Biphasic Flux Profiles of Melatonin: The Yin–Yang of Transdermal Permeation Enhancement Mediated by Fatty Alcohol Enhancers

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Abstract

This study investigates physicochemical processes responsible for the biphasic transdermal flux profiles of melatonin in the presence of saturated fatty alcohols (SFAL) and unsaturated fatty alcohols (USFAL). The first phase melatonin flux ($J_{1st}$) in the presence of USFAL enhancers increased with increase in the number of double bonds and reached a limiting value with two double bonds in the molecule. In case of SFAL enhancers, $J_{1st}$ increased with enhancer chain length and log formulation/skin partition coefficients (log $P_s$), which were calculated using the solubility parameters of various formulation components. But, melatonin flux in the second phase decreased with increase in the enhancer chain length and log $P$ values. On the other hand, the transepidermal water loss (TEWL) from the SFAL treated skin increased drastically in the second phase and correlated with log $P$ value of the enhancer. High TEWL value, indicative of a severely disrupted SC, may help the polar formulation components to accumulate in the SC. As a consequence, the SC polarity could change significantly and reduce the partitioning of lipophilic enhancer and/or melatonin in the second phase. This study demonstrated that an optimal level of barrier disruption enhances the transdermal permeation of drugs, whereas, a drastic barrier disruption impedes transdermal transport.

Keywords

solubility parameters; fatty alcohols; melatonin; transdermal permeation; vehicle; rat skin

Introduction

Disturbance in the circadian pattern of melatonin production might lead to sleep disorders like delayed sleep syndrome,1 jet lag in aircrew and cosmonauts,2 and shift work syndrome.3 A successful clinical treatment can be developed against these sleep disorders, if melatonin is substituted exogenously at a rate sufficient to simulate its endogenous circadian rhythm. Transdermal administration of melatonin in the form of a patch, cream, or solid lipid nanoparticles (SLN) could be considered to provide sustained plasma concentrations of melatonin.4–6 However, inadequate transdermal absorption of melatonin necessitates the use of a suitable vehicle and a penetration enhancer to achieve rapid systemic absorption of the hormone via the skin.

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“Yin” and “Yang” describe opposing qualities in a phenomenon, which are in a dynamic equilibrium. Each advance (Yang) is followed by a retreat (Yin), and every fall (Yin) transforms into a rise (Yang). Any mutable phenomenon is a consequence of Yin and Yang.
Chemical penetration enhancers have been routinely employed to increase the transdermal penetration of therapeutic agents.\textsuperscript{7,8} Previously, several investigators have demonstrated that the transdermal permeation of melatonin could be improved by employing ethanolic vehicles\textsuperscript{9–11} or mixtures of penetration enhancers and ethanolic vehicles.\textsuperscript{12,13} However, some enhancers like saturated fatty alcohols (SFALs) and unsaturated fatty alcohols (USFALs) generate biphasic melatonin flux profiles, which thwart the maintenance of stable plasma melatonin concentrations for an extended period of time. Therefore, the current study was aimed at investigating physicochemical processes responsible for the biphasic transdermal flux profiles by employing melatonin flux data in the theoretical framework of regular solution theory.

**Theoretical**

It is believed that fatty alcohols enhance transdermal drug permeation by increasing the drug solubility in the lipid matrix of stratum corneum (SC). The extent of solubilization and its influence on the percutaneous absorption can be estimated with the help of solubility parameters ($\delta$) of the constituent solutes and solvents. Solubility parameter is equal to the square root of the cohesive energy ($\delta E$) divided by the molar volume ($V$). Barton\textsuperscript{14} observed that the cohesive energy density of a compound is related to its physical properties, such as molecular weight, solubility, and partition coefficient. Of these physical properties, partition coefficient and solubility play an important role in determining transdermal permeation of the compound.

