Pharmaceutical Research, Vol. 25, No. 11, November 2008 (© 2008)
DOI: 10.1007/s11095-008-9696-y

Research Paper

Screening of Chemical Penetration Enhancers for Transdermal Drug Delivery Using Electrical Resistance of Skin

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Received May 2, 2008; accepted July 23, 2008; published online August 6, 2008

Purpose. A novel technique is presented for identifying potential chemical penetration enhancers (CPEs) based on changes in the electrical resistance of skin.
Methods. Specifically, a multi-well resistance chamber was designed and constructed to facilitate more rapid determination of the effect of CPEs on skin resistance. The experimental setup was validated using nicotine and decanol on porcine skin *in vitro*. The multi-well resistance chambers were capable of operating at 37°C in order to simulate the physiological temperature of the human body. Further, the utility of the multi-well resistance chamber technique was validated using standard Franz diffusion cells. Electrical resistance measurements were used to evaluate the potency of seven new potential CPEs, identified using virtual screening algorithms. From the resistance measurements, the chemicals 1-dodecyl-2-pyrrolidinone (P), menthone (M) and R(+)-3-amino-1-hydroxy-2-pyrrolidinone (C) were identified as the better penetration enhancers among the seven tested. Further, traditional permeation experiments were performed in Franz diffusion cells to confirm our findings.
Results. The permeation test results indicated that, of the three CPEs deemed potentially viable using the newly-developed resistance screening technique, both P and M increased the permeation of the test drug (melatonin) through skin in 48 h.
Conclusion. In summary, this resistance technique can be used to effectively pre-evaluate potential CPEs, thereby reducing the time required to conduct the permeability studies.

KEY WORDS: chemical penetration enhancers; Franz cell; multi-well chamber; permeability; resistance.

INTRODUCTION

Transdermal drug delivery is achieving preference over other forms of drug delivery due to its potential advantages, including minimal trauma induction, noninvasiveness, increased patient compliance and potential for continuous or controlled delivery (1,2). It has been successfully exploited for the delivery of low molecular weight and high permeating molecules such as nicotine. However, high molecular weight and low permeating drugs such as melatonin and insulin cannot permeate easily through the Stratum Corneum (SC), the top layer of the skin. This is because the SC acts as an effective barrier between the internal organs of the body and foreign substances.

An approach to breach this skin barrier to drugs is by using chemicals called chemical penetration enhancers (CPEs). Although the exact mechanisms by which CPEs function are not completely understood, Barry and Williams (3) introduced the lipid protein partitioning (LPP) theory, which suggests a chemical can enhance penetration by one or more of the following mechanisms: (a) disruption of SC lipids, (b) interaction with intracellular proteins, or (c) increased partitioning of the drug into the SC (4). The potency of a CPE in increasing the permeation of a drug is determined by quantifying the amount of drug permeated through skin in the presence of the CPE. Typically, these experiments are performed in Franz diffusion cells, and the amount of drug permeated is quantified by using analytical techniques, which include High Performance Liquid Chromatography (HPLC) (5,6) or Liquid Scintillation Counting (LSC) (2,7). Such measurements are resource and labor intensive, cost prohibitive and have limited throughput. In addition, these permeation experiments provide an indirect assessment of the effect of the CPE, which includes CPE–drug interactions. Further, there is no rational design in the criteria for selecting candidate CPEs for study, and this trial-and-error method can be time consuming. Thus, a need exists for a robust, quick alternate technique that can effectively replace the existing techniques and lead to a better understanding of the effect of the CPE alone on the skin.

Previously, electrical resistance of the skin has been used to assess the integrity of skin prior to experiments for *in vitro* dermal testing (8,9) and evaluating the corrosive effects of cosmetics on the skin (10). This suggests that the electrical properties of skin, especially the resistive (or conductive) properties, can be used to determine the effect of potential CPEs on the barrier properties of the skin. Recently,

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electrical conductance of skin was used as a technique to identify potential CPE's from binary mixtures of two chemicals at different concentrations (11). However, these experiments were carried out at conditions different from traditional permeation experiments. Further, detailed comparison of this technique with the traditional Franz diffusion cell has not been done.

In this study, resistive properties of skin were used to determine the changes in its barrier properties in the presence of various chemicals. A high throughput multi-well resistance chamber was designed and constructed, similar to a technique reported recently (12,13). The multi-well resistance chambers were equipped to perform the experiments at conditions identical to permeation experiments. First, experiments were performed using CPEs reported in the literature (14) and then extended to seven new potential CPEs which were identified by a virtual design algorithm (15–17). Herein, we show a significant agreement exists between the resistance technique and the standard permeation experiments; thus, we confirm the efficacy of the resistance technique for screening potential CPEs.

