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AUTOMATED IN VITRO METHOD FOR EVALUATING DIFFUSION CHARACTERISTICS OF TRANSDERMAL NITROGLYCERINE DELIVERY SYSTEMS WITH OR WITHOUT SKIN

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ABSTRACT

A diffusion cell, termed the Patch Cell, suitable for conducting *in vitro* percutaneous absorption testing of transdermal nitroglycerine (TNG) delivery systems using skin from man, hairless mice and Yucatan pigs, is described. In addition, the Patch Cell can be utilized for batch-to-batch quality control testing of transdermal TNG delivery systems. The diffusion cell is incorporated into a laboratory robotics workstation that performs unattended, automated sampling of up to 24 diffusion cells over a 24-hour period. The method, referred to as the Patch Cell Method, has been used to measure the *in vitro* percutaneous absorption of TNG from two commercially available nitroglycerine delivery patches through hairless mouse skin, Yucatan pig skin, and human stratum corneum. TNG flux through hairless mouse skin, 30.2 +/-3.3 $\mu g/cm^2/hr$ from Transderm Nitro[1] patches and 33.4 +/-8.2 $\mu g/cm^2/hr$ from Nitro Dur II[2] patches, were comparable with the previously reported results of Keshary and Chien (9). The percutaneous TNG flux from these same patches, through the Yucatan pig skin, was 25.4 +/-2.4 and 21.9 +/- 1.9 $\mu g/cm^2/hr$ respectively. TNG flux through human stratum corneum from Transderm Nitro patches was measured at 21.2 +/- 4.1 $\mu g/cm^2/hr$. The Patch Cell Method was also compared to the FDA dissolution bath technique for measuring percent drug released directly into saline, as a batch-to-batch

quality control test. Results from the FDA and Patch Cell methods for three commercial nitroglycerine delivery systems were:

Percent of Drug Released

Patch Type	FDA 1	FDA 2	Patch Cell
Transderm Nitro	68 +/- 2.1 (n=6)	56 +/-1.5 (n=6)	74 +/-2.7 (n=10)
Nitro Dur II	94 +/- 1.4 (n=6)	93 +/- 0.7 (n=6)	91 +/- 2.4 (n=5)
Deponit[3]	29 +/- 0.7 (n=6)	28 +/- 1.1 (n=6)	30 +/- 1.0 (n=3)

These results indicate that the Patch Cell method, in addition to being useful for in vitro percutaneous absorption assays, is also useful for direct release testing into water.

INTRODUCTION

There is a need for automated methods to evaluate batch-to-batch release uniformity of transdermal delivery systems and their *in vitro* percutaneous delivery profiles. Traditional diffusion cell methods have measured the rate of drug release from drug solutions or pastes, through a rate-limiting membrane, either biological or synthetic. These methods are not suited for routine, batch-to-batch equivalency or quality control oriented testing because they require manual sampling, are too small to accommodate large, i.e., greater than 5 cm^2, delivery devices and require frequent total replacement of receiver fluid to avoid loss of sink conditions. The dissolution bath, traditionally used for measuring the rate of tablet dissolution, has been successfully adapted for use to determine the release properties of several transdermal nitroglycerine (TNG) delivery devices, and is considered the accepted method for measuring the rate of drug release into water from these devices (1). However, the dissolution bath can not be used for measuring percutaneous absorption.

In developing a transdermal delivery system, the rate of percutaneous absorption from the prototype delivery device must be determined. Additionally, it is often necessary to evaluate the extent of transdermal drug delivery enhancement resulting from the addition of chemical enhancers into the formulation. Enhanced delivery can only be measured through skin, most desirably human skin. Existing methods, i.e., Franz cells (2), Keshary-Chien cells (3), Chien-Valia side

by side cells (4), and Flow-through cells (5), make use of excised hairless mouse skin, pig skin, and human skin, require manual sampling, frequent replacement of receiver fluid, and, because of their small size, are unsuitable for testing whole patches larger than 5 cm^2.

Our laboratory has designed an *in vitro* diffusion cell that is large enough to accommodate nitroglycerine delivery systems up to 20 cm^2, approaches sink conditions for large devices when tested through skin, and will maintain limited sink conditions for the same device when testing directly into water, similar to a dissolution bath. These cells are thus suited for development work, in which a biological membrane is required, and for quality control testing, where release rates into water need to be measured. The cells and the test procedure follow the guidelines as set forth from the FDA and AAPS Workshop, 1987 (6). The integration of laboratory robotics has allowed unattended sampling for extended periods of time, and thus offers an additional advantage over traditional diffusion cells. This paper describes the diffusion cell (Patch Cell), the apparatus, methodology and process validation of the method termed the Patch Cell Method (PCM), using TNG as the model for validation.

Patch Cell Description

The Patch Cell, (Figure 1), is constructed from glass, Teflon™, and stainless steel. The Patch Cell consists of a large receiver compartment[4], with a volume of approximately 200 mL, completely surrounded by a glass water jacket containing inlet and outlet ports for connection to a water bath. There is a depression in the bottom of the receiver in which a Teflon coated magnetic stir bar[5] (1 x 5/16 inch) is located. The receiver compartment is covered by a 1/2 inch thick Teflon template[6] which has a circular aperture in the center (diameter = 2.5 to 3.5 inches, depending upon the type of assay performed). Two 16 gauge, blunted, lab pipetting cannulae[7] in the template serve as sampling ports. The outer assembly[6] is a 1/2 inch thick disk of Lexan™ and is used, along with a stainless steel clamping ring[6], to clamp the cell together. An O-ring[6] between the template and the glass Patch Cell ensures a leak-proof seal. For release testing directly into water, a sheet of polyester[6], 0.004 to 0.007 inches thick, can be cemented to the Teflon template.

