DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY, 11(6&7), 1213-1253 (1985)

MECHANISM OF TRANSDERMAL CONTROLLED NITROGLYCERIN ADMINISTRATION (III) CONTROL OF SKIN PERMEATION RATE AND OPTIMIZATION*

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ABSTRACT

A mathematical model was developed to correlate the drug permeation rate through the skin with the drug release rate from a matrix-type drug delivery system. Experiments were carried out using hairless mouse abdominal skin mounted on a recently-developed and hydrodynamically well-calibrated Keshary-Chien skin permeation system. A matrix-type drug delivery system was designed to contain different loading doses of nitroglycerin and to study the effect of drug loading variation on the rate of drug release, the rate of skin permeation and the equilibrium concentration of nitroglycerin in the skin.

 0363-9045/85/1106-1213$3.50/0

Results indicated that the stratum corneum plays a significant rate-limiting role in the skin permeation of nitroglycerin across the intact skin, yielding a constant skin permeation profile. The permeation rate across the intact skin was observed to increase with the increase in the drug release flux initially and then levelled off in a hyperbolic fashion. Various constants were obtained from the reciprocal plot of skin permeation rate vs. drug release flux. These constants could be used for the prediction of the skin permeation rate. A very good correlation between the predicted and the observed values of skin permeation rates was observed.

After the stratum corneum was removed by stripping technique, the mechanism and the rate of skin permeation became dominated by the mechanism and the release rate of the delivery system.

A linear correlation was observed between the drug permeation rate through the skin and the equilibrium concentration of drug in the skin. This correlation was observed in both intact and viable skins.

INTRODUCTION

During recent years much work has been devoted on the development of rate-control drug delivery systems. It is highly desirable that these delivery systems administer the drug at a rate which is controlled mainly by the system itself and does not vary with the variation in the environment. One of the most

successful means of producing such a drug delivery system has been the use of diffusion process to control the release of drugs (1).

In the treatment of an illness with a drug, it is desirable, from the standpoint of pharmacodynamics, to maintain the concentration of the drug in the target tissue(s) at a constant level and within the therapeutically effective range (2).

One of the major advances in Pharmaceutical R & D in recent years has been the successful development of transdermal therapeutic systems, which utilize the controlled drug release technology to control the infusion of drug to the systemic circulation through the intact skin, the so-called transdermal controlled administration (3). The currently marketed controlled-release transdermal therapeutic systems can be classified in three main categories: Membrane permeation-controlled and matrix diffusion-controlled drug delivery systems and microsealed drug delivery system (4). The matrix-type drug delivery systems are known to have several advantages: one of them is the ease of fabrication as compared to other types of delivery systems, such as the membrane-encapsulated reservoir-type devices (5). Furthermore, for certain applications, like the sustained release of macromolecules (6), the use of a matrix system is essential. One major drawback, however, in the use of a matrix diffusion-controlled drug delivery system is that it generally does not display the desired zero-order

release kinetics (7). The drug release profile from a slab of matrix-type device is reportedly proportional to the square root of time (7, 8). On the other hand, the membrane permeation-controlled drug delivery system provides a constant, zero-order drug release profile, while it is more difficult to fabricate and has a potential of dose dumping due to membrane breakage (7, 9). Chien et al (10-12) developed a hybrid-type drug delivery system, the so-called Microsealed Drug Delivery System, with homogeneous, microscopic dispersion of drug reservoirs in polymer matrix to maximize the advantages and to minimize the disadvantages of both membrane permeation-controlled and matrix diffusion-controlled drug delivery systems. It is a matrix in physical appearance and delivers the drug at a rate profile, which is either $\underline{Q \text{ vs. } t}$ or $\underline{Q \text{ vs. } t^{1/2}}$, depending upon the physicochemical properties of drugs in the system (13).

The skin is one of the most extensive and readily accessible organs of the human body. Traditionally, skin has been the common site of administration for dermatological drugs to achieve a localized phamacological action. More recently, the potential of skin serving as the port of administration for a number of systemically active drugs has also been recognized. Along the course of skin permeation, a drug molecule is known to encounter a number of diffusional resistances which counteract its penetration through various skin tissue layers. For most drugs, the principal diffusional resistance has been found to reside in the stratum corneum layers, i.e., the rate-controlling barrier (14).

The mechanisms of drug release and skin permeation of various transdermal therapeutic systems have been extensively studied in recent years (4, 15-18). It was increasingly realized that the mechanisms of drug release and skin permeation from a transdermal drug delivery system can be assessed by the use of a well-calibrated *in vitro* diffusion cell. A variety of diffusion cells have been proposed and used for studying the percutaneous absorption of drugs (16, 19-22). Among the various diffusion cells developed so far for skin permeation studies, the Franz diffusion cell has been commercialized and widely employed for studying the skin permeation profiles by finite-dosing technique (22, 23).

The most popular method for the evaluation of a rate-control transdermal drug delivery system is to monitor the drug permeation profile across an excised skin mounted on an *in vitro* diffusion cell. Earlier, we reported the design of an *in-vitro* skin permeation system to overcome the deficiencies observed in the Franz diffusion cell and to achieve a better temperature control and hydrodynamic condition required for skin permeation kinetics studies (24). This system was also used in the present investigations.

According to many reports published in the literature, the release profile of a drug from a matrix-type drug delivery system can be described by a linear Q vs. $t^{1/2}$ relationship (4, 7, 13, 23-27).

If the transdermal therapeutic system releases a drug at a rate which is substantially greater than the rate of percutaneous absorption, that means the permeation through the stratum corneum is the primary rate-limiting step in the course of transdermal controlled administration of drug; then the skin permeation profile of the drug should follow a constant zero-order kinetics (4), no matter what is the mechanism involved in the controlled release of drug from a therapeutic system (18).

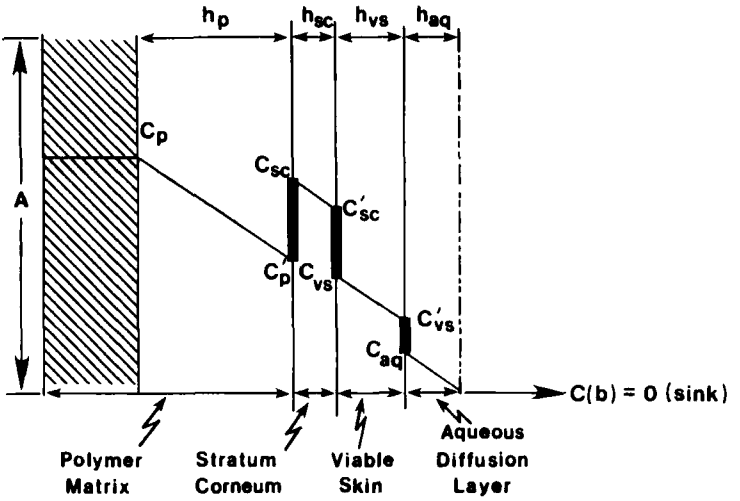
In the present study, a matrix diffusion-controlled drug delivery system was developed to release nitroglycerin at variable rates and was used to study its effect on the rate of skin permeation. A simple mathematical model was derived to establish the relationship among the rate of release, the drug loading dose, the rate of skin permeation and the drug concentration retained in the skin. Experiments were conducted to verify this relationship. The results are discussed in this report.

THEORETICAL ANALYSIS

A physical model of a matrix-type drug delivery system, applied onto the skin is depicted in Figure 1.

In the matrix-type drug delivery system, the drug solids are homogeneously dispersed as discrete crystals or solid particles in a matrix environment formed by the cross-linking of linear polymer chains (7, 9, 27). The dispersed particles or crystals cannot delocalize from their positions in the polymer matrix.

THEORETICAL MODEL FOR SKIN PERMEATION OF NITROGLYCERIN FROM A MATRIX DIFFUSION-CONTROLLED TRANSDERMAL DRUG DELIVERY SYSTEM



A = Drug loading in the polymer matrix
h = Thickness
C,C′ = Concentrations
p = Polymer
sc = Stratum corneum
vs = viable skin
aq = aqueous layer

Figure 1

A theoretical model for the release and the skin permeation of nitroglycerin from a matrix diffusion-controlled transdermal delivery system.

