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IMPROVEMENT IN TRANSDERMAL BIOAVAILABILITY OF NITROGLYCERIN BY FORMULATION DESIGN

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ABSTRACT

Controlled skin permeation kinetics of nitroglycerin delivered by the three once-a-day transdermal therapeutic systems was recently evaluated and compared using the freshly excised hairless mouse abdominal skin mounted in a finite-dosing Frantz diffusion cell assembly. The kinetics of skin permeation from the conventional ointment formulation was also studied using the same *in vitro* skin permeation system.

Six experimental formulations of nitroglycerin were developed, aiming to enhance the permeation rate of nitroglycerin through intact skin. The kinetics of skin permeation of nitroglycerin from these experimental formulations was also investigated, using the same *in vitro* skin permeation system, and compared to the conventional ointment formulation as well as

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the newly marketed once-a-day transdermal therapeutic systems. Results indicated that the rates of skin permeation can be greatly improved by proper formulation design.

INTRODUCTION

Nitroglycerin has been an important drug in the treatment or prevention of anginal pectoris for several decades (1). Several types of pharmaceutical dosage forms of nitroglycerin, mainly the oral, topical and intravenous infusion fluid are commercially available in the prescription market. Among these dosage forms, the topical nitroglycerin ointment is most widely used in prophylactic angina therapy. Effective blood levels can be maintained for 4-8 hours after each topical application of ointment. Unfortunately, the ointment formulation is greasy and a protective wrapping is required to prevent staining. In addition, reports indicated that the ointment must be carefully and consistently applied over a large surface area for maintenance of clinical efficacy (2).

Recent development of the "once-a-day" transdermal nitroglycerin delivery systems, namely Nitrodisc system (Searle Pharmaceuticals, Inc., Skokie, Illinois), Nitro-Dur system (Key Pharmaceutical, Inc., Miami, Florida), Transderm-Nitro system (Ciba Pharmaceutical Corporation, Summit, New Jersey) and Deponit system (Pharm-Schwartz GmbH, Monheim, Germany), provided a better control in both the surface area of medication and the rate of release of nitroglycerin to the skin for at least 24 hours. Although the surface-area-normalized-plasma levels achieved by these transdermal patches were comparable with that after 2% ointment application (3,4), the actual plasma level obtained following the application of a unit of transdermal patch was generally less than that following the usual dose (1-2 inches) of ointment, which can be spread over a much larger skin area (5). For refractory patients, multiple patches may therefore be required to achieve the plasma nitroglycerin level needed.

In our previous report (6), we have evaluated these four transdermal therapeutic systems and the ointment formulation using an *in vitro* skin

permeation system. The results indicated that, although the stratum corneum plays an important barrier effect against the skin permeation of nitroglycerin, the formulation design may also play some roles in the transdermal controlled administration of nitroglycerin.

Our objectives are: (1) to develop an alternate formulation, which would be more aesthetically acceptable than the greasy ointment and could attain a higher plasma level with a small skin area of application; and (2) to develop a transdermal therapeutic system which would represent greater improvements over the currently marketed products in terms of size and plasma level achievable. Six experimental formulations were developed and their skin permeation profiles were evaluated using Frantz diffusion cells and hairless mouse abdominal skin. The results are discussed in this report.

EXPERIMENTAL

Materials:

1. Chemicals and Reagents
   a. Nitroglycerin -- Pure nitroglycerin was extracted from its 10% lactose triturate (ICI, Wilmington, Delaware) by dissolving the triturate in excess of distilled water and then collecting the oily nitroglycerin settlement at the bottom. The purity of the extracted nitroglycerin was determined by phenoldisulphonic acid method (7).
   b. Polyethylene glycol (PEG) 400 and sodium chloride were purchased from Fisher Scientific Co., Fairlawn, New Jersey.
   c. Methanol, HPLC grade, was purchased from Burdick and Jackson Lab. Inc., Muskagon, Michigan.
   d. Water was freshly prepared by "Nanopure" system from Sybron/Barnstead, Boston, Massachusetts.
2. Nitroglycerin formulations

   The following nitroglycerin formulations were prepared:

   a. 2% Nitroglycerin ointment - A lanolin-petrolatum ointment containing 2% nitroglycerin.

b. 2% Nitroglycerin creams - A water-washable o/w cream containing 2% nitroglycerin. The oil phase comprised approximately 45% of the formulation. Formulation A (Lot #W1273-53) contains 1.6% of water-soluble polymer and Formulation B (Lot #W1273-54) contains no polymer.

c. Nitroglycerin-film forming solutions - These solutions contained 2.8% nitroglycerin and an ethanol-soluble polymer. A mixture of glycerin, PEG 75 lanolin oil and a branched-chain ester was used as the plasticizer. Formulation C (Lot #W1273-50) contains 5% polymer and Formulation D (Lot #W1273-51) contains 10% polymer.

