Transfer of Diclofenac Sodium across Excised Guinea Pig Skin on High-Frequency Pulse Iontophoresis. I. Equivalent Circuit Model

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High frequency pulse iontophoresis of diclofenac sodium across excised guinea-pig skin was carried out *in vitro*. An equivalent circuit model was constructed to simulate the time courses of voltage drop across the donor solution and the skin. Parameter values obtained by the least-squares adaptation of the model to observed data were consistent with expectation and validated the proposed model.

Keywords iontophoresis; pulse iontophoresis; diclofenac sodium; voltage drop; equivalent circuit; skin; drug transport; guinea pig

Enhanced transdermal delivery of drugs by means of the technique of iontophoresis has been reported by many authors.[1] This method involves the migration of charged substances into the skin or tissues under a gradient of electrical potential.

Taking the electrical nature of skin reported by Yamamoto and Yamamoto[2] into account, Okabe *et al.*[3] developed a pulse-generating device for iontophoresis and applied it to transdermal administration of metoprolol. We have determined the transport rates of diclofenac sodium across excised guinea-pig skin under electric pulse driving. Time courses of voltage drop across the donor solution, the excised skin and the acceptor solution were recorded, as well as the cumulative amount of the drug transported through the skin.

The aim of this part of the study was to construct an equivalent circuit model for *in vitro* high-frequency pulse iontophoresis and to evaluate the model parameters.

Theory

General Description of the Equivalent Circuit Model The model circuit is shown in Fig. 1. Simplifying the circuit proposed by Yamamoto *et al.*,[2] the electrical features of skin are simulated by a parallel connection of a resistance R_s and a capacitor C_s. Those of the donor or acceptor solution are represented by series connection of a resistance (R_d or R_a) and a cell (E_d or E_a).

Electric cells E_d and E_a represent, among others, charged species which are unable to follow to a quick alternation of the electrical field, due to their moment of inertia. The power source provides an applied voltage, E_0 (1 – 10 V) for t_1 (7.5 μs) followed by a depolarizing period t_2 (17.5 μs).

Mathematical representation of the model is provided by the following set of equations.

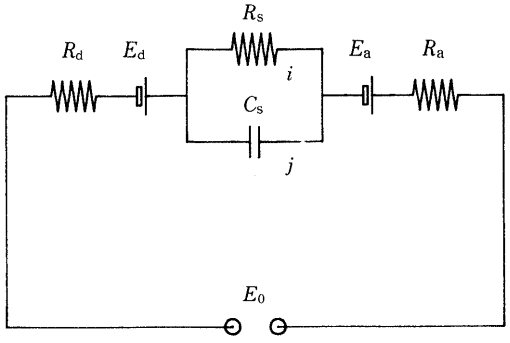

Fig. 1. Equivalent Circuit Model

$$e_d = R_d(i+j) + E_d \quad (1)$$

$$e_a = R_a(i+j) + E_a \quad (2)$$

$$e_s = R_s \cdot i \quad (3)$$

$$j/C_s = R_s \cdot di/dt \quad (4)$$

$$E_0 = e_d + e_a + e_s \quad (0 \le t < t_1) \quad (5)$$

$$0 = e_d + e_a + e_s \quad (t_1 \le t < t_1 + t_2) \quad (6)$$

where e's are the electrical potential difference of the respective sites denoted by the subscript, and i and j are electrical current specified in Fig. 1.

Inserting equations Eq. 1 through Eq. 4 into Eq. 5, and rearranging, we obtain Eq. 7.

$$(R_d + R_a) \cdot R_s C_s \frac{di}{dt} + (R_d + R_a + R_s) i = E_0 - E_d - E_a \quad (7)$$

Solving Eq. 7 for i, and using Eq. 3, the expression for e_s^+, e_s of the charging period, is obtained (Eq. 8).

$$e_s^+ = \frac{R_s(E_0 - E_d - E_a)}{(R_d + R_a + R_s)} + A \cdot \exp\left[-\frac{1}{C_s}\left(\frac{1}{R_s} + \frac{1}{R_d + R_a}\right) t \right] \quad (0 \le t < t_1) \quad (8)$$

where A is an integration constant.