It is assumed that the drug molecules can elute out of the matrix only by dissolution in and then by diffusion through the polymer structure. It is visualized that, microscopically, the drug solids in the surface layer of the matrix, which is in intimate contact with the skin, are first to elute, and when this layer becomes exhausted the drug solids in the next layer begin to

be depleted (27). This is schematically illustrated in Figure 1. There exists, therefore, a drug depletion zone with a thickness of h_p. This thickness becomes greater and greater as more drug solids elute out of the device, leading to an inward advancement of the drug dispersion zone/drug depletion zone interface further into the core of the device.

For the purpose of mechanistical analysis of transdermal controlled drug administration, the skin tissues can be divided into stratum corneum, which is composed of dead, keratinized skin cells, and viable skin, which is considered to consist of viable cell layers of epidermis and dermis. Transport of drug molecules through these layers is believed to be by a consecutive diffusion process. Microscopically, there also exists a thin layer of stagnant solution, called hydrodynamic diffusion layer, on the interface between dermis and solution sink. In the clinical situation, this diffusion boundary layer can be visualized to exist around the capillary network in the dermal papillary layer. The thickness of this diffusion layer, h_{aq}, varies with the blood flow pattern or the fluid hydrodynamics in the solution. In the in-vitro situation, in which the skin is mounted on a skin permeation cell, this thickness can be consistently controlled at a finite value by controlling the angular rotation speed of the stirring magnet in the receptor solution (Figure 2). If this thickness is fixed at a finite constant value and is smaller than the surface area of the skin available for drug permeation, the diffusion of drug molecules to and from the skin surface

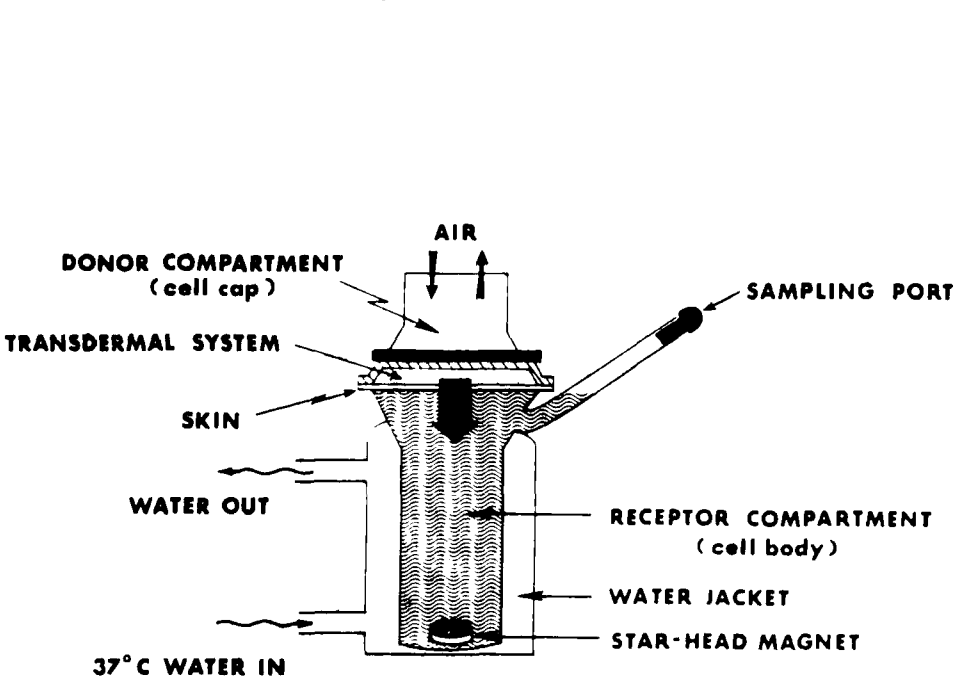


Figure 2

Schematic illustration of the newly developed Keshary-Chien (K-C) skin permeation cell. The cell consists of two compartments in vertical arrangement: a donor compartment, which is exposed to an ambient condition, and a receptor compartment which is maintained at 37°C by circulating a thermostated water through the waterjacket. The solution hydrodynamics in the receptor compartment is kept at constant by a Teflon-coated starhead magnet rotating at 600 rpm by a synchronous motor mounted directly underneath the cell mounting block.

may be treated, for simplicity, as one-dimensional diffusion to a plane surface (27, 28).

For the mechanistic analysis of controlled release of drug molecules from the matrix-type drug delivery system, the following assumptions are made:

1. The dissolution of drug crystals into their surrounding medium is the very first step of the drug release process.
2. A pseudo-steady state exists in the process of controlled release.
3. The diffusion coefficient of drug molecules in a given medium is constant.
4. The partition coefficient, K_1, for the interfacial partitioning of drug molecules from the device towards the stratum corneum is related to its solubility in the polymer, S_p, and in the stratum corneum, S_{sc}, i.e., $K_1 = \frac{S_{sc}}{S_p}$
5. The partition coefficient, K_2, for the interfacial partitioning of the drug molecule from the stratum corneum towards the underlying viable epidermis and dermis is related to its solubility in the stratum corneum, S_{sc}, and in the underlying viable tissue, S_{vs}, $K_2 = \frac{S_{vs}}{S_{sc}}$
6. The partition coefficient, K_3, for the interfacial partitioning of a drug molecule from the viable dermis towards the receptor (dermal) solution is related to its solubility in the viable dermis layer, S_{vs}, and in the receptor solution, S_{aq}, $K_3 = \frac{S_{aq}}{S_{vs}}$
7. Drug in the matrix is finely divided and homogeneously distributed such that matrix dissolution is not a rate-limiting factor. Drug molecules have a finite solubility in the matrix and the total concentration of drug per unit

volume including the undissolved drug is much greater than its finite solubility in the matrix (S_p).

8. A sharp boundary is maintained between the drug dispersion phase and the drug depletion region within the device which recedes into the device as time passes.
9. Drug reaches the matrix surface by diffusion process through the matrix continuum rather than the pores resulted from the dissolution of drug particles.
10. End diffusion is negligible.

Under these conditions, an activity gradient for the drug is established beginning at the receding boundary interface between drug dispersion phase and drug depletion region and is essentially terminating at the outer layer of the aqueous diffusion layer on the dermal side of the skin. This gradient is depicted in Figure 1 as a series of discontinuous concentration gradients from the delivery system to the dermal sink.

The fluxes in the polymer matrix (J_p), stratum corneum (J_{sc}), viable skin (J_{vs}) and the aqueous boundary layer (J_{aq}) are mathematically defined by the following expressions:

$$J_p = \frac{D_p}{h_p}(C_p - C'_p) = \frac{C_p - C'_p}{R_p} \quad (1)$$

$$J_{sc} = \frac{D_{sc}}{h_{sc}}(C_{sc} - C'_{sc}) = \frac{C_{sc} - C'_{sc}}{R_{sc}} \quad (2)$$

$$J_{vs} = \frac{D_{vs}}{h_{vs}}(C_{vs} - C'_{vs}) = \frac{C_{vs} - C'_{vs}}{R_{vs}} \quad (3)$$

$$J_{aq} = \frac{D_{aq}}{h_{aq}} (C_{aq} - C_b) = \frac{C_{aq}}{R_{aq}} \quad (\text{since } C_b \simeq 0) \tag{4}$$

Where J's refer to the fluxes of the drug molecule across various media (in $mg/cm^2/hr$), D's are the diffusivities of drug molecule in the media (in cm^2/hr), h's are the thicknesses of the media (in cm) and R's are the resistances to drug diffusion in the media (in hr/cm), respectively. The subscripts p, sc, vs and aq denote the polymer medium, stratum corneum, viable skin and aqueous hydrodynamic boundary layer, respectively.

Partition coefficients for various interfacial partitioning are defined by the following relationships:

Stratum corneum/polymer matrix:

$$K_1 = \frac{C_{sc}}{C'_p} \tag{5}$$

Viable skin/stratum corneum:

$$K_2 = \frac{C_{vs}}{C'_{sc}} \tag{6}$$

Aqueous layer/viable skin:

$$K_3 = \frac{C_{aq}}{C'_{vs}} \tag{7}$$

Where C and C' refer to the concentrations of drug molecule in a particular medium. Subscripts sc, p, vs and aq have been defined earlier.

