A mechanistic study on the effect of ethanol and importance of water on permeation of drugs through human third-degree burn eschar

Azadeh Ghaffari, Hamid R Moghimi, Ali Manafi, Habibolah Hosseini

ABSTRACT
Ethanol that affects hydration of skin and used in wound treatment formulations was studied here for its effect on permeation of drugs through burn eschar and to investigate the presence of a porous pathway in this barrier. In this study, permeations of clindamycin phosphate (CP, hydrophilic) and diazepam (lipophilic) through human burn eschar were investigated in the presence and absence of ethanol. Permeability coefficients (Kp) of CP and diazepam through hydrated eschar were calculated to be $13.1 \times 10^{-3}$ and $17.4 \times 10^{-3}$ cm/h respectively. These Kp values were decreased by about 1.5–5.3 and 2–10.7 times respectively upon the addition of 20–70% ethanol. Increased amount of ethanol decreased permeation flux of CP (2–20 times) and increased that of diazepam (3–80 times) from saturated solutions. Thermal analysis showed that ethanol dehydrates eschar and also changes its internal proteineous structure. Such changes were concluded to be the main reasons behind decreased Kp of both drugs. Comparison of Kp data suggests the possibility and importance of a pore pathway in permeation of both drugs through the hydrated burn eschar. Present results show that ethanol, and possibly other dehydrating agents, can decrease the permeability of eschar and that this effect should be considered in formulation developments.

Key words: Burn eschar • Drug permeation • Ethanol • Hydration • Thermal analysis

INTRODUCTION
Thermal destruction of the skin barrier and subsequent formation of avascular necrotic tissue (burn eschar), that is suitable environment for microbial colonization and proliferation, can potentially cause morbidity and mortality in patients with burn (1). To prevent sepsis, one of the important strategies is to control burn-wound infection, and for burn-wound infection control topical usage of antibiotics is very crucial. The ability of antibiotics to penetrate
in and beneath the burn eschar, where the microorganisms may proliferate and invade next tissue, is very important in prevention of bacterial or fungal invasions (1,2).

Previous studies have shown that most of antibiotics used in patients with burn could not penetrate the burn eschar in therapeutic amounts when applied topically (1–3). To solve this problem, different penetration enhancers, such as terpenes and surfactants, have been used over the past years to improve permeation of drugs through eschar barrier (3–6). Also, previous studies have shown that hydration state affects drug permeation through burn eschar to a great extent (4,5), the mechanism of which is not well understood yet. Such enhancement methods or conditions had been reported for intact healthy skin before, but the permeation data of normal skin cannot be extrapolated to burn eschar because of prominent level of structural differences between the normal and thermally damaged skin (7,8).

On the other hand, wound is normally subjected to different conditions that might affect its permeation behaviour. For example, it is clear that hydration level of eschar depends on wound-maintenance approach-like hydrotherapy. This technique has been widely used for many years to clean wounds, wash off exudates, facilitate dressing change, wash off topical formulation, debride wounds and facilitate physiotherapy or occupational therapy to make the patients feel better or pain modification, and is performed either by immersion tank or shower sprayer of water with or without antimicrobial solutions (9). Other potential effective conditions are wound dressings and type and content of drug formulations, of which one is ethanol-containing systems.

Ethanol is used as a solvent or cosolvent in different formulations and extracts that are used in wound treatment (10–15). It has been shown that ethanol and other alcohols, like glycerin, dehydrate normal skin and that this phenomenon can affect percutaneous absorption of different drugs (16–18). It has also been shown that in normal skin the effect of hydration depends on different physicochemical properties of the penetrant such as partition coefficient and aqueous solubility (19).

In this study, to investigate the effect of ethanol on permeation of both hydrophilic and lipophilic drugs and also to study the importance of pores as a potential pathway for permeation of drugs through burn eschar, the effect of different concentrations of ethanol (10–70%, v/v) on eschar permeation of clindamycin phosphate (molecular weight; MW = 504.9), as a model hydrophilic drug, and diazepam (MW = 284.7), as a model lipophilic penetrant, was investigated at constant thermodynamic activity (saturated drug concentration). Clindamycin phosphate and diazepam show the log octanol–water partition coefficients of 0.5 and 2.7 respectively (20,21). The effect of ethanol on hydration level and structure of eschar was also investigated by thermal analysis.

