

Electret Enhances Transdermal Drug Permeation

Narasimha Sathyanarayana MURTHY,^{*,a} Vishwanath Anantharamaiah BOGUDA,^b and Kotrappa PAYASADA^c

^aDepartment of Pharmaceutics, The University of Mississippi; University, MS 38677, U.S.A.; ^bBangalore Institute of Pharmacy Education and Research; Bangalore-560064, Karnataka, India; and ^cRad Elec Inc.; Frederick, MD 21704, U.S.A. Received July 16, 2007; accepted October 9, 2007; published online November 7, 2007

Electrets are polymeric discs that carry semi permanent electrostatic charge. These provide electrostatic potentials in the range of 500 to 3000 V. In the current work, the effect of electret exposure on the skin permeability was investigated. Transdermal transport studies were carried out across porcine epidermis in Franz diffusion cells. Salicylic acid, fluorescein labeled dextrans (FD) and propofol were used as test diffusants. The ability of electret to enhance the transdermal permeation of salicylic acid was studied *in vivo* in Sprague Dawley rats. Electret enhanced the permeability of porcine epidermis to salicylic acid. The enhancement factor increased with the surface voltage, however it was independent of the nature of charge (+ or -). The enhancement by electret was cut-off at 1 kDa, as interpreted by studying the transport of FD. The electrets decreased the permeability of skin to propofol, a lipophilic diffusant. Pretreatment of porcine epidermis enhanced the iontophoretic transport of salicylic acid, whereas the same did not enhance the transport of salicylic acid by electroporation. It is most likely that electret exposure renders the lipid domains of stratum corneum more permeable to polar molecules and in turn hampers the diffusion of nonpolar diffusant.

Key words electret; skin; drug delivery; transport; permeation; enhancer

Transdermal drug delivery has been an attractive research area due to its advantages over the other routes of drug delivery. Administration of drugs by transdermal route is also associated with less severe side effects and relatively lesser dose requirement. Transdermal administration provides a port for controlled delivery of drugs. However, transdermal drug delivery is limited by the barrier properties of the upper most layer of the skin, stratum corneum. Stratum corneum consists of closely packed corneocytes and the intercellular lipids. The intercellular lipids are in lamellar arrangement.¹⁾ Not all the drugs can permeate through the skin in therapeutically required quantities. Generally drugs that are lipophilic permeate through the skin successfully, whereas hydrophilic drugs and macromolecules (>600 Da) undergo limited penetration. Therefore, permeation enhancers need to be used to enhance the transdermal permeation of polar molecules. Although several chemical and physical permeation enhancers have been found to be efficient in enhancing the transport of hydrophilic drugs and macromolecules, research is ongoing to explore novel and relatively more efficient and safer methods to enhance the skin permeability to polar drugs.²⁾ Moreover, use of chemical permeation enhancers over prolonged duration would most likely cause local and systemic untoward effects. The major concern with the use of physical forces, such as electric current, ultrasound, magnetic field, and laser has been the risk of hampering the skin barrier permanently.³⁾ In this direction, the possibility of utilizing electrets for enhancing the skin permeability to hydrophilic drugs was investigated. Electret is an electrically charged Teflon[®] disk that carries semi permanent electric charge. It is characterized by the surface potential in volts. These provide surface potentials from 500 to 3000 V, easily measurable using an electret reader. Electrets and the electret readers used in this study are commercially available under the brand name E-PERM[®] (Rad Elec Inc., Frederick, MD, U.S.A.). The active surface of the electret is about 12 cm². These are widely used as components of electret ion chambers used for meas-

uring radon and radiation.^{4–6)} These are also used in electronic devices which require high electrostatic fields without the necessity of high batteries or voltage units.

