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Extended release of lidocaine from linker-based lecithin microemulsions

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ABSTRACT

In a previous article we reported on the use of linker-based lecithin microemulsions as effective transdermal delivery vehicles for lidocaine [Yuan, J.S., Ansari, M., Samaan, M., Acosta, E., 2008. Linker-based lecithin microemulsions for transdermal delivery of lidocaine. Int. J. Pharm. 349, 130–143]. It was determined at that time that the performance of these vehicles was in part due to a permeability enhancement effect, but also due to the amount of lidocaine absorbed in the skin. In the present article we take advantage of this drug absorbed in the skin to produce an extended release profile where the lidocaine-loaded skin is used as an *in situ* patch. The release of lidocaine from the skin is modeled using a differential mass balance that yields a first order release profile. This profile depends on the mass of drug initially loaded in the skin and a mass transfer coefficient. When the release profile of lidocaine was evaluated as a function of the concentration of lidocaine in the microemulsion, application time, and microemulsion dosage; we observed that all these different conditions only change the mass of lidocaine initially loaded in the skin. However, these parameters do not change the mass transfer coefficient. When the release profile of Types I and II microemulsions was compared, it was determined that the mass transfer coefficient of Type II systems was larger than that of Type I. This suggests that the morphology of the microemulsion plays an important role on the release kinetics. These linker microemulsions were able to release 90% of their content over a 24-h period which rivals the performance of some polymer-based patches. Fluorescence micrographs of transversal cuts of skin loaded with Nile red are consistent with the observed release profiles.

1. Introduction

The goal of transdermal drug delivery (TDD) is to achieve better percutaneous absorption and permeation of active ingredients for local treatment (Bronaugh and Maibach, 2005). Microemulsions have been reported to improve the absorption of drugs in the skin significantly compared to aqueous solutions, gels, or cream formulations (Baroli et al., 2000; Delgado-Charro et al., 1997; Williams and Barry, 1992). The enhanced absorption is typically associated with the high surface area of microemulsion aggregates and the presence of surfactant that act as permeation enhancers. A microemulsion is a system that contains water and/or oil nanodomains coexisting in thermodynamic equilibrium due to the presence of a surfactant film at the oil/water interface. There are three microemulsion morphologies depending on the continuous and dispersed media. Type I (o/w) microemulsions consist of oil solubilized in micelles present in an aqueous continuous phase. Type II (w/o) microemulsions consist of water solubilized in reversed micelles present in an oil continuous phase. Types III and IV microemulsions contain bicontinuous channels of oil and water. In the case of a Type III system, besides the microemulsion, there is an excess phase of oil and an excess phase of water, but other than that, it retain the same characteristics of the Type IV (Bourrel and Schecter, 1988).

Microemulsions provide many advantages as topical TDD systems, including high solubilization for both oil- and water-soluble drugs, being thermodynamically stable, transparent, and spontaneous formation. However the use of microemulsions has been limited by the toxicity of ionic surfactants and alcohols used in most formulations. In a previous study (Yuan et al., 2008), alcohol-free lecithin microemulsions were formulated using linker molecules with generally recognized as safe (GRAS) or food additive status.

Linker molecules are defined as amphiphilic molecules that segregate near the oil–water interface but only from one side of the interface (Sabatini et al., 2003). As such, a lipophilic linker is a molecule that approaches the interface from the oil side, but that has weak, if any, interaction with water molecules across the interface. A hydrophilic linker, on the other hand, approaches the interface from the aqueous phase, but it has weak, if any interactions with the oil molecules (Acosta et al., 2002). The main difference between a linker and a cosurfactant, such as medium

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chain alcohols, is that the cosurfactant has substantial interactions with both, oil and water molecules. Furthermore, there is a special "zipper" self-assembly that takes place when combining hydrophilic and lipophilic linkers whereby the lipophilic and hydrophilic linkers come together to the interface guided by their interactions with the surfactant in the formulation (Acosta et al., 2004). It has been found that hydrophilic linkers reduce the rigidity of the surfactant film at the oil/water interface, accelerating several interfacial processes (Acosta et al., 2003).

The formulation of lecithin microemulsions as "green solvents" using sorbitan monooleate as lipophilic linker and hexyl polyglucoside as a hydrophilic linker was reported by Acosta et al. (2005). Yuan et al. (2008) produced a modified version of this formulation introducing a mixture of sodium caprylate and caprylic acid as hydrophilic linkers, and isopropyl myristate as the oil phase. The microemulsions produced in that study had 6–10 nm aggregates, and relative low viscosity (10–40 cP). In that study, it was shown that lecithin-linker formulations containing this mixture of linkers (including sorbitan monooleate as lipophilic linker) were less toxic and produced a faster penetration that the same formulation using pentanol as cosurfactant. Based on those results, it was proposed that the combination of hydrophilic linkers produced an effect beyond simple skin permeation enhancing, and that the low interfacial rigidity of these systems facilitated the penetration of the surfactant aggregates carrying the active to the skin. Furthermore, the presence of the hydrophilic linker was shown to produce rather small aggregates of 6–10 nm that can easily penetrate through the epithelial tissue.

In this work, we hypothesize that by topically administrating an active ingredient using the linker microemulsions previously formulated by Yuan et al. (2008), the drug will be safely absorbed into the skin, thus producing an *in situ* delivery patch. Potential advantages of this *in situ* patch include its application on uneven and exposed parts, its low cost, and customizable dose.