To the best of our knowledge, there is no such data available in the literature.

MATERIALS AND METHODS

Chemicals

Clindamycin phosphate was purchased from Suzhou Pharmaceutical Factory (Suzhou, China). Diazepam was supplied by Changzhou Siyao Pharmaceutical Company (Changzhou, China). Ethanol was purchased from Bidestan (Tehran, Iran). Monobasic potassium phosphate (96%), phosphoric acid (85%) and high performance liquid chromatography (HPLC)-grade acetonitrile were purchased from Merck (Germany).

Eschar samples

Third-degree burn eschar samples, which were separated at the time of surgical debridement (1–2 weeks post-burn) from burned patients, were obtained from Motahari Burn Center (Tehran, Iran). The cause of burning in all patients was flame. Eschar samples were from 10 patients, 6 men and 4 women (32 ± 15 years, mean ± SD). Large pieces of eschar tissues were stored at −20°C until use; not later than 12 months. There is no published data available regarding the effect of freezing on the barrier properties of burn eschar. However, it has been shown that storage of normal human skin at −20°C, even up to 15 months, does not change its permeability to water (22). Besides, preliminary comparison of our data collected over last 2–3 years show that the length of storage at −20°C does not affect the barrier properties of burn eschar.
Permeation studies
For permeation studies, the large pieces of burn eschar were thawed at ambient temperature and cut into appropriate smaller pieces. The eschar samples were fully hydrated by water by placing the samples in water for 12 h at ambient temperature. Permeation studies were performed by home-made diffusion cells with effective surface area of approximately 1.8 cm². Eschar samples were placed between donor and receptor chambers of the cells while the epidermal side faced the donor compartment. Three milliliters of saturated solutions of clindamycin phosphate or diazepam in either water or ethanol/water solutions (10, 20, 50 and 70%) (v/v) were placed in donor chambers. The same solvents (25 ml) were used as the receptor phases.

The cells were then placed in a thermostatically controlled water bath with stirrer. The temperature was kept at (37°C ± 0.5) in the receptor chamber that gives a temperature of approximately 32°C at the surface of the eschar. The speed of stirring in the receptor chamber was 300 rpm.

Serial samples were collected from the receptor chamber for 24 h and their drug contents were analysed by HPLC. Sink condition was maintained throughout the experiments. The cumulative amount of permeated drug was plotted against time and the slope of the linear portion of the plot was measured as the steady-state flux ($J$). Permeability coefficient ($K_p$) was then calculated using $J$ and donor-drug concentration ($C$) using Fick’s law ($K_p = J/C$).

Flux and permeability coefficient were statistically compared using one-way ANOVA followed by the least significant difference (LSD). The level of significance was set at $P < 0.05$. The statistical analysis was computed with the SPSS software version 17.0 (SPSS Inc., Chicago, IL).

HPLC analysis
HPLC analysis of clindamycin phosphate
Clindamycin phosphate, the hydrophilic model drug, was measured by a HPLC method suggested by the United States Pharmacopeia (USP) (23). Samples were analysed by HPLC apparatus (Merck, Germany), using a 25 cm × 4.6 mm RP-18 column with 3 μm particle size (Perfectsil Target ODS-3, MZ-Analysetechnik). The mobile phase was acetonitrile and pH 2.5 phosphate buffer (22.5:77.5, v/v). The flow-rate was 1 ml/min, and clindamycin phosphate was detected using a UV detector at a wavelength of 210 nm (23). The results showed a linear relationship ($r^2 = 0.995$) between area under the curve and the concentration of clindamycin phosphate in the range of 0.01–10 mg/ml. Recovery percentage and inter-day and intra-day studies showed good accuracy and repeatability of this method.