The effects of partition coefficient and solubility on the flux of a permeant are inversely related to each other (Eq. 3). Increased permeant solubility in the donor solution is associated with a reduction in its skin partitioning. In other words solubility and partition coefficient tend to cancel out each other, making it difficult to determine their individual effects on the flux. The most reasonable way out of this impasse is to estimate solubility and partition coefficient independently. The solubility of a particular drug in the formulation can be easily determined; however, the estimation of its formulation/skin partition coefficient could be more involved. Sloan et al.\textsuperscript{15} identified a method for the determination of theoretical partition coefficients by using solubility parameters of the skin, the vehicle, and the drug. The theoretical log partition coefficients (log $P$s) calculated according to their method have been found to correlate well with the experimentally determined permeability coefficients.\textsuperscript{16}

According to the Fick’s law, steady state flux of a drug is given by

$$J=K \times C_v$$  \hspace{1cm} (1)

where $K$ is the permeability coefficient and $C_v$ the concentration of the drug in the formulation. However, $K$ is a product of the partition coefficient $P$ and the diffusion coefficient $D$ over skin thickness $h$.

$$K=P \times \frac{D}{h}$$  \hspace{1cm} (2)

$$J=P \times \left(\frac{D}{h}\right) \times C_v = \frac{C_a}{C_v (D/h)} \times C_v$$  \hspace{1cm} (3)
where \( C_s \) is the concentration of drug in the skin. The value of \( D/h \) the constant as long as the value of \( D \) for a drug in a particular membrane is independent of the vehicle used. Thus the experimentally determined \( K \) value should be directly proportional to \( P \).

\[
K = \text{constant} \times P 
\]  

(4)

Based on the above theoretical background and Hildebrand's expression for the activity coefficient of nonpolar solutes in nonpolar solvents, Sloan et al.\(^\text{16}\) proposed the following equation for the theoretical determination of \( \log P \) utilizing the solubility parameters of the drug (\( \delta_d \)), vehicle (\( \delta_v \)), and the skin (\( \delta_s \)).

\[
\log P = \left[ \frac{(\delta_d - \delta_v) V_d \varphi_v^2}{RT} \right] - \left[ \frac{(\delta_d - \delta_v) V_d \varphi_v^2}{RT} \right]
\]  

(5)

where \( V_d \) is the molar volume of melatonin, \( R \) the gas constant, and \( T \) the absolute temperature. \( \varphi_v^2 \) is the volume fraction of vehicle and was calculated from the following expression

\[
\varphi_v = \frac{V_v (1 - S_v)}{V_v (1 - S_v) + V_s S_v}
\]  

(6)

where \( V_v \) is the molar volume of the vehicle and \( S_v \) the mole fraction solubility of the vehicle. \( \varphi_v^2 \) approaches unity in dilute solutions.

**Experimental**

**Materials**

Melatonin, propylene glycol, and fatty alcohols were obtained from Sigma Chemical Co. (St. Louis, MO). Ethanol USP (200 proof) was obtained from Florida Distillers Co. (Lake Alfred, FL). High performance liquid chromatography (HPLC) grade solvents and nylon filters were purchased from ThermoFisher Scientific (Atlanta, GA). All chemicals were used as received. The Franz type diffusion cells were obtained from PermeGear, Hellertown, PA.

**Methods**

**Animals and Skin Samples**—Six- to nine-week-old male Sprague–Dawley rats with an average weight of 150 ± 20 g were procured from Harlan, Inc. (Indianapolis, IN). The rats were housed in animal care facility with food and water provided \textit{ad libitum}. One day prior to the experiment, hair on the dorsal surface of the rats was removed with an electric clipper fitted with #40 blade. Animals were sacrificed just before the experiment by an overdose of sodium pentobarbital administered intraperitoneally. Previously shaven dorsal skin was harvested after careful excision and cleaning from the subcutaneous tissue. Each experiment was performed in triplicate with skin specimens obtained from three different animals.

**Melatonin Formulations**—Each melatonin formulation had a unique base, which is a combination of a penetration enhancer (5%, w/w) and ethanolic vehicle, WE (water: ethanol, 40:60). Penetration enhancers used were a homologous series of fatty alcohols, where each member differed from the adjacent member in either alkyl chain length or in the degree of unsaturation. SFALS such as octanol, nonanol, decanol, undecanol, dodecanol (Lauryl
alcohol), tridecanol, and tetradecanol (myristyl alcohol); and USFALs such as oleyl alcohol, linoleyl alcohol, and linolenyl alcohol were used in this study. Excess amount of melatonin (∼1.75 g) was added to 5mL of the base and equilibrated in an environmental shaker for 24 h at 37°C. The resultant formulation was filtered through 0.22 μm nylon filter and used as donor solution in the permeability studies.