Likewise, e_s of the depolarizing period, e_s^-, is obtained by using Eq. 6, as Eq. 9.

$$e_s^- = \frac{-R_s(E_d + E_a)}{(R_d + R_a + R_s)} + B \cdot \exp\left[-\frac{1}{C_s}\left(\frac{1}{R_s} + \frac{1}{R_d + R_a}\right) t \right] \quad (t_1 \le t < t_1 + t_2) \quad (9)$$

where B is an integration constant.

Since the end of the charging period is the beginning of the depolarizing period and the end of the depolarizing period is the beginning of the charging period, Eqs. 10 and 11 are obtained.

$$\frac{R_s(E_0 - E_d - E_a)}{(R_d + R_a + R_s)} + A \cdot e^{-\lambda t_1} = \frac{-R_s(E_d + E_a)}{(R_d + R_a + R_s)} + B \quad (10)$$

$$\frac{R_s(E_0 - E_d - E_a)}{(R_d + R_a + R_s)} + A = \frac{-R_s(E_d + E_a)}{(R_d + R_a + R_s)} + B e^{-\lambda t_2} \quad (11)$$

where

$$\lambda = \frac{1}{C_s}\left(\frac{1}{R_s} + \frac{1}{R_d + R_a}\right)$$

Solving these equations for A and B, and substituting Eqs. 8 and 9, final expressions for e_s^+ and e_s^- are obtained as Eq. 12 and Eq. 13, respectively.

$$e_s^+ = \frac{R_s}{(R_d+R_a+R_s)}\left[(E_0-E_d-E_a)-E_0\left\{\frac{1-e^{-\lambda t_2}}{1-e^{-\lambda(t_1+t_2)}}\right\}e^{-\lambda t}\right] \quad (12)$$

$$e_s^- = \frac{R_s}{(R_d+R_a+R_s)}\left[(-E_d-E_a)+E_0\left\{\frac{1-e^{-\lambda t_1}}{1-e^{-\lambda(t_1+t_2)}}\right\}e^{-\lambda t}\right] \quad (13)$$

The following expressions for e_d^+, e_d^-, e_a^+ and e_a^- were derived through similar manipulation.

$$e_d^+ = E_d + \frac{R_d}{R_d+R_a+R_s}\left[(E_0-E_d-E_a) + \frac{R_s}{R_d+R_a}\left\{\frac{1-e^{-\lambda t_2}}{1-e^{-\lambda(t_1+t_2)}}\right\}E_0 e^{-\lambda t}\right] \quad (14)$$

$$e_d^- = E_d + \frac{R_d}{R_d+R_a+R_s}\left[-E_d-E_a-\frac{R_s}{R_d+R_a}\left\{\frac{1-e^{-\lambda t_1}}{1-e^{-\lambda(t_1+t_2)}}\right\}E_0 e^{-\lambda t}\right] \quad (15)$$

$$e_a^+ = E_a + \frac{R_a}{R_d+R_a+R_s}\left[(E_0-E_d-E_a) + \frac{R_s}{R_d+R_a}\left\{\frac{1-e^{-\lambda t_2}}{1-e^{-\lambda(t_1+t_2)}}\right\}E_0 e^{-\lambda t}\right] \quad (16)$$

$$e_a^- = E_a + \frac{R_a}{R_d+R_a+R_s}\left[-E_d-E_a-\frac{R_s}{R_d+R_a}\left\{\frac{1-e^{-\lambda t_1}}{1-e^{-\lambda(t_1+t_2)}}\right\}E_0 e^{-\lambda t}\right] \quad (17)$$

Experimental

Materials Diclofenac sodium was used as received from the manufacturer. Aqueous solutions were prepared with deionized water. To simplify the system, other electrolytes were not used in the donor solution. To aid preservation of the skin function, MEM culture fluid (pH 7.4, Nissui Seiyaku Co., Tokyo) was used as the acceptor solution.

