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# MECHANISM OF TRANSDERMAL CONTROLLED NITROGLYCERIN ADMINISTRATION (II) ASSESSMENT OF RATE-CONTROLLING STEPS

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## ABSTRACT

The mechanisms and the rates of skin permeation of nitroglycerin delivered by four transdermal therapeutic systems were investigated using hairless mouse skin mounted on a newly-developed and well-calibrated Keshary-Chien skin permeation system. Experiments were carried out to identify and characterize the rate-controlling roles of stratum corneum, controlled-release drug delivery system and dermal solution sink in the transdermal controlled administration of nitroglycerin.

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Results indicated that the stratum corneum plays a significant rate-limiting function in the permeation of nitroglycerin across an intact skin, yielding a constant skin permeation profile, i.e., a linear Q vs. t relationship. The rate of skin permeation could vary as great as four folds from 44.7 mcg/cm$^2$/hr for Nitrodisc system to 11.2 mcg/cm$^2$/hr for Deponit system. Without the mediation of trandermal delivery system, pure nitroglycerin had a skin permeation rate of 41.4 mcg/cm$^2$/hr.

As the rate-limiting stratum corneum layers were successively removed by stripping technique, the rate of skin permeation increased in proportion to the number of strippings until a plateau rate was achieved after stripping for 10 times or more. As the stratum corneum was totally eliminated, the viable skin was exposed and the mechanism and the rate of skin permeation became dominated by the mechanism and the rate of drug release from the transdermal therapeutic systems.

Maintenance of dermal solution sink was also found to be crucial to the transdermal controlled administration of nitroglycerin. The rate of blood flow in the dermal microcirculation network was too observed to play a rate-limiting effect on the skin permeation of nitroglycerin.

## INTRODUCTION

Recently, the potential of using readily accessible organ, like the skin, for transdermal controlled administration of

systemically-active drugs was increasingly appreciated. In addition to other benefits, the transdermal route of administration can also achieve a prolonged therapeutic effect, while bypassing the gastrointestinal and hepatic first-pass eliminations. The interests and activities in this new spectrum of Pharmaceutical R & D have been greatly promoted following the simultaneous development and successful marketing of three technologically different controlled-release transdermal drug delivery systems: Nitrodisc system[1], Nitro-Dur system[2] and Transderm-Nitro system[3]. All these systems administer a controlled daily dose of a century old, extensively hepatically metabolized and short-acting drug, nitroglycerin, through an intact skin for at least 24 hours (1).

The epidermis has been consistently found to determine the overall extent of percutaneous absorption. For example, most water-soluble, low-molecular-weight nonelectrolytes applied to the skin surface could diffuse into the blood stream at a rate approximately 1000 times more rapidly if the epidermis were diseased, damaged or removed (2). As early as 1951, Berenson and Burch (3) proved, by conducting water permeability experiments on isolated epidermis and stratum corneum, that the horny layer of the skin is the principal permeability barrier, at least with respect to water.

In the transdermal controlled administration of drugs, a controlled-release transdermal therapeutic system is applied onto and administers a drug at a predetermined rate to the surface

of the skin; the drug then penetrates through the skin to the underlying microcirculation network,, thus entering the systemic circulation (4). Hence, it is critically important to understand the controlled delivery of a drug from a transdermal therapeutic system in relation to the skin permeation of the drug after release, where are the rate-limiting steps in determining the overall rate of transdermal drug administration, and how the mechanism and rate of drug delivery may affect the transdermal bioavailability of a drug.

The technique most frequently employed for the measurement of skin permeation of a drug is the in-vitro system with an excised skin mounted on a diffusion cell. Franz (5) designed a special diffusion cell for the in-vitro study of percutaneous absorption of compounds under a finite-dosing condition to simulate the clinical conditions. The diffusion cell is currently marketed as Franz Diffusion Cell[4], in which a skin sample is sandwiched between a donor and a receptor compartments with the stratum corneum side facing upwards into the open donor compartment and the dermis towards the thermostated receptor solution. A drug or a drug dosage form is then applied onto the stratum corneum and samples of the receptor solution are withdrawn at scheduled time intervals and assayed for drug concentration.

Over the years of use of the Franz diffusion cell for studying the skin permeation of drugs, several deficiencies were discovered (6). It was observed that this cell design could not achieve

the solution hydrodynamics and temperature control needed for accurate evaluations of skin permeation kinetics. To study the kinetics and mechanisms of skin permeation, a new finite-dosing skin permeation system, called Keshary-Chien Skin Permeation Cell was developed in this laboratory to overcome these deficiencies and to accomplish the solution hydrodynamics and temperature control required (6). In this investigation, the well-calibrated Keshary-Chien (K-C) skin permeation cell (Figure 1) was utilized to investigate the mechanisms and the rate-limiting steps involved in the process of transdermal controlled administration of nitroglycerin from various types of controlled-release transdermal therapeutic systems. The results will be analyzed and discussed in this report.

## EXPERIMENTAL

### A. Materials

1. Chemicals and solvents:

   a. Nitroglycerin: Pure nitroglycerin was extracted from the 10% nitroglycerin-lactose triturate[5] by dissolving the triturate in an excess amount of distilled water and then collecting the nitroglycerin precipitate settled at the bottom. The purity of the extracted nitroglycerin was determined by USP Phenoldisulphonic acid method[6].

   b. Methanol: Glass distilled HPLC grade[7].

   c. Water: HPLC grade prepared freshly by "Nanopure"

system[8].

d. Polyethylene Glycol 400[9].

e. Sodium Chloride[9].

2. Transdermal Nitroglycerin-releasing Therapeutic Systems:

a. Nitrodisc system (16mg/8cm$^2$)[1]

b. Nitro-Dur system (51mg/10cm$^2$)[2]

c. Transderm-Nitro system (25mg/10cm$^2$)[3]

d. Deponit system (16mg/16cm$^2$)[10]

3. Animals:

Male hairless mice of HRS/J strain[11] were used in the investigation. They were provided with free access to food and water. The bedding was changed at least once a week. The age of mice was controlled at 5 - 7 week old.

B. Preparations

1. Preparation of Intact Skin

Immediately following sacrifice by cervical dislocation of spinal cord, a 3.5cm x 3.5 cm portion of the full-thickness abdominal skin was carefully excised. The dermal side of the skin was cleaned of any adhering subcutaneous tissue and/or blood vessels.

2. Preparation of Stripped Skin

Immediately following sacrifice by cervical dislocation, the abdominal region of the hairless mouse was stripped with

cellophane tape[12]. The mouse was secured on a dissecting board and the skin was stripped by placing the tape on the skin surface and moving the thumb back and forth a few times with a uniform pressure and then pull off the tape (7). A fresh piece of tape was used for each stripping. About 3.5cm x 3.5cm portion of this stripped skin was carefully excised and used in skin permeation studies as soon as possible to avoid any drying of the skin surface.

3. Preparation of Dermal Solution

A saline solution containing 20% w/w Polyethylene glycol 400 was prepared and used as the dermal solution in the receptor compartment of K-C skin permeation system (Figure 1). The aqueous solubility of nitroglycerin (1.8 mg/ml) was improved by the incorporation of 20% w/w polyethylene glycol 400 (3.2 mg/ml) to maintain an effective sink condition, which simulates the biological sink achieved by hemoperfusion.