Preparations:

1. Preparation of nitroglycerin-impregnated microporous discs:

Saturated nitroglycerin solutions were prepared by suspending excess amount of nitroglycerin/lactose triturate in fatty acid esters. After overnight equilibrium at room temperature, the suspensions were filtered and assayed. Discs measuring 10 cm^2 were cut from sheets of either microporous polyethylene (Porex membrane, pore size of 10 μm) or microporous polypropylene (Accurel membrane, pore size of 1 μm). The discs were soaked in the saturated nitroglycerin solution for one hour. Each disc was weighed before and after soaking to determine the exact amount of nitroglycerin solution absorbed: (i) Porex membrane discs contain a saturated nitroglycerin solution in ethyl hexyl palmitate with a mean nitroglycerin content of 15.8 ± 0.1 mg (SD) in each disc. (ii) Accurel membrane discs contain a saturated nitroglycerin solution in octyl hydroxy stearate with a mean nitroglycerin content of 11.3 ± 0.4 mg (SD) in each disc.

2. Preparation of nitroglycerin-releasing transdermal film patch:

An aluminum plate (11.5 cm x 9.0 cm x 0.1 cm) with a circular hole (19 mm in diameter) at the center was used in this preparation. A piece (5 cm x 5 cm) of household aluminum foil was placed over the plate and a glass plunger (18 mm in diameter) was used to press down the aluminum foil slowly into the hole to form an aluminum petri dish

with a circular depression of a fixed diameter. This aluminum petri dish was used as a mold for the preparation of transdermal film. An aliquot (0.25 ml) of nitroglycerin film-forming solutions (Formulations C & D) was accurately pipetted and slowly delivered into the depression. The solution was dried to form a film, under a continuous stream of dry nitrogen, after each application. The process was continued until a constant weight of the dried film was achieved. After complete drying, the film turned into a milky white patch. This aluminum petri dish with the film patch on it was then stretched very gently to produce a smooth depressionless foil with a circular film patch residing at the center.

3. Preparation of ointment or cream patch:

The aluminum plate used in the preparation of the film patches was also employed as a template to prepare the ointment or cream patch of a fixed thickness and constant surface area. The patch was prepared by first placing the plate on a piece of aluminum foil (5 cm x 5 cm) then filling up the circular hole with ointment or cream. The aluminum plate was then lifted straight up from the aluminum foil, leaving a circular ointment or cream patch of 19 mm in diameter and 1 mm in thickness on the backing aluminum foil.

4. Preparation of skin sample:

A piece (3.5 cm x 3.5 cm) of full-thickness abdominal skin was obtained from hairless mouse as described earlier (8). The dermal side of the skin was carefully cleaned to remove any adhering subcutaneous tissue and/or blood vessels.

5. Preparation of elution solution:

Normal saline containing 20% w/w PEG 400 was used as the dermal solution in the receptor compartment. The aqueous solubility of nitroglycerin was enhanced substantially by the incorporation of PEG 400 in normal saline (8); so, the sink conditions required were maintained throughout the skin permeation studies.

Skin Permeation Studies:

The full-thickness skin prepared as outlined above was mounted on the receptor compartment of the Frantz diffusion cell (Figure 1), with the stratum corneum side facing upward and the dermal side facing downward into the receptor compartment. After overnight (15 hours) soaking of dermal side of the skin in the elution solution (which was withdrawn completely and served as the time zero sample), the nitroglycerin patch prepared above was then applied over the stratum corneum surface of the skin. The donor compartment cap was then placed on the top and the whole assembly was securely clamped together.

