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An automated diffusion apparatus for studying skin penetration

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Summary

An automated diffusion apparatus was developed, holding 24 stainless steel diffusion cells with flow-through receptor solution. The apparatus can be used for pseudo-steady-state or in vivo mimic procedures, and requires only small samples of skin. To validate the apparatus, the absorption was determined of water, methanol, ethanol, butanol and hexanol from aqueous solution under pseudo-steady-state conditions; the permeability coefficients increased with carbon number. We measured the permeation of mannitol from dimethylisosorbide–water (DS : water) vehicle and deposited drug films, and the effects of N-methyl-2-pyrrolidone (NP) and 2-pyrrolidone (2P), using pseudo-steady-state and in vivo mimic conditions. Although penetration from the deposited mannitol film was low, penetration from DS : water and from NP and 2P was similar, indicating that NP and 2P had not accelerated mannitol permeation. Both investigations provided information which correlated favourably with the literature, thus confirming the validity and usefulness of the automated apparatus.

Introduction

After a patient applies a formulation to the skin, many changes can occur, e.g. vehicle components may evaporate, be absorbed into the skin, or tissue fluids may dilute the preparation. Such processes will affect the properties of the stratum

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corneum, the chemical potential and thermodynamic activity of the drug and thus its permeation behaviour (Barry, 1983). The 'in vivo mimic' open-cell (Scheuplein and Ross, 1974; Franz, 1978; Akhter et al., 1982; Southwell and Barry, 1981) which employs a donor chamber exposed to controlled room temperature and humidity and maintains the receptor at body temperature, provides a technique which produces reliable data under conditions closely resembling normal in vivo situations. The material under investigation may be applied as a deposited drug film by evaporation of volatile solvent, as a liquid, as a semisolid or even as a drug device, e.g. transdermal therapeutic system. Cumulative penetration profiles can then be obtained with many data points by regular, frequent sampling (Akhter and Barry, 1984). However, this process entails repetitive sampling and receptor replenishment —which is tedious and time-consuming and has in our work required the presence of the investigator day and night for more than 2 weeks—together with a plentiful supply of human skin.

To overcome some of these problems we modified and developed a system of continuous flow-through cells combined with an automated fraction collector, based on a design of Cooper (1981). To assess its value we investigated the absorption of water, methanol, ethanol, butanol and hexanol under pseudo-steady-state conditions and the penetration of mannitol (under pseudo-steady-state and simulated in vivo conditions).

Materials and Methods

Apparatus

The automated apparatus

The system comprises 24 stainless steel diffusion cells with glass bubble chambers, 4 cell carriers/heating units, 1 circular turntable tray directly linked to a spring ratchet drive unit, 4 quadrant intrays to accommodate 4 × 72 glass scintillation vials, 1 multichannel peristaltic pump and 1 automated multirange resetting timer.

Diffusion cells, bubble chambers and cell carriers

The stainless steel diffusion cells consist of an upper half (donor) and a continuous flow-through lower half (receptor) (Fig. 1). Human skin, either full thickness dermatomed or heat separated stratum corneum, is placed between the two halves which are screwed together to provide a pressure seal for the skin. Because of the spongy nature of the skin, the screws should be tightened 2 or 3 times daily for the first 2 days to maintain a good seal. The donor compartment provides a diffusional area of either 0.13 cm^2 or 0.049 cm^2, and opens out in a cone-shape towards a controlled environment (22 ± 1°C, 60 ± 5% R.H.). The small size is particularly useful for tiny skin specimens, e.g. scalp skin obtained from hair transplant clinics (Bennett and Barry, 1983). The receptors have cylindrical flow-through chambers of diameter 4 mm and height 2.9 mm or 2.5 mm × 2.9 mm. Solution is continuously flushed through the receptor cell from a multichannel peristaltic pump—the rate can

