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# Formulation and In Vitro Evaluation of Transdermal Patches of Melatonin

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## ABSTRACT

The present study was undertaken to prepare and evaluate monolithic drug-in-adhesive type transdermal patches of melatonin containing penetration enhancers such as fatty alcohols, fatty acids, and terpenes. The patches were prepared using Eudragit® E 100 as the adhesive polymer. The release profile of melatonin from control as well as enhancer-containing patches showed an initial burst of melatonin release for up to 4 hours and then a plateau after 8 hours. The release profiles of melatonin from patches containing various enhancers were similar to the control patch. However, the addition of enhancers in the patch increased the permeation of melatonin through hairless rat skin. The flux values of patches containing octanol, nonanoic acid, and myristic acid were higher than the control patch (no enhancer), but the differences were not statistically significant ($P > 0.05$). Decanol, myristyl alcohol, and undecanoic acid at 5% concentrations showed significantly higher flux values through hairless rat skin (enhancement ratios 1.7, 1.5, and 1.6 for decanol, myristyl alcohol, and undecanoic acid, respectively) ($P < 0.05$). Menthol and limonene at 5% w/w showed maximum permeation of melatonin among all enhancers studied (enhancement ratios=2.1 and 2.0 for menthol and limonene, respectively) ($P < 0.001$). In general, there was about 4–6 hours of lag time observed before a steady state flux of melatonin was achieved. Though the flux of melatonin observed in the present study is 5–10 times higher than the required delivery rate in humans, it must be noted that the present study was performed using hairless rat skin, which is generally more permeable compared to human skin. Further studies using human skin would prove the usefulness of these patches.

*Key Words:* Transdermal; Melatonin; Patches; Drug release; Penetration enhancers.

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## INTRODUCTION

Transdermal drug delivery offers several advantages over the conventional dosage forms such as tablets and injections, including elimination of first-pass metabolism, minimization of pain, and possible controlled release of drugs. The success of transdermal delivery depends on the ability of the drug to permeate the skin in sufficient quantities to achieve its desired therapeutic effects. However, the highly organized structure of the stratum corneum forms an effective barrier to the permeation of drugs, which must be modified if poorly penetrating drugs are to be administered. The use of chemical penetration enhancers would significantly increase the number of candidates suitable for transdermal delivery.

Melatonin, an indole hormone secreted from the pineal gland, has been found to be useful in the management of sleep disorders such as delayed sleep syndrome, jet lag, shift work syndrome, and migrane headaches.[1–3] Melatonin is a good candidate for transdermal delivery considering its variable oral absorption, short biological half-life (45 minutes), extensive first-pass metabolism, and a favorable octanol–water partition coefficient (log P=1.20).[4–6] Lee et al.[7] studied the transdermal delivery of melatonin in human volunteers using a solution of melatonin in pH 6.1 phosphate buffer containing propylene glycol. The authors concluded that melatonin could be delivered transdermally in human volunteers, although an intersubject variability was noted. In another study, Benes et al.[4] compared the delivery of melatonin from transmucosal, oral controlled-release, and transdermal administration in human volunteers. It was observed that transdermal administration of melatonin showed a significant lag time and a gradual decline in drug delivery after patch removal, possibly due to the deposition of melatonin in the skin. The residual material left in the skin after removal of patch or sample formulation would gradually leach from the skin over time and this may extend the therapeutic profile of the drug.[8–10] Some of these problems can be overcome, at least in part, by using a more effective penetration enhancer and by applying the patch a few hours before the required time of onset of action.

Previous studies in our laboratory[11–14] and others[15] have shown that the permeation of melatonin across the skin can be increased significantly by using chemical penetration enhancers in solution formulations. Chemical penetration enhancers such as fatty alcohols and fatty acids increased the permeation of melatonin across the skin, and the enhancement of permeation was found to be dependent on the chemical structure of the enhancers. Terpenes are an important class of penetration enhancers and they were found to be very effective in enhancing the penetration of drugs.[16] The present study was undertaken to prepare and evaluate monolithic drug-in-adhesive type transdermal patches of melatonin. Selected penetration enhancers from different chemical classes such as fatty alcohols, fatty acids, and terpenes were incorporated in the patches and their effect on the permeation of melatonin was studied using hairless rat skin.

