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COMPARISON OF DIFFUSION STUDIES OF HYDROCORTISONE BETWEEN THE FRANZ CELL AND THE ENHANCER CELL

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ABSTRACT

The Franz diffusion cell remains a popular method to study diffusion of transdermal drug delivery systems through membranes. Recently, VanKel Industries, Inc., (Edison, NJ) developed the "Enhancer Cell," a new device for in vitro transdermal drug diffusion testing. The purpose of this study was to evaluate the enhancer cell for in vitro transdermal diffusion of hydrocortisone from an ointment using a synthetic membrane and a biological membrane and compare it to the traditionally employed Franz cell. The Enhancer cell utilizes existing USP dissolution equipment (USP Apparatus II). Results show a higher cumulative release from the Enhancer cell as compared to the Franz cell. The Enhancer cell demonstrated more durability and was easier to use during experimentation and after completion of the experiment no apparent change was observed in the condition of the ointment or the skin when compared to the Franz cell.

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INTRODUCTION

In recent years, interest in the development of transdermal dosage forms has grown exponentially[1]. Although dissolution devices exist (USP Apparatus V, VI, and VII) to evaluate final transdermal dosage forms[2], specialized diffusion cells are still popular during the developmental stages to study transfer kinetics through membranes[3]. These cells are available in various types and volumes, and used with associated accessories (special magnetic stirrers, temperature controlled circulators and tubings, and customized automated sampling devices). Thus, such diffusion work requires its own set of dedicated instrumentation and thus investment in new equipment. One of the most widely used diffusion cells is the Franz diffusion cell[4].

Recently, VanKel Industries, Inc., (Edison, NJ) has developed the "Enhancer cell," a new device for in vitro transdermal drug diffusion studies which can be used with existing dissolution equipment (USP Apparatus II). The intent of the Enhancer cell was to allow a laboratory that had existing dissolution equipment to conduct diffusion studies which would normally require the purchase of some type of diffusion cell such as the Franz diffusion cell. Thus this method has the advantage of not only reducing investment but also utilizing technology for which there is an extended data base concerning system variables[5,6]. The purpose of this study was to measure the diffusion of hydrocortisone from an ointment base through a synthetic (cellulose) membrane and a biological membrane (rat skin) using the Enhancer cell and compare the results to those obtained using the Franz diffusion cell. The rationale for using a synthetic membrane was that the use of synthetic membrane minimizes the variability associated with animal or human skin and also the release characteristics determined using a synthetic membrane can be used as a quality-control procedure for assuring batch-to-batch uniformity of the dosage form[7].

MATERIALS AND METHOD

Materials :

Commercially available hydrocortisone 1% in an emollient ointment containing mineral oil, white petrolatum and sorbitan sesquioleate was obtained.

As this is not intended to be a commercial evaluation of the hydrocortisone ointment, the brand name and manufacturer will not be listed. Hydrocortisone powder USP/NF and testosterone powder USP/NF were purchased from Spectrum Chemical Mfg. Corp., Gardena, CA. Acetonitrile (HPLC Grade) was purchased from Fisher Scientific Co., Fair Lawn, NJ. Cellulose membranes (molecular weight cutoff : 6000) were purchased from Bel-Art Products, Pequannock, NJ.

Animal Preparation :

For skin studies, the skin from the dorsal area of male Sprague-Dawley rats were used. The rats were sacrificed with a concentrated potassium chloride injection into the heart. The hair was removed with the aid of an electric shaver. The skin from the dorsal area was excised and placed in 0.9% sodium chloride solution. After the removal of excess subcutaneous fat, samples of skin were cut into appropriate size and used immediately.

Dissolution/Diffusion Studies :

a. Enhancer Cell : Enhancer cells from VanKel Industries, Inc., Edison, NJ were evaluated. The Enhancer cell (Figure 1) consisted of a metal load ring (1), a cap (2), a washer (3), membrane or skin (4), an O-ring (6), and a drug reservoir comprising of a body (5) and a screw (7). The ointment (500 mg) was placed in the drug reservoir. A circular piece of cellulose membrane or rat skin, 3 cm in diameter, was placed on the top of the drug reservoir followed by the washer as shown in Figure 1. The metal load ring was used to keep the membrane or skin and the washer in place during the cap application, after which the metal load ring was removed. Finally, the bottom screw (7) was tightened to bring the ointment in complete contact with the membrane or skin making certain that no entrapped air was present at the interface of the ointment and the cellulose membrane or the rat skin.