Electret was found to enhance the penetration of hydrophilic drugs (but not lipophilic drugs) across the skin.⁷⁾ However, the electret effect disappears when moisture content in the formulation increases. The electrets seem to work well with the topical bases which do not have moisture in them. The surface voltage of electret was not affected significantly by the presence of white petroleum jelly coating on the E-PERM[®] electrets. It is also possible to use a thin layer of removable uncharged Teflon[®] to cover the surface of the electret. This allows electric field to go through and at the same time protects the electret surface from getting contaminated. This feature allows electret to be placed in closer vicinity to experimental objects such as skin. Cui *et al.* have reported the effect of electret on the skin permeability of methyl salicylate.⁷⁾ In the present work, we planned to evaluate the effect of electrets on the skin permeability of salicylic acid *in vitro* and *in vivo* and also investigated the influence of molecular weight of the diffusants on the electret's transport enhancement efficiency.

MATERIALS AND METHODS

Materials Electrets (E-PERM[®]) and the electret reader were provided by Rad Elec Inc., Frederick, MD, U.S.A. Fluorescein labeled dextran (FD) of different molecular weight, the phosphate buffered saline without calcium (PBS, pH 7.1), salicylic acid, methanol and acetonitrile were purchased from Sigma Chemicals (St. Louis, MO, U.S.A.).

Skin Porcine belly skin was obtained freshly from a local abattoir (not scalded as per request). Pieces of the skin wrapped in aluminum foil were heated to 60 °C for 2 min in a water bath and the epidermis was gently peeled off the skin. The fresh epidermis was placed on glass microscope slides and kept dry at 4 °C until used. Prior to use, the epidermis

* To whom correspondence should be addressed. e-mail: murthy@olemiss.edu

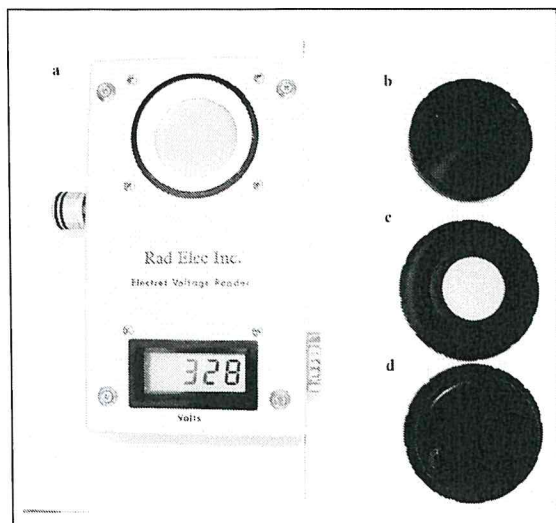


Fig. 1. Electret and Electret Holder

a. Electret reader, b. electret cover, c. middle right is the electret in the holder; d. bottom right is the covered electret.

was hydrated with normal saline (0.9% w/v sodium chloride) for 1 h. A sample was used only if the resistance was greater than $30 \text{ k}\Omega \cdot \text{cm}^2$.

General Experimental Setup A vertical Franz-type diffusion apparatus (Logan Instruments Corp., Somerset, NJ, U.S.A.) was used for transport measurements across the porcine epidermis. The temperature of the chamber was regulated at $37 \pm 1^\circ \text{C}$ by water circulation. A piece of porcine epidermis was placed between two compartments of the diffusion apparatus, one serving as the donor and other as the receiver compartment. The area of epidermis available for diffusion was 0.64 cm^2 . The receiver compartment (5 ml) was filled with PBS (pH 7.1, 150 mM sodium chloride), and $500 \mu\text{l}$ of diffusant solution was placed in the donor compartment.

The resistance of the porcine epidermis was measured before transport studies in a similar diffusion cell containing the electrodes made of 0.3-mm-diameter platinum wires, forming concentric rings. They were placed 2 mm away from the skin in both the donor and the receiver compartments.

Iontophoresis was carried out using Iomed Phoresor II dose controller and electroporation was carried out using a pulse generator (BTX 830M, San Diego, CA, U.S.A.) delivering single or multiple unipolar square pulses.