C. Skin Permeation of Nitroglycerin from Transdermal Therapeutic Systems or Pure Nitroglycerin

1. Intact skin with the presence of dermal solution sink

The full-thickness skin (with stratum corneum intact) prepared freshly as outlined above (section B-1) was mounted on the receptor compartment of the K-C skin permeation cell, with the stratum corneum side facing upward and the dermis side facing downward into the receptor compartment. A unit of the transdermal nitroglycerin-releasing patch was placed onto the skin with the

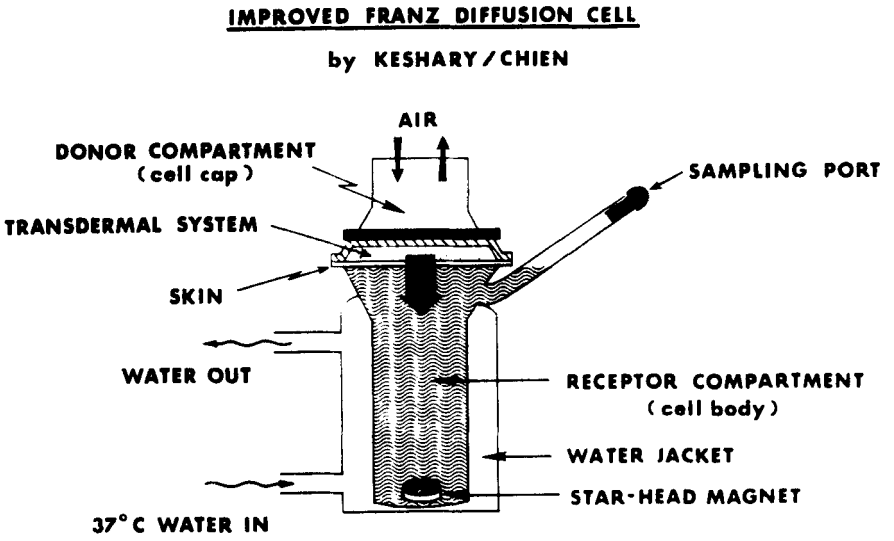


Figure 1: Diagrammatic illustration of one unit of the Keshary-Chien (K-C) skin permeation cell.

drug-releasing disc in intimate contact with the stratum corneum. The donor compartment was placed over the transdermal therapeutic system and the whole assembly was then securely clamped together (Figure 1). In the case where the skin permeation of pure nitroglycerin was studied, a 20 µl (about 32 mg) of the pure nitroglycerin was evenly spread over a 2.72 $cm^2$ surface area of the skin. To prevent any possible droplet formation of the liquid nitroglycerin applied (i.e. uneven spreading) on the stratum corneum due to the relatively high interfacial tension between the stratum corneum and the pure nitroglycerin, a paper disc (2.72 $cm^2$) was cut from a single ply of Kimwipe paper[13] and used as the matrix to achieve a homogeneous spreading of liquid nitroglycerin. This technique produced a rather even contact

of the pure nitroglycerin with the skin surface and at the same time a precise estimate of the area of application, which is very crucial in the calculation of skin permeation profile (in $mg/cm^2$), can be made. To avoid any potential loss of the nitroglycerin dose from the skin surface due to evaporation, entire skin surface was covered with aluminum foil[14]. The donor cap was then placed over and the whole assembly was tightly clamped together.

Following the application of a transdermal therapeutic system or the pure nitroglycerin on the skin, the saline solution containing 20% w/w polyethylene glycol 400 at 37°C was introduced into the receptor compartment, which was thermostatically controlled at 37°C by a circulating waterbath[15]. At the meantime, the donor compartment was maintained at the ambient temperature of 25±1°C.

At a predetermined time interval, one ml sample was withdrawn from the receptor solution, which was replaced immediately with the same volume of the PEG/saline solution to keep the volume in the receptor compartment constant and also to ensure an intimate contact between the dermal surface of the skin and the receptor solution. The concentration of nitroglycerin in the sample was determined by a sensitive HPLC method described later.

2. Stripped Skin in the Presence of Dermal Solution Sink

The stripped skin prepared freshly as outlined above (Section B-2) was mounted onto the receptor compartment of the K-C skin

permeation system. A unit of the transdermal therapeutic system or a dose (32 mg) of pure nitroglycerin was applied onto the skin in the same manner as described above for the Intact skin (Section C-1). The receptor compartment was also filled with the saline solution containing 20% w/w polyethylene glycol 400 at 37°C. At a predetermined time interval, the entire volume of the receptor solution was withdrawn and immediately replaced with the same volume of the fresh, drug-free PEG/saline solution to maintain the sink condition required. The concentration of nitroglycerin in the sample was determined by the HPLC method described later.

3. Intact Skin Without the Presence of Dermal Solution Sink

The same procedure as described above (Section C-1) for the intact skin with the presence of dermal solution sink was used, except that no saline solution was filled into the receptor compartment and a unit of Nitro-Dur system was applied for 6, 12, 18 or 24 hours.

At the completion of each application, the transdermal therapeutic system was removed and the amount of nitroglycerin deposited on the skin surface and bound to the skin tissue was analyzed by an assay procedure described later.

4. Viable Skin with Variable Dermal Solution Sink

The same procedure as described above (Section C-2) was used, except that the receptor solution was withdrawn at various

rates, ranging from 0.5 ml/3 hrs to 12 ml/3 hrs, to achieve different levels of skin condition. In this study, Nitro-Dur system and 25x stripped skin were used.

D. Release Profiles of Nitroglycerin from the Transdermal Therapeutic Systems

In these studies, experiments were conducted without the skin sample sandwiched between the donor and receptor compartments. A unit of a transdermal therapeutic system was directly mounted between the donor and the receptor compartments of the K-C skin permeation system and the whole assembly was then clamped together. The saline solution containing 20%w/w Polyethylene Glycol 400 was introduced into the receptor compartment. At a predetermined time interval, the receptor solution was completely removed and replaced with the same volume of drug-free PEG/saline solution at 37°C. In this way the required sink condition was maintained. Concentration of nitroglycerin in the sample was then determined by the HPLC method described later.

E. Determination of Residual Nitroglycerin on Skin Surface

This was done at the end of each skin permeation experiment. A single piece of kimwipe paper[13] was folded a few times and used to carefully wipe dry the nitroglycerin residue from the skin surface. Nitroglycerin in the Kimwipe paper was dissolved in 5 ml of methanol by vortexing for 2 minutes[16]. The concentration of nitroglycerin in the methanol was determined by the HPLC method described later.

F. Determination of Nitroglycerin Bound to Skin

After the skin surface was wiped dry, the area of skin which was in intimate contact with the drug-releasing disc of the transdermal therapeutic system was carefully cut out and its dermal surface was wiped dry of any adhering solution with kimwipe paper. The skin sample was then homogenized, using a polytron homogenizer[17], in 10 ml of methanol. During homogenization, the temperature of the sample was maintained cold by surrounding it in crushed ice to prevent any possible loss of nitroglycerin due to the heat generated. Homogenized skin was then centrifuged[18] at 2500 rpm for 5 minutes. The clear supernatant was separated and then assayed for nitroglycerin by HPLC.

G. Analytical Methods

For this investigation, a microprocessor-controlled high performance liquid chromatograph[19] equipped with a variable wavelength detector, an automatic sampler, a variable-volume injector, a dual-head reciprocating pump and a dual solvent system was used. Using a combination of methanol and water at a ratio of 60:40 as mobile phase at a flow rate of 1 ml/min and the column temperature at ambient, nitroglycerin in the sample solution (with an injection volume of 10 µl) was resolved by a reversed phase column[20] and detected at a wavelength of 205 nm.