MATERIALS AND METHODS

Materials

Melatonin (≥98%, purity), decanol (≥95%, purity), nicotine (≥99%, purity), sodium dodecyl sulphate (≥99%, purity), 1-(1-adamantyl)-2-pyrrolidinone, R(+)-3-amino-1-hydroxy-2-pyrrolidinone, menthone (≥97%, purity), 1-(4-nitrophenyl)-pyrrolidine-2,5 dione, 1-dodecyl-2-pyrrolidinone (≥99%, purity), 3-methyl-2-oxazolidinone (≥99.5%, purity) were purchased from Sigma Aldrich (St. Louis, MO, USA). 1-Methyl-2-pyrrolidinone was obtained from ConocoPhillips (Bartlesville, OK, USA). Sodium chloride (≥99.5%, purity), and sodium phosphate dibasic heptahydyarte (≥99.5%, purity) were purchased from Fisher Scientific (Fair Lawn, NJ, USA). Potassium chloride (≥99%, purity) was obtained from Spectrum Chemical Mfg. Corp. (New Brunswick, NJ, USA). Potassium phosphate monobasic (≥99%, purity) was purchased from EM Science (Gibbstown, NJ, USA). Absolute ethanol (200 proof) was obtained from Aaper Alcohol and Chemical Co. (Shelbyville, KY, USA). HPLC grade acetonitrile was purchased from Fischer Scientific (Atlanta, GA, USA). All the chemicals were of analytical grade.

Skin Preparation

Porcine whole skin from the abdominal region of female Yorkshire pigs was purchased locally (Ralph's Packing Co., Perkins, OK, USA) prior to steam cleaning. Skin was washed under cold running water and the hair was clipped using an electric clipper (Wahl, Series 8900, USA). The exogenous tissues and subcutaneous fatty layers were removed carefully. The skin was then used immediately or wrapped in aluminum foil and stored at −20°C for future use. Frozen skin was thawed at room temperature for about 2 h before use. Skin membrane integrity was checked before starting the experiment by measuring resistance at a frequency and voltage of 0.1 KHz and 0.2 V, respectively, using a LCR Databridge (Instek, CA, USA) operated in parallel mode. Samples with an initial resistivity of 20 KΩ cm^2 or above with Phosphate Buffer Saline (PBS, pH—7.4, phosphate and sodium chloride concentrations of 0.001 and 0.137 M, respectively) were used in the experiments (18–20). Any skin samples with a lower resistivity than the above values were discarded. Quality of the prepared tissue was also assessed by performing histology on randomly chosen samples.

Validation of Resistance Technique in a Single Well

The resistance technique was validated using 1.77 cm^2 area vertical Franz diffusion cells (Perme Gear Inc., Bethlehem, PA, USA). Donor and receiver chambers were filled with 0.9% NaCl solution and resistance of the skin was measured using two 4 mm Ag/AgCl electrodes (*Invivo* Metrics, Healdsberg, CA, USA), one each in the donor and receiver chamber (through the sampling port). Next, NaCl solution was emptied from the donor chamber and the cell was filled with 500 μL of 15% (by wt) sodium dodecyl sulphate (SDS) in water, as reported in the literature (21). After 20 h incubation with SDS at room temperature, the donor chamber was replaced with fresh 0.9% NaCl, and the resistance was measured.

The Resistance Reduction Factor, RF, (also referred to in the literature (21) as the damage ratio) was calculated. It is defined as the ratio of the initial resistance value at time 0 to the resistance value of the sample obtained at time *t*, or:

$$\mathrm{RF} = \frac{R_0}{R_t}$$

The initial resistance reading, R_0, was taken after incubating the skin with the chemical for at least 10–15 min to reduce the variability in the measurements acquired.

Multi-well Resistance Chamber

A schematic diagram of the resistance chamber is shown in Fig. 1. It consists of two half-inch thick Teflon plates fixed to a Teflon Petri dish. Five holes with a diameter of 0.794 cm were drilled into each Teflon plates. The holes in the top plate serve as donor chambers, and the holes in the bottom plate serve as receiver chambers, as in Franz diffusion cells. Porcine skin was placed between the receiver and donor plates with the stratum corneum facing the donor wells, and the two plates were clamped together tightly. The Petri dish was filled with PBS such that the receiver chambers were completely filled with PBS, which was assured by checking the skin resistance; presence of air pockets between the skin and the receiver chambers showed very high resistance values since air has low conductivity. Resistance readings were taken using a common electrode placed beneath the receiver plate and the other placed sequentially into each donor well, as shown in Fig. 1.

The validation of the electrical resistance technique for identifying potential CPEs was undertaken in two steps, as follows.

