

Report

Validation of a Flow-Through Diffusion Cell for Use in Transdermal Research

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A flow-through finite-dose diffusion cell has been designed for use in transdermal drug delivery research. The diffusion cell consists of an upper donor chamber and a lower receiver compartment through which a continuous supply of fresh solvent flows. The flow is directed to an automatic fraction collector. To validate the flow-through cell, its performance was compared directly against that of a conventional single-reservoir Franz cell. Homologous alkyl *p*-aminobenzoates were diffused through dimethylpolysiloxane membranes, and permeability coefficients increased with increasing chain length, reaching a plateau at the butyrate ester for both types of cells. This behavior suggests a shift from membrane-controlled diffusion to boundary layer control. Permeation of the butyrate and valerate compounds was significantly faster when the flow-through cell was used, suggesting that better mixing is obtained through the flow-through cell design. Considering the advantages offered in terms of time and labor saved through its use, the flow-through cell with automatic fraction collector appears to be a viable alternative to the conventional Franz cell.

KEY WORDS: transdermal; diffusion cells; flow-through; Franz cell.

INTRODUCTION

In vitro experiments are often performed that utilize finite-dose diffusion cells in the development of drug formulations for transdermal administration. Typically, a cell with a self-contained receiver compartment is used. An application containing a drug is layered over the upper, exposed surface of the membrane, and the permeant is collected beneath in a stirred compartment from which samples are periodically withdrawn. Alternatively, a flow-through receiver can be utilized. In this case, drug molecules passing through the membrane are carried away by the solvent flowing against the membrane undersurface to be collected in discrete volumes at a remote location. An automatic fraction collector works well to collect the efflux solvent.

As recently emphasized by Bronaugh and Stewart (1), a flow-through cell offers several advantages over static cells. Sampling is automatic, making the flow-through cell much more convenient to use, especially for experiments that are carried out over long periods of time. In addition, the continuous flow through the receiver helps to maintain sink conditions throughout the course of an experiment, a particularly important feature for compounds having large permeability coefficients through the membrane under consideration.

This paper reports on a flow-through diffusion cell designed and constructed for use in transdermal drug delivery research. In order to test this new cell, a direct comparison with a conventional cell was performed using a homologous series of *p*-aminobenzoate esters.

MATERIALS AND METHODS

Materials

Methyl *p*-aminobenzoate (Aldrich, Milwaukee, Wis.), ethyl *p*-aminobenzoate, and butyl *p*-aminobenzoate (Sigma, St. Louis, Mo.) were used as received. The propyl and pentyl esters were synthesized from *p*-aminobenzoic acid (Sigma) and the respective alcohols (Sigma). Reagent-grade ethyl ether (Sigma) and hexane (Fisher, Fair Lawn, N.J.) were used as solvents for purification and recrystallization. High-performance liquid chromatography (HPLC)-grade methanol (Mallinckrodt, Paris, Ky.) was used for all assays. Deionized water was used in the synthesis and as the receptor fluid in all permeation experiments. Unreinforced 0.0127-cm-thick dimethylpolysiloxane sheets (Silastic Sheeting, gift, Dow Corning, Midland, Mich.) were used as membranes.

Synthesis of *p*-Aminobenzoates

The propyl and pentyl esters were prepared using a modification of the method described by Vogel (2). Dry hydrogen chloride gas was bubbled through 1.37 mol of the respective alcohol until saturation was achieved, as indicated by no further increase in the weight of the solution. The flask containing the alcohol was maintained at 0°C in order to facilitate dissolution of the gas in the alcohol. The acidified alcohol was then poured into a round-bottom flask containing 0.088 mol of *p*-aminobenzoic acid. The flask was fitted with a column condenser and the mixture was refluxed for 6 hr. Upon cooling, the reaction mixture solidified as the hydrochloride of the ester.

