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Evaluation of a novel diffusion cell for in vitro transdermal permeation: effects of injection height, volume and temperature

I.J. Bosman *, S.R. Avegaart, A.L. Lawant, K. Ensing, R.A. de Zeeuw

Groningen Institute for Drug Studies (GIDS), University Centre for Pharmacy, Department of Analytical Chemistry and Toxicology, A. Deusinglaan 1, 9713 AV Groningen, The Netherlands

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Abstract

The objective of this study was to evaluate the performance of a new, compact, dynamic diffusion cell for in vitro transdermal permeation. These so-called Kelder-cells were developed as an automated alternative to the static Franz diffusion cells. The new cells were used in combination with the ASPEC-system (automatic sample preparation with extraction columns) which was initially designed for the automation of solid-phase extractions. Three variables were tested to optimize the performance of the new cell system: injection height into the inlet compartment, volume flowing through the receptor compartment and temperature. Experiments were performed using the tritium labelled anticholinergic [^{3}H]dexetimide permeating through an artificial membrane (Silastic®). The injection height of the needle into the inlet compartment of the cell should be programmed at −34 mm to ensure complete air tightness, thus forcing the buffer to flow through the cell. The volume of buffer flow through the receptor compartment is important in maintaining sink conditions: a volume of 117 μl was chosen to replace the total content of the cell (84 μl) every 2 min. The temperature was precisely controlled in a thermostatic cabinet to minimize variations in experimental conditions. For [^{3}H]dexetimide, an increase in temperature of 20°C reduced the lag time by a factor of approximatley two, however the influence on the flux was negligible. The data for the Kelder-cells were comparable with static Franz diffusion cells at a pseudo-steady state, however Kelder-cells have the advantage of automatic sampling, continuous replacement of the receptor solution, and unattended operation over at least 24 h. © 1998 Elsevier Science B.V. All rights reserved.

Keywords: ASPEC-system; Automated dynamic sampling; Diffusion cell; In vitro; Kelder-cell; Silastic®

1. Introduction

In vitro skin permeation of a drug can be studied using a static diffusion cell or a dynamic flow-through cell [1–3]. The main difference is that in the flow-through cell the receptor fluid flows below the skin, so that saturation of this fluid with the chemical in question does not occur, as may happen with the static system [4].

One of the most widely used static designs for studying in vitro permeation is the Franz diffusion cell [1,2]. Permeation of chemicals is monitored by sampling the stirred receptor solution.

* Corresponding author. Present address: Faculty of Pharmacy, Department of Pharmaceutical Analysis, Sorbonnelaan 16, 3584 Ca, Utrecht, The Netherlands. Tel.: +31 50 3633341; fax: +31 50 3633311.

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This manual procedure is labour intensive, requires constant attention and sampling is often limited to normal laboratory hours.

Recently we developed a new, compact, dynamic diffusion cell for in vitro transdermal permeation [5]. The main objective in designing these so-called Kelder-cells was to develop an automated alternative for the Franz diffusion cells. The Kelder-cells were used in combination with the ASPEC-system (automatic sample preparation with extraction columns) which was initially designed for the automation of solid-phase extractions (SPE) [6]. The cells mimic the blood flow beneath the skin by replacement of the receptor solution every 2 min. The collection of the receptor solution is flexible and reproducible and the volume and flow rate through the receptor compartment of the cell can be varied.

In previous experiments, we focused on the development of the design and experiments which were not temperature controlled [5]. However, the temperature of an in vitro system should be controlled at a target temperature to minimize variation in experimental conditions. Therefore, the ASPEC-system was placed in a temperature controlled cabinet to provide a uniform temperature for the entire system.

The purpose of the present study was to evaluate the performance of the new cell system by testing the following variables: injection height into the inlet compartment, volume flowing through the receptor compartment and temperature. Experiments were performed with the tritium labelled anticholinergic [^{3}H]dexetimide, which was used in previous experiments as an internal standard to correct for variations in the skin [7,8]. An artificial membrane (Silastic®) was used as a model for skin. The results are discussed and compared with those obtained using the static Franz diffusion cell.

