Recovery of Human Skin Impedance In Vivo After Iontophoresis: Effect of Metal Ions

Submitted March 23, 2000; accepted July 3, 2000; published July 27, 2000

Catherine Curdy, Yogeshvar N. Kalia, Françoise Falson-Rieg, and Richard H. Guy

Centre Interuniversitaire de Recherche et d'Enseignement, Pharmapeptides, Campus Universitaire, Parc d'Affaires International, F-74166 Archamps, France

Faculté des Sciences - Section de Pharmacie, Laboratoire de Pharmacie Galénique, Université de Genève, CH-1211 Genève 4, Switzerland

Faculté de Pharmacie, Institut des Sciences Pharmaceutiques et Biologiques, Université Claude Bernard - Lyon 1, F-69373, France

Centre Interuniversitaire de Recherche et d'Enseignement, Pharmapeptides, Campus Universitaire, Parc d'Affaires International, F-74166 Archamps, France

ABSTRACT The objective of this study was to investigate the effect of the counter-ion (cation) on the recovery of human skin impedance after iontophoresis in vivo. A series of metal chloride aqueous solutions (NaCl, KCl, $CaCl_2$, and $MgCl_2$) was investigated: first at the same concentration (133 mmol/L) and then at the same ionic strength as a NaCl solution at 133 mmol/L. The influence of hydration alone was also examined as a control. The recovery of human skin impedance was followed in the frequency range 1-1,000 Hz, over a 30-minute period after iontophoresis during which 3 impedance spectra were recorded. The results revealed that at t = 30 minutes post-iontophoresis, skin impedance was approximately 3 times greater than the value immediately after the cessation of current passage. However, the results showed that the nature of the cation had no effect on recovery, regardless of whether the ions were at the same concentration or at an equivalent ionic strength. A simple parallel RC-equivalent circuit model for skin was used to determine the resistive (R) and capacitive (C) contributions to skin impedance. An analysis of variance on the calculated R and C values did not show any differences between the electrolytes used at the 2 different ionic strengths.

1 **Corresponding author**:Richard H. Guy, Centre Interuniversitaire de Recherche et d'Enseignement, Pharmapeptides, Campus Universitaire, Parc d'Affaires International, F-74166 Archamps, France; telephone: 33-0-4-50.31.50.21; fax: 33-0-4-50.95.28.32; e-mail addresses: rhg@pharma1.cur-archamps.fr; kalia@pharma1.cur-archamps.fr; curdy@pharma1.cur-archamps.fr

INTRODUCTION

The *stratum corneum* (SC), the outermost 10-20 μm of the epidermis, is generally recognized as the principal barrier to transdermal drug transport (1,2). The lipid-protein matrix of the SC not only restricts the passive diffusion of lipophilic molecules but also severely limits the transport of hydrophilic compounds across the membrane. Therefore, several techniques have been examined for their ability to increase drug penetration through the SC and thereby extend transdermal drug delivery to a wider range of molecules. Of these, iontophoresis, which involves the application of a low-level current (<0.5 mA/cm^2) across the skin, provides a highly controllable means of enhancing molecular transport (3,4). However, the future development of this technique must ensure that it does not provoke unacceptable side-effects and that skin barrier integrity is not compromised.