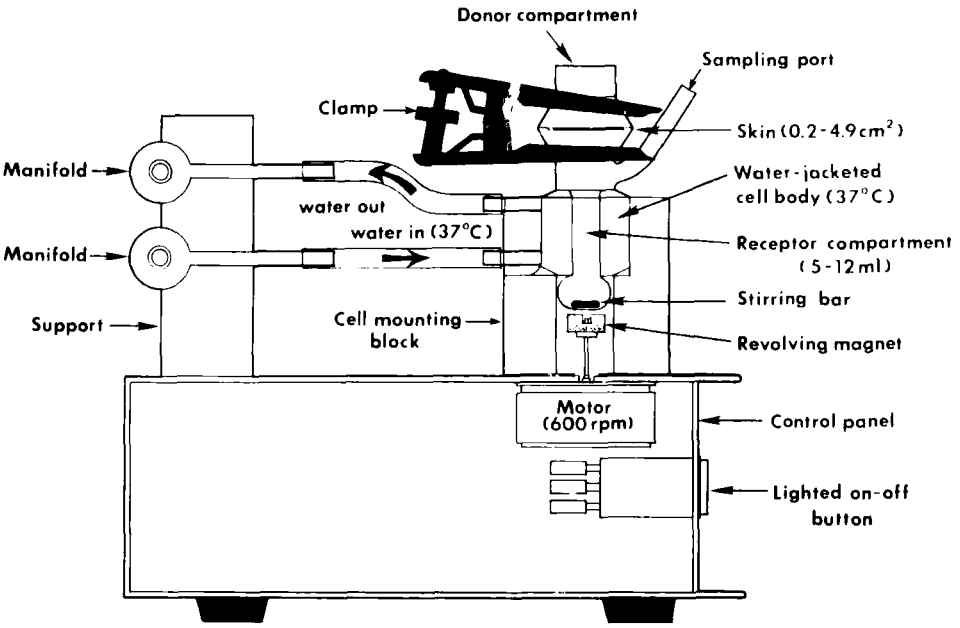
The elution solution at 37°C was then introduced into the receptor compartment of the diffusion cell, which was thermostatically controlled at 37°C by a circulating water bath. In the meantime, the donor compartment was maintained at the ambient temperature of 25 ± 1°C.

At predetermined time intervals, a 1 ml sample was withdrawn from the receptor solution, which was replaced immediately with the same volume of the drug-free elution solution to keep a constant volume in the receptor compartment and to ensure 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 as described earlier (6).

Drug Release Studies:

The same procedure as outlined above for skin permeation studies was also used for the drug release studies, except that no skin specimen was used and the patch was permitted to be in direct contact with the receptor solution. At predetermined times, the receptor solution was totally withdrawn and replaced with the same volume of drug-free elution solution at 37°C. By doing this, the required sink condition was maintained. The concentration of nitroglycerin in the sample was also determined by the HPLC method.

FINITE DOSING DIFFUSION CELLS by FRANZ



[T.J.Franz, Current Problems in Dermatol, 7 58 (1978)]

Figure 1

Schematic illustration of the commercially available finite-dosing Frantz diffusion cell assembly. Each of the diffusion cell is stationed in a cell-mounting block and is consisting of two compartments: a donor compartment, which is exposed to an ambient condition, and a receptor compartment, which is maintained at 37°C by circulating thermostated water through the water-jacketed cell body. The solution hydrodynamics in the receptor compartment is kept constant by a tiny rod-shaped stirring bar rotating at 600 rpm by a synchronous motor mounted underneath.

RESULTS AND DISCUSSION

Ointment Patches:

The release and skin permeation profiles of nitroglycerin from the ointment patch are shown together in Figure 2. The rate of release was higher at the beginning and then became lower in the later part of the study. In fact, the release profile of nitroglycerin from the ointment formulation can be better described by a Q vs. $t^{\frac{1}{2}}$ relationship as expected from Higuchi model (9) (Figure 3):

