Electrochemical Characterization of Human Skin by Impedance Spectroscopy: The Effect of Penetration Enhancers

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The electrochemical properties of human cadaver skin were studied in a diffusion cell with impedance spectroscopy as a function of time in the absence and presence of penetration enhancers dodecyl *N,N*-dimethylamino acetate and Azone. An improved electrochemical model of skin is presented, and combining the novel model with modern fractal mathematics, the effect of enhancers on the surface of skin is demonstrated. The enhancers appeared to open new penetration routes and increase the ohmic resistance, capacitive properties, and fractal dimension of skin, which means a rougher or more heterogeneous surface.

KEY WORDS: transdermal drug delivery; impedance spectroscopy; human skin; penetration enhancers; fractal surface.

INTRODUCTION

Transdermal drug delivery including iontophoresis provides a noninvasive method for the administration of systemically effective drugs, which cannot be orally delivered (1). Understanding of the electrochemical properties of skin helps to evaluate the mechanisms of the drug transport through skin, thus facilitating the design of a device for transdermal therapy.

Electrical properties of skin have previously been studied with impedance spectroscopy by several authors, e.g., Refs. 2–9, of which the papers by Yamamoto and Yamamoto (2–5) are most rigorous. Transport properties and mechanisms have been studied by Burnette and Ongpipattanakul (10,11), Pikal and Shah (12–14), and Sims *et al.* (15,16); numerous iontophoretic flux experiments with constant or pulsed current have been reported; see Ref. 17, for example.

The purpose of this paper is to cast new light on the permeability properties of human cadaver skin using impedance spectroscopy and analyzing the results in a novel way. The applicability and power of the method is demonstrated with two penetration enhancers.

MATERIALS AND METHODS

Method

In the impedance method a low-amplitude sinus excitation signal, $e(t) = e_0\sin(\omega t)$, is applied into the system and the amplitude, i_0, and phase shift, δ, of the outcoming signal, $i(t) = i_0\sin(\omega t + \delta)$ is analyzed:

$$i(t) = \kappa(t) \cdot e(t) \tag{1}$$

$\kappa(t)$ is the conductivity of the system, and ω is the angular frequency, $2\pi f$. When Eq. (1) is Laplace transformed, we obtain in the frequency domain

$$I(s) = Y(s) \cdot E(s) \tag{2}$$

where $Y(s)$—the transform function but *not a transform of* $\kappa(t)$—is the inverse of the impedance $Z(s)$ of the system, and $s = j\omega$, where j is the imaginary unit. The dependence of the amplitude and the phase shift of the outcoming signal on the frequency ω is analyzed. The rather complicated mathematics of the measurement is usually carried out in the equipment. The impedance of the system, $Z(s)$, is a complex quantity, and it is generally presented with either Bode plot or Nyquist plot: in Bode plot log $|Z(s)|$ and the phase angle are presented as a function of $\log(\omega)$; in Nyquist plot the imaginary part of $Z(s)$ is presented as a function of the real part of $Z(s)$, and ω is a parameter.

The next task is to construct, with the aid of electric components with known impedances, a model for the system, an *equivalent circuit,* which fits into the measured data. Usually such a circuit is easily found, but giving physical significance to the components may cause problems, and multiparameter nonlinear fits may result in totally erroneous values of the physical quantities. Therefore, nonlinear mathematical fits should be exactly right that it could be accepted. An equivalent circuit proposed for human skin is discussed later in detail.

Experimental

The excised human abdominal skin was separated from elderly people as a 0.5-cm-thick layer (The University Hospital of Kuopio). Epidermis was separated by heating the skin sample in distilled water at 60°C for 2 min. The epidermis samples were dried at room temperature and ambient moisture and stored at −15°C until used.

The enhancers studied were dodecyl *N,N*-dimethylaminoacetate (DDAA; University of Kansas, Department of Pharmaceutical Chemistry) and Azone (Whitby Research Inc., Irvine CA), which effectively increase percutaneous penetration of both hydrophobic and hydrophilic model drugs (18). Before the impedance measurements the skin samples were pretreated with 10 μL of pure enhancer solution for 3.5 hr and hydrated for 30 min to remove possible wrinkles from the samples.

An isoosmotic 0.15 *M* NaCl solution was used throughout the study at room temperature (23°C). The cell used was tailor-made of Teflon at the workshop of Helsinki University of Technology, Department of Chemistry; the volume of the chambers of the cell was 5 ml, and the area of skin was 1 cm^2.

The measurements were carried out with a Solatron 1286 four-electrode potentiostat and Solatron 1170 frequency response analyzer (FRA) controlled by HP3911 computer which also calculated the impedances in the form of

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either a Bode plot or a Nyquist plot. The potentiostat supplies electric current through the two large outer Ag/AgCl electrodes (area, ca. 10 cm^2) in such a way that the potential drop across the skin measured with the two reference Ag/AgCl electrodes is equal to the signal given by the signal generator in the FRA. The electrodes were prepared electrolyzing pure silver plates and wires in 0.1 *M* HCl solution with a constant current of 0.1 mA cm^{-2}.

The frequency of the input sinus signal was varied between 0.01 Hz and 50 kHz and the root mean square amplitude was 5 mV (peak-to-peak voltage is then ca. 14 mV) in order to preserve close to steady-state conditions, as the theory requires. The impedances were measured at 0-, 0.1-, 0.5-, and 1.0-V potential drops across the skin. The results were fitted into an equivalent circuit presented below using the software by Boukamp (19).