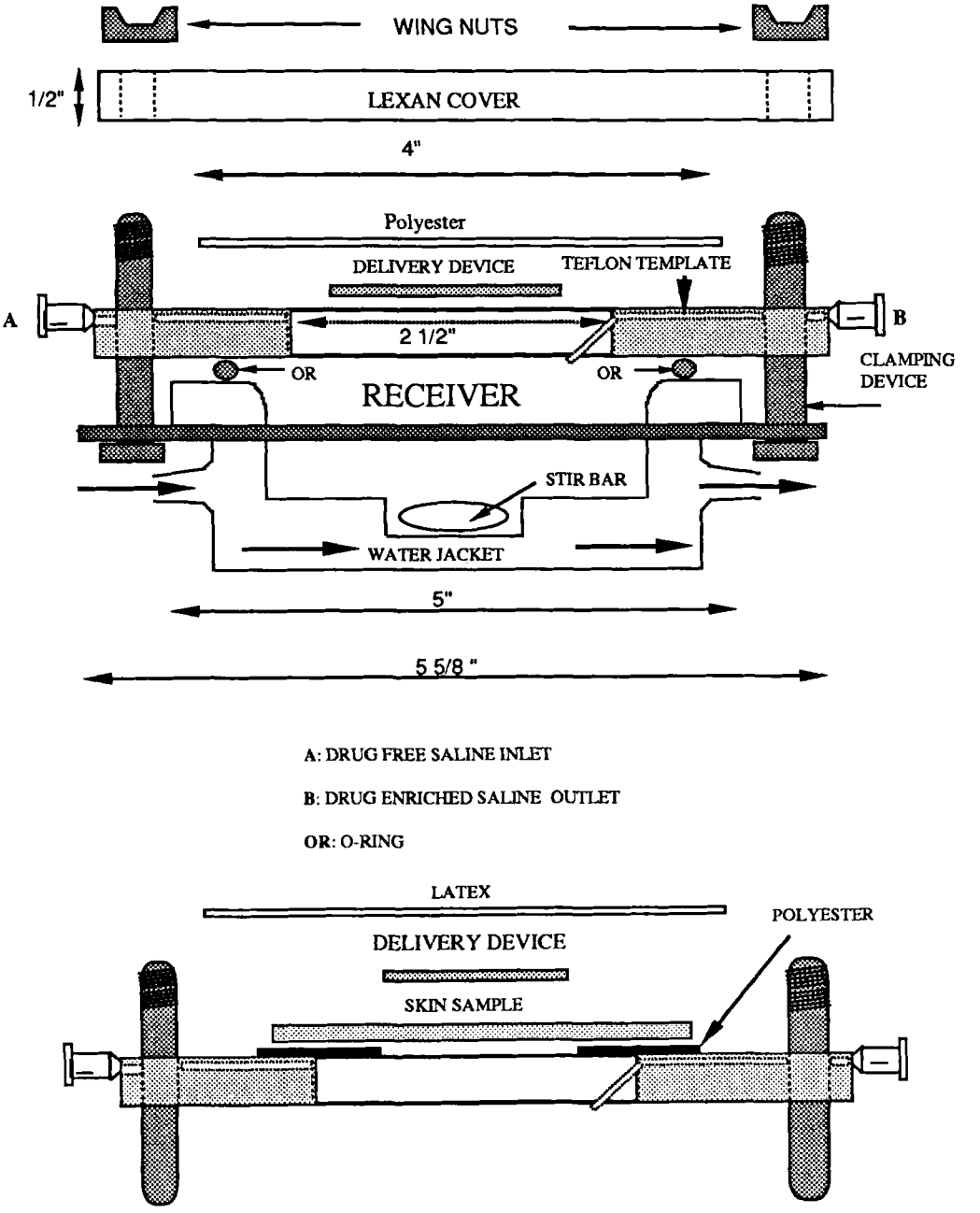


FIGURE 1

Patch Cell Assembly. A: For release testing directly into saline, the delivery device is fastened to the underside of a polyester film using double-sided cellophane tape. **B:** For percutaneous absorption testing, the skin sample is cemented, dermal side down, either to the Teflon template or to the polyester and the delivery system is placed over the epidermis.

Double-sided cellophane tape is used to attach the transdermal delivery system to the polyester with their delivery surface facing into, and in direct contact with, the receiver fluid (Figure 1A).

The Patch Cell can accommodate a large variety of device sizes for studying *in vitro* percutaneous absorption. When using large patches, the skin is mounted directly on the Teflon template with the dermal side in contact with the receiver fluid. The Lexan cover and restraining ring secure the system. When using smaller device sizes, an aperture, smaller than that in the Teflon template, can be punched into the polyester. The polyester can then be cemented to the Teflon template. The skin is then cemented to the polyester. The delivery system can now be placed over the skin and the cell assembly completed as above, (Figure 1B).

Description of Robotic Components

The robotic components[8] of the Patch Cell Method include: a Zymate Laboratory Robot and Controller upgraded with Zymate II software; a General Purpose Hand (GPH) with HPLC vial capping fingers; a printer; six vial racks, customized to hold 50 4mL HPLC vials; three Power and Event Controllers (PEC); one Master Laboratory Station (MLS); one Capping Station; one Balance interface each for the Mettler PE-160 electronic Sample Balance[9] and the Mettler PM-600 electronic Waste Balance[9] twenty-four, 3-way liquid handling valves; Teflon tubing; and a Hamilton Intelligent Valve Processor[10] with three 8-way valves. In addition, the PCM set up includes: one 20 liter water bath containing three 2 liter receiver fluid reservoirs; four Haake circulating water baths[9] (6 cells per bath); and four 6-place Wheaton BioStir-6® magnetic stirring consoles[11]. The layout, (Figures 2 and 3), requires 2 tables at 4 x 7 feet each, a vacuum pump[9] and two 4 liter flasks[9] for waste collection, and one custom made sampling station[6]. The PCM has the capacity for unattended sampling of up to 24 Patch Cells, sampled 11 times each, over a twenty-four hour period. The Patch Cell Method also uses an IBM-PC to collect data during the assay and to position the Hamilton Valves. The IBM-PC and the Zymate Controller are interfaced via the Zymate computer interface.