First, the results from the resistance chamber using porcine skin *in vitro* were compared to results from the Franz diffusion cell experiments (described in the section below) for two systems:

1. Nicotine at a concentration of 100 mg/mL in PBS. Nicotine was selected because it is a low molecular

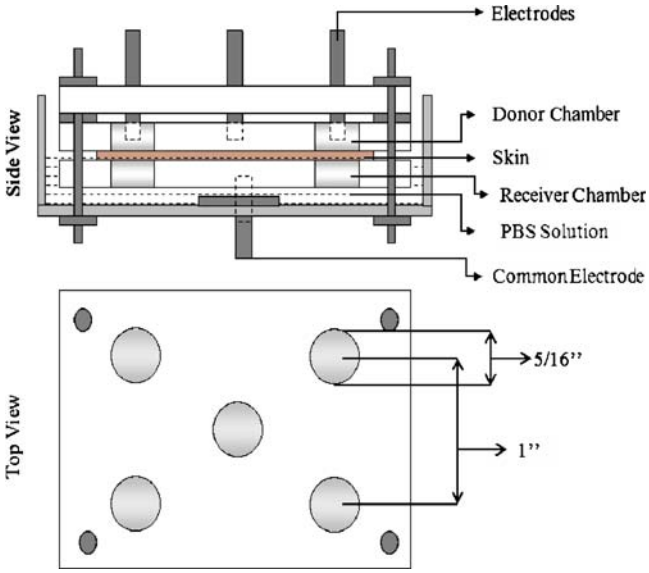

Fig. 1. Schematic diagram of the multi-well resistance chamber (*top* and *side views*).

weight, highly permeating chemical. The experiments with nicotine were performed at room temperature.

2. Decanol at a concentration of 5% (wt/*v*) in 1:1 PBS and ethanol solution. Decanol is reported in the literature (14) as a good permeation enhancer. The experiments with decanol were performed by maintaining the receiver chambers at 37±1°C using 0.3175 cm copper tubing below the receiver plate through which hot water was re-circulated using a peristaltic pump and a constant-temperature water bath.

Second, resistance experiments were performed to evaluate the potency of seven potential CPEs: [1-(1-adamantyl)-2-pyrrolidinone (A), 1-methyl-2-pyrrolidinone (B), R(+)-3-amino-1-hydroxy-2-pyrrolidinone (C), menthone (M), 1-(4-nitro-phenyl)-pyrrolidine-2,5 dione (N), 1-dodecyl-2-pyrrolidinone (P), 3-methyl-2-oxazolidinone (Q)]. The chemical structures of the seven CPEs are given in Fig. 2. These candidate CPEs were generated using computer aided molecular design (CAMD) techniques, in which structure-based property models for skin permeation coefficient (K_P), octanol–water partition coefficients (K_{OW}) (22), melting point (MP) (15), aqueous solubility, and skin sensitization were used in conjunction with genetic algorithms to identify potential CPEs. The physio-chemical properties of the CPEs were given in Table I. All seven potential CPEs were tested at a concentration of 5% (wt/*v*) in 1:1 PBS and ethanol solution with the receiver chambers maintained at 37±1°C.

Vertical Franz diffusion cells with an exposure area of 0.64 cm^2 were used for validating the multi-well resistance chamber measurements. Receiver chambers of the diffusion cells were filled with PBS. Donor chambers were filled with 5% (wt/*v*) of decanol in 1:1 PBS and ethanol solution or 100 mg/mL of nicotine in PBS. Experiments were conducted by maintaining the receiver chamber of the diffusion cells at 37±1°C by a re-circulating water jacket around it. Skin resistance was measured with Ag/AgCl electrodes as mentioned in the validation experiments with SDS. At different time intervals, the RF was determined as described in "Validation of Resistance Technique in a Single Well".

In the above experiments, a donor chamber filled with 1:1 PBS and ethanol solution alone served as a control, except for the experiments with nicotine where the PBS solution was used as control. RF values were calculated at different time intervals (0, 3, 6, 12 and 24 h).