Membrane All diffusion studies involved abdominal skin, freshly excised from 7- to 8-week-old, Hartley male guinea pigs (Shizuoka Jikkendobutsu Co., Hamamatsu) after removal of hair using electric hair clippers and an electric shaver.

Power Source The iontophoretic device (Advance, Tokyo) was conditioned to provide a pulse of 3 to 10 V at 40 kHz with 30% duty (7.5 μs) followed by a 70% depolarizing period (17.5 μs).

Diffusion Cell A schematic diagram of the diffusion cell utilized in these studies is shown in Fig. 2. Excised guinea pig skin was sandwiched between donor and acceptor chambers of horizontal diffusion cells, with the stratum corneum facing the donor compartment. The total area available for transport was 3.14 cm^2. The surface area of the platinum electrode was 0.5 cm^2. The donor and acceptor electrodes were positioned 7 and 17 mm from the skin surface, respectively. The cell halves were held together with a pinch clamp, and the entire assembly was maintained at 37 °C by immersion in a thermostatically controlled water bath. For the purpose of mixing, the acceptor solution was recirculated at 3 ml/min.

At appropriate intervals, samples (1 ml each) were removed from the acceptor compartment and the concentration of diclofenac sodium was determined by high performance liquid chromatography (HPLC). The acceptor compartment was supplemented with fresh solution immediately after sampling.

Time Course of Voltage Drop Voltage drop across the donor and acceptor solution, and the skin were observed as a function of time, using an oscilloscope. Voltage values were read out from the wave pattern displayed on the screen.

Estimation of Model Parameters A nonlinear least squares regression program FKDM which is based on the algorithm of Berman *et al.*[4] was used to fit the model to observed data and to estimate model parameters on a personal computer (NEC PC-9801/Vm2).

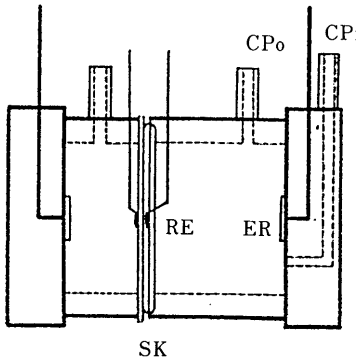

Fig. 2. Schematic Diagram of Diffusion Cell

SK, skin; CPi, CPo, recirculation ports (in and out); ER, electrode; RE, reference electrode.

Results

Model Adaptation The proposed model was fitted to the observed data. The observed time courses of voltage drop across the donor solution and the excised skin are shown in Figs. 3 and 4 with the model estimates. Coincidence of the values with the model estimates verifies the applicability of the proposed model. Estimated parameters are listed in Tables I and II.

Effect of Applied Potential E_0 Table I shows model parameters obtained in drug transport experiments with different applied potentials, in which the drug concentration of the donor solution was kept constant (5.0 mg/ml, Fig. 3). It is clear from Table I that E_d+E_a's are E_0-dependent but $R_d/R_s+R_a/R_s$'s and $R_s \cdot C_s$'s are independent of E_0.

Effect of Donor Concentration Table II shows model parameters obtained in drug transport experiments with different drug concentrations of the donor solution, and with the applied potential kept constant (5 V, Fig. 4). Although $R_s \cdot C_s$'s, and E_a's were independent of the donor concentration, R_d/R_s's, R_a/R_s's and E_d's showed a trend of drug-concentration dependency.