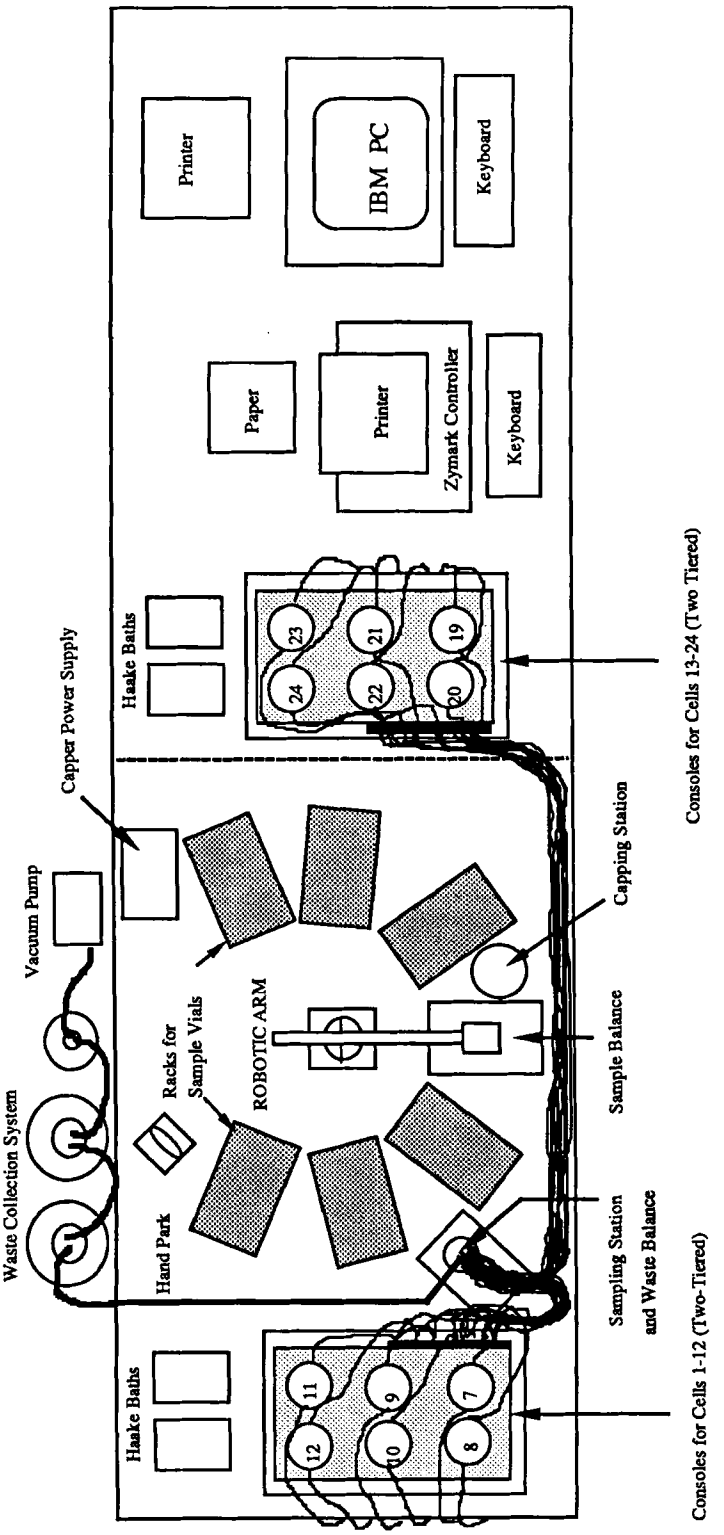
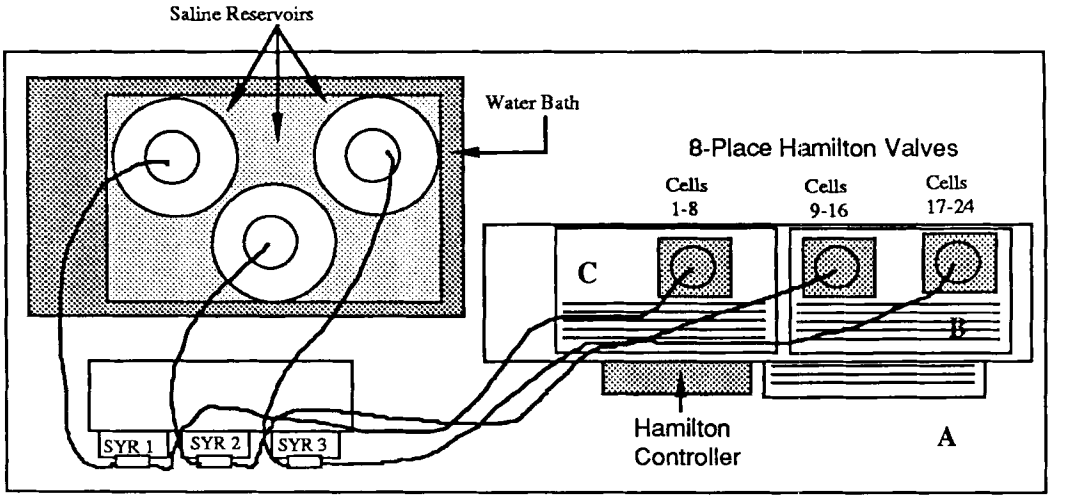


FIGURE 2

Top Level View of The Patch Cell Assay Robotic Workstation. The robot arm is positioned in the center of a circle described by the HPLC vial racks. Six Patch Cells are placed on each of the Biostir-6 consoles which are stacked two-high.



A: Power and Event Controller Cells 1-8

B: Power and Event Controller Cells 9-16

C: Power and Event Controller Cells 17-24

FIGURE 3

Bottom Level View of The Patch Cell Assay Robotic Workstation. The apparatus located beneath the robot arm includes one Master Laboratory Station, the saline reservoirs and the large water bath to maintain temperature at 37°C, three Power and Event Controllers, and three 8-place Hamilton Valves and the Valve Controller.

The Laboratory Unit Operations (LUO), and the Robotic components used in the Patch Cell Method include:

1. Removal of the correct sample vial from the appropriate rack (Robot arm, Controller and GPH)
2. Removal and parking of the cap (Capping Station)
3. Taring the empty vial (Mettler PE-160 Sample Balance) and waste container (Mettler PM-600 Waste Balance)
4. Filling a syringe and opening the correct valves (MLS and PEC)
5. Collection of the sample and dispensing remaining volume to waste (MLS and PEC)

6. Re-weighing the sample and calculating net weight and volume of the sample and waste collected, (Mettler PE-160 and PM-600 respectively)
7. Recapping the vial and returning the vial to the appropriate rack (Robot arm, Controller and GPH)

Throughout the sampling operation, for each cell sampled, the Zymate controller transfers cell number, sample number, time of sampling and the total volume removed to the IBM PC.

Description of Robotic Manipulations

A typical Patch Cell Method is performed at a temperature of 37° C, with constant stirring maintained at 600 rpm. The sample collection procedure consists of removing approximately 21 mL from the receiver by displacement, i.e., 21 mL of drug free saline is pushed into the receiver and the resulting overflow is collected either as waste (approximately 19 mL), or as sample to be analyzed. The sample, approximately 2 mL, is collected into HPLC vials at each time point.

The automated sampling procedure is as follows (refer to Figures 2 and 3 for the Patch Cell Layout). The Patch Cell Method begins with an initialization program which returns all cell and sample tracking counters to their starting values, initializes the Hamilton Valve Controller, and enables the operator to enter the number of cells to be sampled, the sampling interval, and the number of samples to remove from each cell over the course of the assay.