**In Vitro Diffusion Studies**—The excised skin specimens were mounted between donor and receiver compartments of the Franz diffusion cells in such a way that the dermal side of the skin was in contact with the receiver solution (phosphate buffer of pH 7.4). The donor compartment was filled with 1 mL melatonin formulation and sealed tightly to prevent the solvent loss due to evaporation. Throughout the experiment, a circulating water bath maintained the diffusion cells at 37 ± 0.5°C, which in turn maintained the skin surface at physiological temperature of 32 ± 0.5°C. The receiver phase was sampled at regular intervals and replenished with the same amount of fresh phosphate buffer.

**HPLC Analysis**—Chromatographic separation was performed by ODS-AQ column (5μ, 3 × 150 mm, YMC, Inc., Milford, MA). A combination of methanol and water (50:50) was used as the mobile phase pumped at a flow rate of 0.5mL/min. Melatonin was analyzed with UV detector set at a wavelength of 223 nm. The retention time of melatonin was 5.6 min.

**Determination of Transepidermal Water Loss (TEWL) In Vivo**

The TEWL was measured as described previously by Kanikkannan and coworkers. Briefly, 230 μL of WE vehicle or the WE vehicle containing 5% (w/v) SFAL enhancer was incorporated into the Hill top chamber® (surface area 1.04 cm²), which was planted on the previously shaven rat skin with a waterproof tape (Johnson and Johnson, Inc., New Brunswick, NJ). A similar site on the same animal was affixed with a blank Hill top chamber® to evaluate the occlusive effects of the chamber. The Hill top chamber® was removed after 3 h and the treated area was gently wiped. The TEWL was measured using Tewameter TM 210 (Courage/Khazaka, Cologne, Germany) at 0, 1, 2, 4, 6, 24, 48, 72, and 96 h after the removal of the chamber. The rate of change of TEWL (ΔTEWL) was expressed as g/m² h⁻¹.

**Data Analysis**

The plots of cumulative amount of melatonin permeated versus time exhibited biphasic behavior. The flux of the first phase was usually obtained over a 3–18 h period by employing linear regression and the second phase flux was obtained from the remaining portion. Permeability coefficients of either phase were obtained by dividing the fluxes by the solubility of melatonin in the donor formulation.

**Solubility Parameter and log P Calculations**

Solubility parameters of melatonin (Tab. 1) and other fatty alcohols (Tab. 1) were obtained using the method of Fedors¹⁷ as demonstrated previously by Martin et al.¹⁸ For WE vehicle the volume shrinkage was taken into consideration. However, when fatty alcohol was added to the vehicle, it was assumed that there was no significant change of volume on mixing. The solubility parameter of the formulation was calculated as the sum of the products of the volume fraction and solubility parameter of each component. The log P of each formulation component was estimated by considering the entire formulation, excluding the solute of interest, as a donor phase. For example, when SFAL enhancer was considered as the solute of interest, a mixture of WE vehicle and melatonin was regarded as the donor phase. Alternatively, if melatonin was the solute of interest, the mixture of WE vehicle and SFAL enhancer was considered as the donor phase.

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Results and Discussion

A transdermal delivery device (TDD) is expected to generate square-wave plasma pharmacokinetic profile of the permeant with a rapid absorption phase; steady state plasma levels for an extended period; and a rapid decline of plasma concentrations after the TDD removal. Rapid transdermal absorption is conventionally achieved by transiently disrupting SC with various vehicles and penetration enhancers added to the TDD. However, the SC disruption may sometimes cause significant changes in its polarity that may drastically alter drug partitioning between the formulation and the skin. Under these circumstances, it may be very difficult to maintain transdermal delivery at a constant rate. The biphasic melatonin flux profiles observed in the presence of fatty alcohol enhancers are most likely a consequence of such a phenomenon.

The biphasic flux profiles are not specific to melatonin or the type of skin used in the transport experiments. Kanikkannan and Singh have shown similar biphasic flux profiles of melatonin in human, porcine as well as in hairless rat skins. Furthermore, biphasic flux profiles, in the presence of fatty alcohol enhancers, were previously observed with other drugs such as indomethacin, tefagur, and captopril. Nevertheless, the physicochemical factors responsible for the biphasic profiles were not thoroughly investigated.

