Investigation of microemulsion and microemulsion gel formulations for dermal delivery of clotrimazole

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Abstract

Dermal delivery of hydrophobic drugs by microemulsion (ME) formulations and effect from ME microstructures were studied. Anti-fungal drug, clotrimazole (CLOT), was used as the model compound. ME formulations possessing different microstructures were prepared using a ME system that contains isopropyl myristate as oil, Labrasol and Cremophor EL as surfactant and co-surfactant, and water. Permeation experiments on human cadaver skin were conducted for ME and the control formulations of different CLOT concentrations. Dermal delivery of CLOT assessed by the dermal tissue drug concentration was found to be significantly higher for MEs when compared with the control formulation, evidenced by dermal retention Enhancement Ratio (ERd) of 5.1, 2.8, and 3.0 for tested O/W, bi-continuous, and W/O MEs, respectively. The highest concentration was observed with O/W ME, suggesting the ME microstructure is an important formulation variable for enhancing dermal delivery efficiency. ME gel formulations prepared by incorporating 1.0%(w/w) of Carbopol980 showed comparable dermal CLOT concentration to MEs, but up to 2.4 fold higher than the commercial CLOT cream product, Lotrimin®. Furthermore, Fluorescein Isothiocyanate (FITC), used as a model compound for highly hydrophobic drugs, was also studied for dermal delivery by MEs, and results show consistent ME microstructure effect, suggested by significantly higher FITC concentrations in all skin layers, SC, viable epidermis, and dermis, from O/W ME over bi-continuous and W/O MEs.

1. Introduction

Microemulsions (MEs) are single optically isotropic and thermodynamically stable dispersions of oil, water, and surfactants with droplet sizes in the submicron range (Lee et al., 2003). As formulation vehicles, they offer high drug solubilizing capacity, long term stability, ease of preparation, and capability to enhance skin permeation for hydrophobic and hydrophilic drugs (Heuschkel et al., 2008; Kreilgaard, 2001). For more than two decades, extensive studies have been conducted on MEs for transdermal and dermal drug delivery, although the drug skin permeation enhancement effect from MEs has been well established, the mechanism is still not completely elucidated. One main reason is that a microemulsion is a complex system that has many variables. Recent research provided more insights, from the point of view of understanding mechanisms, they can be categorized into the following: (a) formulation constituent/excipient permeation enhancer effect: oil is a critical excipient that impacts drug solubility and skin permeation (Lopes et al., 2010; Montenegro et al., 2011; Pepe et al., 2012; Ren et al., 2014; Thomas et al., 2014) and so are the surfactant and co-surfactant (Hoppel et al., 2015; Montenegro et al., 2011; Todosićević et al., 2015). Additional excipients can be formulated in order to improve drug solubility and skin permeation, e.g. β-cyclodextrin (Mura et al., 2014), and cholesterol (Schmalfub et al., 1997); (b) ME composition and its optimization: Response-Surface Model (RSM) in conjunction with experimental design has been increasingly used by different research groups for ME formulation optimization (Ge et al., 2014; Tsai et al., 2015; Zhao et al., 2015). Results from composition studies generally support the observation that for hydrophobic drugs, increasing water or oil content and decreasing surfactant content help to achieve higher drug skin permeation (Bhattia et al., 2013; Ge et al., 2014; Hoppel et al., 2014, 2015; Kreilgaard, 2001; Tsai et al., 2015; Zhang and Michniak-Kohn, 2011); (c) microstructure influence: ME droplet shape or size may have effects on drug permeation enhancement (Mahrhauser et al., 2015; Sintov, 2015). Model drug skin permeation study results have been correlated with ME microstructures in several publications (Cavalcanti et al., 2016; Sintov and Greenberg, 2014; Zhang and Michniak-Kohn, 2011); and (d) ME-gel formulations: many studies were conducted on ME-gel formulations in order to explore transdermal or dermal product development (Foudad et al., 2013; Ge et al., 2014; Wan et al., 2015; Zhu et al., 2009; Valenta and Schultz, 2015).
2. Materials and methods