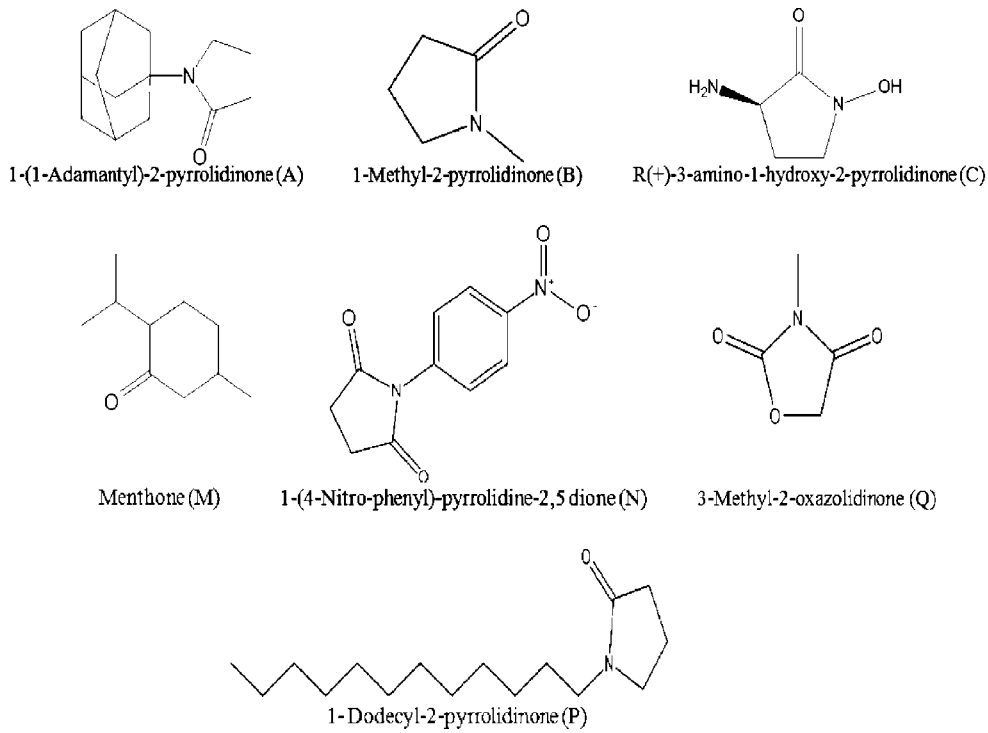

Fig. 2. Chemical structures of the seven potential CPEs investigated in this study.

Table I. Physio-chemical Properties of the Potential CPEs

Legend	CPE	CAS no.	MW (Da)	MP (K)	log(K_P)	log(K_{OW})	Aqueous solubility (n) (mg/mL)
A	1-(1-Adamantyl)-2-pyrrolidinone	NA	219.36	330.46	−1.89	3.24	0.24
B	1-Methyl-2-pyrrolidinone	872-50-4	99.13	239.51	−3.32	−0.006	273.03
C	R(+)-3-Amino-1-hydroxy-2-pyrrolidinone	123931-04-4	116.12	260.95	−3.79	−1.01	298.47
M	Menthone	10458-14-7	154.28	298.17	−1.44	2.93	0.44
N	1-(4-Nitro-phenyl)-pyrrolidine-2,5 dione	35488-92-7	220.19	415.92	−2.36	1.42	0.44
P	1-Dodecyl-2-pyrrolidinone	2687-96-9	253.43	260.82	−1.46	4.07	0.01
Q	3-Methyl-2-oxazolidinone	19836-78-3	115.00	302.18	−2.67	0.28	97.88

NA Not available

Permeation Experiments

Melatonin, a weakly hydrophobic and low permeating drug was used for performing the permeation studies. It is used to treat sleep disorders and alleviate jetlags after flight journeys. It has an octanol/water partition coefficient, log (K_{OW}) of 1.2, molecular weight of 232.28 Da and aqueous solubility of 3.176±0.06 mg/mL (23). Due to its short biological half-life and hepatic first pass metabolism during oral administration, transdermal delivery of melatonin was extensively studied during the last decade (14).

Preparation of Solutions

Melatonin solution (175 mg/mL) was prepared in a mixture of 40:60 (by volume) of water and ethanol. Enhancers were added to these solutions to give 5% (wt/*v*) concentrations. Round bottomed flasks containing the solutions were sealed with parafilm, wrapped in aluminum foil, and placed in an environmental shaker at 37°C for 24 h. The solutions were then filtered using 0.45 μm nylon filters (Fischer, Atlanta, GA, USA). These solutions were stored at −20°C until used. Phosphate Buffer (PB, pH—7.4, monosodium phosphate monohydrate and disodium phosphate heptahydrate concentrations of 13.55 and 46.41 mM, respectively) was used in the permeation measurements instead of PBS as in the case of resistance experiments. This was done to compare the permeation experimental set up with the literature results for melatonin using PB in the receiver chamber (14).

Experiments

The permeation experiments were carried out using 0.64 cm^2 vertical Franz diffusion cells having donor and receiver chambers capacities of 1 and 5 mL, respectively. The receiver chamber temperature was maintained at 37±1°C by re-circulating hot water through the water jacket around the receiver chamber.

The receiver chambers of the Franz diffusion cells were filled with the PB and stirred continuously using a magnetic stir bar. The skin sample was placed (stratum corneum facing the donor chamber) between the donor and receiver chambers, and a 1 mL solution containing melatonin and the enhancer in the carrier was placed inside the donor chamber. At different times (3, 6, 9, 12, 18, 24, 36 and 48 h), 0.1 mL samples were retrieved from the receiver chamber using a syringe. Fresh PB was added to fill the receiver chambers.

