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The Enhanced Iontophoretic Transport of TRH and Its Impedance Study

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ABSTRACT

The transdermal drug delivery of iontophoresis provides a noninvasive method for the administration of effective drugs. The enhanced iontophoretic transport of thyrotropin-releasing hormone (TRH), a tripeptide with molecular weight of 362 and a pK_a of 6.2, through an excised rabbit skin has been achieved by in vitro iontophoresis. The results indicate that the steady-state flux of TRH in a diffusion cell is proportional to the current density. In addition, the electrochemical properties of rabbit skin were studied with impedance spectroscopy, and it was found that the skin impedance decreased to a low and stable value with respect to its initial skin impedance while a current was applied through the rabbit skin. This is in good agreement with our experimental results on iontophoretic transport. As compared to passive diffusion, the iontophoresis dramatically increased the transport fluxes of TRH, and ethanol pretreatment further enhanced its iontophoretic transport. A practical implication of these results is that iontophoresis with a chemical permeation enhancer (ethanol pretreatment) can be applied to enhance and control the transdermal delivery of peptides.

INTRODUCTION

The transdermal administration of drugs has gained much attention in recent years. Traditional transdermal delivery systems transport drugs through the skin mainly by the laws of passive diffusion. The stratum corneum in the skin is considered to be the major barrier for passive transdermal diffusion. The problem of poor transdermal transport of drugs can be overcome by providing an external electrical energy source which will facilitate the transport processes. Many polypeptides normally carry electrical charges at pH values away

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from their isoelectric points. Therefore, one promising enhancement technique of applying an electrical field or current through the skin, called iontophoresis, can induce an increased electrical migration of charged drug molecules into tissues in an electrolyte medium (1). This technique has been used clinically in delivering medication to surface tissues and has been recognized to be an effective, safe, and painless noninvasive way to administer ionized drugs.

Peptides and proteins are biopolymers and their therapeutic function has gained growing recognition in recent years (2–4). Many of these biopolymers are increasingly used for the treatment of various chronic diseases. Unfortunately, these biopolymers are highly susceptible to degradation by proteolytic enzymes in the gastrointestinal tract and are subject to the extensive hepatic metabolism when taken orally. Parenteral administration is the preferred route, but they are therapeutically effective only for a short duration, and repeated injections not only are inconvenient and fearful to patients, but also increase the risk of contamination by unsanitary needles. The intrinsic permeabilities of these polypeptides through the skin may be too low to allow significant amounts of the drug to permeate. Due to their hydrophilic nature and large molecular sizes, polypeptides do not readily penetrate the skin by passive diffusion. Therefore, it is essential to develop an innovative technique and nonparenteral route to promote the transdermal permeation of hydrophilic peptides and proteins through the rate-limiting barrier of the hydrophobic stratum corneum. The pretreatment with ethanol (this procedure is to simulate the function of a chemical permeation enhancer) accompanied by iontophoresis was also investigated in this study, with the aim of evaluating the potential of the enhancer plus iontophoresis as a means for controlled transdermal delivery of these polypeptides.

Understanding the electrochemical properties of skin helps to evaluate the mechanism of the drug transport through skin and thus facilitates the design of a device for transdermal therapy. Electrical properties of skin have previously been studied with impedance spectroscopy by some authors (5–7), of which the papers by Yamamoto and Yamamoto (5,6) are most rigorous. The skin transport properties and mechanism have been investigated by Burnette and Ongpipattanakul (8), and by Pikal and Shah (9).

The purpose of this paper is to cast a new focus on the permeability properties of rabbit skin by using impedance spectroscopy. The iontophoretic transport of a model peptide (TRH) was investigated in this study and was compared with its passive transport. In addition, the iontophoretic transport of TRH across skin, with and without ethanol pretreatment, was also investigated. Ethanol pretreatment shows the enhanced effect of a chemical permeation enhancer. Thus, the synergetic effect of a chemical permeation enhancer and iontophoresis can be regarded as a potential means for the controlled transdermal drug delivery. In this study, the flux–current relationship for TRH through a rabbit skin was characterized in the in vitro experiments, and the electrical properties of skin during iontophoresis was monitored by simultaneous impedance spectroscopy.