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To remove unreacted acid, the solidified mass was dissolved in ethyl ether and washed three times with 300 ml of a 10% solution of sodium carbonate. The ether was evaporated, the resulting oily substance was placed into 300–500 ml of hexane, and the solution was allowed to sit overnight. The resultant crystals were filtered and placed in hexane. This medium was heated to solubilize the ester, whereupon activated charcoal was added. The charcoal was filtered out, and the resulting clear solution was placed in an ice bath for 2 hr. The formed crystalline product was vacuum filtered. The resultant white, needle-like crystals were placed in a desiccator under vacuum for 1 day to remove residual solvent. The propyl and pentyl esters melted sharply at 73 and 53°C, respectively, in agreement with literature values (3).

Assay

The alkyl *p*-aminobenzoates were assayed by HPLC, using UV detection at 285 nm. This technique was advantageous in that ester degradation could easily be detected through the appearance of *p*-aminobenzoic acid. The methanol:water ratio of the mobile phase varied for each ester: 45:55 (v/v) for methyl and ethyl *p*-aminobenzoate, 50:50 for the propyl ester, and 60:40 for the butyl and pentyl esters. Quantitation was by peak height ratio to a known internal standard which was chosen from the homologous series. Linear standard curves were obtained by first dissolving a known amount of a particular ester in 1000 ml of water. Appropriate dilutions were made and 100- μ l samples were injected onto the column. Peak height ratios were plotted against concentration, and linear regressions of the data were performed.

Solubility Studies

An excess of ester was mixed with water in a jacketed flask, and the resulting suspension was stirred at 25°C for 3 days. Samples were obtained via warmed, glass-wool-tipped pipettes, and each was then passed through a 0.45- μ m filter with the aid of a warmed plastic syringe. Five to ten milliliters of each sample solution was passed through a filter (Millipore, Bedford, Mass.) and then discarded prior to collection of a sample to condition the filter and minimize loss by adsorption. Samples were appropriately diluted with water and set aside for assay.

Permeation Studies

The Franz diffusion cell (Fig. 1) (Crown Glass, Somerville, N.J.) was used for the non-flow-through experiments. The cell had an effective area for diffusion of 0.785 cm², and the receiver compartment volumes ranged from 4.6 to 5.0 ml. The receiver compartment was filled with solvent, and the membrane was placed over the upper opening of the receiver, in contact with the liquid. A rubber o-ring was placed around the outer edge of the membrane and the upper cell cap was clamped into place. Small magnetic stirrers at the bottom of the receiver compartment stirred the contents.

The flow-through cell (Crown Glass) is also pictured in Fig. 1. The 1.0-ml receiver compartment has an inlet and an outlet to allow flow of solvent. In the operation of the flow-through cell, the receptor fluid (water) was pumped from a temperature-controlled reservoir into and through the cell

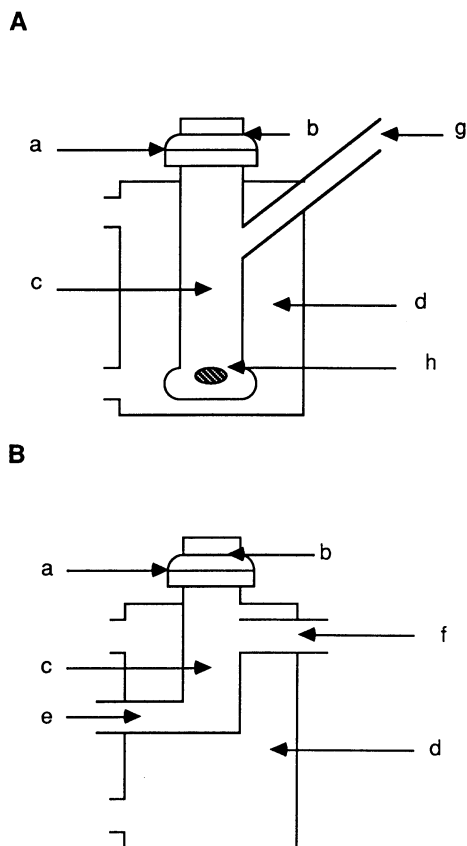


Fig. 1. Diagrammatic representations of the Franz diffusion cell (A) and flow-through cell (B). Cell components are as follows: (a) membrane; (b) donor compartment; (c) receiver compartment; (d) water jacket; (e) solvent inlet; (f) solvent outlet; (g) sampling port; (h) magnetic stir bar.

