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Research paper

Post-iontophoresis recovery of human skin impedance in vivo

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Abstract

The objective of this study was to better understand the recovery of human skin impedance following iontophoresis in vivo. Volunteers were subjected to a 15-min period of iontophoresis in the presence of aqueous solutions of either NaCl, KCl, $CaCl_2$ or $MgCl_2$ at 133 mM. Subsequently, the low-frequency impedance (at 1 Hz) recovery was followed for a further 30 min. Assuming direct proportionality between the reciprocal impedance and the ion concentration in the membrane, the experimental data were fitted to the appropriate solutions of Fick's second law of diffusion to derive characteristic diffusion parameters (D/L^2), apparent diffusivities (D), diffusion pathlengths (L) and mobilities, and ion concentrations in the skin immediately post-iontophoresis. Ion fluxes out of the membrane after termination of current flow were also deduced. In general, recovery was relatively independent of the background electrolyte as previously reported, and the data were consistent with ion transport in predominantly aqueous pathways. Compared to its mobility in aqueous solution, however, the apparent Cl^- mobility in the skin was smaller, presumably due to the fact that, under normal physiological conditions, the human skin barrier supports a net negative charge. In parallel, the initial 'release' of Na^+ and K^+ from the skin post-iontophoresis was faster than that of Ca^{2+} and Mg^{2+}, the latter cations of higher charge density being able to associate more strongly, it seems, with the negatively-charged skin. The simple physicochemical analysis of the data presented serves to emphasize that a decrease in skin impedance is *not* a manifestation of damage to the barrier – rather, it is a natural response to the relevant electrical potential and ion concentration gradients involved.

Keywords: Impedance spectroscopy; Iontophoresis; Skin barrier function; Transdermal drug delivery

1. Introduction

The uppermost layer of the skin, the stratum corneum, severely restricts the transport of charged compounds and hydrophilic molecules [1]. Iontophoresis involves the application of a small electrical current at current densities of less than 0.5 mA/cm^2 [2], and is an efficient and non-invasive technique that can be used to overcome the skin barrier [3]. However, the clinical application of iontophoresis demands minimal or no side-effects as well as the rapid recovery of the barrier after termination of current flow. Several non-invasive biophysical techniques have been used to assess the effects of iontophoresis on skin integrity in vivo [4] and impedance spectroscopy, in particular, has proven suitable for monitoring changes in the electrical properties of the stratum corneum as a function of the iontophoretic current density or time of current application [5,6]. For example, increasing the current density or current application time caused a greater decrease in impedance and delayed the recovery of skin impedance [6]. Previously [7], the influence of the electrolytes present in the buffer solution on the recovery of human skin impedance (over the frequency range 1–1000 Hz) was evaluated in vivo after the application of an iontophoretic current. In particular, the impact of the physicochemical properties of the specific cation employed (molecular weight, ionic radius, charge) was examined. It was found, 30 min after termination of current flow, that skin impedance had recovered by about 2.5–3 times that immediately after iontophoresis. Moreover, there was no difference in the impedance recovery on the nature of the electrolyte employed, nor did the ionic strength or the concentration of the metal chloride present in the buffer solution have any influence on the recovery process.

The goal of the present study was to investigate the influence of the metal chloride present in the buffer on the kinetics of impedance recovery. Given that the decrease in skin impedance during iontophoresis is probably due to the increased local ion concentration within the membrane (in

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particular, in the stratum corneum), it is reasonable to assume that the recovery of skin impedance after current passage is due to the 'loss' of these 'extra' ions, i.e. skin impedance is inversely related to the local ion concentration, previous in vitro experiments having shown that skin resistance can be changed simply by altering the level of ions available to carry charge within the membrane [8]. It was decided to track this diffusive movement of ions from the skin (that is, their egress from the low-resistance conducting pathways across the membrane) by monitoring the recovery of the low-frequency (1 Hz) impedance [7,9] following a 30-min period of iontophoresis in the presence of different metal chloride solutions (at 133 mM). These impedance data were then subsequently fitted to appropriate solutions of Fick's second law of diffusion adapted to the experimental conditions used (either with the same, or different, ionic concentrations on the 'donor' and 'receiver' sides of the membrane). Apparent diffusivities (D), diffusional pathlengths (L) and ionic mobilities were determined, and fluxes were deduced for each separate ion.