HPLC Analysis

Melatonin concentration was quantified using a HPLC system (Dionex Co., Sunnyvale, CA, USA) following the procedure reported in the literature (24). In brief, a Waters Symmetry 300 column (4.6×150 mm dimensions, 5 μm and 90 Å packing) and a mobile phase consisting of 40:60 (by volume) acetonitrile and water were used. Flow rate was set at 1.0 mL/min, injection volume at 30 μL and temperature at 30°C.

The retention time observed was near 4 min. Melatonin was detected at 304 nm and quantified using a standard curve developed as follows. Melatonin (211 g) stock solution was prepared by dissolving 211 mL of filtered PB on a shaker at 37°C for 24 h. The solution was free of precipitates since the solubility of Melatonin in buffer solutions is ~1.6 mg/mL (25). This solution was diluted serially to give solutions of 0.5, 0.25, 0.1, 0.05, 0.025, 0.01, 0.005, 0.0025, 0.001, 0.0005 and 0.0001 (all in milligram per milliliter) concentrations. These solutions were used for developing a calibration curve on each day of the analysis prior to analyzing the samples.

A representative data set relating area under the curves from HPLC analysis to various concentrations of melatonin is showed in Fig. 3. A linear regression was fitted to the data with a correlation coefficient (R^2=0.99). This calibration curve was used to calculate the concentration of melatonin from each experiment. The percent difference from actual value (%DFA) and the percent relative standard deviation

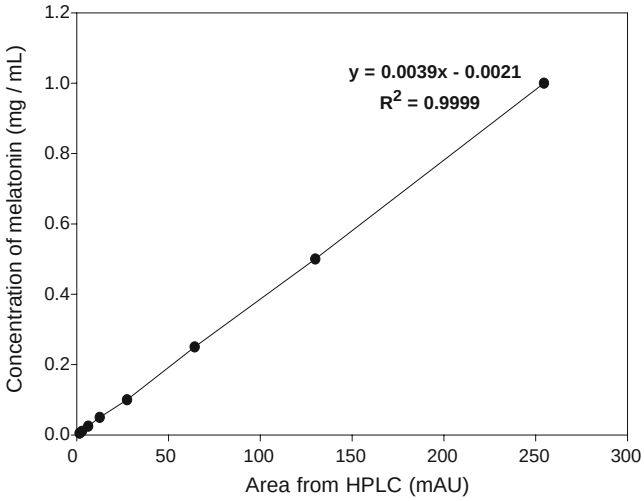

Fig. 3. Representative calibration curve for the HPLC analysis of melatonin.

(%RSD, percent ratio of standard deviation to the mean of multiple determinants) were calculated to determine the accuracy and precision of the HPLC analysis and both were found to be less than 5% (data shown in Table II).

Determining the Permeability Coefficient

The following steady-state equation was used to calculate permeability of the skin:

$$\text{Amount of drug permeated} = A_m \times C_0 \times P \times t \qquad (1)$$

where A_m is the exposure area of the skin sample (=0.64 cm^2), C_0 (mM) is the initial concentration in the donor chamber, and P is the permeability $\left(\frac{D_m K_m}{L}\right)$ of the membrane. The latter is given in terms of D_m the diffusion coefficient, K_m the partition coefficient, and L the thickness of the skin sample. In this study, the amount of drug permeated was calculated as the total amount of drug permeated through skin during the steady-state permeation period (from t=24 h to t=48 h) and the amount sampled from the receiver chamber at 24 and 36 h.

The permeability factor, defined as the ratio of the permeability coefficient obtained from the potential enhancer to that of the control, was calculated as follows:

$$\text{Permeability Factor} = \frac{P_{CPE}}{P_{Control}}$$

Then the permeability factors in the presence of the CPEs considered were compared to the RFs obtained from the resistance chamber.

Statistical Analysis

All experiments were performed at least three times. The coefficient of variation, CV, was calculated by the ratio of the standard deviation to the mean of multiple experiments in order to compare the results from the SDS validation studies to the reported literature value (21). Single factor one-way analysis of variance (ANOVA) with a 95% confidence interval was used to determine the significant difference between the RFs of the candidate CPEs tested.

RESULTS

Validating the Resistance Technique

Validation of the resistance technique was performed using SDS. The results showed that the RF was 10 (CV=0.15) after 20 h. This was comparable to the reported literature value of 11.3 (CV=1) (21). Moreover, the CV was significantly lower than the reported value. Variability in the experimental measurements was reduced by the following precautions:

1. Using skin samples which had similar initial resistance value in all the experiments,
2. Using porcine abdominal skin instead of the dorsal skin since it has less variation in thickness and hair density, and
3. More importantly, not washing the skin with detergent and not allowing it to dry prior to final resistance reading. To accomplish this, the SDS solution was wiped off using tissue paper, because washing with soap may result in accumulation of the surfactants, which may give false resistance value by interacting with the NaCl solution. Also, allowing the skin to dry before taking the resistance readings may damage its integrity.