HPLC analysis of diazepam
Diazepam, the lipophilic model drug, was measured by a modified USP method (23), developed for the current investigation. The HPLC apparatus and column were as described above for clindamycin phosphate. Acetonitrile and water mixture (6:4, v/v) was used as eluent. The flow-rate was 1 ml/min. Detection was performed using a UV detector at 254 nm. The results exhibited good linear relationship ($r^2 = 0.997$) between area under the curve and the concentration of diazepam over a concentration range of 0.1–10 µg/ml. This method showed good inter-day and intra-day repeatability and recovery percentage.

Protein precipitation
As the eschar samples release traces of proteins in the receptor phase, it was necessary to precipitate these proteins before injection to HPLC. Acidic protein precipitation was performed for clindamycin phosphate samples by adding 20 µl of perchloric acid 60% (v/v) to 0.5 ml of sample followed by vortexing for 5 min and centrifugation at 9000 × g for 10 min. The supernatant was then used for drug analysis. As the control, ethanolic clindamycin phosphate solution was diluted with water and then acidic precipitation was performed as mentioned above. Protein precipitation of diazepam samples were carried out using acetonitrile. Acetonitrile was added 1.5 times of the sample volume and were then vortexed and centrifugated as above. The supernatant was then analysed by HPLC.

Method validation
To evaluate possible interaction of the material released from the eschar with the assay methods, control permeation experiment (using donor phases without drugs) were performed as explained above and the corresponding receptor phases were analysed by HPLC using...
both diazepam and clindamycin phosphate assay methods. The obtained chromatograms did not show any peak at the place where drugs appear.

Also the samples taken from the receptor phases of the control experiments were spiked with clindamycin phosphate and diazepam standard solutions at 0.1 mg/ml and 0.4 μg/ml respectively. These solutions were then treated for protein precipitation as mentioned before and recoveries of the drugs were measured afterwards. The recoveries of clindamycin phosphate and diazepam were calculated to be 93–98% (n = 9) and 93–99% (n = 9) respectively.

**Solubility studies**

Saturated solutions of diazepam and clindamycin phosphate in water and ethanol/water (10, 20, 50, 70%, v/v) at 32°C were prepared by adding excess amount of drugs to the vehicles and stirring for 24 h at room temperature, and then 24 h at 32°C. After this period, the excess drugs were filtered using PTFE 0.45-μm membrane filter (Chromafil® Xtra PTFE-45/25, Macherey-Nagel GmbH & Co. KG, Duran, Germany) and the solutions were diluted and analysed by HPLC.

**Thermogravimetric analysis (TGA)**

Thermogravimetric analysis (TGA-50, Shimadzu, Japan) were used to determine the moisture content of eschar samples before and after hydration. Small pieces of third-degree burn eschar were hydrated with water for different times of 0.5, 2, 12 and 24 h and were then blotted dry, and the samples (16 mg) were analysed by TGA by heating between 10 and 500°C at the rate of 10°C/min with constant N2 gas flow at 30 ml/min.

**RESULTS AND DISCUSSION**

**Solubility studies**

The solubility of clindamycin phosphate at 32°C was measured to be 95 mg/ml (Table 1) that is very close to what is reported by Resman et al., 80 mg/ml (24), although higher solubilities are also reported in the literature (25). Water solubility of diazepam at 32°C was measured to be 55 μg/ml (Table 1) which is comparable to what is reported; 50 μg/ml (26). As is shown in Table 1, the solubility of clindamycin phosphate decreases upon increasing the amount of ethanol in the system, which is opposite to that of diazepam where ethanol increases the solubility of this lipophilic drug in a concentration-dependent manner, as expected.