Substituting Eq. (7) for C_{aq} in Eq. (4) gives

$$J_{aq} = C'_{vs} K_3 / R_{aq} \tag{8}$$

$$\text{or} \quad C'_{vs} = \frac{J_{aq} R_{aq}}{K_3} \tag{9}$$

Substituting Eq. (9) for C'_{vs} in Eq. (3) yields

$$J_{vs} = \frac{C_{vs} K_3 - J_{aq} R_{aq}}{K_3 R_{vs}} \tag{10}$$

Assuming that the continuity of flow across the series of barriers establishes a quasi-steady state:

$$J_p = J_{sc} = J_{vs} = J_{aq} = J_s \tag{11}$$

where J_s is the flux of drug permeation across the skin tissue (composite skin tissue layers)

Substituting Eq. (10) for J_{vs} in Eq. (11) and rearranging give:

$$J_s = \frac{C_{vs}}{R_{vs}} - \frac{J_s R_{aq}}{R_{vs} K_3} \tag{12}$$

$$\text{or} \quad J_s\left(1 + \frac{R_{aq}}{R_{vs} K_3}\right) = \frac{C_{vs}}{R_{vs}} \tag{13}$$

$$\text{or} \quad J_s = \frac{C_{vs}}{R_{vs}\left(1 + \dfrac{R_{aq}}{K_3 R_{vs}}\right)} \tag{14}$$

$$\text{or} \quad C_{vs} = J_s\left[R_{vs} + \frac{R_{aq}}{K_3}\right] \tag{15}$$

From Eq. (6),

$$C'_{sc} = \frac{C_{vs}}{K_2} \tag{6a}$$

Substituting Eq. (15) for C_{vs} in Eq. (6a) yields:

$$C'_{sc} = \frac{J_s}{K_2}\left[R_{vs} + \frac{R_{aq}}{K_3}\right] \tag{16}$$

Replacing Eq. (16) for C'_{sc} and Eq. (11) for J_{sc} in Eq. (2) gives:

$$J_s = [C_{sc} - \frac{J_s}{K_2}(R_{vs} + \frac{R_{aq}}{K_3})]\frac{1}{R_{sc}} \quad (17)$$

$$\text{or} \quad J_s\,[1 + \frac{R_{vs}K_3 + R_{aq}}{K_2K_3R_{sc}}] = \frac{C_{sc}}{R_{sc}} \quad (18)$$

$$\text{or} \quad C_{sc} = R_{sc}[J_s(1 + \frac{R_{vs}K_3 + R_{aq}}{K_2K_3R_{sc}})] \quad (19)$$

From Eq. (5)

$$C_p' = \frac{C_{sc}}{K_1} \quad (5a)$$

Substituting Eq. (19) for C_{sc} in Eq. (5a)

$$C_p' = \frac{R_{sc}}{K_1}[J_s\,(1 + \frac{R_{vs}K_3 + R_{aq}}{K_2K_3R_{sc}})] \quad (20)$$

Replacing Eq. (20) for C_p' and Eq. (11) for J_p in Eq. (1) yields:

$$J_s = \frac{1}{R_p}[C_p - \frac{R_{sc}}{K_1}\,J_s\,(1 + \frac{R_{vs}K_3 + R_{aq}}{K_2K_3R_{sc}})] \quad (21)$$

$$\text{or} \quad J_s\,[1 + \frac{R_{sc}}{K_1R_p}\,(1 + \frac{R_{vs}K_3 + R_{aq}}{K_2K_3R_{sc}})] = \frac{C_p}{R_p} \quad (22)$$

$$\text{or} \quad J_s = \frac{C_p}{R_p + \frac{R_{sc}}{K_1}(1 + \frac{R_{vs}K_3 + R_{aq}}{K_2K_3R_{sc}})} \quad (23)$$

The amount of drug, Q, released from polymer matrix to form a depletion zone with a thickness of h_p is given by:

$$Q = A_c h_p\,[A - \tfrac{1}{2}\,(C_p - C_p') - C_p'] \quad (24)$$

$$\text{or} \quad Q = A_c h_p\,[A - (\tfrac{1}{2}C_p + \tfrac{1}{2}\,C_p')] \quad (24a)$$

For a case where $A >> C_p$, $[A-(\frac{1}{2}C_p + \frac{1}{2}C_p')]$ can be approximated to A since $C_p > C_p'$ or $C_p > (\frac{1}{2}C_p + \frac{1}{2} C_p')$, so, Eq. (24a) is simplified to:

$$Q = A_c h_p . A \tag{25}$$

The rate of release is then defined by:

$$\frac{dQ}{dt} = \frac{d}{dt} (A_c h_p . A) \tag{26}$$

When the diffusion through stratum corneum is the rate-limiting step and a pseudo-steady state is established:

$$\frac{dQ}{dt} = 0 \tag{27}$$

or

$$\frac{dQ}{dt} = A_c . A . \frac{dh_p}{dt} = 0 \tag{28}$$

which implies that

$$A_c . A . h_p = \text{constant} = k \tag{29}$$

or

$$h_p = \frac{k}{A} \text{ (as } A_c \text{ is constant)} \tag{30}$$

Where k is a constant; The experimental evidence for the validity of Eq. (27) and (30) is provided in the Results and Discussion section later.

Since $R_p = h_p/D_p$ (31); substituting Eq. (30) for h_p in Eq. (31) and Eq. (31) for R_p in Eq. (23) gives:

$$J_s = \frac{\frac{C_p D_p}{\frac{R_{sc} D_p A}{K_1 k}(1 + \frac{R_{vs} K_3 + R_{aq}}{K_2 K_3 R_{sc}})} \; A/k}{} \tag{32}$$

or $J_s = \frac{\alpha \cdot A}{1 + \beta \cdot A}$ (33)

where α and β are composite constants and are defined as follows:

$$\alpha = C_p D_p / k \tag{34}$$

$$\text{and } \beta = \frac{R_{sc} D_p}{K_1 k} \left(1 + \frac{R_{vs} K_3 + R_{aq}}{K_2 K_3 R_{sc}}\right) \tag{35}$$

$$\text{or } \beta = \frac{\alpha R_{sc}}{K_1 C_p} \cdot \left(1 + \frac{R_{vs} K_3 + R_{aq}}{K_2 K_3 R_{sc}}\right) \tag{35a}$$

Eq. (33) suggests that the rate of skin permeation, J_s, should increase as increasing the drug loading in the polymer device, A, and a hyperbolic relationship should be observed as one plots J_s vs. A.

According to Higuchi (8) and Chien (14), the release of a drug, at steady state, from a matrix-type drug delivery system into a sink should follows the relationship of:

$$\frac{Q}{t^{1/2}} = [(2A - C_p)\, C_p \cdot D_p]^{1/2} \tag{36}$$

where Q is the cumulative amount of drug released from a matrix-type drug delivery system into the sink (in mg/cm^2); and t is the time elapsed. All other terms are the same as defined earlier.

$$(Q/t^{1/2})^2 = (2A - C_p)\, C_p D_p \tag{37}$$

$$\text{or } (Q/t^{1/2})^2 = 2AC_p D_p \qquad (\text{since } 2A >> C_p) \tag{38}$$

$$\text{or } A = (Q/t^{1/2})^2 / 2C_p D_p \tag{39}$$

Substituting Eq. (39) for A in Eq. (32),

$$J_s = \frac{(Q/t^{1/2})^2 / 2k}{1 + \frac{R_{sc}(Q/t^{1/2})^2}{2K_1 k C_p}\left[1 + \frac{R_{vs} K_3 + R_{aq}}{K_2 K_3 R_{sc}}\right]} \tag{40}$$

$$\text{or} \quad J_s = \frac{\alpha'(Q/t^{\frac{1}{2}})^2}{1 + \beta'(Q/t^{\frac{1}{2}})^2} \tag{41}$$

Where α' and β' are composite constants and defined as follows:

$$\alpha' = \frac{1}{2k} \tag{42}$$

and

$$\beta' = \frac{R_{sc}}{2K_1 k C_p}\left(1 + \frac{R_{vs}K_3 + R_{aq}}{K_2 K_3 R_{sc}}\right) \tag{43}$$

$$\text{or} \quad \beta' = \frac{\alpha' R_{sc}}{K_1 C_p} = \left(1 + \frac{R_v K_3 + R_{aq}}{K_2 K_3 R_{sc}}\right) \tag{43a}$$

All other terms are the same as defined earlier.

Equation (41) suggests that the rate of skin permeation, J_s, should increase as increasing the square of the *in vitro* release flux of drug in the sink, $(Q/t^{\frac{1}{2}})^2$, and a hyperbolic relationship should be obtained as one plots J_s vs. $(Q/t^{\frac{1}{2}})^2$.