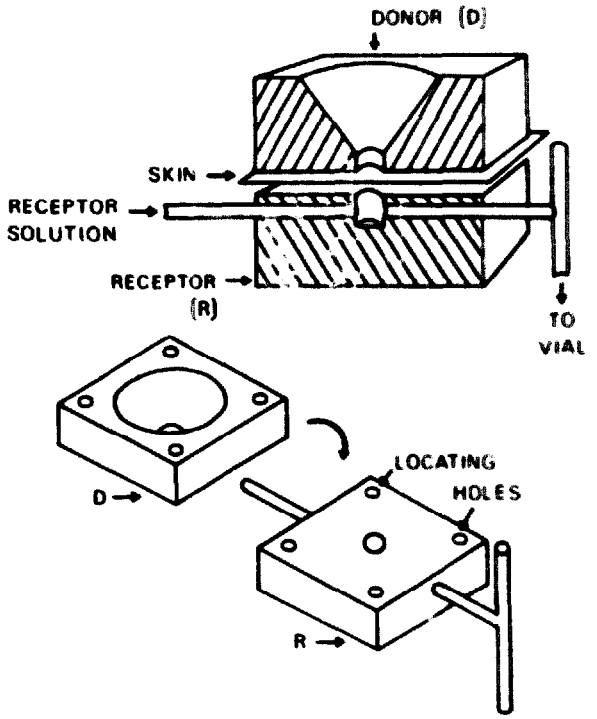

Fig. 1. Individual stainless steel diffusion cell.

be adjusted to maintain sink conditions (thus a flow rate of 1 ml · h $^{-1}$ would replace the receptor about 70 times per hour in the small cell). An additional refinement is to place small metal rods across the receptor cavity to reduce further the receptor volume and to promote turbulent flow to the dermal side of the skin and thus reduce static diffusion layers.

The receptor solution can develop air bubbles—even a single bubble trapped beneath the skin would significantly reduce the area available for drug permeation. Hence glass bubble chambers were designed to trap any air and they were linked to the cells and a 30-channel peristaltic pump (Watson Marlow-501S) via PTFE tubing. This design of bubble chamber works efficiently; however, it is also advisable to de-gas aqueous receptor solution. When using mixed solvent systems (e.g. ethanol/water) air bubbles become a major hindrance; the receptor solution may then be refluxed or sonicated and helium passed through the solvent before and during the experiment.

The diffusion cells are mounted on hollow copper carriers through which thermostated water passes; each carrier can support 6 diffusion cells and bubble chambers (Fig. 2). In our system 4 such carriers hold a total of 24 cells.

Rotating turntable, motor drive unit and vial trays

The receptor solution is collected in glass (or plastic mini) scintillation vials, accommodated in 4 quadrant vial trays (Fig. 3). Each tray holds 12 × 6 vials, is removable and made from Tufnol for ease of decontamination. They are slotted into