**Permeation studies**

Clindamycin phosphate permeation flux and permeability coefficient through burn eschar

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Solubility of clindamycin phosphate and diazepam in water and ethanol/water solutions at 32°C. Data are mean ± SD, n = 3</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Clindamycin phosphate</strong></td>
<td><strong>Diazepam</strong></td>
</tr>
<tr>
<td>Treatment</td>
<td>Solubility (mg/ml)</td>
</tr>
<tr>
<td>Water</td>
<td>95.09 ± 2.56</td>
</tr>
<tr>
<td>Ethanol 10%</td>
<td>–</td>
</tr>
<tr>
<td>Ethanol 20%</td>
<td>60.34 ± 1.38</td>
</tr>
<tr>
<td>Ethanol 50%</td>
<td>45.23 ± 1.96</td>
</tr>
<tr>
<td>Ethanol 70%</td>
<td>23.65 ± 1.76</td>
</tr>
<tr>
<td>Ethanol 96%</td>
<td>1.20 ± 0.18</td>
</tr>
</tbody>
</table>

*Ethanolic solution/water.*
from water and different ethanol/water solutions are shown in Table 2. Permeation flux and permeability coefficient of clindamycin phosphate from water (absence of ethanol) through burn eschar were calculated to be 1.25 mg/cm²/h and 13.1 × 10⁻³ cm/h respectively. Increasing the concentration of ethanol decreased the flux by about 2–4–20 times in the range of 20–70% ethanol content of the system. Permeability coefficient was also decreased by about 1.5–5.3 times in the same range (Table 2).

Clindamycin phosphate was used as a saturated solution in all used donor phases, which results in equal thermodynamic activities in all systems. In equal thermodynamic activities and as far as vehicles do not affect the properties of the barrier, the flux is expected to stay constant in spite of changes in the drug concentration in the donor phases. As is seen in Table 2, by increasing the alcohol content of the system (decreasing the clindamycin phosphate concentration, while thermodynamic activities are constant), flux decreases. This might show that ethanol reduces the diffusion coefficient or increases the length of permeation pathway through the eschar. As is seen later, a complementary mechanism could possibly be the presence of a porous pathway that is filled with the donor phase. In such cases, the flux is expected to decrease by decrease in the penetrant concentration but \( K_p \) is expected to stay constant, that is, \( K_p \) will be independent to partition coefficient unless diffusion coefficient and pathlength are affected. Considering this hypothesis, and the results that show the reduction of \( K_p \), might indicate that volume fraction of pores is highly decreased upon the addition of ethanol (reduction in effective diffusion coefficient). As is seen later, ethanol removes water from burn eschar and decreases its hydration level that can be considered as a reason behind this possible volume reduction.

To the best of our knowledge, there is no data available in the literature regarding the effect of ethanol on permeation of drugs through third-degree burn eschar. However, Hatanaka et al. have studied the effect of ethanol (20–60%) as a hydroethanolic solution on the permeation of seven hydrophilic drugs (5-fluorouracil, diclofenac sodium, nicorandil, antipyrine, morphine hydrochloride, isoproterenol hydrochloride and dopamine hydrochloride) through intact hairless rat skin. All the drugs were saturated in the vehicle. Their data show that except for diclofenac sodium, ethanol does not affect permeability coefficient of studied molecules and any change in the flux is due to change in the concentration. The same study also investigated the effect of polyethylene glycol (PEG) 400 and the results showed that PEG decreases both flux and permeability coefficient of the mentioned drugs. They concluded that these drugs permeate through hydrophilic pores, hence constant permeability coefficient and the decrease caused by PEG 400 are due to increased viscosity of pores medium (17). These data are partially different from what is found here, and this might be because of the differences between the structure and composition of the membranes. Normal stratum corneum pathways include mainly of lamellar lipid structure (27), while burn eschar is a protein–water semisolid membrane with some lipid component (8,28).