The objective of the current study was to elucidate various factors responsible for the biphasic transdermal flux of melatonin by conducting the following sets of experiments: (a) Permeability studies to determine biphasic flux of melatonin in the presence of various vehicles and penetration enhancers; (b) Investigation of partitioning processes at the formulation/skin interface using solubility parameters; and (c) Transepidermal water loss measurements (TEWL) that provide a quantitative determination of the extent of barrier disruption (Tab. 2).

Biphasic Melatonin Flux across Rat Skin in the Presence of SFAL Enhancers

Saturated Fatty Alcohols (SFALs)—In the presence of SFALs, melatonin exhibited biphasic flux profiles (Fig. 1A), with higher flux in the first phase than in the second phase (Tab. 3). The melatonin permeability increased in the first phase with an increase in the SFAL chain length. But the second phase permeability was inversely related to the enhancer chain length (Tab. 3). A cursory examination of the flux data may seem that the change of melatonin flux in the second phase is due to the depletion of melatonin concentration in the donor compartment with time. But the donor solutions used in this study were saturated with melatonin at 37°C. Moreover, less than 3% of melatonin in the donor solutions was ultimately transported to the receiver solution. Hence, it is unlikely that the decrease of melatonin flux in the second phase was due to reduction in the thermodynamic activity of melatonin in the donor solutions.

To expose the physicochemical parameters contributing to the differences between first and second phase melatonin fluxes, the ratio of the fluxes ($R_J$) was calculated using the following equation:

$$ \frac{J_{1\text{st phase}}}{J_{2\text{nd phase}}} = R_J = \frac{[(D_1P_1/h_1) \times C_{v1}]}{[(D_2P_2/h_2) \times C_{v2}]} \quad (7) $$

Based on the previous conclusion that the melatonin concentration in the formulation remains unchanged throughout the experiment and assuming no significant changes in the skin thickness, the Eq. (7) could be reduced to
Eq. (8) suggests that the first and second phase melatonin fluxes are directly proportional to
the product of the diffusion coefficient and the formulation/skin partition coefficient of
melatonin.

The octanol/water partition coefficients (log \( P_{o/w} \)) of permeants have been classically used to
predict drug partitioning into skin. However, they are of little value in describing partitioning
processes at the formulation/skin interface. Therefore, theoretical formulation/skin partition
coefficients (log \( P \)) were calculated using the solubility parameters of the permeant
(\( \delta_{\text{MELATONIN}} \)), the vehicle (\( \delta_{\text{vehicle}} \)), the enhancer (\( \delta_{\text{c8–c14}} \)), and SC (\( \delta_{\text{SC}} \)). Hildebrand initially
applied solubility parameters for the regular solutions of nonpolar solutes in nonpolar solvents.
However, Sherertz et al.\(^{22}\) demonstrated that they could be extended to mixed vehicles of polar
solvents like oleic acid and propylene glycol. The log \( P \) values of melatonin determined using
their approach remained unchanged in various formulations.

Incorporating this information in Eq. (8) results in

\[
R_j = \frac{D_1 P_1}{D_2 P_2}
\]

Eq. (9) indicates that the ratio of melatonin flux in the first and second phases \( (R_j) \) is
proportional to the ratio of melatonin diffusion coefficients in those phases. Moreover, the
\( R_j \) value was found to increase with the chain length of the enhancer (Tab. 3). Is it possible
that these biphasic flux profiles are due to alterations in the skin barrier mediated by various
formulation components including the SFAL enhancers? To address this question, it is
important to look at the partitioning behavior of various formulation components between the
transdermal formulation and skin and the effect they may have on the SC. When a transdermal
formulation is in contact with the skin, other formulation components also copermeate the skin
along with the drug as dictated by their physicochemical properties. Kai et al.\(^{23}\) demonstrated
that the penetration of alkanols through the skin exhibited a pattern very similar to the
permeation profile of the model penetrant (nicotinamide). In addition, ethanol could
copermeate into the skin with the SFAL enhancer.\(^{24}\) Once in the SC, ethanol has been found
to extract SC lipids, while SFALs disrupt the densely packed SC lipids.\(^{13}\) Together, they could
alter the nature of SC as a permeation barrier.