EXPERIMENTAL

A. Materials:

1. Nitroglycerin: Pure nitroglycerin was extracted from its 10% lactose triturate[1] by dissolving the triturate in an excess 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[2].
2. Silicone elastomers - Dow Corning MDX4-4210 clean grade elastomer[3] and curing agent[3] were used to prepare polymer matrix.

3. Other chemicals and reagents - methanol[4] (glass-distilled HPLC grade), polyethylene glycol[5] (PEG) 400 and sodium chloride[5] were used as obtained. HPLC grade water was freshly prepared by Nanopure system[6].

B. Preparations:

Preparation of intact skin - Immediately following sacrifice by cervical dislocation of spinal cord, a portion (about 3.5cm x 3.5cm) of the full-thickness abdominal skin was carefully excised from the hairless mouse (5-7 week old, male). The dermal side of the skin was carefully cleaned of any adhering subcutaneous tissue and/or blood vessels.

Preparation of stripped (viable) skin - Immediately following sacrifice by cervical dislocation, the abdominal region of the hairless mouse skin was stripped with cellophane tape[7]. The mouse was secured on 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 pressure as uniform as possible (29). 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 for skin permeation studies as soon as possible to avoid the drying of this viable skin surface.

Preparation of elution solution - 20% w/w Polyethylene glycol 400 solution in normal saline was used as the dermal solution in the receptor compartment of the K-C skin permeation system[8].

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) as the solubilizer to maintain an effective sink condition required in the investigation.

Preparation of matrix-type nitroglycerin-releasing transdermal patch - Pure nitroglycerin was accurately weighed and homogeneously dispersed with an equal weight of lactose powder. Care was taken to avoid possible loss of nitroglycerin by evaporation. This nitroglycerin/lactose blend was mixed thoroughly with the required amount of MDX4-4210 elastomer. Curing agent for the elastomer was added to this mixture (0.1 part by weight of the MDX4-4210 elastomer). The mixture was mixed well for about 5 minutes by a laboratory stirrer[9] and was then deaerated under a vacuum of about 30 mm Hg for about 10 minutes with intermittant release of vacuum to break the entrapped air bubbles.

A plastic cup[10] (19.5mm in inner diameter and 5 mm in depth) was used as the moulding device and holder. The inside of the cup was lined with a 2" x 2" piece of aluminum foil[11] to prevent a direct contact of silicone device with the plastic cup and, therefore, any possible diffusion of nitroglycerin from the device to the plastic material. About 1.4 gm of the deaerated blend of nitroglycerin/elastomer was accurately weighed into the aluminum foil-lined plastic cup holder. After covering the holder with a 1" x 1" piece of aluminum

foil[11], the nitroglycerin/elastomer blend was then cured at 75°C for 30 minutes.

After curing, the cured discs were removed from the oven, the aluminum foil was discarded and the edge of each disc was carefully trimmed to produce a transdermal disc with a fixed surface area. These discs were later used in the skin permeation and release studies.

C. Skin Permeation Profile of Nitroglycerin from Transdermal Discs

1. Intact skin - The freshly excised full-thickness skin sample (contains stratum corneum) was mounted on the receptor compartment of the K-C cell[8], with the stratum corneum side facing upwards into the donor compartment and the dermal side facing downwards into the receptor compartment. One unit of the transdermal disc was placed with the nitroglycerin-releasing surface in intimate contact with the skin and the donor cap was positioned and clamped.

Following the application of transdermal discs on the skin, the receptor solution containing 20% w/w polyethylene glycol 400 in normal saline was introduced into the receptor compartment, which was thermostated at 37°C by a circulating waterbath[12], while the donor compartment was maintained at the ambient temperature of 25±1°C.

At a predetermined time interval, 1 ml of sample from the receptor solution was withdrawn and replaced immediately with a same volume of the fresh, drug-free PEG 400/saline solution

to keep the volume in the receptor compartment constant and to ensure a good contact between the dermal side of the skin and the receptor solution. The nitroglycerin concentration in the sample was then determined by a sensitive HPLC method. The amount of nitroglycerin retained in the skin was also determined at the end of each experiment (24 hours) by a method described later.

2. Viable skin - The same procedure as outlined above for intact skin was used, except that the stripped skin was used and at a scheduled time interval, the entire volume of the receptor solution was sampled and renewed with the same volume of drug-free PEG/saline solution.

D. Release Profiles of Nitroglycerin from the Transdermal Discs

The same procedure as outlined above for skin permeation studies was also applied, except that no skin sample was sandwiched between the transdermal disc and the receptor solution and at each scheduled sampling time, the receptor solution was completely withdrawn and replaced with fresh drug-free PEG/saline solution. In this way the required sink condition was maintained on the dermis side. The nitroglycerin concentration in the samples was then assayed by the HPLC method.

E. Determination of Nitroglycerin in the Skin

After the skin surface was wiped dry with a single ply of folded kimwipe paper[13], the area of skin which was in intimate contact with the drug-releasing surface of the transdermal disc 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[14], in 10 ml of methanol. During homogenization, the sample temperature was maintained cold by surrounding it in crushed ice to prevent any possible loss of nitroglycerin due to the heat generated. Homogenized skin samples were then centrifuged at about 2,500 rpm[15] for 5 minutes. The clear supernatant was separated and assayed for nitroglycerin content by HPLC.

F. Analytical Methods

For this investigation, a microprocessor-controlled high performance liquid chromatograph[16] was used. It is equipped with a variable wavelength detector, an automatic sampler, a variable-volume injector, a dual-head reciprocating pump and a dual solvent system. Using methanol and water (at a composition of 60/40) as the 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[17] and detected at a wavelength of 205 nm.

Under the HPLC conditions outlined above, nitroglycerin produced a sharp 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 times of 3.1 and 2.9 min., respectively. This stability-indicating method has a detection sensitivity of 75-100 ng/ml for nitroglycerin.

G. Data Analysis

From the concentration profiles of nitroglycerin in the receptor solution, flux (in mg/cm^2) was calculated using a computer program (in Appendix) 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 cm^3 of 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 [30].

RESULTS AND DISCUSSION

Skin permeation profile of nitroglycerin across intact skin

The skin permeation profiles of nitroglycerin from the transdermal discs containing various loading doses of nitroglycerin are shown in Figure 3. Results indicate that nitroglycerin permeates through the intact hairless mouse abdominal skin at constant rate (linear Q vs. t relationship).

The observed constant rate of skin permeation could be the result of the fact that the thickness of drug depletion zone formed during the 24-hr skin permeation does not add substantially to the total thickness of the diffusional path (i.e., drug depletion zone + skin + hydrodynamic diffusion layer). From the total amount of nitroglycerin released during the 24-hr skin permeation studies, the thickness of drug depletion zone formed

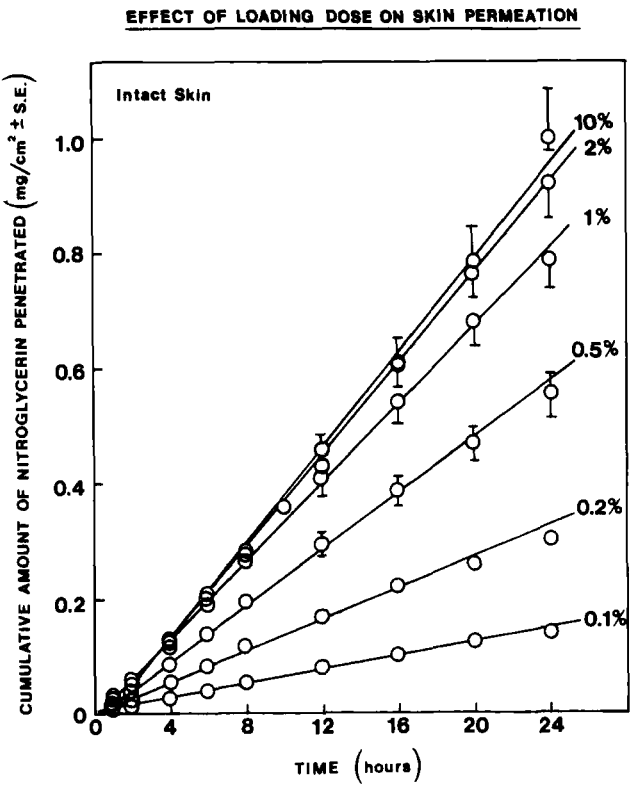


Figure 3

Time course for the 24-hr permeation of nitroglycerin across the intact skin from the matrix diffusion-controlled transdermal delivery system containing various loading doses (% w/w). A constant (Q vs. t) skin permeation profile was obtained. The rate of skin permeation increased in proportional to the increase in drug loading up to a loading dose of about 2%. No significant increase in skin permeation rate was observed as the loading dose increased beyond the 2% level. Each data point represents the mean value ± standard error of 4 determinations.