by a peristaltic pump (Rainin Rabbit, Rainin, Woburn, Mass.). After exiting the cell, the fluid enters a length of Teflon tubing, and the drops which emerge from the end of each of the tubings are collected in test tubes situated in an automatic fraction collector (Isco, Lincoln, Nebr.). The collector allows for simultaneous collection from a number of cells and replacement of test tubes with a fresh set at predetermined intervals. The distance traversed by the fluid in the outlet tubing was minimized so that the time of fraction collection correlates well with the time of membrane absorption. The effective area for diffusion for the flow-through cells ranged from 0.709 to 0.866 cm². Both the Franz cells and the flow-through cells were made of glass and were jacketed for temperature control. All experiments were carried out at 25°C. The receptor fluid consisted of filtered and degassed deionized water. Seven hundred microliters of an aqueous slurry of each compound was used as the donor phase. These slurries, which had been equilibrated to saturation by continuous stirring of an excess of the compound in water for 3 days at 25°C, were used in order to maintain a constant permanent concentration in the donor phase. In the Franz-cell experiments, the entire contents of a cell were exchanged for fresh solvent each time a sample was taken. For the flow-through cell, studies were performed at two different flow rates, 12 and 24 ml/hr, to ascertain the influ-

ence of flow rate on permeation. In each cell configuration sink conditions were approximated and steady-state kinetics were observed.

Data Reduction

In order to compare quantitatively the results obtained for both cell types, permeability coefficients were calculated via the following equation:

$$P_T = \frac{J_s}{C_s A} \quad (1)$$

where P_T is the permeability coefficient (cm/hr), J_s is the steady-state flux (mg/sec), C is the aqueous solubility of the permeant at 25°C (mg/ml), and A is the area for diffusion (cm²). The steady-state flux was obtained by performing linear regression of the cumulative amount permeated against time. Solubility experiments were performed at 25°C for the methyl and ethyl esters, and C_s values were found to be 1.57 and 1.02 mg/ml, respectively. Since these values were in excellent agreement with those reported by Shah and Nelson (4), the C_s values reported by these authors were used for the remaining esters in this study. At no time did the concentration of any compound in the receiver phase exceed 8% of the donor-phase concentration, and thus sink conditions were suitably approximated.

THEORETICAL CONSIDERATIONS

The permeability coefficients of homologous permeants diffusing through a lipid membrane follow (5):

$$P_T = \frac{KD_m D_{aq}}{h_m D_{aq} + \Sigma(h_{aq} K D_m)} \quad (2)$$

where D_m and D_{aq} are the membrane and aqueous diffusion coefficients, respectively; h_m and h_{aq} are the membrane and aqueous layer thicknesses, respectively; and K is the membrane/water partition coefficient. For polar compounds, K is small, and $h_m D_{aq} \gg \Sigma(h_{aq} K D_m)$. In this case,

$$P_T = \frac{K D_m}{h_m} \quad (3)$$

As the alkyl chain length is extended and K becomes large, $\Sigma(h_{aq} K D_m) \gg h_m D_{aq}$, and the aqueous layers control the diffusional process. In this circumstance, Eq. (1) reduces to

$$P_T = \frac{D_{aq}}{\Sigma h_{aq}} \quad (4)$$

RESULTS AND DISCUSSION

Relative concentrations vs time for the methyl through pentyl esters using both the Franz cell and the flow-through cell are shown in Figs. 2A and B, respectively. In all experiments the concentration buildup was normalized to the unit concentration by dividing the accumulated concentration of the ester by its solubility. Permeability coefficients calculated from steady-state rates are shown in Fig. 3. These initially increase with increasing chain length and reach a constant value at the butyrate ester. Within the cell types, no significant difference (Student's t test, $P < 0.05$) was observed between the permeability coefficients for the butyl

