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DEVELOPMENT OF A SKIN PERMEATION CELL TO SIMULATE CLINICAL STUDY OF IONTOPHORETIC TRANSDERMAL DELIVERY[1]

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ABSTRACT

A new single-compartment iontophoretic skin permeation cell was developed to simulate the clinical application of iontophoretic devices. The stability and permeation kinetics of LHRH, the model peptide, were investigated in this new permeation cell (cell design A) using two analytical methods and compared with a commonly used skin permeation cell (cell design B). A polyacrylamide-based hydrogel device was fabricated as the drug reservoir for the iontophoretic transdermal delivery of LHRH. The skin permeation profiles of LHRH were found identical for both permeation cells when assayed by radioactivity measurement. However, cell design A gave a skin permeation profile that was substantially higher than that obtained in cell design B when assayed by HPLC. This is because the electrochemical degradation of LHRH occurred in the receptor compartment of the Valia-Chien permeation cell (cell design B). This degradation could be overcome by using the new single-compartment iontophoretic permeation cell.

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INTRODUCTION

Currently, there are two major types of permeation cells have been used to conduct in-vitro iontophoretic permeation study. One is a vertical type, and another is a horizontal-type. The vertical-type permeation cell can be further classified into two-compartment and three-compartment models. The two-compartment permeation cell is a modification of the Keshary-Chien skin permeation cell (1). The problems encountered with this type of cell design include difficulty in placing electrodes into the chambers, especially in the donor (upper) chamber, poor temperature control in the donor chamber and the possibility of solvent evaporation from the donor solution, interruption of current flow during sampling, and potential of drug degradation at the electrode surface. The three-compartment vertical permeation cell can avoid the interruption of current flow during sampling and the electrochemical degradation of drugs in the receptor, since both electrodes are located in the upper chambers. However, this type of cell design has a problem of its own. Both electrodes are located next to each other, which may cause an electric short circuit due to current flow directly from the anode to the cathode. This can be resolved by incorporating an additional chamber between the anode and the cathode chambers to separate the two electrodes (2).

The horizontal-type permeation cell has been increasingly used in iontophoresis-facilitated skin permeation studies. This type of cell design also has two- and three-compartment models. A typical example of the two-compartment horizontal permeation cell is the Valia-Chien skin permeation cell which consists of a donor and a receptor half-cell, in mirror image, with a skin specimen sandwiched between them (3). For conducting an iontophoretic skin permeation study, a pair of electrodes are immersed directly into the donor and receptor solutions. This in-vitro setup has been successfully applied to study the iontophoretic skin permeation of vasopressin (4), AZT (5), and insulin (6), but it does not simulate the clinical situation as well as one would expect. Also, there is a potential electrochemical degradation occurs at the electrodes in donor and receptor compartments during the course of iontophoretic permeation study. A three-compartment iontophoretic permeation cell, claimed to mimic the in-vivo condition more closely, has been developed and evaluated (7). However, this cell design requires a total immersion of the cell in a water bath to maintain a constant temperature.

A new design for an iontophoretic permeation cell, a single compartment horizontal type, has been recently developed in our research center. LHRH was used as a model peptide which is a decapeptide. A series of investigations were performed to compare this new skin permeation cell with the Valia-Chien permeation cell. The solution stability of LHRH under an electric field and the iontophoretic skin

permeation of LHRH from a hydrogel formulation were evaluated in both cell designs. The results are reported and discussed in this article.