2.1. Materials

Clotrimazole, Fluorescein Isothiocyanate (FITC), dioxane, and pro-pylene carbonate (PC) were all purchased from Sigma-Aldrich, St. Louis, MO, USA. Labrasol was a sample provided by Gattefosse. Isopropyl myristate (IPM), Cremophor EL, propylene glycol (PG), triethanolamine (TEA), methanol, acetonitrile, water, and PBS tablets were purchased from Fisher Scientific, Waltham, MA, USA. Carbopol980 was purchased from Lubrizol, Wickliffe, OH, USA. Lotrimin® was purchased as the gelling agent at 1.0% (w/w). Model drugs, CLOT or FITC, were dissolved in the mixture of IPM/(Labrasol/Cremophor EL, 4/1 (w/w)) with the aid of sonication, then, the right amount of water was added in based on the corresponding water content and mixed by vortex shaking to obtain the formulation which was an isotropic and transparent solution. Table 1 lists formulations used in the study, their labels, compositions and corresponding micro-structures.

2.2. Preparation of ME formulations

A microemulsion system used was developed, and its ternary phase diagram construction and microstructure characterization were reported in the previous study (Zhang and Michniak-Kohn, 2011). Briefly, the system used IPM as oil phase, Labrasol (caprylic/capric polyglycerol-8 glycerides) and Cremophor EL (polyethylene-35 castor oil) as surfactant and co-surfactant, and water as aqueous phase. ME formulations were prepared along the water dilution line of Oil/(Surfactant and co-surfactant) 1/9 (w/w). Model drugs, CLOT or FITC, were dissolved in the mixture of IPM/(Labrasol/Cremophor EL, 4/1 (w/w)) with the aid of sonication, then, the right amount of water was added in based on the corresponding water content and mixed by vortex shaking to obtain the formulation which was an isotropic and transparent solution. Table 1 lists formulations used in the study, their labels, compositions and corresponding micro-structures.

2.3. Preparation of ME gel formulations

ME-gel formulations were prepared based on ME_5/5 and ME_65/35. Carbopol980 was used as the gelling agent at 1.0% (w/w). The preparation followed partially the method reported by Zhu et al. (2009). The drug loaded ME formulation was first prepared, 1.0% (w/w) of Carbopol980 was added and allowed to swell overnight at room temperature, then, the formulation was gelled by adjusting its pH to 6.8 ± 0.2 with the addition of TEA.

2.4. Measurement of CLOT solubility in formulation excipients and various vehicles

CLOT was weighed into a 4-ml glass vial, then, 2 ml of the tested excipient or vehicle was added in obtaining a suspension. The sample vial was put in a shaker and agitated under constant speed of 600 rpm at 37.0 ± 0.1°C overnight. One mL of the sample was pipetted into a micro-centrifuge tube and this was centrifuged at 14,000 rpm at 37 °C for 10 min. One hundred μL of the supernatant was taken and diluted using methanol appropriately, and then placed in a HPLC vial. The prepared sample was analyzed using HPLC to determine solubility of CLOT.

2.5. Skin permeation study

2.5.1. Skin permeation experiment

Dermatomed human cadaver skin was defrosted, cut into 14 × 14 mm pieces, and soaked in PBS buffer to hydrate for 15 min before permeation experiments. Skin pieces from two human donors were used in the study, which were harvest from their body backs. The donors are male Hispanic and White, aged 47 and 50, and death due to atherosclerotic corona and hypertension, respectively. For the study comparing ME formulations of the same CLOT load, skin samples from the same human donor were used in order to reduce data variability. Permeation experiments were conducted using Franz diffusion cells (Permegear, Hellertown, USA), which had a donor diffusion area of 0.64 cm² and receptor volume of 5.1 mL. In the permeation experiment, the skin samples were sandwiched between the donor and the receptor with the stratum corneum layer facing the donor, 0.15 mL of solution formulation or 0.3 mL of gel/cream formulation was added into the donor, and this was occluded using Parafilm®. The receptor was filled with medium under constant stirring, and was kept at 37.0 ± 0.2°C by circulating water bath jacket. PBS buffer/Dioxane, 9/1 (v/v) was used as the receptor medium, which provided sink conditions for permeation of CLOT (Ning et al., 2005). Skin permeation experiments for all formulations were run for 24 h unless otherwise specified. Replicates 3–6 were conducted for each formulation.