Relatively few studies have addressed the assessment of post-iontophoretic barrier function in vivo (5,6). In our work, impedance spectroscopy, a noninvasive biophysical tool (7), has been used to evaluate the electrical properties of the skin. Yamamoto and Yamamoto have demonstrated in vivo (8) that the skin's electrical resistance resides primarily in the SC: skin impedance decreased as layers of the SC were sequentially removed by tape stripping. Normally, human skin in vivo is characterized by relatively high impedance. If the barrier function is reduced in some way, the skin becomes more permeable and the passage of ions into and across the SC is more facile, thereby leading to a decrease in impedance (9). The utility of in vivo impedance measurements for the characterization of the barrier function has been established (8,10-13). Kalia and Guy (11) recently investigated the effects of iontophoresis on the electrical characteristics of human skin in vivo. The influence of iontophoretic current density, duration of current application, and hydration were studied. The authors noted that increasing both current density and time of application caused a greater impedance drop and delayed the post-iontophoretic recovery. Another factor of importance is the composition of the electrolyte used in the iontophoretic experiment; in particular, bearing in mind the skin's net negative charge and consequent permselectivity, the nature of the cations present in a drug-containing formulation is of particular relevance. These ions may be present to enhance drug stability and hence improve delivery; however, they also compete with the drug to carry the charge flowing across the skin and may reduce, therefore, the extent of iontophoretic drug delivery. In another sense, the nature of the electrolyte may also affect the recovery of skin impedance post-iontophoresis, and cations that favor this process would be considered valuable formulation components. The earlier study (11), for example, suggested that the return of skin impedance towards pre-iontophoretic levels was apparently favored by calcium as compared to sodium. It has also been suggested that the disruption of ion gradients within the epidermis may impair skin barrier function and may be manipulated to play a role in altering the membrane's permeability (14).

The principal objective of this work was to extend our preliminary measurements to a larger group of cations and to determine whether skin impedance recovery is related to ion charge or to other physical properties of the ion involved, such as ionic mobility, ionic radius, or molecular weight. Therefore, we have evaluated the effect of a series of metal chlorides on the recovery of human skin impedance in vivo after iontophoresis. In addition, we examined the impact of ionic strength on the rate of recovery. Finally, to control for the effect of hydration alone, impedance was also monitored when the skin was bathed in an electrolyte solution without passage of an iontophoretic current.

MATERIALS AND METHODS

Chemicals

$CaCl_2$ and $MgCl_2$ were obtained from Aldrich Chemical (Gillingham, England). NaCl was purchased from Fluka Chemie AG (Buchs, Switzerland) and KCl from Sigma Chemical (St. Louis, MO). Deionized water (resistivity $18 mol/L Ωcm^{-1}) purified by a Millipore System (Milli-Q Ufplus; Bedford, MA) was used to prepare all solutions.

Electrodes

Ag/AgCl electrodes were used for the application of both the alternating current, required for the impedance measurements, and the iontophoretic current.

The electrodes were prepared by dipping silver wire (1 mm diameter, 99.99% pure, Aldrich Chemical Company) in molten AgCl. Afterwards, they were immersed in 133 mmol/L NaCl and conditioned against a Pt cathode for approximately 12 hours at an applied current of 0.1 mA/cm^2.

Human Subjects

The studies were conducted in healthy volunteers (age 24-32 years), with no history of dermatological disease. They were required to maintain the skin sites under investigation (on the ventral forearm) free from

application of topical formulations. Informed consent was obtained from all participants. We used both the right and left arms, previous studies having shown that there was no significant difference in passive skin impedance between corresponding sites on both arms. The study was approved by the Commission d'Ethique, Département des Neurosciences Cliniques et Dermatologie, Hôpitaux Universitaires de Genève.

Experimental Apparatus

The equipment used to record the impedance spectrum of the skin in vivo was identical to that used in our previous study (11).

Experimental Procedure

Two glass chambers (1.76 cm^2 area, 2 cm apart) were adhered to the ventral forearm of healthy human volunteers and were rendered watertight by the application of silicone grease. The chambers were filled with 2.8 mL of electrolyte solution into which Ag/AgCl electrodes were inserted. A series of metal chlorides (NaCl, KCl, $CaCl_2$, and $MgCl_2$) was investigated at both the same concentration (133 mmol/L, pH: 5.8, 5.7, 10.2, and 9.5, respectively) and the same ionic strength (133 mmol/L NaCl or KCl, 44.3 mmol/L $CaCl_2$ or $MgCl_2$, pH: 5.8, 5.7, 8.2, and 6.7, respectively). An initial impedance spectrum (passive impedance) was recorded over the frequency range 1-1,000 Hz. Next, the skin was allowed to hydrate for 20 minutes. An iontophoretic current of 0.1 mA/cm^2, delivered using a Kepco Power Supply APH 100 M (Flushing, NY), was then applied for 15 minutes. Further impedance measurements were performed immediately after stopping the iontophoretic current flow and at t = 30 minutes after iontophoresis. The experiments were conducted in an air-conditioned room at an ambient temperature of 22° to 24°C.

