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.

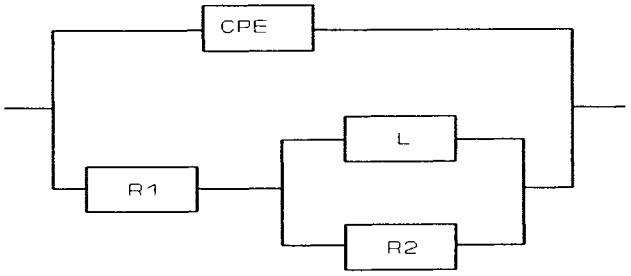
The physical meaning of α is not completely clear—except the values mentioned above—but it is proposed that the values between 0.5 and 1 are connected to the fractal dimension, D_F, of the surface studied. In our case D_F can be estimated by a simple relationship (22):

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

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-

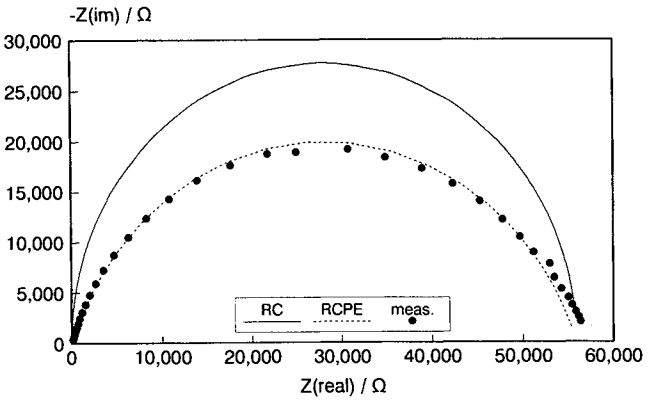


Fig. 1. A Nyquist plot of human skin treated with Azone; time of hydration is 18 hr. Points are measured values and the dashed line is the best fit for a parallel combination of a resistor and a CPE; the solid line represents a RC-circuit which has the same parameter values as the RCPE-circuit.

ture of skin justifies our choice for the circuit: stratum corneum forms the other side of the skin sample and it may "throw a shadow" so deep on epidermis that the entire response to the input signal is formed in stratum corneum due to its great transport barrier (2,24).

RESULTS AND DISCUSSION

The resistance of human skin is variable: in Ref. 9 values between 4.3 and 86.7 kΩ cm^{-2} are presented, but according to our experience, values below 10 kΩ cm^{-2} correspond to samples with fractions or other macroscopic appendages; values above 50 kΩ cm^{-2} were also encountered but those samples showed an anomalous behavior and were left out from the analysis at this early stage. The values presented here are not average values but from single measurements because the mathematical procedure is rather tedious. Although the numerical values of the components do vary from one person to another, the trend remains.

In Fig. 2 it can be seen that, surprisingly, enhancers increase the resistance of skin. Because of the low relative permittivity of the lipoidal matrix, it is obvious that current penetrates skin through aqueous pores and other hydrophilic channels (11), which means that enhancers block these routes. Thus, the flux enhancement of drugs must take place due to increased partitioning, i.e., solubility of lipophilic solutes in the lipoidal matrix. It has been found in differential scanning calorimeter studies that the main mechanism of the action for DDAA and Azone is the disordering of the lipids of stratum corneum (25), which probably causes the increase in the resistance. In the case of Azone an isolating layer on the skin surface may also cause the increase.

The value of the frequency exponent α (Fig. 3) is decreased as the resistance is increased, and according to Eq. (3), the fractal dimension is increased, i.e., the surface is roughened. In order to visualize the effect of enhancers on

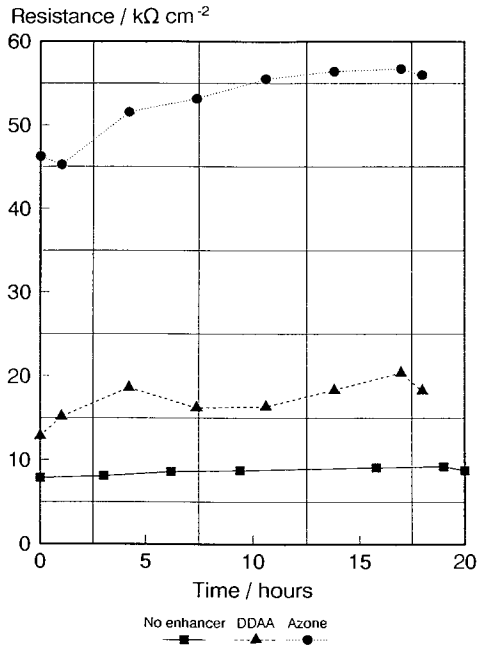


Fig. 2. The resistance of skin as a function of time.