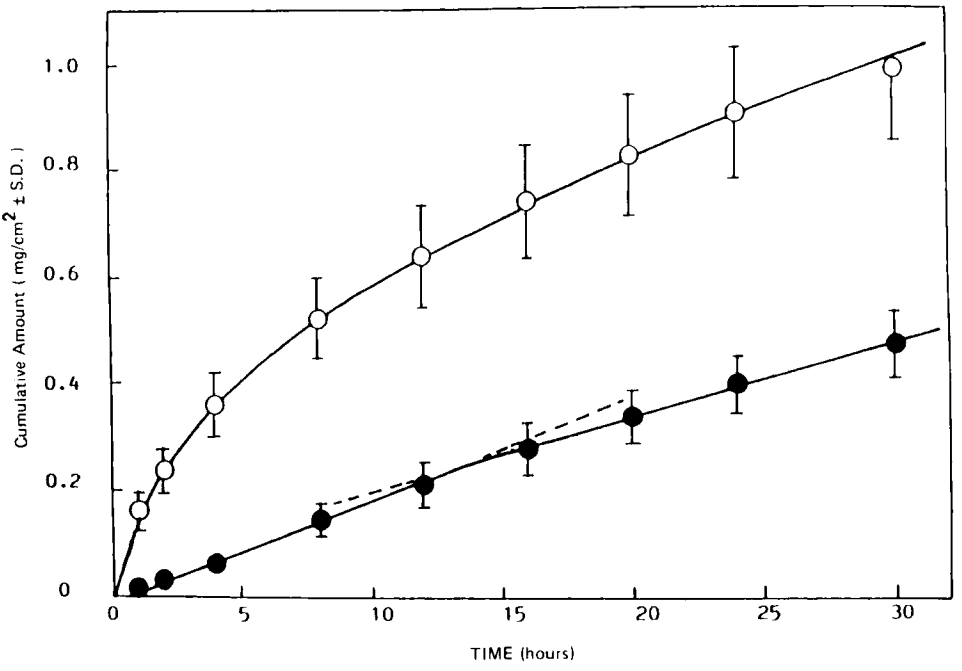


Figure 2

Release profile (O) and the skin permeation profile (●) of nitroglycerin from the ointment formulation (petrolatum base, 2% nitroglycerin). The release of nitroglycerin from the ointment formulation showed a non-linear Q vs. t profile, while the skin permeation of nitroglycerin produced a biphasic zero-order kinetics: 18.2 ± 3.4 μg/cm^2/hr (<12 hr) and 14.4 ± 5.9 μg/cm^2/hr (>12 hr). [N=8]

$$Q = [D_p (2A - C_p) C_p t]^{1/2}$$

Where Q is the cumulative amount of nitroglycerin released after time t, D_p is the diffusivity of nitroglycerin in the ointment matrix, A is the initial loading of nitroglycerin in the matrix, and C_p is the solubility of nitroglycerin in the ointment. Also shown in Figure 3 for comparison are the release profiles from Nitro-Dur and Nitrodisc systems. Apparently, the release fluxes of nitroglycerin from the Nitrodisc system (498.5 μg/cm^2/hr$^{1/2}$) and Nitro-Dur system (841.6 μg/cm^2/hr$^{1/2}$) were both significantly higher than the release flux from the 2% ointment formulation (187.3 μg/cm^2/hr$^{1/2}$).

While the results indicated that the release of nitroglycerin from the ointment patch is under a matrix diffusion-controlled process (Q vs.

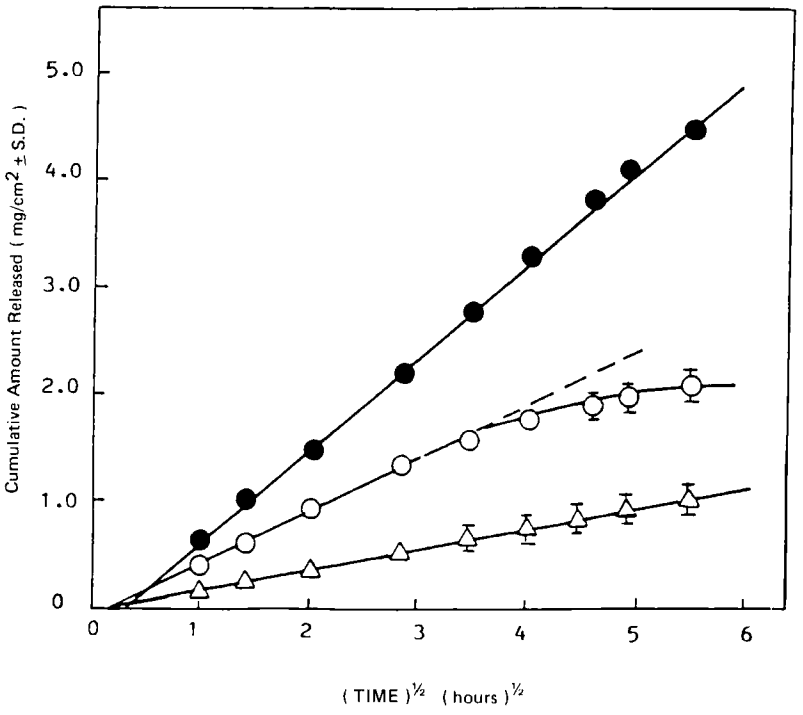


Figure 3

Release profile of nitroglycerin from the ointment formulation (△) is defined by a linear Q vs $t^{1/2}$ relationship as expected from matrix diffusion-controlled process. The release flux of nitroglycerin from the ointment formulation (187.3 ± 24.8 μg/cm²/hr$^{1/2}$) [N=8] is significantly less than those from Nitrodisc system (○) (498.5 ± 27.8 μg/cm²/hr$^{1/2}$) [N=8] and Nitro-Dur system (●) (841.6 ± 9.5 μg/cm²/hr$^{1/2}$) [N=8].