Under the HPLC conditions outlined above, nitroglycerin produced a very sharp, clear absorption peak at a retention time of 4.9 min., while the two primary degradation products, 1,2-

and 1,3- dinitroglycerin, had yielded characteristic peaks at retention time of 3.1 and 2.9 min., respectively (8). This stability-indicating method has a detection sensitivity of 75-100 ng/ml for nitroglycerin.

H. Data Analysis

From the concentration profiles of nitroglycerin in the receptor solution, the flux (in $mg/cm^2$) of skin permeation was calculated using a computer program and then plotted as a function of time (in hours) or square root of time (in square root of hours).

The amount of nitroglycerin bound to skin was calculated as the amount per skin volume ($mg/cm^3$). The literature value on the thickness of the abdominal skin with and without the stratum corneum was used to calculate the volume of the intact and viable skins (4).

The residual amount of nitroglycerin on the skin surface was calculated as $mg/cm^2$ of skin surface area in contact with the drug-releasing disc of the transdermal therapeutic system.

## RESULTS AND DISCUSSION

A. Assessment of the Rate-controlling Role of Stratum Corneum

The rate-controlling role of stratum corneum in the skin permeation of nitroglycerin was evaluated by successively stripping various layers of stratum corneum using Scotch tape stripping technique (7). To minimize any potential complication from the

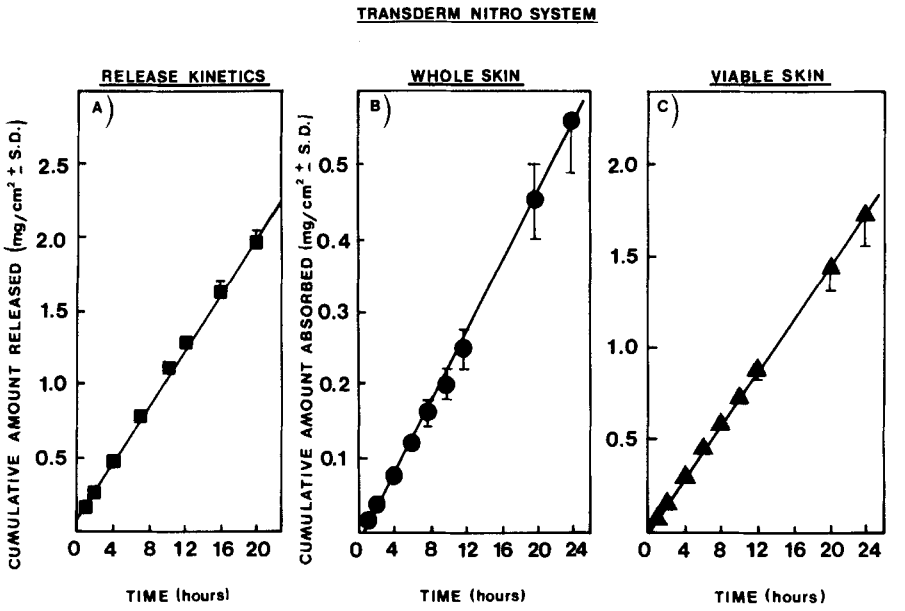


Figure 2: Release and skin permeation profiles of nitroglycerin from Transderm-Nitro system. Nitroglycerin released from Transderm-Nitro system at zero-order rate of 94.6 (±4.1) mcg/cm$^2$/hr (A) and penetrated through the hairless mouse skin also at zero-order kinetics with permeation rate of 23.6 (±2.9) mcg/cm$^2$/hr for intact skin (B) and of 71.5 (±5.6) mcg/cm$^2$/hr for the skin after stripping 25 times (C). Each data point represents the mean value ± one standard deviation of 4 determinations.

drug release mechanism of a drug delivery system, a membrane permeation-controlled drug delivery system with zero-order drug release kinetics, like Transderm-Nitro system (Figure 2), was first used to study the effect of stratum corneum stripping on

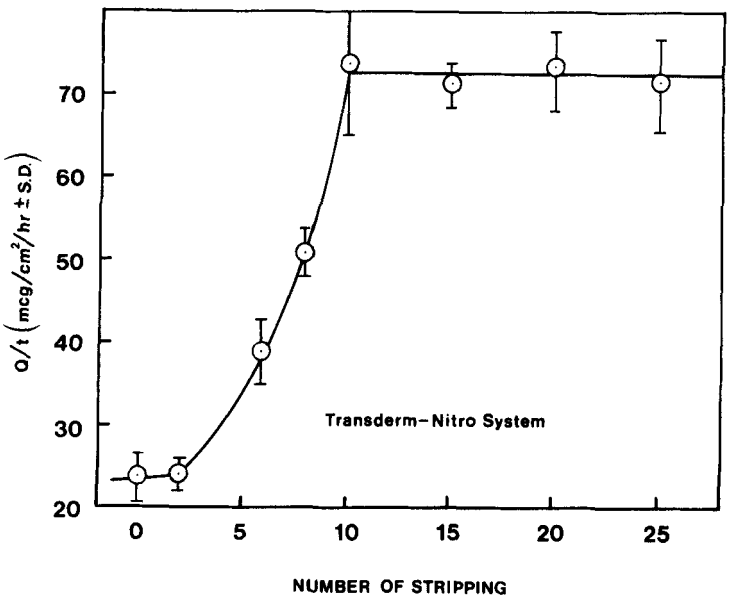


Figure 3: Effect of successive stripping on the rate of permeation (Q/t) of nitroglycerin across hairless mouse skin. The plateau level of 72.4 (±1.2) mcg/cm$^2$/hr was reached after stripping for 10 to 25 times. Each data point represents the mean value ± one standard deviation of 4 determinations.

the skin permeation rate profile of nitroglycerin (Figures 2 and 3).

Results indicated that the skin permeation profile of nitroglycerin delivered by Transderm-Nitro system still maintains the same zero-order kinetics, i.e., linear Q vs. t relationship, even after stripping the skin for 25 times (Figure 2). The rate of skin permeation was noted to increase by 3 times from 23.6(± 2.9) mcg/cm$^2$/hr for the intact skin to 71.5(± 5.6) mcg/cm$^2$/hr

for the completely stripped skin (25 x stripping). The rate of skin permeation across viable skin (71.5 mcg/cm$^2$/hr) is quite close to the rate of release (94.6 ± 4.1 mcg/cm$^2$/hr) of nitroglycerin from the Transderm-Nitro system. It was interesting to note that the skin permeation rate of nitroglycerin increases proportionally with the number of stripping until a plateau level of 72.4 (±1.2) mcg/cm$^2$/hr is attained after 10x stripping of the skin surface (Figure 3). No further increase in the skin permeation rate was observed beyond the peak rate (72.4 ± 1.2 mcg/cm$^2$/hr) when the skin was stripped more than 10 times. This observation clearly suggested that the stratum corneum is the rate-controlling tissue in the process of skin permeation of nitroglycerin; and, by stripping the skin surface for more than 10 times with scotch tape, the rate-controlling stratum corneum layers are effectively removed from the skin surface. Following the removal of stratum corneum, the rate of skin permeation across the viable skin (which consists of viable epidermis and dermis) became controlled by the drug release mechanism of transdermal therapeutic system. In other words, the rate-limiting step in the transdermal controlled administration of drugs has now shifted from the stratum corneum layers to the transdermal therapeutic system. No sex dependency was detected in the effect of stratum corneum stripping on the rate of skin permeation (Table 1).