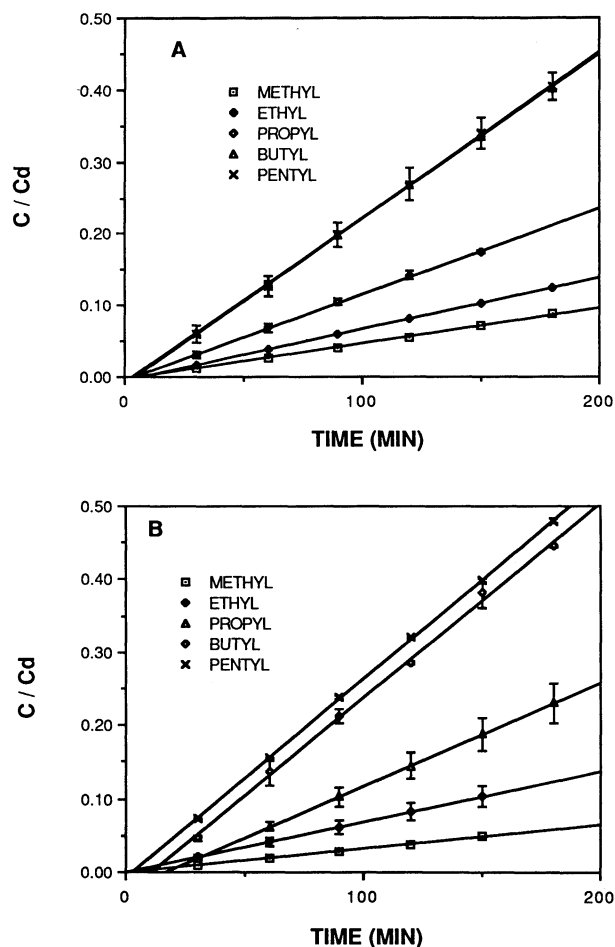


Fig. 2. Normalized concentration versus time plots for the methyl through pentyl esters using the Franz cell (A) and flow-through cell (B). Each data point is the mean value of three experiments. The bar denotes the standard deviation. See text for experimental details.

and pentyl esters. Comparison of permeability coefficients obtained for the two diffusion cells indicates that the values for the methyl and ethyl compounds are not significantly different between the cell types. The permeability coefficient calculated for the propyl ester at the 24-ml/hr rate was significantly higher than that observed for either the 12-ml/hr flow rate or the Franz cell. Permeability coefficients calculated at both flow rates in the flow-through design are much higher than those obtained with the Franz cell for the butyl and pentyl esters ($P < 0.05$).

Studies by Flynn and Yalkowski (5) indicate that there is a parabolic relationship between the chain length of the *p*-aminobenzoates and their permeabilities through silicone rubber membranes from saturated solutions. This behavior suggests that as the homologous series is ascended, there is a shift from a situation in which diffusion is controlled predominantly by the membrane to one in which permeation is controlled by aqueous diffusion layers. As the chain length is increased, a plateau region is reached for permeability coefficient values, signifying that diffusion across the diffusion layers has become the rate-controlling event. The permeability coefficient in the diffusion layer control region is inversely proportional to the summed thicknesses of both