The typical controlled-release form for topical treatment of skin diseases is drug-in-adhesive patches. Since the transdermal absorption occurs through a slow process of diffusion, driven by a concentration gradient, the patches must be kept in continuous contact with the skin for a considerable time (hours and days) (Scheindlin, 2004). This often causes skin irritation at the site of administration (Shah and Skelly, 1987; Kurihara-Bergstrom et al., 1991). For extreme situations such as patients with painful skin irritations, blisters, burns, or other skin wounds, the use of sprayable therapeutical microemulsion formulations may represent a significant advance over the patches that require direct contact with the skin. Since these microemulsions are transparent, the formulation could be used to treat various skin conditions without affecting the appearance of the patient.

Another advantage of *in situ* patches over conventional ones is that a large excess of drug has to be placed in the conventional patches to maintain the concentration gradient. For example, the label of lidocaine patch (Lidoderm®) states that only 3% of the applied dose is absorbed (Scheindlin, 2004). The cost of excess drug in the patches may be significant for numerous drugs.

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

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

However, there is no previous report to study extended transdermal release of lidocaine from microemulsions. The aim of the study is to test the *in situ* delivery patch hypothesis by evaluating the skin absorption and the release profile of lidocaine *via* linker microemulsions. Lidocaine-laden linker microemulsions were applied on pieces of excised pig ear skin and *in vitro* extended release studies were conducted. The release profile from these studies were analyzed by a controlled-release model (described in the next section) and compared to the release profile of traditional transdermal patches, gels and polymer films reported in the literature. Furthermore, fluorescence microscopy was used to study the permeation and location of a lipophilic fluorescent dye (Nile red) within the skin.

2. Materials and method

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%), sodium chloride (99%+, Fluka brand), Dulbecco's phosphate buffered saline (PBS), Nile red and lidocaine powder (base form, 98%+). Laboratory grade soybean lecithin (99%+) was purchased from Fisher Scientific (Fairlawn, NJ, USA). Soybean lecithin is a mixture of phospholipids (mainly phosphatidyl cholines) produced by acetone purification of soybean gum residues. Sodium phosphate monobasic, monohydrate (ACS grade) and acetonitrile (HPLC grade) were purchased from EMD Chemicals Inc. (Darmstadt, Germany), and they were used as received. Anhydrous ethyl alcohol and methanol were purchased from Commercial Alcohols Inc. (Brampton, ON, Canada). Unless otherwise stated, the composition is expressed on weight basis (i.e. wt.%) throughout this paper.

2.1.2. Skin

Pig ears were purchased from the local market and frozen overnight. Prior to use, they were thawed by rinsing with running water for 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 11.4 mm diameter ready for use.

Table 1
Compositions of Types II and I linker-based lecithin microemulsions.

Formulation	LE	SM	SC	CA	NaCl	Water	10% or 20% lido-IPM
Type II	4.0	12.0	1.0	3.0	0.9	41.1	38
Type I	4.0	12.0	7.0	3.0	0.9	35.1	38

LE, lecithin; SM, sorbitan monooleate; CA, caprylic acid; SC, sodium caprylate.
All compositions are expressed as wt.%.

2.2. Microemulsion preparation

Microemulsion formulations were prepared using equal volumes of aqueous solution and oil (5 ml of each) in flat bottom test tubes at constant temperature (25 ± 1 °C), electrolyte concentration (0.9% NaCl in the aqueous solution), and pressure (1 atm) (Yuan et al., 2008). The oil used in this study is IPM. Lidocaine was loaded in the microemulsion formulations by predissolving this drug in IPM to a concentration of 10% (10% lido-IPM) and 20% (20% lido-IPM, near the solubility limit of lidocaine in IPM). After introducing all the ingredients, the test tubes were thoroughly vortexed, then vortexed once a day for 3 days, and left to equilibrate for 2 weeks. The composition of the linker-based lecithin microemulsions is shown in Table 1. Additional information regarding the phase diagram, viscosity, and particle size for these linker formulations have been previously reported (Yuan et al., 2008).

2.3. In vitro extended release studies

The *in vitro* extended release experiments were conducted in MatTek Permeation Devices (MPD) supplied by MatTek Corporation (Ashland, MA, USA). The exposed area of tissue for the MPD is 0.256 cm^2. The pig ear skin was placed in the MPD, with the epidermis facing up. A test microemulsion formulation (400 μl) was applied in the donor compartment. The receptor compartment was filled with 5 ml of PBS (0.01 M phosphate, 0.137 M NaCl, pH 7.4). After 30 min (unless stated otherwise), the donor microemulsion was withdrawn and the skin surface was blotted dry with Kimwipes and then used for extended release. This loading time of 30 min was selected based on a previous study that shows that the continuous release reaches steady state after 30 min (Yuan et al., 2007). At predetermined times (1, 3, 6, 12, 24 and 48 h), the receiver solution was withdrawn completely from the receptor compartment and was immediately replaced by fresh PBS solution. At 48 h, the experiment was terminated. All permeation experiments were conducted in triplicate at room temperature.

2.3.1. In vitro skin absorption

The pig ear skin samples at the end of the *in vitro* extended release studies were used to test the final concentration of the drug absorbed in the skin (C_s^f). Prior to measuring the absorbed lidocaine, the pig skin was rinsed with a PBS solution and placed into 2 ml methanol for overnight extraction of lidocaine (Yuan et al., 2008). The equivalent lidocaine concentration absorbed in skin was calculated as the mass of lidocaine extracted from the skin divided by the volume of the skin (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 initial lidocaine concentration (C_s^0) absorbed into skin after 30 min application of a microemulsion was then calculated by adding the cumulative amount of drug permeated to the receiver and the final amount of drug extracted from the skin and dividing this value by the volume of the skin.