can be determined. The results (Table I) indicated that depending upon the drug loading (A) in the transdermal discs, a thickness of drug depletion zone, ranging from 0.11 to 1.54 mm, is formed after skin permeation studies for 24 hours. This range of drug depletion zone thickness accounted for 2.2 ~ 30.8% of the total

Table I - Effect of Drug Loading (A) on the Formation of Drug Depletion Zone (h_p) in the Polymer Matrix

$\frac{2A}{(mg/cm^3)}$	$2A/C_p$ [1)]	h_p at 24 hrs (mm)	h_p/matrix thickness [2)]
2.84	0.85	1.54	30.8%
6.76	2.02	1.71	34.2%
9.82	2.94	1.24	24.8%
12.84	3.82	1.28	25.6%
18.20	5.42	1.00	20.0%
22.88	6.84	0.85	17.0%
34.40	10.34	0.63	12.6%
43.90	13.10	0.54	10.8%
67.90	20.26	0.34	6.8%
112.26	33.52	0.20	4.0%
222.28	66.34	0.11	2.2%

1) $C_p = 3.35\ mg/cm^3$

2) Thickness of polymer matrix = 5 mm

thickness of the polymeric matrix and was, as expected from Equation (30), a function of the reciprocal of drug loading. The larger the drug loading, the smaller the thickness of depletion zone. Analysis of the data suggested that for a disc containing a loading dose greater than 22 mg/cm^3, i.e. $2A > 43.9$ mg/cm^3 in Table I, the ratio of drug loading to polymer solubility ($2A/C_p$) is maintained at a level greater than 13.10, i.e., $2A > 13.1\ C_p$ or $2A >> C_p$, a thickness of only 10% (or less) of the total matrix thickness is formed as a result of drug release for skin permeation during the 24-hr period. So, a pseudo-steady-state (Equation 27) was established and a constant apparent rate of skin permeation was obtained (Figure 3).

The permeation rate of nitroglycerin through intact skin as indicated by the magnitude of the slope (Q/t), was observed to increase steadily as increasing the loading of nitroglycerin in the silicone disc from 0.1% to 2% (Figure 3). Skin permeation rate appeared to reach a plateau level at the loading dose of 2%, beyond which any further increase in the loading dose only resulted in an insignificant increase in the skin permeation rate. When the skin permeation rate ($mcg/cm^2/hr$) was plotted against the loading dose (Figure 4), a hyperbolic relationship was resulted as predicted from the Equation 33.

By plotting the experimental data on the skin permeation rate at various loading doses by $(J_s)^{-1}$ vs. $(A)^{-1}$ relation, the values of the constants α and β in Equation (33) can be determined.

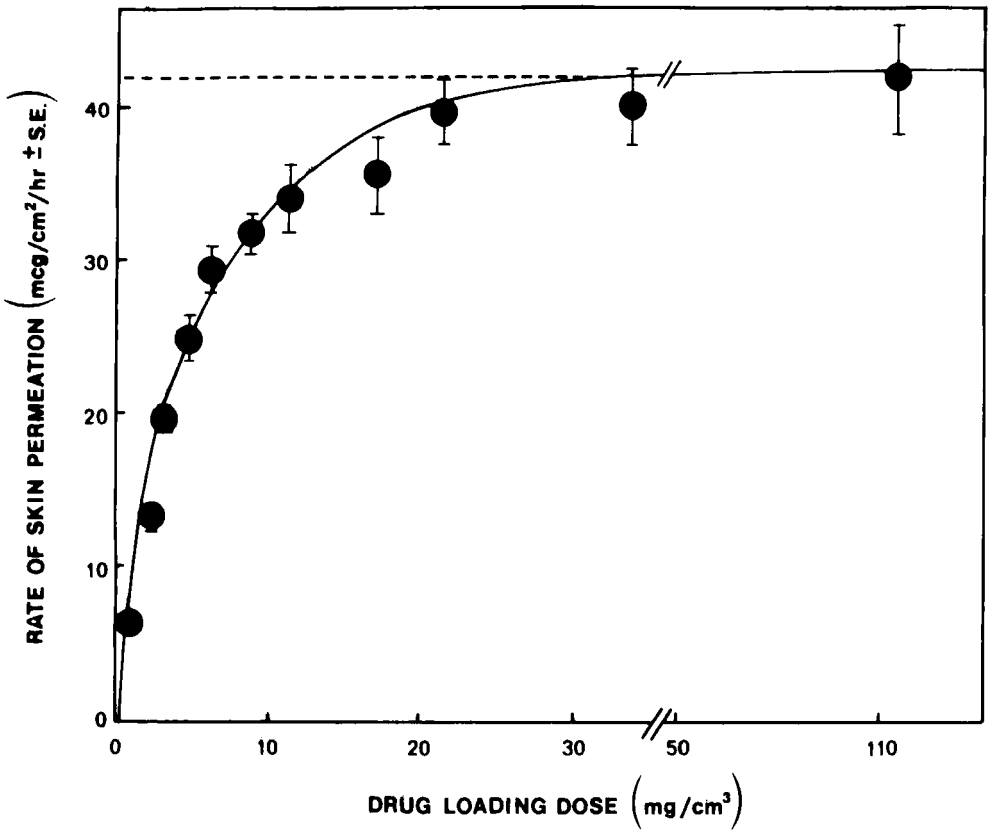


Figure 4

Effect of drug loading dose on the permeation rate of nitroglycerin across the intact skin. As expected from Equation (33), a hyperbolic relationship was observed between the skin permeation rate and the drug loading. Each data point represents the mean value ± standard error of 4 determinations.

The slope of the linear $(J_s)^{-1}$ vs. $(A)^{-1}$ plot gives the value of $1/\alpha$ and the intercept gives the value of β/α. From the slope and the intercept, the values of α and β were calculated to be 0.0145 and 0.3333, respectively. The hyperbolic relationship between the permeation rate of nitroglycerin (J_s) across the intact skin of male hairless mouse and the loading dose (A) of

nitroglycerin in the matrix diffusion-type drug delivery system can, therefore, be quantitated by:

$$J_s = \frac{0.0145A}{1 + 0.3333A} \quad (44)$$

Furthermore, it was noted that initially the concentration of nitroglycerin retained by the intact skin also increased steadily with the increase in the loading dose of nitroglycerin in the polymer matrix and finally attained a steady-state level of about 1.83 mg per unit volume of skin tissue at high loading doses.

Skin permeation profiles of nitroglycerin through viable skin

The results are shown in Figure 5. It was noted that the skin permeation of nitroglycerin through the viable skin followed a linear Q vs. $t^{1/2}$ relationship, which is different from the Q vs. t linearity observed earlier for intact skin. The skin permeation flux ($Q/t^{1/2}$) through the viable skin, as indicated by the magnitude of the slope, was also noted to increase steadily as increasing the loading dose of nitroglycerin in the silicone disc from 0.1% to 5%. Skin permeation flux appeared to reach a plateau level at the loading dose of 5%, beyond which any further increase in the loading dose only resulted in an insignificant increase in the skin permeation flux. The increase in skin permeation flux ($Q/t^{1/2}$) was found to be linearly dependent upon the $(2A\text{-}Cp)^{1/2}$ for up to a $(2A\text{-}C_p)^{1/2}$ value of 8 $mg^{1/2}/cm^{3/2}$ (Figure 6), indicating that the permeation across the viable skin is controlled by the release mechanism from the polymer matrix.