2. Experimental

2.1. Materials

[^{3}H]Dexetimide hydrochloride (12.6 Ci mmol^{-1}) was obtained from Janssen (Beerse, Belgium). 1-Dodecylazacycloheptan-2-one (Azone®) was kindly supplied by Nelson Research (Irvine, CA). Viton® O-rings were obtained from Eriks (Alkmaar, The Netherlands). All other chemicals and solvents were of analytical grade and obtained from Merck (Darmstadt, Germany). Polyethylene tubes (12 ml) were obtained from Greiner (Alphen, The Netherlands). Rialuma, used as the scintillation liquid, was obtained from Lumac (Olen, Belgium), in combination with mini-scintillation counting vials from Packard (Groningen, The Netherlands).

2.2. Preparation of solutions

Isotonic phosphate buffered saline pH 7.4 (PBS-buffer) was prepared by dissolving 8.00 g NaCl, 0.20 g KCl, 0.20 g KH_2PO_4 and 1.44 g $Na_2HPO_4 \cdot 2H_2O$ in 1 l distilled water. The drug solution of [^{3}H]dexetimide was prepared by mixing 20 μl of an ethanolic stock solution of [^{3}H]dexetimide (3 MBq ml^{-1}) with 430 μl of ethanol–propylene glycol–PBS-buffer–Azone® 60:20:15:5 v/v/v/v [9].

2.3. Preparation of Silastic® membranes

Non-reinforced silicone membrane (Silastic®, polydimethylsiloxane, type 500-1, Laboratoire

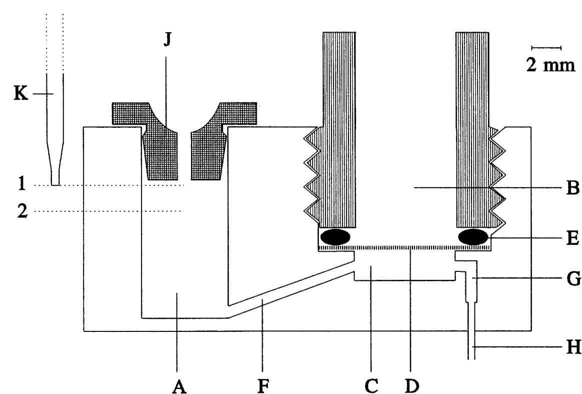

Fig. 1. Cross-section of the Kelder-cell: (A) inlet compartment; (B) donor compartment; (C) receptor compartment; (D) membrane; (E) O-ring; (F) inlet channel; (G) outlet channel; (H) outlet tube; (J) polypropylene cap; (K) needle; 1, injection height of − 32 mm; 2, injection height of − 34 mm.

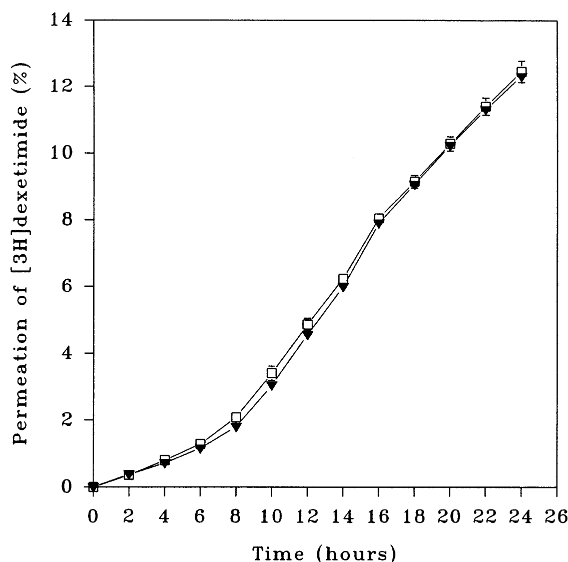

Fig. 2. Effect of injection height on the permeation of [^{3}H]dexetimide through Silastic® membranes at 22°C. □, − 32 mm ($n = 3$); ▼, − 34 mm ($n = 2$); each point represents the mean and SEM.

Perouse Implant, Bornel, France) of 0.125 mm thickness was extensively rinsed in hot distilled water (60°C) until all sodium bicarbonate (present on the surface to facilitate handling) was removed. This was followed by a thorough rinse in distilled water (20°C) for 1 h [10].