A USP Six Spindle Dissolution Tester (Vanderkamp® 600, VanKel Industries, Chatham, NJ) was used for evaluation of the Enhancer cell. The flask assembly was modified (Figure 2) because 200 ml capacity flasks were used instead of the standard 900 ml flasks. It was essential to use smaller

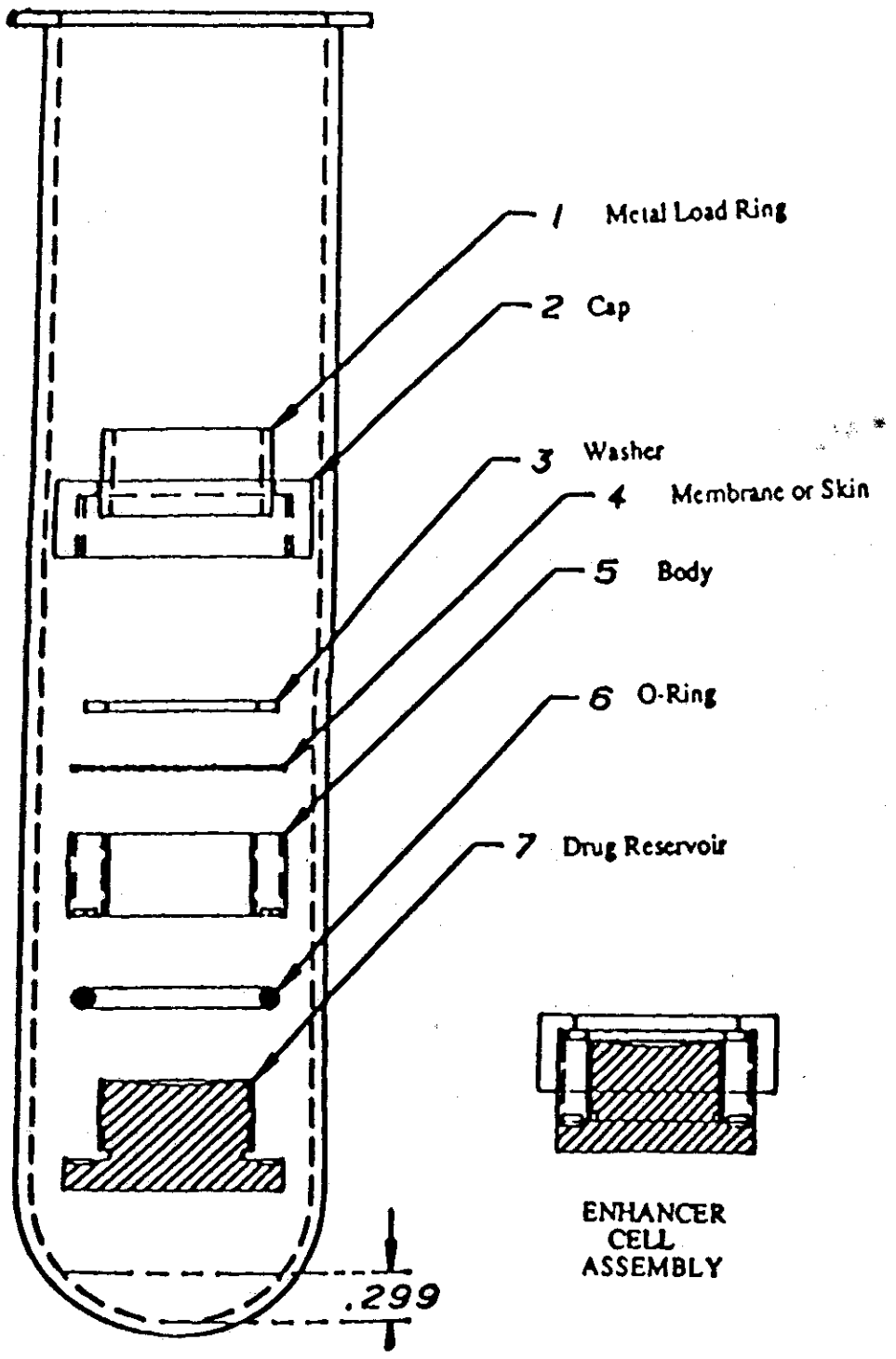

FIGURE 1
Schematic Diagram of the Enhancer Cell

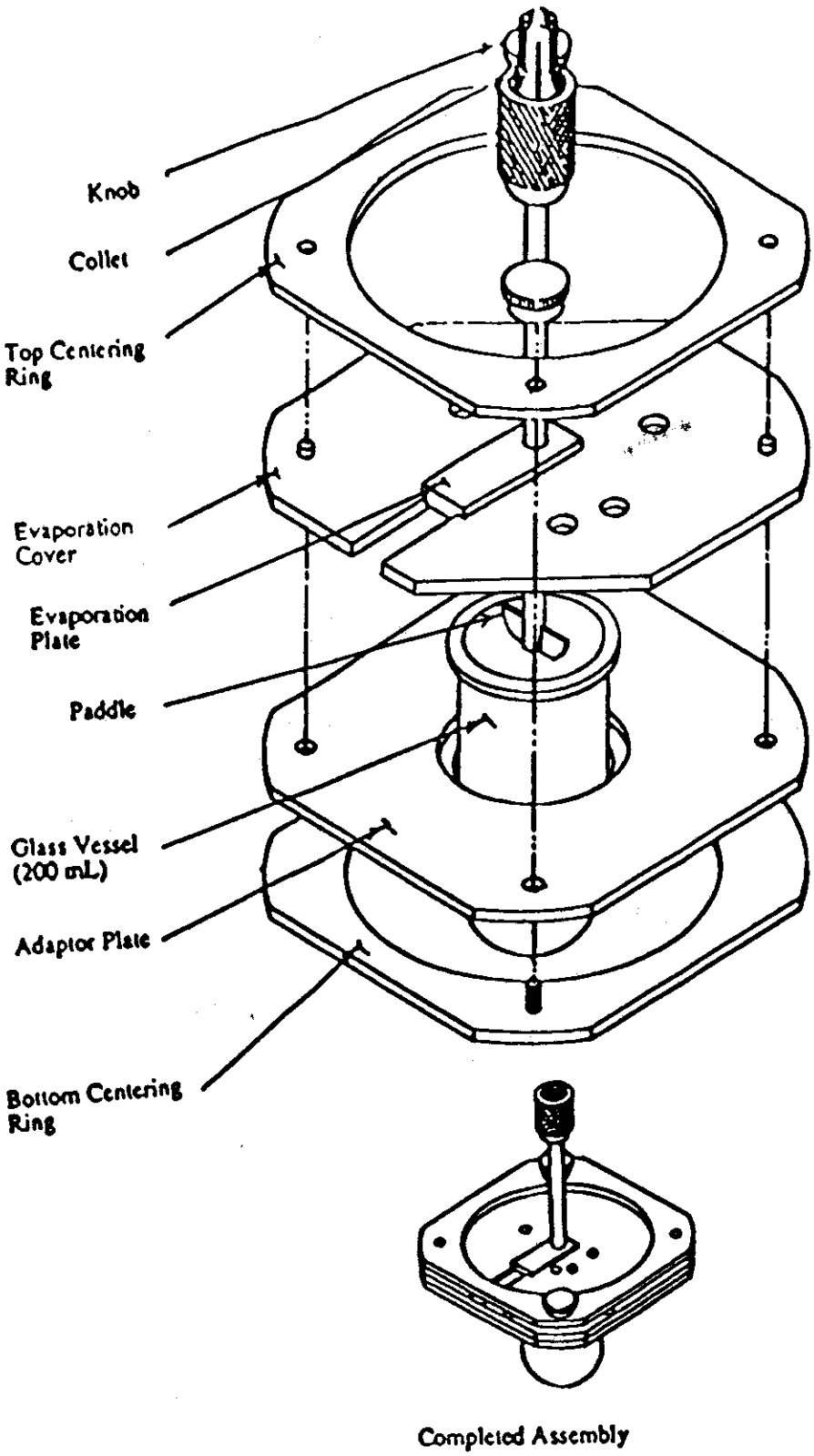

FIGURE 2
Schematic Diagram of 200mL Flask Centering Ring Assembly

receptor volumes in this experiment in order to obtain samples of detectable concentrations of hydrocortisone for the HPLC analysis. The flask centering ring assembly modification differed from the standard flask assembly in that it included an additional adapter plate to hold the smaller sized 200 ml flask in the center, an evaporation plate that fits on the evaporation cover such that together they cover the flask completely at the top, smaller sized paddles (1/4" shaft) and 1/4" collets to fit the paddles.