## MATERIALS AND METHODS

### Materials

Melatonin, fatty alcohols (octanol, decanol, myristyl alcohol), fatty acids (nonanoic acid, undecanoic acid, myristic acid), acetone, isopropyl alcohol, and succinic acid were procured from Sigma Chemical Co. (St. Louis, MO). Ethanol USP (200 proof) was obtained from Florida Distillers (Lake Alfred, FL). Terpenes (limonene, menthol) were procured from Aldrich Chemical Co., Inc. (Milwaukee, WI). Water and methanol [both high-performance liquid chromatography (HPLC) grade] and nylon filters were obtained from Fisher Scientific (Atlanta, GA). Eudragit® E 100 was obtained from Rohm America (Piscataway, NJ). Acetyl tributyl citrate (ATBC) was obtained from Morflex, Inc. (Greensboro, NC). Transdermal patch components such as backings and release liners were generously supplied by 3M Pharmaceuticals (St. Paul, MN).

### Preparation of Patches

Drug-in-adhesive type patches were prepared using Eudragit E 100 as the adhesive polymer.[17] A mixture of acetone, isopropyl alcohol, and ethanol (6.4:0.6:3.0) was used as the solvent. Acetyl tributyl citrate and succinic acid were used as plasticizer and crosslinker, respectively. Trial patches were prepared by varying formulation parameters (concentration of polymer, plasticizer, and drug) and procedures (change in drying time and thickness of the patch). The optimized patch composition was used for further studies using penetration enhancers. Eudragit E 100 was dissolved in portions in a mixture of acetone, isopropyl alcohol, and ethanol. Under continuous stirring, ATBC was added to this solution followed by succinic acid, melatonin, and penetration enhancer. The weight of the penetration enhancer was adjusted by the polymer (Eudragit E 100) so as to keep the total weight (25 g)

*Table 1.* General formula for the preparation of transdermal patches.

| Ingredient | Quantity |
|---|---|
| Eudragit® E 100 | 8.70 g |
| ATBC | 3.50 g |
| Succinic acid | 0.32 g |
| Acetone | 7.64 g |
| Isopropyl alcohol | 0.69 g |
| Ethanol | 3.71 g |
| Melatonin | 0.44 g |
| Enhancer[a] | |

[a]2.5% or 5% enhancer was added and this weight was adjusted by Eudragit E 100.

constant. The solution was coated on the Polyester type backing membrane (Scotchpak™ 1006, 3M Pharmaceuticals, St. Paul, MN) using a mechanical drive (Resource, Jamesburg, NJ) set at wet film thickness of 200 μm. The films were dried in an oven at 60° C for 30 minutes to evaporate the solvents. The release liner (Scotchpak™ 1022, 3M Pharmaceuticals) was attached to the adhesive side of the film. The final patches were wrapped with aluminium foil and stored at ambient temperature until further use. The composition of the optimized patch is shown in Table 1.

## Melatonin Assay

The melatonin content in the patch was determined by HPLC analysis. The adhesive layer of the patch (area 4 $cm^2$) was dissolved in methanol. This solution was diluted further with methanol and analyzed as described below under HPLC analysis. The analysis was performed in triplicate.

## Melatonin Release Studies

The USP dissolution test apparatus 5 (VK7000, VanKel, Cary, NC) was used to study the release profile of melatonin from the patches. The dissolution vessels were filled with 750 mL of phosphate buffered saline, pH 7.4. The temperature of the dissolution medium was maintained at 32±0.5° C. A circular patch was cut and placed on the disc with the release surface facing up, and the disc was placed at the bottom of the dissolution vessel. The diffusional surface area of the patch was 12.5 $cm^2$. The paddles were stirred at a speed of 50 rpm. At various time intervals, 10-mL samples were collected up to 24 hours using an autoampler (VK 8000, VanKel, Cary, NC). The samples were analyzed for melatonin content by HPLC. All experiments were performed in triplicate.