2.4. Permeation experiments with Kelder-cells

2.4.1. Kelder-cell

The Kelder-cells (University Centre for Pharmacy, Groningen, The Netherlands) were made compatible with the ASPEC-system (Gilson Medical Electronics, Villiers le Bel, France) and the system was placed in a temperature controlled cabinet. Five racks were placed in the polypropylene tray and five blocks, each containing four cells, were placed in these racks. The Kelder-cell consists of: (A) an inlet compartment; (B) a donor compartment; and (C) a receptor compartment (Fig. 1). A circular piece of membrane (D) with a diameter of 12 mm was cut and placed into the receptor compartment which was filled with PBS-buffer. A Viton® O-ring (E) was used to position the membrane between the donor and receptor compartment. The cell is very compact with a contact area of 0.51 cm^2 and a receptor compartment volume of 77 μl [5]. After 30 min of equilibration of the membrane with the PBS-buffer, 75 μl of the drug solution was applied in the donor compartment by means of a pipet. The donor compartment was covered with parafilm to prevent evaporation of the solvent. The inlet compartment of the cell was sealed with a polypropylene cap, suitable for injection purposes (J). For each cell, 12 polyethylene tubes (12 ml) were positioned below the cell, to collect subsequent fractions of the receptor solution with permeated chemical. The interval time between subsequent fractions, the dispensing volume and the flow rate of the buffer through the cell could be varied.

22.4.2. Permeation experiments

The ASPEC-system was programmed to run permeation experiments for 24 h, which means that samples were collected in each polyethylene tube every 2 h. To optimize the performance of the system, different variables were tested: injection height of the needle into the inlet compartment, dispensing volume of receptor fluid flowing through the cell, and temperature.

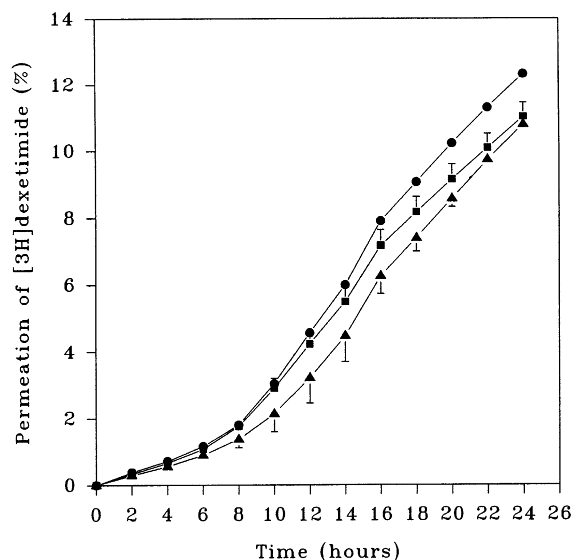

Fig. 3. Effect of collection volume on the permeation of [^{3}H]dexetimide through Silastic® membranes at 22°C. ▲, 4.5 ml ($n = 2$); ■, 7 ml ($n = 4$); ●, 9 ml ($n = 2$) collected in 2 h; each point represents the mean and SEM.

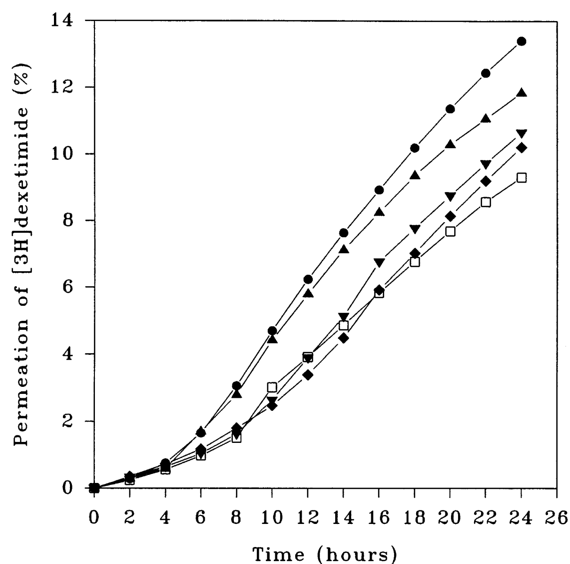

Fig. 4. Effect of temperature on the permeation of [^{3}H]dexetimide through Silastic® membranes. ▼, 22°C ($n = 3$); ◆, 27°C ($n = 4$); □, 32°C ($n = 5$); ▲, 37°C ($n = 4$); ●, 42°C ($n = 5$).

