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# DEVELOPMENT OF A TRANSDERMAL DELIVERY DEVICE FOR MELATONIN IN VITRO STUDY

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## ABSTRACT

The present study was undertaken to develop a transdermal delivery device for melatonin and to determine the effects of system design on the release of melatonin. Melatonin(MT) diffusion characteristics from 2 solvents through a series of ethylene vinyl acetate membranes with 4.5%, 9%, 19%, 28% vinyl acetate were characterized using vertical Franz® diffusion cells. The solvent used were 40% (v/v) propylene glycol (PG) and 40%(v/v) propylene glycol with 30%(w/v) 2-hydroxypropyl-β-cytrodextrin. The best release rate (Jss = 0.795 μg/h/cm$^2$) was obtained from the 40% PG vehicle through the 28% vinyl acetate membrane. Melatonin diffusion through this membrane with an acrylate pressure sensitive adhesive (PSA) with and without MT loading was also studied. The data revealed an interaction between MT and the PSA in the systems with MT-loaded adhesive. A MT transdermal delivery device was constructed based on the above data. Effect of storage time (1 day, 2 days, and 3 days) on the developed device was also investigated. Steady state flux values of MT did not vary significantly with storage time (p-value = 0.14). The steady state flux was 1.88 ± 0.6 μg/hr/cm$^2$(n=9). However, storage time did affect the burst effect of MT. Total amount of MT released in the first hour was 137.4 ± 25.7 μg after 3 days, 61.5 ± 8.9 μg after 2 days, and 43.8 ± 20.9 μg after 1 day.

## INTRODUCTION

Melatonin (N-Acetyl-5-Methoxytryptamine) is an indole neurohormone secreted by the pineal gland. Although the usefulness of administering melatonin to produce physiological or pharmacological plasma concentrations is still unknown (1), it appears that melatonin administration may be beneficial in patients with below normal melatonin blood concentrations. Therefore, it is desirable to develop drug delivery systems for melatonin which are able to mimic the physiological, endogenous plasma melatonin concentration in patients whose melatonin profile needs to be reestablished . Endogenous melatonin secretion is generally high during nighttime producing a bell-shaped plasma profile with a peak concentration of approximate 60 pg/ml. Daytime melatonin plasma concentrations are generally less than 20 pg/ml (2). Drug delivery systems for controlled release of melatonin such as transdermal, oral (3,4), and transmucosal (5) have been developed in order to deliver melatonin in a physiological pattern.

A previous study provided evidence that transdermal administration of melatonin was possible (4). The present study was undertaken to determine the effects of system design on the release of melatonin, using Franz® diffusion cells. Experiments were designed to (1) determine the flux of melatonin through a series of ethylene vinyl acetate (EVAc) membranes using a donor solution of either (a) 40% propylene glycol (PG) or (b) 40% PG with 30% (w/v) 2-hydroxypropyl-β-cyclodextrin (2-HPCD) (2) determine the flux of melatonin through an acrylate transfer adhesive with and without "melatonin loading" of the adhesive and (3) fabricate and evaluate, *in vitro*, a transdermal delivery device (TDD) based on the above.

## MATERIALS AND METHODS

### Materials

Melatonin was purchased from Regis Chemical Co. (Morton Grove, IL). HPLC grade, methanol, analytical grade, isopropanol, and ethyl acetate were obtained from Mallinckrodt Specialty Chemicals Co. (Paris, KY). Methylparaben was purchased from Sigma Chemical Co. (St. Louis, MO). Analytical grade propylene glycol (PG) and heptane were obtained from J.T. Baker, Inc. (Phillisburg, NJ). 2-Hydroxypropyl-β-cyclodextrin (2-HPCD) was provided courtesy of American Maize-Product Company (Hammond, ID, USA). The 3M Company (St. Paul. MN) provided ethylene vinyl acetate membranes (0.05 mm thickness) with 28%, 19%, 9%, and 4.5% vinyl acetate content (3M CoTran™ controlled caliper film), neutral acrylate pressure sensitive adhesive (3M CoTran™ PGTA #9871), backing film (heat sealable, tan polyester film laminate, Scotchpak™ film #1006), and fluoropolymer coated release liner (Scotchpak™ film #1022).