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 consisting of single pump, detector, AS40 automated sampler, AD25 absorbance detector and Chromeleon chromatography software (Dionex). Lidocaine was separated by a reverse phase column (Genesis, C_{18}, 4 μm, 150 mm × 4.6 mm) and detected through its absorbance at 230 nm (AD25 detector). 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 a mobile phase with a flow rate at 1.0 ml/min. The column temperature and the injection volume were 25 °C and 20 μl, respectively.

2.3.3. Statistical data analysis

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

2.4. Fluorescence microscopy

To visualize the permeation of the microemulsions into the skin tissues, the linker-based lecithin microemulsions containing a fluorescent dye, Nile red (0.001%), were prepared. The dye was simply dissolved into the Types II and I systems with 10% lido-IPM (Table 1). The systems were vortexed and left for equilibrate for overnight. The microemulsions containing Nile red were then used to conduct *in vitro* extended release studies. After 1 h, 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 (λ_{exc} = 450–500 nm) to visualize Nile red. All photographs were taken with 63× objectives and exposure time 2.0 s. Solutions of 0.001% Nile red in IPM was considered as the control.

3. Results and discussion

3.1. In vitro extended release

To investigate the potential of linker-based lecithin microemulsions absorbed in skin as *in situ* patches for extended delivery, we examined the release profile of these systems with different formulation and application conditions. In terms of formulation conditions, we evaluated the effect of drug loading in the microemulsion (10% lidocaine in IPM and 20% lidocaine in IPM) and the effect of microemulsion morphology (Type I-o/w-microemulsion vs. Type II-w/o-microemulsion). These effects are described in Section 3.1.1, and the first order release kinetics (Eq. (7)) is applied to these results in Section 3.1.2. To evaluate the effect of application conditions (Section 3.1.3), the release profile was examined as a function of dosage (mg of microemulsion/cm^2 skin) and application time.

3.1.1. Effect of formulation conditions: drug loading and microemulsion morphology

Fig. 1a and b reports the drug release profiles obtained by plotting the cumulative amount of lidocaine released as a function of time. For all of the *in situ* patches, the cumulative release profiles

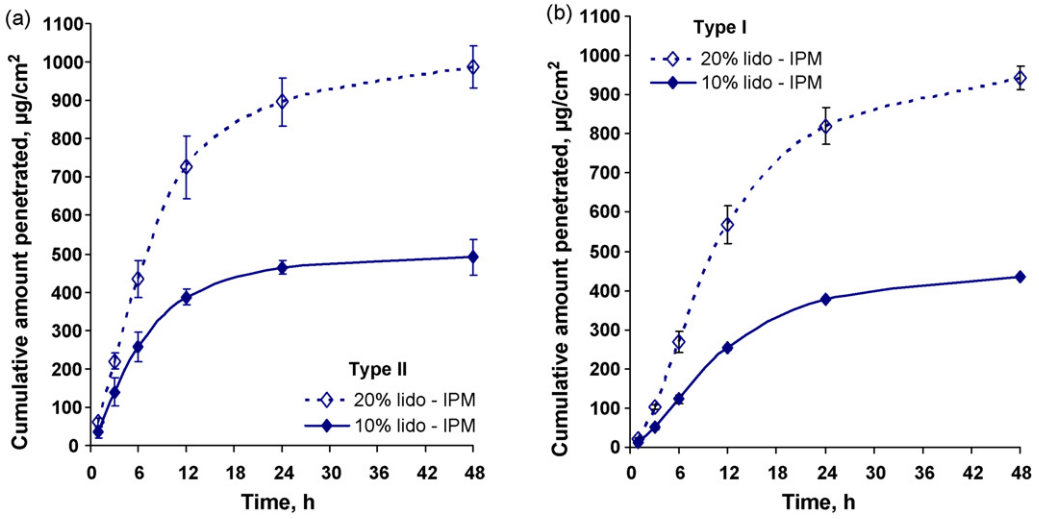

Fig. 1. *In vitro* release profiles of lidocaine from the *in situ* patches produced with (a) Type II and (b) Type I microemulsions formulated with 10% and 20% of lidocaine in IPM.

typically show a large increase in the first 24 h. When increasing the drug loading from 10% lido-IPM to 20% lido-IPM in either the Type II or Type I formulation, it shows that the lidocaine released also doubled. The increase in drug released from the *in situ* patches with increasing lidocaine loading in the microemulsion is attributed to the larger lidocaine concentration absorbed in the skin. For the Type II formulation with 10% lido-IPM, the lidocaine absorption in the skin was 24.2 ± 3.3 mg/ml, while that for the Type II formulation with 20% lido-IPM doubled to 50.2 ± 4.6 mg/ml (Table 2). Similarly, the data in Table 2 also show that for Type I systems, increasing the concentration of lidocaine from 10% to 20% in IPM, the concentration of lidocaine absorbed in the skin increased from 21.0 ± 1.9 mg/ml to 46.6 ± 5.4 mg/ml. These observations are in agreement with the results obtained by Kreilgaard et al. (2000) where they determined that transdermal drug delivery increases as the drug loading in the microemulsions increases.

