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Comparison of In Vitro Release Rates of Nitroglycerin by Diffusion Through a Teflon Membrane to the USP Method

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ABSTRACT

The in vitro release profile of nitroglycerin (GTN) from different transdermal patches through synthetic membranes has been determined and compared to the USP adapted release rate method. Five different nitroglycerin transdermal test formulations were compared to commercially available Nitro-Dur®. All formulations display similar release rate profiles when tested by the USP adapted release rate method. In contrast, significant differences among the tested formulations were observed by using a synthetic Teflon membrane. In these studied an attempt was made to develop a simple, reliable, and reproducible method for testing the release of GTN from different transdermal patches in vitro.

INTRODUCTION

One of the aims of in vitro experimentation for a transdermal system is to predict delivery and penetration of a drug molecule from the patch into the body. Drug release or dissolution testing helps in assuring product quality but may not predict in vivo performance. A test which can be used to predict in vivo drug delivery is the investigation of drug penetration through human cadaver or animal skin. However, the supply of human skin is limited, and noticeable variations among skin samples from different anatomic sites as well as from donor to donor have been cited in the literature (1–3). Several investigators have studied the usefulness of different artificial membranes for predicting in vitro transdermal delivery for a number of drugs (4–6). Selection of a suitable synthetic membrane requires careful consideration. The appropriate membrane should be without significant barrier effects. Wu et al. (7) studied GTN release through various artificial membranes. They reported that GTN release was very fast when polysulfone (Supor 450) membrane was used because

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this hydrophilic-type membrane has no barrier effect in the release. Polytetrafluoroethylene (PTFE) membrane, on the other hand, did not show satisfactory release of GTN either from solution or from ointment formulations (7). The authors attributed these observations to the very poor wetting property of the membrane by the tested formulations. The objective of this investigation was to test the utility of PTFE membrane for determining the release rates of nitroglycerin from different GTN transdermal patches, assuming the hydrophobic character of the PTFE membrane could mimic the rate-limiting step of the human skin lipids.

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MATERIALS AND METHODS

Chemicals

The materials used in these studies were obtained from the following sources: sodium chloride, high-performance liquid chromatography (HPLC) grade methanol, and acetonitrile from Fisher Scientific (Fair Lawn, NJ); nitroglycerin reference standard from United States Pharmacopeial Convention, Inc. (Rockville, MD); PTFE (Zylon®) and polysulfone (Supor 450®) membranes from Gelman Sciences (Ann Arbor, MI); Nitro-Dur® transdermal nitroglycerin patches from Key Pharmaceutical (Miami, FL); and experimental nitroglycerin transdermal test patches from our laboratory (Schering-Plough Research Institute, Kenilworth, NJ).

Permeation Studies

Permeation studies were conducted with Franz diffusion cells (Crown Glass Company, Sommerville, NJ). The cell diameter was 15 mm and the cross-sectional area for diffusion was 1.76 cm^2 with a 7.5 ml cell volume. The membranes were presoaked in 0.9% saline solution for 30 min before mounting on the diffusion cells. To avoid entrapment of air bubbles, the patches were immediately placed on the membranes. The entire content of receptor fluid (0.9% saline) was removed and replaced with equal volume of fresh saline at predetermined time points (1, 2, 4, 6, 24, and 30 hr) to maintain a sink condition. The receiver compartment was stirred with a magnetic bar, and the temperature of the cells was maintained at 32 $\pm$ 0.2°C. The samples were analyzed by HPLC.

Release Rate Testing

A Hanson Research Model 72 SL dissolution apparatus was used for the release rate study (modified USP paddle method). The dissolution fluid used was deionized water and it was maintained at 33 $\pm$ 1°C. The patches (10 cm^2 each) were held between two wide mesh (10 mesh) screens. The samples were withdrawn at 0.5, 1, 2, 3, and 4 hr and analyzed by HPLC.

Chromatographic Instrumentation

The chromatographic system consisted of a dual-pump multisolvent delivery system (Waters 600), an automatic sample injector (WISP Waters 712), a programmable multiwavelength UV detector (Waters 490), and a single-channel integrator (Waters 740).

HPLC Analysis

The nitroglycerin (GTN) amount in the receptor fluid was determined by reverse-phase HPLC analysis. The mobile phase (15% MeOH, 45% deionized water, and 40% acetonitrile) was pumped isocratically at flow rate 1.0 ml per min. The method used a Perkin-Elmer C18-CR 3 micron column (4.6 mm $\times$ 8.0 cm) and 214 nm wavelength. The injection volume was 20 µl. The retention time for nitgroglycerin was approximately 3.6 min. Quantification was performed by comparing peak surface area with calibration curves obtained with known amounts of GTN (concentration range: from 1.6–160 µg/ml) under identical analytical conditions.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANVOA). The differences between paired data were compared using Student's t test, and $p < 0.05$ was taken as the level of significance for all analysis.