To test the validity of this inference, we determined the magnitude of SC disruption by
measuring the TEWL \( \text{in vivo} \) from a patch of rat skin exposed to various fatty alcohol enhancers
dissolved in WE vehicle (5%, v/v). Numerous investigators have demonstrated quantitative
Correlation between TEWL and percutaneous absorption; while few other investigators
reported a lack of correlation, most likely due to assumptions made in the experimental design,
measurement methods, and nature of the permeant (reviewed by Levin and Maibach\(^{25}\)). It must
also be pointed out that in many occasions, the correlation between TEWL and percutaneous
absorption was reported in the skin treated with harsh chemicals. For example, Tsai et al.\(^{26}\)
have shown good correlation between TEWL and the transdermal permeability of a moderately
lipophilic compound, such as hydrocortisone (log \( P = 1.5 \)) in acetone treated mouse skin.
Although, melatonin has a log \( P \) value (1.2) similar to that of hydrocortisone, it is not safe to
assume correlation between TEWL and the transdermal permeability of melatonin in the
presence of SFAL enhancers. Closer look at the TEWL and melatonin permeability data reveal

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some interesting relationship between these variables. No significant differences were observed in the mean rate of change of TEWL ($\Delta$TEWL) from the skin areas exposed to various SFAL enhancers within the first 9 h after exposure (Fig. 2). This first 9 h may correspond to the movement of SFALs, which closely resemble skin components, into the SC; the associated increase in the permeability of melatonin could be due to the ability of SFAL enhancers to interact with SC lipids. The increase of $\Delta$TEWL values in the later phase (9–48 h) from the skin exposed to longer chain SFALs (C$_{12}$–C$_{14}$) suggests barrier disruption. Surprisingly, the transdermal flux of melatonin in the presence of longer chain SFALs decreased. Therefore, it is important to investigate physicochemical phenomena driving these intriguing trends.

The magnitude of SFAL effect on the SC could be dependent upon the concentrations at which they accumulate in the SC, which in turn is dependent upon: the log $P$ value of the enhancer, the enhancer concentration in the formulation, and the mobility of the enhancer in the SC. The log $P$ values of SFAL enhancers increase with the enhancer chain length (Tab. 4). The lag time of melatonin skin permeation decreased with an increase in the enhancer chain length, which suggests that the penetration enhancers with higher log $P$ values partition into SC and increase its permeability to melatonin quicker than the enhancers with lower log $P$ values (Fig. 4A). To determine the impact of enhancer concentration on the biphasic melatonin flux, the transdermal permeability studies were conducted at various concentrations of representative SFAL enhancers (Fig. 3). When octanol (C$_8$) concentration in the formulation was increased from 5% to 10%, the melatonin flux increased substantially in the first phase. However, the average melatonin flux ($\langle J_{1st} + J_{2nd}\rangle/2$) did not change with the enhancer concentration, which suggests that the gain of melatonin flux in the first phase was compensated by the loss of melatonin flux in the second phase. Increase in nonanol (C$_9$) concentration from 5% to 10% did not affect melatonin flux in either phases significantly. However, a reduction in C$_9$ concentration from 5% to 2.5% drastically reduced first phase melatonin flux. In addition, the average of 1st and 2nd phase melatonin flux also decreased when the C$_9$ concentration decreased from 5% to 2.5%. Increasing the concentrations of either lauryl (C$_{12}$) or myristyl (C$_{14}$) alcohol in the formulation had no effect on either the first phase or the second phase melatonin flux. Moreover, melatonin exhibited biphasic flux profiles in the presence of all the SFAL enhancers at the concentrations tested. These results do not purport an obvious relationship between the biphasic melatonin flux and enhancer concentration in the donor solution.

The extent of accumulation in the SC barrier is also dependent upon the mobility of SFAL enhancer. The mobility of short chain alkanols, such as C$_8$ and C$_9$, is higher through SC lipid matrix. Hence, their accumulation in the lipid matrix is low. Consequently, higher concentration of short chain alkanols is required in the formulation to achieve the desired enhancement effect (Fig. 3). On the other hand, SC mobility of long chain alkanols like C$_{14}$, whose melting points are above the skin temperature (32°C), is low. Hence, larger quantities of long chain alkanols could accumulate in the SC causing perturbation and permeation enhancement. In the current study, the concentration of SFAL enhancer in the formulation is kept constant at 5% (v/v). Therefore, magnitude of the transition time point ($T_{trans}$) between first and second phases could be a consequence of the ability of the enhancer to partition and accumulate in SC. As shown in Figure 4B, the $T_{trans}$ changed in a parabolic fashion with increase in the enhancer chain length.

