DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 10(4), 575-599 (1984)

DEVELOPMENT OF A DYNAMIC SKIN PERMEATION SYSTEM FOR LONG-TERM PERMEATION STUDIES

Yie W. Chien and Kirti H. Valia

Controlled Drug Delivery Research Center
Rutgers University, College of Pharmacy
Piscataway, New Jersey 08854

ABSTRACT

A dynamic skin permeation system was developed aiming to eliminate the deficiencies observed in the apparatus currently available. It consists of two half-cells in mirror image, each contains a stirring platform to permit a starhead magnet to rotate at a synchronous speed. The solution chamber is equipped with a sampling port, which can be tightly closed with glass stopper. The design characteristics of this new system was calibrated, using the commercially available Franz diffusion cell as the reference. The results clearly suggested that this newly developed skin permeation system shows consistently superior than the Franz diffusion cell in terms of the control of skin surface temperature and the efficiency of solution mixing. To investigate the potential effect of environment, the temperature on the stratum corneum surface was varied, while the dermal solution was maintained at constant body temperature, and its effect on the kinetics of skin permeation was studied. The energy requirements for various steps of skin permeation were determined. The results suggested that the skin permeation system developed in this investigation can be useful for the mechanistic analysis of long-term skin permeation kinetics.

0363-9045/84/1004-0575$3.50/0

For the direct measurement of skin permeation kinetics, the technique commonly employed is to mount a suitable size of skin sample in between two diffusion chambers. The assembly is then thermostated at a constant temperature. A volume of drug solution is filled into the donor chamber and another equal volume of drug-free solution is added to the receptor chamber. Then, the appearance of the drug species in the receptor solution and/or the disappearance of the drug species from the donor solution is monitored, by an appropriate assay method, as a function of time. The skin sample could be excised from either humans or animals.

An early model of in vitro diffusion cell was designed by Scheuplein (1) in 1965 to study the routes of skin penetration, using radiolabeled compounds, and the effect of solubility. Later, Menczel and Maibach (2) used a modified electrodialysis unit to investigate the percutaneous absorption of drugs. Michaels et al (3) fabricated a skin permeation cell which was equipped with a pair of Teflon impellers to stir the solutions in both donor and receptor compartments, through the open sampling ports, at a synchronous speed by the external motors. Recently, Durrheim et al (4) developed a miniature diffusion cell, in which donor and receptor compartments had a volume of only 1.5 ml each and was equipped with two open ports: one was used to accommodate the externally driven impellers and another for solution filling and sampling. Review of these skin diffusion cells indicate that they apparently have the following drawbacks: (a) both the donor and receptor solutions are maintained at the same temperature by either immersing the whole cell in a temperature regulated waterbath or retaining it in thermally controlled oven. This setup could not simulate exactly the clinical setting, in which the donor compartment is exposed to an ambiant environment; and, (b) the solution in the donor and receptor compartments is constantly exposed to the atmosphere through the openings for stirring and sampling. The extent of solvent loss could be significant and may affect the drug concentration in the solutions. These deficiencies need to be corrected for the long-term skin permeation studies of transdermal therapeutic systems.

Most recently, an upright-type diffusion cell was designed by Franz (5)

and a commercial model was marketed. It consists of an upper (donor) compartment, which has a wide opening at the top exposing to the air without any temperature control, and a lower (receptor) compartment, in which the solution in the inner chamber is thermostated at 37°C by circulating water in the outer jacket and maintained at a constant hydrodynamic condition by a tiny magnetic bar rotating at 600 rpm. It was designed to simulate the clinical conditions. A skin sample is also sandwiched between the donor and the receptor compartments and the skin permeation of drug is followed by sampling the receptor solution, also via an open sampling port, at a scheduled interval for assay. This laboratory has utilized this cell design to evaluate and to compare the long-term skin permeation kinetics of nitroglycerin delivered by the three (3) recently approved nitroglycerin-releasing transdermal therapeutic systems (6).

Our experience with Franz diffusion cell indicates that it has several critical drawbacks: (a) it is sensitive to any variations in the atmospheric temperature, which could become severe in a laboratory without a well controlled room temperature or due to the implementation of energy conservation policy in the laboratory; and, (b) it is not suitable for the evaluation of solution or suspension-formulations or volatile drugs due to its inherent upright, open donor compartment design, which does not permit the use of any stirring setup as in the receptor compartment.

After reviewing all the skin permeation apparatus currently available, it becomes apparent to us that for accurate assessment of long-term skin permeation kinetics, a new in vitro skin permeation system should be developed to minimize the potential deficiencies outlined above and, in the meantime, to incorporate all the advantages realized in the existing systems. With these considerations in mind, this laboratory has developed such a skin permeation system (Figure 1). We have also calibrated the system and found that it is capable of simulating a clinical setting by maintaining the receptor solution at body temperature, while varying the temperature on skin surface. The horizontal design with the skin sandwiched at an upright position in between two half-cells appears to simulate more closely the position the humans normally take under the clinical conditions, with the exception of

the lay down position during the bedtime. It is a totally closed system with a matched pair of magnets rotating in a fixed position at a synchronous speed in the circular, depressed platforms. In addition, it permits the skin permeation study of drugs under either finite-or infinite-dosing condition. The results of our evaluation will be discussed in this report.

EXPERIMENTAL

Materials:

Estradiol[2], benzoic acid[3], polyethylene glycol (PEG) 400[3] and acetonitrile[4] were used as obtained. HPLC grade water was freshly prepared in the laboratory[5].

HPLC Method:

Instrumentation: A high performance liquid chromatographic system equipped with a reciprocating pump[6], an injector[7], an UV detector[8] (with a cell volume of 15.6 µl), a reverse-phase µ Bondapak C_{18} column (with a guard column)[9] and a chart recorder[10] was used in this investigation.