$t^{1/2}$ relationship), the skin permeation of nitroglycerin from the ointment showed a biphasic zero-order kinetics (18.2 μg/cm²/hr [<12 hr]; 14.4 μg/cm²/hr [>12 hr]) (Figure 2). This permeation rate profile is very similar to that from the Nitro-Dur system (8).

The rate of skin permeation can be described by a linear Q_s vs. t relationship (10):

$$Q_s = P_s (C_d - C_r) \quad t$$

Where Q_s is the cumulative amount of nitroglycerin penetrating through the skin, P_s is the skin permeability coefficient, C_d is the concentration of nitroglycerin in the donor compartment, e.g., on the surface of the

stratum corneum, and C_r is the concentration of nitroglycerin in the receptor solution. If the sink condition in the receptor solution is maintained (i.e., $C_r << C_d$), the above equation can be simplified to:

$$Q_s = P_s C_d t$$

Therefore, a constant skin permeation rate is observed.

Cream Patches:

The nitroglycerin delivered by the water-washable o/w cream formulations was also found to penetrate through the hairless mouse skin at a biphasic zero-order rate process (Figure 4). However, the rate of skin permeation of nitroglycerin from the cream formulations are 2-3 times greater than that from the ointment formulation [47.0 and 50.2 (<15 hr) vs. 18.2 (<12 hr) $\mu g/cm^2/hr$]. The higher skin permeation rates obtained from the cream patch could be the result of the change in formulation from an oily ointment base to a hydrophilic o/w emulsion base and the underlying thermodynamic factors. It is generally recognized (11, 12) that drug release from a topical vehicle will usually be faster from a formulation in which the drug concentration approaches saturated solubility. The solubility of nitroglycerin in the cream (at least in the liquid component of both oil and water phases) is approximately 2%. This saturated solution represents maximum thermodynamic activity. Although the solubility of nitroglycerin in the ointment formulation could not be determined accurately, it has been reported (13) that the drug's thermodynamic activity in the ointment is not unusually high. To extend the duration of action and to prevent dose-dumping, the cream was designed such that the drug was distributed primarily in the internal or oil phase. On the other hand, the addition of 1.6% water-soluble polymer in the cream formulation only slightly reduces the rate of skin permeation from 50.2 to 47.0 $\mu g/cm^2/hr$ (<17 hr) and from 24.0 to 22.8 $\mu g/cm^2/hr$ (>17 hr).

Film Patches:

Nitroglycerin released from the film patch was found to penetrate through the hairless mouse skin under a monophasic zero-order process (Figure