Taken together, these studies indicate that the correlation between melatonin flux in the first phase and the enhancer chain length is a reflection of the barrier perturbation caused by the alkanols, which is function of their lipophilicity and the degree of accumulation in the SC. The inverse correlation between the second phase melatonin flux and the enhancer chain length could be due to an increase in the polarity of the skin barrier caused by the diffusion of polar formulation components like water and ethanol into the perturbed skin. This brings the polarity of skin closer to that of the formulation and reduces the formulation/skin partitioning of
melatonin; due to which the transdermal flux of melatonin could be reduced in the second phase.

**Biphasic Melatonin Flux across Rat Skin in the Presence of USFAL Enhancers**

Even in the presence of USFALs, melatonin exhibited biphasic flux profiles (Fig. 1B), with higher flux in the first phase than in the second phase (Tab. 3). The log $P$ values of USFALS are similar to that of C$_{12}$, because of the proximity of their solubility parameters ($\delta_{\text{OLA}} = 9.47$; $\delta_{\text{LOA}} = 9.5$; and $\delta_{\text{LONA}} = 9.53$). However, the first and the second phase melatonin fluxes in the presence of oleyl alcohol (OLA) were significantly lower than those in the presence of C$_{12}$ (Tab. 3), which may reflect the mechanistic differences in their barrier disruption. The enhancement effect of SFALs is mediated by the solubilization and extraction of SC lipids. On the other hand, OLA might not be able to interact with SC lipids well, because most of the lipids present in the SC are saturated. It is likely that OLA increases melatonin permeability by inserting itself into SC lipid packing and disrupting the SC lipids with its “kinked” structure.

A kink in USFAL structures is mainly due to presence of double bond in cis-configuration. An increase in the number of double bonds increases kinking in the structure, which may enhance SC disruption. The impact of the degree of USFAL unsaturation on melatonin permeation was studied using OLA, Linoleyl alcohol (LOA), or linolenyl alcohol (LONA) which have one (cis-9-octadecyl), two (cis,cis-9,12-octadecyl), or three cis double bonds (all cis-9,12,15-octadecyl), respectively. An increase in the number of double bonds to two (LOA), increased first and the second phase melatonin flux significantly. The maximum rate of penetration probably reached a limiting value when the double bond number increased from 2 to 3 in the molecule. Hence, the melatonin permeation did not increase any further when LONA was used in the formulation instead of LOA.

In conclusion, the current study has demonstrated that the theoretical partition coefficients calculated from the solubility parameters of various formulation components are useful in understanding the partitioning processes occurring at skin/formulation interface. Both SFAL and USFAL enhancers modulate the SC and cause melatonin flux enhancement. However, disrupting the SC to a point where epidermal water as well as polar formulation components move into SC and increase its polarity, could result in decreased formulation/skin partitioning of melatonin and reduce its transdermal flux. The biphasic skin absorption could significantly impact the pharmacokinetic profile of the permeant in vivo. In transdermal applications intended for prolonged use, shorter chain SFALs (nonanol or decanol) or USFALs with lower degree of unsaturation (OA) can ensure constant drug penetration over a longer time span.

**Acknowledgments**

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**References**


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Figure 1.
Biphasic permeation of melatonin through rat skin in the presence of: (A) saturated; and (B) unsaturated fatty alcohols. Each data point represents the mean ± SD of three experiments.
Figure 2.
Rate of transepidermal water loss (TEWL) from the in vivo rat skin treated with various saturated fatty alcohols. Each bar represents the mean ± SD of three experiments. ***Denotes that the rate of change of TEWL between 9 and 48 h is significantly different ($p < 0.001$) from the rate between 0 and 9 h.
Figure 3.
Effect of fatty alcohol enhancer concentration on the first and second phase melatonin flux across rat skin in vitro. The first phase flux profiles of C\textsubscript{8}, octanol; C\textsubscript{9}, nonanol; C\textsubscript{12}, lauryl alcohol; C\textsubscript{14}, myristyl alcohol were indicated by filled symbols connected with solid lines. The second phase flux profiles of C\textsubscript{8*}, C\textsubscript{9*}, C\textsubscript{12*}, and C\textsubscript{14*} are indicated by unfilled symbols connected with dotted lines.
Figure 4.
(A) Lag times of first phase melatonin flux in the presence of various saturated fatty alcohol (SFAL) enhancers. (B) Time of transition from first phase to second phase melatonin flux in the presence of various SFAL enhancers. C₈, octanol; C₉, nonanol; C₁₀, decanol; C₁₁, undecanol; C₁₂, lauryl alcohol; C₁₃, tridecanol C₁₄, myristyl alcohol.
Table 1
Group Contribution Method for Calculating Molar Volume and Solubility Parameter of Melatonin