Conditions: A combination of acetonitrile and water (50:50) was used as the mobile phase which, at ambient condition and a flow rate of 1.5 ml/min, yielded an operating pressure of 2,000 psi. The UV detector operated at the wavelength of 280 nm and the sensitivity of 0.005 AUFS, which had a detection limit of 0.5 µg/ml (or, equivalently, 25 ng at an injection volume of 50 µl). A volume of 50 µl drug solution or sample was injected. Under this HPLC condition, a well-defined estradiol peak appeared at the retention time of 4.9 min. Result of the multiple injections indicated a coefficient of variation of $\pm$ 1%.

Determination of estradiol concentration in the sample solutions was carried out by measuring the height of the estradiol peak at 4.9 minutes following each injection and then calculating the concentration in µg/ml from the standard curve constructed from a series of standard solutions with known estradiol concentrations.

Skin Permeation System:

The skin permeation system developed in this laboratory is composed of a skin permeation cell and a magnetic driving unit (Figure 1).

The skin permeation cell consists of two half-cells, which are fabricated from Pyrex glass in mirror image. Each of the half-cells contains a solution chamber, which measures 0.9 cm in diameter and 3.8 cm in length and can hold a solution up to 4.0 ml. A depressed, circular platform, which measures 10 mm in diameter and 4 mm in depth, has been built in at the center bottom of the solution chamber to permit a starhead-shaped magnet to rotate at a synchronous speed in a fixed physical location. The solution chamber is equipped with a sampling port, which is situated at a location distant from the area where skin will be mounted and can be closed tightly with a matched piece of glass stopper (℔ 7/25). The whole unit of solution chamber is then completely sealed inside a water jacket and can be regulated at a given temperature by a circulating waterbath.[11] With the use of two units of circulator baths, one half-cell can be maintained at a temperature to simulate the variation in atmospheric temperature on skin surface, while another half-cell can be thermostated at constant body temperature maintained by hemoperfusion.

After mounting a skin sample in between the two half-cells, the wholly assembled skin permeation cell becomes a totally closed cell system, which yields a skin surface of 0.64 cm^2 available for drug permeation. The assembly can then be easily clamped onto the magnetic driving unit, which is equipped with a pair of revolving magnets sitting perpendicularly underneath the stirring platform in each of the half-cells. The revolving magnets can be driven to spin at 600 rpm by a pair of synchronous motors, which make the starhead magnets to rotate at a synchronous speed in both half cells by magnetic force. It produces a constant hydrodynamic property in the solution with a thin thickness of diffusion layer on the surface of both sides of the skin.

Six (6) units of the skin permeation system have been successfully connected in series and controlled at the same experimental condition.

Two triplicate experiments can be easily run simultaneously.

Calibration of Skin Permeation System:

This newly designed skin permeation system, which is designated as the Valia-Chien permeation system for the purpose of easy identification, was calibrated in three areas: (a) dynamics of skin surface temperature, (b) efficiency of solution mixing and (c) thickness of hydrodynamic diffusion layer. The commercially available model[1] of the Franz diffusion cell was also evaluated as the reference system.

a) Dynamics of Skin Surface Temperature - Experiments were designed to determine the length of time required for an excised skin sample in the in vitro permeation system to reach an equilibrium temperature and the stability of the skin surface temperature. It is known that on the living body the skin is maintained at a temperature close to the body temperature by the microcirculation in the dermal tissue. To do this, a full-thickness of skin sample was freshly excised from the abdomen of a 5-7 week old male hairless mouse,[12] using the technique reported previously (4, 6), and was mounted in between the two half cells of the Valia-Chien permeation cell (Figure 1). The half cell, to which the stratum corneum part of the skin was exposed, was designated as the donor compartment and another half cell, to which the dermis part of the skin was in contact, was called the receptor compartment. During the course of study, the receptor compartment was maintained at 37°C by a circulating waterbath,[11] while the donor compartment was kept at 25°C byanother circulating waterbath.[13] The temperature on the stratum corneum was taken by a temperature probe[14], which was introduced through the sampling port of the donor compartment (contains no solution) and was in close contact, at perpendicular position, with the skin surface. Then, a room-temperature saline solution containing 20% v/v of PEG400 (to achieve a sink condition for the drug) was delivered into the receptor compartment and the skin surface temperature was continuously measured every 30 or 60 seconds until the equilibrium temperature was reached and every 5 minutes afterward to check the stability of skin surface temperature.

Same study was also carried out in Franz diffusion cell as the reference system. The same experimental procedure as outlined above was used, except that the donor compartment of the Franz diffusion cell was exposed, due to its inherent design, to a laboratory with controlled room temperature (at 25°C) and cares were taken to minimize any potential draft during the skin temperature measurements.

b) Efficiency of Solution Mixing - It was designed to determine the length of time the system had to take to achieve a homogeneity in drug concentration in the solution. To do this, the Valia-Chien permeation cell was assembled by connecting the half cells together without a skin sample sandwiched; and the whole cell was then filled with 7.8 ml of saline solution (which contains 20% v/v PEG 400 to achieve a sink condition for the drug) and maintained at 37°C throughout the studies by a circulating waterbath.[11] Then, a 50 µl of methanolic solution containing 10 µg of estradiol was instilled, at time zero, into the saline solution from the sampling port in the donor compartment with both magnets rotating at 600 rpm. Samples, in 50 µl each, were withdrawn from the sampling port in the receptor compartment (two sampling ports have a physical separation of 6 cm) at predetermined intervals and assayed immediately for estradiol level by the HPLC method outlined earlier. The final estradiol concentration in the solution when homogeneity was reached should have a theoretical level of 1.28 µg/ml.