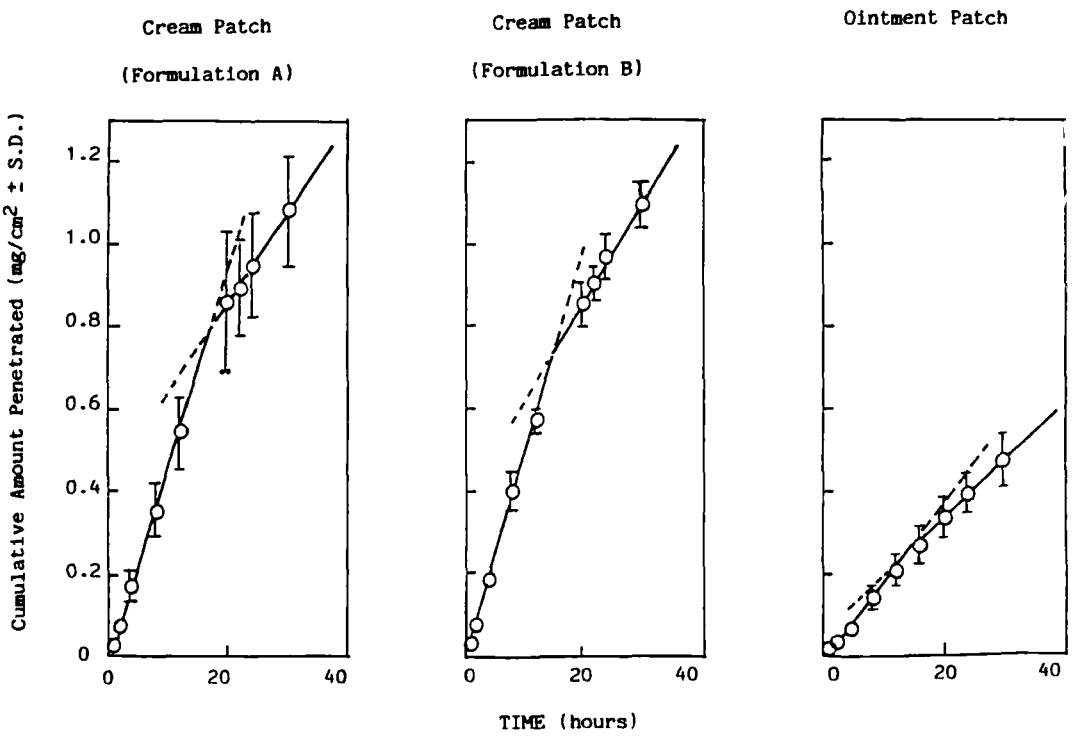


Figure 4

Skin permeation profiles of nitroglycerin from the water-washable cream formulations as compared to that from the ointment formulation. As in the case of ointment formulation, the permeation profiles of nitroglycerin from cream formulations also followed a biphasic zero-order kinetics. The rate of skin permeation of nitroglycerin from the cream formulation is only slightly reduced by the addition of 1.6% water-soluble polymer in the formulation: Formulation B [50.2 ± 3.5 (<15 hr); 24.0 ± 1.8 μg/cm^{2}hr (>15 hr)] [N=8] vs Formulation A [47.0 ± 8.0 (<17hrs): 22.8 ± 3.3 μg/cm^2/hr(>17hr)] [N=8]. The rates of skin permeation from these cream formulations are about 2-3 folds greater than that from the ointment formulation [18.2 ± 3.4 (<12 hr); 14.4 ± 3.9 μg/cm^2/hr (>12 hr)] [N=8].

5). It is interesting to note that the rate of skin permeation of nitroglycerin delivered by either Formulations C or D, which contains an ethanol-soluble polymer, is substantially greater than that from the marketed Nitro-Dur system, in which nitroglycerin/lactose triturate is dispersed in a hydrophilic polymer matrix of PVP/PVA/glycerol/water; the rates of skin permeation were 42.0 and 25.2 μg/cm^2/hr for Formulations C and D, respectively, as compared to 17.0 μg/cm^2/hr (<12 hr) and 10.3 μg/cm^2/hr (>12 hr) for Nitro-Dur system (Figure 5). Apparently, the skin permeation