<table>
<thead>
<tr>
<th>Atom or Group</th>
<th>Number of Groups</th>
<th>ΔE (cal/mol)</th>
<th>ΔV (mL/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>–CH=</td>
<td>4</td>
<td>4120</td>
<td>54.0</td>
</tr>
<tr>
<td>–C=</td>
<td>4</td>
<td>4120</td>
<td>–22.0</td>
</tr>
<tr>
<td>–CH₃⁻</td>
<td>2</td>
<td>2360</td>
<td>32.2</td>
</tr>
<tr>
<td>–CH₃</td>
<td>2</td>
<td>2250</td>
<td>67.0</td>
</tr>
<tr>
<td>O</td>
<td>1</td>
<td>800</td>
<td>3.8</td>
</tr>
<tr>
<td>–NH⁻</td>
<td>2</td>
<td>4000</td>
<td>9.0</td>
</tr>
<tr>
<td>–C=O</td>
<td>1</td>
<td>4150</td>
<td>10.8</td>
</tr>
<tr>
<td>Ring closure</td>
<td>2</td>
<td>500</td>
<td>32.0</td>
</tr>
<tr>
<td>Olefinic bonds</td>
<td>5</td>
<td>2000</td>
<td>–11.0</td>
</tr>
</tbody>
</table>

ΣΔE = 24,300  ΣΔV = 24,300
δD = (24,300/175.8)¹/²
   = 11.75 (cal/mL)¹/²
Table 2

Physical Properties of Fatty Alcohols

<table>
<thead>
<tr>
<th>Fatty Alcohols</th>
<th>Molecular Weight (g/mol)</th>
<th>Density (g/cm³)</th>
<th>Molar Volume (cm³/mol)</th>
<th>Solubility Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octanol</td>
<td>130.22</td>
<td>0.827</td>
<td>31.45</td>
<td>10.30</td>
</tr>
<tr>
<td>Nonanol</td>
<td>144.26</td>
<td>0.828</td>
<td>31.53</td>
<td>10.12</td>
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<tr>
<td>Decanol</td>
<td>158.28</td>
<td>0.823</td>
<td>31.56</td>
<td>9.78</td>
</tr>
<tr>
<td>Undecanol</td>
<td>172.30</td>
<td>0.830</td>
<td>31.58</td>
<td>9.61</td>
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<tr>
<td>Lauryl alcohol</td>
<td>186.34</td>
<td>0.832</td>
<td>31.60</td>
<td>9.51</td>
</tr>
<tr>
<td>Myristyl alcohol</td>
<td>214.38</td>
<td>0.824</td>
<td>31.64</td>
<td>9.16</td>
</tr>
</tbody>
</table>
Table 3