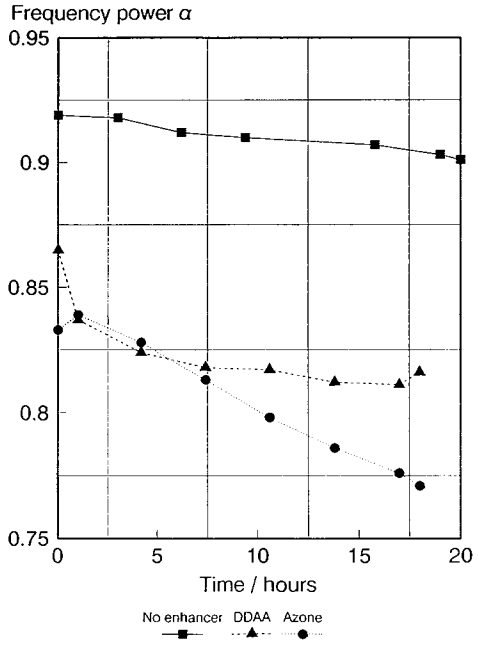


Fig. 3. The frequency exponent of the CPE as a function of time.

the surface roughness, the simulation in Fig. 5 was carried out as follows.

The cross section of a fractal surface with the dimension D_F is a fractal curve with the dimension $D_F - 1$ (26). If this curve is Fourier transformed, its power spectrum in the frequency domain is proportional to $f^{-\beta}$, with β and D_F related by (23)

$$D_F = E + \tfrac{1}{2} \cdot (3 - \beta) \quad (4)$$

where E is the corresponding euclidean dimension. In Fig. 5 two fractal curves, with $D_F = 1.1$ and 1.3 ($\beta = 2.8$ and 2.4 when $\alpha = 0.9$ and 0.775), are presented. The curves were generated by simulating white noise, which has a uniform power spectrum, with random numbers, Fourier transforming the white noise, multiplying its power spectrum with a weight function $f^{-\beta}$, and calculating the inverse Fourier transform of the thus obtained spectra. All the calculations were carried out in an IBM PC using the PC-MATLAB software (27).

The values of D_F of 1.1 and 1.3 correspond approximately to the cross sections of skin without enhancers and with Azone after 18 hr of hydration. Figure 5 illustrates in a qualitative way the effect of Azone on the surface roughness; it must be emphasized that it is not a picture of real skin but presents two curves with the same fractal dimension as the cross section of skin, i.e., we have no measure for the width or depth of the peaks and valleys. Since the origin of our data is in the ion transport across the surface of skin,the fractal dimension reflects its chemical heterogeneity rather than the shape of the surface (e.g., hydrophilic and hydrophobic lipid domains) (24), as well. Nevertheless, Fig. 5 shows that enhancers roughen the surface of skin, which means that they open new channels in the lipoidal matrix; this is confirmed by the increased water flux (28). However, these new channels obviously do not permit the flux of electrolytes because the resistance is increased. We have also

noticed that the iontophoretic flux of sotalol is decreased after the treatment with enhancers (28).

What these new channels are is not evident, but since water is a rather small molecule (diameter, 0.28 nm) and an exceptional chemical compound, it is possible that it can penetrate through microscopic pores between lipid molecules. Ions are always hydrated in aqueous solutions, i.e., surrounded by water molecules which are bound to an ion with a relatively strong bond. Therefore, the effective radii of hydrated ions are greater than their crystallographic radii, which may cause their inability to use these pores.

The admittance of the CPE represents the capacitive properties of skin and its increase due to enhancers can be seen in Fig. 4, which means an increase in the relaxation time. It is rather difficult to name the reason for this increase but enhancers have an effect on the relative permittivity of the tissue and change its effective surface area (Fig. 5): both of these affect the relaxation time.