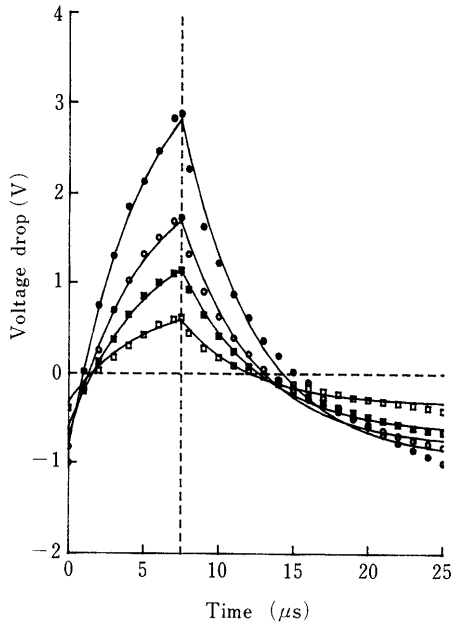

Fig. 3. Time Courses of Voltage Drop across the Skin with Various Applied Voltages

□, 3; ■, 5; ○, 7; ●, 10 V. Curves: calculated values.

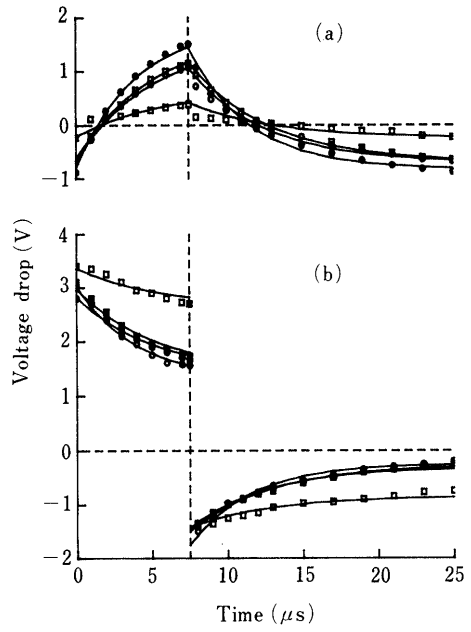

Fig. 4. Time Courses of Voltage Drop across the Skin and the Donor Solution with Various Donor Concentrations

□, 1.0; ■, 5.0; ○, 7.5; ●, 10.0 mg/ml. Curves: calculated values.
(a) Skin. (b) Donor solution.

Discussion

Since $R_s \cdot C_s$'s are inherent characteristics of the excised skin, it is reasonable that $R_s \cdot C_s$'s are not dependent on either applied potential or drug concentration. The electric resistances of donor and acceptor solutions, R_d's and R_a's, are expected to differ according to the concentration of ionized species in the solution. The results obtained were consistent with this expectation.

If the E_d's and E_a's correspond to ionized species in the solution as suggested above, the magnitude of E_d and E_a should depend both on applied potential and on drug concentration. However, compared with the donor solution, the drug concentration is negligibly small in the acceptor solution, and this explains the ambiguity of observed dependency of E_a's on drug concentration.

Consequently, the proposed equivalent-circuit model is considered to be suitable for evaluations of experimental conditions and environments.

TABLE I. Parameters Obtained by Model Adaptation (1)

E_0 (V)	E_d+E_a (V)	$R_d/R_s+R_a/R_s$	$R_s \cdot C_s$ (μs)	Concn. (mg/ml)
3.0	0.890	1.589	7.417	5.0
5.0	1.418	1.134	9.730	5.0
7.0	1.780	1.205	8.956	5.0
10.0	1.989	1.084	9.506	5.0

TABLE II. Parameters Obtained by Model Adaptation (2)

E_0 (V)	E_d (V)	E_a (V)	R_d/R_s	R_a/R_s	$R_s \cdot C_s$ (μs)	Concn. (mg/ml)
5.0	0.1184	1.270	3.918	0.7113	6.64	1.0
5.0	0.2168	1.214	0.683	0.3549	10.39	5.0
5.0	0.2337	1.335	0.769	0.4417	9.11	7.5
5.0	0.2250	1.321	0.565	0.2926	8.66	10.0

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