## Skin Permeation Studies

Male CD® (SD) hrBi hairless rats (Charles River Laboratories, Wilmington, MA) were sacrificed using halothane and the skin was excised from the dorsal surface. The subcutaneous tissue was removed carefully with scissors and scalpel. A flow-through cell with a diffusional surface area of 0.636 $cm^2$ (PermeGear, Riegelsville, PA) attached to Ismatec® IPC multichannel peristaltic pump and fraction collector (Retriever IV®) operated by an index controller was used for the skin permeation studies. A circulating water bath was used to maintain the temperature at 37° C. Phosphate buffered saline at pH 7.4 was used as the diffusion medium. The patches were cut into 1-$cm^2$ pieces using a biopsy punch, and applied to the epidermal side of the hairless rat skin with slight pressure before mounting on the receiver cell. Samples were programmed for collection at various time intervals up to 24 hours. The samples were analyzed by HPLC.

## HPLC Analysis

The analysis of melatonin was performed using a Waters HPLC operated by Millennium $^{32}$ software. The system consisted of a pump (model 515), autosampler (model 717), and photodiode array UV detector (model 996). A reversed phase $C_{18}$ (ODS-AQ™ S5 μm 120 A $3.0 \times 150$ mm) column along with a guard column (ODS-AQ S5μ 120 A $4.0 \times 23$ mm) were used in the analysis. A combination of methanol and water (50:50) was used as the mobile phase at a flow rate of 0.5 mL/min. The detector wavelength was set at 223 nm and the chromatographic peak for melatonin was detected at 4.9 minutes.

## Data Analysis

For each experiment skins from at least three rats were used. The cumulative amount of melatonin permeated through the skin was plotted as a function of time. The slope of the linear portion of the plot was calculated as the flux (μg/$cm^2$/h). The enhancement ratio was calculated by dividing the flux of the patch (with enhancer) with that of flux without enhancer. Student's t-test was performed to determine the level of significance. The differences were considered to be significant at $P<0.05$.

## RESULTS AND DISCUSSION

Adhesive matrix patches are easy to fabricate and provide thin and small transdermal patches. Eudragit E 100 polymer has gained wide commercial acceptance; hence we used it to formulate adhesive matrix patches for melatonin. The optimized patch composition was used for further studies using penetration enhancers. The criteria to define the optimized composition were films of good flexibility and adhesiveness at a predetermined wet film thickness of 200 μm. This was achieved by altering the plasticizer and polymer concentrations per unit area of the patch. The film adhesiveness and flexibility were qualititatively evaluated. The acrylic polymers have several desirable features, such as resistance to oxidation, thermal degradation, and moderate cost. They are permeable to water vapor and oxygen and generally exhibit good tack.[18]

### Release of Melatonin from Patches

Figure 1 shows the release profiles of melatonin from patches containing 2.5% and 5% octanol as penetration enhancer. The release profile of melatonin from control patches showed an initial burst of melatonin release for up to 4 hours and then a plateau after 8 hours. The addition of octanol (2.5% and 5%) did not alter the release profile of melatonin compared to the control patch. The amount of melatonin released up to 24 hours from the patch containing octanol 2.5% (391.29±36.75 μg/cm$^2$) and octanol 5% (398.89± 21.14 μg/cm$^2$) was not significantly different from the control patch (400.12±39.39 μg/cm$^2$) ($P>0.01$). The estimated melatonin content of the patches was 398.14–442.56 μg/cm$^2$ and over 95% of melatonin was released from the patch within 24 hours of the release study. The release profile of melatonin from patches containing other fatty alcohols, fatty acids, and terpenes was also similar to the control patch (data not shown).