At the start of each sampling interval, a timer is activated which ensures that the samples are removed at the appropriate times during the assay. The robot arm is then instructed to remove the appropriate sample vial from the corresponding rack. The vial is placed into the capping station where the cap is removed and subsequently "parked". The vial is then removed from the capping station and placed in the vial holder located on the weighing pan of the Mettler PE-160 electronic sample balance. At this time, the balance is instructed to electronically tare the vial. The robotic arm then removes the vial from the balance and proceeds to a safe position in front of the sample station. The

Mettler PM-600 waste balance then electronically tares the waste receptacle. Concurrently, the Master Lab station (MLS) is instructed to fill the appropriate syringe with 9.0 mL of fresh saline from the reservoir. (The MLS has 3 syringes, each is plumbed, via the Hamilton valves, to 8 Patch Cells.) Two mL are then pushed back to the reservoir to purge the syringe of air. The MLS is directed to close the valve to the reservoir and open the valve leading to the Hamilton valves. Simultaneously, the Hamilton valve controller receives instructions to open the valve to the correct cell, while the Power and Event Controller (PEC) opens the valve between the appropriate cell and the sampling station. When all the valves are in the sampling configuration, the MLS is instructed to pump the 7.0 mL of saline to the Patch Cell via sampling port A. The fresh saline displaces drug-enriched saline through sampling port B, which is collected in the waste receptacle on the Mettler PM-600 waste balance. After emptying the syringe, the valve between the Patch Cell and the sampling station is closed. The MLS is instructed to fill the syringe as before and the valves are once again cycled to the sample collection position. The MLS pumps 3.0 mL of fresh saline to the Patch Cell and then stops and waits while the robot arm positions the sample vial under the correct port of the sampling station. Once at the correct location, the MLS pumps 2.0 mL to the Patch Cell. This displaced 2.0 mL sample is collected into the sample vial. After collecting the 2.0 mL sample, the robot arm returns to the safe position and the MLS dispenses the remaining 2.0 mL to the Patch Cell, with the displaced volume of receiver solution collected into the waste receptacle. The cycle is repeated once more, with the entire displaced volume being collected in the waste receptacle.

When the cycle is completed, all valves are returned to pre-sampling positions, and the robot arm returns the sample vial to the sampling station to collect the last drop from the spout. The vial, containing the collected sample, is then returned to the PE-160 sample balance. The balance weighs the vial plus sample, subtracts the vial tare weight and returns a sample weight to the controller. The waste balance re-weighs the waste receptacle, subtracts the tare weight and returns a waste weight to the controller. The controller combines the sample and

waste weights and converts the total weight to a total sample volume. The sample vial is removed from the balance and placed in the capping station, the cap is retrieved and the vial is capped. After capping, the vial is returned to the rack. During the sampling operation, the controller has sent the cell number, sample number, sample volume and sampling time to the IBM-PC where it is stored in an array. The sampling cycle continues onto the next cell in sequence until all Patch Cells have been sampled. After the completion of the sample cycle, i.e., all cells in the assay have been sampled, the waste receptacle is emptied via vacuum and the system waits for the timer to count down before starting the next sampling cycle.

Results from the PCM, i.e., sample ID, collected volume and collection time, along with the HPLC results, are entered into a LOTUS™ spreadsheet[12] which calculates flux from the patch and total amount of drug delivered at each time point, see Appendix I for equations.

In order to ascertain the level of performance of the Patch Cell System, three commercially available transdermal nitroglycerine patches were tested releasing drug directly into saline, and the results obtained with the Patch Cell Method were compared to those reported using the FDA approved dissolution bath technique (7). In addition, the Patch Cell was evaluated for reproducibility in sample collection and in determining the flux rate from Transderm Nitro-5 patches. *In vitro* percutaneous absorption from the same patches was measured using the Patch Cell and skins obtained from hairless mice, Yucatan miniature pigs, or human donors.

MATERIALS AND METHODS

A 1x Dulbecco's phosphate buffered saline solution was prepared from 10x concentrate[13]. HEPES 1.0 M buffer solution[13] was added to a final concentration of 25mM. The saline solution was then titrated to pH 7.4 using 5.0 N NaOH[14]. The saline was degassed, under vacuum, prior to usage, using a Bransonic 8200 Ultrasonic Bath[9] Transdermal nitroglycerine patches, release tested directly into saline, are listed in Table 1.

TABLE 1

Transdermal Nitroglycerine Patches Used for Patch Cell Method Evaluation

Manufacturer	Product Name	Surface area (cm^2)	Labeled content (mg)
Ciba	Transderm-Nitro	20	50
Key	Nitro Dur II	20	80
Wyeth	Deponit	30	32

Patch cells were prepared as shown in Figure 1A, and as follows: a sheet of polyester film was cemented to the Teflon template using GE RTV™ silicone rubber adhesive. The device under study was adhered to the film, active surface down, by using Scotch™ double-sided cellophane tape on the delivery device backing. The receiver compartment was almost completely filled with de-gassed saline before the Teflon template was put in place. Once the Teflon template, containing the delivery device, was in place, the Lexan cover was placed over the clamping device and the Patch Cell assembly was secured by tightening the 4 wing nuts. Residual air was purged by connecting a 20 mL syringe filled with de-gassed saline to Port B and purging the air through Port A.

Once assembled, each Patch Cell was placed on a Wheaton Biostir console (See Figure 2 for Patch Cell Placements). Water bath connections were made and the sampling ports connected to the robotic system so that fresh, drug-free saline could be delivered from the reservoir to the Patch Cell via port A, and the drug-enriched receiver fluid would be displaced through port B for collection at the Sample Collection Station. Samples were then collected over a 24 hour period, as described above. For the reproducibility studies, 24 Patch Cells were prepared as above using the 10 cm^2 Transderm Nitro-5 patches.

The Patch Cell Method was used to measure the rate of *in vitro* percutaneous absorption of nitroglycerine from commercial transdermal delivery systems using Yucatan miniature pig skin, hairless mouse skin and human stratum corneum-epidermis. Pig skin was attained frozen from Charles River Laboratories and was maintained at -70° C until needed. The pig skin was thawed and dermatomed to a thickness of 0.030 to 0.050 inches using a Zimmer air-driven dermatome. Male hairless mice, strain Skh, were obtained from Charles River. Skin was prepared fresh from mice euthanized by CO_2 asphyxiation. Dorsal skin was used throughout the testing. Human skin was obtained from NDRI (National Disease Research Interchange), of Philadelphia. Human skin came from autopsy (abdominal) and/or surgical (reduction mammaplasty) procedures. Stratum corneum-epidermis (SCE) was harvested according to a previously described technique (8), and stored frozen until needed.

In vitro percutaneous absorption testing required a modification in the Patch Cell set-up, as shown in Figure 1B. A proper sized aperture (just slightly larger than the delivery device area) was cut in the polyester film. The film was then cemented to the Teflon template using GE RTV silicone rubber adhesive. The hairless mouse skin or dermatomed pig skin was then cemented, dermis down, onto the polyester film using Permabond 910 adhesive. Care was taken not to have adhesive spill onto the dermal side of the skin. Hair was carefully scissored away from the pig skin in the area in which the delivery device would be adhered. The delivery device was then adhered to the skin and the Patch Cells were assembled and sampled as described above. For human SCE testing, the patch was first placed over the SCE, and then the SCE-patch laminant was cemented to the polyester film.