Equipment and Analytical Method

Vertical Franz® Diffusion cells (Crown Glass Co.) were used for all diffusion studies. The cross sectional area and receptor volume for these cells were 3.14 $cm^2$ and 15 ml, respectively. Melatonin concentration in non-biological fluids was determined using an HPLC system with UV detection. The HPLC was equipped with an M-45 pump, WISP® 710B injector, reversed-phase C18 (4μ) radial compression column and Model 441 detector with a 229-nm light source (all from Waters® Associates). Retention time and sensitivity for melatonin (mobile phase: methanol/water, 50:50) were 4 minutes and 10 ng/ml, respectively, at a flow rate of 1.2 ml/minute. Methylparaben was used as the internal standard. Its retention time was 6.5 minutes.

Data Analysis

The flux (μg/hr/$cm^2$) and lag time of melatonin released from each system were calculated from the slope and the intercept of the plot of the cumulative amount of melatonin in the receptor solution at steady state against time using linear regression analysis. After logarithm transformation, independent groups of calculated flux values were statistically analyzed by one-way analysis of variance as initial comparisons of means and by Tukey-Kramer intervals multiple comparison using STATGRAPHIC® version 5.0.

Membrane Studies

Melatonin diffusion through four different ethylene vinyl acetate membranes was determined in triplicate. Two different donor solutions of melatonin were prepared as follows (4):

- 40%(v/v) propylene glycol vehicle

| | |
|---|---|
| Melatonin | 0.01438 g |
| Propylene Glycol (PG) | 0.6 ml |
| Phosphate Buffer pH 6.1 | 0.9 ml |

- 40%(v/v) propylene glycol with 30%(w/v) 2-hydroxypropyl-β-cyclodextrin (2-HPCD)

| | |
|---|---|
| Melatonin | 0.03061 g |
| Propylene Glycol (PG) | 0.6 ml |
| 2-hydroxypropyl-β-cyclodextrin[a] (2-HPCD) | 0.45 g |
| Phosphate Buffer pH 6.1 | 0.9 ml |

[a] Molecular weight, 1542, average degree of molar substitution, 6.5 moles of hydroxypropyl/mole of β-cyclodextrin, range of molar substitution, 5-8.

One and a half (1.5) ml of donor solution was placed in the donor cell. Normal saline was employed as the receptor solution and was maintained at 30°C throughout the study. Two hundred microliters were collected from the receptor chamber via a sampling port at 1,2,3,4,6,8 and 12 hours after adding the melatonin solution to the donor cell. An equal volume of saline was replaced

through the sampling port after each sample collection. Samples were kept frozen until HPLC analysis.

Adhesive Studies

Ethylene vinyl acetate membrane, 28% vinyl acetate, (28%VAc), was used in these adhesive studies. Neutral acrylate adhesive was applied to a 2.5X2.5 cm area of the membrane by either using an air brush (spray method) or by a pressure transfer method. With the transfer method the adhesive with paper backing was pressed against the membrane. The backing was then carefully removed leaving the adhesive on the membrane. In the spraying method, adhesive was physically removed from the backing paper, weighed, and dissolved in either isopropanol or a 50%(v/v) mixture of ethyl acetate/heptane. The adhesive was then directly sprayed on the membrane under the hood. The sprayed surface was even and smooth with good tackiness. Details of each diffusion system are summarized in Table 1. Diffusion studies for each system were conducted using the method described in the Membrane Studies section. The 40% propylene glycol melatonin donor solution was used for these studies.