The results of the present study are in agreement with the release kinetics of the marketed drug-in-adhesive type patches (Deponit®, Nitro-Dur®) of nitroglycerin.[19] In drug-in-adhesive type patch, the drug and excipients (e.g., enhancer) are directly loaded into the polymer adhesive. The adhesive provides several functions, including skin adhesion, matrix for the drug and excipients, and control of drug release. Drug-in-adhesive type patches are generally thin, which increases patient compliance. Monolithic drug-in-adhesive type patch is easy to formulate, and the permeation of drug from this type of patch will be mostly controlled by the skin rather than the patch.

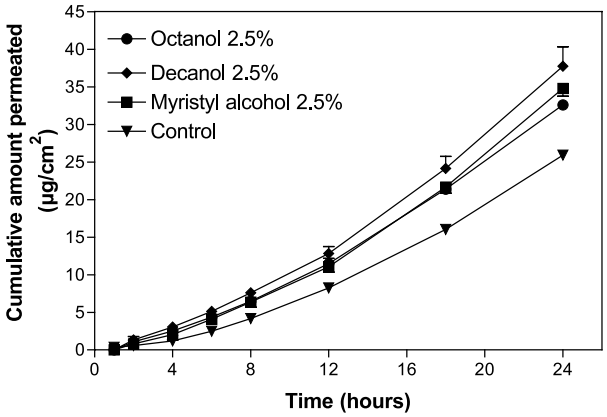


*Figure 2.* Effect of fatty alcohols (2.5%) on the permeation of melatonin from patches across hairless rat skin.

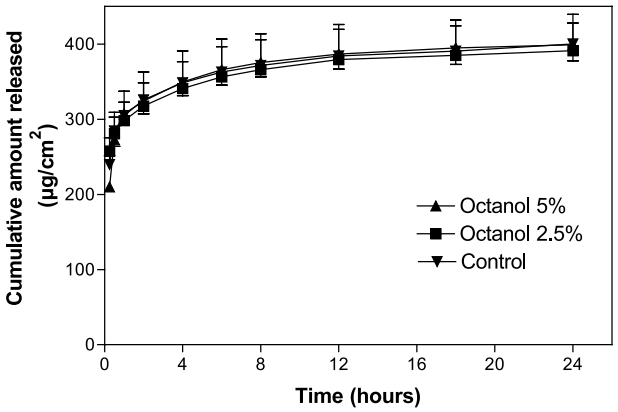


*Figure 1.* Release profiles of melatonin from patches containing 2.5% and 5% of octanol as penetration enhancer.

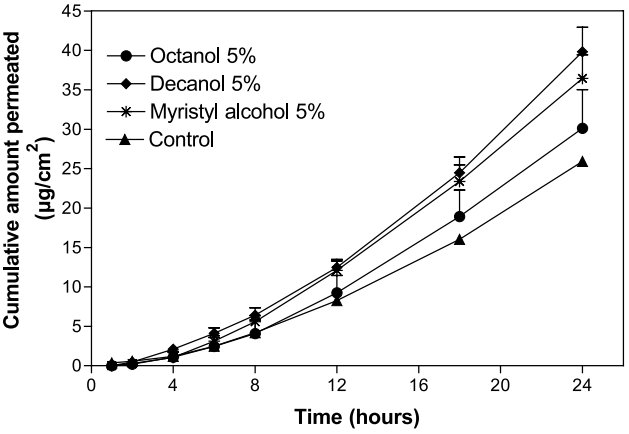


*Figure 3.* Effect of fatty alcohols (5%) on the permeation of melatonin from patches across hairless rat skin.

This helps in achieving maximum permeation rate, though the delivery will be for a relatively short duration. Many of the transdermal patches introduced recently were of drug-in-adhesive type (e.g., Combi-Patch®, FemPatch®, Climara Patch®).