The injection height can be programmed to move the needle vertically up or down and this is important to ensure air tightness when the needle enters the polypropylene cap of the inlet compartment. To position the needle (Fig. 1K) at a lower level than height 0, which is defined as the position of the needle in the home position, a negative value should be programmed. The tested injection heights were −32 and −34 mm as shown in Fig. 1 (temperature 22°C, collection volume 9 ml).

The tested volumes collected during the 2 h in a polyethylene tube were 4.5, 7 and 9 ml, respectively (temperature 22°C, height −34 mm). This corresponds with 60 aliquots of 75, 117 and 150 μl injected into the cells sequentially every two min. The temperature experiments were performed at 22, 27, 32, 37 and 42°C (height −34 mm, collection volume 7 ml).

In all experiments, the flow rate through the injection needle was programmed at 50 μl s^{-1} and the interval time between subsequent rinsings of one cell was in the order of 2 min (discontinuous flow-through) [5].

2.5. Permeation experiments with Franz diffusion cells

These experiments were performed according to a previously described procedure [5]. The drug solution of [^{3}H]dexetimide was prepared by mixing 10 μl of an ethanolic stock solution of [^{3}H]dexetimide (3 MBq ml^{-1}) with 500 μl of ethanol–propylene glycol–PBS-buffer–Azone® 60:20:15:5 v/v/v/v and 200 μl of this dosing solution was applied to the donor compartment [9].

2.6. Analytical procedure

To determine the concentration of [^{3}H] dexetimide in the receptor solution, 1 ml of the receptor solution sample was added to mini-scintillation vials and mixed with 3.5 ml Rialuma. The vials were counted for 40000 counts or 5 min in a liquid scintillation counter (Minaxi, Packard, Groningen, The Netherlands), whichever came first.

2.7. Data analysis

Using Kelder-cells, the amount of permeated drug was calculated based on the measured concentrations in the receptor compartment, and the collected volume. Using Franz diffusion cells, the measured concentrations were corrected for the dilution during sampling. The results of the permeation experiments were plotted in graphs showing the percentage of permeated [^{3}H]dexetimide versus time. Flux values were calculated by linear regression from the steady state portion of the permeation curve. The lag time was determined by extrapolation of the steady state portion of the curve to the intercept of the time axis. The calculated values were presented as means and SEM.

3. Results and discussion

3.1. Effect of injection height, volume and temperature

To optimize the performance of the Kelder-cells, different parameters were varied in the pro-

Table 1
Permeation data of [^{3}H]dexetimide at different temperatures

Sampling time (h)	Percentage of permeated [^{3}H]dexetimide				
	22°C ($n = 3$)	27°C ($n = 4$)	32°C ($n = 5$)	37°C ($n = 4$)	42°C ($n = 5$)
2	0.33 (0.01)	0.35 (0.05)	0.25 (0.01)	0.30 (0.06)	0.28 (0.02)
4	0.65 (0.01)	0.736 (0.06)	0.56 (0.02)	0.59 (0.12)	0.75 (0.04)
6	1.04 (0.01)	1.17 (0.10)	1.00 (0.04)	1.70 (0.39)	1.65 (0.07)
8	1.62 (0.08)	1.80 (0.06)	1.51 (0.08)	2.80 (0.67)	3.06 (0.10)
10	2.63 (0.23)	2.47 (0.12)	3.02 (0.09)	4.42 (0.89)	4.70 (0.10)
12	3.89 (0.33)	3.39 (0.16)	3.92 (0.08)	5.80 (1.06)	6.24 (0.12)
14	5.13 (0.36)	4.48 (0.17)	4.86 (0.22)	7.12 (1.14)	7.63 (0.20)
16	6.76 (0.34)	5.92 (0.35)	5.84 (0.38)	8.24 (1.22)	8.92 (0.31)
18	7.77 (0.31)	7.02 (0.30)	6.77 (0.54)	9.35 (1.31)	10.18 (0.38)
20	8.74 (0.27)	8.14 (0.26)	7.68 (0.67)	10.28 (1.38)	11.37 (0.42)
22	9.71 (0.28)	9.19 (0.26)	8.56 (0.67)	11.06 (1.46)	12.44 (0.47)
24	10.64 (0.31)	10.20 (0.29)	9.30 (0.68)	11.84 (1.52)	13.40 (0.51)
Flux[a] (pmol cm^{-2} h^{-1})	0.244 (0.013)	0.230 (0.008)	0.200 (0.025)	0.236 (0.025)	0.271 (0.017)
Lag time[b] (h)	4.87 (0.45)	5.30 (0.39)	3.73 (0.62)	2.17 (0.06)	2.49 (0.42)