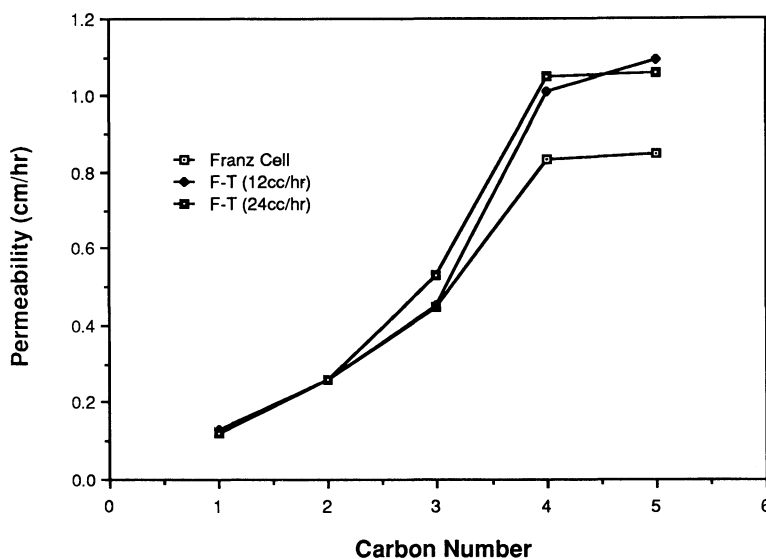


Fig. 3. Calculated values for permeability coefficients (cm/hr) as a function of carbon-chain length for the methyl through pentyl esters. F-T denotes flow-through cell. The permeability coefficients were calculated from Eq. (1).

boundary layers (one on each side of the membrane). The effective thickness can be estimated providing that the diffusivity, which varies only slightly through the homologous series, can be reasonably estimated. The plateaus which are observed in the permeability vs carbon number plots (Fig. 3) are indicative of a shift from membrane control to aqueous diffusion layer control at the butyrate ester for both cell types. The high plateau values observed for flow-through cell experiments at each of the flow rates indicate that the effective thickness of the aqueous boundary layer is less when the flow-through cell is used. Thus, it appears that at the flow rates used in these experiments, mixing in the fluid region adjacent to the membrane is more efficient than when the permeant is collected in the discrete receiver chamber.

In order to examine the sensitivity of the results to the flow rate in the flow-through cell, experiments were performed at flow rates of 40 and 60 ml/hr. At these high flow rates results were erratic, with large standard deviations within sets of data. This observation suggests that at excessive flow rates, liquid passes through the collection area in a turbulent fashion, forming eddies in the current and fluid channeling through the cell. This short-circuiting paradoxically results in a large hydrodynamic layer. Thus, it appears that there exists a range of flow rates within which reliable and reproducible data can be obtained. The flow rates should be fast enough to ensure rapid removal of permeant but not so fast as to create turbulence and possible distention of the membrane. It appears that for the cell in question, a flow rate in the vicinity of 12 ml/hr is sufficient to maintain sink conditions with even the fastest-permeating compound studied. For the flow-through cell operating at this rate, the average concentration buildup in the receiver compartment within a sampling interval was 7% of the donor concentration, which is within the 10% limit often cited as necessary for the maintenance of sink conditions (6).

Although hydrodynamic influences were readily apparent in these experiments with silicone rubber membranes, one might argue that diffusion layers are far less likely to be important with full-thickness skin membranes.

While the latter is the general expectation, transdermal delivery circumstances are sometimes encountered in which the contributions of boundary layers are consequential. Physicochemical compromise of the stratum corneum as the result of disease, mechanical damage, or the use of skin penetration enhancers markedly reduces the diffusional resistance of the stratum corneum. Such skin conditioning results in an appreciable upgrading of the importance of other strata in series with the stratum corneum, including the hydrodynamic layers in diffusion-cell experiments. For example, stratum corneum-stripped skin is often used to determine a permeant's summed diffusional resistance in the viable epidermis and dermis and it becomes important to know the hydrodynamic characteristics of the cell to assign correctly a value to the tissue itself. Situations also exist with the intact skin where the partition coefficient (K) is of sufficient magnitude that the aqueous boundary layer resistance is consequential in diffusion-cell studies. And in a recent review (7), the implementation of standard *in vitro* methods to assess the effects of vehicles on drug delivery through the skin was advocated. Techniques of study to compare a generic product with the innovator's and to test for lot-to-lot variations in performance almost certainly will include flow-through cell designs and they may involve the use of highly permeable artificial membranes. Thus it becomes additionally important to know the magnitude of the diffusion layers in flow-through cells as described in this paper.

Of importance, the flow-through cell yielded results comparable to those obtained with the traditional static cell. Considering the advantages offered in terms of time and labor saved through its use, the flow-through cell is preferable to the static cell for most purposes and especially when permeation is rapid.

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