In Vitro Transport of Salicylic Acid by Direct Exposure to the Electret This is a permeability study of salicylic acid across the excised porcine skin epidermis. Salicylic acid ointment of 1% w/v (in white petroleum jelly) was weighed (250 mg) and filled in the donor compartment. The electret was placed close (*ca.* 1 mm distance) from the surface of the formulation in the donor compartment. The receiver compartment was filled with PBS (pH 7.1). In the case of propofol transport studies, $200 \mu\text{l}$ of the propofol was placed in the donor compartment and the electret was placed at the vicinity of the surface of propofol.

In Vitro Transport Studies across Pretreated Porcine Epidermis The pretreated porcine epidermis was prepared by placing an electret above the surface of the skin at a dis-

tance of 1 mm. The porcine epidermis was sandwiched between the donor and the receiver compartment, receiver compartment was filled with PBS and the donor with the drug solution prepared in PBS. The amount of drug diffused into the receiver compartment was measured by sampling the medium at hourly interval for 10 h. The steady state flux was calculated as the slope of the linear part of the cumulative transport-time graph. In control experiments, the epidermis was exposed to blank electret (electret with zero surface voltage).

In Vitro Permeability Studies of High Molecular Weight Dextran Transport studies of fluorescein isothiocyanate (FITC), dextrans of molecular weight 1 kDa, 4 kDa, 10 kDa were (used as test permeants in aqueous solution at a concentration 2 mg/ml) carried out across the porcine epidermis pretreated with -3000 V electret. The transport experiments were carried out for 4 h and the cumulative transport in 4 h was determined.

Iontophoresis and Electroporation The two electrically mediated techniques, iontophoresis and high voltage electroporation were applied across the porcine epidermis pretreated with electret (-3000 V). Iontophoresis was carried out at 0.5 mA/cm^2 and the electroporation protocol was 30 pulses of 1 ms duration at 80 V and 1 Hz. Ag/AgCl electrodes were used as electrodes. The donor chamber electrode was connected to cathode in iontophoresis and electroporation as well. The cumulative transport was determined in a sample drawn after 4 h.

In Vivo Studies The *in vivo* studies were carried out in Sprague Dawley rats. The rats were anesthetized by ketamine and xylazine i.p. injection. Five hundred milligrams (1% w/v in white petroleum jelly) of salicylic acid ointment was applied on the shaved area (2 cm^2) of the skin. The control group of rats was exposed to blank electrets and the test group to the charged electrets at 1 mm from the surface of the skin for 2 h. The blood samples were obtained *via* jugular vein and collected in heparin coated tubes, centrifuged and the plasma was separated. The salicylic acid was extracted and analyzed.⁸⁾

Analytical Methods The diffusants in the receiver compartments were measured by fluorescence spectroscopy using Hitachi 2500 fluorimeter. Salicylic acid was analyzed by fluorescence emission intensity at 396 nm with excitation at 308 nm.⁹⁾ FITC dextrans were measured by the fluorescence emission intensity at 520 nm with excitation at 494 nm and propofol was measured by measuring the emission intensity at 310 nm with excitation at 276 nm.^{10,11)} There was no background fluorescence in the receiver compartment during any of these experiments. Salicylic acid (SA) in blood samples was measured by HPLC. The blood samples were centrifuged and the plasma was separated. SA in the plasma was extracted in benzene:ethyl acetate (1:1 v/v) after acidification of the samples with a drop of 5% *o*-phosphoric acid.^{8,9)} The solvent was evaporated at $4-8^\circ \text{C}$ under a stream of nitrogen and the residue was dissolved in PBS (pH 7.1). The recovery of SA by this method was $89.3 \pm 4.7\%$. Twenty microlitres of the sample was injected into HPLC. The mobile phase was 60:40: methanol: phosphate buffer (pH 7.1). The drug was detected by UV at 270 nm.