Table II. Precision and Accuracy of the HPLC Analysis for Melatonin

Concentration of melatonin (μg/mL)	Percent DFA[a]	Percent RSD[b]	n[c]
7.50	4.00	1.90	5
300.00	0.94	2.17	5

[a] Percent DFA: ((mean value − actual value)/actual value)×100
[b] Percent RSD: (standard deviation/mean value)×100
[c] n: Number of replications

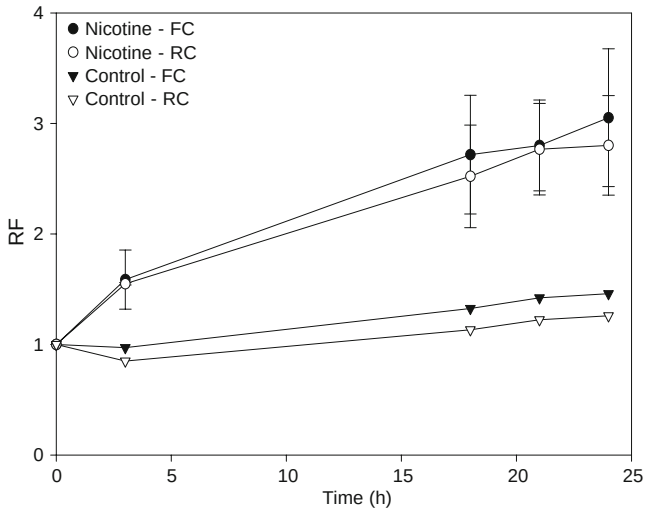

Fig. 4. Comparison of RFs obtained from the resistance chamber and the Franz diffusion cell in the presence of nicotine. The *error bars* correspond to the standard deviation (n=3) of replicate measurements. *FC* Franz diffusion cell, *RC* resistance chamber.

Validating the Multi-well Resistance Chamber

The multi-well resistance chamber was validated against the results from the Franz diffusion cell using nicotine and decanol.

For nicotine, a linear increase in the RF with time was observed in the resistance chamber (Fig. 4), while there was no significant change in RF of the control. RF values from Franz diffusion cells showed essentially identical behavior. This suggested that there is no influence of the adjacent wells on skin resistance, and it confirmed the possibility of using the multi-well resistance chamber to perform multiple experiments simultaneously.

For decanol, interestingly, no significant change (Fig. 5) was observed in RFs between decanol and control even after 48 h. Since these experiments were performed at room temperature (21°C), unlike the permeation experiments, we questioned whether the temperature of the receiver chamber

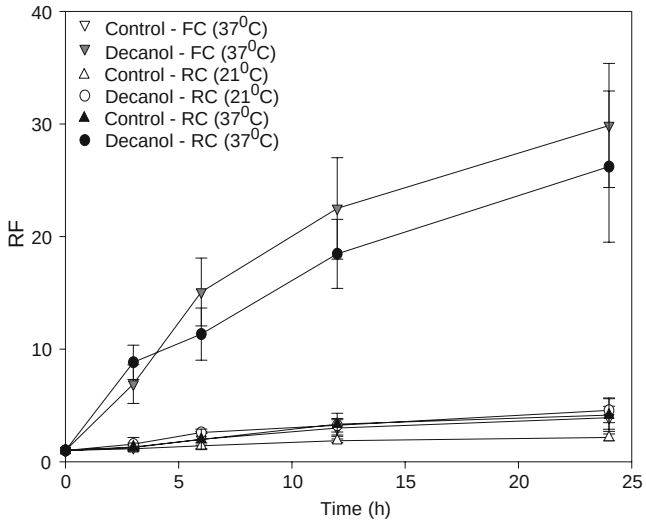

Fig. 5. Effect of temperature on RF in the presence of decanol in comparison to Franz diffusion cell experiments. The *error bars* correspond to the standard deviation for triplicate measurements. *FC* Franz diffusion cell, *RC* resistance chamber.