Water was used as the receptor phase medium. The Enhancer cell including the ointment was placed into the dissolution flask. The flask assembly was then completed, the receptor phase medium (200 ml) was poured into the flask, and the paddles rotated at 100 rpm. Samples were withdrawn at 0.33, 0.66, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 hours. At each sample interval, an exact volume of the sample was withdrawn from each flask and replaced immediately with an identical volume of fresh medium. A correction factor was included in the calculations to account for the drug lost in the samples.

Diffusion studies were conducted using cellulose membranes in the Enhancer cell. A set of six diffusions was run to obtain the cumulative release profile. The experiment was repeated using the skin from the dorsal area of the rat in the Enhancer cell instead of the cellulose membrane, with the outer stratum corneum in contact with the ointment. A control Enhancer cell with the skin but without the ointment was also run in the skin studies.

b. Franz Cell : A six-unit system of standard open cap, Franz diffusion cell were utilized. Water was used as the receptor phase medium. The lower part of the diffusion cell was filled with the receptor phase medium and stirred by means of a constant spinning bar magnet. Cellulose membrane was placed between the lower and upper part of the Franz cell and clamped. Hydrocortisone ointment (500 mg) was placed on the membrane in the donor cell cap and spread uniformly with the aid of a flat surface. The receptor compartment was checked for air bubbles and if present removed by manual tipping of the cell. Samples were withdrawn at 0.33, 0.66, 1, 2, 3, 4, 5, 6, 7, 8, 10 and 12 hours. At each sample interval, an exact volume of the sample was withdrawn from each diffusion cell and replaced immediately with an identical volume of fresh

medium. A correction factor was included in the calculations to account for the drug lost in the samples.

The experiment was repeated using the skin from the dorsal area of the rat instead of the cellulose membrane, with the outer stratum corneum in contact with the ointment (donor compartment). A control diffusion cell with the skin but without the ointment was also run in the skin studies.

HPLC Analysis :

HPLC analysis was performed on all samples in this study. A 712 WISP autoinjector and M-45 Solvent Delivery System (Waters Associates, Inc., Milford, MA); Spectroflow 783 UV detector (Kratos Analytical, Ramsey, NJ) and a Hewlett-Packard 3392A Integrator (Hewlett-Packard, Avondale, PA) were utilized. Testosterone (500 ng/ml) was used as an internal standard. A Medsil-5-C18 column, 4.6 mm i.d. x 25 cm long, was used with a mobile phase consisting of acetonitrile:water (50:50 v/v) mixture. Using a flow rate of 1 ml/min, hydrocortisone was eluted between 3-4 min and testosterone was eluted between 6.5-7.5 min. Both were detected at 242 nm.

RESULTS AND DISCUSSION

a. Ease of use : The intent of the Enhancer cell was to allow a laboratory that had existing dissolution equipment the ability to conduct diffusion experiments that would normally require the purchase and training in the use of some type of diffusion cells (for e.g. Franz cells) and associated equipment (special stir plates, custom circulation equipment etc.), which can be quite expensive and fragile, since many are made of glass. These new Enhancer cells would allow a lab to conduct diffusion studies using existing equipment (for e.g. USP Apparatus paddle over disk or reciprocating disk), the operation of which would already be familiar to the lab staff.