Values are means and SEM.
[a] No significant differences between temperatures: one-way ANOVA, $P = 0.15$.
[b] Significant differences between temperatures: one-way ANOVA, $P = 0.012$.

gramming of the ASPEC-system. One important parameter is the injection height of the needle into the inlet compartment of the cell. The range in varying this height is narrow because if the needle moves too deep into the inlet compartment, it will be jammed. However, if the needle does not move deep enough, the buffer cannot be forced to flow through the cell. Fig. 2 shows the mean cumulative percentage of [^{3}H]dexetimide permeation, using two different injection heights. For the tested heights, -32 and -34 mm, no significant differences were found in lag times (4.6 ± 0.7, 5.2 ± 0.1 h, Student's t-test, $P = 0.30$) nor in flux values (0.25 ± 0.02, 0.26 ± 0.01 pmol cm^{-2} h^{-1}, Student's t-test, $P = 0.59$). In further experiments, we used a height of -34 mm to ensure complete air tightness of the cells.

Another important parameter is the volume of buffer pumped through the receptor compartment of the cell. In order to maintain sink conditions, the volume pumped through the cell in a given time should be significantly greater than the volume of the receptor compartment [11,12]. In Fig. 3 the permeation of [^{3}H]dexetimide is shown, using three different volumes of 4.5, 7 and 9 ml, respectively, collected in 2 h. The results indicate that with an increase in collection volume, the permeation of [^{3}H]dexetimide increases. However, comparison of the three volumes, 4.5, 7 and 9 ml, showed no significant differences in lag times (6.3 ± 1.2, 4.9 ± 0.6, 5.2 ± 0.1 h, one-way ANOVA, $P = 0.51$) nor in flux values (0.24 ± 0.02, 0.23 ± 0.02, 0.26 ± 0.01 pmol cm^{-2} h^{-1}, one-way ANOVA, $P = 0.14$). In further experiments we collected 7 ml fractions in 2 h, which corresponds with 60 injections of 117 μl aliquots. Using this volume, the total content of the cell (84 μl) is replaced by fresh buffer every 2 min.

Temperature control is also an important factor in permeation experiments. Many of the diffusion cells in use have a water jacket to control the temperature. However, only the receptor compartment is thermostated when using these type of cells [1,2]. We performed temperature experiments with [^{3}H]dexetimide in a thermostatic cabinet which controlled the temperature of the entire system precisely (± 1°C). Fig. 4 shows the mean cumulative percentages of [^{3}H]dexetimide permeation at five temperatures, and for reasons of clarity the corresponding SEM's are presented in

Table 1. The results show that at 37°C, the SEM's are much higher compared to other temperatures, which may be explained by a swelling of the Silastic® membrane [10].

With an increase in temperature, the lag time decreased from approximately 5 h at 22°C to 2.5 h at 42°C, however, the influence on the flux was negligible (Table 1). The decrease in lag time indicates that the diffusivity of [^{3}H]dexetimide through the membranes increases at higher temperatures (Table 1). As the flux values did not differ significantly, it is more likely that partitioning in the membrane changed due to alterations in thermodynamic activities in the donor compartment. Our results are not in line with the theoretical guideline which describes a 10°C rise in temperature producing a doubling in permeation [1,10,13]. However, other experiments showed that changes in permeability coefficients appear to be small for temperatures up to 70°C indicating that the overall effect on in vitro skin permeation is small [14].