2. Materials and methods

Calcium and magnesium chlorides were obtained from Aldrich Chemical Company (Gillingham, England). Sodium chloride was purchased from Fluka Chemie AG (Buchs, Switzerland), potassium chloride from Sigma Chemical Company (Saint-Louis, MO). De-ionized water (resistivity≥18 MΩ cm) purified by a Millipore System (Milli-Q Ufplus), was used to prepare all solutions.

Four healthy subjects, without history of dermatological disease took part in the study. The volunteers maintained the skin site of study free from topical formulations before and

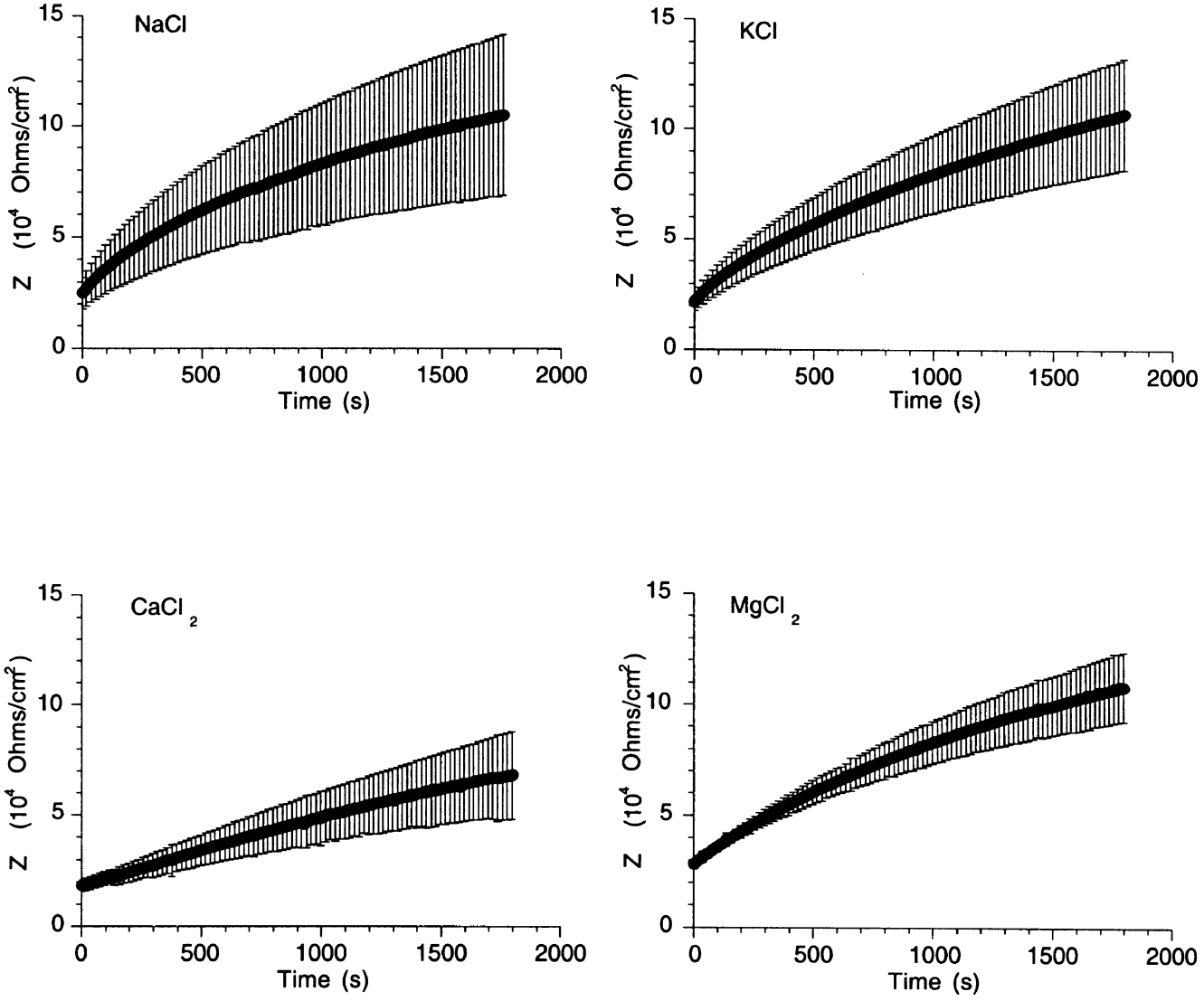

Fig. 1. Low-frequency (1 Hz) skin impedance recovery during 30 min following iontophoresis in the presence of a background electrolyte consisting of either NaCl, KCl, $CaCl_2$ or $MgCl_2$ (mean ± SD, $n = 4$). For purposes of clarity, only the SD of every fifth data point is shown.

during the investigation. The study was approved by the Commission d'Ethique, Département des Neurosciences Cliniques et Dermatologie, Hôpitaux Universitaires de Genève.

The equipment used to record skin impedance spectra in vivo was identical to that used previously [6]. Ag/AgCl electrodes were used both for the application of the iontophoretic current and for impedance monitoring [7].

Two glass chambers (area 1.76 cm^2, 2 cm apart) were affixed to the ventral forearm of the subject. The use of silicone grease and adhesive tapes assured a water-tight seal between the chambers and the skin. The chambers were filled with a 133 mM solution of either NaCl, KCl, $CaCl_2$ or $MgCl_2$, and an Ag/AgCl electrode was inserted into each chamber. A 0.1 mA/cm^2 iontophoretic current (delivered using a Kepco Power Supply APH 100 M, Flushing, NY) was then applied for 15 min. After termination of current flow, the recovery of low-frequency skin impedance was monitored at 1 Hz over the next 30 min.

3. Results and discussion

Low-frequency skin impedance recovery in the 30 min following iontophoresis using 133 mM solutions of either NaCl, KCl, $CaCl_2$ or $MgCl_2$ is shown in Fig. 1. The values displayed are the actual (non-normalized) impedance data ($\Omega.cm^{-2}$). The recovery profiles are similar, and essentially independent of the metal chloride employed. In each case, impedance recovers in the 30 min post-iontophoresis to a value which is almost three times that measured immediately after stopping the current. Therefore, and consistent with our previous results [7], the impedance recovery subsequent to the application of an iontophoretic current is independent of the cation present in the electrolyte solution. Nevertheless, it appeared that the standard deviations in the data were smaller in the experiments carried out using $CaCl_2$ and $MgCl_2$.