MATERIALS AND METHODS

The Diffusion Cell

Almost all of the in vitro apparatus used for iontophoresis consist of simple two-compartment diffusion cells [10–12]. A four-electrode, two-chamber, and horizontal electrochemical diffusion cell was designed and employed in all iontophoretic transport studies. Figure 1 is a schematic diagram of a four-electrode, two-chamber, and horizontal diffusion cell with an impedance measurement system. A tissue membrane of $\pi/4$ cm^2 area was exposed to the donor and receptor compartments of the diffusion cell, which were magnetically

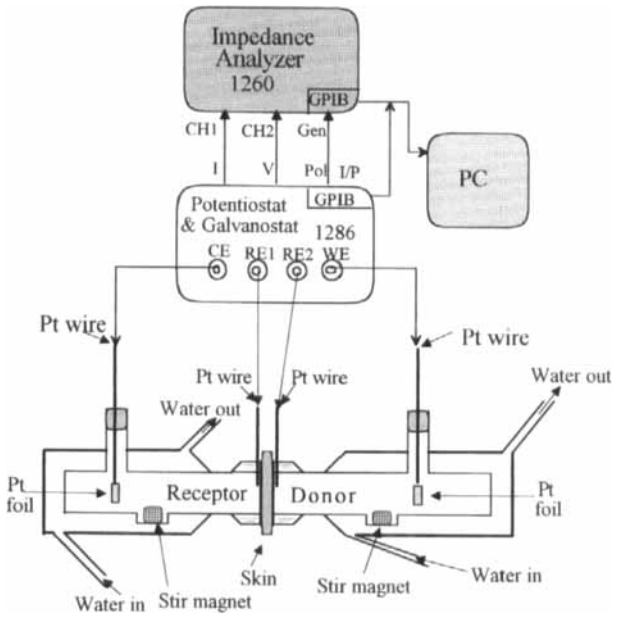

Figure 1. Four-electrode diffusion cell with impedance measurement system.

stirred and water jacketed with the volume of each half-cell of approximately 4 ml. The temperature of the diffusion cell was controlled by circulating water continuously through the water jacket from a constant temperature bath, which was maintained at 37° ± 0.2°C.

Solution Preparation

The thyrotropin-releasing hormone (TRH, Sigma Co.) used in the in vitro iontophoretic experiments in a weakly basic tripeptide with a molecular weight of 362.42 and a pK_a value of 6.2. All peptide experiments were carried out in a phosphate/citrate buffer with an ionic strength of ~0.17 M. The donor citrate buffer solution holding at pH 4 was consisted of 0.0771 M Na_2HPO_4 and 0.0615 M citric acid. Based on the pK_a values of the ionizable TRH, its charge at the donor solution (pH 4.01) can be protonated to be positive. The positively charged drug molecule is facilitated in its transport across the skin while the anode is placed in the donor compartment. An isotonic Sorensen phosphate buffer of pH 7.4, consisting of 0.019 M NaH_2PO_4 an 0.081 M Na_2HPO_4, was used as the receptor buffer solution to simulate tissue buffer system. The concentration of the drug (TRH) in the donor buffer solution was about 2.76 mM. A full-thickness skin was excised from the inner side of the rabbit ear skin dermatomed to about 100 μm thickness and was placed in deionized distilled water. Then the ultrasonic cleaner (Branson 1200) was employed for 20 min to remove gas bubbles entrapped in the skin.

Electrodes

Most of the in vitro apparatus utilize a system of two electrodes, one in each compartment of the apparatus, and the conventional two-electrode system is merely maintained at a applied current by the power supply, while the skin resistance dominates the voltage drop across the skin. In order to investigate the skin electrochemical characteristics, sets of two electrodes each are placed on both sides of the skin, as shown in Fig. 1. Hence, a four-electrode system was designed in this study to proceed the impedance measurement and drug delivery during the iontophoretic experiments. Platinum wires (or foil) were generally used as electrodes in most in vitro studies (13). Silver–silver chloride electrodes could also be used in some in vitro studies. However, the silver–silver chloride electrode may precipitate peptides/protein. So platinum electrodes are preferred for use in the delivery of peptides. In this work, platinum wires (99.99% purity, D = 0.5 mm) were selected as the reference electrodes, which were placed 2 ~ 3 mm from either side of the tissue membrane (in order to observe the impedance variation across the skin). The anode in the donor compartment and the cathode in the receptor compartment, placed in each sampling port of the electrochemical cell, 2 cm from either side of the tissue membrane, were made of platinum foil (7.5 × 5 × 0.5 mm). Before installation of the electrodes, these electrodes were pickled in 0.1 N HCl solution for 20 min in order to clean the surface of the electrode, and then rinsed in the double-distilled water.