A control experiment was also performed to evaluate simply the effect of hydration on skin impedance. After recording an initial impedance spectrum, the skin was hydrated for 65 minutes (ie, the total duration of the iontophoretic experiments) using either 133 mmol/L NaCl, 133 mmol/L $CaCl_2$, or 44.3 mM $CaCl_2$. Subsequently, a second impedance spectrum was measured.

RESULTS AND DISCUSSION

The recovery of skin impedance following iontophoresis from several metal chloride solutions at a fixed concentration of 133 mmol/L is shown in Figure 1.

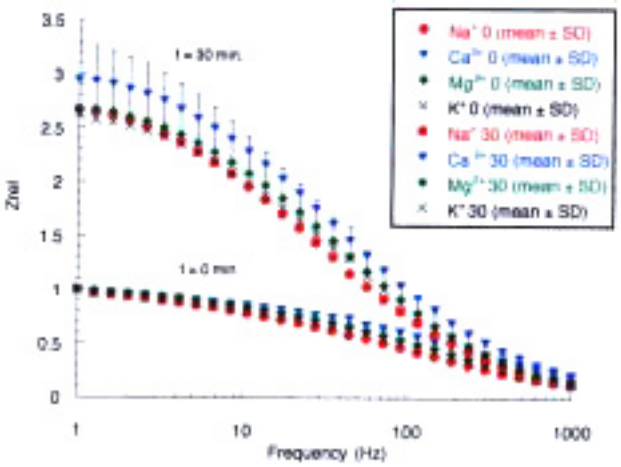

Figure 1. Skin impedance recovery (Z_{rel}) versus frequency (Hz) at t = 0 and t = 30 minutes after iontophoresis of a series of metal chloride solutions at the same concentration (mean ± SD, n = 5). For purposes of clarity, SD values are only shown for K^+, which had the highest variability.

The data are expressed as relative impedance (Z_{rel}) as a function of frequency. Z_{rel} is the absolute value of skin impedance (at each frequency) divided by the impedance at 1 Hz measured immediately after the termination of iontophoresis. On average, there is a 2.5- to 3-fold recovery of the low-frequency skin impedance (ie, resistance) within 30 minutes of the termination of iontophoresis. However, the differences in the recovery observed for the different metal cations employed are negligible. Similarly, the Z_{rel} versus frequency profiles are essentially superimposable. Figure 2 presents the corresponding results obtained under conditions of constant ionic strength. The recovery of Z_{rel} is very similar in profile and magnitude to that presented in Figure 1, and, again, there is no distinction apparent between the different metal cations used. When the raw

impedance data at the lowest frequency (1 Hz), obtained either after iontophoresis or following a 65-minute period of skin hydration were compared, it was found that the recovery 30 minutes after iontophoresis attained only approximately 10% to 20% of the value observed at the end of 65 minutes of simple hydration (data not shown).