The loading of the ointment and setup of the dissolution equipment is very easy with the Enhancer cell. The thickness of the ointment was very uniform which was facilitated by the screw mechanism of the Enhancer cell compared to the Franz cell where obtaining an uniform ointment layer poses a serious

problem. Also, the manual manipulation of the receptor phase to avoid air bubbles while withdrawing samples becomes very tedious in Franz cells[3,8]. Comparatively, utilization of Enhancer cell requires sample withdrawal in a standard manner similar to those in standard dissolution studies. Also, complete unattended automation of Franz cell is not currently available[3,8], a feature that can be obtained for the Enhancer cell. A Franz cell setup requires a lot of bench space along with a clutter of water flow tubes (one inlet and one outlet for each cell), temperature controlled circulation equipment and stirring plates. It requires care and experience with the various components on part of the investigator lest causing breakage of some part of the diffusion cell. These are problems that we have encountered when using glass diffusion cells. On the other hand, Enhancer cells require the use of a standard dissolution apparatus, it needs less space and minimizes the problem of breakage, since the cells themselves are made of teflon and sampling does not require direct contact with the glass portions of the dissolution apparatus which due to the design of the dissolution apparatus are held in a more sturdy manner compared to the Franz diffusion cells (as shown in Figure 2).

b. Viability of Experimental Conditions : In the Franz cell, the ointment and the skin were directly exposed to the atmosphere which caused degradation and subsequent degradation products which could interfere with the HPLC analysis. However, in the Enhancer cell, the isolation of the ointment inside the device and the skin immersed in the receptor phase medium at all times decreased the formation of degradation products and increased the viability of the skin and the ointment. The ointment turned from white to translucent in the Franz cell and over the course of the experiment the skin hardened and had a putrid odor. In the Enhancer cell, no apparent change in the consistency of the ointment at the end of the diffusion study or in the condition of the skin (except hydration) was observed. Thus, the Enhancer cell minimizes variability and interference in analysis by canceling the effect of atmospheric exposure.

c. Diffusion studies : It can be seen from Figures 3 and 4 that the release of hydrocortisone was higher from cellulose membrane studies than the skin

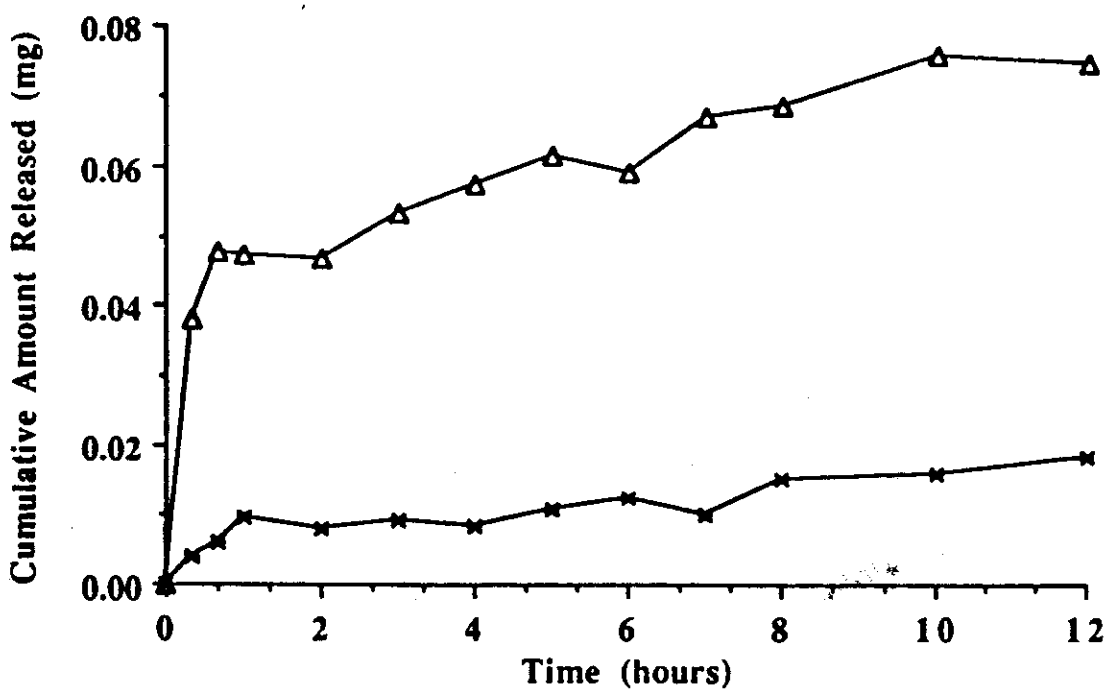

FIGURE 3
Diffusion of Hydrocortisone through the Enhancer Cell ;
Cellulose Membrane = Δ ; Rat Skin = *