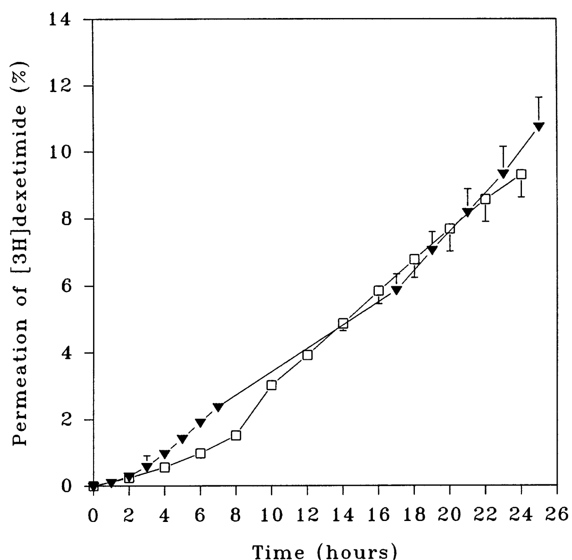

Fig. 5. Permeation of [^{3}H]dexetimide through Silastic® membranes using static Franz diffusion cells and Kelder-cells. □, Kelder-cell ($n=5$); ▼, Franz diffusion cell ($n=6$); each point represents the mean and SEM.

Table 2
Variability in [^{3}H]dexetimide permeation through Silastic® membranes using Franz diffusion cells and Kelder-cells

Sampling time (h)[a]	Coefficient of variation[b]	
	Kelder-cells ($n=5$)	Franz diffusion cells ($n=6$)
1	—	38.5
2	9.2	22.9
3	—	13.7
4	8.0	12.3
5	—	7.9
6	9.4	10.0
7/8	12.4	8.8
10	7.0	—
12	4.3	—
14	9.9	—
16/17	14.7	19.9
18/19	17.7	19.0
20/21	19.4	20.5
22/23	17.4	21.4
24/25	16.4	20.0

[a] Sampling time is every 2 h for the Kelder-cells; other time intervals are sampling times for Franz diffusion cells.
[b] Coefficient of variation = SD/mean × 100%.

3.2. Comparison of Kelder-cell and Franz diffusion cell

The Kelder-cell and the static Franz diffusion cell were compared using the compound [^{3}H]dexetimide. The receptor compartments of the Franz diffusion cells were kept at 37°C through an external water jacket which may result in a membrane temperature of approximately 32°C [13]. Therefore, we compared these data with the experiments on the Kelder-cells performed at 32°C (Fig. 5). In the beginning of the curves, the two cell types differed ($t=4$ and 6 h, Student's t-test, $P<0.05$), however if non-sink conditions should occur using Franz-cells, this would result in a lower rather than a higher permeation. In the latter end of the curves no significant differences were found (Student's t-test, $P>0.05$). It is likely that the amount of [^{3}H]dexetimide which permeated through the membrane was too small to require continuous replacement of the receptor solution. The variability in the data expressed as

the coefficient of variation was also comparable for the two cell types (Table 2).

Thus, the data of the Kelder-cells were comparable with static Franz diffusion cells in a pseudo-steady state. However, Kelder-cells offer the advantage of automatic sampling, continuous replacement of the receptor solution and unattended operation over at least 24 h. Further studies with other drugs are warranted.

4. Conclusions

The injection height of the needle into the inlet compartment of the cell should be programmed at -34 mm to ensure complete air tightness and force the buffer to flow through the cell. The volume of buffer pumped through the cell is important to completely remove the permeated chemical: a volume of 117 μl was chosen to replace the total content of the cell (84 μl) by fresh buffer every 2 min. The temperature of the entire system was precisely controlled in a thermostatic cabinet. Equivalent data in pseudo-steady state were produced using Kelder-cells and Franz diffusion cells.

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