Impedance Measurements

The impedance measurements during iontophoresis experiments were carried out by a Schlumberger 1260/1286 system (Schlumberger Instruments), which can be seen from Fig. 1. The 1286 electrochemical interface is a programmable potentiostat/galvanostat, which was used for the four-probe measurements in this study. The 1260 frequency response analyzer analyzes the magnitude and phase shift of the response signal, which is compared to the input signal by a correlation process. The frequency response analyzer was performed in the range of 1 Hz ~ 1 MHz at the increment of 10 frequencies per logarithmic decade. The impedance was obtained by superimposing a sinusoidal alternating potential of a small amplitude (10 mV) on a DC potential. The impedance of the system, Z, is a complex quantity, generally presented with either a Bode plot or a Nyquist plot. In the Bode plot, Log $|Z|$ and the phase angles are presented as functions of frequency (log ω); in the Nyquist plot, the imaginary part of Z versus the real part of Z is plotted with ω as a parameter. Software called "ZPLOT" was employed to analyze impedance data by using curve fitting, linear regression, and polarization resistance functions.

Ethanol Pretreatment

The diffusion cell was assembled with the rabbit skin clamped between the two half-cells. The donor and receptor sides were filled with ethanol and this system was maintained at 37°C with stirring for 1 hr. The ethanol pretreatment procedure has been described in details by Srinivasan et al. (14). Then, the ethanol was drained, the two chambers were washed thoroughly with the buffer, and the iontophoresis experiment was carried out.

Experimental and Analytic Procedures

The volume of all samples, which were taken periodically every 2 hr over 10 ~ 12 hr, was about 0.1 ml. Each sample was taken from the receptor cell at each designated time and an equal volume of fresh buffer solution was replaced after each withdrawal. All the samples were stored immediately in a 0° ~ 4°C refrigerator until analyzed. For every 120 min the iontophoretic experiment was paused, and then scan of the impedance spectroscopy of the epidermis was performed for 2 ~ 3 min.

The concentration of TRH was analyzed by a capillary electrophoresis (CE) instrument (P/ACE system 5010, Beckman Instrument Inc.), which is a relatively new separation method that has proven to be especially useful for the analysis of biomolecules. This technology involves the migration of a charged solute in a narrow bore capillary under high electric field. The method is primarily analytical because of the exceedingly small sample capacity (only nanoliter volumes are required); the basic configuration of the capillary electrophoresis system is illustrated in Fig. 2. The basic instrument configuration for CE is consisted of a fused-silica capillary (50 to 100 cm in length and 50 to 100 μm in internal diameter) with an optical viewing window, a controllable high-voltage power supply (up to 30 kV), two electrode assemblies, two buffer reservoirs, and an ultraviolet (UV) detector. In the analysis, the run buffer solution for TRH was 0.01 M phosphate buffer at pH 2.38. Because CE uses very high voltages in a <100 μm internal diameter capillary, the efficiency is very high with the number of theoretical plates up to 5×10^5, and a potential theoretical limit of 2.8×10^6 plates/m reported (15). The UV detector was set at 214 nm for detecting TRH, and the temperature was kept at 25°C. A 25 kV was applied across capillary (57 cm in length and 75 μm in internal diameter), and the relative retention time for TRH was found to be 7.6 min. All TRH in CE chromatographic peaks were quantified by using peak areas. Linear regressions were calculated for the peak area of measured responses versus known concentrations. The curves were linear with an average slope of 0.012 ml/μg, and an average intercept of 0.00663.

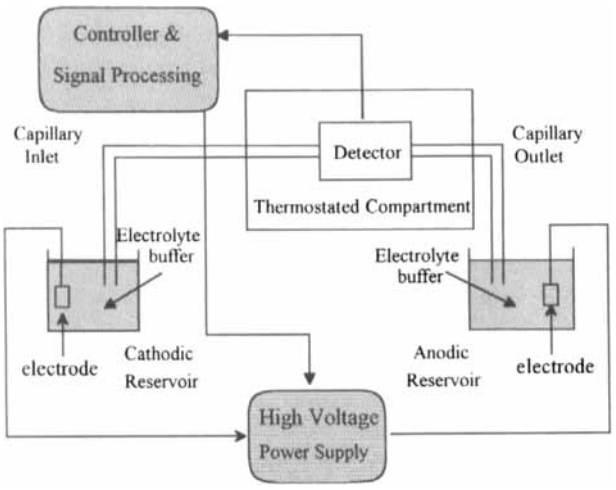

Figure 2. Basic configuration of the capillary electrophoresis system.