During the extended release experiments, most of the drug absorbed in the skin was released. Fig. 2a and b helps to illustrate this observation. In Fig. 2a and b, the fraction of lidocaine released is plotted as a function of time for the *in situ* patches of Fig. 1. The fraction release curves show that approximately 80–90% of the drug was released by 24 h and nearly 100% released by 48 h. According to Fig. 2a and b, increasing the drug loading from 10% lido-IPM to 20% lido-IPM had no impact on the fraction of lidocaine released (ANOVA test—*P* ranges from 0.08 to 0.74 for Type I systems, and from 0.11 to 0.57 for Type II systems). These observations are consistent with Eq. (7): (introduced in section 3.1.2) in that the fraction of drug release is not a function of the mass of drug loaded in the skin (M_∞).

It is also important to highlight that the shapes of the fractional release curves of Fig. 2a and b are different for Types I and II microemulsions. The release from Type I microemulsions (Fig. 2b) is slower than Type II microemulsions (Fig. 2a) either for 10% or 20% lido-IPM. After 12 h of release, the Type I microemulsion has released almost 60% of its load whereas the Type II systems has released approximately 75%. Furthermore, the Type I system seems to approach a linear release profile during the first 12 h of release. These observations are consistent with those of Yuan et al. (2008). At this point there is no clear explanation for the lower rate of penetration for Type I systems, but this may be related to the higher viscosity of these systems. According to the data of Yuan et al. (2008), the viscosity for the Type I formulation is approximately 35 cP, and for Type II, approximately 15 cP. If one considers that the formulation penetrated the skin as a fluid that moves through a porous media, and that the Darcy or Washburn penetration equations apply, then the flux is proportional to 1/viscosity. Therefore, the higher the viscosity, the lower the flux of the carrier through the porous media.

3.1.2. Drug release kinetics

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

In this study, drug release data obtained were analyzed in terms of the fraction of lidocaine released as a function of time. To this end, we propose a simple mass transfer mechanism illustrated in Fig. 3. As shown in Fig. 3, the drug transport in the experiment can be divided into two parts: (1) the flux F_{ds} from the donor solution to the

Table 2
Formulation and release kinetic parameters: lidocaine loading in microemulsions, initial drug absorption in the skin (C_s^0, mg/ml), release rate constants of lidocaine (k_{sr}, $\times 10^3$ cm/h) and the time required to reach 50% release ($t_{50\%}$, h) calculated by the first-order model fitted to the 24 h release data.

Microemulsion systems	Drug loading (mg/ml)	Drug absorption C_s^0 (mg/ml)	k_{sr} ($\times 10^3$ cm/h)	$t_{50\%}$ (h)
Type II				
10% lido-IPM	55.8 ± 0.8	24.2 ± 3.3	8.4 ± 0.6	6.2 ± 1.1
20% lido-IPM	104.0 ± 0.0	50.2 ± 4.6	7.2 ± 1.3	7.7 ± 0.4
Type I				
10% lido-IPM	40.8 ± 1.6	21.0 ± 1.9	6.0 ± 0.6	9.5 ± 0.3
20% lido-IPM	71.0 ± 0.1	46.6 ± 5.4	6.0 ± 0.7	9.2 ± 0.4

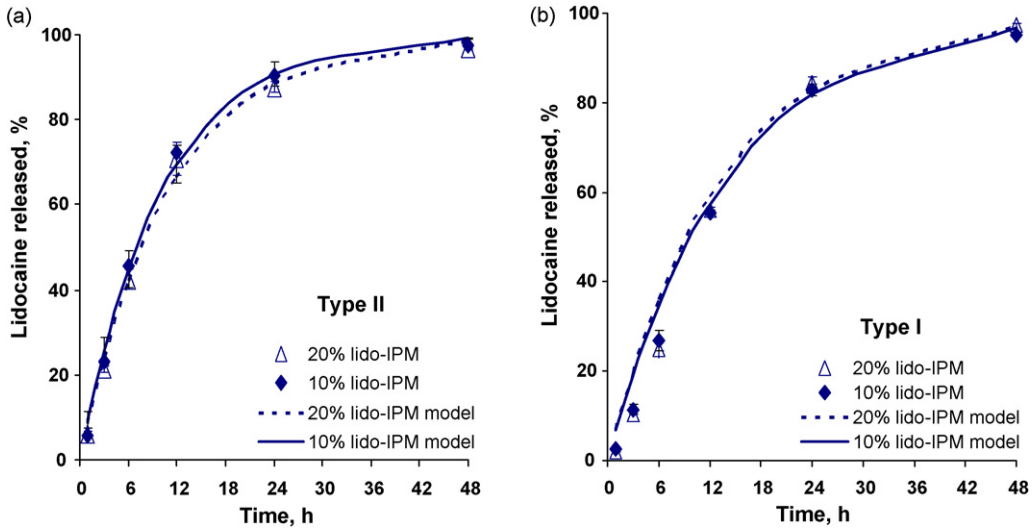

Fig. 2. Fraction of lidocaine released from *in situ* patches produced with (a) Type II and (b) Type I microemulsions formulated with 10% and 20% of lidocaine in IPM. The solid and dashed lines represent the fits (24-h data) to the first-order model (Eq. (7)).

skin, and (2) the flux F_{sr} from the skin to the receiver solution. The differential mass balance of lidocaine yields the following equation:

$$\frac{dC_s}{dt}V = (F_{ds} - F_{sr})A \quad (1)$$

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

In the *in vitro* extended release studies, the applied microemulsion was withdrawn from the donor compartment before the extended release. In this case, $F_{ds} = 0$, and Eq. (1) is then can be written as,

$$\frac{dC_s}{dt}l = -F_{sr} \quad (2)$$

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

$$F_{sr} = k_{sr}(C_s - K_{sr}C_r) \quad (3)$$

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

Substituting F_{sr} in Eq. (2) by Eq. (3) we obtain that:

$$dt = \frac{dC_s}{k_{sr}(K_{sr}C_r - C_s)}l \quad (4)$$

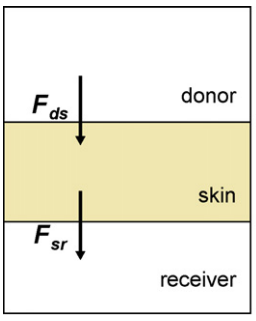

Fig. 3. Schematic of the mechanism of drug transport for *in vitro* extended release studies.