RESULTS AND DISCUSSION

In these studies different experimental transdermal test formulations of 10 cm^2 surface area were tested. The composition of the test formulations varied only in the enhancer concentration. The concentration of drug and the thickness of the transdermal patches were held constant. Test formulations were always compared to the control, Nitro-Dur.

USP Dissolution Test

The in vitro release rate profiles of nitroglycerin from various patches using USP adapted release rate method are shown in Fig. 1. The results show a very

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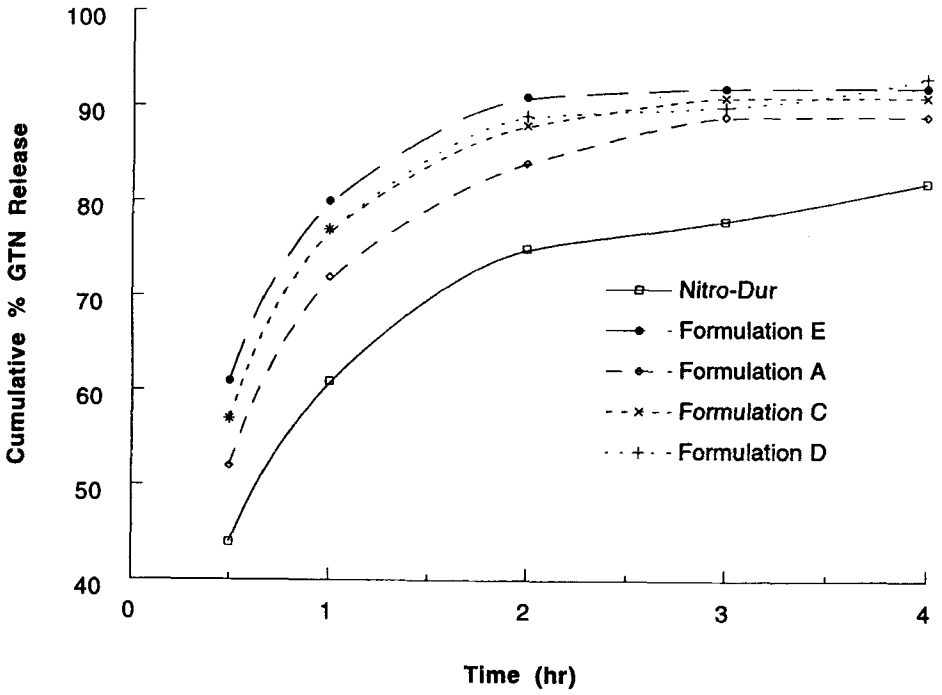

Figure 1. Cumulative percent nitroglycerin (GTN) released using USP paddle method. Each point represents an average of 3 samples.

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fast initial burst release for all the formulations tested. Ninety percent of the drug was detected in the dissolution media within 4 hr. Analysis of variance (ANOVA) was performed to determine statistical significance in the release rates among the screened formulations using the dissolution method. No significant difference was observed ($p > 0.05$) in the release rates. The USP adapted release rate method was incapable of differentiating between the various formulations.

In Vitro Permeation Studies

The in vitro permeation profiles of nitroglycerin from different formulations through polysulfone and PTFE membrane are shown in Figs. 2 and 3, respectively. The permeation through polysulfone membrane was similar to the USP release rate method. The permeation through polysulfone membrane was very fast and most of the drug permeated through the membrane in 4 hr. The very fast diffusion through this membrane is an indication that it has no barrier resistance to drug release and hence is not a good discriminating membrane. In contrast, the cumulative amount of nitroglycerin permeated through PTFE membrane showed a linear relationship for the formulations without exhibiting any marked lag time or burst effect phenomena (Fig. 3). The steady-state flux values were calculated from this linear relationship. The flux values are listed in Table 1. The flux values for all the formulations were statistically different from the control patch (Nitro-Dur, $p < 0.05$) and also from the enhancer-free formulation (Formulation A).

Comparison of In Vitro Steady-State Flux Results: Human Cadaver Skin and PTFE Membrane

Figure 4 shows the amount of GTN permeated across PTFE membrane and full-thickness human skin from test formulation (Formulation D) and the control Nitro-Dur. The steady-state flux values through PTFE membrane and human cadaver skin are listed in Table 2. These data indicate that there is a very good correlation between the PTFE membrane and skin permeation results. Comparison of flux ratios of the experimental formulation and Nitro-Dur using PTFE membrane and human cadaver skin is listed in Table 3. Using a Student t test for paired data, no significant difference in the steady-state flux of GTN through either PTFE membrane or human cadaver skin was observed.