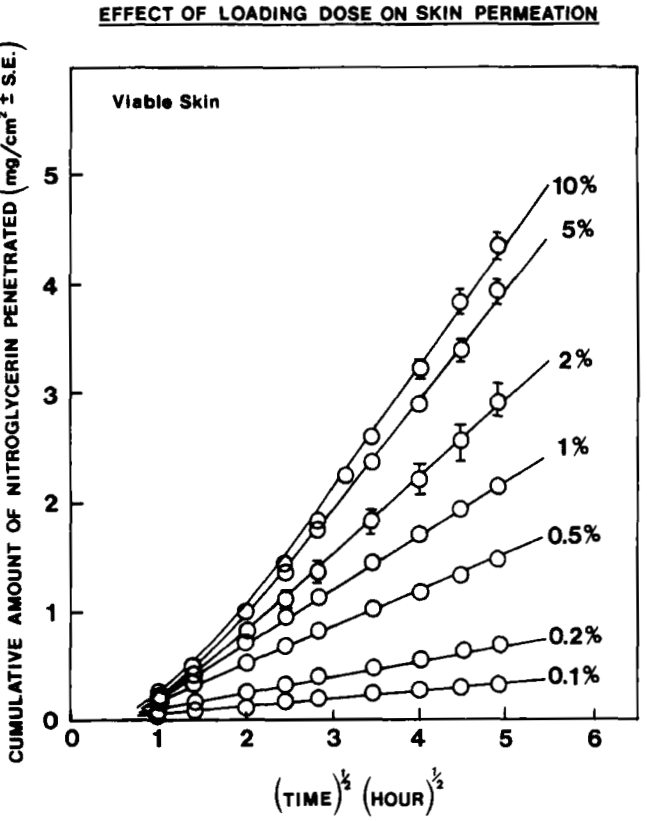


Figure 5

Time course for the 24-hr permeation of nitroglycerin across the viable skin from the matrix diffusion-controlled transdermal delivery system containing various loading doses (% w/w). The permeation profiles of nitroglycerin was found to follow a linear Q vs. $t^{\frac{1}{2}}$ relationship, suggesting that by removing the stratum corneum, the rate-limiting step shifts from the stratum corneum to the drug delivery system. Now, the permeation across the viable skin is controlled by the release mechanism of nitroglycerin from the polymer matrix. Each data point represents the mean value ± standard error of 4 determinations.

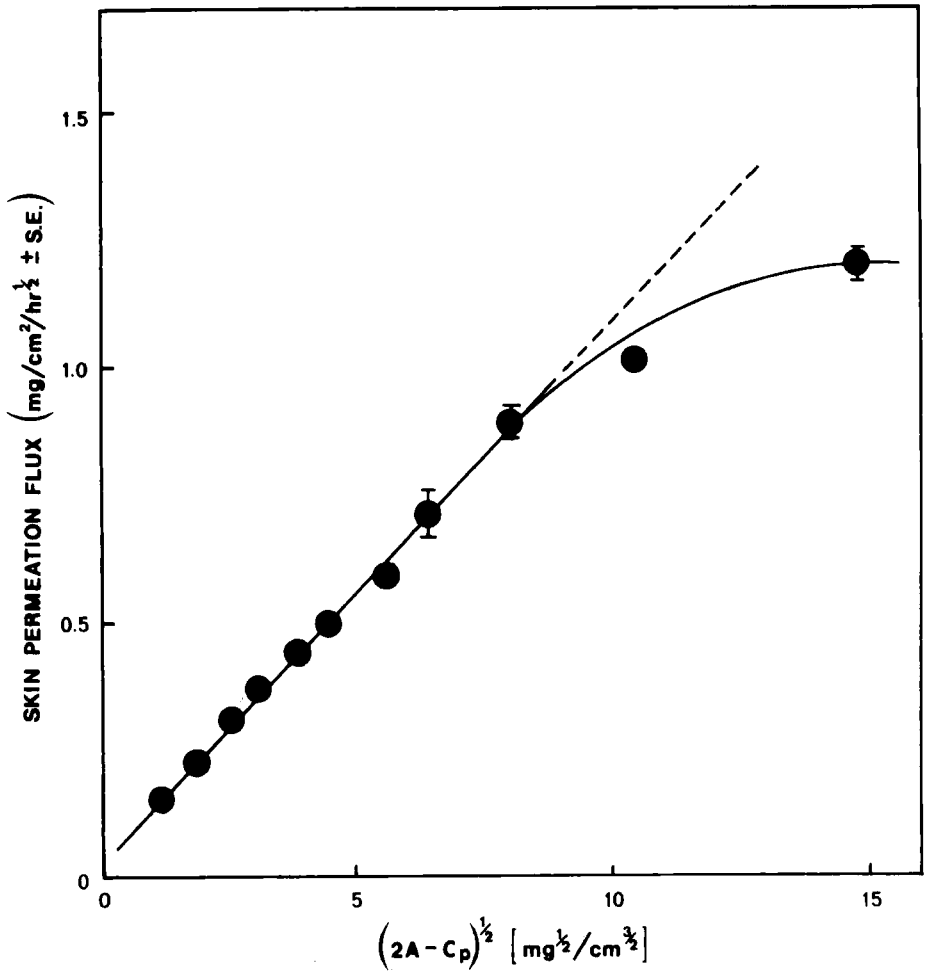


Figure 6

Dependence of the permeation flux of nitroglycerin across the viable skin on $(2A-Cp)^{1/2}$, a relation similar to Equation (36).

It was observed that the concentration of nitroglycerin in the viable skin also increased steadily with the increase in loading doses and finally reached a plateau level, also at 1.8 mg per unit volume of the skin tissue.

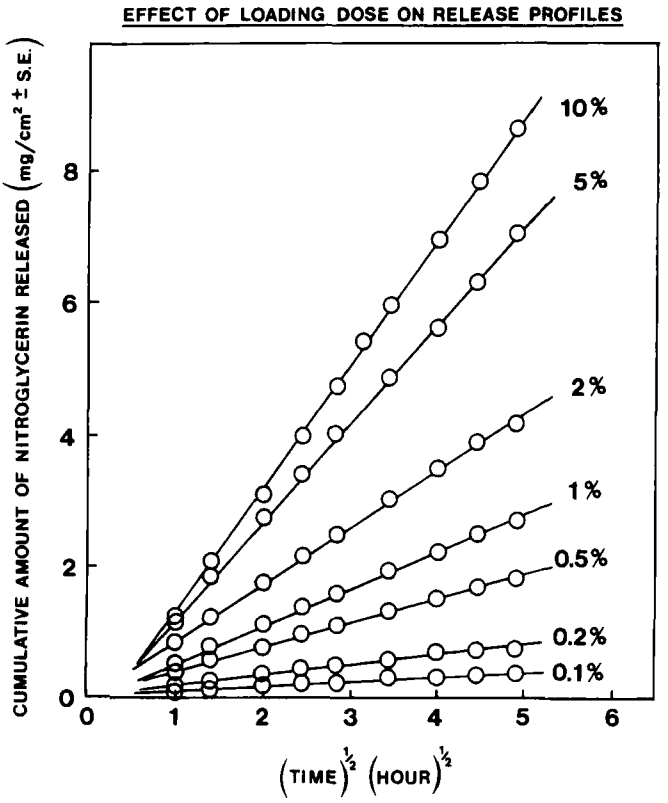


Figure 7

Time course for the 24-hr release profiles of nitroglycerin from the matrix diffusion-controlled transdermal delivery system containing various loading doses. The drug release flux ($Q/t^{\frac{1}{2}}$) increased as increasing the drug loading in the polymer matrix. Each data point represents the mean value ± standard error of 4 determinations.

Release profiles of nitroglycerin from the silicone disc

As expected from Equation (36), the release profile of nitroglycerin from the silicone discs followed a Q vs. $t^{\frac{1}{2}}$ profile (Figure 7). The release flux ($Q/t^{\frac{1}{2}}$) of nitroglycerin was observed to increase in proportional to the increase in the loading dose of nitroglycerin. A plot of drug release flux ($Q/t^{\frac{1}{2}}$) vs. square

root of (2A-Cp) yielded a straight line (Figure 8), a characteristic drug release profile of matrix-type drug delivery system.

From the linear $Q/t^{\frac{1}{2}}$ vs. $(2A-Cp)^{\frac{1}{2}}$ plot, a slope, which is $(CpDp)^{\frac{1}{2}}$, of 0.1339 $mg^{\frac{1}{2}}/cm^{\frac{1}{2}}/hr^{\frac{1}{2}}$ was obtained. Nitroglycerin was determined to have a Cp value of 3.35 mg/cm^3 in MDX4-4210 polymer, so Dp was calculated to be $5.35 \times 10^{-3} cm^2/hr$.