Integrating between $t = 0$ at the beginning of the extended release and any "t":

$$\int_0^t dt = \int_{C_s^0}^{C_s} \frac{dC_s}{k_{sr}(K_{sr}C_r - C_s)}l \quad (5)$$

where C_s^0 is the drug concentration in the skin at time $t = 0$, i.e., the initial lidocaine concentration absorbed into skin. In our extended release experiments, at predetermined times, the receiver solution was withdrawn completely from the receptor compartment and was immediately replaced by fresh PBS solution. In this way, we can assume that the drug concentration in the receiver is negligible as each time the receiver the receiver medium is being replaced with fresh medium, that is, $C_r \sim 0$. From Eq. (5), the drug concentration in the skin at time t can be written as,

$$C_s = C_s^0 \ \exp\left(-\frac{1}{l}k_{sr}t\right) \quad (6)$$

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

$$\frac{M_t}{M_\infty} = 1 - \exp\left(-\frac{1}{l}k_{sr}t\right) \quad (7)$$

where M_t is defined as the mass of drug released at time t, and M_∞ is the mass of drug released at time approaches infinity (this is also the mass of drug loaded in the skin). This equation follows the classic first-order drug release model: $M_t/M_\infty = 1 - \exp(-kt)$ (Cussler, 1997).

We proceeded to fit the fractional release profiles of Fig. 2a and b to the first-order release model (Eq. (7)). Fig. 4a and b presents the value $\log(1 - M_t/M_\infty)$ against time (t) for the 24 and 48-h release data. The high correlation factor ($R^2 > 0.95$) of all the fits in Fig. 4a and b is consistent with the hypothesis that the mechanism of drug release from all of the *in situ* patches applying either Type II or I microemulsions obey the first-order release kinetics. However, the 48-h fit (Fig. 4a) of Eq. (7) show substantial deviations from the release data for Type I microemulsions. The 24-h fit (Fig. 4b), on the other hand, show good agreement with the data. Even when the 24-h fit is extended to 48-h release, shown as solid and dashed lines in Fig. 4a and b, the error obtained at 48-h is not substantial.

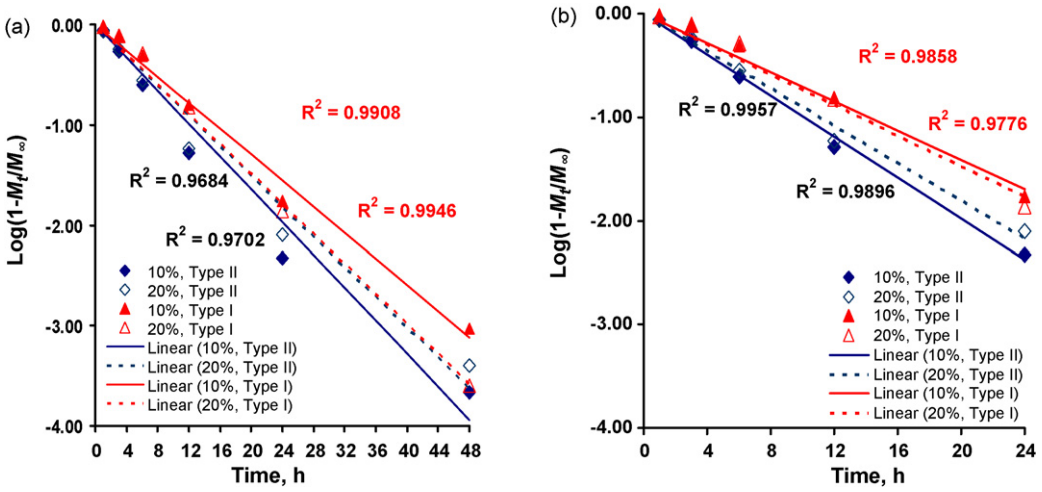

Fig. 4. Log $(1 - M_t/M_\infty)$ against time for the (a) 48-h data and (b) 24-h data, in which M_t/M_∞ is the same data of Fig. 2.

Table 2 presents the release rate constants (k_{sr}) obtained after fitting the 24-h fractional release data with Eq. (7). The time to release 50% of the load ($t_{50\%}$) was determined by Eq. (7) and is also presented in Table 2. We were able to confirm that the values of k_{sr} do not depend on the loading of lidocaine in Type I ($P = 0.98$) or Type II ($P = 0.31$) microemulsions. Furthermore, we were able to determine that the value of k_{sr} was different for Types I and II microemulsions formulated with 10% lidocaine in IPM ($P = 0.045$), but not distinctly different for formulations produced with 20% lidocaine in IPM ($P = 0.21$). The analysis of these fits suggests that, consistent with the earlier observations, the loading of lidocaine in the microemulsion does not affect the fractional release. However, the morphology of the microemulsion (Type I vs. Type II) does have an impact on the release kinetics. The slower lidocaine release from Type I microemulsion is consistent with previous observations in continuous delivery studies (Yuan et al., 2008). In that work, it was shown that the "skin" permeability of Type II microemulsions loaded with lidocaine was almost twice that of Type I microemulsions. This "skin" permeability can be interpreted as a mass transfer coefficient for continuous delivery when the donor is not removed from the permeation device. The ratio between k_{sr} for Type II and k_{sr} for Type I systems formulated with 10% of lidocaine in IPM (Table 2) is close to 1.4, which approaches the ratio observed for the "skin" permeability by Yuan et al. (2008). Using the argument of viscosity (35 cP for Type I and 15 cP for Type II), and Darcy's law or Washburn equation (flux $\sim$ 1/viscosity) then the ratio of "skin" permeabilities should be close to 2. Further studies to explore this phenomenon and to explain the effect of microemulsion morphology are still needed.