As observed in the membrane-moderated Transderm-Nitro system, the skin permeation profile of nitroglycerin delivered by Deponit system, a multilaminate-type transdermal therapeutic system with

TABLE 1: EFFECT OF SEX ON SKIN PERMEATION RATE OF NITROGLYCERIN[1)]

| Sex[2)] | Rate of Skin Permeation ($mcg/cm^2/hr \pm S.D.$) | |
|---|---|---|
| | Intact Skin | Viable Skin |
| Male | 23.6 ± 2.9 | 71.5 ± 5.6 |
| Female | 23.4 ± 2.9 | 79.3 ± 12.3 |

1) Transderm-Nitro System

2) Hairless Mouse

zero-order drug release profile (Figure 4-A), is also defined by the constant Q vs. t relationship. The rate of skin permeation across the intact hairless mouse skin (11.2 ± 1.8 $mcg/cm^2/hr$) was found to be two times slower than that from Transderm-Nitro system (23.6 ± 2.9 $mcg/cm^2/hr$), which agreed with the observation that the Deponit system releases nitroglycerin at a rate (Q/t = 18.0 ± 1.0 $mcg/cm^2/hr$) which is substantially slower than the rate of release from Transderm-Nitro system (94.6 ± 4.1 $mcg/cm^2/hr$). Following the elimination of the rate-limiting stratum corneum, the skin permeation profile for Deponit system still followed the linear Q vs.t relationship and the rate of permeation across the stratum corneum-free viable skin increased by only 56% from (11.2 ± 1.8 $mcg/cm^2/hr$ to 17.5 ± 0.9 $mcg/cm^2/hr$)

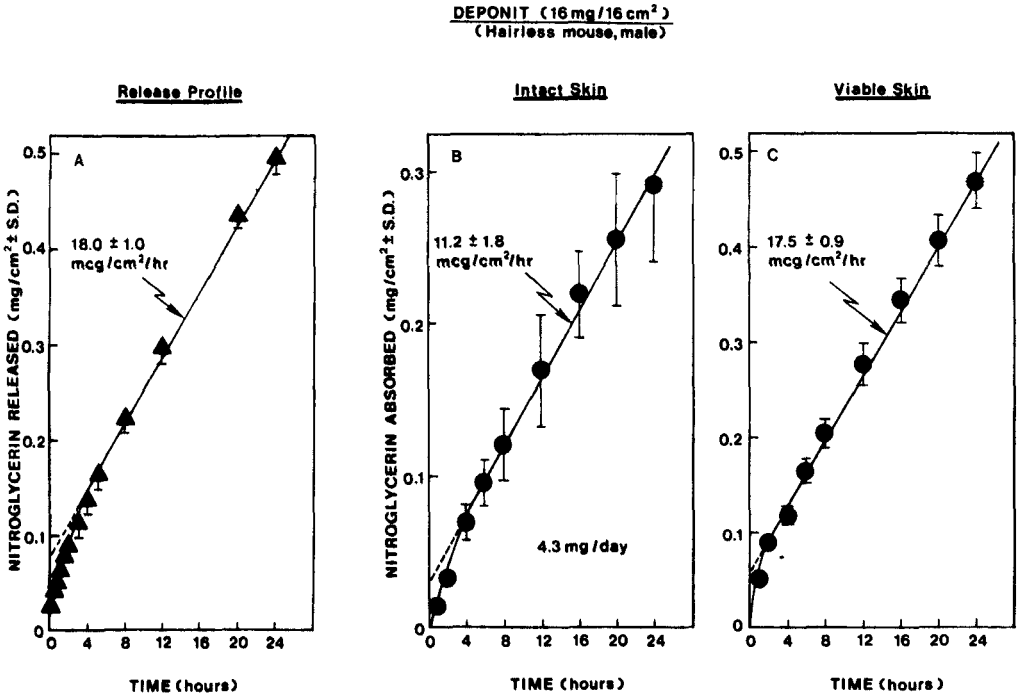


Figure 4: Release and skin permeation profiles of nitroglycerin from Deponit system. Nitroglycerin released from Deponit system at zero-order kinetics with a release rate of 18.0 (±1.0) mcg/cm$^2$/hr (A) and permeated through the hairless mouse skin also at zero-order kinetics with permeation rate of 11.2 (±1.8) mcg/cm$^2$/hr for intact skin (B) and of 17.5 (±0.9) mcg/cm$^2$/hr for the skin after 25x stripping (C). Each data point represents the mean value ± one standard deviation of 4 determinations.

(Figure 4), as compared to a 3-fold increase seen earlier in the case of Transderm-Nitro system (Figure 2). And, the rate of permeation across the viable skin (17.5 ± 0.9 mcg/cm$^2$/hr) was almost the same as the rate of release from the Deponit system (18.0 ± 1.0 mcg/cm$^2$/hr). The results suggested that the mechanism and rate of skin permeation of nitroglycerin are in greater control by the mechansim and rate of release from Deponit system, as illustrated by the slower rates of release and skin permeation as well as only a small increase (56%) in the rate of permeation when the stratum corneum was totally stripped from the skin.

The effect of stratum corneum on the transdermal controlled administration of nitroglycerin delivered by matrix diffusion-type drug delivery systems, like Nitrodisc and Nitro-Dur systems, was also investigated. Results indicated that the skin permeation profile of nitroglycerin from Nitrodisc system is also defined by the same linear Q vs. t relationship as observed earlier for Transderm-Nitro and Deponit systems. A skin permeation rate of 44.7 (±6.4) mcg/cm$^2$/hr was achieved, which is almost twice greater than the rate of skin permeation for Transderm-Nitro system (23.6 ± 2.9 mcg/cm$^2$/hr). As the rate-limiting stratum corneum was totally removed by stripping, the rate of skin permeation increased considerably (Figure 5). The linear Q vs. t relationship was maintained for only up to 12 hours after the medication. At the 12-hr point, approximately 85% of the loading dose in the Nitrodisc system had already been released, and beyond