MATERIALS

Luteinizing hormone-releasing hormone (LHRH-AcOH 2.5 H_2O) was obtained from Bachem Bioscience Inc. (Philadelphia, PA). The radiotracer (I^{125}-Tyr^5-LHRH) with a specific activity of 2200 μCi/mmol was purchased from NEN Research Product/Dupont (Wilmington, DE). Deionized water, after purification by filtering through a Nanopure system (Barnstead, Dubuque, IA), was used to prepare all buffered solutions. Albumin, acrylamide, N' N' methylene bis-acrylamide, catalyst system, citric acid, and sodium phosphate were purchased from Sigma Chemical Company (St. Louis, MO). Platinum wires and foils (99.95 % purity) used to fabricate electrodes, were obtained from Johnson Mattey (Seabrook, NH). Female hairless rats (6 - 8 weeks old), the animal model, were ordered from Harlan Sprague Dawley Inc. (Indianapolis, IN). The programmable Keithley 500 A measurement and control system (Keithley, Cleveland, OH) was used as the power source.

METHODS

Designs of skin permeation cell

Cell design A : Clinically, one of the suitable sites for the application of an iontophoretic device is the lower portion of the arm, just around the wrist. In such a case, the electrode pads are expected to be placed on the opposite sides of the wrist. A cell design was recently developed in this laboratory to simulate the clinical situation of wearing a wristwatch-type iontophoretic device. This cell design consists of a single solution compartment, which represents the central compartment for systemic circulation, and two openings (0.64 cm^2 each), which are used for the mounting of skin specimens, on opposite ends (Figure 1). The central compartment has a solution volume of 4.5 ml, in which the fluid hydrodynamics can be maintained by a starhead-shaped magnet rotating at a constant speed (600 rpm) and the temperature can be kept at 37 °C by circulating thermostated water through the water jacket surrounding the central compartment. Two hydrogel devices : one, a LHRH-containing device, is applied to the abdominal skin and acts as the anode and the other, a placebo device, is applied to the dorsal skin and functions as the cathode. The iontophoretic hydrogel device is shown in Figure 2. The samples can be withdrawn from the sampling port with no interruption in current flow.

Cell design B : This system was modified from the hydrodynamically well-calibrated Valia-Chien skin permeation cell (3) by replacing its donor half-cell with a LHRH-containing hydrogel device,

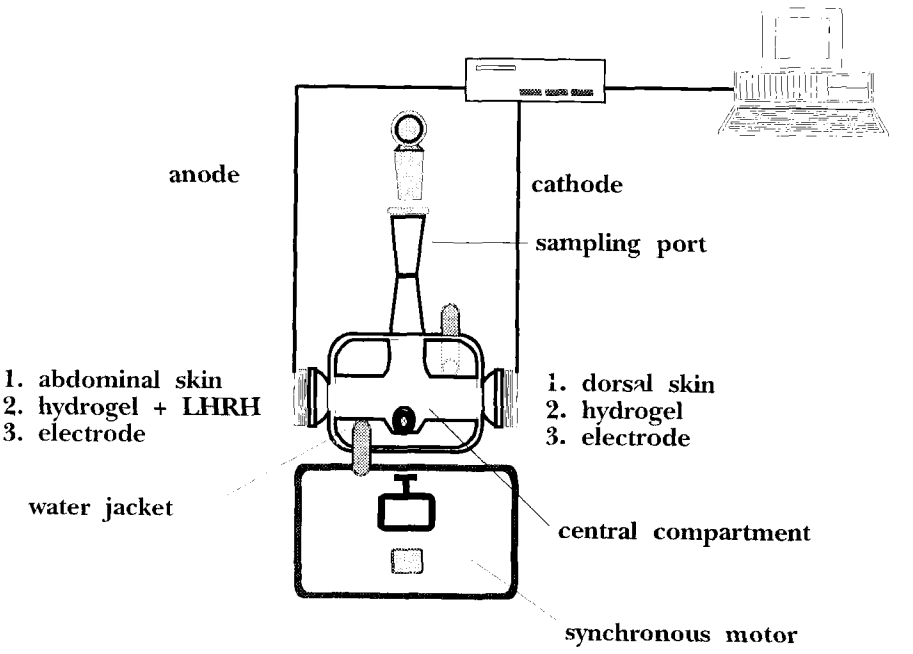

FIGURE 1
Schematic illustration of the clinically-relevant skin permeation cell (cell design A)

that is applied onto the abdominal skin (Figure 3). The solution compartment (3.5 ml) is also well stirred by a starhead-shaped magnet rotating at a constant speed of 600 rpm and the temperature is also maintained at 37 °C by circulating thermostated water through a specially-designed water jacket surrounding the solution compartment.