RESULTS AND DISCUSSION

The Iontophoretic Transport of Thyrotropin-Releasing Hormone

Before TRH is used in the iontophoresis experiment, it is essential to investigate the stability of the drug in the diffusion cell under applied voltage or current. Considering the pain threshold of skin, a generally agreed limit for tolerable current intensity if about 0.6 ~ 0.7 mA/cm^2. So the current is performed below 0.7 mA/cm^2 for the iontophoretic experiments. Figure 3 reveals that the drug concentration versus duration time at various currents (0.1, 0.25, 0.5 mA and no current). It can be seen obviously from the figure that the drug concentration does not decrease with increasing time except at

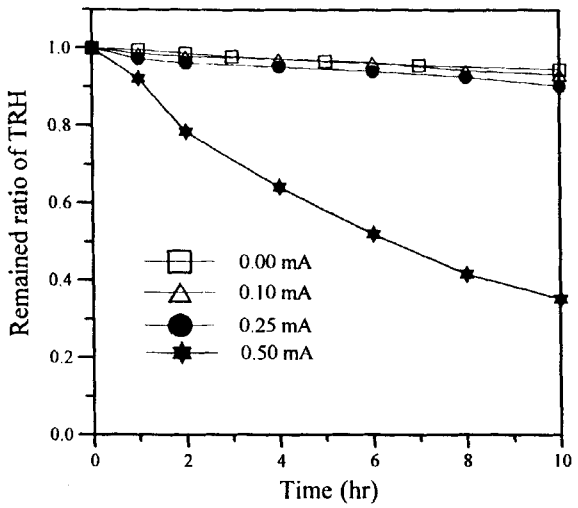

Figure 3. TRH stability test at 37°C for various currents in pH 4.01 citrate/phosphate buffer solution with platinum electrode. Initial TRH concentration: 1 mg/1 ml.

the current of 0.5 mA, in which the attenuation percentage for drug concentration is about 65% for 10 hr.

A representative set of data for TRH have been obtained by measuring the accumulation amounts versus iontophoresis time for various current intensities, and these are shown in Fig. 4. A linear relationship between the TRH amount in the receptor and iontophoresis time was observed for TRH across the rabbit skin. It is clear that an increase in current intensity during iontophoresis entails an augment of the in vitro transdermal accumulation amount of drug transport. However, the passive cumulative amount (no applied current) cannot permeate enough to be detected by the CE; the reason is that the hydrophilic drug is hardly transported across the hydrophobic skin. The mass fluxes of TRH were calculated by taking the amount of TRH present in the receptor compartment and then dividing this amount by both the collection time interval and the permeable skin area (0.785 cm^2). The flux of TRH versus iontophoresis time under various current intensities is depicted in Fig. 5. It is observed that, the higher current intensities we applied, the more flux transport across the excised rabbit skin was produced. These mass fluxes increase conspicuously in the beginning 2 hr and then tend to reach a stable value, which indicates that the constant steady-state fluxes were achieved during iontophoresis.

The effect of current density on the in vitro transdermal steady-state flux of TRH is shown in Fig. 6. A

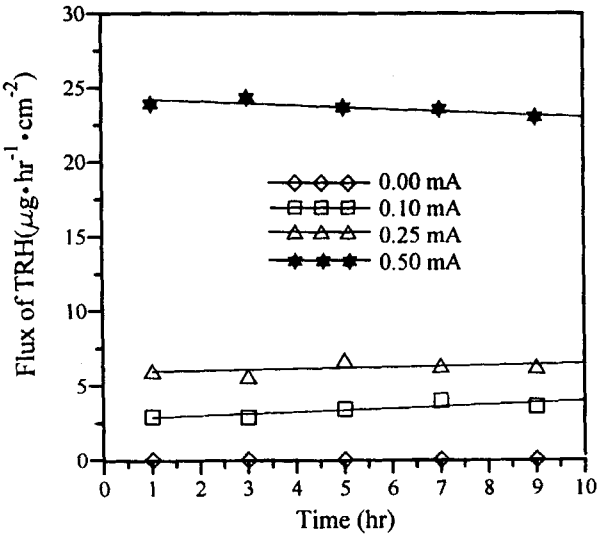

Figure 5. The flux of TRH versus time at various currents for rabbit skin at 37°C. Donor: 4 mg TRH/4 ml, pH 4.01 citrate/phosphate buffer. Receptor: pH 7.4 phosphate buffer, skin area: 0.785 cm^2.