2.5.2. Permeation analytical sample preparation

At the end of the permeation experiment, three different types of analytical samples were prepared for HPLC assay to assess CLOT dermal retention and transdermal permeation: (1) Dermal samples, (2) Stratum Corneum Epidermis (SCE) samples, and (3) transdermal permeation samples. One mL of the receptor medium was filled into a HPLC vial which served as the transdermal permeation sample. The skin sample was wiped clean (twelve times using Kimwipes®), and the skin that had been treated with the formulation was cut out using a 10 mm biopsy punch. This sample was then subjected to 60°C water treatment for 60 s, and peeled to separate SCE and dermal layers according to an established method (Puglia et al., 2001). The SCE sample was placed in a 50-mL plastic centrifuge tube and 10 mL of methanol was added to extract the sample by vortex shaking for 2 min and sonicating in a water bath for 1 h. The sample was then filtered through a 0.22 μm PTFE syringe filter (Whatman) and filled into a HPLC vial, which served as SCE sample. The weight of dermal sample was recorded and it was then cut into 12–14 pieces and placed in a plastic homogenization tube which contained metal milling beads. Then 1 mL of methanol was added and the sample was homogenized at speed of 600 rpm for 15 min. Following this treatment, 0.5 mL of the methanol solution was taken and filtered using a 0.2μm syringe-less filter device (Mini-UniPrepTM, polypropylene filter medium, Whatman), and this then served as the Dermal sample. HPLC analyses of these samples were conducted in order to determine CLOT concentrations.
2.6. HPLC analytical method

Clotrimazole concentrations were assayed using a HPLC method. An Agilent Zorbax Eclipse XDB phenyl column 4.6 × 250 mm and a mobile phase of 0.005% H₃PO₄/acetonitrile, 45/55 (v/v), were used. The method was operated under the following conditions: column at ambient temperature, flow rate at 1 mL/min, UV detection at 210 nm, injection volume of 10 μL, and run time of 8 min. The method was validated for reproducibility and linearity with a dynamic linear range from 0.1 μg/mL to 100 μg/mL. Skin permeation sample concentrations were determined by the CLOT peak area calibrated by bracketing external CLOT standards.

2.7. FITC skin deposition study by fluorescent microscopy

FITC ME formulation skin penetration experiments were conducted using porcine skin, which was harvested by dermoming the skin to a thickness of 800 μm, using porcine skin, which was harvested by dermoming the skin to a thickness of 800 μm, using porcine skin, which was harvested by dermoming the skin to a thickness of 800 μm, using porcine skin, which was harvested by dermoming the skin to a thickness of 800 μm, using porcine skin, which was harvested by dermoming the skin to a thickness of 800 μm. The skin samples were stored at −80 °C until use. The experiment was similar to the human skin permeation experiment described in Section 2.5.1. Replicates of three Franz cell tests for each formulation were conducted. Receptor medium was PBS buffer, 0.15 mL of ME formulations containing 0.1% (w/w) FITC was added into the donor, and skin samples were treated for 12 h, then were cleaned from excess formulation, rinsed under running DI H₂O for 10 s, then blotted dried using a paper towel. The skin samples were frozen in OCT medium on a sample holder placed on dry ice, and then sectioned to obtain the cross-sectional slices of 8 μm thickness at −22 °C using a cryostat microtome (LeicaCryoStat, CM3050S, Wetzlar, Germany). The skin slices were collected on glass slides and were examined by fluorescent microscopy under GFP light (Zeiss Fluorescence microscope with AxioCamMRmCmcamera). Fluorescent intensity of different skin layers on images were quantitatively analyzed by Image-J software, which were correlated to FITC deposition concentrations in the SC, viable Epidermis (vE),, and Dermal (D) layers. Small integration boxes of 400–600 squared pixel areas were drawn in the different skin layers for fluorescent intensity quantification, box locations were at the skin depth (counted from SC surface down into the dermis) of 0–20 μm, 70–100 μm, and 200–250 μm for SC, vE, and D layers, respectively. Results were reported as (means ± S.D.) that were calculated based on n = 30 readings from 6 skin slices of 3 Franz cell treated skin samples for each formulation.