Delivery Device Studies

A 3X3 cm device (TDD) was prepared by heat sealing 28% VAc membrane to a backing film (see Materials section). Adhesive was then applied using the spray method to the membrane surface. Ethyl acetate/heptane was used as the adhesive solvent. One, two or three days prior to the diffusion studies, 1.5 ml of the 40% propylene glycol vehicle with melatonin was placed in the device. A release liner was applied, the device was wrapped in foil and then stored at room temperature prior to the diffusion study. After 1, 2, or 3 days of storage, the device was evaluated using the method described in the Membrane Studies section. These studies were conducted in triplicate. The melatonin content of the device was determined after the conclusion of the diffusion study.

## RESULTS AND DISCUSSION

Membrane Studies

Permeation profiles of melatonin through ethylene vinyl acetate (28%,19%, 9%, and 4.5% vinyl acetate) membranes from 40%PG vehicle and 40%PG with 30% 2-HPCD are shown in Figure 1. The associated physicochemical parameters are shown in Table 2. Flux increased with increasing VAc content of the membranes for both vehicles (Figure 2). ANOVA indicated significant differences among the resulting flux values (p-value=0). The flux rate obtained from the system of 40% PG vehicle through 28% VAc membrane was almost twice that of 40% PG with 30% 2-HPCD vehicle through the same membrane type. The 40% PG vehicle provided a greater flux compared to the 40% PG with 30% 2-HPCD vehicle, since 2-HPCD decreased the permeability coefficient through the membrane despite the higher drug solubility in this vehicle.

TABLE 1.

Adhesive studies:
Preparation of the studied diffusion systems.

| System | Donor MT | Adhesive on the membrane | Solvent used |
|---|---|---|---|
| 1 | yes | spray method | isopropanol |
| 2 | yes | spray method | EtAc/heptane |
| 3 | yes | pressure transfer | none |

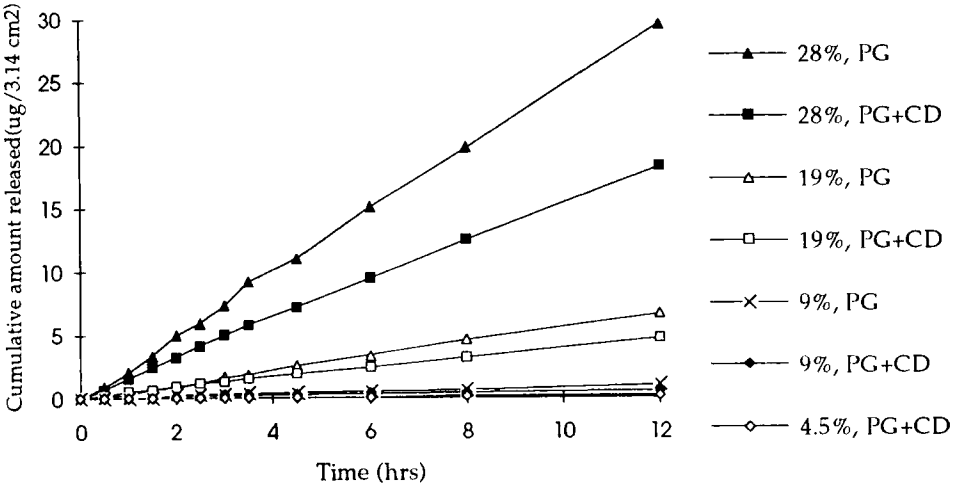


FIGURE 1.
Melatonin diffusion profile through a series of ethylene vinyl acetate membranes with different vinyl acetate content (28%, 19%, 9%, and 4.5%) using a donor solution of (a) 40% propylene glycol(PG) or (b) 40% propylene glycol with 30% cyclodextrin (PG+CD).