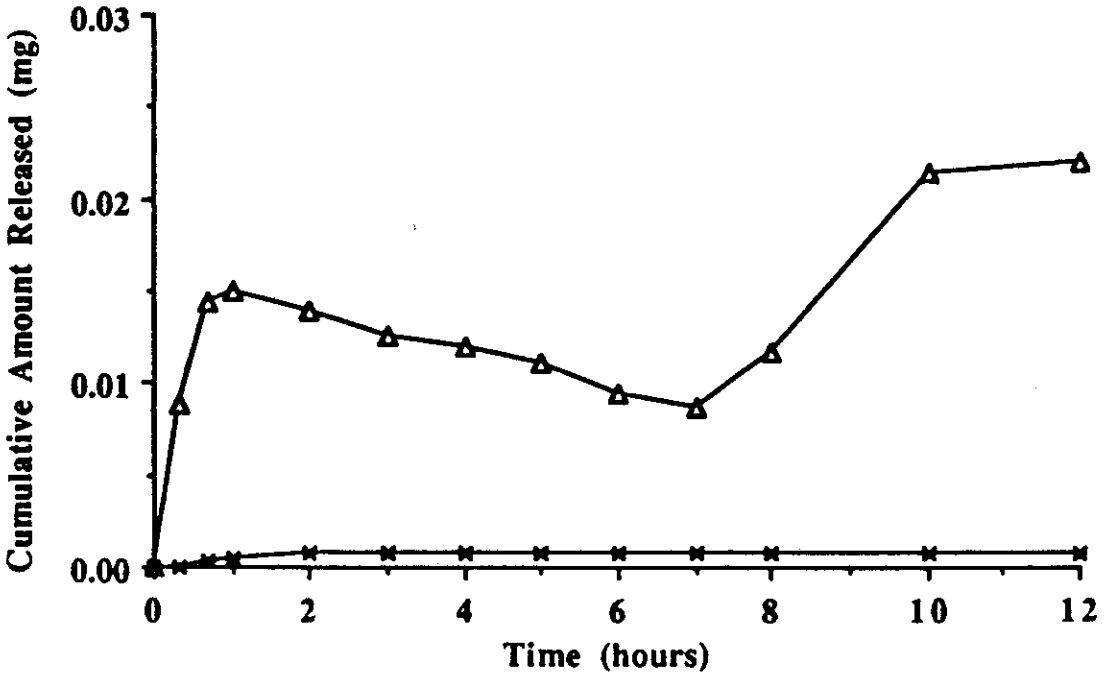

FIGURE 4
Diffusion of Hydrocortisone through the Franz Cell ;
Cellulose Membrane = Δ ; Rat Skin = *