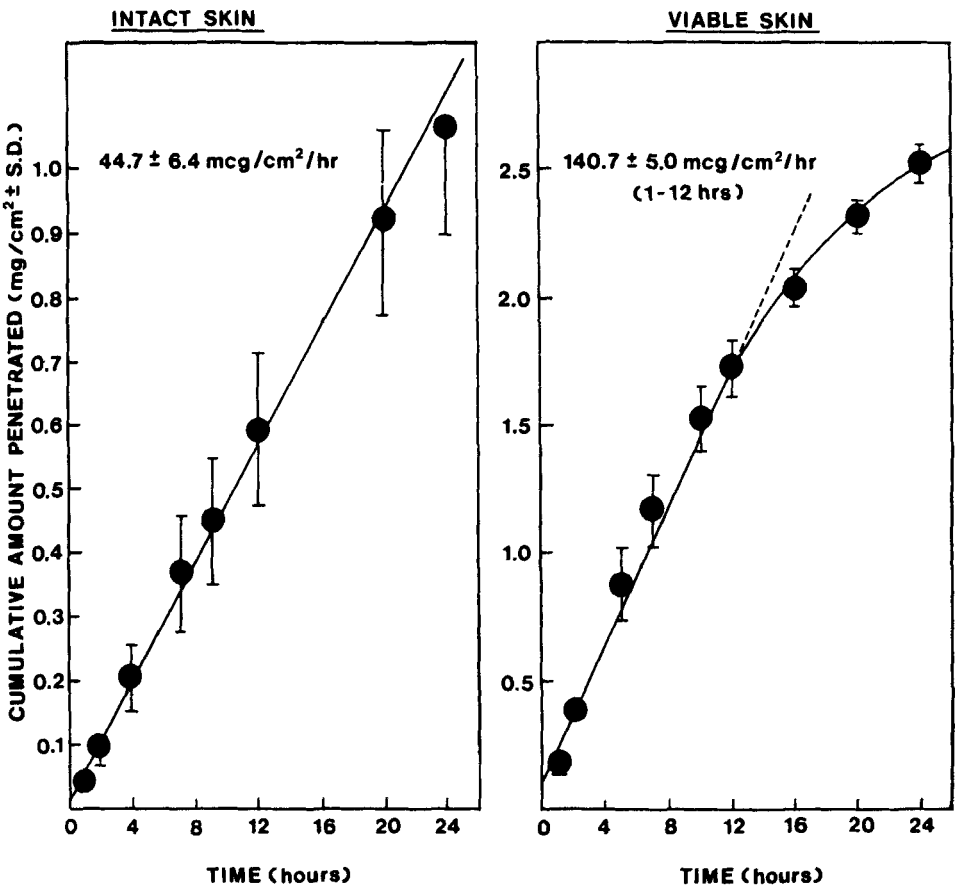


Figure 5: Permeation profiles of nitroglycerin from Nitrodisc system. Nitroglycerin permeated through the intact skin at zero-order kinetics with a permeation rate of 44.7 (±6.4) mcg/cm$^2$/hr. After 25x stripping, the permeation profile across the viable skin was also constant for the initial 12-hr period with a permeation rate of 140.7 (±5.0) mcg/cm$^2$/hr. Each data point represents the mean value ± one standard deviation of 4 determinations.

that the skin permeation rate gradually declined. The rate of skin permeation across the viable skin (140.7 ± 5.0 mcg/cm$^2$/hr) as calculated from the first 12-hour data points was found to be 3 folds greater than the rate of permeation across the intact skin (44.7 ± 6.4 mcg/cm$^2$/hr) for 24 hours, indicating that the stratum corneum adds a significant diffusional resistance to the permeation of nitroglycerin across the intact skin.

On the other hand, if the permeation of nitroglycerin across the viable skin is controlled by its release mechanism from the Nitrodisc system, then all the skin permeation data points for the viable skin (Figure 5) should follow a linearity defined by the Q vs. $t^{1/2}$ relationship as expected from the matrix diffusion-controlled drug release process. As expected, the results suggested that the permeation profile of nitroglycerin across the viable skin can be described by the same linear Q vs. $t^{1/2}$ relationship as the release of nitroglycerin from the Nitrodisc system (Figure 6). The mean flux of skin permeation ($Q/t^{1/2}$ = 612.0 mcg/cm$^2$/hr$^{1/2}$) was found to be only slightly lower than the flux of release (645.8 mcg/cm$^2$/hr$^{1/2}$). The observation suggested that as the stratum corneum is totally removed, the mechanism and rate of skin permeation across the viable skin become controlled by the mechanism and rate of release from the Nitrodisc system.

The same phenomenon was also encountered in the Nitro-Dur system, which is also a matrix diffusion-controlled drug delivery

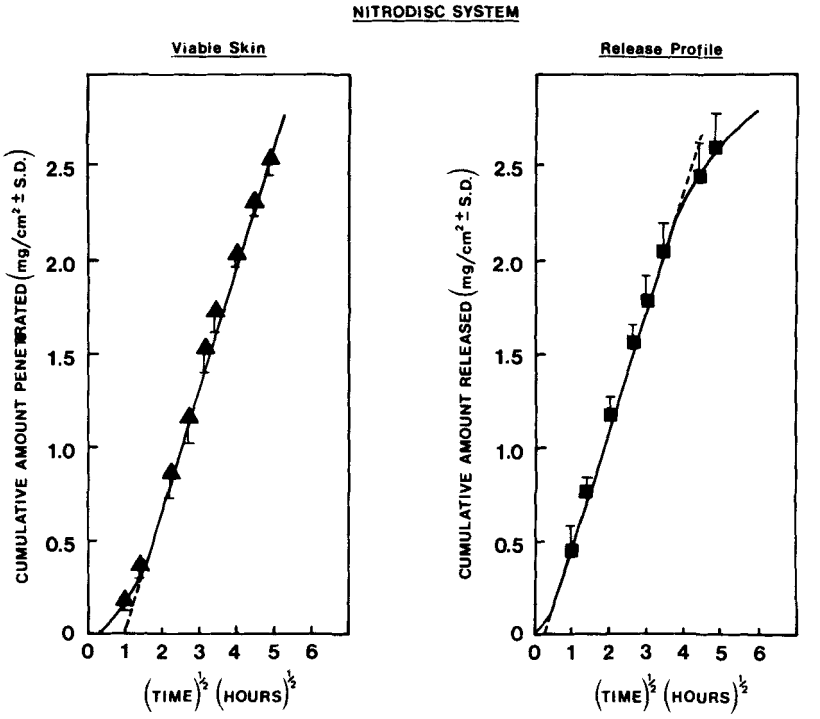


Figure 6: The 24-hr permeation profiles of nitroglycerin across the viable skin were found to yield the same linear Q vs. $t^{\frac{1}{2}}$ relationship as observed for the release profiles from Nitrodisc system. A permeation flux of 612.0 (±67.3) mcg/$cm^2$/$hr^{\frac{1}{2}}$ was obtained as compared to the release flux of 645.8 (±62.9) mcg/$cm^2$/$hr^{\frac{1}{2}}$. Each data point represents the mean value ± one standard deviation of 4 determinations.

system. Similar to Transderm-Nitro, Deponit and Nitrodisc systems, the nitroglycerin delivered by Nitro-Dur system also penetrated through the intact skin at a rate profile which is well characterized by the linear Q vs. t relationship (Figure 7).

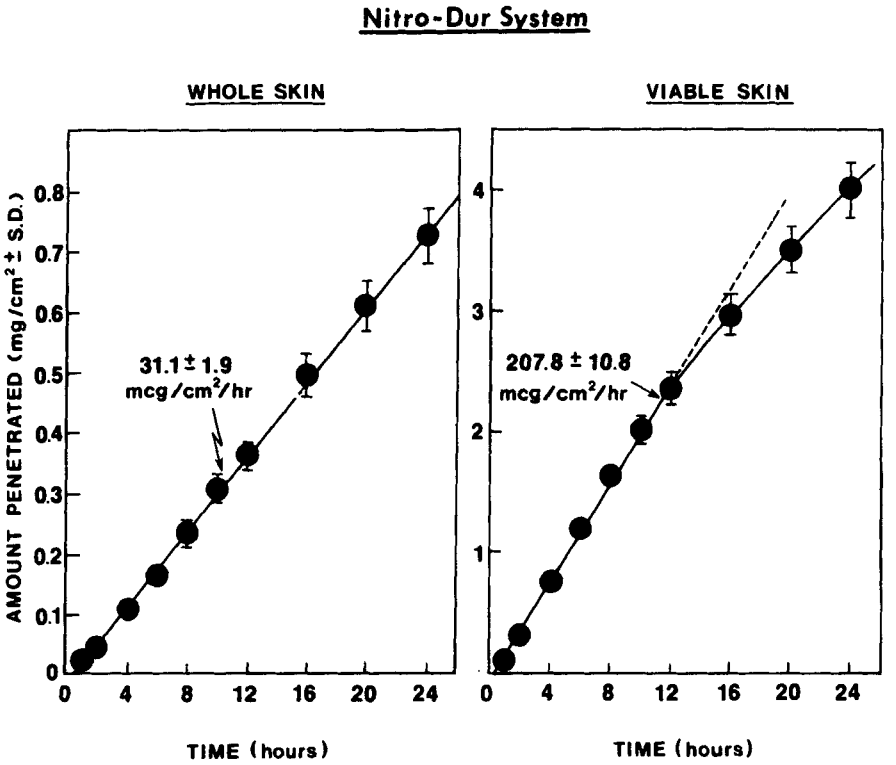


Figure 7: Permeation profiles of nitroglycerin from Nitro-Dur system. Nitroglycerin permeated through the intact skin at zero-order kinetics with a permeation rate of 31.1 (±1.9) mcg/cm$^2$/hr. After 25x stripping, the permeation profile across the viable skin was also constant for the initial 12-hr period with a permeation rate of 207.8 (±10.8) mcg/cm$^2$/hr. Each data point represents the mean value ± one standard deviation of 4 determinations.