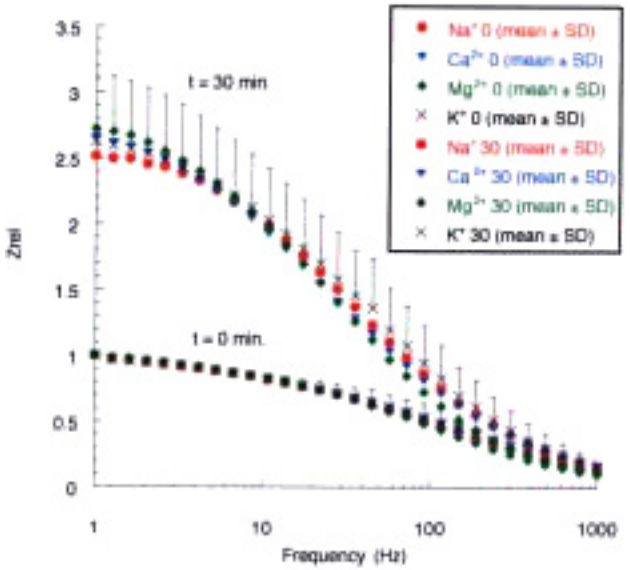

Figure 2. Skin impedance recovery (Z_{rel}) versus frequency (Hz) at t = 0 and t = 30 minutes after iontophoresis of a series of metal chloride solutions at the same ionic strength (mean ± SD, n = 6). For purposes of clarity, SD values are only shown for K^+, which had the highest variability.

Parenthetically, it should be noted that the pH of the metal ion solutions examined in this work was never less than 5.5; that is, for all experiments, the concentration of H_3O^+ was always at least 10^4-fold smaller than the metal ion concentration and, in addition, the skin always retained its cation permselectivity and a net negative charge (15).

The impedance spectra were used to generate model-dependent resistance and capacitance parameters characterizing the skin as an equivalent electrical circuit comprising a resistance (R) and a capacitance (C) in parallel (8). The data were fitted to the following equation:

$$\frac{1}{Z_{real} - R_1} = \frac{1}{R} + (2\pi f)^2 RC^2 \qquad \textit{(Eq. 1)}$$

where f is the frequency (Hz) and R_1 is the resistance of the electrodes and buffer solutions in series with the parallel R-C circuit of the skin (16,17).

Plots of $(Z_{real} - R_1)^{-1}$ versus $(2\pi f)^2$ according to Equation 1 were used to calculate the capacitive and resistive components of the stratum corneum at t = 0 and t = 30 minutes after iontophoresis for both series of experiments. The R and C values (mean +/- SD) so determined (Table 1) are typically on the order of approximately 20 kΩ/cm^2 and approximately 50 nF/cm^2.

Table 1. Resistance (R) and capacitance (C) (mean ± SD, n = 5 or 6) of human skin in vivo determined immediately and at 30 minutes post-iontophoresis in the presence of different electrolyte solutions

Electrolyte	Concentration (mmol/L)	Time post-iontophoresis (min)	R (kΩ)	C (nF.cm^{-2})
1. Fixed concentration				
NaCl	133	0	21 ± 6	53 ± 6
		30	42 ± 5	41 ± 8
KCl	133	0	21 ± 3	50 ± 12
		30	44 ± 9	38 ± 9
$CaCl_2$	133	0	16 ± 6	55 ± 16
			29 ± 8	47 ± 10
$MgCl_2$	133	0	28 ± 4	38 ± 11
		30	63 ± 15	31 ± 10
2. Fixed ionic strength				
NaCl	133	0	21 ± 6	49 ± 9
		30	43 ± 6	38 ± 7
KCl	133	0	22 ± 5	46 ± 11
		30	48 ± 10	35 ± 8
$CaCl_2$	44.3	0	21 ± 6	47 ± 10
		30	42 ± 14	38 ± 8
$MgCl_2$	44.3	0	37 ± 9	37 ± 10
		30	77 ± 23	31 ± 9

The resistance parameter approximately doubled during the 30 minutes following termination of current flow. This recuperation was statistically significant ($P < .05$). On the other hand, the changes in capacitance observed 30 minutes post-iontophoresis were too small to achieve statistical significance. Both findings concur with the results of

previous, less detailed studies (11,18). Statistical analysis using ANOVA and the Fisher test (95% confidence interval) was performed on the R and C values obtained for each ion. There were no statistically significant differences between the uni- and divalent ions, nor was there any apparent influence of ionic strength. Moreover, plots of the difference between the R values at t = 30 and t = 0 after iontophoresis (ΔR) and between the C values at those 2 time points (ΔC) versus the ionic radii, mobility, and molecular weight of the cations investigated (Figures 3 and 4) do not highlight any effect of those parameters on the skin impedance recovery after iontophoresis.