Analytical

Collected samples were analyzed for TNG using the following reverse phase, isocratic HPLC separation method: The mobile phase was 50:50 methanol:water pumped at 1.5 mL/minute[15]. Samples (40 µL) were injected onto a 15 cm x 4.6 mm ID, Supelcosil, LC-8, 5 micron analytical column[16] using an autoinjector with a 200 µL sample loop[17]. Detection

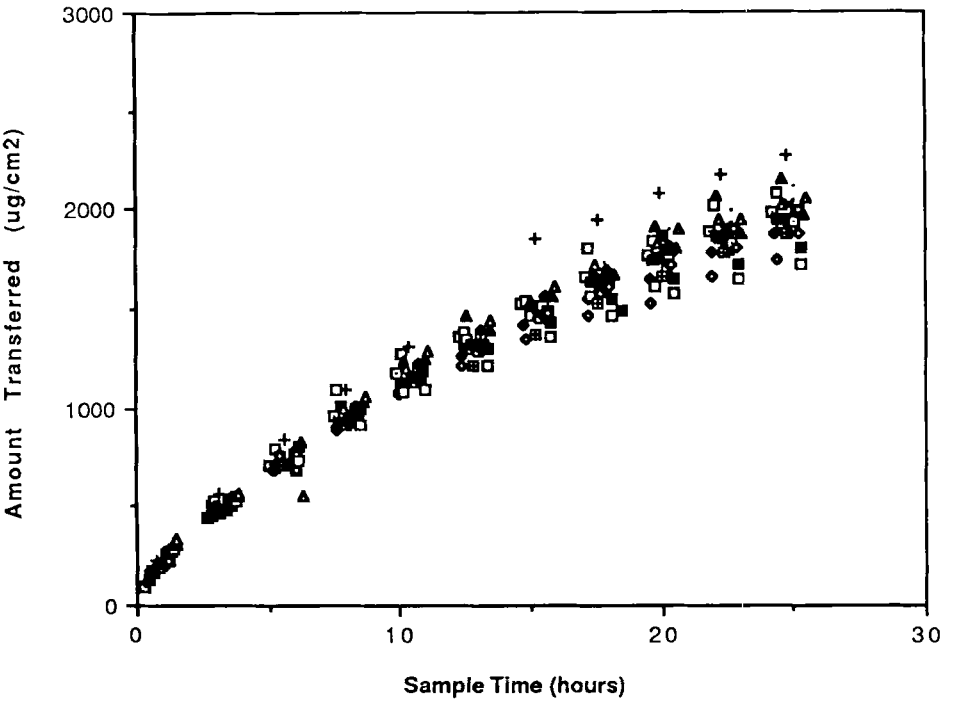


FIGURE 4

Reproducibility of TNG Flux Measurements Directly into Saline. Transderm Nitro-5 ($10cm^2$) patches used in 24 Patch Cells, 11 samples of 21ml each were taken from each Patch Cell.

of the TNG peak at 210nm was accomplished using a UV detector equipped with a deuterium lamp[18]. The TNG peak typically eluted between 4.5 and 5 minutes.

RESULTS and DISCUSSION

The reproducibility of the Patch Cell Method was evaluated using Transderm Nitro-5 releasing directly into water (Figure 4).

An analysis of the variance components (Table 2) indicates that there is significant between cell variability ($p < 0.001$). However, the between cell variability is significant only because the within cell variability is so small and the total system variability is only 3%. The

TABLE 2

Sample Volume Reproducibility
Using 24 Transderm Nitro Patches

Sample Time (hr)	Intercellular Mean	Intercellular Std Dev	Intercellular % CV
1.0		0.34	1.61%
3.4		0.51	2.43%
5.8		0.55	2.66%
8.2		0.61	2.94%
10.6		0.62	3.00%
13.0		0.65	3.16%
15.4		0.68	3.28%
17.8		0.71	3.43%
20.2		0.70	3.38%
22.6		0.73	3.53%
25.0		0.72	3.48%

Overall Mean: 20.73 (n=262)

Breakdown of Variance Components:

Estimate	% Total	Relative S.D.	
Between Cell	0.3425	86%	2.8%
Within Cell	0.0571	14%	1.2%
Total Variation	0.3996	100%	3.0%

total system variability is an indication of the sampling procedure precision. The overall mean volume of 20.73 +/- 0.62 mL represents 262 samples, taken over 24 hours from 12 Patch Cells, and is greater than 98% of the targeted 21 mL sample volume.

The Patch Cell Method was used to evaluate the release of nitroglycerine from three commercially available patches directly into water. These results were then compared with the results reported for these same delivery system using the FDA dissolution bath method (1,7), Figure 5. There is some agreement between the results obtained with the two methods. Transderm Nitro patches, using membrane diffusion to control the rate of delivery, released 55-60% of the contained TNG over