2.8. Statistical analysis of data

Skin permeation experiments for each formulation were conducted in three to six replicates. Results were expressed as means ± standard deviation (S.D.). Student t-tests were employed to assess differences between mean values of formulations, and the statistically significant differences were defined as p < 0.05.

3. Results and discussion

3.1. CLOT solubility in formulation excipients and vehicles

CLOT solubility data at 37 °C in formulation excipients and various vehicles are presented in Table 2. As expected, CLOT solubility is high in surfactant/co-surfactant mixture, Labrasol/Cremophor EL (73.8 mg/mL), moderate in oil, IPM (9.3 mg/mL), and extremely low in aqueous PBS (5 × 10⁻⁵ mg/mL). In ME vehicles, CLOT solubility decreased approximately linearly when the water content increased from 20% to 70% (w/w) as shown in Fig. 1. It can be derived that at the same CLOT load, if the water content increases in MEs in the same range, resulting in ME microstructure changes from W/O to bi-continuous, then to O/W, the thermodynamic activity of CLOT in ME formulations will increase accordingly due to decreasing CLOT solubility in the ME vehicle. It is necessary for a ME vehicle to have solubility capacity higher than 10 mg/mL in order to prepare a formulation of 1.0% (w/w) of CLOT load, therefore, ME_65/35 of O/W microstructure was used to prepare 1.0% (w/w) of CLOT formulation instead of ME_7/3 due to its lower solubility capacity.

PBS/Dioxane (9/1, v/v) was used as the receptor fluid in the CLOT permeation study. CLOT solubility in the fluid was found to be 7.1 μg/mL which meets the sink condition due to overall CLOT concentration

<table>
<thead>
<tr>
<th>Table 2</th>
<th>CLOT solubility at 37 °C in formulation excipients and vehicles.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation Excipients</td>
<td>Solubility (mg/mL)</td>
</tr>
<tr>
<td>Labrasol/Cremophor EL (4/1, w/w)</td>
<td>73.8</td>
</tr>
<tr>
<td>IPM/(S + CoS) (1/9, w/w)</td>
<td>69.9</td>
</tr>
<tr>
<td>IPM (oil)</td>
<td>9.3</td>
</tr>
<tr>
<td>Propylene Carbonate (PC)</td>
<td>39.7</td>
</tr>
<tr>
<td>PBS</td>
<td>5.0 × 10⁻⁵</td>
</tr>
<tr>
<td>PBS/Dioxane (9/1, v/v)</td>
<td>7.1 × 10⁻⁵</td>
</tr>
</tbody>
</table>

Note: IPM = isopropyl myristate; PBS = phosphate buffer saline; S = surfactant; CoS = co-surfactant.
3.2. Skin permeation tests for MEs containing 0.5% (w/w) of CLOT

Human cadaver skin permeation test results for ME formulations containing 0.5% (w/w) of CLOT are presented in Table 3 and Fig. 2. CLOT dermal delivery can be directly observed based on CLOT concentrations in skin SCE and dermal layers. In this study, dermal layer CLOT concentration is used as the index for evaluating CLOT dermal delivery efficiency. As can be seen, dermal CLOT concentration from ME_7/3 is significantly higher than other MEs (\( p < 0.05 \)), being 2.0, 2.2, and 1.8 fold of that of ME_2/8, ME_4/6, and ME_5/5, respectively. ME_7/3 of O/W microstructure provided the highest CLOT dermal delivery with a moderate increase when compared to ME_2/8, ME_4/6, and ME_5/5, which were of W/O, bi-continuous, and bi-continuous microstructures, respectively, and were not significantly different in CLOT dermal delivery. Therefore, O/W type ME shows to be superior to W/O and bi-continuous MEs in CLOT dermal delivery.