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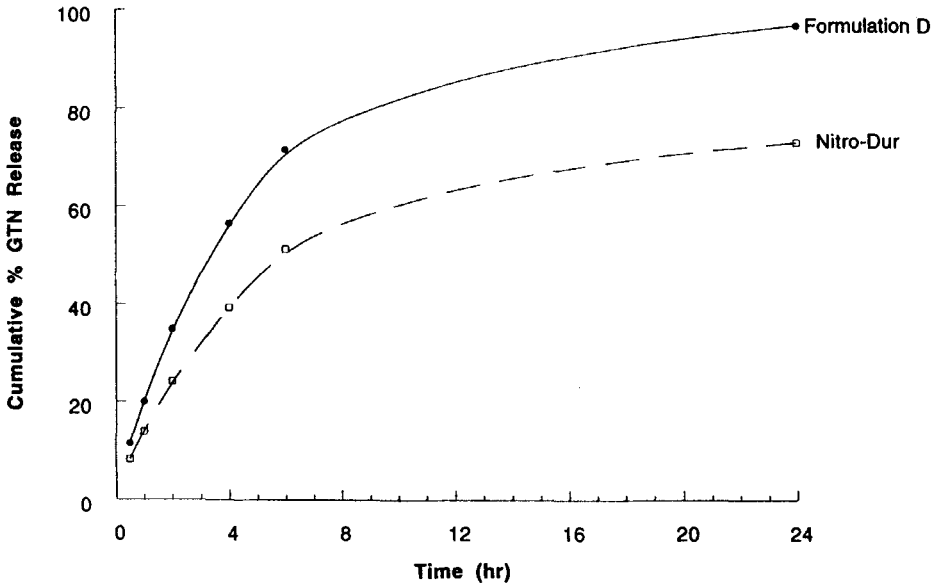

Figure 2. Cumulative percent nitroglycerin (GTN) released through polysulfone membrane using Franz diffusion cells. Each point represents an average of 5 samples.

The aim of this work was to determine an in vitro method sensitive enough to be used during the development of a transdermal drug delivery system without using human cadaver skin. The use of a hydrophilic membrane such as polysulfone failed to distinguish between different formulations because the release profiles were rapid and similar to the dissolution profile (Fig. 2). On the contrary, the PTFE membrane could differentiate between the different formulations evaluated and showed an excellent correlation to skin permeation stud-

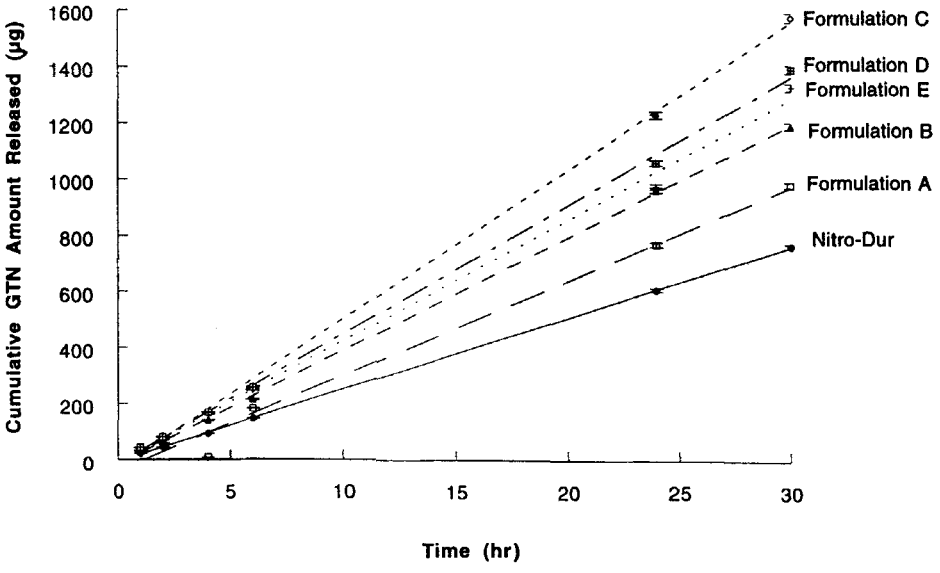

Figure 3. Release rate of nitroglycerin (GTN) from different test formulations and Nitro-Dur patches through PTFE membrane. Each point represents the mean of ± *SD* of 5 experiments ($n = 5$).