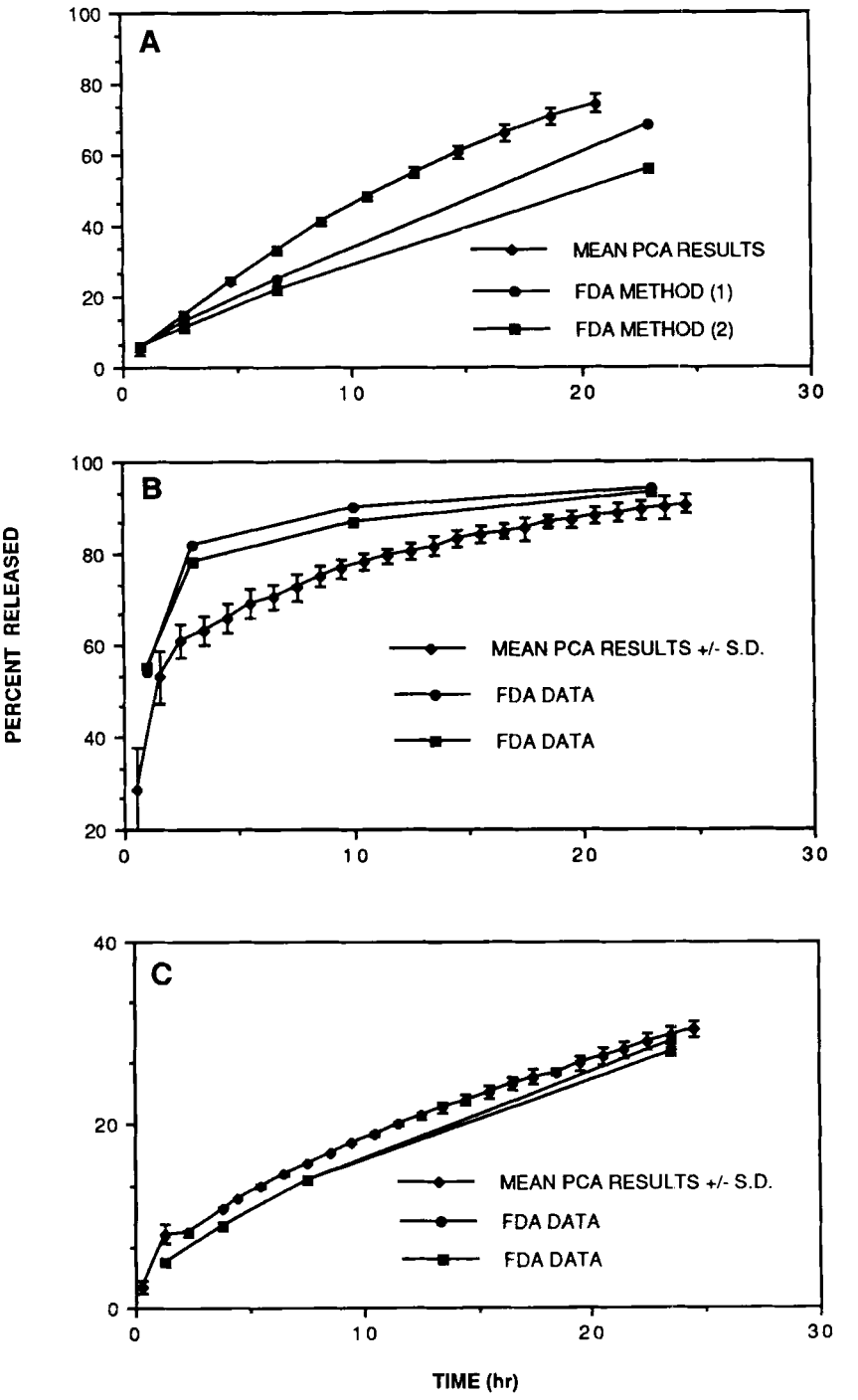


FIGURE 5

Mean Percent Label Release, +/- S.D. **A:** From Transderm Nitro-10 patch (50 mg nitroglycerine per patch). **B:** From Nitro Dur II patch (80 mg nitroglycerine per patch). **C:** From Deponit patch (32 mg nitroglycerine per patch).

TABLE 3

Release Rates ($\mu g/cm^2/hr$) into saline		
Patch Type	Keshary and Chien	Patch Cell Method
Transderm Nitro	94.0 +/- 4.1	86.6 +/-3.6
Nitro Dur II	1303.3 +/- 18.7*	1003.2 +/-139.9*
Deponit	18.0 +/- 1.09	14.3 +/-4.6

Release Rates ($\mu g/cm^2/hr$) Through Hairless Mouse Skin		
Patch Type	Keshary and Chien	Patch Cell Method
Transderm Nitro	23.6 +/- 2.9	30.2 +/- 3.3
Nitro Dur II	31.2 +/- 1.9	33.4 +/- 8.2 +/- 4.4

* = $\mu g/cm^2/hr^{1/2}$

the 24 hour test period with the diffusion bath method and 75% when tested with the Patch Cell Method, (Figure 5A). The matrix diffusion devices, Nitro Dur, are designed to rely on the intrinsic barrier properties of the skin to control the rate of transdermal delivery in a linear fashion (Q vs t), however, in the absence of the skin barrier, these devices release drug in a Q vs $t^{1/2}$ relationship. Release profiles obtained using the FDA dissolution bath and the Patch Cells were similar (Figure 5B), with approximately 90% of the nitroglycerin being released over the test period. The Deponit patches, representing another membrane controlled delivery device, released only 30% of the available TNG over the test period and represented the closest agreement obtained (Figure 5C). There is very good agreement with release testing into saline, relative to the results of Keshary and Chien (9), using their small volume diffusion cells, Table 3.

The results of *in vitro* percutaneous absorption from Transderm-Nitro and Nitro Dur II patches through pig and mouse skins, are shown in Figures 6 and 7. The results of testing the TNG delivery profile through human SCE are shown as Figure 8. The average release rate obtained for

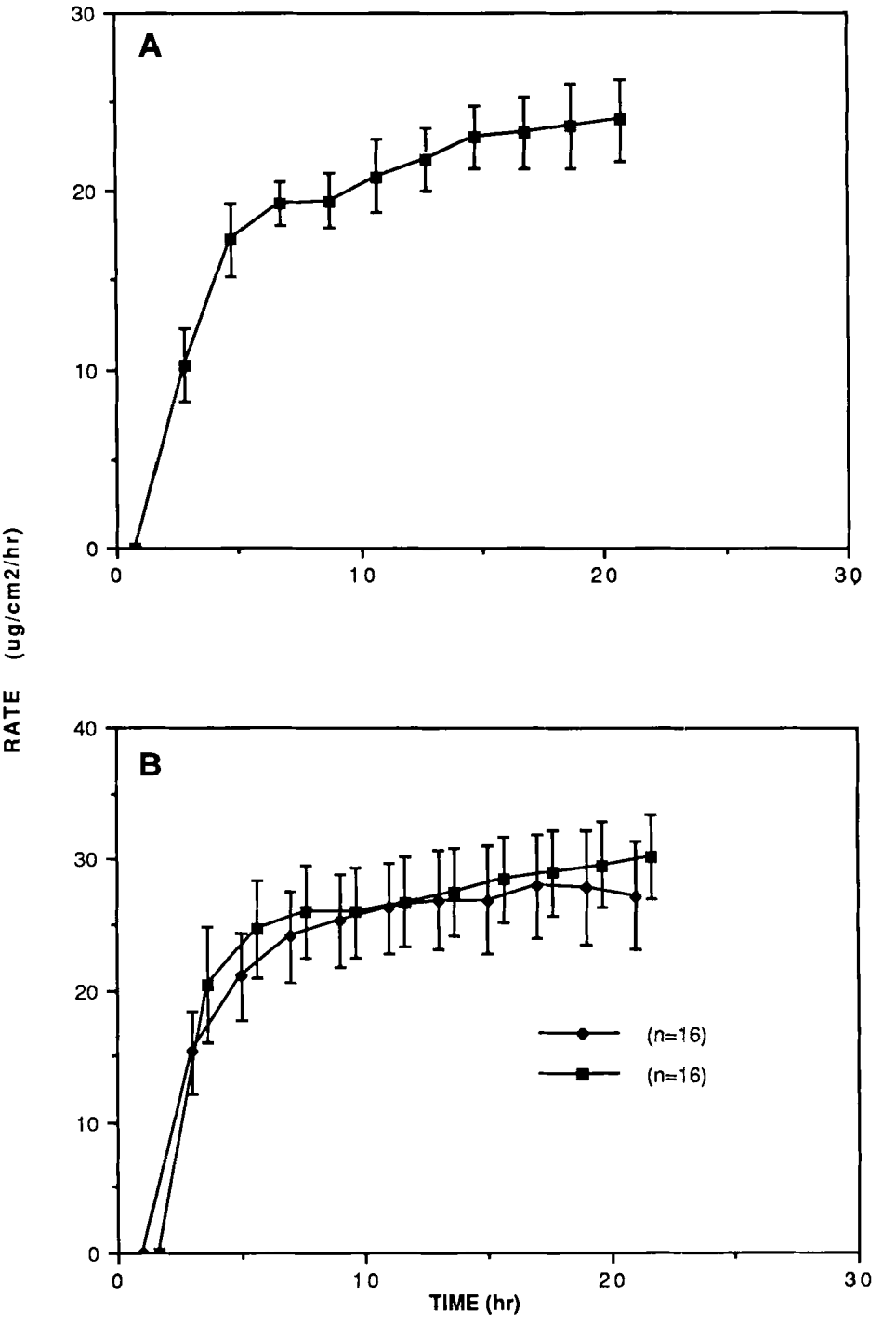


FIGURE 6

Mean Rate of TNG Absorption, +/- S.D., From Transderm Nitro-2.5 Patches. A: Through Yucatan Pig Skin (0.030 to 0.050 inches thick). **B:** Through Hairless Mouse skin.