TABLE 2.
Flux ($\mu g/hr/cm^2$), permeability coefficient (cm/hr), and lag time of melatonin from vehicles through ethylene vinyl acetate membranes.

| Vehicle: 40% PG (v/v) in Phosphate Buffer pH 6.1 | | | | |
|---|---|---|---|---|
| VAc content | Flux(n=3) ($\mu g/hr/cm^2$) | MT Solubility (mg/ml)[b] | Permeability ($cm/hrx10^{-5}$)[a] | Lag time |
| 28 % | 0.795 ± 0.14 | 9.587 | 8.292 | none |
| 19 % | 0.183 ± 0.0041 | 9.587 | 1.909 | 15.7 ± 5.4min |
| 9 % | 0.023 ± 0.0032 | 9.587 | 0.240 | 1.5 hrs |
| Vehicle: 40% PG (v/v), 30% 2-HPCD (w/v) in Phosphate buffer pH 6.1 | | | | |
| VAc content | Flux(n=3) ($\mu g/hr/cm^2$) | MT Solubility (mg/ml)[b] | P ($cm/hrx10^{-5}$)[a] | Lag time |
| 28 % | 0.487 ± 0.026 | 20.408 | 2.386 | none |
| 19 % | 0.123 ± 0.0032 | 20.408 | 0.603 | none |
| 9 % | 0.011 ± 0.0041 | 20.408 | 0.054 | 1.5 |
| 4.5 % | - [c] | 20.408 | - | - [c] |

[a]Permeability coefficient (P) = flux ($\mu g/hr/cm^2$) ÷ melatonin solubility ($\mu g/ml$).
[b]From reference 4.
[c]Not enough data points to calculate flux and lag time. Melatonin released in the receptor phase was first detected at 8 and 12 hrs, respectively, in the triplicate samples.

Diffusivity and solubility are the two key properties of membrane systems that influence diffusion and membrane substance selection for dosage form device. Diffusivity in polymer systems is affected by the size and shape of the active ingredient, the flexibility of the polymer chains, and the packing density or crystallinity of the polymer chains (6). Solubility of solutes in polymers involves many complex interactions between the solute and polymer such as polarity, hydrogen bonding, and steric effects. Solute solubility is low in polymer crystallites. Solubility decreases proportionately to increases in polymer crystallinity (7 and 8). In these experiments with melatonin, increasing vinyl acetate content of the EVAc membrane may reduce polymer crystallinity, and the polymer becomes rubbery and more permeable. This change in

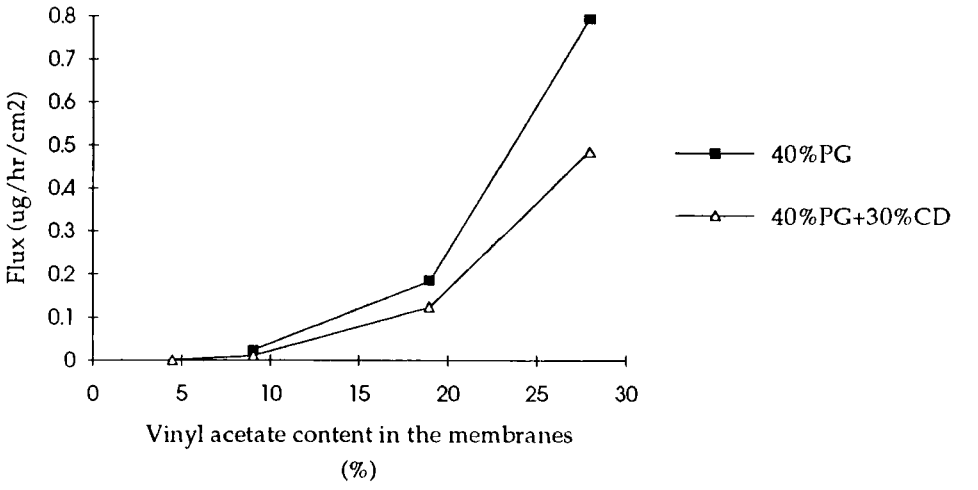


FIGURE 2.
Relation between flux of melatonin through ethylene vinyl acetate copolymer membranes and percent content of vinyl acetate in the membranes using a donor solution of (a) 40% propylene glycol (40%PG) or (b) 40% propylene glycol with 30% cyclodextrin (40%PG+30%CD).



membrane property may facilitate the movement of melatonin through the membrane.