Flux and Permeability of Melatonin in the Presence of Fatty Alcohol Enhancers

<table>
<thead>
<tr>
<th>Formulation</th>
<th>$J_{1st}$ (μg/cm² h)</th>
<th>$K_{1st}$ (cm/h × 10⁵)</th>
<th>$J_{2nd}$ (μg/cm² h)</th>
<th>$K_{2nd}$ (cm/h × 10⁵)</th>
<th>$p$ ($J_{1st}$ vs. $J_{2nd}$)</th>
<th>$R_J = J_{1st}/J_{2nd}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>WE vehicle</td>
<td>1.8 (0.2)</td>
<td>0.62</td>
<td>8.32 (0.58)</td>
<td>2.92</td>
<td>n.s.</td>
<td>0.21</td>
</tr>
<tr>
<td>Saturated fatty alcohols (SFALs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% C8 + WE</td>
<td>8.8 (1.5)</td>
<td>2.92</td>
<td>49.80 (2.7)</td>
<td>16.62</td>
<td>***</td>
<td>0.18</td>
</tr>
<tr>
<td>5% C9 + WE</td>
<td>17.7 (2.9)</td>
<td>5.80</td>
<td>32.21 (2.75)</td>
<td>10.556</td>
<td>*</td>
<td>0.55</td>
</tr>
<tr>
<td>5% C10 + WE</td>
<td>28.9 (4.5)</td>
<td>10.01</td>
<td>30.17 (5.65)</td>
<td>10.43</td>
<td>n.s.</td>
<td>0.96</td>
</tr>
<tr>
<td>5% C11 + WE</td>
<td>38.9 (6.2)</td>
<td>15.55</td>
<td>26.43 (7.02)</td>
<td>9.21</td>
<td>n.s.</td>
<td>1.47</td>
</tr>
<tr>
<td>5% C12 + WE</td>
<td>58.0 (14.4)</td>
<td>20.77</td>
<td>17.14 (0.85)</td>
<td>6.13</td>
<td>***</td>
<td>3.39</td>
</tr>
<tr>
<td>5% C13 + WE</td>
<td>63.6 (8.02)</td>
<td>22.14</td>
<td>14.81 (0.26)</td>
<td>5.15</td>
<td>***</td>
<td>4.30</td>
</tr>
<tr>
<td>5% C14 + WE</td>
<td>64.3 (10.5)</td>
<td>23.05</td>
<td>7.77 (2.84)</td>
<td>2.78</td>
<td>***</td>
<td>8.29</td>
</tr>
<tr>
<td>Unsaturated fatty alcohols (USFALs)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5% OA + WE</td>
<td>41.1 (8.4)</td>
<td>14.22</td>
<td>25.7 (4.9)</td>
<td>8.89</td>
<td>*</td>
<td>1.60</td>
</tr>
<tr>
<td>5% LOA + WE</td>
<td>65.4 (7.2)</td>
<td>23.33</td>
<td>24.3 (5.4)</td>
<td>8.67</td>
<td>***</td>
<td>2.69</td>
</tr>
<tr>
<td>5% LONA + WE</td>
<td>62.2 (3.5)</td>
<td>21.82</td>
<td>20.1 (2.5)</td>
<td>7.05</td>
<td>***</td>
<td>3.10</td>
</tr>
</tbody>
</table>

Saturated fatty alcohols: C₈, octanol; C₉, nonanol; C₁₀, decanol; C₁₁, undecanol; C₁₂, lauryl alcohol; C₁₃, tridecanol; C₁₄, myristyl alcohol. Unsaturated fatty alcohols: OA, oleyl alcohol; LOA, linoleyl alcohol; and LONA, linolenyl alcohol. $J_{1st}$, flux of melatonin in the 1st phase; $J_{2nd}$, flux of melatonin in the 2nd phase; $K_{1st}$, permeability coefficient of melatonin in the 1st phase; $K_{2nd}$, permeability coefficient of melatonin in the 2nd phase; $R_J$, melatonin flux ratio between first and second phases. The data is presented as mean (SEM). n.s., not significant.

* $p < 0.05$.
*** $p < 0.001$. 

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Table 4
Formulation/Skin log P Values of SFAL Enhancer and Melatonin

<table>
<thead>
<tr>
<th>Vehicle</th>
<th>log $P_{\text{Formulation/Skin}}$ SFAL Enhancer</th>
<th>log $P_{\text{Formulation/Skin}}$ Melatonin</th>
</tr>
</thead>
<tbody>
<tr>
<td>WE vehicle</td>
<td>4.08</td>
<td>2.64</td>
</tr>
<tr>
<td>Octanol + WE</td>
<td>4.74</td>
<td>2.19</td>
</tr>
<tr>
<td>Nonanol + WE</td>
<td>5.64</td>
<td>2.17</td>
</tr>
<tr>
<td>Decanol + WE</td>
<td>6.37</td>
<td>2.13</td>
</tr>
<tr>
<td>Undecanol + WE</td>
<td>7.15</td>
<td>2.11</td>
</tr>
<tr>
<td>Lauryl alcohol + WE</td>
<td>9.12</td>
<td>2.14</td>
</tr>
<tr>
<td>Myristyl alcohol + WE</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>