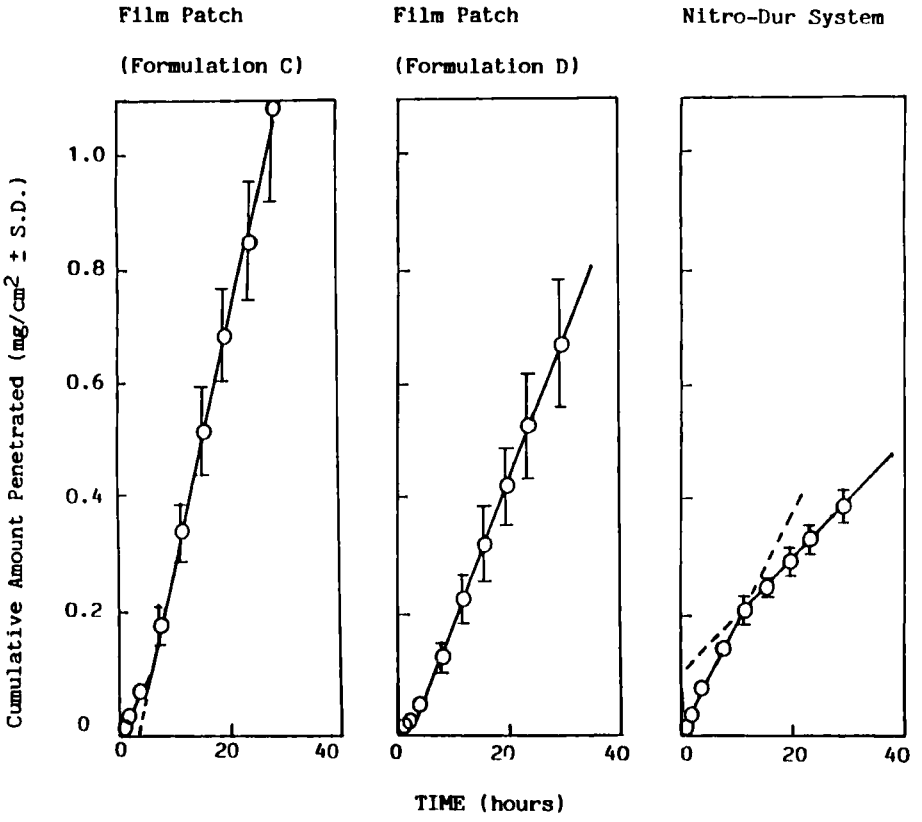


Figure 5

Skin permeation profiles of nitroglycerin from the film patches as compared to that from Nitro-Dur system. The steady-state rate of skin permeation from the film-forming Formulation C (42.0 ± 3.9 μg/cm^2/hr) is significantly greater than the rate of skin permeation for film-forming Formulation D (25.2 ± 3.6 μg/cm^2/hr), which is the result of doubling the concentration of polymer in the formulation from 5% to 10% [N=8]. Both film-forming formulations produced the rate of skin permeation which is significantly greater than Nitro-Dur system [17.0 ± 2.8 (<12 hr); 10.3 ± 2.1 (>12 hr) μg/cm^2/hr].

patterns of nitroglycerin are rather different between the film patch and the Nitro-Dur system, though both contain ethanol-soluble polymers.

The dispersion of nitroglycerin at molecular level in an ethanol-soluble polymer has been used to advantage in the development of a film-forming liquid. The combination of glycerin, water-soluble lanolin and a branched-chain ester serves both as a plasticizer for the film and a solvent for the nitroglycerin. In the film formed, approximately 20% of the drug

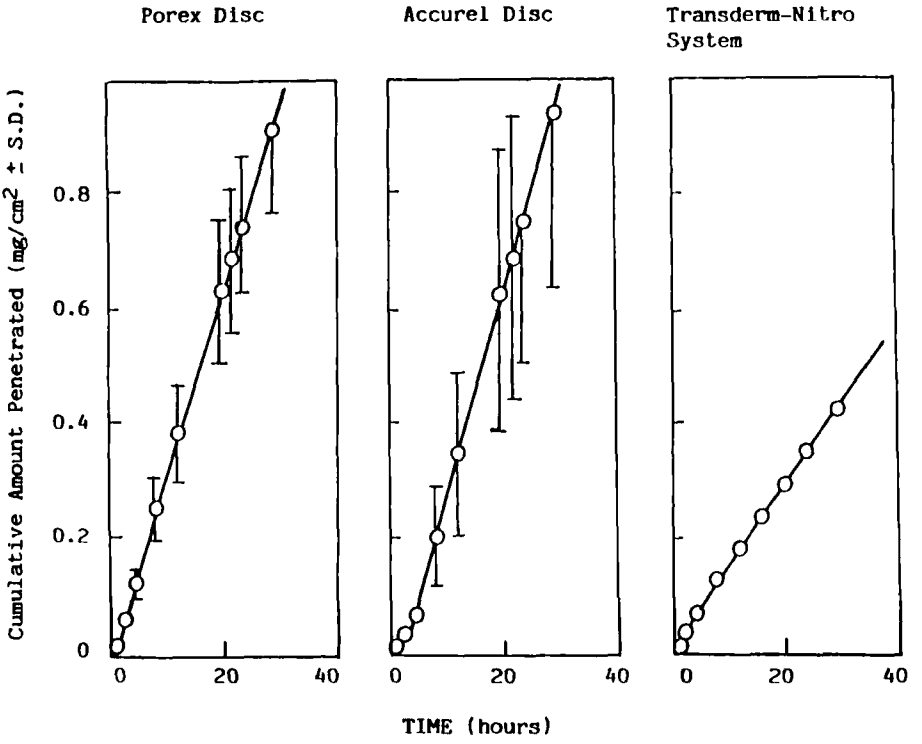


Figure 6

Skin permeation profiles of nitroglycerin from microporous membrane as compared to that from Transderm-Nitro system. The rates of skin permeation of nitroglycerin from the Porex disc (polyethylene membrane with ethyl hexyl palmitate, pore size 10 μm) [30.9 ± 5.0 μg/cm^2/hr; N=4] and from Accurel disc (polypropylene membrane with octyl hydroxy stearate, pore size 1 μm) [32.7 ± 11.5 μg/cm^2/hr; N=8] are very similar. These rates of skin permeation are significantly higher than that from the membrane permeation-controlled Transderm-Nitro system [14.1 ± 0.7 μg/cm^2/hr; N=12].