In the case of Franz diffusion cell, the same experimental procedure as outlined above was carried out, except that 10 ml of the same solution was required to fill the receptor compartment to the rim (which also has a distance of 6 cm from the lower end of the receptor compartment, from where samples will be taken). In addition, a 50 µl of methanolic solution containing 50 µg of estradiol was instilled at time zero from the surface of the solution with the magnet also rotating at 600 rpm. Samples, in 50 µl each, were withdrawn from the bottom of the solution as close to the rotating magnet as possible. The final estradiol concentration in the solution when the homogeneity was reached should have a theoretical

level of 5 μg/ml.

c) Determination of Hydrodynamic Diffusion Layer - To determine the thickness of hydrodynamic diffusion layer on the dermal tissue, which is immersing in the receptor solution during the skin permeation kinetics studies, the dissolution of benzoic acid in the saline solution containing various volume fractions of PEG 400 was conducted. It was done by preparing a benzoic acid disc (1.8 cm in diameter and 0.2 cm in thickness) in a metal mold from a fused benzoic acid and then mounting it in between the two half cells of the Valia-Chien permeation cell (secured firmly with parafilm). At zero time, the saline solution containing 0-40% $^{v}/_{v}$ of PEG 400 at 37°C was introduced into the receptor compartment (also thermostated at 37°C) with the magnet rotating at 600 rpm. The rate of dissolution of benzoic acid from the disc was determined by monitoring the concentration profile of benzoic acid in the receptor solution by an UV spectrophotometer[15] as a function of time.

Same experimental procedure was also carried out in Franz diffusion cell.

Temperature Dependence of Skin Permeation Kinetics:

The hairless mouse skin was mounted in between the two half cells of the Valia-Chien permeation cell in the same way as described earlier (Figure 1). The receptor compartment was thermostated at 37°C by a circulating waterbath[11] to simulate the body temperature and the temperature in the donor compartment was maintained at either 15, 25, 30 or 37°C throughtout the study by another circulating water-bath.[13] A 3.4 ml of drug-free saline solution containing 20% of PEG 400 at 37°C was filled into the receptor compartment and another 3.4 ml of estradiol suspension (3 mg/ml) in the same saline solution at a specific temperature was added into the donor compartment. At each of the predetermined intervals (1, 2, 4, 6, 8, 10, 12, 16, 20, 24, 28, 32, 40 and 48 hours), a 50 μl of sample was withdrawn from the receptor solution and assayed immediately for estradiol concentration by the HPLC method outlined earlier. The experiments were carried

out in triplicate for each temperature. A standard curve with a correlation coefficient of 0.9999, using the same saline/PEG solution and known estradiol concentrations was established during and after the completion of each run of the experiment. The sample was injected into the HPLC column immediately following the sampling and, due to the size of the sample (only 50 µl), no internal standard was incorporated. The same syringe was used throughout each of the triplicate experiments and the injection-to-injection reproducibility for each syringe had been calibrated before use by multiple injections and found to be within the acceptable limit.

The estradiol concentration (in µg/ml) in each sample was determined from the standard curve and the amount of estradiol permeating through the skin was calculated from the estradiol concentration in the receptor solution at each sampling time point after correcting for the total volume of the solution and the amount of estradiol withdrawn for assay in the preceeding sample.

Determination of Estradiol Solubility:

At the end of each 48-hour skin permeation study, the estradiol suspension in the donor compartment was quickly filtered through a swinnex filter equipped with a Teflon filter membrane[16] and the estradiol concentration in the filtrate was also assayed by the HPLC method, after proper dilution, to determine the equilibrium solubility of estradiol in the saline/PEG solution at each specific temperature. Triplicate determinations were done for each temperature.

Determination of Skin/Solution Partition Coefficient:

Skin preparations of known weight (100 ∿ 200 mg each) were equilibrated with 3 ml of estradiol solution (1.5 µg/ml in saline solution containing 20% v/v of PEG 400) in a shaking waterbath at 15, 25, 30 and 37°C for a duration of up to 5 days. The estradiol concentration in the solution was sampled every day and assayed by HPLC until the equilibrium state was reached. The skin/solution partition coefficient at various temperatures was then determined from

the concentration of estradiol in the skin and the equilibrium concentration of estradiol in the solution.

RESULTS AND DISCUSSION

Development of Skin Permeation Cell:

For the investigation of skin permeation kinetics, the effect of solution hydrodynamics in the vicinity of skin surface should be minimized as much as possible and the solution mixing should be efficient. Under these conditions, the real mechanisms involved in the skin permeation of drugs can then be realized and the intrinsic rate of skin permeation, in which only the skin tissue plays the rate-limiting role in the whole process of skin permeation (not the hydrodynamic diffusion layers on both sides of the skin), can be determined.

It is also important to study the effect of temperature variation above the skin surface on the rate of skin permeation. As we all know that under a normal clinical situation, the skin on the body surface is constantly regulated by the microcirculation in the dermal layer and, in the meantime, it is also subjected to a cooling effect from the atmosphere. The skin surface temperature may vary from one part of the body to another, depending on the degree of microcirculation underneath the skin and also on the extent of covering on the surface of the skin, and may also change with environmental temperature. This temperature effect should be characterized quantitatively by systematically varying the temperature on skin surface, while maintaining the dermal solution at body temperature; so, the rate of skin permeation of drugs from a transdermal therapeutic system at different temperature conditions can be projected.

The skin permeation cell designed should also be a totally close system, so the potential of solvent loss, due to evaporation at elevated temperatures can be minimized. It is particularly critical in running a long-term skin permeation study, in which the solvent loss could be significant and might potentially affect the drug concentration in the solution.

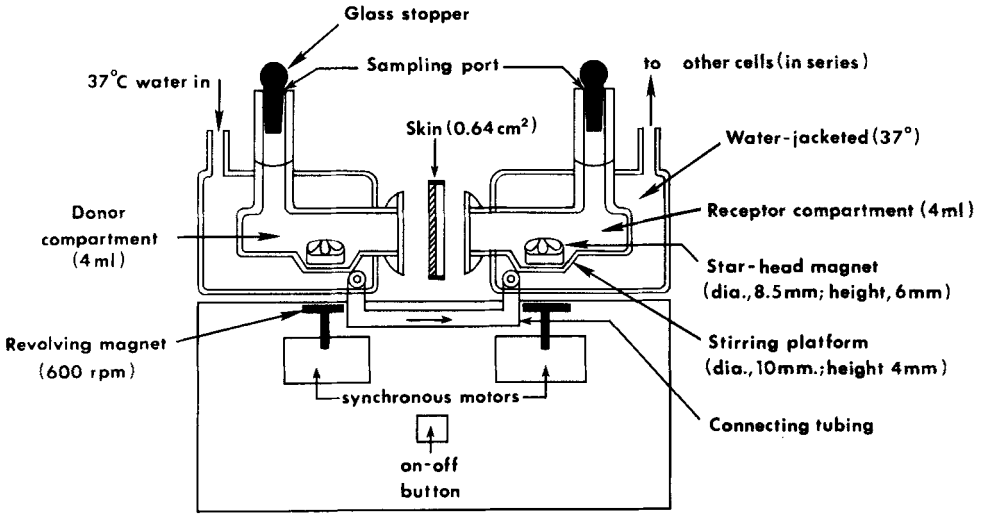

FIGURE 1

Diagrammatic illustration of the skin permeation system developed. See the text for details of the design.