The results of the no-current control experiment in which the skin was simply hydrated with 133 mM NaCl for 45 min (a period equivalent to the with-current measurements, i.e. 15 min of iontophoresis plus 30 min recovery) are shown in Fig. 2. As observed before [7], the low frequency skin impedance falls gradually over this time to a more or less constant value which is, on average, 5 times greater than that attained by the skin 30 min after a 15-min period of iontophoresis.

Human skin in vivo is characterized by a relatively high impedance which is principally associated with the stratum corneum [10,11]. During iontophoresis, the concentration of ions in the membrane (i.e. the stratum corneum) is increased, and skin resistance falls. After termination of current flow, the locally increased ionic concentration gradually returns towards the normal physiological level meaning that ions have diffused out of the iontophoresed site either into the electrolyte solution remaining in contact with the skin or into the deeper layers of the skin. Pathways of iontophoretic current flow have been associated with routes of low resistance in the stratum corneum, including the appendages [12,13]. It seems reasonable to postulate that ions leaving the membrane after iontophoresis diffuse via the same routes, a hypothesis consistent with the relatively rapid recovery of skin impedance within the first min following iontophoresis.

To model the recovery rate of low-frequency skin impedance post-iontophoresis, i.e. the diffusive 'release' of ions from the skin once current passage is terminated, and hence to characterize this ion transport process, two assumptions are required. First, that the reciprocal of the skin's impedance ($1/Z$) and the ion concentration in the membrane are directly proportional and, second, that the cation used and Cl^- contribute to the measured $1/Z$ values in a manner proportional to their corresponding transport numbers (t_i). The latter were obtained from the literature [9]: for NaCl, $t_{Na^+} = 0.6$ and $t_{Cl^-} = 0.4$; for KCl, $t_{K^+} = 0.6$ and $t_{Cl^-} = 0.4$; for $CaCl_2$, $t_{Ca^{2+}} = 0.4$ and $t_{Cl^-} = 0.6$; for $MgCl_2$, $t_{Mg^{2+}} = 0.3$ and $t_{Cl^-} = 0.7$.