## Skin Permeation of Melatonin from Patches

Figures 2 and 3 show the permeation profiles of melatonin from transdermal patches containing 2.5% and 5% fatty alcohols, respectively. The addition of fatty alcohols increased the permeation of melatonin across the hairless rat skin compared to the control. Decanol showed the maximum permeation of melatonin from the patch followed by myristyl alcohol and octanol. Table 2 presents the steady-state flux values of melatonin from patches containing various enhancers. The flux of melatonin from patches increased 30–40% by the addition of 2.5% fatty alcohols, but these increases were not statistically significant ($P>0.05$). At 5% concentration, decanol and myristyl alcohol increased the flux of melatonin ($P<0.01$ and $P<0.05$, respectively), but there was no increase in the flux observed with octanol ($P>0.05$). Overall, 5% decanol showed the maximum permeation of melatonin across hairless rat skin (1.7 fold as shown by the enhancement ratio). The pattern of permeation enhancement effect of different saturated fatty alcohols (5%) observed in the present study was similar to the results obtained using solution formulations of melatonin with fatty alcohols (C-8 to C-14).[12] There was a parabolic relationship between the chain length of alcohols and theophylline flux, but increased flux was associated with skin damage caused by the longer chain alcohols.[20] A parabolic relationship between carbon chain length of fatty alcohol and permeation enhancement was also observed for other drugs including naloxone[21] and Tegafur.[22]

***Table 2.*** Steady-state fluxes of melatonin from patches containing various enhancers across the hairless rat skin.

| Enhancer | Flux (μg/cm$^2$/h) | Enhancement ratio |
|---|---|---|
| Control | 1.30±0.05 | 1.0 |
| Fatty alcohols | | |
| Octanol, 2.5% | 1.69±0.07 | 1.3 |
| Octanol, 5% | 1.42±0.19 | 1.1 |
| Decanol, 2.5% | 1.83±0.12 | 1.4 |
| Decanol, 5% | 2.17±0.09[b] | 1.7 |
| Myristyl alcohol, 2.5% | 1.69±0.08 | 1.3 |
| Myristyl alcohol, 5% | 1.98±0.13[a] | 1.5 |
| Fatty acids | | |
| Nonanoic acid, 2.5% | 1.78±0.09 | 1.4 |
| Nonanoic acid, 5% | 1.65±0.09 | 1.3 |
| Undecanoic acid, 2.5% | 1.76±0.12 | 1.4 |
| Undecanoic acid, 5% | 2.05±0.18[a] | 1.6 |
| Myristic acid, 2.5% | 1.72±0.07 | 1.3 |
| Myristic acid, 5% | 1.70±0.07 | 1.3 |
| Terpenes | | |
| Menthol, 2.5% | 2.13±0.10[b] | 1.6 |
| Menthol, 5% | 2.71±0.19[c] | 2.1 |
| Limonene, 2.5% | 1.64±0.14 | 1.3 |
| Limonene, 5% | 2.59±0.09[c] | 2.0 |

Note: Each value represents the mean±SD from three replicates. The enhancement ratio is calculated by dividing the flux with enhancer with that of flux without enhancer (control).
[a]$P<0.05$.
[b]$P<0.01$.
[c]$P<0.001$ vs. control.