As shown in Figure 1, the skin permeation cell developed in this investigation aims to incorporate all the aforementioned considerations into the cell design. It consists of a pair of half cells in mirror image and it becomes a totally close cell system after assembled. The sampling ports can be tightly closed with glass stoppers, so the potential of solvent loss by evaporation during the course of skin permeation studies can be reduced to the minimum. Each of the half cells is constructed with a circular depression at the central bottom area of the solution chamber to provide a platform for the starhead-shaped magnet to spin at a constant, synchronous speed in a fixed position. This stirring platform design permits the maintenance of a constant solution hydrodynamics and a rapid achievement of solution

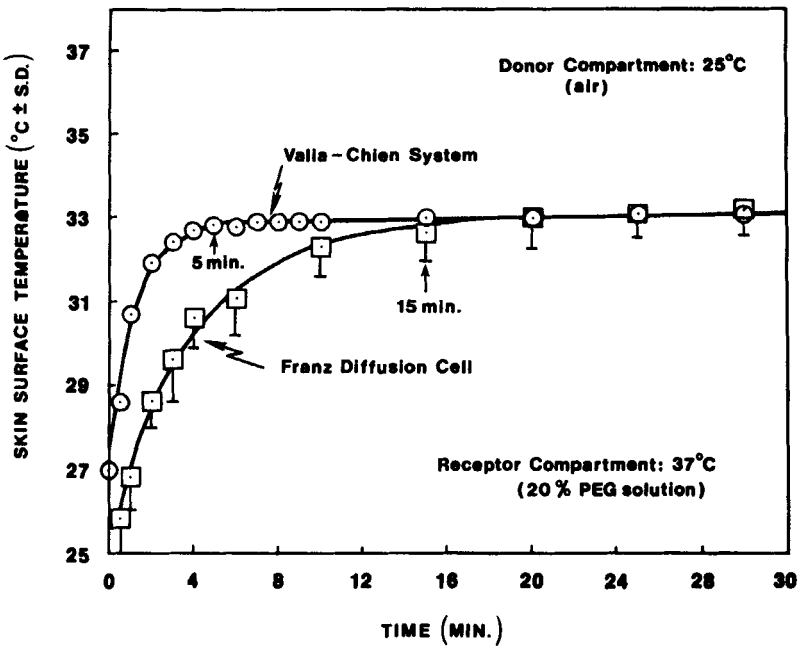

FIGURE 2

The time course for the skin surface temperature. The equilibrium temperature of 32.8°C was reached within 5 minutes in the Valia-Chien system and after 10 minutes in the Franz diffusion cell. The bar represents the range of one standard deviation.

homogeneity without the risk of exposing the drug solution to the open air. Each of the two half cells is totally sealed in a water jacket individually. This individualized water jacket design permits the solution temperature in donor and receptor compartments be controlled at a same or a different temperature, so the studies can be run under an isothermal or a nonisothermal condition as desired. Therefore, the effect of temperature variation on the rate of skin permeation can be assessed quantitatively.

Dynamics of Skin Surface Temperature:

The dynamics of skin surface temperature was studied under the conditions described earlier in the "experimental" section. The results outlined in Figure 2 indicated that a skin surface temperature of 32.8°C was reached at equilibrium under the condition that the dermis was bathing

in a 37°C saline solution and the stratum corneum is exposed to an atmosphere controlled at 25°C. It was found that the equilibrium skin surface temperature was reached within 5 minutes in the Valia-Chien skin permeation system, while in the Franz diffusion cell it took 10-15 minutes to reach the same temperature. But, in any case, a stable skin surface temperature was maintained throughout the course of study as long as the dermal solution was controlled at 37°C and the environment surrounding the surface of the skin was kept at 25°C.

The temperature in the bulk of the dermal solution was also monitored and found that an equilibrium temperature of 36.8°C was reached in the Valia-Chien system within 3 minutes. It was only 0.2°C lower than the target body temperature of 37°C. On the other hand, there exists a temperature gradient in the Franz diffusion cell between the skin and the region enveloped within a water jacket. The solution in the water-jacketed region was able to maintain the target body temperature throughout the study, while the solution above the water-jacketed region but immediately below the dermal tissue has an equilibrium temperature that is almost 4°C lower than the body temperature.

If both the donor and the receptor compartments (which were thermally controlled at 37°C) were filled with a 22°C saline solution, it was observed that it takes only 3 minutes for the Valia-Chien system to reach the equilibrium temperature of 36.8°C in both compartments; in the meanwhile, the skin surface (stratum corneum) achieves an equilibrium temperature of 36.7°C, which is only 0.1°C lower than the solution temperature (36.8°C). The result suggested that the skin surface can be controlled at a temperature that is in the vicinity of body temperature if the environmental temperature on the skin surface is also thermally regulated at 36.8°C as in the dermal solution.

Efficiency of Solution Mixing:

Efficiency of solution mixing determines the thickness of hydrodynamic diffusion layer on the skin surface as well as the time course

of reaching a homogeneous drug concentration in the solution. It is critically important in the skin permeation kinetics studies that the permeation of drug molecules should encounter only the barrier effect imposed by the skin layers and the drug molecules should be quickly distributed throughout the receptor solution as soon as they have penetrated through the skin layers. The hydrodynamic diffusion layer on either side of the skin surface should not play any significant barrier effect.