The change in $1/Z$ as a function of time could then be fitted by an appropriate solution to Fick's second law of diffusion using the appropriate boundary conditions. Crank [14] provides expressions for the concentration profile across a membrane when the initial concentrations of diffusant at the outer and inner surfaces of the barrier are initially either the same (the case in our experiments when the electrolyte was NaCl) or different (the situation for KCl, $CaCl_2$ and $MgCl_2$). Integration of these expressions over the membrane thickness permits the total amount (Q) of ions in the membrane as a function of time (t) to be found.

Therefore, for the surface concentrations equal:

$$Z_{1Hz}^{-1} \propto Q = 2AL\left[C_1 - \frac{8}{\pi^2}(C_1 - C_0)\exp\left\{\frac{-D\pi^2 t}{4L^2}\right\}\right] \quad (1)$$

where C_0 is the assumed uniform ion concentration in the membrane at the end of iontophoresis (i.e. at $t = 0$), C_1 is the

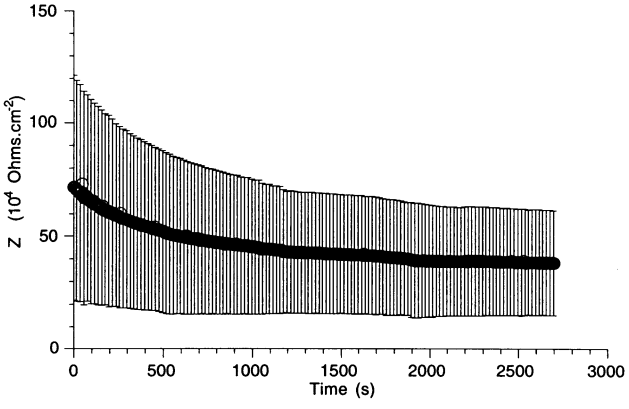

Fig. 2. Decrease of low-frequency skin impedance during a 45-min hydration period with 133 mM NaCl solution (mean ± SD, $n = 4$). For purposes of clarity, only the SD of every fifth data point is shown.

ion concentration at the inner and outer membrane surfaces, A is the area of skin exposed, D is the ion diffusivity across the membrane and L is the diffusion pathlength.

For the surface concentrations different:

$$Z_{1Hz}^{-1} \propto Q = AL\left[(C_1 + C_2)\left[\frac{1}{2} - \frac{4}{\pi^2}\exp\left\{\frac{-D\pi^2 t}{L^2}\right\}\right] + \frac{8C_0}{\pi^2}\exp\left\{\frac{-D\pi^2 t}{L^2}\right\}\right] \quad (2)$$

where C_1 and C_2 are the initial concentrations at the inner and outer surfaces of the membrane respectively.

The contributions to the measured $1/Z$ data as a function of time post-iontophoresis for each ion were fitted to either Eq. (1) or (2) as appropriate, and values for the characteristic kinetic transport parameter, D/L^2, and the *apparent* C_0, D and L were obtained (see Table 1). Note that C_0, D and L are *apparent* values since Z_{1Hz}^{-1}, rather than Q, is the measured parameter and the proportionality constant between them is not exactly known. As mentioned above, Eq. (1) was fitted to the data for Na^+ and Cl^- (when the background electrolyte was either NaCl or KCl) with $C_1 = 133$ mM; for the other electrolytes, Eq. (2) was used with $C_2 = 133$ mM for K^+, Ca^{2+} and Mg^{2+}, and 266 mM for Cl^-, and C_1 obtained for each cation from the literature [15]; C_1 was, of course, equal to 133 mM for Cl^- for each electrolyte.