relationship between current density and steady-state flux was obtained with the regression line $Y = 38.58X^{1.311}$ for TRH. The linearity implies that the iontophoresis enhances the flux of drugs without losses due to polarization. However, the curve in Fig. 6 show a

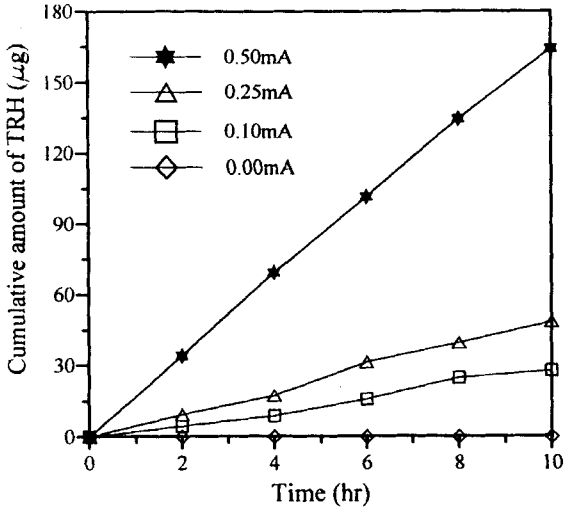

Figure 4. The cumulative amount of TRH versus time at various currents for rabbit skin at 37°C. Donor: 4 mg TRH/4 ml, pH 4.01 citrate/phosphate buffer. Receptor: pH 7.4 phosphate buffer, skin area: 0.785 cm^2.

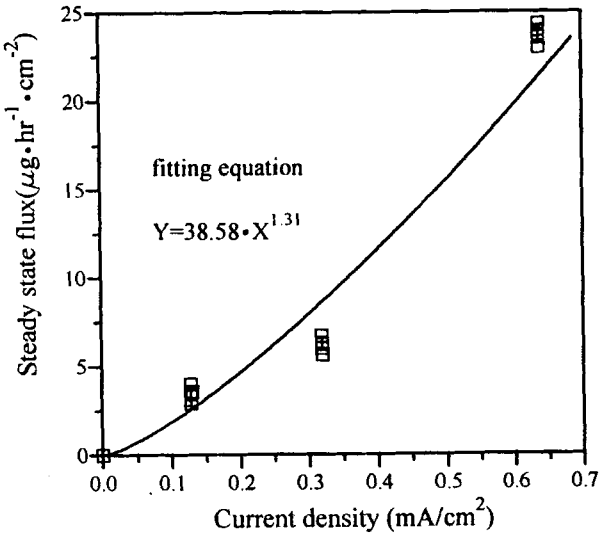

Figure 6. A plot fo mass flux versus current density at steady state for TRH at 37°C. Donor: 4 mg TRH/4 ml, pH 4.01 citrate/phosphate buffer. Receptor: pH 7.4 phosphate buffer, skin area: 0.785 cm^2.

little concave upward tendency. This means that the skin resistance seems to be reduced as the current density is raised. In order to investigate and explain this phenomena, the impedance spectroscopy was introduced for the impedance measurement of the transdermal iontophoretic transport.

Impedance Spectroscopy

Skin is often modeled as a parallel combination of a resistor R_p (skin resistance) and a capacitor C_d (capacitance). In fact, it is assumed that an equivalent circuit for an epidermis system is regarded as a resistor (solution resistance) in series with the skin (R–C circuit) shown in Fig. 7. The impedance data for the rabbit skin were measured for the circuit displayed, and a semicircle is plotted in this complex plane plot, which is called the Nyquist plot. At high frequencies, the impedance is shunted with the double-layer capacitance, and the impedance becomes reduced the solution resistance, R_Ω. At middle frequencies, there appears a capacitive loop whose shape is nearly a semicircle, which corresponds to the circuit formed by the capacitance in parallel with the resistance. At low frequencies, the capacitance offers a high impedance, and hence the current passes mostly through R_p and R_Ω, so the impedance appears to be the value of $R_\Omega + R_p$. The individual data fall on a semicircle of a diameter equal to the skin resistance. It has been found that the skin resistance appeared to a large value (60 ~ 100 kΩ) while no current was applied. However, when we applied currents, the skin resistance was decreased to a low value with respect to its initial skin resistance; and the higher the current intensity we applied, the less skin resistance resulted. Another important plot for evaluating the impedance data is the analysis of the frequency dependence of the absolute value

$$|Z| = \sqrt{(Z')^2 + (Z'')^2}$$

The plot of log $|Z|$ as a function of log(ω), called a Bode plot, is shown in Fig. 8. At very high and very low frequencies, $|Z|$ becomes independent of frequency. From these horizontal regions, the values of R_p and R_Ω can also be determined.