Statistical Analysis The curve fitting and statistical analysis was carried out using GraphPad Prism 3.03 soft-

ware. Student's *t*-test was used as the test for significance and a *p* value of less than 0.05 was considered statistically significant. The data points provided in the graphs are an average of 3–5 trials. The error bars represent standard deviation.

RESULTS AND DISCUSSION

In Vitro Transport Studies of Salicylic Acid The transport flux of salicylic acid in control was $3.12 \pm 0.53 \mu\text{g/h/cm}^2$. Three negative and positive electrets of 1000, 2000, 3000 V were used for the experiments. The surface voltage of the electrets did not vary by more than $\pm 5\%$. The transport of salicylic acid was enhanced significantly by the presence of electrets (Fig. 2). The enhancement factor increased with the increase in surface voltage of the electret. However, the nature of surface voltage (+ or -) did not make any difference on the transport enhancement ability. That is, at a given surface voltage, there was no significant difference in the enhancement factor between positive and negative electrets.

Cui *et al.*⁷⁾ used rat skin in their studies on electret mediated delivery of methyl salicylate. Moreover, methyl salicylate is relatively less polar and therefore is likely more permeable across the skin than salicylic acid. These two major differences explain the difference in the enhancement factor reported for methyl salicylate by Cui *et al.* and that observed for salicylic acid in the current work.

Generally, the physical permeation enhancer mediated drug delivery systems enhance the transdermal transport of drugs by electrokinesis of the diffusant and/or by electropermeabilization of the membrane. In the present experimental conditions, salicylic acid exists absolutely in unionized form in the paraffin medium. Therefore no driving force contributes to the enhanced transport due to charge repulsion. Moreover, the transport enhancement was of the same order in case of both positive and negative electrets. Therefore it is most likely that the electret enhanced the transport of salicylic acid by rendering the skin relatively more permeable.

In Vitro Permeation Studies of Propofol Propofol is a general anesthetic drug administered by i.v. infusion. It is a liquid at room temperature and has poor water solubility. It is highly lipophilic in nature and therefore we have chosen propofol as a candidate for this study. It is most likely that propofol transports across the porcine epidermis *via* lipid pathway. Propofol transport across the porcine epidermis pretreated with electrets was significantly decreased and was proportional to the surface voltage (Fig. 3). From this data one can speculate that electrets are likely to affect the lipophilic pathways in the stratum corneum. However more structural studies are required to demonstrate the actual mechanism of electret on the lipid domains of the stratum corneum. This could be the reason for decrease in the transport of propofol across the porcine epidermis when exposed to electrets.

In Vivo Studies The plasma concentration of salicylic acid at different time points were fitted to a noncompartment pharmacokinetic model and the C_{max} , T_{max} and AUC_{0-t} were calculated (Fig. 4). The T_{max} (6 h) of salicylic acid did not differ between the control and the experimental group. C_{max} in the case of control group was $5.18 \pm 1.3 \text{ mg/l}$ and in the case of electret exposed group it was $12.27 \pm 3.07 \text{ mg/l}$. The

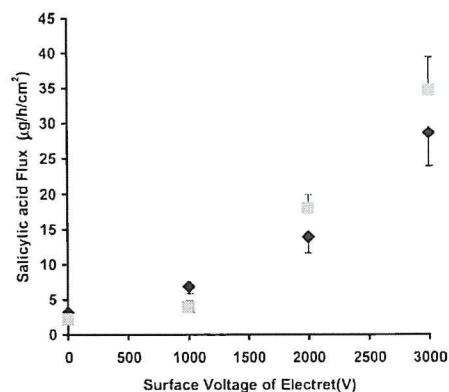


Fig. 2. Transport Flux of Salicylic Acid across the Porcine Epidermis When Exposed to Positive Charge Electret (■) and Negative Charge Electret (◆) of Different Surface Voltage