In the Valia-Chien system, the solution mixing in both donor and receptor compartments is achieved by a pair of star-head magnets rotating synchronously at 600 rpms in a specially designed stirring platform (Figure 1). On the other hand, in the Franz diffusion cell, due to the vertical design, only the solution in the receptor compartment can be stirred by a "rod"-shaped magnet, which is also rotating at 600 rpms.

The efficiency of solution mixing was evaluated by studying the time required for the solution to achieve an homogeneous distribution of a drug dose following the input of the drug. Under the conditions outlined earlier in the Experimental section, the results indicated that it takes only one (1) minute to reach the theoretical estradiol level in the Valia-Chien system as compared to 30 minutes required in the Franz diffusion cell (Figure 3). It should be pointed out that the distance between the site for drug input and the site for sampling in both systems are very much the same (6 cm apart). Hence, the data obtained here demonstrate that the Valia-Chien system has a greater efficiency of solution mixing than the Franz diffusion cell. The results may also suggest that the hydrodynamic diffusion layer underneath the dermal tissue could be substantially smaller in thickness in the Valia-Chien system than in the Franz diffusion cell.

The hydrodynamic diffusion layer on the immediate surface of the skin can be determined by studying the dissolution profiles of benzoic acid under the same experimental conditions. The following relationship should be followed for the dissolution kinetics (7):

$$\ln \left(\frac{C_s}{C_s - C_t}\right) = \frac{K_m A}{V} (t - t_1) \qquad (1)$$

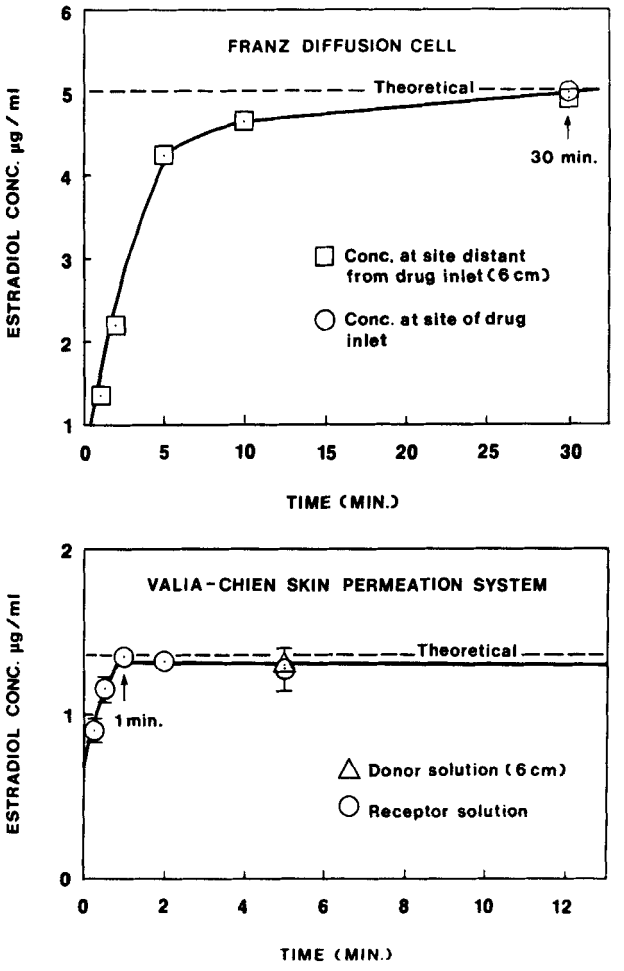

FIGURE 3

The time course for the solution to achieve an homogeneous distribution of a drug dose following the instillation of the drug. The bar represents the range of one standard deviation.

where C_s is the aqueous solubility of benzoic acid in the saline solution containing various volume fractions of PEG 400, C_t is the benzoic acid concentration at time t, t_l is the lag time extrapolated from the steady-state dissolution profile, A is the surface area of the benzoic acid disc available for dissolution, V is the volume of the receptor solution used, and Km is the mass transfer coefficient as defined by:

$$K_m = \frac{D_s}{h_{aq}} \qquad (2)$$

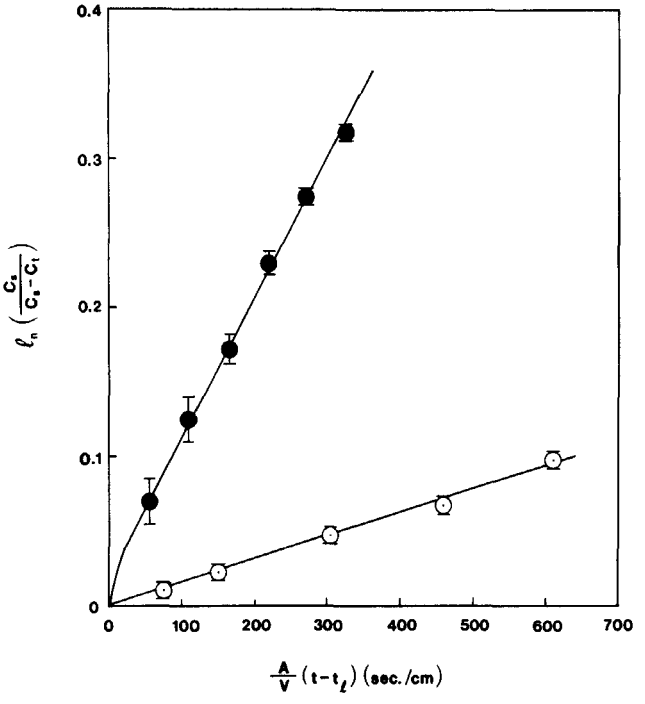

FIGURE 4

Linear relationship between ln (C_s/C_s-C_t) and $A(t-t_l)/V$ as described by Equation (1). Key: ● Valia-Chien permeation system, ○ Franz diffusion cell. The bar represents the range of one standard deviation.

where D_s is the aqueous diffusivity of benzoic acid and h_{aq} is the thickness of hydrodynamic diffusion layer. From the slope of the ln $(C_s/C_s - C_t)$ versus $A(t - t_l)/V$ and the known values of D_s in the solution, the thickness of hydrodynamic diffusion layer (h_{aq}) can be determined.