CLOT transdermal delivery was assessed by CLOT cumulative permeated amount over 24 h, \( Q_{24h} \), in the study. The \( Q_{24h} \) value for ME_7/3 is significantly higher than other MEs (\( p < 0.05 \)), being 21.4, 2.7, and 1.7 fold of that of ME_2/8, ME_4/6, and ME_5/5, respectively. These results of significantly increased CLOT transdermal permeation from MEs of increasing water content are expected, and are in concordance with literature reports on many other drugs in ME transdermal studies (Hoppel et al., 2014; Lee et al., 2003; Zhang and Michniak-Kohn, 2011).

3.3. Skin permeation tests for ME and ME gel formulations containing 1.0% (w/w) of CLOT

Human cadaver skin permeation test results for ME, ME-gel formulations, the control formulation, and Lotrimin® cream that all contained 1.0% (w/w) of CLOT, are presented in Table 4 and Fig. 3.

CLOT 1.0% (w/w) solution in Propylene Glycol (PG) was first chosen as the control formulation in the study, the results (not reported here) showed that PG had noticeable dermal delivery enhancement effect for CLOT and was not suitable for being a control. Then, CLOT 1.0% (w/w) solution in Propylene Carbonate (PC) was chosen as the control formulation, because PC provided high CLOT solubility, and had been used previously as the control vehicle in transdermal study (Sintov, 2015).

Fig. 3 shows dermal and SCE CLOT concentrations from ME formulations. They are higher than the control formulation. ME_65/35 with O/W microstructure showed the highest dermal and SCE CLOT concentrations and a dermal delivery Enhancement Ratio (ER_D) of 5.1. While dermal CLOT concentrations and ER_D from W/O ME_2/8 and bi-continuous ME_5/5 are not significantly different from each other (ER_D being 3.0 and 2.8, respectively). When CLOT load in MEs increased from 0.5% to 1.0% (w/w), the differences in thermodynamic activity of CLOT in MEs of increased water content increased, however, the overall results of 1.0% (w/w) MEs show closely comparable extent of change to those from 0.5% (w/w) MEs in term of dermal and SCE drug concentrations when water content increased, suggesting that CLOT thermodynamic activity is not the main cause for observed change in dermal and SCE drug concentrations when water content increased from 20% to 65%, and rather microstructure change is the cause. In other words, ME microstructure has a significant impact on CLOT dermal delivery, and O/W ME enables higher CLOT skin retention than W/O and bi-continuous MEs (\( p < 0.05 \)). The observed phenomena may potentially be explained based on two facts: (a) O/W ME’s higher water content results in a higher degree of skin hydration which in turn increases CLOT skin penetration (Hoppel et al., 2014); and (b) CLOT, which is located at interface with surfactant/co-surfactant, will have higher mobility in O/W ME vehicle (Fanun, 2009).

For transdermal delivery as assessed by CLOT cumulative permeated amount, \( Q_{24h} \), the values for MEs were significantly higher than control (\( p < 0.05 \)), and increased significantly with water content. Data of CLOT dermal concentrations and transdermal \( Q_{24h} \) from this study are in good agreement with the previous literature report on CLOT human skin permeation and its dermal CLOT concentration is significantly lower than ME_5/5 (\( p < 0.05 \)). The result suggests that oil is a critical component in ME for drug skin retention enhancement, and demonstrates the superiority of ME over a micelle vehicle for dermal delivery. The results are in concordance with literature reports on such comparisons (Sintov, 2015; Sintov and Greenberg, 2014). One possible explanation is that oil lowers surface tension between the ME and SC, and allows better contact and penetration into SC by surfactants and the associated drug.

Additionally, Micelle_5/5 formulation that contained only surfactant/co-surfactant and water, but no oil, was also tested for CLOT human skin permeation and its dermal CLOT concentration is significantly lower than ME_5/5 (\( p < 0.05 \)). The result suggests that oil is a critical component in ME for drug skin retention enhancement, and demonstrates the superiority of ME over a micelle vehicle for dermal delivery. The results are in concordance with literature reports on such comparisons (Sintov, 2015; Sintov and Greenberg, 2014). One possible explanation is that oil lowers surface tension between the ME and SC, and allows better contact and penetration into SC by surfactants and the associated drug.