**Table 2** Clindamycin phosphate permeation flux and permeability coefficient from different ethanol–water solutions through burn eschar

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Flux (mg/cm²/h)</th>
<th>Flux ratio*</th>
<th>P-value†</th>
<th>Permeation coefficient ((K_p))</th>
<th>Permeability coefficient ((K_p)) ratio*</th>
<th>P-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>1.25 ± 0.24</td>
<td>1</td>
<td></td>
<td>13.15 ± 2.48</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Ethanol 20%</td>
<td>0.53 ± 0.11</td>
<td>0.42</td>
<td>0.000</td>
<td>8.78 ± 1.88</td>
<td>0.67</td>
<td>0.007</td>
</tr>
<tr>
<td>Ethanol 50%</td>
<td>0.18 ± 0.02</td>
<td>0.14</td>
<td>0.000</td>
<td>3.88 ± 0.39</td>
<td>0.30</td>
<td>0.000</td>
</tr>
<tr>
<td>Ethanol 70%</td>
<td>0.06 ± 0.02</td>
<td>0.05</td>
<td>0.000</td>
<td>2.50 ± 0.85</td>
<td>0.19</td>
<td>0.000</td>
</tr>
<tr>
<td>Ethanol 96%</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
<td>ND</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Data are mean ± SD, \( n = 3–7 \).

*Ethanolic solution/water.
†In comparison to water by one-way analysis of variance (ANOVA) followed by least significant difference (LSD) post hoc test.
§Not applicable.
Diazepam permeation flux and permeability coefficient from water and ethanolic solutions through burn eschar were found to be 0.96 μg/cm²/h and 17.37 × 10⁻³ cm/h respectively (Table 3). Increasing the amount of ethanol increased the permeation flux by about 3–81 times in the range of 10–70% ethanol in the system. Increased flux is due to increased concentration, although much less than increased solubility (3–850 times) (Table 3). This can be better explained by $K_p$. Increased amount of ethanol decreased the permeability coefficient of diazepam in the same range up to 11 times (Table 3) that is in reasonable comparison with clindamycin data as explained before and again show that ethanol increases barrier properties of burn eschar.

In a previous study, the effect of ethanol/water systems (over 0–90% w/w) on oestradiol, as a lipophilic model drug, across normal human skin was investigated (18). Saturated oestradiol solutions were used and it was shown that up to 60% ethanol, increasing the amount of ethanol in the solution, increase the oestradiol flux that was attributed by the authors to increased solubility of drug in the stratum corneum. At higher ethanol concentrations, oestradiol flux was decreased by increasing the ethanol concentration which was attributed to dehydration effect of ethanol on the stratum corneum (18). These data are in partial correlation with the present results.

In another study, the effect of ethanol/water systems on skin permeability of seven lipophilic model drugs (estradiol, ibuprofen, flurbiprofen, indomethacin, isosorbide dinitrate, cyclobarbital and aminopyrine) through hairless mouse skin was studied, and it was shown that the permeation fluxes of saturated lipophilic drugs were increased with increasing the amount of ethanol (0–60% v/v) in ethanol/water systems, while the permeability coefficient decreased by increasing the amount of ethanol. These results were attributed to the enhancement effect of ethanol towards permeation of lipophilic drugs (17). As mentioned earlier, the same study for hydrophilic drugs indicated that ethanol do not show any enhancement effect towards hydrophilic drugs. The difference is that lipophilic drugs prefer lipophilic pathways that can be altered by fluidization by ethanol. The results of lipophilic drugs permeation through normal skin are somewhat in correlation with the present eschar data, and this shows that both mechanisms, permeation through pores and lipid domains, are still possible in eschar for lipophilic drugs.

Comparison of the permeability coefficients of clindamycin phosphate and diazepam through burn eschar shows that there is no significant difference between these values either from water or ethanolic vehicles (Tables 2 and 3). These similarities might suggest the presence and importance of a pore pathway in the eschar for both drugs. On the basis of such a mechanism, continual decrease of permeability coefficient of clindamycin phosphate and diazepam in ethanolic vehicle, as compared to water, can be attributed partly to decreased porosity or increased tortuosity of pore pathways through burn eschar that can be due to dehydration and protein shrinkage of the burn eschar.