The D/L^2 values for each electrolyte were very similar, differing by less than a factor of two. In the case of Cl^-, the derived apparent D and L values were independent of electrolyte (one-way ANOVA) as would be expected. However, the apparent C_0 for Cl^- was significantly and understandably higher (one-way ANOVA followed by the Student–Newman–Keuls test at $P < 0.05$) for $CaCl_2$ and $MgCl_2$ than for the univalent 1:1 electrolytes (NaCl and KCl). Between the different cations studied, the values for C_0 were generally consistent, and the results for L were in reasonable agreement with those determined for Cl^-. Some statistical differences between the cation apparent diffusivities were apparent, despite the higher standard deviations associated with these values. However, once again, the range in the results was less than 3-fold suggesting that the values themselves were not reflecting substantive mechanistic differences between the transport behaviour of the different cations.

The apparent diffusivities (D) were transformed into apparent mobilities (u) via Eq. (3):

$$u = \frac{D \cdot z \cdot F}{R \cdot T} \quad (3)$$

where z is the charge on the ion, F is Faraday's constant, R is the universal gas constant and T is the absolute temperature. The relative mobilities obtained for the four cations are in Table 2, and differed by less than two-fold, similar to the values of the corresponding ionic mobilities in aqueous solution which are similarly not very sensitive to the specific cation. This finding is consistent, therefore, with the proposed aqueous nature of the iontophoretic transport pathways across the skin. An important difference found, however, was that the deduced relative mobility of Cl^- in the skin was, on average, 2–3 times smaller than that of the cations studied. In contrast, the mobility of Cl^- in aqueous solution at 25°C is greater than that of the four cations considered. The reason for this observation probably lies in the fact that, at physiological pH, human skin carries a

Table 1
Fitted parameters[a] (characteristic diffusion parameter, D/L^2; apparent ion concentration within SC at the end of iontophoresis, C_0; apparent ion diffusivity, D; and apparent diffusion pathlength across the skin, L) from the interpretation of post-iontophoretic, low-frequency skin impedance data, as a function of time using either Eq. (1) or Eq. (2) as appropriate (see text for details).

	Electrolyte	$10^4 * D/L^2$ (s^{-1})	C_0[b] (M)	$10^7 * D$[b] ($cm^2.s^{-1}$)	L[b,c] (mm)
NaCl	Na^+	3.2 ± 0.3	0.54 ± 0.07	2.7 ± 1.2	0.14 ± 0.04
	Cl^-	3.2 ± 0.3	0.54 ± 0.07	1.2 ± 0.5	0.095 ± 0.02
KCl	K^+	3.0 ± 0.2	0.32 ± 0.01	5.0 ± 2.4	0.53 ± 0.1
	Cl^-	3.0 ± 0.3	0.60 ± 0.03	1.2 ± 0.8	0.092 ± 0.03
$CaCl_2$	Ca^{2+}	1.9 ± 0.6	0.32 ± 0.05	4.0 ± 1.1	0.47 ± 0.08
	Cl^-	1.9 ± 0.6	0.92 ± 0.15	1.1 ± 0.3	0.24 ± 0.04
$MgCl_2$	Mg^{2+}	2.6 ± 0.3	0.27 ± 0.05	1.6 ± 0.5	0.25 ± 0.04
	Cl^-	2.6 ± 0.3	0.81 ± 0.16	1.0 ± 0.3	0.20 ± 0.03

[a] Mean ± SD, $n = 4$.

[b] The values presented *assume* $Z_{1Hz}^{-1} = Q$ (see Eqs. (1) and (2)), i.e. that the proportionality constant between these parameters = 1 cm^2 $(\Omega.mol)^{-1}$. In fact, this parameter is not known and the values of C_0, D and L are therefore *apparent*, not absolute.

[c] Note that the solutions to Fick's second law presented assume that, in the case of equal surface concentrations, the stratum corneum thickness is actually equal to $2L$. In the 'asymmetric' situation, the SC thickness is simply L.