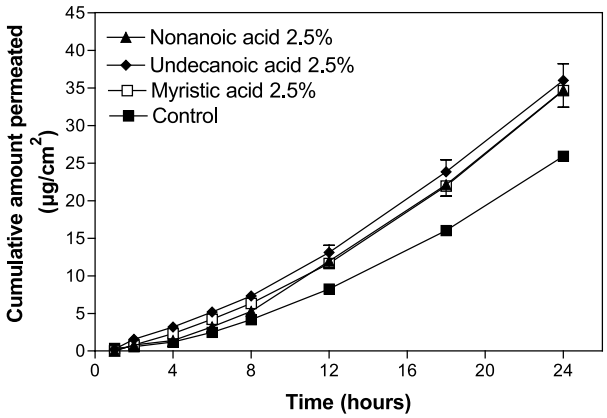


***Figure 4.*** Effect of fatty acids (2.5%) on the permeation of melatonin from patches across hairless rat skin.

Figures 4 and 5 show the permeation profiles of melatonin from transdermal patches containing 2.5% and 5% fatty acids, respectively. The presence of fatty acids increased the permeation of melatonin across the hairless rat skin compared to the control. The maximum permeation of melatonin was shown by undecanoic acid at 5% concentrations. As observed with fatty alcohols, the permeation of melatonin from patches increased 30–40% by the addition of 2.5% fatty acids (Table 2), but these increases were not statistically different vs. control ($P>0.05$). At 5% concentration, undecanoic acid significantly increased the flux of melatonin ($P<0.05$, enhancement ratio 1.6), but no increase in the flux was observed with 5% nonanoic acid and myristic acid ($P>0.05$). The parabolic effect of carbon chain length on the permeation of melatonin

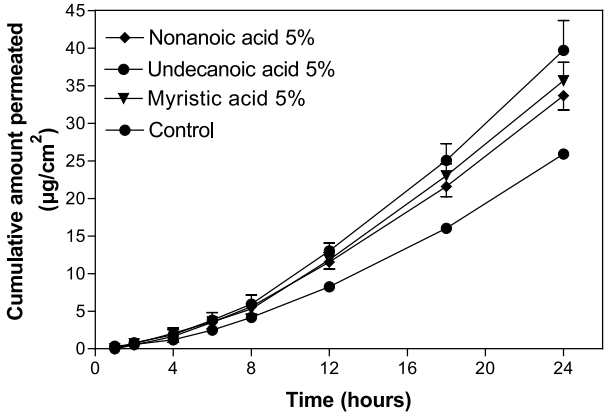


*Figure 5.* Effect of fatty acids (5%) on the permeation of melatonin from patches across hairless rat skin.

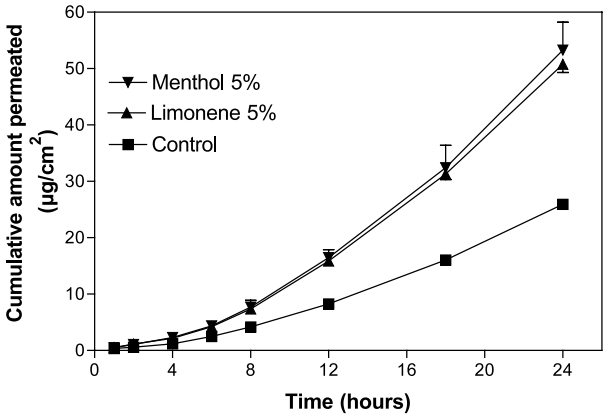


*Figure 7.* Effect of terpenes (5%) on the permeation of melatonin from patches across hairless rat skin.

in the present study with patches was also similar to the solution formulations.[11] The mechanism by which fatty alcohols and fatty acids increase skin permeability appears to involve disruption of the densely packed lipids that fill the extracellular spaces of the stratum corneum and fluidization effects of stratum corneum.[23] The change in the physical structure of stratum corneum lipids has been assessed using differential scanning calorimetric and infrared spectroscopic techniques.[24] It has been reported that C-10 to C-12 fatty acids possess an optimal balance between partition coefficient or solubility parameters and affinity to skin.[25]

Figures 6 and 7 show the permeation profiles of melatonin from transdermal patches containing 2.5% and 5% of terpenes as enhancers, respectively. At 2.5% concentration, the permeation of melatonin with menthol was higher than control ($P<0.01$), whereas limonene did not show a statistically significant increase in the permeation of melatonin ($P>0.05$). At 5% concentration, the permeation profile of melatonin was similar with both enhancers. The concentration of the enhancer played a significant role in the permeation enhancement effect of menthol and limonene. Out of all the enhancers, menthol and limonene (5%) showed maximum permeation enhancement effect ($P<0.001$, enhancement ratios 2.1 and 2.0 for menthol and limonene, respectively). Terpenes such as menthol and limonene have been widely used as skin penetration enhancers for a variety of compounds.[26] The terpenes act by disrupting the lipid structure of the stratum corneum, thereby increasing the diffusion coefficient of the polar drug in the membrane.[27,28] Overall, as shown in Table 2, the presence of skin penetration enhancers in the formulation increased the flux maximum up to two-fold as shown by the enhancement ratios. Our earlier studies[11,12] showed that the flux from solution formulations containing these enhancers at 5% w/w concentration increased the flux several times (four- to ten-fold) over control solution with no enhancers. This indicates that the release of enhancer from the patch and its action on the skin to reduce the barrier property is an important factor for permeation enhancement of melatonin by these enhancers. The target delivery rate of melatonin ($K_o$) from a transdermal patch can be calculated from the pharmacokinetic parameters using the following equation:

$$AK_o = [C_p \cdot V_d \cdot K_{el}] \text{ or } [C_p \cdot CL]$$

The average endogenous plasma concentration ($C_p$) of melatonin during day time is low (<10 pg/mL), and at night the melatonin levels rise to 30–120 pg/mL.[29,30] Our aim is to deliver exogeneous melatonin to achieve

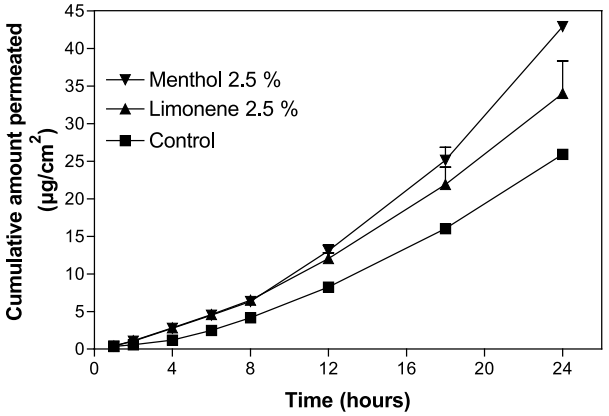


*Figure 6.* Effect of terpenes (2.5%) on the permeation of melatonin from patches across hairless rat skin.

the plasma levels to be maintained in the above range of 30 to 120 pg/mL. For calculating the target delivery rate of melatonin from a transdermal patch, the midpoint of 75 pg/mL (30 to 120 pg/mL) was considered. The total body clearance (CL) of melatonin (normalized to body weight) is 1.2 L/h/kg.[31] From the above equation, the target delivery rate ($K_o$) of melatonin from a patch size (A) of 25 $cm^2$ can be calculated as 0.25 μg/$cm^2$/h. In the present study, the flux of melatonin from the control patch (without enhancer) was 1.30±0.05 μg/$cm^2$/h. The addition of penetration enhancer increased the flux significantly with menthol (5%) for up to 2.71±0.19 μg/$cm^2$/h. In general, there was about 4–6 hours of lag time observed before a steady-state flux was achieved. Benes et al.[4] observed a long lag time (~13 hours) before a peak level of melatonin was observed in human volunteers treated with transdermal patches of melatonin with transcutol and N,N-dimethyldodecylamide-N-oxide as penetration enhancers. In the present study, however, the lag time was 6 hours. Though the flux of melatonin observed in the present study is 10 times higher than the required delivery rate in humans, it must be noted that the present study was performed using hairless rat skin, which is generally more permeable compared to human skin. Further studies using human skin would prove the usefulness of these patches. The skin toxicity of these patches containing penetration enhancers must also be evaluated.

## CONCLUSIONS

Monolithic drug-in-adhesive type transdermal patches of melatonin were prepared using Eudragit E 100, and the effects of different penetration enhancers on the release of and permeation of melatonin were studied. Release of melatonin from the patches followed a first-order kinetics. Addition of enhancers in the patch increased the permeation of melatonin through hairless rat skin. Decanol and undecanoic acid showed the maximum permeation of melatonin among the fatty alcohols and fatty acids, respectively. A lag time of 4–6 hours was observed before a steady-state flux of melatonin was observed. Menthol showed the maximum permeation of melatonin among all the enhancers studied.

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