Analytical methods

Two analytical methods were used in this investigation :

HPLC assay : The HP1090 system with a multi-wavelength UV detector and a MOS C_8 reversed phase column [100 mm * 4.6 mm (i.d.)] (Hewlett Packard, Avondale, PA) were used. A wavelength of 220 nm was chosen to detect LHRH, since its Trp^3 and Tyr^5 have UV absorption maxima at 219 nm and 222 nm, respectively (8). The mobile phase used was phosphate buffer (0.5 M), which was adjusted by NaOH to pH 6.5, and acetronitrile, with the ratio of 80 to 20, at a flow rate of 1 ml/min. (9). The sample was injected into the system at a volume of 20 ul.

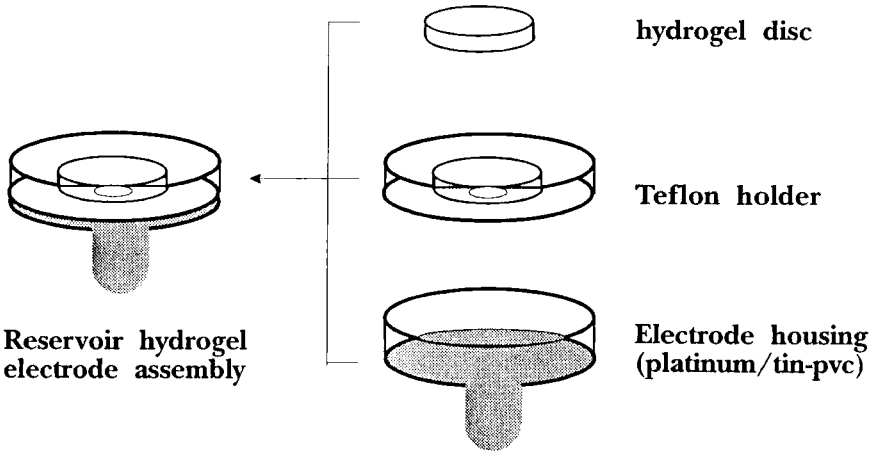

FIGURE 2
Schematic illustration of an iontophoretic hydrogel device

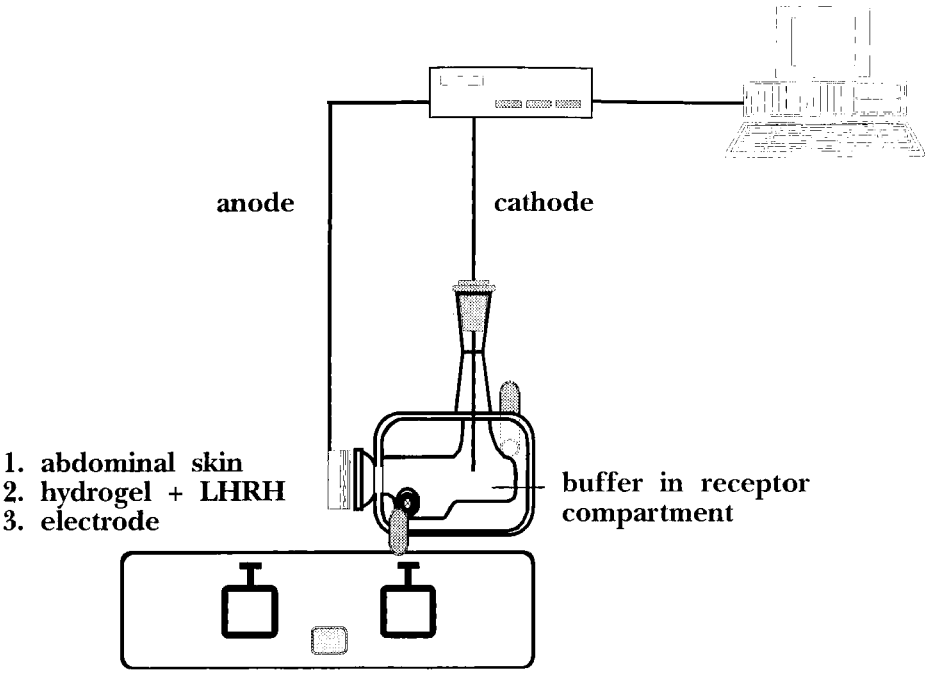

FIGURE 3
Schematic illustration of the cell design B

Radioactivity assay : The gamma counter (Beckman, Somerset, NJ) was used to measure I^{125} radioactivity. The sample (50 µl each) was counted for a duration of one minute.

Solution stability of LHRH

Two pieces of freshly-excised skin of a hairless rat were mounted onto both openings of cell design A with the dermal sides facing the central compartment. LHRH solution (10 µg/ml) was prepared in isotonic citrate/phosphate buffer (pH 3 to 7) containing albumin (0.2 %) and thimerosal (0.01 %) (10). A volume of 4.5 ml was placed into the central compartment. Stability studies were conducted for 6 hours with no current application. The control studies were run without skin. Samples (50 µl each) were taken from the solution compartment at predetermined time intervals and analyzed by HPLC.

Effect of cell designs on electrical stability of LHRH