It is important to point out that, in contrast to our earlier measurements (n = 2) (11), no differences were observed in the present work (n = 5) between the recoveries of skin impedance following iontophoresis in the presence of sodium and calcium ions. This difference may reflect the fact that we have now used a larger cohort of subjects in this more detailed study, allowing appropriate statistical comparisons between the R and C parameters derived from fitting the impedance data as a function of frequency to Equation 1.

In conclusion, it would appear that there are no ion-specific factors that directly influence the impedance drop observed during iontophoresis or the subsequent recovery. This is the case despite the sometimes wide differences in the physicochemical properties of the cations tested (size, mobility, ionic radii, transport number, etc.). It follows that, at least for the short period of iontophoresis evaluated here, there is no specific cation-dependent impact, either positive or negative, on the recovery of skin impedance. Clearly, however, the range of potential excipients that may contribute to the background electrolyte of an actual iontophoretic delivery system is much broader than the different metal cations examined here, and a systematic evaluation of additional formulation components is therefore warranted.

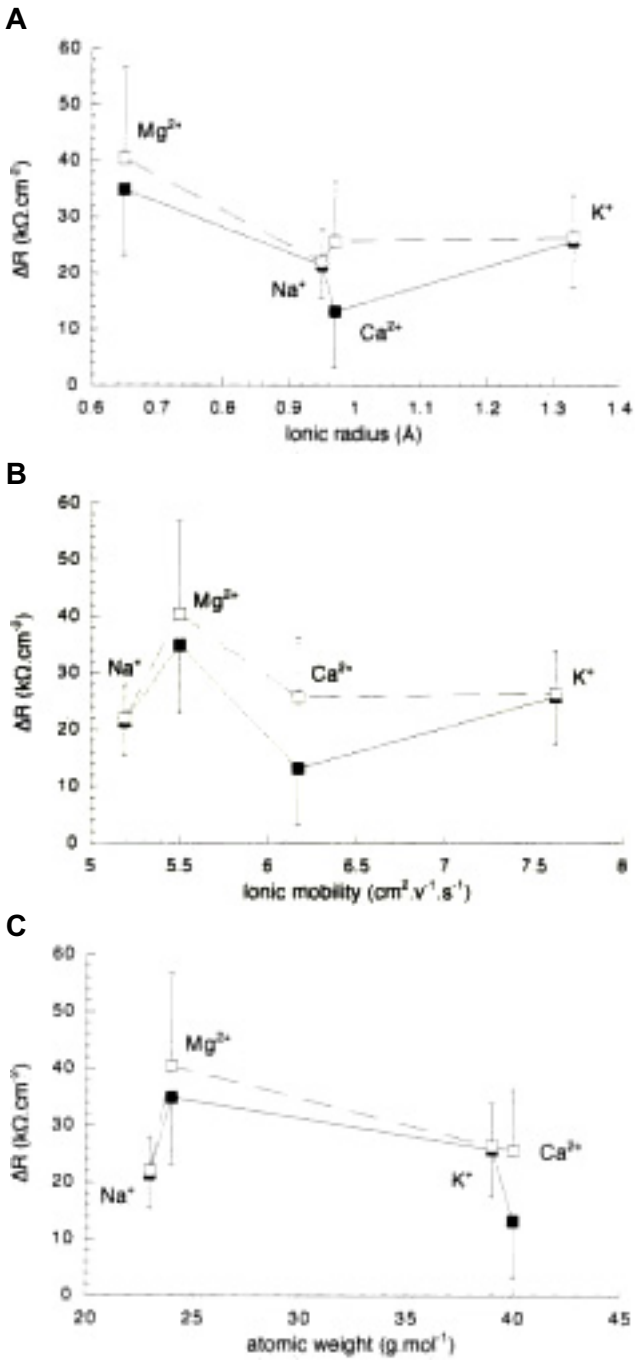

Figure 3. Changes in skin resistance (ΔR = [resistance at t = 30 minutes post-iontophoresis] - [resistance immediately following current passage]) versus (A) the ionic radii of the metal cations, (B) their ionic mobilities, and (C) their atomic weights, at both fixed concentration (closed symbols, mean ± SD, n = 5) and fixed ionic strength (open symbols, mean ± SD, n = 6).

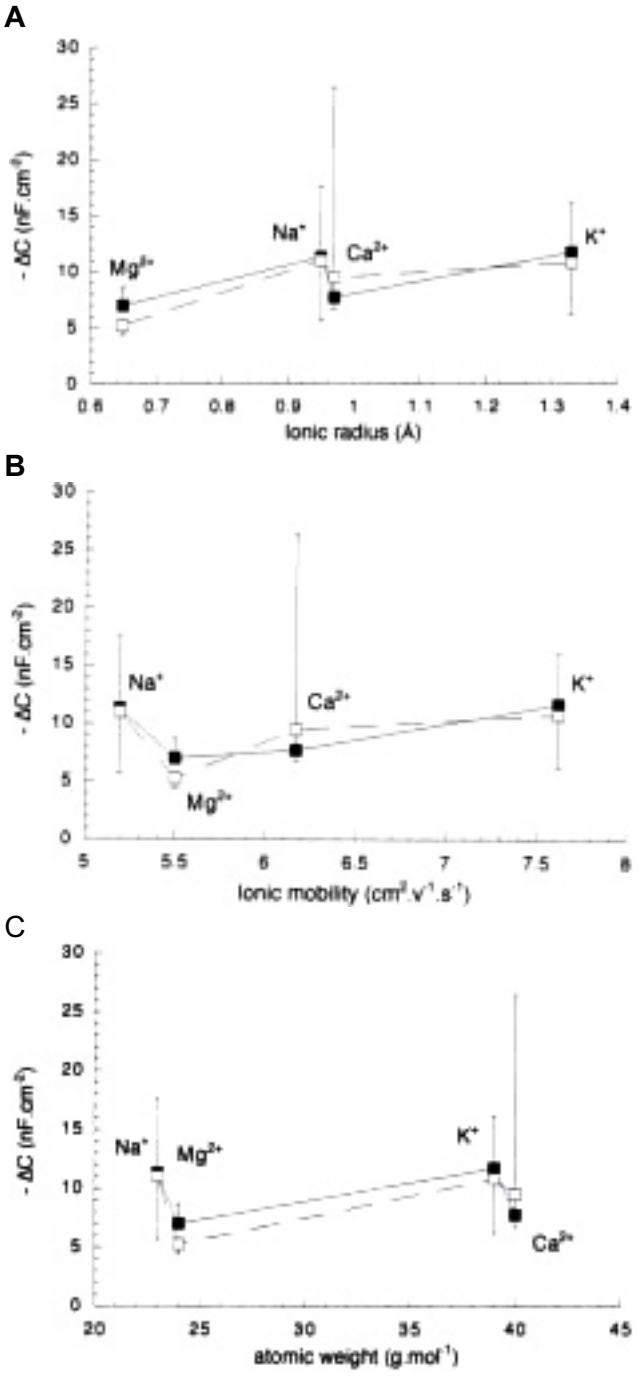

Figure 4. Changes in skin capacitance (ΔC = [capacitance at t = 30 minutes post-iontophoresis] - [capacitance immediately following current passage]) versus (A) the ionic radii of the metal cations, (B) their ionic mobility, and (C) their atomic weights, at both fixed concentration (closed symbols, mean ± SD, n = 5) and fixed ionic strength (open symbols, mean ± SD, n = 6).