is soluble in these additives; the remainder is homogenously dispersed in the polymer matrix. It is postulated that the plasticizers function as a drug reservoir and that desorption from the polymer matrix and subsequent solubilization in the plasticizer controls the release of drug from the film patch. The data from another in vitro release studies (14) clearly indicated the rate-controlling effect of the polymer. The results from the skin permeation studies seemed to correlate well with the data from the release studies. By reducing the polymer concentration from 10 to 5%, the steady-state rate of skin permeation was increased from 25.2 to 42.0 μg/cm^2/hr.

Table 1

Comparison of skin permeation rate profiles of nitroglycerin from various experimental formulations

Formulations	Rate of Skin Permeation[a] ($\mu g/cm^2/hr$)	Dose absorbed at 24 hours	
		$mg/10\ cm^2$	% loading dose
Experimental Formulations:			
Ointment patch	18.2 ± 3.4 (<12 hr) 14.4 ± 3.9 (>12 hr)	3.9 ± 0.5	21.1 ± 2.6
Cream patch			
A	47.0 ± 8.0 (<17 hr) 22.8 ± 3.3 (>17 hr)	9.5 ± 0.9	50.1 ± 4.9
B	50.2 ± 3.5 (<15 hr) 24.0 ± 1.8 (>15 hr)	9.8 ± 1.1	53.4 ± 6.3
Film patch			
C	42.0 ± 3.9	8.5 ± 1.2	38.1 ± 5.5
D	25.2 ± 3.6	6.5 ± 0.9	23.6 ± 4.1
Microporous discs			
Accurel disc	30.9 ± 4.9	7.3 ± 1.2	15.7 ± 2.6
Porex disc	32.7 ± 11.5	7.5 ± 2.7	20.1 ± 7.3
Reference Formulations[b]:			
Nitrodisc	17.7 ± 2.8	4.3 ± 0.7	21.9 ± 3.8
Nitro-Dur	17.0 ± 2.8 (<12 hr) 10.3 ± 2.1 (>12 hr)	3.3 ± 0.6	6.4 ± 1.2
Transderm-Nitro	14.1 ± 2.5	3.5 ± 0.7	13.9 ± 2.6

a: Mean ± standard deviation of at least four determinations

b: From Chien, Keshary, Huang and Sarptodar, 1983.

Microporous Discs:

Nitroglycerin delivered by the microporous plastic membranes penetrates through the hairless mouse skin at a zero-order rate (Figure 6). The rates of skin permeation of nitroglycerin from Porex membrane and from Accurel membrane are very similar (30.9 vs 32.7 $\mu g/cm^2/hr$). The pore size of the

membrane and the solvent used for the deposition of nitroglycerin in the pores seem to make very little difference in affecting the skin permeation of nitroglycerin. However, the rates of skin permeation of nitroglycerin delivered by these two microporous discs are both significantly higher than that from the Transderm-Nitro system (14.1 $\mu g/cm^2/hr$), which controls the skin permeation of nitroglycerin by a rate-controlling membrane of ethylene/vinyl acetate copolymer (Figure 6).

The results of the present study are summarized in Table 1, the rates of skin permeation of nitroglycerin from Nitrodisc, Nitro-Dur and Transderm-Nitro systems are also included for comparison.

In summary, nitroglycerin delivered by these six experimental formulations penetrates through the abdominal skin of hairless mouse at a rate profile which is significantly greater than those from the ointment formulation, Nitrodisc, Nitro-Dur or Transderm-Nitro systems currently available in the market. It, therefore, suggests that a transdermal patch can be developed to deliver nitroglycerin from these six experimental formulations to achieve a higher plasma level than the transdermal therapeutic system currently available in the market. The results also demonstrate the feasibility of improving the skin permeation of nitroglycerin through the proper formulation design.

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14. In these studies, the release of nitroglycerin into isopropyl myristate was investigated. The results indicated that the release rate of nitroglycerin from the film patches decreases as increasing the polymer content. At a polymer concentration of 2.5%, virtually the entire drug load was released in 2 hours; while at a polymer concentration of 15%, very little drug was release after 7 hours.