Adhesive Studies

The resulting flux from the three adhesive systems is displayed in Table 3. Flux through the three adhesive system was not significantly different (p-value=0.06) and did not differ from the flux through the 28% VAc membrane without adhesive. The pressure transfer method of adhesive application resulted in a lag time of approximately 2.5 hours, while the spray method of application resulted in no lag time. Using ethyl acetate/heptane (50/50) as a solvent in the spraying method provided the highest flux. This combination solvent was also convenient to use because it evaporated quickly during spraying. Both solvents, isopropanol and EtOAc/heptane, provided a smooth, even, and tacky adhesive layer. The pressure transfer technique resulted in a rather rough adhesive layer, which separated from the membrane overnight.

When both melatonin and adhesive were dissolved in the same solvent prior to application to the membrane, there was very low melatonin flux (data not shown). The HPLC chromatogram of the receptor solution showed an unidentified peak.

TABLE 3.
Flux values and lag time of melatonin using a 28% vinyl acetate membrane with and witout adhesive.

| System[a] | Flux value ($\mu g/hr/cm^2$)[b] | Lag time (hrs) |
|---|---|---|
| 1 | $0.927 \pm 0.104$ | 0 |
| 2 | $1.01 \pm 0.082$ | 0 |
| 3 | $0.743 \pm 0.016$ | $2.451 \pm 0.021$ |
| No adhesive | $0.795 \pm 0.14$ | 0 |

[a] See Table 1.
[b] The flux values were statistically compared to the flux of melatonin through an identical system witout adhesive ($0.795 \pm 0.14$ $\mu g/hr/cm^2$). They was no significant difference (p=0.06).

TABLE 4.
Flux values of melatonin from transdermal delivery devices as a function of storage time.

| Storage time | Flux ($\mu g/hr/cm^2 \pm$S.D.)[a] | Lag time (hrs) |
|---|---|---|
| 1 day | $2.53 \pm 1.10$ | 0 |
| 2 days | $1.68 \pm 0.27$ | 0 |
| 3 days | $1.55 \pm 0.25$ | 0 |

[a]There was no significant difference.

Delivery Device Studies

Melatonin flux from aged TDD's is displayed in Table 4. Flux values from TDD's aged 1-day had a relatively high variation compared to those of the other two groups. There was no significant difference in flux (p-value=0.14) between the devices aged from one to three days. Mean steady-state flux was $1.88 \pm 0.6$ $\mu g/hr/cm^2$.

Although, the flux of melatonin was not significantly different, the cumulative amount of melatonin released over 12 hours was notably different (Figure 3). The highest burst effect was obtained from 3-day patches, following by that of 2-day patches and 1-day patches, respectively. This result is reasonable since a TDD aged by storage will have a membrane and adhesive