The skin specimens, freshly excised from hairless rats, were mounted on both openings of cell design A or on the opening of cell design B with the dermal sides all facing the solution compartment. A blank polyacrylamide-based hydrogel disc housed in a Teflon device (which has a platinum foil as the electrode, Figure 2) was applied onto the stratum corneum surface. A LHRH solution (10 µg/ml) was prepared in isotonic citrate/phosphate buffer (pH 3) containing 0.2 % albumin. An appropriate volume was placed into the solution compartment (4.5 ml for cell design A and 3.5 ml for cell design B). Electrical stability of LHRH in both cell designs was studied by applying a direct current (with intensity of 0.6 mA) for 3 hours. The control studies were also conducted in both cells without any current application. Samples (50 µl each) were taken from the solution compartments at predetermined time intervals and analyzed for LHRH concentration by HPLC.

Comparison between new iontophoretic permeation cell and Valia-Chien permeation cell

The two types of skin permeation cells were compared in terms of the skin permeation of LHRH facilitated by iontophoresis. The skin specimens, freshly-excised from hairless rats, were mounted onto the openings of cell designs A and B. Hydrogel devices (with pH at 3.6 and ionic strength of 0.64 mM) were fabricated to contain 175 µg of LHRH and 1 µCi of I^{125} LHRH each. The LHRH-containing hydrogel device was applied onto the stratum corneum surface of the abdominal skin, as the anode, for both cell designs since LHRH carries a positive charge at pH 3.6 (11). A placebo (drug-free) hydrogel device, was also prepared from the hydrogel formulation, without LHRH, and was applied onto the stratum corneum surface of the dorsal skin as the cathode for cell design

A. A direct current with a current intensity of 0.6 mA was applied for 3 hours. Samples (50 µl each) were taken from solution compartments and the LHRH concentration was measured by first counting the radioactivity in each sample followed by HPLC assay.