The results in Figure 4 indicate that the dissolution profiles of benzoic acid disc in the receptor solution in both Valia-Chien permeation system and Franz diffusion cell do follow Equation (1) fairly well. K_m values were determined from the slope of the lines and then used to calculate h_{aq} by Equation (2). Results are tabulated in Table 1. The data indicate that the Valia-Chien system has a hydrodynamic diffusion layer with thickness ranging from 0.0054 ($\pm$ 0.0006)

cm to 0.0101($\pm$ 0.0021) cm as the PEG 400 concentration in the solution increases from 0% to 40%·^{v}/v. The thickness of hydrodynamic diffusion layer maintained in the Valia-Chien system was found to be 3-7 times smaller than that achieved in the Franz diffusion cell, which ranges from 0.0287 ($\pm$ 0.0027) cm to 0.0389 ($\pm$ 0.0091) cm.

The results generated above clearly demonstrate that the efficiency of solution mixing in the Valia-Chien permeation system is significantly better than that in the commercially available Franz diffusion cell; so, a much thinner thickness of hydrodynamic diffusion layer is maintained in the Valia-Chien system. In other words, the effect of hydrodynamic diffusion layer on the skin permeation kinetics can be substantially reduced when one uses the Valia-Chien skin permeation system and the skin becomes the principal barrier in the skin permeation of the drugs.

Skin Permeation Kinetics and Temperature Dependence:

The rate of skin permeation ($^{Q}s/t$) at steady state can be described by the following relationship (8):

$$\frac{Q_s}{t} = P_s \ (C_D - C_R) \qquad (3)$$

where Q_s is the cumulative amount of drug permeating through a unit surface area of the skin, P_s is the skin permeability coefficient of the drug, C_D and C_R are the drug concentrations, respectively, in the donor and receptor solutions. The results in Figure 5 indicate that the skin permeation profiles of estradiol through the skin follow fairly well the linear relationship defined by Equation (3). The ^{Q}s vs. t linearity was achieved at all temperatures for a duration of up to 48 hours. However, the rate of skin permeation (Q_s/t) was observed to increase as increasing the temperature in donor solution.

In this investigation, the drug concentration in the donor solution (C_D) is maintained at a saturation solubility (C_D^S) level (by incorporating an excess amount of estradiol in the solution), while the drug concentration in the receptor solution (C_R) is maintained below a sink condition ($C_R < 10\% \ C_D^S$). So, Eq. (3) can be simplified to:

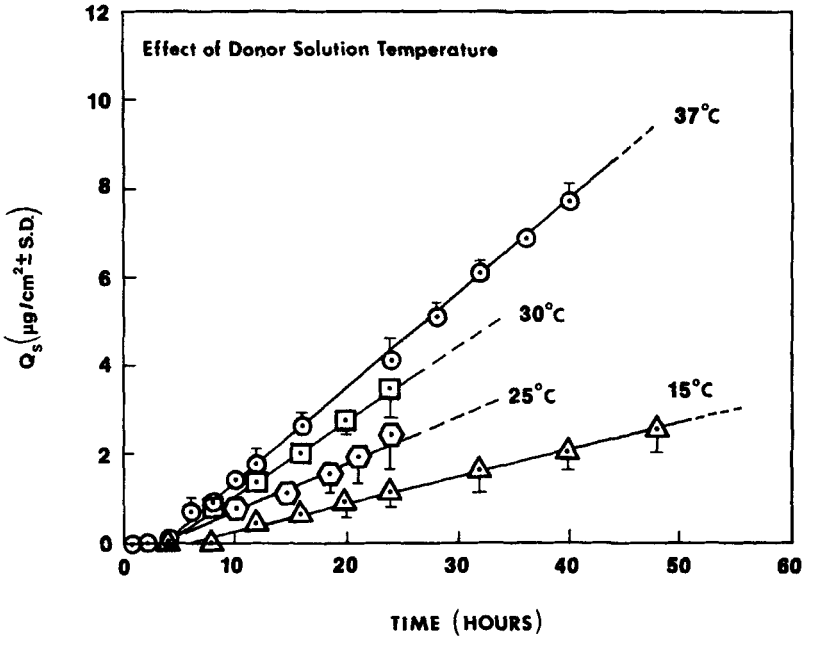

FIGURE 5

Linear Q_s-t relationship at various temperatures. Key: △, 15°; ⬡, 25°; □, 30°; and ○, 37°C. The Q_s/t of estradiol was calculated to be 0.062, 0.114, 0.168, and 0.209 μg/cm²/hr, respectively. The bar represents the range of one standard deviation.

$$\frac{Q_s}{t} = P_s C_D^s \qquad (4)$$

where P_s, the permeability coefficient across a skin barrier, is determined by the skin/solution partition coefficient (K_s), the skin diffusivity at the steady-state (D_{ss}) and the thickness of the skin (h_{sb}) as expressed by the relationship of:

$$P_s = \frac{K_s D_{ss}}{h_{sb}} \qquad (5)$$

The steady-state skin diffusivity (D_{ss}) can be determined from Equation (6) if P_s, h_{sb} and K_s values are known or predetermined:

$$D_{ss} = \frac{P_s h_{sb}}{K_s} \qquad (6)$$

and the nonsteady-state skin diffusivity (D_{ns}) can be calculated from the lag time (t_l) and the skin barrier thickness (h_{sb}) by the following relation:

$$D_{ns} = \frac{h_{sb}^2}{6t_l} \tag{7}$$

It is known that the saturation solubility (C_D^S), the steady-state diffusivity (D_{ss}) and the nonsteady-state diffusivity (D_{ns}) are temperature-dependent variables (9) as defined, respectively, by the following relations:

$$\log C_D^S = \text{constant} - \frac{\Delta H}{2.303R} \cdot \frac{1}{T} \tag{8}$$

$$\log D_{ss} = \text{constant} - \frac{(Ed)ss}{2.303R} \cdot \frac{1}{T} \tag{9}$$

$$\log D_{ns} = \text{constant} - \frac{(Ed)ns}{2.303R} \cdot \frac{1}{T} \tag{10}$$

were ΔH is the energy of solvation, $(E_d)_{ss}$ and $(E_d)_{ns}$ are the energies of activation for diffusion at steady-state and nonsteady-state, respectively.