### Table 3  Diazepam permeation flux and permeability coefficient from water and ethanolic solutions through burn eschar

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Flux (μg/cm²/h)</th>
<th>Flux ratio*</th>
<th>$P$-value†</th>
<th>$K_p \times 10^3$ (cm/h)*</th>
<th>$K_p$ ratio*</th>
<th>$P$-value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>0.96 ± 0.61</td>
<td>1</td>
<td>0.537</td>
<td>17.37 ± 11.01</td>
<td>1</td>
<td>0.880</td>
</tr>
<tr>
<td>Ethanol 10%</td>
<td>2.64 ± 0.98</td>
<td>2.75</td>
<td>0.000</td>
<td>16.82 ± 6.33</td>
<td>0.97</td>
<td>0.004</td>
</tr>
<tr>
<td>Ethanol 20%</td>
<td>2.65 ± 1.12</td>
<td>2.76</td>
<td>0.000</td>
<td>8.42 ± 3.57</td>
<td>0.49</td>
<td>0.031</td>
</tr>
<tr>
<td>Ethanol 50%</td>
<td>32.20 ± 3.79</td>
<td>33.54</td>
<td>0.000</td>
<td>3.15 ± 0.37</td>
<td>0.18</td>
<td>0.007</td>
</tr>
<tr>
<td>Ethanol 70%</td>
<td>77.38 ± 16.59</td>
<td>80.60</td>
<td>0.000</td>
<td>1.62 ± 0.35</td>
<td>0.09</td>
<td>0.004</td>
</tr>
</tbody>
</table>

Data are mean ± SD, $n = 3–8$.

*Ethanolic solution/water.

† In comparison to water by one-way analysis of variance (ANOVA) followed by least significant difference (LSD) post hoc test.

**Thermal analysis**

Water uptake and hydration level of eschar as a function of exposure time were investigated by...
TGA (Table 4). Results showed that dry eschar shows about 11% moisture content. Here, dry eschar is defined as eschars that are cleaned from underlying tissues and fat after surgical debridement and left for equilibrium with the ambient condition (25°C, 45% RH) for more than about 1 h, and there was no significant change in dry eschar water content between different storage times of 1 and 3 h. Hydration level of eschar samples increased rapidly to about 50% within 30 min after soaking in water with no further increase even up to 24-h exposure (Table 4).

DSC studies showed that fully hydrated eschar shows two endothermic peaks (Figure 1C). The first transition (T1) with a midpoint of about 1°C and onset of around −0.7°C should be related to the melting of ice. The second transition (T2) was appeared around 65°C. Transitions T1 and T2 are absent in the dry eschar (Figure 1A). But both can be seen in normal skin.

The percentage of free water in 12-h hydrated burn eschar was measured by enthalpy of T1 transition. The enthalpy of ice melting that was measured for deionized double distilled water is reported to be 331.9 J/g (27). Considering the enthalpy of transition T1 (116.14 ± 23.91 J/g) and that hydrated eschar contains about 50% water, it was calculated that 37.2% of water content of eschar is bound. Dry eschar did not show the melting point of ice and it might show that there is no free water in dry burn eschar and all of the moisture (about 11%) is in the form of bound water.

DSC thermogram of dry eschar showed two endothermic peaks which were absent in the hydrated eschar. These peaks are called T3 and T4 to discriminate from hydrated samples. The first transition T3 appeared at about 113°C and T4 was at about 155°C (Figure 1A; Table 5).

To the best of our knowledge, there is no available data in the literature on the thermal analysis of burn eschar. Burn eschar is a proteinous structure with some lipid components (28). It has been shown for zein (the most abundant protein in the vitreous region of the corn endosperm) that hydration decreases enthalpy of protein glass transition temperature (29). Therefore, it might be concluded here that both T3 and T4 are related to proteins and their transition fade upon hydration and possible formation of a gel structure. Transition T2 which was observed in hydrated eschar (Figure 1C) and was absent in dry eschar may be as a result of liquid crystal structures of lipids of burn eschar which were formed by the hydration of dry lipids. It has been reported that dry lipids such as cholesterol and fatty acids, which show high transition temperature, can form liquid crystalline structures with lower transition temperatures (27). In reheated dry and hydrated burn eschar samples, all the transitions were disappeared except for T1 (Figure 2A and 2C).

