase in lidocaine concentration in the skin is mainly due to the increase in the solubilization capacity of lidocaine in the microemulsion with increasing surfactant concentration. In Type II systems the increase in lidocaine absorption in the skin is related to the penetration of lidocaine associated to the reverse micelles. In both microemulsions, the presence of micelles or reverse micelles improves the skin-donor partition of lidocaine. In Type I systems, however, increasing the surfactant concentration between 0 and 2.4% LE reduces the skin-donor partition coefficient of lidocaine, because the activity of the free drug in the continuous phase reduces with increasing surfactant concentration. With respect to mass transfer coefficients, increasing the surfactant concentration does not produce a substantial change in donor-skin and skin-receiver mass transfer coefficients, suggesting that lecithin and linkers do not act as permeation enhancers. Comparing these mass transfer coefficients one concludes that the skin-receiver transport is the rate-limiting step.

The three-compartment mass balance model introduced in this work decoupled the partition and mass transport effects, but it uses assumptions that need to be revisited in the future. For example, the model assumes that skin is a homogenous phase where lidocaine is homogenously distributed. A realistic lidocaine concentration profile could produce values of mass transfer coefficients that can be related to diffusion coefficients, and equations of transport in porous media. Another assumption is that the microemulsion is a single phase where the lidocaine is homogeneously distributed. Future versions of this model should consider the lidocaine dissolved in the continuous phase and the lidocaine associated with the microemulsion droplets as two different sources of lidocaine. Taking into account such effects it will be possible to decouple the transport via molecular diffusion and the transport via the proposed microemulsion-mediated transport mechanism. To introduce such sophistications in the model it will be necessary to introduce methods to measure the free and the micelle-associated lidocaine in microemulsion and in the skin, along with the penetration profile in the skin.

The fluorescence microscopy studies support the proposed mechanism of microemulsion-mediated transport whereby the microemulsion droplets penetrate the skin, carrying with it the active ingredient.

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