In the equivalent circuit presented above the extra impedance in the low-frequency region is modeled as a parallel combination of a resistor, R2, and an inductor, L, but giving physical significance for these elements is not evident. The individual values of R2 or L are not of importance, but their combined effect is:

$$Z_{R2,L} = \frac{j\omega\ R2\ L}{R2 + j\omega\ L} \qquad (5)$$

Several combinations of R2 and L would yield the same result in the narrow frequency region where this subcircuit is sensed. The most important feature is that R2 and L have zero values without enhancers and are smaller with DDAA than with Azone.

This circuit is sensed in the low-frequency region and therefore it has to be related to a relatively slow process. Since the enhancers loosen the structure of skin, dipolar molecules in skin begin to orientate with an alternating field in such a way that they create a current which opposes the external alternating current, as in self-induction. With Azone the resistance of skin is also decreased with the increased potential drop across the skin, which implies that the electric field forces the molecules to align with itself. However, this orientation-relaxation process is so slow that at high frequencies, molecules do not have enough time to orientate and the phenomenon disappears.

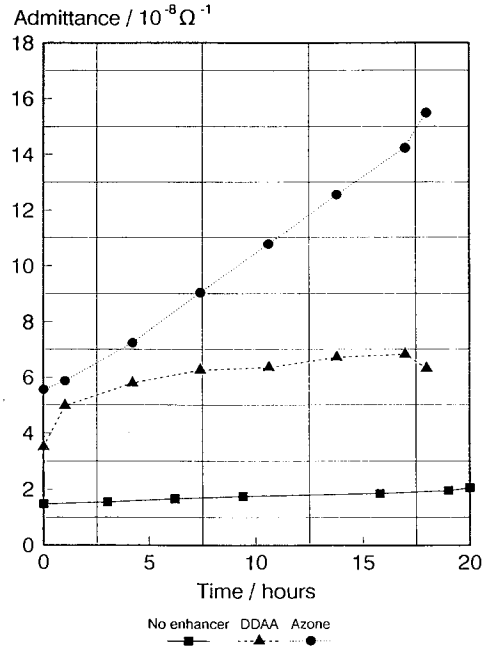


Fig. 4. The admittance of the CPE as a function of time.

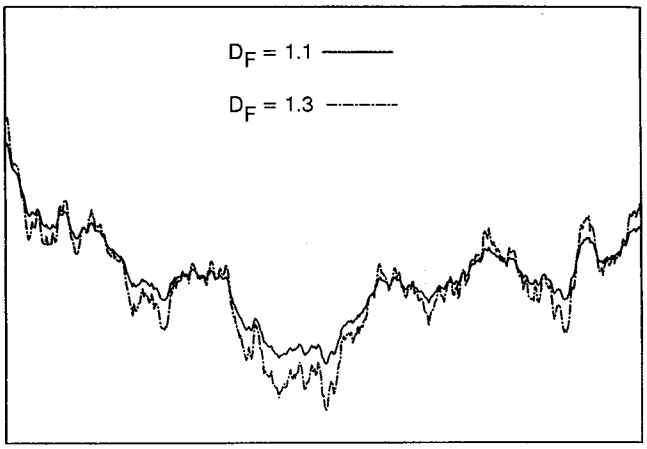


Fig. 5. Fractal curves with fractal dimensions equal to the values of the cross sections of human skin in the absence, D_F = 1.1, and presence, D_F = 1.3, of Azone.

Recently it has been presented (29) that low-frequency inductive effects at electrodes can be explained with a two-step electron transfer process where an intermediate species is adsorbed on the electrode. It is not evident what kind of analogous process at the membrane/solution interface would be responsible for the inductive effects, but this possibility must be taken into consideration.

CONCLUSIONS

It is shown that the modern fractal mathematical approach can be applied in the interpretation of the impedance spectra of human cadaver skin. This is done by introducing a constant phase element (CPE) into the equivalent circuit, instead of a capacitor, and relating the frequency exponent of the CPE to the fractal dimension of the surfaces.

Penetration enhancers appeared to increase the heterogeneity of skin by opening new penetration routes and increasing the disorder of the lipoidal matrix. As a consequence the ohmic resistance, capacitive properties, and fractal dimension of skin are increased. At the same time another relaxation process takes place, which is proposed to be due to the alignment of the lipid molecules in the alternating electric field.

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