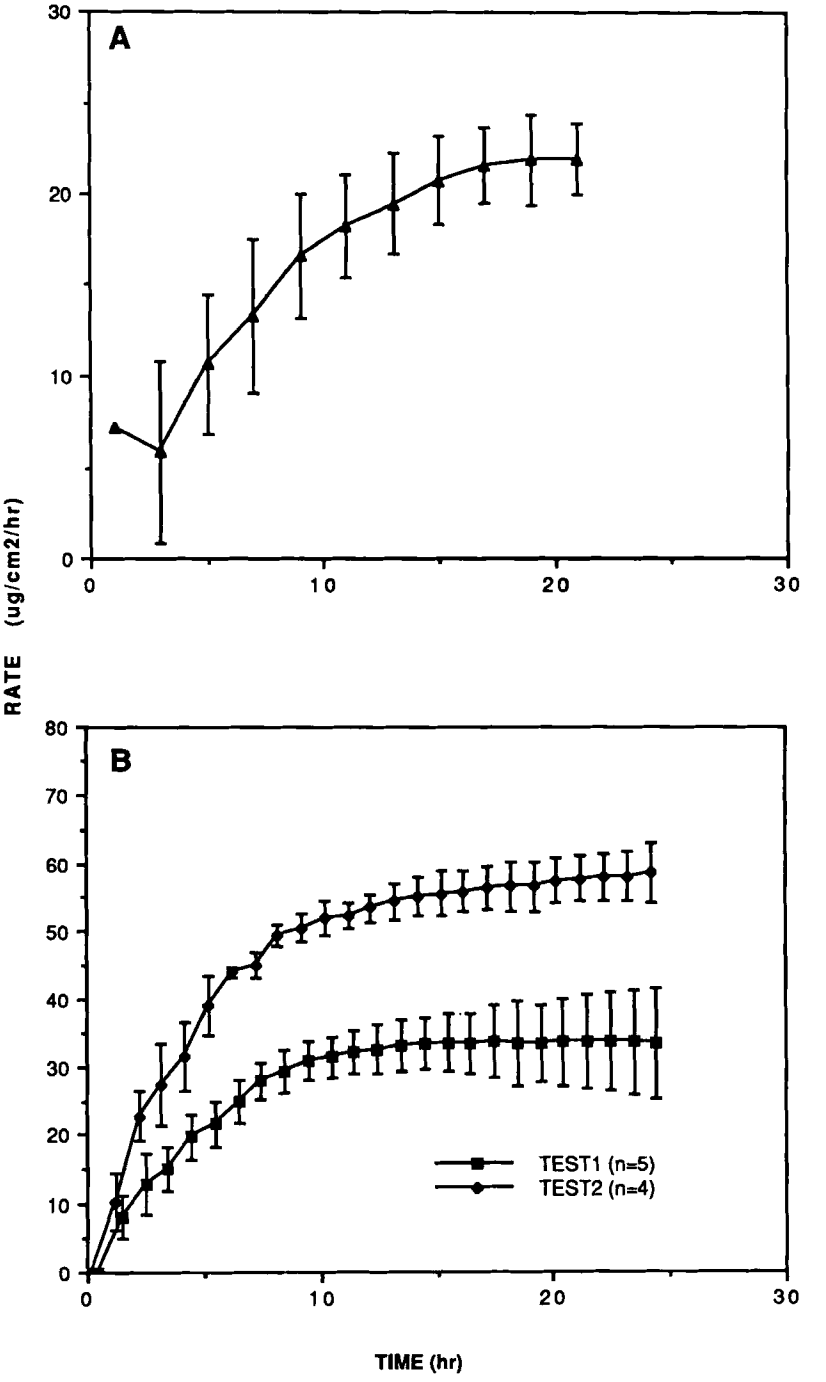


FIGURE 7

Mean Rate of TNG Absorption, +/- S.D., From Nitro Dur II Patches. A: Through Yucatan Pig Skin (0.030 to 0.050 inches thick). These data represent results from 6 different days using different skins each day. **B:** Through male hairless mouse skin. Five separate skins were used in TEST1 and 4 separate skins were used in TEST2.

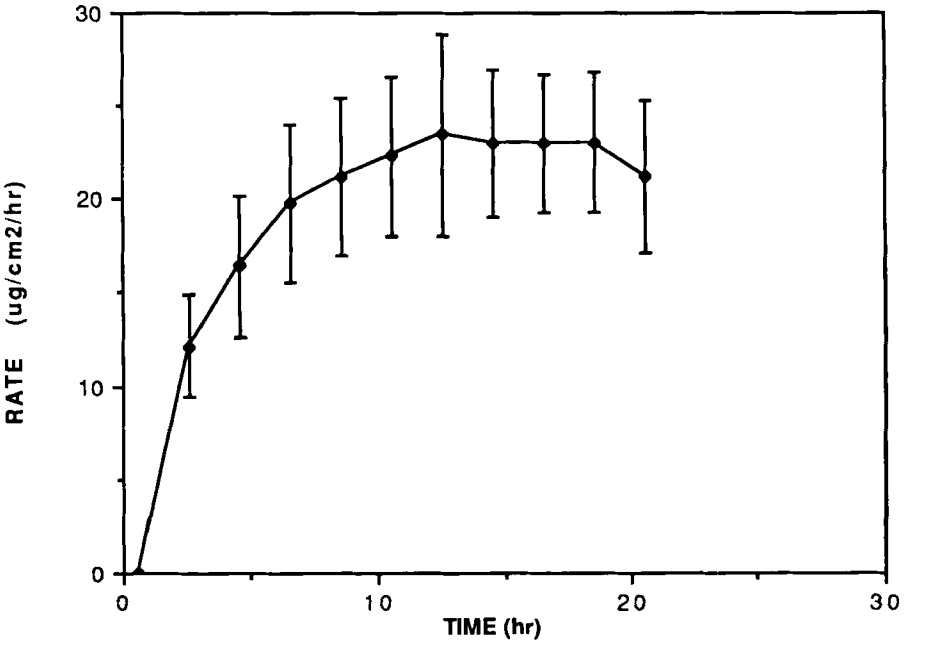


FIGURE 8

Mean Rate of TNG Absorption, +/- S.D., From Transderm Nitro-2.5 Through Human Stratum Corneum-Epidermis. Transderm Nitro-2.5 patches tested over human abdominal stratum corneum-epidermis sections.

the Transderm Nitro patches through pig skin was 25.4 +/- 2.37 $\mu g/cm^2/hr$, with a % CV of less than 10%. This agrees well with the rate of 21 $\mu g/cm^2/hr$ claimed on the label.