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Table 1

Steady-State Flux Values of Various Nitroglycerin Formulations

Formulation	% of Enhancer Used	PTFE Membrane Flux (μg/cm²/hr)
A	0	11.61 ± 1.07 (n = 5)
B	2.3	14.29 ± 0.99 (n = 5)
C	5	18.80 ± 0.73 (n = 4)
D	10	16.28 ± 2.25 (n = 4)
E	15	15.32 ± 3.59 (n = 4)
Nitro-Dur		9.28 ± 0.88 (n = 28)

Note. All experimental patches were made on a laboratory scale. Number of replicates performed in parentheses.

ies using human cadaver skin (Table 2). PTFE membrane probably mimics the hydrophobic property of human skin and may be used successfully to replace human cadaver skin in screening formulations during development of transdermal formulation.

Table 2

Steady-State Flux Through PTFE Membrane and Human Cadaver Skin

Formulation	PTFE Membrane Flux[a] (μg/cm²/hr)	Whole-Skin Flux[a] (μg/cm²/hr)
D	15.21 ± 1.47	14.41 ± 2.84
Nitro-Dur	9.05 ± 1.59	8.24 ± 0.67

[a] n = 5.

CONCLUSION

An in vitro release rate test procedure through Teflon PTFE membrane can distinguish between the release rates of GTN from various experimental transdermal formulations. Consequently, this method is preferable over the present USP adapted dissolution method. This simple technique also makes it possible to predict the in vivo permeation rate from nitroglycerin patches, since

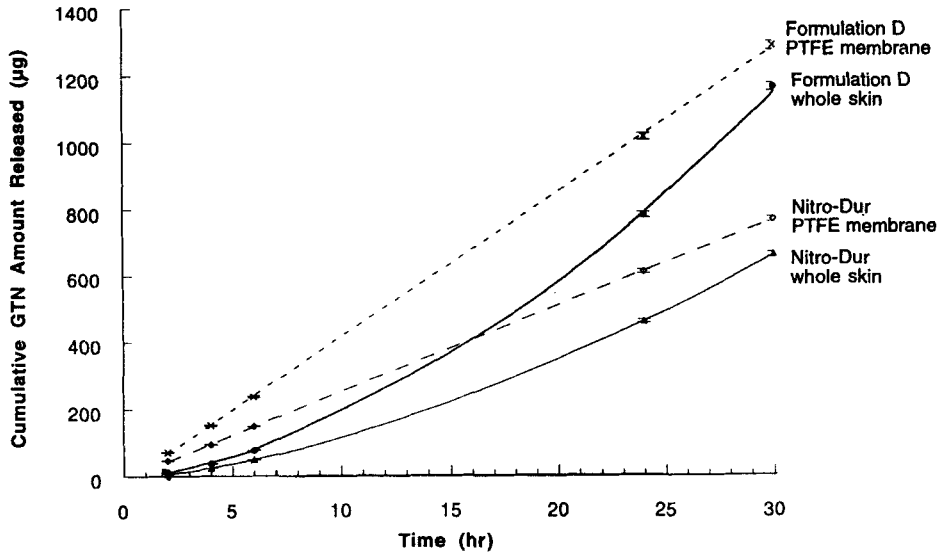

Figure 4. Release rate of nitroglycerin (GTN) from test formulation and Nitro-Dur patches through PTFE membrane and whole skin, using Franz diffusion cells. Each point represents the mean of ± *SD* of 5 experiments (n = 5).

Table 3

Flux Ratios: PTFE/Skin

	Formulation D	Nitro-Dur
Flux ratio: PTFE membrane/whole skin	1.05	1.1

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the transfer rates of the GTN across this synthetic membrane and whole skin are similar.

REFERENCES

1. R. J. Feldmann and H. I. Maibach, Regional variation in percutaneous penetration of ^{14}C cortisol in man, J. Invest. Dermatol., 48, 181 (1967).
2. H. I. Maibach, R. J. Feldmann, T. H. Milby, and W. F. Serat, Regional variations in percutaneous penetration in man, Arch. Environ. Health., 23, 208 (1971).
3. A. Rougier, D. Dupuis, C. Lotte, R. Roguet, R. C. Wester, and H. I. Maibrach, Regional variation in man: Measurement by the stripping method, Arch. Dermatol. Res., 278, 465 (1986).
4. A. Orbe and L. O. Sundelölf, A new in vitro model for quantitative study of cream permeability, Int. J. Pharm., 41, 49 (1988).
5. W. J. Addicks, G. L. Flynn, and N. Weiner, Drug transport from thin applications of topical dosage forms: Development of methodology, Pharm. Res., 5, 377 (1988).
6. V. P. Shah, J. Elkins, S. Y. Lam, and J. P. Skelly, Determination of in vitro drug release from hydrocortisone creams, Int. J. Pharm., 53, 53 (1989).
7. S. T. Wu, G. K. Shiu, J. E. Simmons, R. L. Bronaugh, and J. P. Skelly. In vitro release of nitroglycerin from topical products by use of artificial membranes, J. Pharm. Sci., 81, 1153 (1992).

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