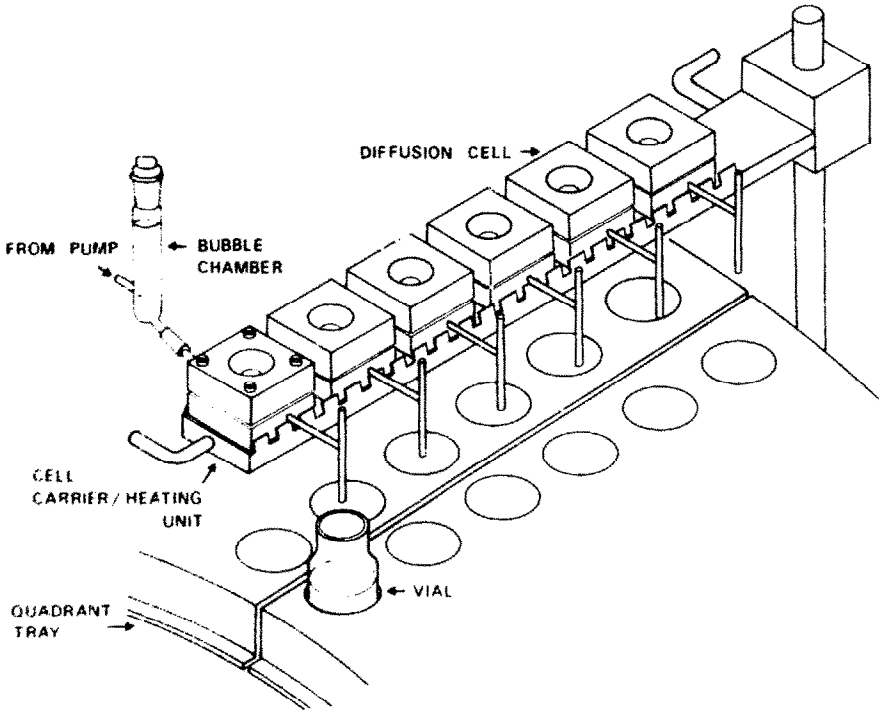

Fig. 2. Section of automated apparatus showing six diffusion cells in position on one cell carrier.

Fig. 3.

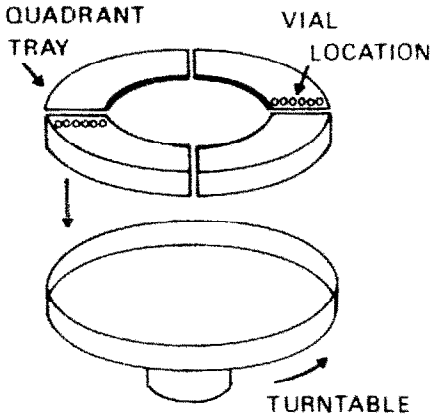

Fig. 3. Quadrant trays positioned in turntable.

Fig. 4.

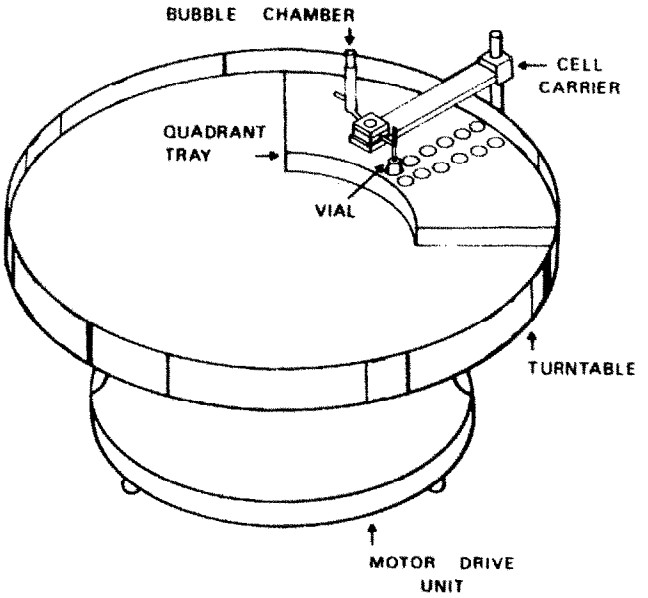

Fig. 4. The automated apparatus—quarter assembly of diffusion cells.

position on a main circular turntable, linked to a spring-loaded ratchet drive unit which moves the turntable only the distance of one vial row, with minimum vibration.

The overall system is represented in Fig. 4 which shows how a quarter of the total diffusion cells are set up.

The functioning system

To automate the system, a delay timer (Omron H2C—resetting) was linked to the peristaltic pump and the motor drive unit. The timer has minimum and maximum sampling times of 0.5 min and 30 h, respectively, and thus provides adequate time to preset the system to function by itself, for each day. The pump switches off for 1.0 min while the turntable moves on to collect the next sample; this prevents droplets of receptor solution falling onto the trays and missing the vials. Magnetic sensors prevent movement of the turntable beyond a preset angle, e.g. 90° or 360°. This stops samples becoming contaminated with further receptor solution from the next diffusion cell carrier, when more than one carrier is used.

Preparation of human skin

Strips of caucasian abdominal skin obtained at autopsy were stored at −24°C, then thawed, washed with warm water, clamped between two metal plates and refrozen so as to provide an even surface. The plates were removed and full thickness skin (~ 430 μm deep) was obtained by cutting the skin, when the dermis and epidermis were just mobile to the touch, with a Davies Dermatome (Duplex Electro Dermatome). The skin was hydrated in water for 4 h to avoid excess swelling when mounted in the diffusion cells.