In order to compare the extended release performance of Types I and II formulations with other systems, we plotted the release profile of the microemulsions, and the release profile of other lidocaine patches reported in the literature (Repka et al., 2005; Glavas-Dodov et al., 2002; Burgalassi et al., 1996; Gref et al., 1994). These release profiles are presented in Fig. 5 for Types I and II microemulsions formulated with 10% lido-IPM. The lidocaine release from the *in situ* patch is slower than that from lidocaine embedded in a polymer (HPC:HPMC) film (Repka et al., 2005) and in polymeric nanospheres (Gref et al., 1994), however, it has similar performance to buccal patches produced with chitosan gels (Burgalassi et al., 1996). It is necessary to clarify that the *in situ* patches rely, for the most part, on the properties of the skin and the interactions between the drug and the surfactants/oil in the microemulsion to regulate the transport of the active. In the polymer film of Repeka et al. or in the buccal patch, the mechanism of controlling the rate of release is different because there is a physical film/membrane to regulate the release of the drug. While the mechanisms of delivery are different, we confirmed the possibility of using linker-based lecithin microemulsions absorbed in skin as *in situ* patches for extended delivery.

On the other hand, the drug release from the *in situ* patch is relatively fast when compared to the released profile of lidocaine from a cross-linked hydrogel (Glavas-Dodov et al., 2002). One possible reason is because the microemulsion does not have the high viscosity (Yuan et al., 2008) or the fixed 3D pore network structure of the polymer that help regulate the fraction of lidocaine release.

The comparisons discussed above should be interpreted in light of the fact that the release profile not only depends on the formulation and properties of the polymer, but also on the flow dynamics and concentration profiles around the film reservoir. With the exception of the work of Glavas-Dodov et al. (2002), all the other profiles were obtained using USP dissolution tests. In those tests, the higher shear provided by the agitation of the paddles may have increased the rate of release. However, it is important to remember

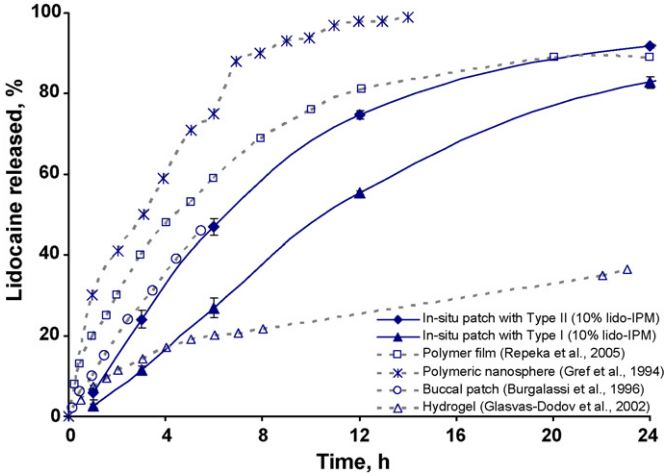

Fig. 5. Comparison of lidocaine release from *in situ* patches produced with Type II and linker microemulsion (10% lido-IPM) to other delivery systems in the literature: a polymer (HPC:HPMC) film (Repka et al., 2005), polymeric nanosphere (Gref et al., 1994), a buccal patch (Burgalassi et al., 1996) and a hydrogel (Glavas-Dodov et al., 2002).

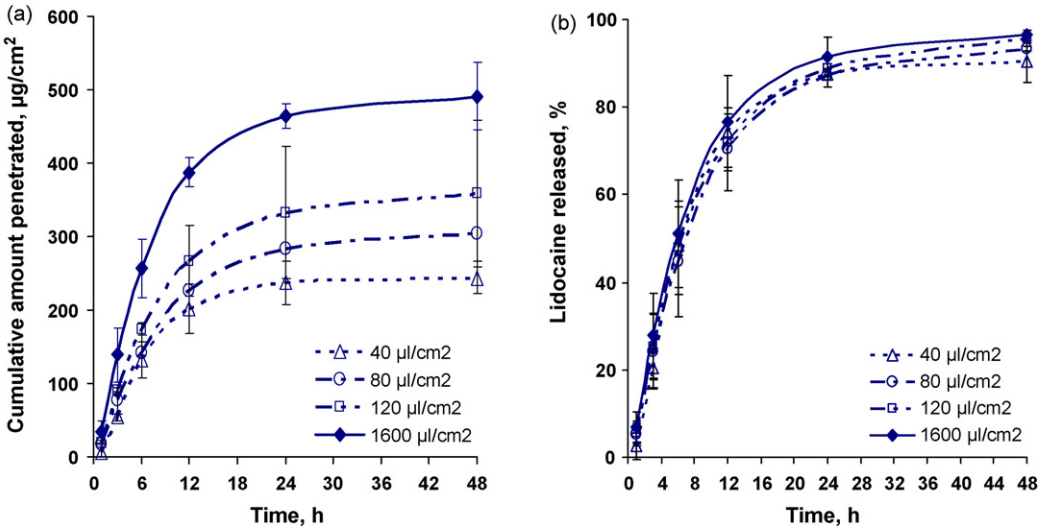

Fig. 6. (a) *In vitro* release profiles of lidocaine and (b) fraction of lidocaine released from the Type II microemulsion formulated with 10% lido-IPM. Different curves present different dosages.

that we also maintained "sink" conditions by periodically replacing the receiver solution with fresh buffer solution.