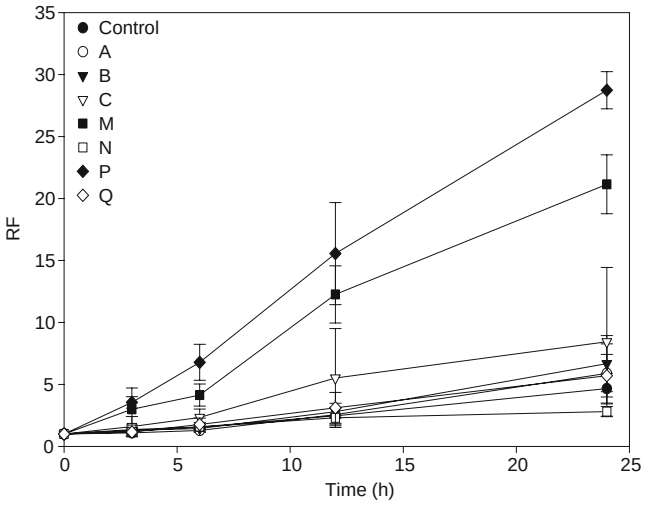

Fig. 6. RF at 24 h for seven CPEs. The *error bars* correspond to the standard deviation for triplicate measurements. 1-(1-adamantyl)-2-pyrrolidinone (*A*), 1-methyl-2-pyrrolidinone (*B*), R(+)-3-amino-1-hydroxy-2-pyrrolidinone (*C*), menthone (*M*), 1-(4-nitro-phenyl)-pyrrolidine-2,5 dione (*N*), 1-dodecyl-2-pyrrolidinone (*P*), 3-methyl-2-oxazolidinone (*Q*).

could affect the permeability or the RFs value. To test this possibility, the temperature of the receiver chamber of multi-well resistance was increased to 37°C (physiological body temperature), similar to permeation experiments. The RF in the presence of decanol at 37°C was significantly higher than the control value. To check the consistency of these results, they were compared with those of the Franz diffusion cell. The RFs obtained in the multi-well chambers in the presence of decanol and control at 37°C were comparable to those obtained using Franz diffusion cell (Fig. 5). Further, no increase in RF was observed when experiments were performed by maintaining the receiver chamber at 21°C in Franz diffusion cells. This suggests that the temperature of the receiver chamber significantly influences RF values. At lower temperatures, (less than 37°C) the skin resistance might be higher due to increased rigidity of the lipid bilayers. At higher temperatures, the individual lipid molecules may have more vibrational energy, which makes the lipid bilayers more fluidic and may offer less resistance. All subsequent experiments were performed maintaining the receiver chamber at 37°C.

Evaluation of New CPEs

The behaviors of the seven new potential CPEs were evaluated in the multi-well chamber using the resistance technique. The results given in Fig. 6 indicate that three CPEs (C, M and P) increased the RFs with respect to the control sample. The other four CPEs showed no significant effect on RFs with respect to the control ($P>0.05$). Although RF value had increased in the presence of CPE C, it was statistically similar to the control ($P>0.05$). Potential CPEs P and M were relatively more potent than C in increasing the RF value. The difference between the RF values of P and M with respect to the control were statistically significant ($P<0.05$). Samples P and M also show a continuous increase in RF- without reaching saturation, even after 24 h.

Experiments were performed to test the abilities of the CPEs to enhance the permeation of a drug through porcine skin, using melatonin as the test drug for 48 h. The amount of melatonin permeated through the skin was quantified using HPLC technique. The flux of melatonin through porcine skin was 4.21±0.21 μg $(cm)^{-2}$ h^{-1}, which is comparable to 4.5± 0.8 μg $(cm)^{-2}$ h^{-1} reported by Andega *et al.* (5). There was significant enhancement in the permeation of melatonin through skin in the presence of CPEs P and M, which is consistent with the results from the multi-well resistance chamber. The only exception was in skin exposed to CPE C, where no significant permeation of the drug was observed relative to the control (K_P in the presence of CPE C is almost equal to the control value). The permeability coefficient, K_P of melatonin in the presence of seven CPEs individually was calculated and given in Table III.

To correlate the RFs from the resistance chamber to the permeability factors from the permeation experiments, RF values obtained after 24 h were plotted against the permeability factors for the seven CPEs. A regression line was drawn through all the data points. The results obtained (Fig. 7) show a good correlation ($R^2=0.98$) between the permeability factors and the RFs of the CPEs. This suggests

Table III. Permeability Coefficient (K_P) of Melatonin for the Seven CPEs

Legend	CPE	K_P (10^{-5} cm/h)
	Control	3.4±0.5
A	1-(1-Adamantyl)-2-pyrrolidinone	3.8±0.3
B	1-Methyl-2-pyrrolidinone	4.7±1.1
C	R(+)-3-Amino-1-hydroxy-2-pyrrolidinone	3.7±0.1
M	Menthone	11.0±2.2
N	1-(4-Nitro-phenyl)-pyrrolidine-2,5 dione	3.6±0.5
P	1-Dodecyl-2-pyrrolidinone	15.6±3.6
Q	3-Methyl-2-oxazolidinone	4.5±1.0