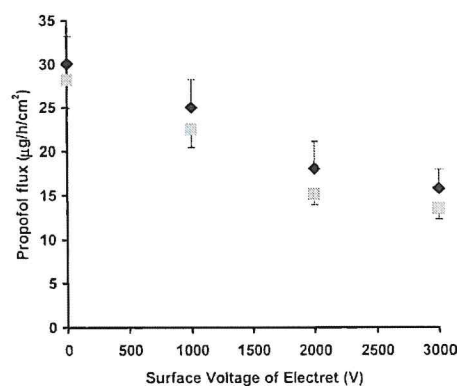


Fig. 3. Transport Flux of Propofol across the Porcine Epidermis When Exposed to Positive Charge Electret (■) and Negative Charge Electrets (◆) of Different Surface Voltage

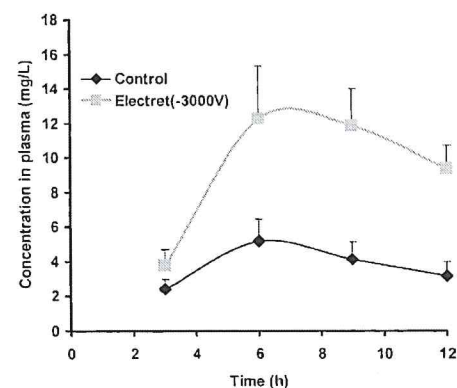


Fig. 4. Plasma Concentration–Time Profile of Salicylic Acid in Rat Model. Control group was exposed to blank electret (◆) and the experimental group (■) was exposed to the electret of surface voltage -3000 V.

AUC_{0-t} data indicate that the bioavailability of salicylic acid was *ca.* 2.5 fold higher in case of electret group ($97.7 \pm 13.87 \text{ mg} \cdot \text{h/l}$) of rats over the control group ($39.9 \pm 10.87 \text{ mg} \cdot \text{h/l}$).

Macromolecular Transport The objective of this experiment was to assess the efficiency of electrets in enhancing the transdermal penetration of macromolecules. The transport of FITC Dextran 1 kDa was enhanced by *ca.* 4 folds

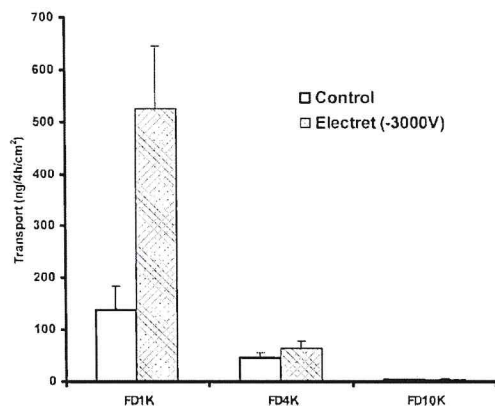


Fig. 5. Transport of Fluorescein Labeled Dextrans across the Electret Treated Porcine Epidermis Pretreated with Electret of Surface Voltage -3000 V

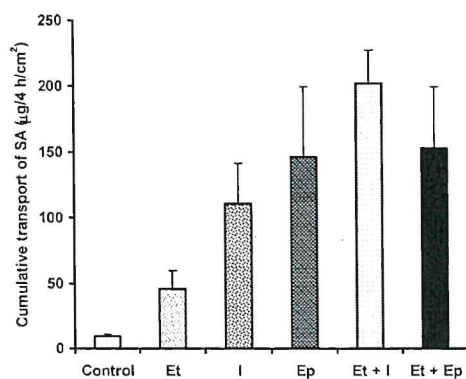


Fig. 6. Transport of Salicylic Acid by Different Techniques

"Control" was treated with blank electret, pretreated with -3000 V electret (Et), iontophoresis (I), electroporation (Ep), iontophoresis across the porcine epidermis pretreated with -3000 V electret (Et+I), electroporation of the porcine epidermis pretreated with -3000 V electret (Et+Ep).

by electret (Fig. 5). However, the transport of higher molecular weight dextrans was not enhanced significantly. In this case also the enhancement factors due to positive and negative electret remained similar.