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

3.1.3. *Effect of application conditions: dosage and application time*

In order to investigate the influence that microemulsion dosage has on extended release, the release profiles of Type II microemulsions formulated with 10% lidocaine in IPM were obtained for dosages of 40, 80 and 120 µl of microemulsion per cm^2 of skin, and placed on the top of the skin for 30 min. We concentrated on Type II microemulsions because they are less toxic than Type I systems (Yuan et al., 2008), and therefore more likely to be used as delivery systems. The results were compared with that for a dosage of 1600 µl/cm^2 (400 µl applied on the MPD mentioned in Section 2.3). The release profiles for different dosages are given in Fig. 6a. According to these cumulative release profiles, the larger the microemulsion dosage, the larger the amount of lidocaine released.

To understand the data in Fig. 6a, it is important to consider the initial lidocaine concentration absorbed in the skin after different dosages. The initial lidocaine concentration in the skin was 11.1 ± 3.2, 16.0 ± 4.0, and 21.4 ± 6.5 mg/ml for the dosage of 40, 80 and 120 µl/cm^2 respectively. Based on this information, the larger the microemulsion dosage, the larger the initial lidocaine concentration in the skin, and the larger the cumulative amount of drug released. The fractional release profile (Fig. 6b) confirms that the fraction of drug release is not a function of the mass of lidocaine initially absorbed in the skin (M_∞), as predicted by Eq. (7).

To explain the effect of microemulsion dosage, one can consider that transdermal delivery with microemulsions resembles the general phenomena of imbibition of liquids into porous media, which is explained by the Washburn equation (Washburn, 1921). According to this equation, a wetting fluid (such as microemulsions) will penetrate a capillary tube as long as there is liquid in the mouth of the

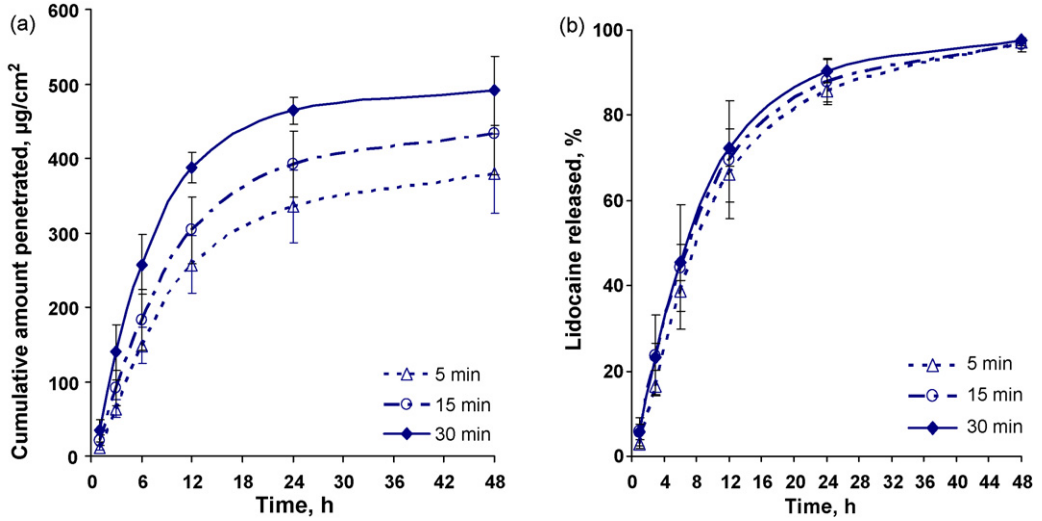

Fig. 7. (a) *In vitro* release profiles lidocaine and (b) fraction of lidocaine released from the Type II microemulsion formulated with 10% lido-IPM. Different curves present different application times.

tube. Larger doses provide a larger reservoir of fluid on the mouth of the pores, thus imbibing more microemulsion into the skin. In reality, dosages of 10–40 μl/cm^2 can be used for topical applications. However, a dosage of 1600 μl/cm^2 is unrealistic because the microemulsion, having the relatively low viscosity of 15 cP and a wetting contact angle on the skin (less than 10°), would quickly spread over the skin. In this work most of the studies were conducted at 1600 μl/cm^2 for the purpose of evaluating effects other than dosage in order to ensure that the pores are filled during the time that the microemulsion is in contact with the skin.

Besides dosage, application time is also a factor that may affect the extended release of lidocaine. To evaluate this effect, the released profile was obtained for skins dosed with 1600 μl/cm^2 of Type II systems (10% lidocaine in IPM) and exposed for 5 and 15 min. Fig. 7a shows the cumulative lidocaine release profiles for 5, 15 and 30 min of application time. As expected, longer application times resulted in more drug permeation through the skin. The drug initially absorbed in the skin also increases from 21.2 ± 4.2 to 24.2 ± 3.3 mg/ml as the application time increases from 5 to 30 min. This observation is consistent with the full differential mass balance equation (Eq. (1)), where the concentration in skin is expected to increase from zero, when the donor is placed on top of the skin, to a steady state value when the flux of lidocaine received from the donor equals the flux of lidocaine released from the skin. According to a previous study (Yuan et al., 2007), it was found that the time for linker-based lecithin microemulsions to reach steady state during continuous permeation is close to 30 min. For application times shorter than 30 min, it is expected that the concentration of the drug in the skin to be lower than the steady state value, which is consistent with the experimental observation. However, even for an application time of 5 min, the amount of lidocaine initially absorbed and later released from the skin is comparable to that obtained with a 30 min application time.

