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An improved in vitro method for measuring skin permeability that controls excess hydration of skin using modified Franz diffusion cells

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ABSTRACT

When liquid donors/receivers are used for in vitro skin permeation studies, excess hydration can change skin properties compared to in vivo conditions. A novel in vitro method of determining the permeability of drugs through skin was developed that avoids exposing the membrane to dilute donor/receiver solutions. The drug is dissolved in an unstirred donor gel, and diffuses through a membrane into an unstirred gel receiver that can potentially be adjusted to mimic physiological conditions. Pulsatile microdialysis (PMD) was used to sample local concentrations in the receiver medium, and a model was developed to allow the determination of permeability. For Doxepin HCl, permeabilities through artificial membranes and human cadaver skin were determined using the new and previously reported methods. For artificial membranes that minimally hydrate, the new method gave consistent but slightly lower permeability values. For human cadaver skin, the permeability determined using the new method was 1/6 that of the fully hydrated skin. Limitations of the model, their relations to experimental design and data analysis were evaluated. It was concluded that this method can be applied to characterize membrane permeabilities using experiments that may avoid membrane breakdown and more closely mimic physiological conditions.

1. Introduction

The introduction of drugs to the systemic circulation via transdermal delivery through the skin is an important alternative to the oral route of administration. The potential advantages of the transdermal route are well documented, and include bypassing the hepatic first pass metabolism, and avoiding gastrointestinal side effects and/or incompatibility (Schaefer and Redelmeier, 1996).

In vitro permeation experiments are a valuable adjunct to in vivo percutaneous absorption studies, and provide a convenient means for evaluating the permeation characteristics of drugs (Bronaugh and Maibach, 1991). A variety of passive diffusion systems for in vitro transdermal experiments have been developed for use with different kinds of membranes (Nicolettos, 1998). For in vitro transdermal studies, modified Franz diffusion cells are perhaps the most commonly used setups. These cells have two chambers, one containing the active agent (donor vehicle) and the other containing a receiver solution; a membrane separates the two while the receiver chamber is jacketed to maintain temperature control. In the use of these cells, the receiver is typically a stirred solution, and the donor may be a stirred solution or unstirred gel. Experiments are conducted in which the receiver solution is sampled to obtain the mass of a drug that has crossed the membrane from the donor to the receiver vs. time, and parameters such as the membrane permeability are then determined (Mazzo et al., 1986).

Setups that use a liquid receiver and/or donor do not mimic the available water in physiological systems, which can be a significant issue when studying biological membranes such as skin. It has been reported that using a liquid receiver in transdermal experiments with human cadaver skin may result in skin breakdown, unrealistic swelling, microbial growth, and leaching out of important skin components, especially if these experiments last more than 24 h or so (Van Hal et al., 1996). Another important factor is hydration. The uptake of water causes corneocytes to be swollen and develop water pools in the intercellular lamellar region. Also, the stratum corneum hydrates in the presence of excess liquids (Holbrook and Wolff, 1993). Since crossing the stratum corneum is the rate-determining step in drug penetration, hydrating it could significantly alter the permeability of drugs. As an example, exposing skin to a liquid receiver and/or donor would lead to much greater hydration than would occur in vivo with a transdermal patch drug delivery system, which could lead to problems in establishing in vitro–in vivo correlations (IVIVC).

These points suggest that it is potentially beneficial to use more physiologically relevant in vitro experiments, particularly with regard to the excessive hydration effects of the membrane resulting from water exposure. It would be of interest to study the effects of various media on the permeability of drugs with a range of

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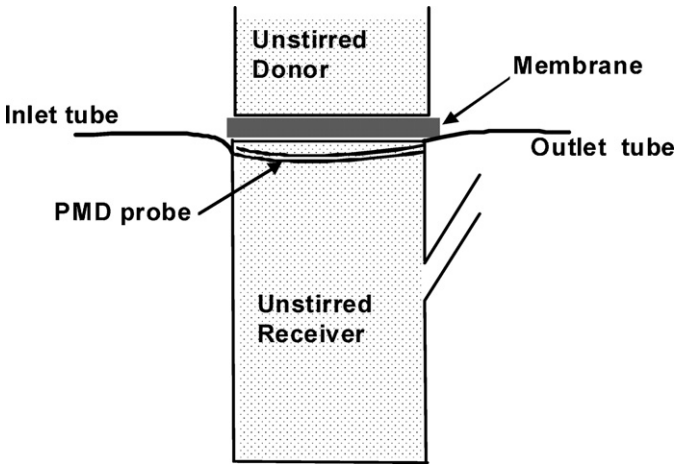

Fig. 1. Schematic diagram of the experimental setup with modified Franz cells.

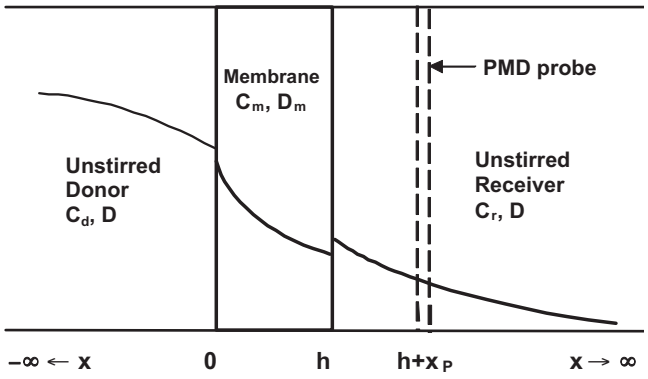

Fig. 2. Schematic diagram of the diffusion model. In the actual modified Franz diffusion cell setup, $x \rightarrow \infty$ is in the vertically downward direction.

physicochemical properties, especially as compared with current methods that use liquid donor and/or receiver media, with the goal of determining how to best mimic in vivo conditions.