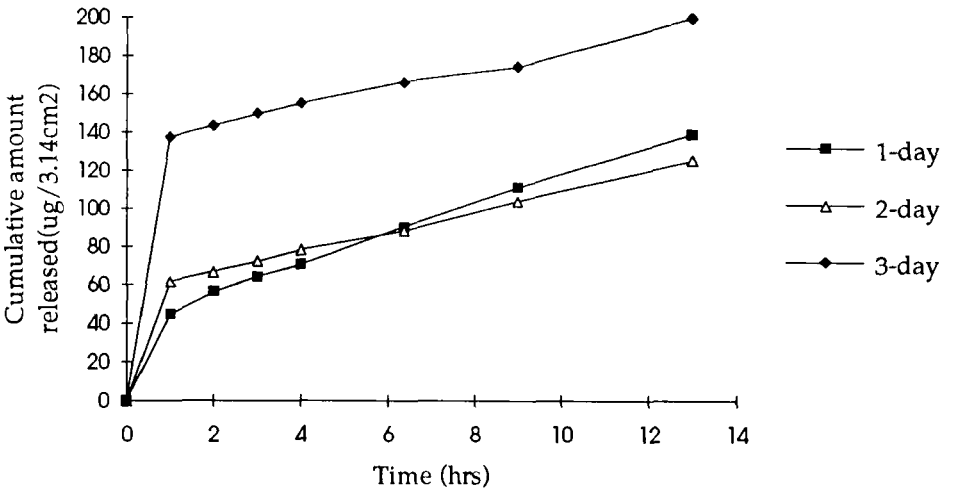


FIGURE 3.
Cumulative amount (μg) of melatonin released from transdermal delivery devices with storage. Values are expressed as mean (n=3).



saturated with melatonin, which will release at an initially high rate when placed in an desorbing environment (9). Thus, the longer storage time resulted in more melatonin penetrating into the membrane and the adhesive. The changing of the burst effect with storage time resulted in a different overall release rate (total amount released/12 hrs)as 3-day patches (4.89 ± 0.39 μg/hr/cm$^2$), followed by 1-day patches (3.40 ± 1.24 μg/hr/cm$^2$), 2-day patches (3.07 ± 0.29 μg/hr/cm$^2$), and freshly prepared patches (1.18 ± 0.10 μg/hr/cm$^2$).

Determination of the amount of melatonin remaining in the TDD reservoir showed that approximately 3.7 ± 0.8% of melatonin was probably trapped in the membrane and adhesive portion of the device as shown in Table 5.

Melatonin flux value from the developed TDD is higher than the flux of the drug through human skin, which has been estimated to be about 0.83 μg/hr/cm$^2$ (4). Therefore, patch size may be appropriately adjusted to deliver the drug to obtain desired plasma concentrations.

## CONCLUSIONS

The study has provided information for a reservoir type of melatonin TDD. The membrane studies showed increased permeability coefficients with increasing VAc content of the membranes for both vehicles. The highest flux

TABLE 5.
Mass balance of melatonin in transdermal delivery device. Values expressed as means plus standard deviation(n=3).

| Storage time (day) | Applied melatonin (mg) | Recovered melatonin in reservoir (mg) | Recovered melatonin in receptor (mg) | Trapped melatonin in the membrane and adhesive (mg)[a] |
|---|---|---|---|---|
| 1 day | 13.11 | 12.47 ± 0.16 | 0.14 ± 0.052 | 0.50 ± 0.21 |
| 2 day | 13.11 | 12.42 ± 0.06 | 0.12 ± 0.011 | 0.57 ± 0.052 |
| 3 day | 13.11 | 12.54 ± 0.27 | 0.20 ± 0.021 | 0.37 ± 0.28 |

[a] Applied MT minus recovered MT in device reservoir and receptor solution.



rate was obtained from the system of 40% PG vehicle through 28% VAc membrane. The 40% PG vehicle provided a greater flux compared to the 40% PG with 30% 2-HPCD vehicle, despite the higher melatonin solubility in the 40% PG with 30% 2-HPCD vehicle. The study of adhesive application onto the 28% vinyl acetate membrane either by the spray or pressure transfer method showed no significant difference in melatonin flux. However, the pressure transfer method was not acceptable. When melatonin was physically mixed with adhesive solution prior to the application, very low flux occurred, and the HPLC chromatogram had an unidentified peak. Delivery devices were constructed, and the effect of storage was studied. There was no significant difference in steady state flux among TDD's aged from one to three days. Nonetheless, the highest burst effect was obtained from 3-day patches, followed by that of 2-day patches and 1-day patches, respectively. Melatonin flux values from the developed TDD were approximately twice the estimated melatonin steady state flux through human skin, which has been estimated to be 0.83 $\mu g/hr/cm^2$.

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