Combination with Electrically Mediated Techniques

The transport of salicylic acid across the blank electret treated porcine epidermis (control) was $9.36 \pm 1.56\text{ mg}/\text{cm}^2$. Pretreatment with -3000 V electret enhanced the cumulative transport of salicylic acid by ca. 5 fold over transport across the control (Fig. 6). The iontophoresis and electroporation alone across the untreated epidermis porcine epidermis resulted in an enhancement of transport by 11 and 16 folds respectively. Pretreatment of epidermis with -3000 V electret enhanced the iontophoretic transport significantly by ca. 2 fold, whereas the same did not enhance the electroporation mediated transport of salicylic acid.

Iontophoresis is known to enhance the appendageal transport of drugs.¹² From the results of this work, it appears that the electret enhanced the permeability of salicylic acid by compromising the barrier property of stratum corneum. Fur-

ther, it is likely that electret rendered the lipid region of the stratum corneum relatively more permeable. Therefore when combined with the electret, the iontophoretic effects are additive. Electroporation is known to enhance the transport of polar molecules by creating transient aqueous pathways in the lipid domain in the stratum corneum.¹³ The electret effects are not apparent when combined with electroporation likely due to the overlapping mechanisms of penetration enhancement by electret and electroporation.

CONCLUSION

Electret could be used as permeation enhancer for the delivery of drugs across the skin. However, electret exposure could not enhance the transport of high molecular weight diffusants ($>1\text{ kDa}$). The present study is phenomenological and requires extensive structural studies to demonstrate the actual mechanisms involved in permeation enhancement. In combination with iontophoresis the enhancement effect was additive but not with electroporation most likely due to overlapping of mechanisms of barrier alteration. Electret effects are highly influenced by the distance and moisture content in the formulation. Therefore extensive research needs to be carried out towards standardization of the technique to derive optimal benefits of this phenomenon.

Acknowledgements The authors would like to thank the Rad Elec Inc., Bethesda, MD for the generous samples of E-PERM[®] and other instruments. The authors would also like to thank Dr. Cui Lili of Second Military Medical University, Shanghai, China for useful discussions and Dr. Shuangqing Zhang for helping with the pharmacokinetic studies.

REFERENCES

- Matoltsy A. G., Downes A. M., Swency T. M., *J. Invest. Dermatol.*, **50**, 19–26 (1968).
- Fang J. Y., Hwang T. L., Huang Y. B., Tsai Y., *Int. J. Pharm.*, **235**, 95–105 (2002).
- Murthy S. N., Sen A., Hui S. W., *J. Controlled Release*, **98**, 307–315 (2004).
- Kotrappa P., Dempsey J. C., Stieff L. R., Ramsey R. W., *Health Phys.*, **58**, 461–467 (1990).
- Kotrappa P., Stieff L. R., *Radiation Protection Dosimetry*, **47**, 461–464 (1993).
- Kotrappa P., *Health Phys.*, **89**, 164–167 (2005).
- Cui L., Jiang J., Zhang L., Song C., Zhao W., Lin J., *J. Electrostat.*, **51–52**, 153–158 (2001).
- Yue T. L., Varma D. R., *Drug Metab. Dispos.*, **10**, 147–152 (1982).
- Murthy S. N., Zhao Y., Hui S. W., Sen A., *J. Controlled Release*, **105**, 132–141 (2005).
- Murthy S. N., Sen A., Zhao Y., Hui S. W., *J. Pharm. Sci.*, **93**, 1062–1064 (2004).
- Takahashi Y., Yamato K., Akiyama H., Tsuji K., Onishi H., Machida Y., *Biol. Pharm. Bull.*, **28**, 870–875 (2005).
- Kalia Y. N., Merino V., Guy R. H., *Dermatol. Clin.*, **16**, 289–299 (1998).
- Pliquett U., Gallo S. A., Hui S. W., Neumann E., *Bioelectrochemistry*, **67**, 37–46 (2005).