Furthermore, dermal CLOT concentrations of O/W ME_65/35.gel and bi-continuous ME_5/5.gel show to be equivalent to corresponding MEs, but are significantly higher compared with CLOT commercial cream, Lotrimin®. Their \( E_{TD} \) increased to 2.4 and 1.5 fold that of Lotrimin®, respectively. The results indicate that ME-gel formulations, which possess additional advantage of easier application vs. MEs, have the potential of providing improved dermal delivery over a conventional cream formulation. The results on ME-gels from this study are in concordance with reports of numerous previous studies that investigated ME-gel formulations for dermal delivery of various drugs.
3.4. FITC dermal deposition from ME formulations in porcine skin permeation study

FITC is a hydrophobic fluorescent dye which has been used as a probing compound in skin penetration studies previously (Lopes et al., 2010). ME formulations possessing different microstructures containing 0.1% (w/w) FITC were used to treat porcine skin samples. The treated samples were then examined under a fluorescent microscope for FITC skin deposition profiles in different skin layers, namely the stratum corneum (SC), viable epidermis (vE), and dermis (D). FITC dermal deposition concentrations are estimated based on fluorescent intensity in skin layers which were quantitatively analyzed by Image-J software. The results are summarized in Table 5 and plotted in Fig. 4.

Table 4  
Skin permeation study results of ME and control formulations containing 1.0% (w/w) of CLOT.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Dermal CLOT Conc. (mg/cm²)</th>
<th>SCE CLOT Conc. (mg/cm²)</th>
<th>Q_{24h} (μg/cm²)</th>
<th>Dermal ER_{D}</th>
<th>Transdermal ER_{TD}</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>0.022 ± 0.035</td>
<td>1.22 ± 0.69</td>
<td>0.07 ± 0.16</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>ME_2/8</td>
<td>0.065 ± 0.018</td>
<td>1.71 ± 0.56</td>
<td>0.50 ± 0.34</td>
<td>3.0</td>
<td>7.1</td>
</tr>
<tr>
<td>ME_5/5</td>
<td>0.062 ± 0.007</td>
<td>2.61 ± 0.77</td>
<td>0.82 ± 0.61</td>
<td>2.8</td>
<td>11.7</td>
</tr>
<tr>
<td>ME_65/35</td>
<td>0.112 ± 0.022</td>
<td>4.53 ± 1.90</td>
<td>1.43 ± 0.30</td>
<td>5.1</td>
<td>20.4</td>
</tr>
<tr>
<td>Micelle_5/5</td>
<td>0.037 ± 0.010</td>
<td>1.71 ± 0.68</td>
<td>0.77 ± 0.30</td>
<td>1.7</td>
<td>11.0</td>
</tr>
<tr>
<td>Lotrimin®</td>
<td>0.046 ± 0.024</td>
<td>1.57 ± 1.07</td>
<td>0.70 ± 0.58</td>
<td>2.1</td>
<td>10.0</td>
</tr>
<tr>
<td>ME_5/5_gel</td>
<td>0.070 ± 0.007</td>
<td>1.96 ± 0.77</td>
<td>1.04 ± 0.82</td>
<td>3.2</td>
<td>14.9</td>
</tr>
<tr>
<td>ME_65/35_gel</td>
<td>0.112 ± 0.055</td>
<td>2.47 ± 0.90</td>
<td>1.36 ± 0.80</td>
<td>5.1</td>
<td>19.4</td>
</tr>
</tbody>
</table>

Data reported as means ± S.D.; n = 3–6.

Q_{24h}: CLOT cumulative permeated amount over 24 h.

ER_{D} stands for “dermal delivery Enhancement Ratio”, and is calculated as ((Dermal Conc. of the formulation) / (Dermal conc. of the control)).

ER_{TD} stands for “transdermal delivery Enhancement Ratio”, and is calculated as ((Q_{24h} of the formulation) / (Q_{24h} of the control)).
Representative fluorescent microscopic images of skin samples treated by different formulations are showed in Fig. 5.