Equivalent Circuit

Skin is often modeled as a parallel combination of a resistor and a capacitor (RC-circuit), if any model is given. In fact, it is rather a good equivalent circuit for most purposes, and provided a starting point in this study: the resistor stands for the ohmic resistance of skin, while the capacitor describes the double-layer capacitance of the penetration routes of skin. These routes are not sweat glands, hair follicles, etc., because their sizes are far too great to account for the ion selective properties of skin (10). However, if this model were a proper one, a complete semicircle should be obtained in a Nyquist plot. In our measurements with or without enhancers, this never took place, but an incomplete, depressed semicircle always appeared. This phenomenon has also been recognized by Cole (20), for example.

The depressed semicircle can be described using a constant phase element (CPE), which replaces the capacitor in the equivalent circuit (20). The impedance of a capacitor is given by $Z_{cap} = (j\omega C)^{-1}$, while that of a CPE is $Z_{CPE} = Y^{-1} \cdot (j\omega)^{-\alpha}$, i.e., if $\alpha = 1$ a CPE is equal to a capacitor with $Y = C$. If $\alpha = -1$ a CPE is equal to an inductor with $Y = 1/L$; $\alpha = 0$ makes a CPE equal to a resistor. In our measurements α always had values between 0.5 and 1, and the CPE can be described as a "leaking" or pseudo capacitor. A RC-circuit is characterized with one relaxation time $\tau = RC$, while a circuit with the CPE is characterized with a continuous distribution of relaxation times (2,21) because a CPE can alternatively be described with a network of capacitors and resistors. It is impossible to evaluate such a network, e.g., "a transmission line" (21), but the problem is overcome using the fractal approach as follows.

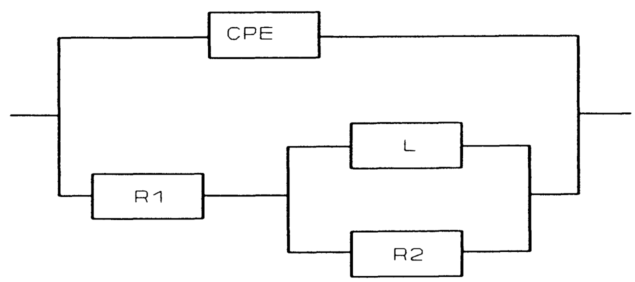
The physical meaning of α is not completely clear—except the values mentioned above—but it is proposed that the values between 0.5 and 1 are connected to the fractal dimension, D_F, of the surface studied. In our case D_F can be estimated by a simple relationship (22):

$$\alpha = 1/(D_F - 1) \qquad (3)$$

The fractal dimension can be considered as a measure of the roughness of the surface: a completely smooth surface corresponding to a capacitor has a fractal dimension 2, while a surface with the dimension 3 is so wrinkled that it fills the entire space. The dimension of natural surfaces, e.g., the surface of the earth, is 2.2–2.3 (23). Equation (3) has a profound significance: a CPE not only explains the depressed semicircles, but introduces the fractal character of skin, excluding the use of a capacitor because physical surfaces cannot be completely smooth.

Further, for the data obtained with enhancers not even this equivalent circuit was satisfactory. First, the size of the semicircle was greater than without enhancers because of the greater ohmic resistance, and second, in the low-frequency region, the semicircle began to stretch alongside the real axis, implying some new phenomenon to take place. In Fig. 1 a Nyquist plot of a sample treated with Azone is shown after 18 hr of hydration. It can be seen that a parallel RC-circuit fits very poorly to the data; a parallel RCPE-circuit almost fits but still is not good enough.

Using the software of Boukamp an equivalent circuit could be found, and it is presented in the following scheme.



R1 is the ohmic resistance of skin, and CPE is the capacitive component including α; R2 and L are components which exist only after the treatment with enhancers and are explained later. This circuit fits almost perfectly to Fig. 1, although the fitted curve has not been drawn for the sake of clarity.

The equivalent circuit presented here is probably not the only possible one, and for skin a circuit where two parallel RC-circuits are in series representing the two surfaces of the membrane has been proposed (2). In our case, however, the relaxation times would be so close to each other that separating them would be ambiguous. Also, the struc-

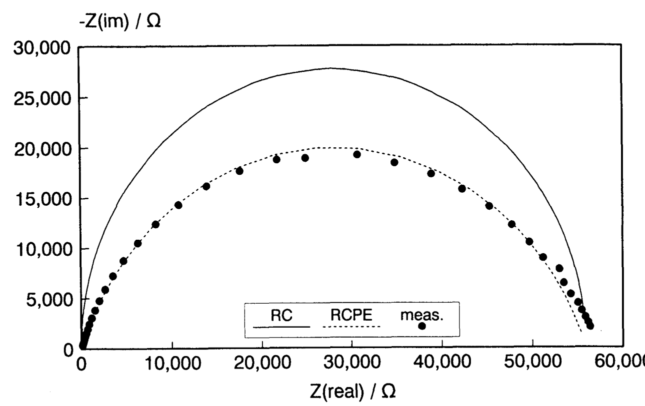


Fig. 1. A Nyquist plot of human skin treated with Azone; time of hydration is 18 hr. Points are measured values and the dashed line is the best fit for a parallel combination of a resistor and a CPE; the solid line represents a RC-circuit which has the same parameter values as the RCPE-circuit.