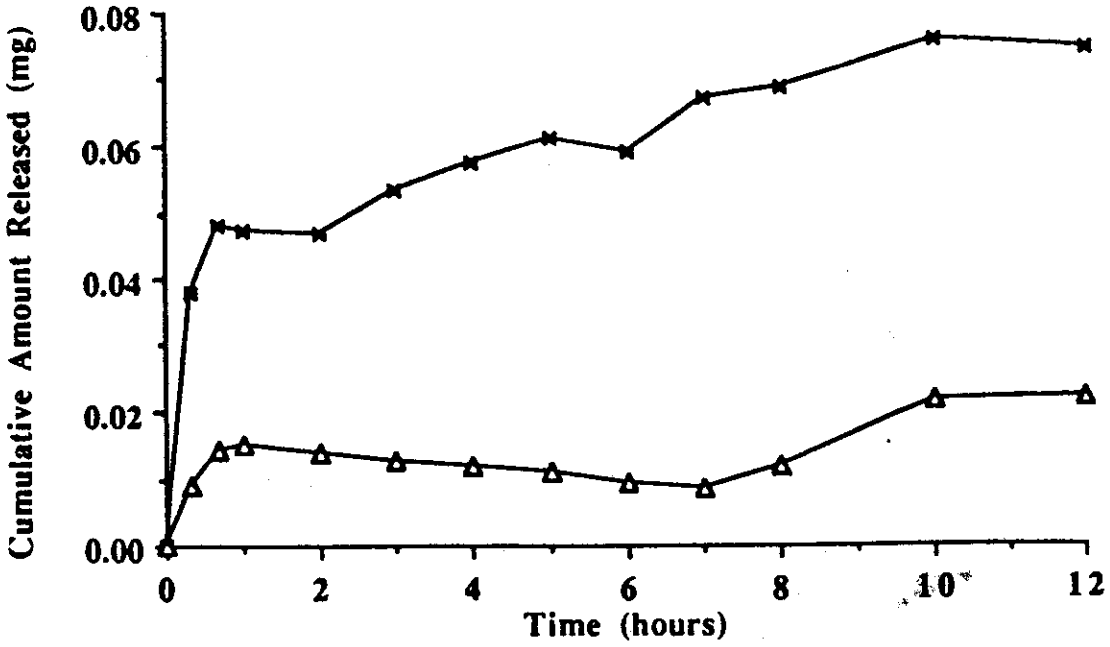

FIGURE 5
Diffusion of Hydrocortisone through the Cellulose Membrane ;
Franz Cell = Δ ; Enhancer Cell = *

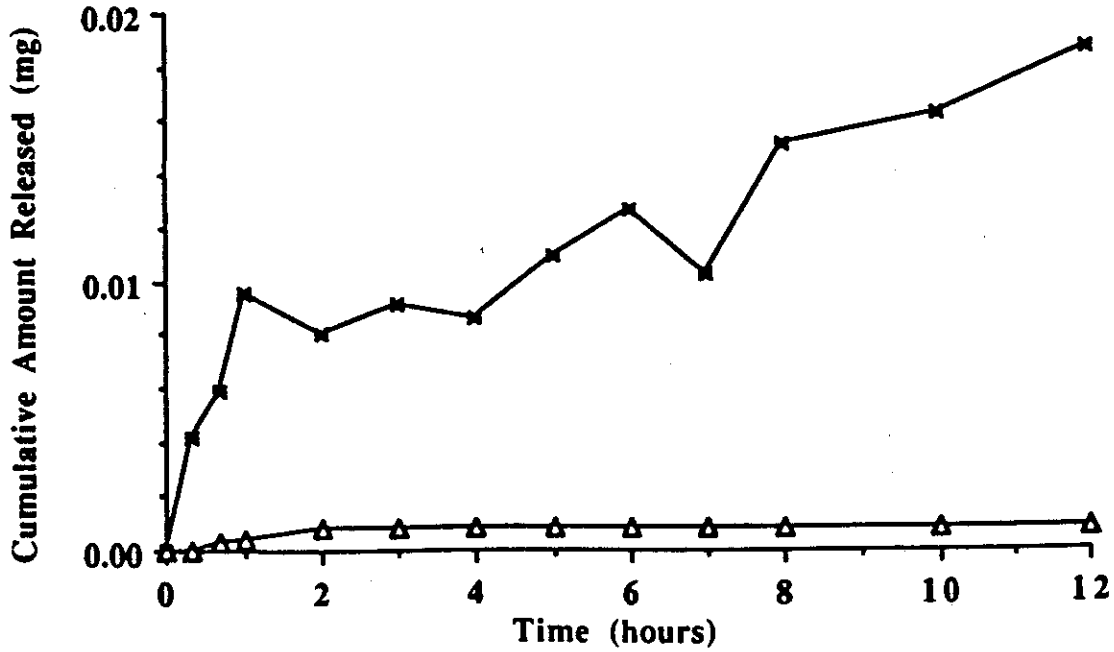

FIGURE 6
Diffusion of Hydrocortisone through the Rat Skin ;
Franz Cell = Δ ; Enhancer Cell = *