The stratum corneum, which dominates the impedance in the system, is not of uniform thickness for every experiment so that the skin shows different impedance values in different parts of the same skin. In order to analyze more consistent impedance data in our studies, we introduced a dimensionless parameter, called polarization ratio, which is defined as the resistance at certain iontophoresis time (R_{po}) normalized to its initial resistance (R_{pi}). Figure 9 plots polarization ratio versus

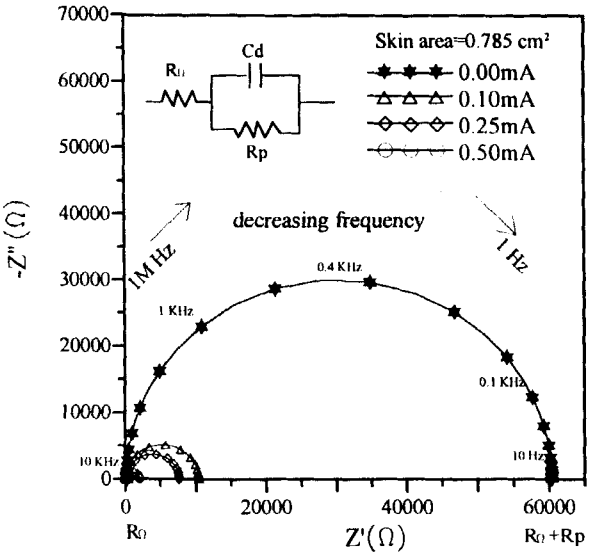

Figure 7. Nyquist plots of fitting data for rabbit skin with various currents at 2 hr. Donor: 4 mg TRH/4 ml, pH 4.01 citrate/phosphate buffer. Receptor: pH 7.4 phosphate buffer, skin area: 0.785 cm².

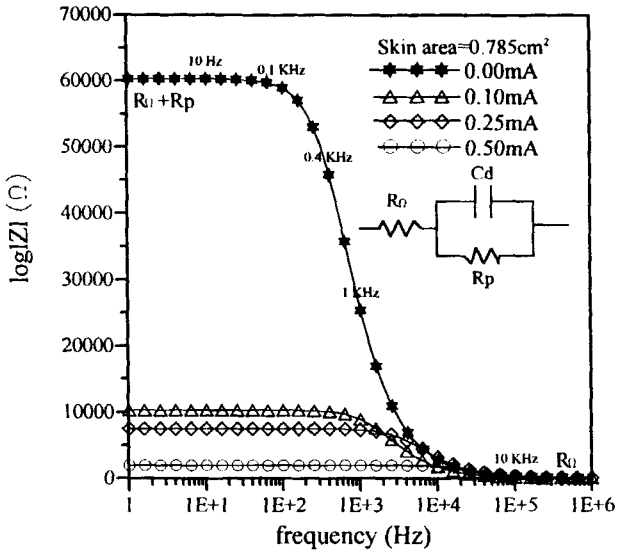

Figure 8. Bode plots of fitting data for rabbit skin with various currents at 2 hr. Donor: 4 mg TRH/4 ml, pH 4.01 citrate/phosphate buffer. Receptor: pH 7.4 phosphate buffer, skin area: 0.785 cm².

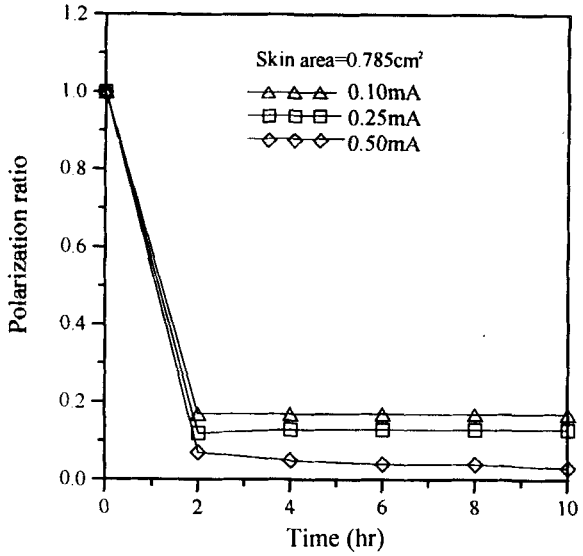

Figure 9. *Comparison of polarization ratio versus time for* TRH at various currents with rabbit skin at 37°C. Donor: 4 mg TRH/4 ml, pH 4.01 citrate/phosphate buffer. Receptor: pH 7.4 phosphate buffer, skin area: 0.785 cm^2.