RESULTS AND DISCUSSION

Solution stability of LHRH

The solution stability of LHRH in the new iontophoretic skin permeation cell (cell design A) was investigated at three pH's (pH 3, 5 & 7) with and without skin. The results shown in Figure 4 indicate that in the absence of skin, LHRH was very stable at all pH's studied over the 6-hr observation period. With exposure to the skin, LHRH was stable at pH 3, but it was degraded at pH 5 and 7. The degradation of LHRH observed at neutral pH might be caused by the enzymes in the skin, since the LHRH solution was in direct contact with the dermis (the possibility of bacterial degradation was reduced by the addition of 0.01 % thimerosal, an antimicrobial agent) (10). At acidic pH, the activity of cutaneous enzymes might be retarded due to the possibility of their denaturalization or the change in the ionic state and/or the conformation of enzymes or substrates which results in decreased affinity between enzymes and substrates. Thus, the extent of LHRH degradation was very low at pH 3 and increased with the increase in solution pH. In order to avoid the enzymatic degradation of LHRH and to determine the actual skin permeation of LHRH following its delivery from the hydrogel device, the citrate/phosphate buffer at pH 3 was chosen as the medium for the following investigations.

Stability of LHRH under an electric field

The effect of an electrical field on the solution stability of LHRH in cell design A and B is compared in Figure 5. The results indicate that at pH 3, LHRH was very stable in the central compartment of cell design A and the application of a direct current had no adverse effect on its solution stability (Figure 5 A). However, it was degraded in the receptor solution of cell design B when a current was applied, which could have resulted from the electrochemical degradation of LHRH at the cathodic electrode (Figure 5 B). At the cathode, a reduction reaction can occur and cause LHRH to degrade. In the case of cell design A, the electrochemical degradation of LHRH was avoided because LHRH did not come into direct contact with the electrode following its skin permeation into the central compartment. The electrical stability of LHRH in the central compartment of cell design A also implies that there was no loss of LHRH or an insignificant amount, if any, by migration from the central compartment to the cathodic hydrogel device. This might be due to the presence of a high concentration of competitive ions in the central compartment.

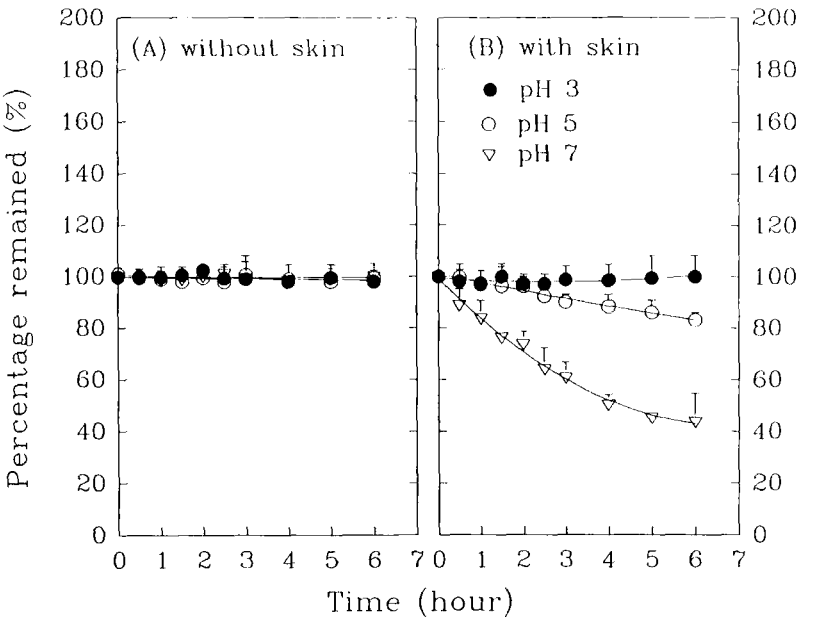

FIGURE 4
Solution stability profiles of LHRH in the solution compartment of the new iontophoretic skin permeation cell (cell design A), with and without skin, at various pH's (n=3).