Taking logarithm of Equations (4) and (5) and then substituting Equations (8) and (9) for $\log C_D^S$ and $\log D_{ss}$ terms give:

$$\log P_s = \log \left(\text{const.} \cdot \frac{K_s}{h_{sb}}\right) - \frac{(Ed)ss}{2.303R} \cdot \frac{1}{T} \tag{11}$$

$$\log \frac{Q_s}{t} = \log \left(\text{const.} \cdot \frac{K_s}{h_{sb}}\right) - \frac{[(Ed)ss + \Delta H]}{2.303R} \cdot \frac{1}{T} \tag{12}$$

Equations (11) and (12) can be established only if the magnitudes of K_s, the skin/solution partition coefficient, and h_{sb}, the thickness of skin barrier, are constant and independent of temperature variation. To verify this, the skin partitioning studies of estradiol were conducted at temperature ranging from 15°C to 37°C. Results indicated that the K_s value does not vary significantly (i.e. K_s value is 9.60 $\pm$ 0.93, 8.64 $\pm$ 1.22, 8.34 $\pm$ 1.83, and 9.02 $\pm$ 0.48, respectively, at 15°C, 23°C, 30°C, and 37°C). The observations suggest that Equations (11) and (12) are valid in this temperature range studied.

According to Equations (8) ~ (12), the rate of skin permeation

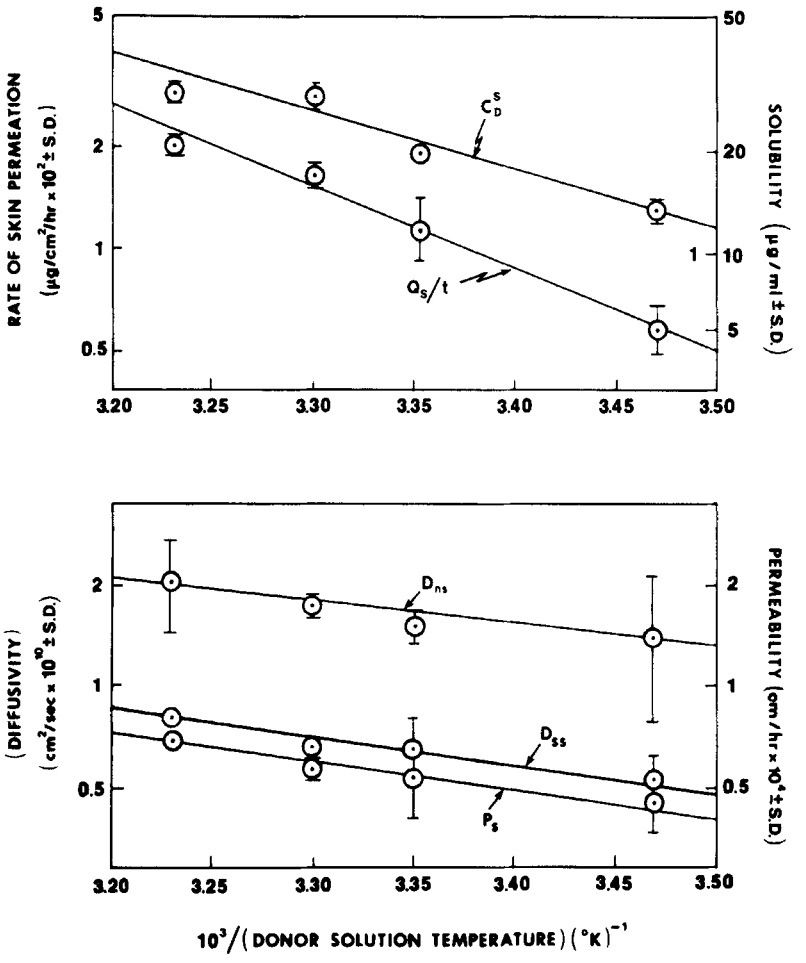

FIGURE 6

Temperature dependency of the rate of skin permeation (Q_s/t), drug solubility (C_D^S), skin permeability (P_s) and diffusivity at steady-state (D_{ss}) and at nonsteady-state (D_{ns}). The lines were drawn using linear regression analysis. The bar represents the range of one standard deviation.

(Q_s/t), skin permeability coefficient (P_s), saturation solubility (C_D^S) in the donor solution, diffusivities at steady-state (D_{ss}) and at non-steady-state (D_{ns}) should be exponentially dependent upon the reciprocal of the absolute temperature $(T)^{-1}$. As shown in Figure 6, the data indicate that these linear relationships as defined in Equations (8) ∿ (12) are followed fairly well experimentally.

From the slope of the linear relationships in Figure 6, the magnitude

TABLE 1

Effect of Polyethlylene Glycol 400 on Mass Transfer Coefficient (K_m) and Thickness of Hydrodynamic Diffusion Layer (h_{aq}) at 37°C

		Valia-Chien Permeation System[b]		Franz Diffusion Cell[b]	
PEG 400[a] (%v/v)	D_s ($cm^2/sec x 10^5$)	K_m ($cm/sec x 10^4$)	h_{aq} (cm)	K_m ($cm/sec x 10^4$)	h_{aq} (cm)
0	1.4500	26.85 (± 3.35)	0.0054 (±0.0006)	4.3767 (±0.7702)	0.0338 (±0.0060)
10	0.9864	18.11 (± 3.72)	0.0055 (±0.0010)	2.5994 (±0.3397)	0.0384 (±0.0053)
20	0.5489	7.60 (± 1.36)	0.0072 (±0.0011)	1.4605 (±0.3216)	0.0389 (±0.0091)
40	0.2157	2.14 (± 0.56)	0.0101 (±0.0021)	0.7570 (±0.0695)	0.0287 (±0.0027)

a. % PEG 400 - %v/v polyethylene glycol 400 in saline solution.

b. The mean (± standard devitation) of 3 determinations.