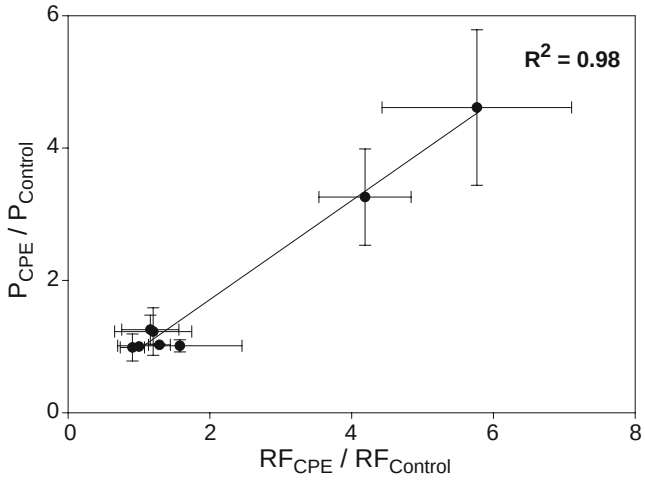

Fig. 7. Comparison of the resistance and permeation measurements. The *error bars* correspond to the standard deviation for triplicate measurements.

that the resistance technique could be used as an alternative to permeation experiments to evaluate CPEs. However, more data distributed uniformly over the correlation range would be required to confirm this finding.

The current experiments also indicate that the testing time can be reduced from 24 to 6 h without a significant change in the outcome. As shown in Fig. 6, using a reduced experimentation time (6 h), chemicals M and P showed significant increase in RFs with respect to the control. This would permit more experiments to be performed in a given time period, thus leading to a higher throughput.

DISCUSSION

In this study, the utility of using the electrical resistance of skin in a multi-well resistance chamber was investigated to identify potential CPEs and to increase the rate at which data can be obtained. First, the highly permeating, well investigated nicotine (26) was used to compare the RF values from the multi-well resistance chamber and a Franz diffusion cell. There was a good agreement between the two (Fig. 4). The results also demonstrated that there is no influence of the adjacent wells on the resistance measurements. Also, a higher difference could be observed in the RFs of control and nicotine at 37°C than at room temperature.

To investigate the effect of temperature on RF values, experiments were performed on decanol while maintaining the receiver chambers at 37°C and 21°C. As shown in Fig. 5, a significant difference in the RF values was observed for 37°C and 21°C. Others have reported the utility of the resistance technique in screening the CPEs (12,13). These studies did not compare to the traditional Franz diffusion cell experiments. Moreover, they did not maintain the receiver chamber at 37°C, similar to the permeation experiments.

Our findings support similar observations in the literature (27) that temperature plays a significant role in assessing the barrier characteristics of skin in the presence of CPEs. Therefore, experiments designed to identify potential CPEs should be performed at temperature conditions consistent with those of permeation experiments.

The electrode set-up reported in the literature (12) to measure the electrical properties may introduce significant variability since the common electrode was inserted into the dermis of the skin. The length of insertion, as well as the path-length of electrical conductivity, could vary from well to well. Thus, this type of electrode set-up could have significant influence on the resistance measurements. The potential problem was avoided in our study by inserting the common electrode in an electrolyte bath as shown in Fig. 1, ensuring that length of current travel is equal in all the wells.

Using the multi-well resistance chamber, three potential CPEs were identified from the seven tested. The results from the multi-well resistance chamber were confirmed by testing the enhancement of the drug melatonin in the presence of the CPEs individually. From the permeation experiments, CPEs M and P showed positive results by increasing the permeation of melatonin through skin. These results indicate that, using the resistance technique, potential CPE's can be effectively pre-screened from a larger pool of chemicals, thus reducing the time required to conduct the permeability studies. For example, in our study CPEs A, P, Q and B can be avoided during the permeation experiments since they were not effective at the concentrations used. Actually, a better strategy would be to examine the behavior of these chemicals at higher concentrations. Also, by using the resistance technique, CPEs can be tested in the absence of the drug considered, which can be useful in understanding the CPE interactions with the skin.

The permeation experiments were performed only with melatonin, which is a weakly hydrophobic and low molecular weight drug, in order to confirm the results from the resistance technique. Drugs with a range of lipophilic behaviours and molecular weights should be carefully selected and experiments have to be performed to fully understand the utility of resistance technique with different classes of drugs. However, the main aim of our study was to explore a methodical approach to screen the CPEs for their potency using the resistance of skin, and the study of enhancement abilities of CPEs with drugs having different properties is beyond the scope of this paper.

Experimental variability usually associated with biological experiments was minimized by storing the skin at −20°C instead of −80°C and by checking the integrity of the skin samples prior to each experiment. Porcine skin from the same breed and sex was maintained throughout the experiments. The experiments were performed in a saturated environment to avoid the evaporation of the PBS from the Petri dish. By this technique, potential CPEs can be identified prior to evaluating their potency in increasing the permeation of a drug. A good correlation was developed between the RFs of the CPEs from resistance technique and the permeability factors obtained from permeation experiments. Nevertheless, additional experiments are required to include more chemicals and assess the viability of potential CPEs at different concentrations. Toxicity of the potential CPEs should be evaluated using histology or *in vitro* cell interaction studies.

ACKNOWLEDGEMENT

Financial support for this research was provided by the National Institutes of Health (#1R21EB005749-01A1).

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