ACKNOWLEDGMENTS

Financial support was provided by Tilderm (Chenôve, France) and the Fonds National Suisse de la Recherche Scientifique (Switzerland). We thank Frédérique Hueber and Catherine Gentil for their input in this work and our colleagues at Archamps for their participation as volunteers in these experiments.

REFERENCES

1. Elias PM. Epidermal barrier function: intercellular lamellar lipid structures, origin, composition and metabolism. J Control Release. 1991;5:199-208.
2. Lévêque JL, Ribaud CH, Garson JC. Caractérisation biophysique du stratum corneum. Relation entre sa structure et ses propriétés. Pathologie-Biologie. 1992;40:95-108.
3. Burnette RR. Iontophoresis. In: Hadgraft J, Guy RH, eds. *Transdermal Drug Delivery.* New York: Marcel Dekker; 1989:247-291.
4. Green PG, Flanagan M, Shroot B, Guy RH. Iontophoretic drug delivery. In: Walters KA, Hadgraft J, eds. *Pharmaceutical Skin Penetration Enhancement.* New York: Marcel Dekker; 1993:311-333.
5. van der Geest R, Elshove DAR, Danhof M, Lavrijsen APM, Boddé HE. Non-invasive assessment of skin barrier integrity and skin irritation following iontophoretic current application in humans. J Control Release. 1996;41:205-213.
6. Kalia YN, Merino V, Guy RH. Transdermal drug delivery. Clinical aspects. Dermatologic clinics. 1998;16:289-299.
7. Burnette RR, De Nuzzio JD. Impedance spectroscopy: applications to human skin. In: Potts RO, Guy RH, eds. Mechanism of Transdermal Drug Delivery. New York and Basel: Marcel Dekker; 1997:215-230.
8. Yamamoto T, Yamamoto Y. Electrical properties of the epidermal stratum corneum. Med Biol Eng. 1976;151-158.
9. Tregear RT. *Physical Functions of the Skin.* London and New York: Academic Press; 1966.
10. Oh SY, Guy RH. Effect of iontophoresis on the electrical properties of human skin *in vivo.* Int J Pharm. 1995;124:137-142.
11. Kalia YN, Guy RH. The electrical characteristics of human skin *in vivo*. Pharm Res. 1995;12:1605-1613.
12. Kalia YN, Nonato LB, Guy RH. The effects of iontophoresis on skin barrier integrity: non-invasive evaluation by impedance spectroscopy and transepidermal water loss. Pharm Res. 1996;13:957-960.
13. Turner NG, Kalia YN, Guy RH. The effect of current on skin barrier function *in vivo:* recovery kinetics post-iontophoresis. Pharm Res. 1997;14:1252-1257.
14. Lee SH, Elias PM, Feingold KR, Mauro T. A role for ions in barrier recovery after acute perturbation. J Invest Dermatol. 1994;102:976-979.
15. Burnette RR, Ongpipattanakul B. Characterization of the permselective properties of excised human skin during iontophoresis. J Pharm Sci. 1987;76:765-773.

16. DeNuzzio JD, Berner B. Electrochemical and iontophoretic studies of human skin. J Control Release. 1990;11:105-112.
17. Burnette RR, Bagniefski TM. Influence of constant current iontophoresis on the impedance and passive Na^+ permeabiility of excised nude mouse skin. J Pharm Sci. 1988;77:492-497.
18. Oh SY, Leung L, Bommannan D, Guy RH, Potts RO. Effect of current, ionic strength and temperature on the electrical properties of the skin. J Control Release. 1993;27:115-125.