When the permeation rate of nitroglycerin (Q/t) across the intact skin was plotted against the square of the release flux $(Q/t^{\frac{1}{2}})$ of nitroglycerin from the polymer matrix, a hyperbolic relationship was established (Figure 9) as predicted theoretically from the Equation 41.

The values of the constants α' and β' in Equation (41) could be determined by plotting the inverse of skin permeation rate $(J_s)^{-1}$ against the inverse of the square of drug release flux $(Q/t^{\frac{1}{2}})^{-2}$. The slope of such plot should give the value of $1/\alpha'$ and the intercept gives the value of β'/α'. From the slope and the intercept, the values of α' and β' were calculated to be 0.5 and 11.63, respectively. The hyperbolic relationship observed between the permeation rate of nitroglycerin (J_s) across the intact skin of male hairless mouse and the square of release flux $(Q/t^{\frac{1}{2}})^2$ of nitroglycerin from the matrix diffusion-controlled drug delivery system can then be quantitated by:

$$J_s = \frac{0.5(Q/t^{\frac{1}{2}})^2}{1 + 11.63\ (Q/t^{\frac{1}{2}})^2} \qquad (45)$$

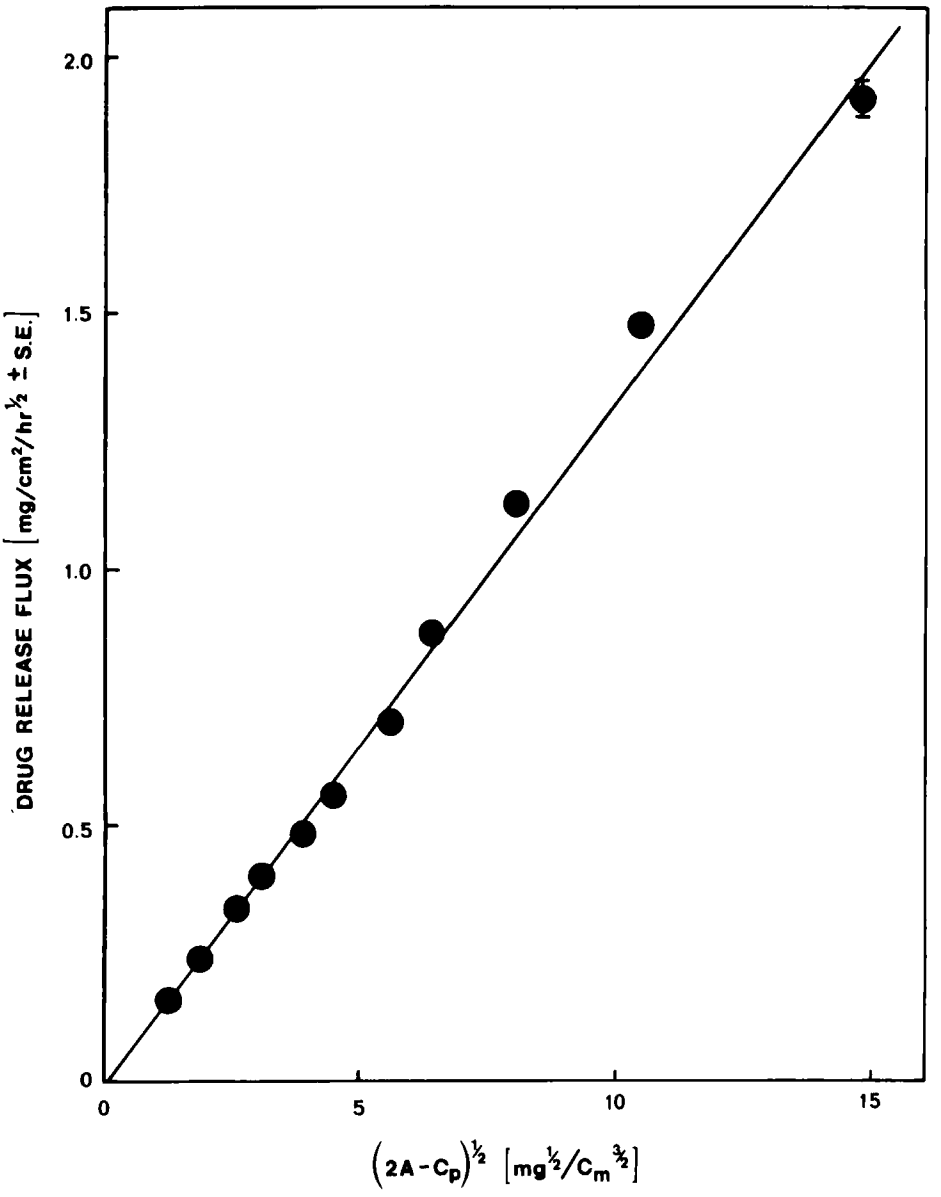


Figure 8

Linear relationship between the drug release flux from the polymer matrix, $Q/t^{1/2}$ ($mg/cm^2/hr^{1/2}$) and $(2A-Cp)^{1/2}$ as expected from Equation 36. From the slope, a value of 13.389×10^{-2} $mg^{1/2}/cm^{1/2}/hr^{1/2}$ was determined for $(DpCp)^{1/2}$. Since Cp was determined to be 3.35 mg/cm^3, so Dp was calculated to be 5.35×10^{-3} cm^2/hr or 1.4865×10^{-6} cm^2/sec. Each data point represents the mean value ± standard error of 4 determinations.

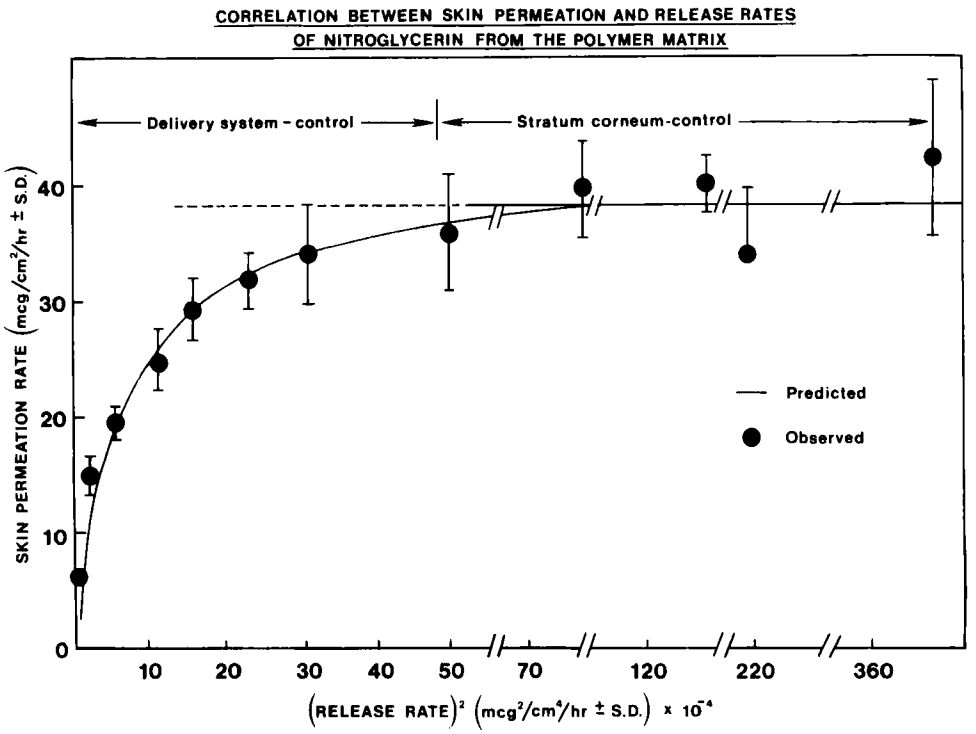


Figure 9

The hyperbolic relationship between the permeation rate across the intact skin and the square of the release flux of nitroglycerin delivered by the matrix diffusion-controlled drug delivery system, as prediced from Equation 41. It was observed that when $(Q/t^{1/2})^2$ value is equal to or less than 48 $mcg^2/cm^4/hr$, the skin permeation rate of nitroglycerin is controlled by the delivery system; when the $(Q/t^{1/2})^2$ value is greater than 48 $mcg^2/cm^4/hr$ the skin permeation rate becomes limited by the stratum corneum. Each data point represents the mean value ± standard error of 4 determinations.