Chemicals

[^{14}C]Mannitol, spec. act. 59 mCi · mmol^{-1}, [^{14}C]methanol, spec. act. 56.9 mCi · mmol^{-1}, [^{14}C]ethanol, spec. act. 61.6 mCi · mmol^{-1} and [^{3}H]water, spec. act. 5 mCi · ml^{-1} were obtained from The Radiochemical Centre (Amersham). [^{14}C]Butanol, spec. act. 10 mCi · mmol^{-1} and [^{14}C]hexanol, spec. act. 5.4 mCi · mmol^{-1} were obtained from ICN Pharmaceutics (California). The non-labelled compounds—mannitol, methanol, ethanol, butanol, hexanol, N-methyl-2-pyrrolidone and 2-pyrrolidone were from BDH Chemicals. Dimethylisosorbide was from ICI Americas; FisoFluor-1 liquid scintillator was supplied by Fisons Scientific Apparatus.

Investigations

Permeation of water and n-alcohols from aqueous solution; pseudo-steady-state diffusion

Full-thickness dermatomed skin was mounted in 4 stainless steel diffusion cells (diameter 2.5 mm) on one cell carrier in the automated apparatus (Fig. 2). The skin was covered with water for 3 days to hydrate fully, with frequent replenishment; cells were covered with Parafilm. The receptor phase, water, was pumped through the cells (flow rate 2.5 ml · h^{-1}), heated to 30 ± 1°C by the cell carrier.

Donor water was removed and a 0.75% v/v aqueous solution of hexanol (10 μl) was applied to each donor compartment and penetration monitored every 30 min for 6 h, with hourly replacement of donor solution.

Remaining hexanol solution was removed from the donor compartment and the skin was washed 5 times with distilled water, covered with water overnight and finally rinsed 5 times with water next morning. Butanol (10 μl, 0.75% v/v aqueous solution) was added to the washed skin and the penetration monitored as per hexanol. Similarly the permeation of ethanol, methanol and tritiated water was investigated in that order, using the same diffusion cells. Receptor samples were analyzed by liquid scintillation counting (Packard Liquid Scintillation Counter, Model Tri-carb 460C) in FisoFluor-1 scintillator. Control experiments confirmed that the alcohols did not damage the skin (Bennett and Barry, 1983).

From the pseudo-steady-state flux data (J) the permeability coefficients (k_p) were calculated using Fick's law, represented by eqn 1:

$$J = k_p \Delta C \quad (1)$$

where ΔC represents the alcohol concentration difference across the skin, (as sink conditions are assumed in the receptor, ΔC = donor concentration). The k_p values thus obtained were compared with those reported by Scheuplein and Blank (1973).

Penetration of mannitol from aqueous solution, deposited drug films and the effect of N-methyl-2-pyrrolidone (NP) and 2-pyrrolidone (2P); pseudo-steady-state and in vivo mimic diffusion

Full-thickness skin, mounted in 4 stainless steel diffusion cells (diameter 4 mm), was placed in the automated apparatus on one cell carrier (Fig. 2). The skin was equilibrated to room temperature (22 ± 1°C) and humidity (60 ± 5% R.H.) for approximately 20 h using a mixture of 50 : 50 ethanol–water for the receptor solution (flow rate 1 ml · h^{-1}).

A saturated solution (15 μl) of mannitol (0.05 μCi) in 50 : 50 dimethylisosorbide–water (DS : water) was placed in the donor compartment of the cells, which were covered with glass coverslips to minimize evaporation. The penetration of mannitol was monitored for 60 h with frequent donor replacement, samples being collected every 2 h. The coverslips were removed and mannitol penetration monitored for a further 60 h but without donor solution replenishment. During this period vehicle components evaporated and penetrated the skin, leaving a solid film of mannitol on the surface. Finally 10 μl of either NP or 2P was added to each of 2 diffusion cells, dissolving the remaining mannitol, and the penetration was monitored for 20 h at hourly intervals.