Table 2
Ionic mobilities in aqueous solution[a], and as experimentally determined in the stratum corneum (SC), expressed relative to the value for the Na^+ ion

	Ion	Aqueous mobility[a] relative to Na^+	Ionic mobility[b] in SC relative to Na^+
NaCl	Na^+	1.0	1.0
	Cl^-	1.5	0.45
KCl	K^+	1.5	1.9
	Cl^-	1.5	0.45
$CaCl_2$	Ca^{2+}	1.2	2.0
	Cl^-	1.5	0.41
$MgCl_2$	Mg^{2+}	1.1	1.2
	Cl^-	1.5	0.37

[a] Mobilities for Na^+, K^+, Ca^{2+}, Mg^{2+} and Cl^- at 25°C being, respectively, 5.19×10^{-4}, 7.62×10^{-4}, 6.17×10^{-4}, 5.50×10^{-4} and 7.91×10^{-4} $cm^2\ s^{-1}\ V^{-1}$ [18].
[b] Average values ($n = 4$).

net negative charge [16]. The presence of such lining the aqueous iontophoretic pathways would be anticipated, therefore, to inhibit anion movement and result in a lower mobility relative to that in simple aqueous solution.

Finally, it was possible to estimate the apparent fluxes (J) of the ions out of the skin post-iontophoresis by fitting the impedance recovery data to the appropriate equations for either equal surface concentrations [14] (in which case, the membrane is assigned a thickness of $2L$ with its surfaces at $x = -L$ and $x = +\mathrm{L}$):

$$J_{total} = |J_{x=-L}| + J_{x=L} = \left|-D\left(\frac{dC}{dx}\right)_{x=-L}\right| + \left(-D\left(\frac{dC}{dx}\right)_{x=L}\right)$$

$$= \left|\frac{2D(C_1 - C_0)}{L}\exp\left[\frac{-D\pi^2 t}{4L^2}\right]\right| + \frac{2D(C_0 - C_1)}{L}\exp\left[\frac{-D\pi^2 t}{4L^2}\right] \quad (4)$$

or surface concentrations different (where the membrane thickness equals L and its surfaces are located at $x = 0$ and $x = L$):

$$J_{total} = |J_{x=0}| + J_{x=L} = \left|D\left[\frac{C_1 - C_2}{L} + \frac{2}{L}((C_1 + C_2) - 2C_0)\exp\left\{\frac{-D\pi^2 t}{L^2}\right\}\right]\right|$$

$$+D\left[\frac{C_1 - C_2}{L} - \frac{2}{L}((C_1 + C_2) - 2C_0)\exp\left\{\frac{-D\pi^2 t}{L^2}\right\}\right] \quad (5)$$