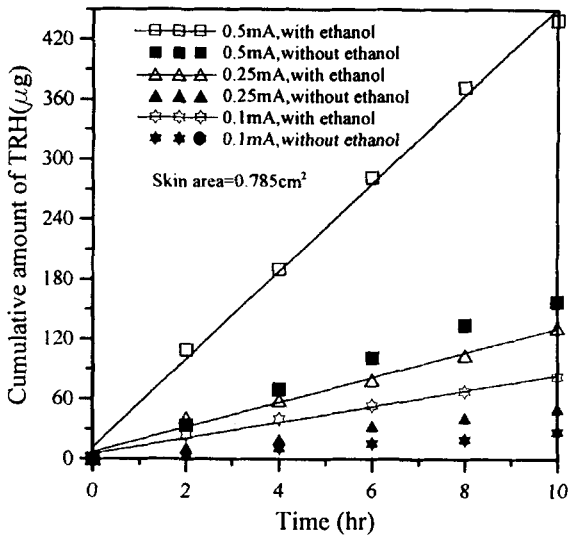

Figure 10. Comparison of the cumulative amount of TRH versus time with various treatments of rabbit skin for different currents at 37°C. Donor: 4 mg TRH/4 ml, pH 4.01 citrate/phosphate buffer. Receptor: pH 7.4 phosphate buffer, skin area: 0.785 cm^2.

operation time at various currents (0.1, 0.25, 0.5 mA). It can be seen that the polarization ratio is lower conspicuously in the beginning 2 hr and then tends to reach a stable value. The polarization ratio decreases with increasing current intensity. Thus, the enhanced permeation of TRH has been confirmed by reducing the resistance of the rate-limiting barrier (the skin) from the impedance results. From the impedance analysis of the skin, such a result is consistent with the relationship between the current densities and steady-state fluxes.

Effect of Ethanol Pretreatment

Figure 10 shows the result of the cumulative amount versus time with and without ethanol pretreatment. The rate of the iontophoretic transport with ethanol pretreatment is greater than that without ethanol pretreatment at each applied current. The enhanced effect due to the ethanol pretreatment is like the effect of a chemical enhancer for passive permeation. As can be seen from Fig. 10, the transport rate of TRH with ethanol pretreatment is about 2.5 ~ 3 times of that without ethanol pretreatment for the same iontophoretic conditions. Generally, in passing through the unpretreated skin, hydrophilic molecules like TRH need to traverse hydrophobic stratum corneum, and the intercellular lipids remain the final rate-limiting barrier to the permeation of hydrophilic drugs. Nevertheless, with a chemical enhancer (ethanol pretreatment) the horny layer in the stratum corneum can be regulated so that the fraction of hydrophilic region is increased, or its resistance to drug passage is reduced. Therefore, the diffusion resistance of the skin decreases with increasing hydrophilic content, and any pretreatment (ethanol pretreatment) that raises the hydration of stratum corneum can promote drug delivery.

The impedance variations of skin with ethanol pretreatment were also monitored during the iontophoretic experiments. While the skin was pretreated with ethanol, its initial impedance values were decreased dramatically from 60 ~100 kΩ to 1 ~ 4 kΩ. Figure11 presents polarization ratio versus operation time at various currents (0.1, 0.25, 0.5 mA). It can be easily seen that the polarization ratio has about the same value during iontophoresis time at 0.1 and 0.25 mA, and is lower in the beginning 2 hr and then tends to reach a stable value at 0.5 mA. The impedance results of skin with ethanol pretreatment are somewhat different from those without ethanol pretreatment, as shown in Fig. 9. the reason is that the initial diffusion resistance of the skin is already reduced with ethanol pretreatment (from 60 ~ 100 kΩ to 1 ~ 4 kΩ) and even when we apply small currents, the skin impedance seems to have a similar value with respect to initial skin impedance unless more current is applied.