Thermogram of the eschar samples treated with hydroethanolic solution (70%) showed a mixed dry and hydrated eschar pattern in which T1 was absent, but T2−T4 were seen. Transition T1 was absent in hydroethanolic systems and might show drying effect of ethanol on eschar samples and reduction of its water content, the same effect has been reported for normal skin by ethanol (16,18). However, reduction of freezing point of water by ethanol cannot be dismissed. T2 is still seen in the presence of hydroethanolic solution (70%). Transitions T3 and T4 were broader and partially

### Table 4

<table>
<thead>
<tr>
<th>Exposure time (h)</th>
<th>Water content (%)</th>
<th>Bound water (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (dry eschar)</td>
<td>11.88 ± 1.63</td>
<td>–</td>
</tr>
<tr>
<td>0.5</td>
<td>54.51 ± 11.81</td>
<td>NS*</td>
</tr>
<tr>
<td>2</td>
<td>52.98 ± 8.86</td>
<td>NS</td>
</tr>
<tr>
<td>12</td>
<td>57.50 ± 4.52</td>
<td>37.2</td>
</tr>
<tr>
<td>24</td>
<td>52.63 ± 2.23</td>
<td>NS</td>
</tr>
</tbody>
</table>

Data are mean ± SD, n = 3–7.

*Not studied.

Figure 1. Sample differential scanning calorimetry (DSC) thermograms of dry burn eschar (A), burn eschar in ethanol 70% (B) and fully hydrated burn eschar (C).
Effect of ethanol and hydration on burn eschar permeation

Table 5 Transition temperatures and enthalpies of dry and hydrated burn eschar

<table>
<thead>
<tr>
<th>Transition</th>
<th>Temperature (°C)</th>
<th>Enthalpy (J/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dry eschar</td>
<td>Hydrated eschar</td>
</tr>
<tr>
<td>$T_1$</td>
<td>$-1.42 \pm 0.49$</td>
<td>$64.58 \pm 1.09$</td>
</tr>
<tr>
<td>$T_2$</td>
<td>$53.79 \pm 1.09$</td>
<td>$64.58 \pm 1.09$</td>
</tr>
<tr>
<td>$T_3$</td>
<td>$113 \pm 0.69$</td>
<td>$64.58 \pm 1.09$</td>
</tr>
<tr>
<td>$T_4$</td>
<td>$155 \pm 1.46$</td>
<td>$64.58 \pm 1.09$</td>
</tr>
</tbody>
</table>

Data are mean ± SD, $n = 3$.

Figure 2. Sample differential scanning calorimetry (DSC) thermograms of reheated dry burn eschar (A), reheated burn eschar in ethanol 70% (B) and reheated fully hydrated burn eschar (C).

merged (Figure 1A and 1B). The enthalpies of the merged transitions $T_3$ and $T_4$ were by about five times of that of dry eschar, that might imply that shrinkage and collapse of burn eschar proteinous structure in ethanol 70% is more than dry eschar. No transition was seen in reheated burn eschar treated within ethanol 70% (Figure 2B). This might explain reduction of permeation of drugs through eschar by ethanol, as discussed previously.

In summary, this investigation shows that permeability of hydrophilic and lipophilic drug models, clindamycin phosphate and diazepam, through third-degree burn eschar are reduced by ethanol, which is concluded to be because of the dehydration of burn eschar, and consequently ends to collapse of protein structure and reduced porosity of permeation pathway. It seems that pores play an important role in permeation of drugs through burn eschar. However, other pathways cannot be dismissed and further investigations are required in this area.

In practical point of view, this data suggest that if ethanol is to be applied to burn tissue (due to any reason or purpose) and permeability of the medicaments is of concern, the reduction effect of ethanol should be compensated by other methods such as application of penetration enhancers [e.g. see (5,6)] or increasing the concentration of the penetrant in the vehicle.

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Effect of ethanol and hydration on burn eschar permeation