Comparison between new iontophoretic permeation cell and Valia-Chien permeation cell

In Figure 6, the iontophoresis-facilitated transdermal permeation profiles of LHRH studied in both cell designs and assayed by two different analytical methods are compared. The skin permeation profiles of LHRH, following its delivery from a hydrogel device, were found to be identical for both cell designs when the assay of LHRH was done by radioactivity measurement (Figure 6 A) since this analytical method was not able to distinguish intact and degraded LHRH. On the other hand, when HPLC assay, which detects only the intact molecules, was used, cell design A yielded a higher skin permeation profile of LHRH than cell design B (Figure 6 B). The results suggest that the new iontophoretic skin permeation cell (cell design A) had potential of stabilizing LHRH by minimizing its degradation that occurred in the receptor solution of cell design B. Since the electrochemical degradation of peptides/proteins often

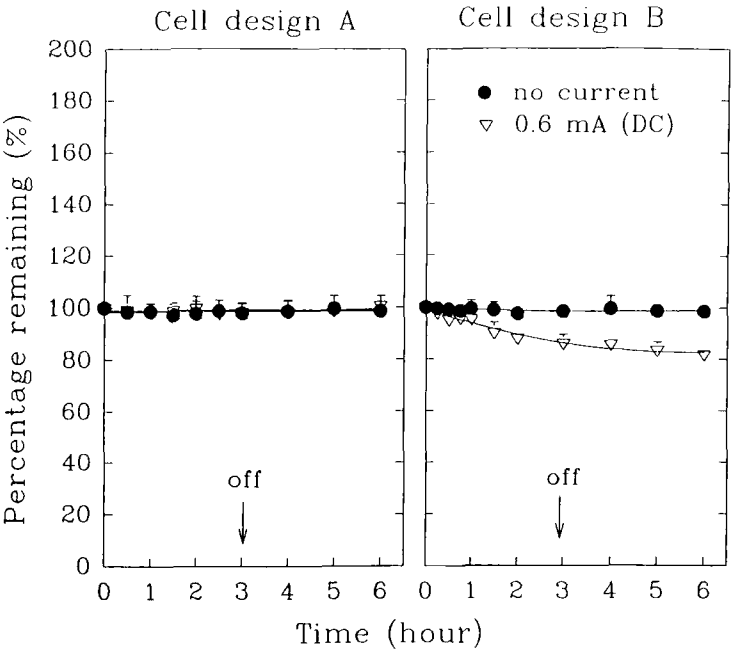

FIGURE 5

Electric stability of LHRH in the solution compartments of cell design A and B. Initial concentration of LHRH was 10 μg/ml in isotonic citrate/phosphate buffer at pH 3. A direct current with a current intensity of 0.6 mA was applied for 3 hours (n=3).

occurs in the receptor solution and can jeopardize the results of skin permeation, the observation suggests that the cell design is critically important in the in-vitro studies of the iontophoretic transdermal permeation of peptides/proteins, such as LHRH in this investigation.

The permeation profile of LHRH obtained with the new iontophoretic permeation cell (cell design A) as measured by HPLC assay was slightly lower than that measured by radioactivity assay. The results are in contrast to the observation reported earlier in Figure 5, in which LHRH has been found to be relatively stable at pH 3, even under current application, in the new iontophoretic skin permeation cell. Therefore, the observed difference in the permeation profiles determined by two analytical methods could be attributed to the possibility that some of the LHRH loading dose degraded inside the hydrogel disc due to the platinum foil prior to its release for transdermal permeation. The results