Fig. 7b presents the fraction of drug release as a function of time for different application times. According to this figure, the application time has no effect on the fractional drug released profile, suggesting that the differences observed in Fig. 7a (absolute release) are only due to the differences in the mass of lidocaine loaded into the skin (M_∞), as would be predicted by Eq. (7).

3.2. Microscopic observations

A fluorescent lipophilic dye, Nile red, was used to visualize the absorption and permeation of linker microemulsions into the skin. The Types II and I linker microemulsions formulated with 10% lidocaine in IPM were applied on the skin surface for 30 min and then withdrawn afterwards. For comparison, 10% lidocaine dissolved in IPM only (no microemulsion) was also included. The skin samples were cross-sectioned and viewed by fluorescence microscopy. The micrographs showing the presence of Nile red (red fluorescent dye) in the skin are presented in Fig. 8.

Fig. 8a shows the location of Nile red formulated in IPM after 1 h of release. Fig. 8b and c shows the location of Nile red formulated in the Types II and I microemulsions remaining in the pig skin after 1 h. After 1 h of extended release, all formulations showed Nile red absorption in the superficial level of stratum corneum. In comparison to the IPM system (Fig. 8a), both Type II (Fig. 8b) and Type I (Fig. 8c) linker formulations absorbed more of the hydrophobic fluorescent compound in the superficial layer and more of the dye permeated deeper into skin. This observation is consistent with the larger absorption values (lidocaine skin concentrations) of Table 2 and those obtained in a previous study (Yuan et al., 2008). The Type II microemulsion had the highest deposition of Nile red on the uppermost skin layer because its external phase is oil which contains more hydrophobic Nile red.

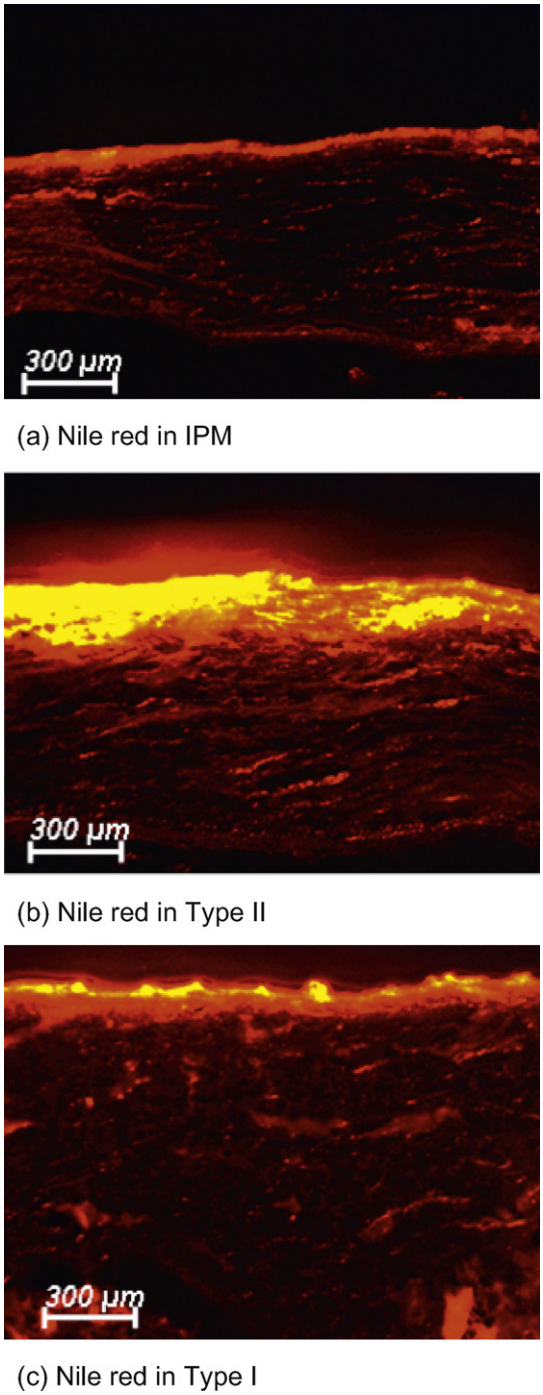

Fig. 8. Penetration of Nile red into pig ear skin from (a) Nile red in IPM after 1 h, (b) Type II linker microemulsion after 1 h, (c) Type I linker microemulsion after 1 h.

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

4. Conclusion

We observed the extended release of lidocaine from the *in situ* skin patches produced with linker-based lecithin microemulsions. After applying the microemulsions for a period of time, lidocaine was absorbed in the skin. The microemulsion imbibed in the skin acted as drug reservoir and provided extended release for over

24 h. The lidocaine release from the microemulsions *in situ* patches follows a first-order kinetics. Increasing the drug loading in the microemulsion, the microemulsion dosage, and the application time increases the drug uptake in the skin, and the cumulative amount of drug release. However, the mass transfer constant (k_{sr}) is not affected by these formulation/application conditions, and depends on the morphology of the microemulsion, and quite likely on the properties of the skin. Microscopic observations illustrated the uptake of a lipophilic dye by the upper layers of the skin.

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

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