Receptor samples were analyzed by liquid scintillation counting as before. From the cumulative penetration profiles, rate (flux) and diffusion coefficient data (D) were obtained (the latter from lag time (τ) measurements using Eqn. 2, assuming the thickness (ℓ) of the stratum corneum to be 15 μm)

$$\tau = \ell^2/6D \quad (2)$$

This procedure assumes that the stratum corneum provides the rate-limiting step in the diffusion process.

Results and Discussion

Water and n-alkanol permeation

The permeability coefficients (k_p) for water and the *n*-alcohols obtained in this investigation (at $30 \pm 1°C$) and by Scheuplein and Blank (1973) at 25 °C are illustrated in Fig. 5 as a function of carbon number (zero carbon represents water). Fig. 5 shows that the k_p values increased exponentially with the molecular weight, because of the increased partition coefficient (stratum corneum : water) with chain length. The good agreement between our data and that of Scheuplein and Blank (1973) confirms the validity and usefulness of the automated system.

Mannitol permeation

Fig. 6 illustrates an example cumulative penetration profile for mannitol over 140 h permeation. It shows that the initial low penetration of mannitol from DS : water mixture reached steady-state after approximately 25 h. Removal of the cover slips allowed the vehicle components to evaporate as well as to permeate through the skin, and a mannitol film deposited on the skin surface. The penetration rate was reduced to a minimum due to slow dissolution of the deposited film, i.e. dissolution was the rate-limiting step during this period of permeation. However, addition of 2P

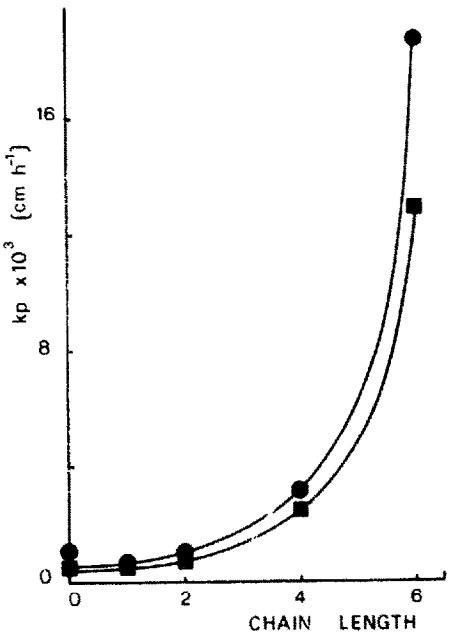

Fig. 5. Plot of permeability coefficients (k_p) for the *n*-alkanols, diffusing through human skin illustrating experimental data (●, $30 \pm 1°C$) and data of Scheuplein and Blank (1973) (■, 25 °C) as a function of chain length (zero carbon represents water).