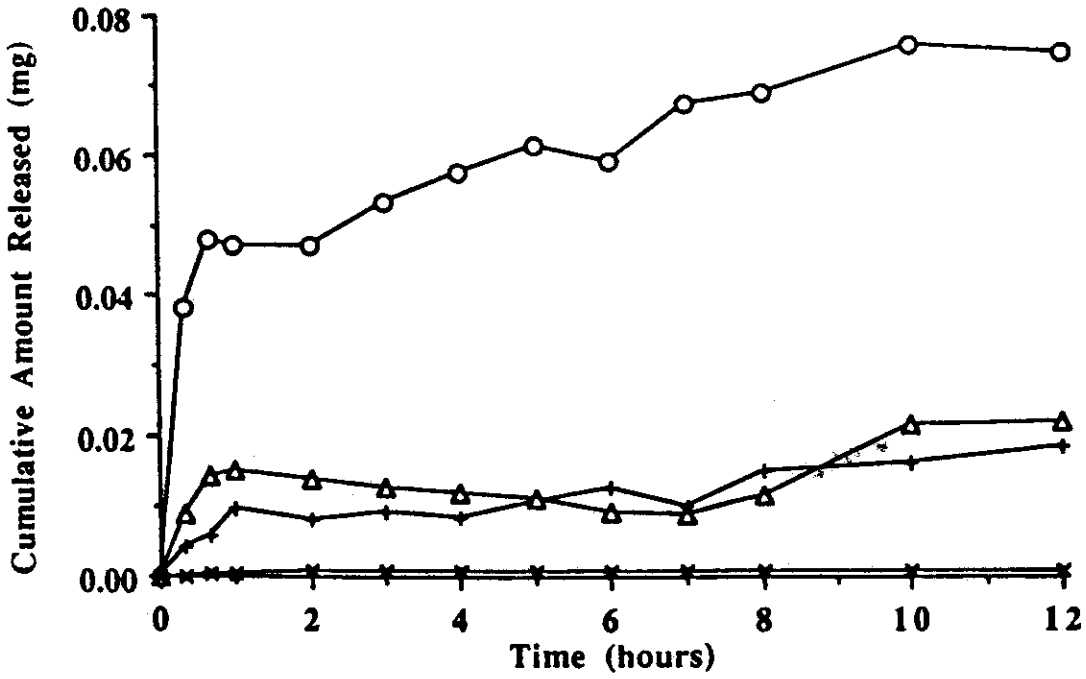

FIGURE 7
Comparison of Hydrocortisone Diffusion through the Enhancer Cell and the Franz Cell ; Franz Cell-Cellulose Membrane = Δ ; Franz Cell-Rat Skin = * ; Enhancer Cell-Cellulose Membrane = O ; Enhancer Cell-Rat Skin = +

studies. This is due to the smaller thickness of the membrane and also the lower permeability of hydrocortisone through the skin. From Figures 5 and 6, it is evident that the release rates for hydrocortisone were higher with the Enhancer cell as compared to the Franz cell irrespective of the diffusion membrane. The cumulative release using a cellulose membrane was more than 3 fold higher with the Enhancer cell compared to that seen with the Franz cell. The cumulative release results in the rat skin studies portrays a much larger difference, with the Enhancer cell showing almost 180 fold higher release than with the Franz cell. Although in general the release is higher through the cellulose membrane compared to the skin, the results of the cumulative release from cellulose membrane with the Franz cell are comparable to those of skin studies with the Enhancer cell (Figure 7). However, 100% release of hydrocortisone was not observed within 12 hours either with the Enhancer cell or the Franz cell, in part due to the poor solubility of hydrocortisone in the receptor phase and the hydrophobicity of the ointment base[7,9].

CONCLUSIONS

From the preceding results and discussion, it can be concluded that Enhancer cell has certain advantages when compared to the Franz cell for in vitro transdermal drug diffusion studies such as reduced investment, loading the cells and set up of the equipment and sampling techniques, especially with automation. Also, the Enhancer cell being an adaptation of the USP Apparatus II can be employed with relative use. The Enhancer cell can also be easily adapted to the Reciprocating Disk (USP Apparatus VII). The Enhancer cell, by the nature of its design, would extrapolate more readily to a transdermal drug delivery system in vivo, with an impermeable cover (the cell itself) holding a drug reservoir next to the skin, which becomes hydrated. This increases the efficiency and thus might reduce the time of diffusion studies. Another important aspect of the Enhancer cell was the improved viability of the rat skin which led to a reduction in the degradation products, with no interference with the HPLC analysis as was observed with the Franz cell.

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