The delivery of nitroglycerine from a Transderm Nitro patch, through human, mouse, and pig skin is similar (Figures 6 and 8). However, mouse skin delivery rates of 30.2 +/- 3.3 $\mu g/cm^2/hr$ are consistently higher than those observed through pig skin. The data are in good agreement with the results reported by Keshary and Chien for release TNG flux through mouse skin from Transderm Nitro (Table 3). In addition, the *in vitro* delivery rate obtained through the human SCE, 21.16 +/- 4.11 $\mu g/cm^2/hr$, is in good agreement with the estimated *in vivo* delivery rate of 21 $\mu g/cm^2/hr$ claimed on the label.

The mouse skin results for Nitro Dur II (Figure 7B) show that the mouse skin consistently overestimated the label claim and produced poor day to day reproducibility. In the first experiment, TEST 1, the average rate at 24 hours was 33.40 +/- 8.22 $\mu g/cm^2/hr$, and for the second experiment, TEST 2, the average rate was 58.55 +/- 4.45 $\mu g/cm^2/hr$. Keshary and Chien measured the flux of TNG from the Nitro Dur system, through mouse skin, at 31.1 +/- 1.9 µg/cm2/hr (Table 3).

The Nitro Dur II patches tested through pig skin (Figure 7A) show good agreement with the label claimed delivery rate of 21 $\mu g/cm^2/hr$. Delivery rate for these patches was measured at 21.92 +/- 1.92 $\mu g/cm^2/hr$. The Nitro Dur II patches require a longer period of time to reach the steady delivery rate when pig skin is tested. This may be an artifact of skin preparation, i.e., if the dermatomed pig skin sample is too thick, or from a site on the pig with low TNG permeability, the rate of penetration through that skin may require a longer period of time to attain a steady-state.

CONCLUSIONS

The Patch Cell Method has demonstrated a very reproducible sampling technique. The method accurately measures TNG release rates from a variety of transdermal TNG delivery systems, directly into saline in a manner comparable to the FDA dissolution bath method, and percutaneously, through various skin types. Because of its size, the Patch Cell Method is particularly suited for testing using large samples of pig skin and thereby able to accommodate a large range in size of commercial and developmental nitroglycerine delivery devices, an advantage over current, smaller diffusion cells, which use hairless mouse skin. The Patch Cell Method therefore represents an acceptable method for measuring *in vitro* percutaneous absorption of nitroglycerine.

The integration of laboratory robotics has allowed unattended, continuous operation, freeing the operator from tedious sample collection procedures. Integrating the Zymate robotics with an IBM-PC has reduced the amount of manual data manipulation, thus improving

accuracy and productivity. The Patch Cell Method shows a high degree of reproducibility, both of sample volume withdrawn and flux values obtained from repeated sampling.

FOOTNOTES

1. Ciba Pharmaceutical Corporation, Summit, New Jersey.

2. Key Pharmaceuticals Inc. Miami, Florida.

3. Wyeth Laboratories, King of Prussia, Pennsylvania.

4. Glass Patch Cells, Glass Fabrication Laboratory, Building 82, Kodak Park, Rochester, New York.

5. Teflon-Coated Stir Bars, Cole-Parmer, Chicago, Illinois.

6. Teflon template, Lexan Covers, Stainless Steel Retaining Rings, Custom Sampling Port, and Polyester Sheets, Auto Machine Systems, Building 605, Kodak Park, Rochester, New York.

7. Gauge Lab Pipetting Cannula, Popper and Son, New Hyde Park, New York.

8. Robotic Components, Zymark Corporation, Hopkinton, Massachusetts.

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16. Analytical Columns, Supelco, Bellefonte, Pennsylvania.

17. Gilson Model 231 Autosampler with Model 401 Diluter, Gilson Medical Electronics, Middleton, Wisconsin.

18. Kratos Spectroflow 757 Detector, Kratos Analytical, Ramsey, New Jersey.

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APPENDIX I

Equations used in the Patch Cell Assay

Receiver Concentration (μg/mL):

For the initial time point: $RC_{(ti)} = (SV_{(ti)} \times SC_{(ti)})/SV_{(ti)} - TV)$

For time points where (ti) > 1:

$$RC_{(ti)} = ((SV_{(ti)} \times SC_{(ti)}) - (TV \times RC_{(ti-1)}))/(SV_{(ti)} - TV)$$

Where $RC_{(ti)}$ = Receiver concentration (μg/mL) at time (ti)
$SV_{(ti)}$ = Sample Volume (mL) at time (ti)
$SC_{(ti)}$ = Sample concentration (μg/mL) at time (ti)
TV = Tube Volume (mL)

(μg) of Drug in Sample, $ST_{(ti)}$: $ST\ (ti) = SV_{(ti)} \times SC_{(ti)}$

Where $ST_{(ti)}$ = μg in sample removed at time (ti)
$SV_{(ti)}$ = Sample volume (mL) at time (ti)
$SC_{(ti)}$ = Sample concentration (μg/mL) at time (ti)

Drug Released (mg/cm^2):

For the initial sample (ti=1): $Total_{(ti)} = ((CV \times RC_{(ti)})/A)/1000$

For time points where (ti) > 1:

$$Total_{(ti)} = (((CV \times RC_{(ti)}) + (TV \times RC_{(ti-1)}) + \Sigma ST_{(ti)})/A)/1000$$

Where $Total_{(ti)}$ = Total drug released at (ti) in mg/cm^2
CV = Cell volume in mL
$ST_{(ti)}$ = μg of drug in sample removed at time (ti)
$RC_{(ti)}$ = Receiver drug concentration (μg/mL) at (ti)
TV = Tube volume, from cell to sample port, in mL
A = The area of the patch (or aperture) in cm^2
n = Number of sample intervals performed at (ti)

Total Drug Delivered (mg): $TD(ti) = Total \times A$

Where $TD_{(ti)}$ = Total mg of drug delivered at (ti)

Total = total mg of drug delivered per cm^2

A = Area of patch or aperture in cm^2

Rate ($mg/cm^2/hr$): (for first order releasing devices)

$$DR = (Total_{(ti)} - Total_{(ti-1)})/((ti)-(ti-1))$$

$$DR = Total_{(ti)} / ti$$ (for steady state devices)

Where $DR_{(ti)}$ = Drug delivery rate at ti in $mg/cm^2/hr$

$Total_{(ti)}$ = Cumulative amount of drug delivered up to time = ti