To check the validity of Equation 45, experimental values of $Q/t^{1/2}$ were substituted into the equation to calculate the J_s values predicted; and the predicted J_s values were then compared with the experimental J_s values. The results in Figure 9 indicated that all the experimental data points of J_s fall onto the theoretically predicted line constructed from the calculated

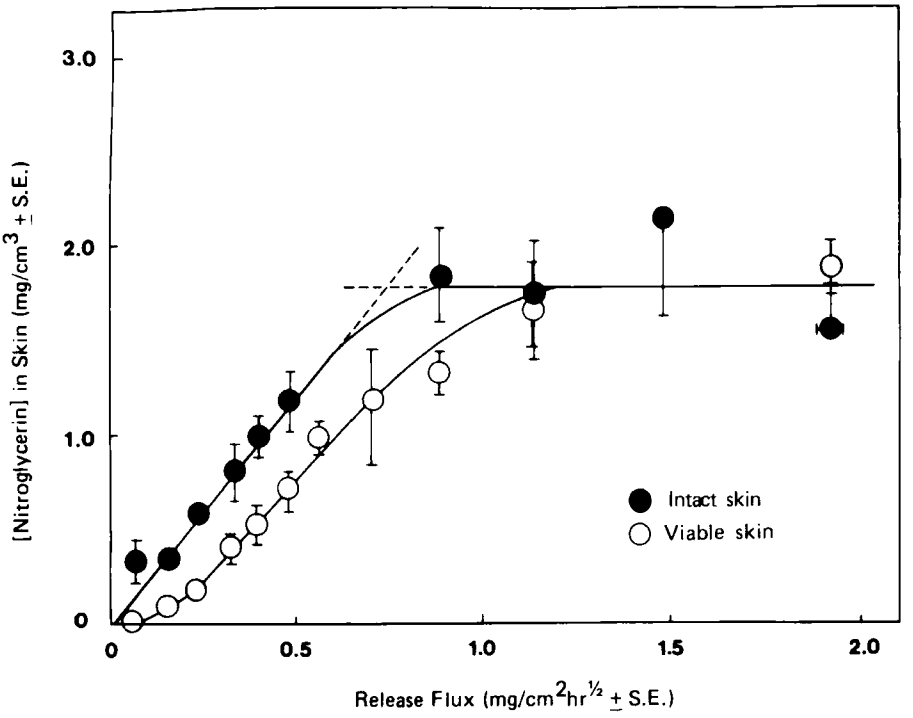


Figure 10

Relationship between the equilibrium nitroglycerin concentration in the skin and the release flux of nitroglycerin from the polymer disc. A plateau skin concentration, which suggests the attainment of saturation concentration of nitroglycerin in the skin, was reached for both intact skin and viable skin when the release rate is greater than 0.75 mg/cm^2/hr$^{1/2}$ for intact skin or 0.95 mg/cm^2/hr$^{1/2}$ for viable skin. Each data point represents the mean value ± standard error of 4 determinations.

J_s values. The results varify the validity of Equations (41) and (45).

Correlation between the skin permeation rate of nitroglycerin and the concentration in the skin

The equilibrium concentration of nitroglycerin in both the intact and the viable skin was observed to increase linearly

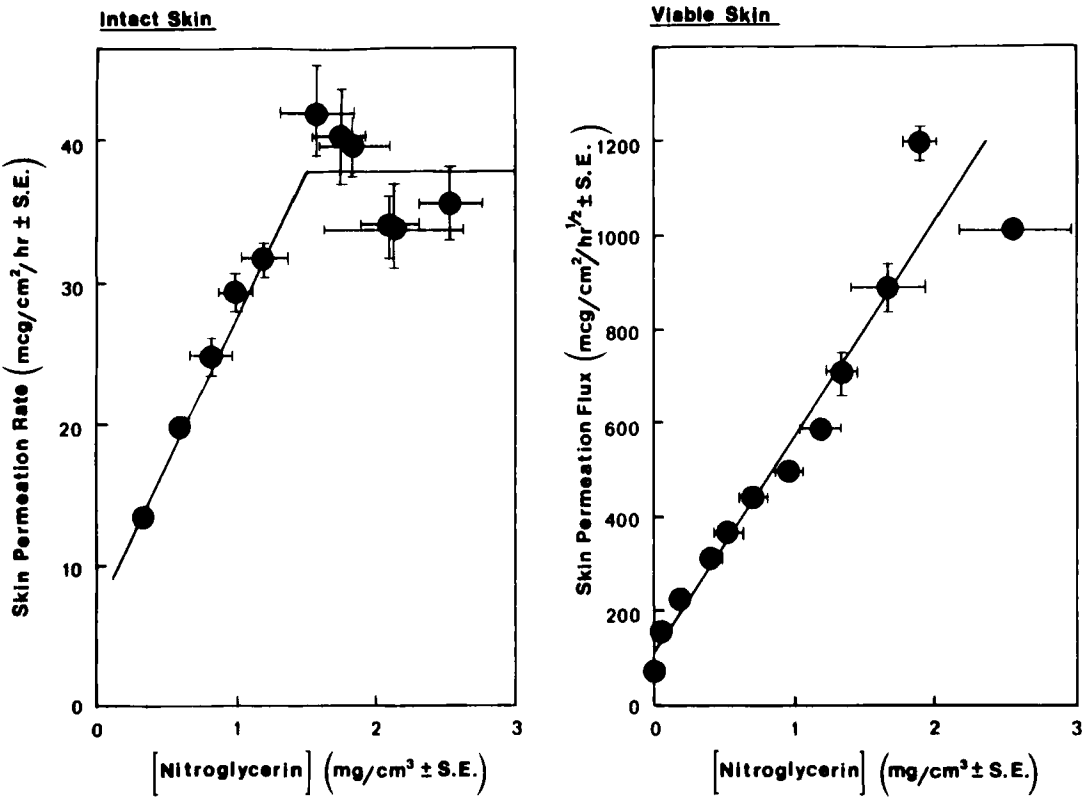


Figure 11

Dependency of the permeation rate across the intact or viable skin on the equilibrium concentration of nitroglycerin in the skin. Each data point represents the mean value ± standard error of 4 determinations.

with the increase in the release flux of nitroglycerin from the silicone discs (Figure 10). This attained a plateau level, beyond which any increase in the release flux did not produce any significant increase in the skin concentration of nitroglycerin. The observation suggests the attainment of the saturated concentration of nitroglycerin in the skin.

Skin permeation rates were found to be dependent upon the equilibrium concentration of nitroglycerin in the intact skin as well as in the viable skin (Figure 11). The rate of permeation across the intact skin was observed to increase as increasing the equilibrium skin concentration of nitroglycerin and a plateau value was reached once the concentration of nitroglycerin in the skin tissue attained a level of around 1.5 mg/cm^3, while the flux of permeation across the viable skin continued to increase even after the saturated nitroglycerin concentration was reached at around 1.8 mg/cm^3.

ACKNOWLEDGMENT

*This series of research articles have been extracted from the thesis submitted by P. R. Keshary to the Graduate School of Rutgers - The State University of New Jersey in partial fulfillment of the requirements for the Degree of Doctor of Philosophy in Pharmaceutical Sciences with specialization in Controlled Drug Release Technology.

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The authors wish to thank Ms. M. Boslet for manuscript preparation.

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FOOTNOTES

1) ICI America, Wilmington, Delaware
2) United States Pharmacopeia XX, page 552.
3) Dow Corning Corp. Midland, Michigan
4) Burdick & Jackson Lab, Inc., Muskagon, Michigan
5) Fisher Scientific Co., Fairlawn, New Jersey
6) Sybron/Barnstead, Boston, Massachusetts
7) 3M Corp., St. Paul, Minnesota
8) Custom fabricated at a local glassblower shop
9) Lab stirrer model 4380-00, Cole-Palmer, Chicago, Illinois
10) Red Dot Electrode (Product #2259 T), 3M, St. Paul, Minnesota
11) Reynold's aluminum household wrap, Reynolds Metals Co., Richmond, Virginia
12) Model #80, Fisher Scientific Co., Fairlawn, New Jersey
13) Kimberly-Clark Corp., Roswell, Georgia
14) Brinkman Instruments, Westbury, New York
15) "Damon", Model CU-5000, International Equipment Co., Needham Heights, Massachusetts
16) HP Model 1084B HPLC, Hewlett-Packard, Palo Alto, California
17) Zorbax C-8 column, Dupont & Co., Wilmington, Delaware

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