A skin permeation rate of 31.1 (±1.9) mcg/cm$^2$/hr was calculated. As the stratum corneum was eliminated by 25 x stripping, the linear Q vs. t relationship was maintained also for only up to

12 hrs. At the 12-hr point, approximately 50% of the loading dose in the Nitro-Dur system had already been released and beyond that the rate of skin permeation gradually decreased. The skin permeation rate across the viable skin (207.8 ± 10.8 mcg/cm$^2$/hr) as determined from the first 12-hr data points was found to be about 7 times greater than the rate of skin permeation across the intact skin (31.1 ± 1.9 mcg/cm$^2$/hr). Once again, the observations suggested that the stratum corneum also plays the rate-limiting role in the permeation of nitroglycerin delivered by Nitro-Dur system.

If the skin permeation of nitroglycerin across the viable skin is determined by the controlled release mechanism of the Nitro-Dur system, then all the skin permeation data for the viable skin (Figure 7) should also follow the $Q$ vs. $t^{1/2}$ linearity defined for the matrix diffusion-controlled drug release process. Results indicated that the skin permeation profile of nitroglycerin across the viable skin does follow the same $Q$ vs $t^{1/2}$ linearity as does the release profile of nitroglycerin from the Nitro-Dur system (Figure 8). The flux of skin permeation ($Q/t^{1/2}$ =1129.4 ± 67.8 mcg/cm$^2$/hr$^{1/2}$) was found to be slightly lower than the flux of release (1303.2 ± 18.7 mcg/cm$^2$/hr$^{1/2}$). The observation suggested that as the rate-limiting stratum corneum is totally removed, the mechanism and rate of skin permeation across the viable skin become dominated by the matrix diffusion-controlled drug release process from the Nitro-Dur system.

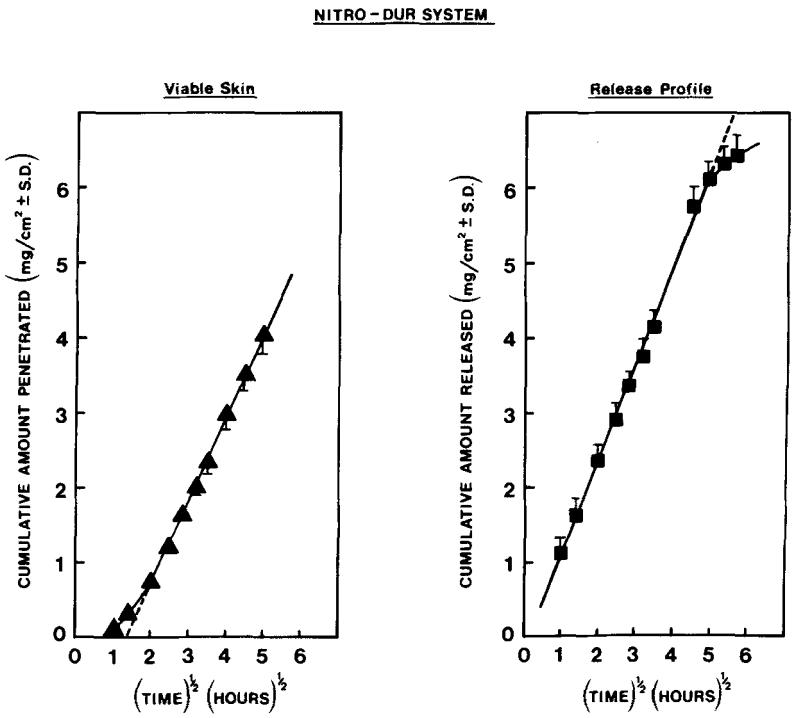


Figure 8: The 24-hr permeation profiles of nitroglycerin across the viable skin were found to produce the same linear Q vs. $t^{1/2}$ relationship as observed for the release profiles from Nitro-Dur system. A permeation flux of 1,129.4 (±67.8) mcg/cm$^2$/hr$^{1/2}$ was obtained as compared to the release flux of 1,303.2 (±18.7) mcg/cm$^2$/hr$^{1/2}$. Each data point represents the mean value ± one standard deviation of 4 determinations.

The results outlined above lead us to conclude that stratum corneum plays a significant rate-limiting role in the skin permeation of nitroglycerin across the intact skin. As the rate-limiting stratum corneum is removed by stripping, the rate

of skin permeation increases as a function of the number of stripping. As the stratum corneum is totally eliminated from the skin, the mechanism and the rate of skin permeation becomes controlled by the mechanism and the rate of drug release from the transdermal therapeutic systems.

B. Assessment of the Effect of Drug Delivery System

To evaluate the effect of drug delivery system on the transdermal controlled administration of nitroglycerin, the skin permeation kinetics of pure nitroglycerin across intact skin and stratum corneum-free viable skin was studied. In this investigation, the skin permation of nitroglycerin is totally free from the effect of controlled drug release mechanism of the drug delivery systems.

Results indicated that without the mediation of drug delivery systems, the pure nitroglycerin penetrates through the intact hairless mouse skin at constant, zero-order kinetics (linear Q vs. t relationship) (Figure 9). A skin permeation rate of 41.4 (±4.9) mcg/cm$^2$/hr was obtained, which is almost two times greater than the skin permeation rate from Transderm-Nitro system (23.6 ± 2.9 mcg/cm$^2$/hr), four times greater than from Deponit system (11.2 ± 1.8 mcg/cm$^2$/hr), 33% greater than from Nitro-Dur system (31.1 ± 1.9 mcg/cm$^2$/hr). On the other hand, Nitrodisc system, which is known to release a skin permeation promotant like isopropyl palmitate, along with nitroglycerin was found to produce a skin permeation rate (44.7 ± 6.4 mcg/cm$^2$/hr) which