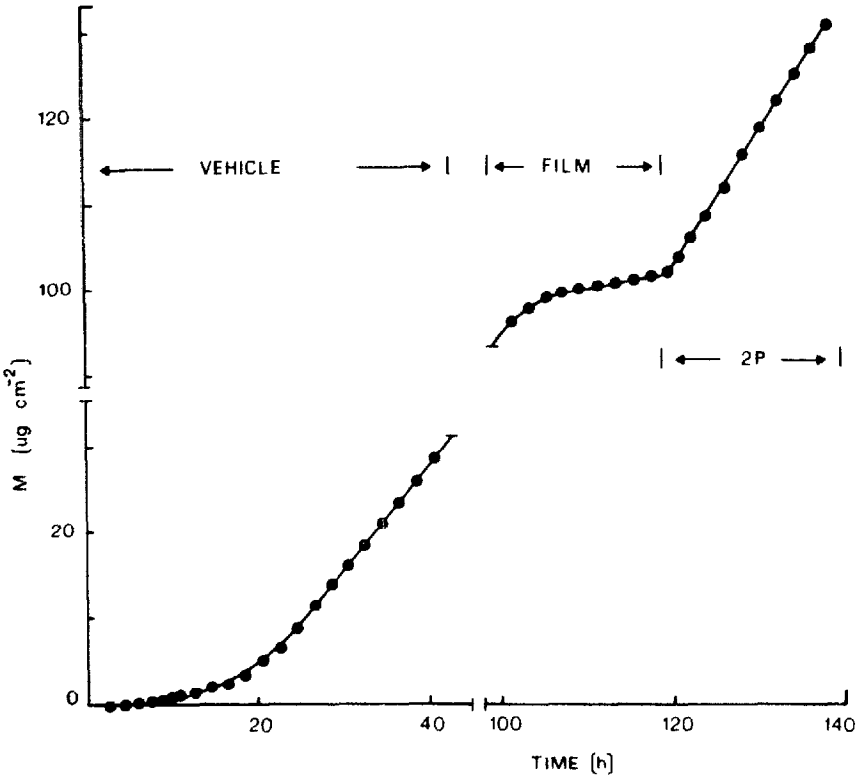

Fig. 6. Penetration of mannitol from saturated dimethylisosorbide–water vehicle (VEHICLE), deposited drug film (FILM) and the effect of 2-pyrrolidone (2P) treatment. Example data illustrate cumulative penetration profiles (M) as a function of time. (For clarity only every alternate experimental point shown.)

TABLE I

PENETRATION OF MANNITOL FROM SATURATED DIMETHYLISOSORBIDE–WATER VEHICLE (Vehicle), DEPOSITED DRUG FILM (Film) AND THE EFFECT OF N-METHYL-2-PYRROLIDONE (NP) AND 2-PYRROLIDONE (2P) TREATMENT. DATA OBTAINED BY LINEAR REGRESSION AND EXPRESSED AS FLUX (J), DIFFUSION COEFFICIENT (D) AND NUMBER OF REPLICATES (n)

Treatment	$J \times 10^{11}$ ($g \cdot cm^{-2} \cdot s^{-1}$)	$D \times 10^{12}$ ($cm^2 \cdot s^{-1}$)	n
Vehicle	26 ± 4.2 (0.999; 32–39) *	8.6 ± 2.1	4
Film	6.9 ± 2.2 (0.990; 7–9)		4
NP	31 (0.999; 19)	32	2
2P	42 (0.999; 17–21)	29	2

* Figures in parentheses refer to correlation coefficient and number of data points per experiment, respectively.

returned the steady-state flux to near its original value, as crystals redissolved and drug partitioned into the skin. The overall effect is displayed in Table 1, which reports pseudo-steady-state (from DS : water) and deposited drug film penetration rates, and diffusion coefficient (D) values. Table 1 also shows that NP and 2P had similar effects and they did not markedly enhance the flux of mannitol compared with DS : water steady-state data—although D values for NP and 2P were larger than DS : water values. This may have been because NP and 2P remained on the skin providing an unsaturated solution from which the mannitol permeated, but there was little penetration enhancement because of unfavourable partitioning of mannitol into the skin. Akhter and Barry (1983) have also reported this effect.

Conclusions

Some benefits of the automated apparatus are listed.

(1) The system is usable for pseudo-steady-state investigations, where the donor concentration is constant, with sink receptor conditions, and the experimental design is a typical physicochemical one.

(2) In vivo conditions can also be simulated, where the donor is exposed to a controlled room environment, with receptor maintained at body temperature. The compound may be applied as a drug film or as a thin liquid or semisolid layer which is allowed to change in concentration; the effect of dose size variation, multiple application, washing of skin and addition of vehicles can also be studied under conditions in which temperature and water gradients develop across the skin.

(3) Many replicates can be run, day and night, without investigator attendance.

(4) The diffusion cells use tiny amounts of skin (full-thickness or stratum corneum); therefore a typical autopsy specimen from the abdomen supplies samples for about 20 cells, instead of for only 3–4 conventional glass cells. Hence, a complete investigation may only require one specimen and thus have the additional benefit of no inter-specimen skin variation (Southwell and Barry, 1984).

(5) The apparatus can be used when tissue is available only in small pieces e.g. punch biopsies and hair transplant pieces.

(6) Since total receptor solution collects, sampling errors are reduced and also the amount of drug required for absorption studies.

Acknowledgements

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