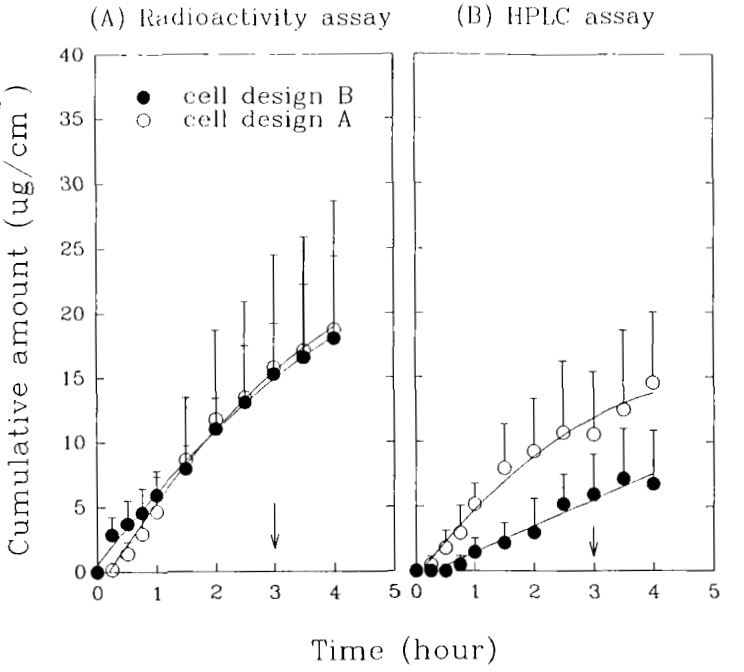

FIGURE 6
Comparison in the iontophoretic permeation profiles of LHRH from hydrogel devices studied in different cell designs as monitored by two analytical methods : radioactivity measurement (A) and HPLC assay (B) (n=3).

demonstrate that the selection of proper analytical methods, which have stability-indicating capability, is crucial to the measurement of actual permeation profiles of peptide/protein drugs.

CONCLUSIONS

In conclusion, the new iontophoretic permeation cell developed in this investigation is capable of minimizing the electrochemical degradation of peptide-based pharmaceuticals in solution by electric current and avoids the interruption of current application during sampling. Additionally, this cell design is closer to the real clinical application of iontophoretic devices for iontophoresis than any other design.

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REFERENCES

1. Banga, A.K. *Ph. D.Thesis* : Transdermal iontophoretic delivery of peptide drugs. Rutgers University, New Brunswick, NJ (1990)
2. Glikfeld, P., Cullander, C., Hinz, R.S., and Guy, R.H., A new system for in-vitro studies of iontophoresis. *Pharm. Res.*, 5, 443 - 446 (1988)
3. Chien, Y.W., and Valia, K.H., Development of a dynamic skin permeation system for long-term permeation studies, *Drug Develop. Ind. Pharm.*, 10, 575 - 599 (1984)
4. Lelawongs, P., Liu, J.C., Siddiqui, O. and Chien, Y.W., Transdermal iontophoretic delivery of arginine-vasopressin (I) : Physicochemical considerations. *Int'l. J. of Pharm.*, 56, 13 - 22 (1989)
5. Wearley, L. and Chien, Y.W., Enhancement of the in vitro skin permeability of Azidothmidine (AZT) via iontophoresis and chemical enhancer, *Pharm. Res.*, 7, 34 - 40 (1990)
6. Banga, A.K. and Chien, Y.W., Characterization of transdermal iontophoretic delivery of insulin. (submitted to *J. Control Release*, 1992)
7. Bellantone, N.H., Rim, S., Fancoeur, M.L. and Rasadi, B., Enhanced percutaneous absorption via iontophoresis I. Evaluation of an in-vitro system and transport of model compound. *Int'l. J. Pharm..*, 30, 63 - 72 (1986)
8. Freifelder, D., Application to biochemistry and molecular biology in *Physical Biochemistry* , W. H. Freeman and company, San Francisco, (1983)
9. Serti, D.C., Johnson, R.N. and Kho, B.T., An accurate, specific HPLC method for the analysis of a decapeptide in a lactose matrix, *J. Liq. Chromat.*, 4, 1135 - 1156 (1981)
10. Chen, L.H. and Chien, Y.W., Solution stability and stabilization of LHRH, in preparation (1993)
11. Mabrey, S., and Klotz, I.M., Conformation of Gonadotropin Releasing Hormone. *Biochem.*, 15, 234 - 242 (1976)