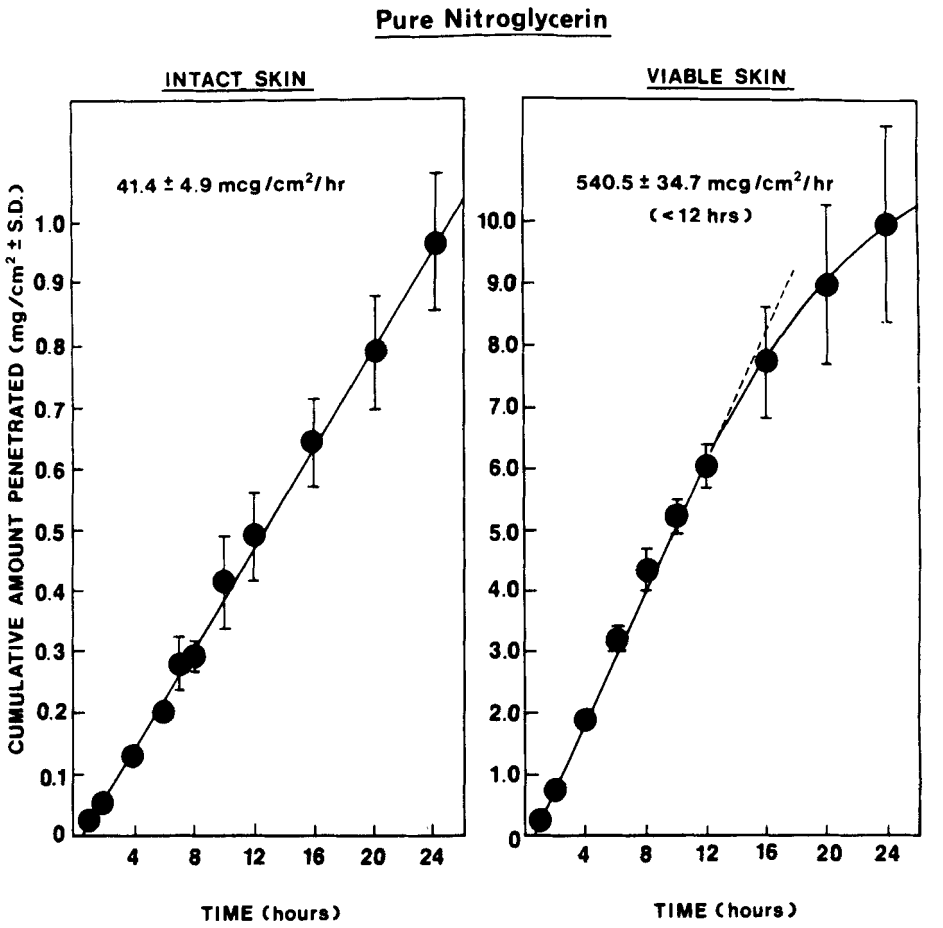


Figure 9: Permeation profiles of pure nitroglycerin. Nitroglycerin permeated through the intact skin at zero-order kinetics with a permeation rate of 41.4 (±4.9) mcg/cm$^2$/hr. After 25x stripping, the permeation profile across the viable skin was also constant for the first 12 hrs of permeation with a permeation rate of 540.5 (±34.7) mcg/cm$^2$/hr. Each data point represents the mean value ± one standard deviation of 7 determinations for intact skin and 4 determinations for viable skin.

is comparable to the rate of skin permeation for pure nitroglycerin (41.4 ± 4.9 mcg/cm$^2$/hr). The effect of drug delivery system on the transdermal controlled administration of nitroglycerin is thus demonstrated.

After stripping off the stratum corneum, the linear Q vs. t relationship was still followed for the permeation of pure nitroglycerin in the first 12 hours of the study and a skin permeation rate of 540.5 (±34.7) mcg/cm$^2$/hr was resulted (Figure 9). This rate of permeation (540.5 mcg/cm$^2$/hr) was 13 times greater than the rate of permeation across the stratum corneum-covered intact skin (41.4 mcg/cm$^2$/hr). The observed increase in the rate of skin permeation after total removal of the rate-limiting stratum corneum was substantially greater for pure nitroglycerin (13 folds) than for the nitroglycerin delivered by a controlled-released transdermal therapeutic system: Deponit system (1.6x increase), Transderm-Nitro system (3x increase), Nitrodisc system (3.1x increase), and Nitro-Dur system (6.7x increase). The rate-controlling role of the controlled release drug delivery system in the transdermal administration of nitroglycerin is becoming more evident as the rate-limiting stratum corneum is eliminated (Table 2). After total removal of the stratum corneum, the mechanism and the rate of skin permeation is predominately controlled by the mechanism and the rate of drug release from the controlled-release transdermal therapeutic systems.

TABLE 2: EFFECT OF STRATUM CORNEUM AND FORMULATIONS ON THE RATE OF SKIN PERMEATION OF NITROGLYCERIN

| Formulations | Rate of Skin Permeation | | | Rate of Release ($mcg/cm^2$) |
|---|---|---|---|---|
| | Intact Skin ($mcg/cm^2/hr$) | Viable Skin <12 hrs ($mcg/cm^2/hr$) | Viable Skin 1-24 hrs ($mcg/cm^2$) | |
| Pure Nitroglycerin | 41.4 ± 4.9 | 540.5 ± 34.7 | --- | --- |
| Therapeutic Systems: | | | | |
| 1) Transderm-Nitro | 23.6 ± 2.9 | 71.5 ± 5.6 | 71.5 ± 5.6 ($hr^{-1}$) | 94.6 ± 4.1 ($hr^{-1}$) |
| 2) Deponit | 11.2 ± 1.8 | 17.5 ± 0.9 | 17.5 ± 0.9 ($hr^{-1}$) | 18.0 ± 1.0 ($hr^{-1}$) |
| 3) Nitrodisc | 44.7 ± 6.4 | 140.7 ± 5.0 | 612.0 ± 67.3 ($hr^{-0.5}$) | 645.8 ± 62.9 ($hr^{-0.5}$) |
| 4) Nitro-Dur | 31.1 ± 1.9 | 207.8 ± 10.8 | 1129.4 ± 67.8 ($hr^{-0.5}$) | 1303.2 ± 18.7 ($hr^{-0.5}$) |

C. Assessment of the Effect of Dermal Solution Sink

It is known that a biological sink is constantly maintained in the dermis by hemoperfusion through the capillary network in the papillary layer (9). To simulate the blood flow in the dermal microcirculation system and to study its effect on the sink condition in the dermis and the permeation rate across the skin, experiments were carried out to study the permeation rate of nitroglycerin through the stratum corneum-free viable skin with the dermal solution sampled at various rates, ranging from 0.5 ml/3 hrs to 12 ml/3 hrs. Results indicated that the sampling rate does influence the flux of permeation (Table 3). The lower the nitroglycerin concentration accumulated in the dermal solution in relative to its saturation solubility, the higher the flux of skin permeation. The permeation flux (1,113.1 ± 36.3 to 1,176.7 ± 50.6 mcg/cm$^2$/hr$^{½}$) achieved under sink condition (i.e., <10% of saturation solubility) was found to be in fairly good agreement with the 1,129.4 ± 67.8 mcg/cm$^2$/hr$^{½}$ obtained independently by variable sampling schedule (compared Table 3 with Table 2).

The effect of dermal solution sink on skin permeation profile of nitroglycerin was also investigated using stratum corneum-covered intact skin. Results indicated that with the nitroglycerin concentration in the dermal solution maintained below 10% of its saturation solubility, a constant rate of skin permeation (24.3 mcg/cm$^2$/hr) is yielded, while the concentrations of nitroglycerin on the skin surface and in the skin tissue reach equilibrium levels of 25 mcg/cm$^2$ and 1.64 mg/cm$^3$, respectively,

TABLE 3: EFFECT OF DERMAL SOLUTION SINK ON PERMEATION FLUX OF NITROGLYCERIN[1] THROUGH VIABLE SKIN[2]

| Sampling Rate (ml/3 hr) | Flux of Permeation ($mcg/cm^2/hr^{\frac{1}{2}}$ ± S.D.) | Extent of Saturation[3] (% Cs) |
|---|---|---|
| 0.5 | 802.3 ± 51.1 | 20.6 ± 1.6 |
| 1.0 | 963.4 ± 35.1 | 19.8 ± 1.2 |
| 2.0 | 1,067.8 ± 102.4 | 15.4 ± 0.6 |
| 5.0 | 1,113.1 ± 36.3 | 8.1 ± 0.4 |
| 12.0 | 1,176.7 ± 50.6 | 3.3 ± 0.3 |

1) Delivered by Nitro-Dur system.

2) Hairless mouse skin after 25x stripping.