The mathematical manipulation, therefore, is to determine

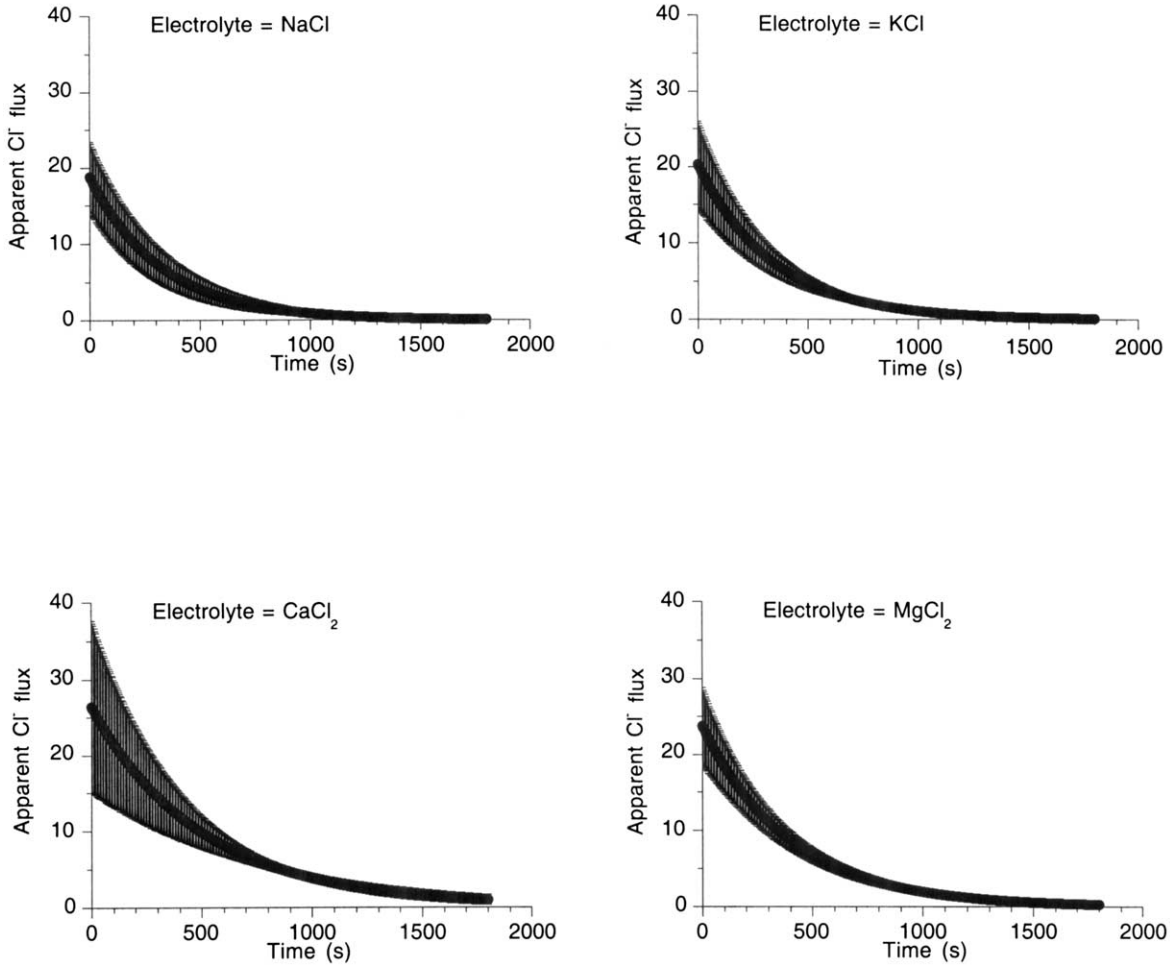

Fig. 3. Calculated outward fluxes (arbitrary units) of Cl^- from the skin following a 30-min period of iontophoresis in the presence of different background electrolytes (mean ± SD, $n = 4$). Data were fitted appropriately to either Eqs. (4) or (5) (see text for details).