TABLE 2

Energy Requirements in Skin Permeation Process

	Energy term	Energies (Kcal/Mole)
Rate of Skin Permeation ($^{Q}s/t$)	$\Delta H + (Ed)_{ss}$	10.11
Drug Solubility (C_D^s)	ΔH	6.78
Skin Permeability (P_s)	$(Ed)_{ss}$	3.34
Diffusivities		
Steady-State (D_{ss})	$(Ed)_{ss}$	3.34
Non-Steady-State (D_{ns})	$(Ed)_{ns}$	2.87

TABLE 3

Effect of Donor Solution Temperature on the Skin Diffusivity of Estradiol

Temperature	Skin Diffusivity (cm^2/sec x 10^8 ±S.D.)		
	D_{ns}	D_{ss}	D_{ns}/D_{ss}
15°C	1.48 (±0.67)	0.54 (±0.10)	2.75
25°C	1.56 (±0.16)	0.66 (±0.16)	2.36
30°C	1.76 (±0.12)	0.68 (±0.04)	2.60
37°C	2.13 (±0.64)	0.83 (±0.01)	2.56
		$\bar{x}$(±S.D.)	2.57 (±0.16)

of ΔH, the energy of solvations, $(Ed)_{ss}$, the energy of activation for diffusion at steady-state, and $(Ed)_{ns}$, the energy of activation for diffusion at nonsteady-state can be determined by using Equations (8) - (12). The results are tabulated in Table 2. The data indicate that the energy required for the skin permeation of estradiol (10.11 $^{Kcal}/_{mole}$) is very much equal to the summation of the energy for solvation (ΔH=6.78 $^{Kcal}/_{mole}$) and the energy of activation for diffusion at steady-state [$(E)_{ss}$= 3.34 $^{Kcal}/_{mole}$]. The magnitude of ΔH value is two folds greater than the $(E)_{ss}$ value. The observations suggest that the skin permeation kinetics of estradiol has a greater dependency on the solubility profile than on the diffusion property of the drug.

It is interesting to observe that the steady-state diffusion of estradiol in the skin requires an energy [$(E)_{ss}$ = 3.34 $^{Kcal}/_{mole}$] which is 30% greater than the energy needed for the diffusion at the nonsteady-state [$(E)_{ns}$ = 2.87 $^{Kcal}/_{mole}$]. In addition, the data in Table 3 illustrate that both D_{ss} and D_{ns} values are increased as increasing the temperature of the donor solution, to which the skin surface is exposed. Interestingly, the D_{ns} values are consistently higher than the D_{ss} values at all temperatures and a constant ratio of D_{ns}/D_{ss} of 2.57 ($\pm$ 0.16) is resulted.

FOOTNOTES

1. Franz Diffusion Cells (FDC-129), Crown Glass Co., Somerville, New Jersey.
2. Roussel-UCLAF, Paris, France.
3. Fisher Scientific Company, Fair Lawn, New Jersey.
4. Distilled-in-glass HPLC grade. Burdick & Jackson, Muskegon, Michigan.
5. Prepared by Nanopure, Sybron/Barnstead, Boston, Massachusetts.
6. Model 6000A. Waters Associates, Milford, Massachusetts.
7. Model U6K. Waters Associates, Milford, Massachusetts.
8. Model 440. Waters Associates, Milford, Massachusetts.
9. Contains 37-50 µm Bondapak C18/Corasil packing material. Waters Associates, Milford, Massachusetts.

10. Omniscribe. Houston Instruments, Austin, Texas.

11. Circulator Bath (Model 80), with a flow rate of 7-15 liters/min. Fisher Scientific Co., Fair Lawn, New Jersey.

12. HRS/J Strain, Jackson Laboratories, Bar Harbor, Maine.

13. Refrigerated Circulator Bath (Model 90), with a flow rate of 7-15 liters/min. Fisher Scientific Company, Fair Lawn, New Jersey.

14. Thermalert (Model TH-6D) equipped with a Thermometer sensor (Type PT-6) Kapton insulated, copper/constantan thermocouple. Bailey Instruments, Inc., Saddle Brook, New Jersey.

15. Model 559A UV/VIS Spectrophotometer, Perkin Elmer Company, Elmwood Park, New Jersey.

16. Type FHLP, Millipore Corporation, Bedford, Massachusetts.

ACKNOWLEDGMENT

The authors wish to thank Dr. K. Tojo, Mr. J. A. Masi and Mr. P. R. Keshary for their technical assistance and to Mrs. V. Gallino for manuscript preparation.

All inquiries should be directed to Yie W. Chien, Controlled Drug Delivery Research Center, College of Pharmacy, Rutgers University, Busch Campus, Piscataway, New Jersey 08854.

REFERENCES

1. R. J. Scheuplein, J. Invest. Dermatol., 45, 334 (1965).

2. E. Menczel and H. I. Maibach, J. Invest. Dermatol., 54, 386 (1970).

3. A. S. Michaels, S. K. Chandrasekaran, and J. E. Shaw, AICHEJ, 21, 985 (1975).

4. H. Durrheim, G. L. Flynn, W. I. Higuchi, and C. R. Behl, J. Pharm. Sci., 69, 781 (1980).

5. T. J. Franz, Curr. Probl. Dermatol., 7, 58 (1978).

6. Y. W. Chien, P. R. Keshary, Y. C. Huang, and P. P. Sarpotdar, J. Pharm. Sci., 72, 968 (1983).

7. C. D. Yu, J. L. Fox, N. F. H. Ho and W. I. Higuchi, J. Pharm. Sci., 68, 1347 (1979).

8. Y. W. Chien, "Novel Drug Delivery Systems: Fundamentals, Developmental Concepts and Biomedical Assessments," Marcel Dekker, New York, N. Y. (1982), pp. 149-218.

9. Y. W. Chien, "Novel Drug Delivery Systems: Fundamentals, Developmental Concepts and Biomedical Assessments," Marcel Dekker, New York, N. Y., (1982), pp. 465-574.

10. R. J. Scheuplein and I. H. Blank, Physiol. Rev., 51, 702 (1971).