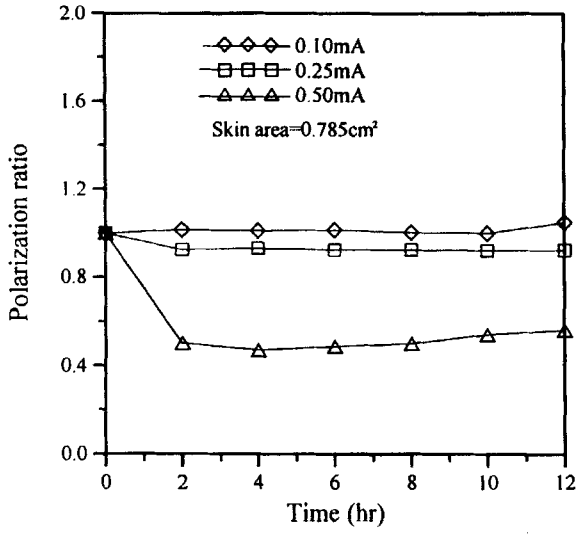

Figure 11. Comparison of polarization ratio versus time for TRH at various currents with rabbit skin of ethanol treatment at 37°C. Donor: 4 mg TRH/4 ml, pH 4.01 citrate/phosphate buffer. Receptor: pH 7.4 phosphate buffer, skin area: 0.785 cm^2.

CONCLUSIONS

The enhanced transport of a model peptide (TRH) through an excised rabbit skin has been investigated by the use of iontophoresis. The resulted steady-state flux of TRH is found to be proportional to the applied current density. Simultaneously, the impedance analysis of skin electric characteristics indicates that skin resistance is reduced to a low and stable value with respect to initial resistance while applying a current. The result of the impedance measurements is in good agreement with our experimental results of iontophoretic transport for TRH. In addition, we also explored the feasibility of combination of iontophoresis and ethanol pretreatment as a technique for controlled transdermal delivery of TRH.

REFERENCES

1. R. Harris, Iontophoresis, in *Therapeutic Electricity and Ultraviolet Radiation* (S. Licht, ed.), John Wiley & Sons, Baltimore, MD 1967.
2. Y. W. Chien, Advances in transdermal systemic drug delivery, Drugs Future, 13(4), 343-362 (1988).
3. A. K. Banga and Y. W. Chien, Iontophoretic delivery of drugs: Fundamentals, developments and biomedical applications, J. Contr. Release, 7, 1-24 (1988).
4. R. Ronald, R. R. Burnette, and Diana Marrero, Comparison between the iontophoretic and passive transport of TRH across excised nude mouse skin, J. Pharm. Sci., 75, 738-743 (1986).
5. T. Yamamoto and Y. Yamamoto, Electrical properties of the epidermal stratum corneum, Med. Biol. Eng., 14, 151-158 (1976).
6. T. Yamamoto and Y. Yamamoto, Dispersion and correlation of the parameters for skin impedance, Med. Biol. Eng. Comput., 16, 592-594 (1978).
7. J. D. DeNuzzio and B. Berner, Electrochemical and iontophoretic studies of human skin, J. Contr. Release, 11, 105-112 (1990).
8. R. R. Burnette and B. Ongpipattanakul, Characterization of the permselective properties of excised human skin during iontophoresis, J. Pharm. Sci., 76, 765-773 (1987).
9. M. J. Pikal and S. Shah, Transport mechanisms in iontophoresis II. Electroosmotic flow and transference number measurements for hairless mouse skin, Pharm. Res., 7, 213-221 (1990).
10. N. H. Bellantone, S. Rim, M. L. Francoeur, and B. Rasadi, Int. J. Pharm., 30, 63 (1986).
11. M. J. Pikal and S. Shah, Pharm. Res., 7, 213 (1990).
12. R. R. Burnette and B. Ongpipattanakul, J. Pharm. Sci., 77, 132 (1988).
13. P. Lelawongs, J. Liu, O. Siddiqui, and Y. W. Chien, Int. J. Pharm., 53, 13 (1989).
14. V. Srinivasan, W. I. Higuchi, S. M. Sims, A. H. Ghanem, and C. R. Behl, Iontophoresis of polypeptides, J. Pharm. Sci., 49, 57-62 (1989).
15. T. Psuda, G. Nakagawa, M. Sato, and K. Yagi, Separation of nucleotides by high-voltage capillary electrophoresis, J. Appl. Biochem., 5, 330 (1983).