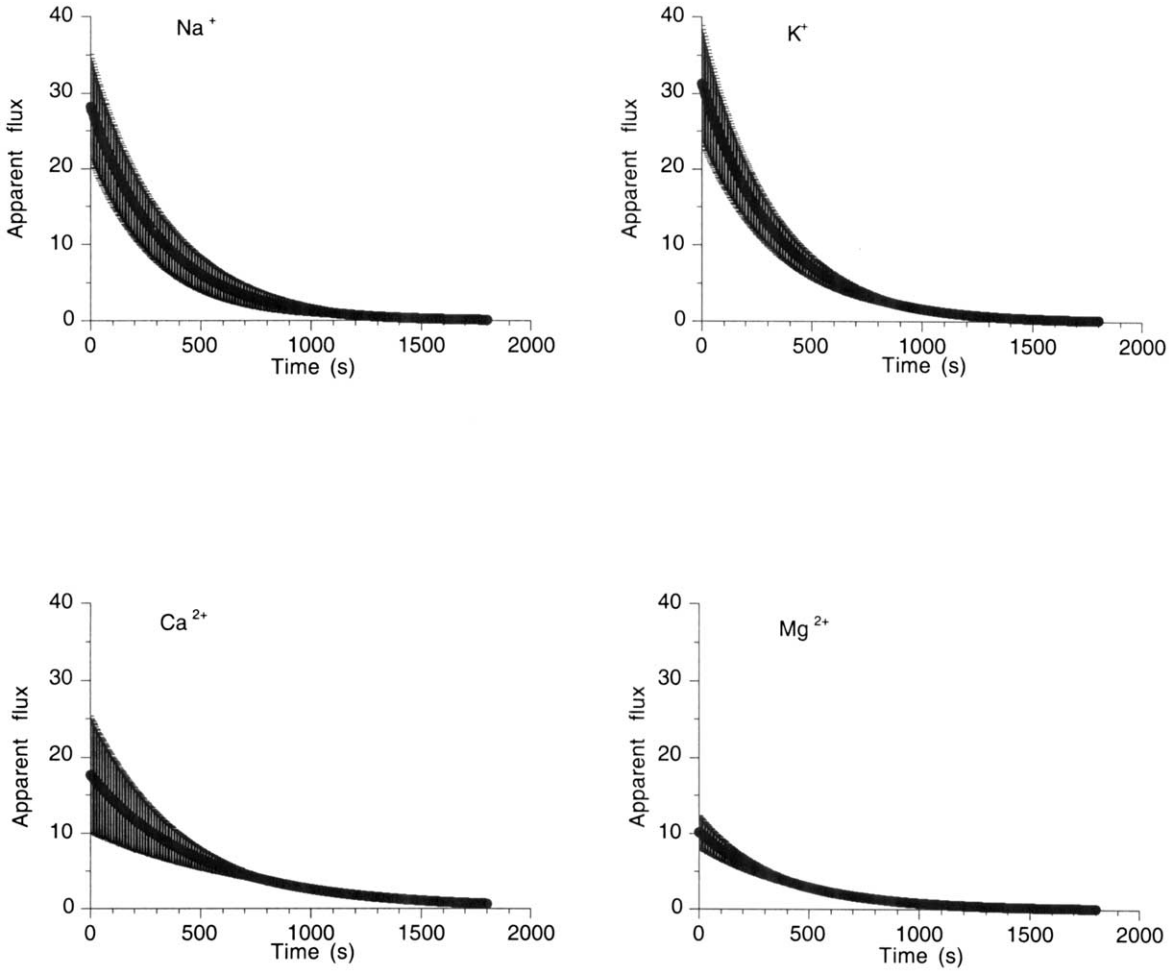

Fig. 4. Calculated outward fluxes (arbitrary units) of Na^+, K^+, Ca^{2+} and Mg^{2+} following a 30-min period of iontophoresis in the presence of, respectively, NaCl, KCl, $CaCl_2$ and $MgCl_2$ at a concentration of 133 mM (mean ± SD, $n = 4$). Data were fitted appropriately to either Eqs. (4) or (5) (see text for details).

from the concentration profiles [14], the ion concentration gradients at the membrane surfaces, and hence to calculate the corresponding fluxes out of the barrier.

The results for Cl^- are in Fig. 3 and, consistent with the data presented in Table 1, the flux profiles are independent of the nature of the background electrolyte. For the cations studied, it is clear that Na^+ and K^+ are different from Ca^{2+} and Mg^{2+}, in that the initial fluxes of the monovalent ions are greater than those of the divalent species (Fig. 4). Again, this observation can be reasonably attributed to the net negative charge of the skin and the potential therefore for Ca^{2+} and Mg^{2+} to bind more tightly with (and hence to be released more slowly from) the membrane. The conclusion follows logically from the measured ion transport numbers across the skin [9] and is consistent with similar deductions from other types of experiment in the literature [16] and with the higher charge/radius ratio of Ca^{2+} and Mg^{2+} (2.02 and 3.07, respectively) compared to Na^+ and K^+ (1.11 and 0.75, respectively) [17].

In conclusion, the results of this work first of all confirm our previous observations [7] that skin impedance recovery is relatively insensitive to the nature of the background electrolyte present. In addition, it has been shown that the return of low-frequency skin impedance post-iontophoresis is quite consistent with a model based on the outward diffusion of ions down their concentration gradients, and that the appropriate solutions to Fick's second law of diffusion were suitable for fitting the data. A lower apparent mobility of Cl^- in the skin (relative to that in aqueous solution) and the slower initial release of Ca^{2+} and Mg^{2+} from the membrane (compared to Na^+ and K^+) post-current passage were reasonably attributed to the established and well-recognized net negative charge of the skin under normal physiological conditions. Overall, then, these findings serve to reinforce, therefore, the fact that decreased skin impedance following iontophoresis is explainable in relatively straightforward physicochemical terms and results from the movement of ions in response to the electric field and to the relevant concentration gradients involved. There is no evidence from our work, at the level and duration of current application employed, to associate diminished impedance with 'damage' to the barrier.

Acknowledgements

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