Fig. 4 illustrated that O/W ME formulation, ME_7/3_FITC-0.1%, provided significantly higher FITC concentrations (fluorescence intensity, p < 0.05) in all skin layers, SC, vE, and D than bi-continuous ME_4/6 and W/O ME_2/8. The results are consistent with those observed on CLOT dermal delivery in previous sections, which indicated ME microstructure plays an important role in ME dermal delivery of hydrophobic drugs, and O/W MEs enable higher dermal delivery efficiency over bi-continuous and W/O MEs when all other formulation variables are kept the same.

Fluorescent image of the skin sample that was treated by blank PBS is shown in Fig. 3.

Table 5
FITC skin deposition from ME formulations quantified by fluorescent intensity using Image-J software.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>FITC Fluorescent Intensity (ABU)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SC</td>
</tr>
<tr>
<td>ME_2/8_FITC-0.1%</td>
<td>26.2 ± 14.5</td>
</tr>
<tr>
<td>ME_4/6_FITC-0.1%</td>
<td>28.9 ± 17.3</td>
</tr>
<tr>
<td>ME_7/3_FITC-0.1%</td>
<td>55.8 ± 18.3</td>
</tr>
</tbody>
</table>

n = 30 for each formulations. Data reported as means ± S.D.
SC = stratum corneum; vE = viable epidermis; D = Dermis.
* Intensity was measured by the integration of pixel brightness (arbitrary units, ABU) using Image J software.
in Fig. 5e shows only skin auto-fluorescence. The image of the same skin sample by regular microscope (under bright field and the identical magnification) in Fig. 5f illustrates normal skin structures. Image of the skin sample treated using Propylene Glycol (PG) solution containing 0.1% FITC in Fig. 5a shows FITC mainly penetrated into and stayed at SC layer. Images of the skin samples treated by W/O ME_2/8_FITC-0.1% and bi-continuous ME_4/6_FITC-0.1% in Fig. 5b and c, respectively, show some level of deeper penetration into SC and deposition at SC layer with slightly increased depth. Finally, image of the skin sample treated by O/W ME_7/3_FITC-0.1% in Fig. 5d demonstrates much deeper FITC penetration through SC, and into the viable epidermis and the dermis layers, as evidenced by relatively strong fluorescent light in wide area of the full thickness skin sample.

4. Conclusions

The dermal delivery of the hydrophobic drug CLOT using an alcohol-free ME formulation system and the influence of the ME microstructure were studied. Results show significant CLOT skin retention enhancement by MEs when compared with the control formulation. The dermal delivery is affected by microstructures of MEs, suggested by significantly higher CLOT deposition in dermis by O/W ME vs. W/O and bi-continuous MEs. Relatively high water content in a formulation is critical in order to form O/W microstructure and to achieve the higher dermal delivery. However, enhancement of dermal delivery as water content increase is moderate when compared with that of transdermal delivery. For example, ER_D and ER_TD for CLOT delivered by O/W ME_65/35 are 5.1 and 20.4, respectively. Therefore, the results suggest that for dermal delivery using ME formulations, it is necessary to consider microstructure influence on drug percutaneous retention and also to find a balance between percutaneous retention and transdermal permeation, in order to achieve the best therapeutic outcome.

Additionally, micellar formulations at the same water content showed lower dermal drug concentrations than the corresponding ME formulation, suggesting the superiority of ME vs. micellar vehicle for drug dermal delivery. Furthermore, ME-gel formulations based on O/W and bi-continuous MEs, which improved the formulation applicability, enabled significantly higher dermal drug concentration than the commercial CLOT cream, Lotrimin®. Results of the study suggest ME and ME-gel formulations are potentially useful for dermal drug delivery product development.

Finally, FITC skin penetration study by MEs showed consistent results as CLOT study. O/W ME provided significantly higher FITC deposition in porcine skin layers of SC, viable epidermis, and dermis than bi-continuous and W/O MEs. Overall, the present study highlight that ME microstructure is a critical variable in ME dermal delivery of hydrophobic drugs for skin retention enhancement. The results provide further insight toward understanding of ME permeation enhancement mechanism and also offer meaningful guiding information for ME formulation development in dermal drug delivery.
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