3) The data represent the nitroglycerin concentration in the dermal solution at 24-hr point relative to its saturation solubility (Cs = 3.2 mg/ml in saline solution containing 20%w/w of PEG 400 at 37°C).

around 6-12 hrs after administration (Figure 10). It resulted in a release rate of 31.9 $mcg/cm^2/hr$. On the other hand, without the presence of a dermal solution sink, an equilibrium level of nitroglycerin (1.54 $mg/cm^3$) was also achieved and maintained 12 hrs after medication, while the concentration of nitroglycerin on the skin surface was observed to increase continuously at a rate of 11.9 $mcg/cm^2/hr$ (Figure 11). It translated into a release rate of 14.6 $mcg/cm^2/hr$. The results suggested that

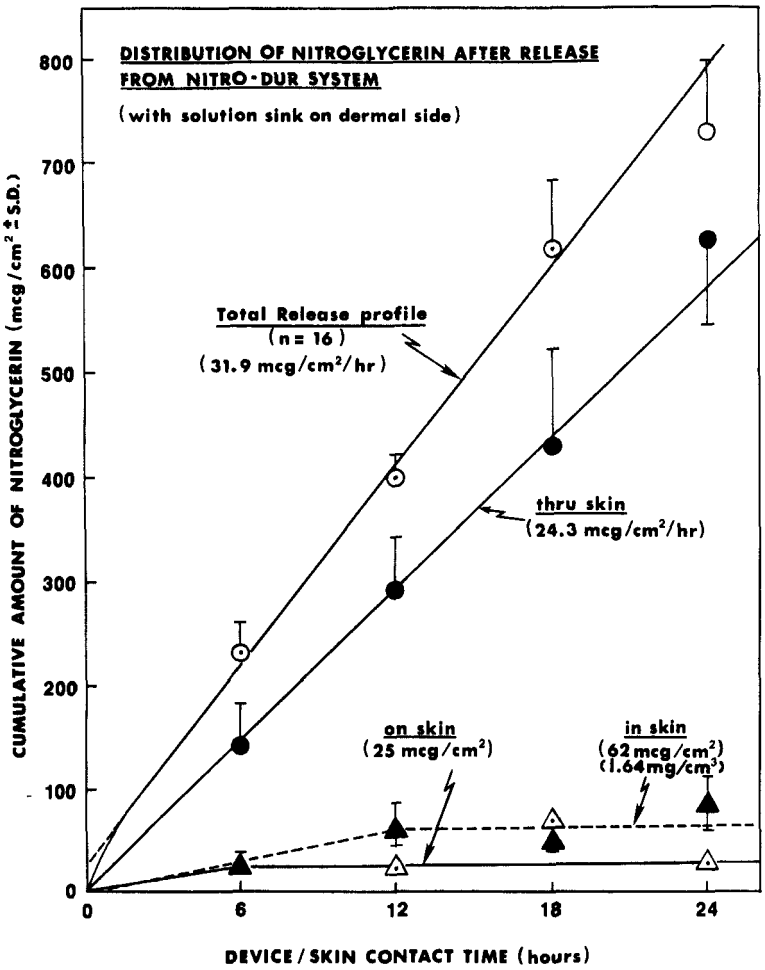


Figure 10: Permeation and distribution profiles of nitroglycerin in the intact skin with solution sink maintained on the dermal side. Equilibrium nitroglycerin concentrations of 25 mcg/cm$^2$ and 1.64 mg/cm$^3$ were achieved on and in the skin within a contact time of 12 hrs. Nitroglycerin permeated through the skin at a zero-order rate of 24.3 mcg/cm$^2$/hr. By calculation, the Nitro-Dur system released nitroglycerin to the skin at a rate of 31.9 mcg/cm$^2$/hr. Each data point represents the mean value ± one standard deviation of 4 determinations.

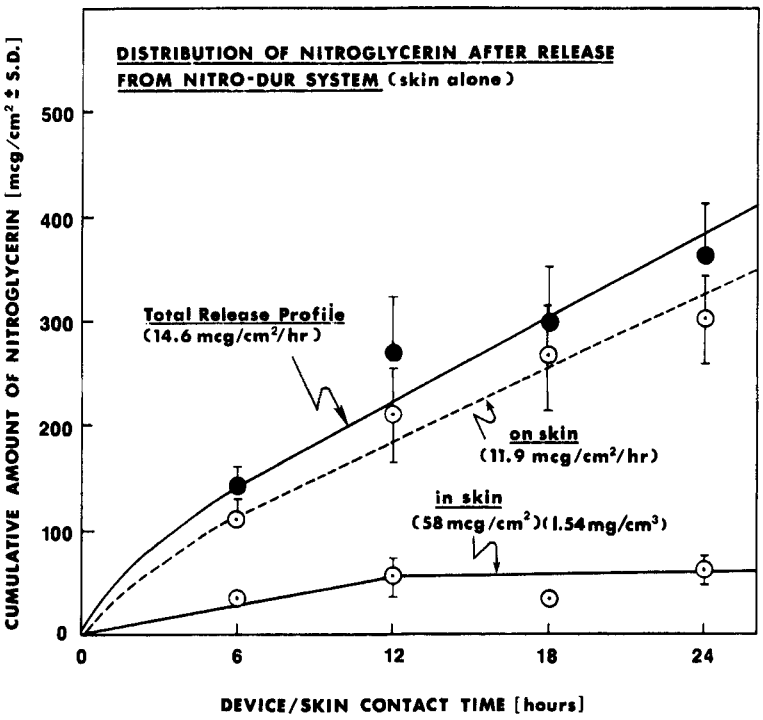


Figure 11: Permeation and distribution profiles of nitroglycerin in the intact skin without any solution sink maintained on dermal side. An Equilibrium nitroglycerin concentration of 1.54 $mg/cm^3$ was achieved in the skin, while the nitroglycerin concentration on skin surface continued to increase at a rate of 11.9 $mcg/cm^2/hr$. By calculation, the Nitro-Dur system released nitroglycerin to the skin at a rate of 14.6 $mcg/cm^2/hr$. Each data point represents the mean value ± one standard deviation of 4 determinations.

without the dermal solution to maintain a sink condition for the permeation of nitroglycerin, the nitroglycerin still continues to release from Nitro-Dur system, even after an equilibrium nitroglycerin concentration (1.54 mg/cm$^3$) is reached in the skin tissue. The excess amount of nitroglycerin was retained on the skin surface. With the presence of dermal solution sink, the nitroglycerin released to the skin surface was carried away by permeation through the skin at a rate of 24.3 mcg/cm$^2$/hr; So, the rate of release of nitroglycerin from Nitro-Dur system was increased by more than two folds from 14.6 mcg/cm$^2$/hr to 31.9 mcg/cm$^2$/hr. The observations led us to conclude that maintenance of a sink condition in the dermal solution is also critical to the transdermal controlled administration of nitroglycerin and the rate of blood flow in the microcirculation network, which determines the extent of sink condition and the degree of drug accumulation in the skin, will play the rate-limiting effect on the rate of skin permeation.



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## FOOTNOTES

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13. Kimberly-Clark Corp., Roswell, Georgia
14. Reynolds #655, household aluminum foil, Reynolds Metals Company, Richmond, Virginia
15. Model #80, Fisher Scientific Co., Fairlawn, New Jersey
16. "Magnestir", Scientific Glass Apparatus Co., Inc., Bloomfield, New Jersey
17. Brinkman Instruments, Westbury, New York
18. Damon Model CU-5000, International Equipment Co., Needham Heights, Massachusetts
19. HP Model 1084B HPLC, Hewlett-Packard, Palo Alto, California
20. Zorbax C-8, 6 μm particle size [15 cm x 4.6 mm], DuPont Company, Wilmington, Delaware

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