The osmotic pressure in the donor and/or receiver can be adjusted using small molecules, but they can enter the membrane and alter its properties, and possibly interact with the permeating drug. Thus, it is postulated that controlling the receiver environment might better be done using larger molecules, such as polymers or gelatins. However, these would increase the viscosity of the receiver, making stirring difficult or impractical. Thus, to allow assessment of the effects of various receiving media, it would be necessary to first develop a method to determine the permeated mass vs. time profiles by sampling unstirred receivers.

This paper presents a method that was developed to determine membrane permeabilities using unstirred donor and receiver media. Sampling of the unstirred receivers is done using a variation of microdialysis referred to as pulsatile microdialysis (PMD), which has recently been developed (Kabir et al., 2005). While PMD has been used as an accurate technique for sampling drug concentrations, it had not been applied to setups such as modified Franz cells before this work. Experimental modifications to the PMD method and mathematical models for data analysis have been developed, which allow calculations of the mass vs. time profiles for drug permeation.

This paper has three main objectives: (1) describe the experimental setup and physical process and present the associated mathematical model; (2) describe the particular experiments and present the resulting calculated permeability; (3) compare the values obtained using this method with those obtained using previously published methods using liquid donor/receiver media, using both artificial membranes that would not be expected to show significant hydration effects, and human cadaver skin (which would be expected to show hydration effects). The model drug Doxepin HCl was used, which has been studied previously in experiments using artificial membranes and skin.

2. Description of the method and model

2.1. Physical description of the setup and experiment

A schematic diagram of the experimental setup is shown in Fig. 1. A membrane is placed between an unstirred donor and an unstirred receiver, using a modified Franz diffusion cell for which the area separating the donor and receiver is circular with a diameter of approximately 1.5 cm. Initially, the drug is dissolved in the donor with a uniform concentration while the membrane and receiver contain no drug. During the experiment, the drug diffuses out of the donor, through the membrane and into the receiver.

A microdialysis probe is placed in the receiver as close as possible to the membrane–receiver interface. The length of the probe is approximately 1 cm, and it runs straight across and as close to the center of the membrane as possible. The probe is a small, highly permeable tube connected to impermeable inlet and outlet tubing and is used to determine the local concentration in the receiver near the probe at various times.

During the drug permeation experiments, two quantities were experimentally measured.

- The local receiver concentrations in the vicinity of the microdialysis probe at various times, which were calculated from data obtained using pulsatile microdialysis (PMD).
- The mass of drug remaining in the donor at the end of the experiment, which is used to determine the amount of drug in the receiver at the time of the last PMD sample. The donor was collected because it was easier to completely collect than the receiver in setup used in this study.

Separate experiments were done to determine the diffusion coefficient of the drug within the donor and receiver media, and the diffusional area of the membrane. These were assumed to remain constant during each permeation experiment.

Because the receiver is unstirred, the concentration varies with position as well as time. Thus, the local receiver concentration obtained from PMD data are not the same as the average concentration in the receiver, and it is necessary to develop a means to determine the permeability of the drug through the membrane in terms of the local receiver concentration vs. time profiles. The mathematical model and numerical methods are presented in Sections 2.2 and 2.3.

2.2. Mathematical model

From the physical description above, drug migration occurs by diffusion in the three unstirred regions (donor, membrane and receiver). It is assumed the membrane behaves like a homogeneous membrane. Although skin is a heterogeneous structure, since permeation through the stratum corneum is the rate limiting step, it has been noted that the behavior can be approximated as that of a homogeneous system (Bellantone et al., 2001).

Fig. 2 shows the donor, membrane and receiver regions, which are labeled with the subscripts "*d*", "*m*" and "*r*", respectively. The donor and receiver are taken to be semi-infinite, and the membrane is of finite thickness *h*. The donor occupies the region $x < 0$, the membrane occupies the region $0 < x < h$, and the receiver occupies

the region $x > h$. A PMD probe is placed in the receiver at $x = h + x_P$, where x_P represents the average distance between the probe and the membrane. (Modeling the donor and receiver as semi-infinite regions imposes limitations on the data that can be used in analyses, which are discussed in Section 2.3 below.) The concentrations in each region are denoted by C_d, C_m and C_r, and the diffusion coefficients are denoted by D_d, D_m and D_r. The partitioning between regions is given by $C_m = K_{dm}C_d$ at $x = 0$ and $C_m = K_{rm}C_r$ at $x = h$, where the K's represent the membrane/donor and membrane/receiver partition coefficients. In the experiments performed in this work, the donor and receiver media were the same except for the drug concentration, so it was approximated that $D_d = D_r$ (simply labeled D) and $K_{dm} = K_{rm}$ (simply labeled K). Sampling of the receiver was done using PMD, and those data were used to determine the concentration in the receiver at $x = h + x_P$ at various times.

Transport by diffusion follows Fick's Second Law, which is a partial differential equation that is first order in time and second order in space, and requires one initial and two boundary conditions. Since there are three unstirred regions, Fick's Second Law must be written and solved in each region, so the complete solution for this setup requires a total of three initial and six boundary conditions (Crank, 1975). The initial and boundary conditions come from the following physical considerations.

- Initially, the donor is uniformly loaded with an initial concentration C_0, and the membrane and receiver are void of drug.
- In the donor, the concentration is finite everywhere, including $x \to -\infty$.
- At the donor–membrane interface ($x = 0$), the flux leaving the donor is equal to the flux entering the membrane.
- Across the donor–membrane interface, the concentrations in the two regions are related by partitioning.
- In the receiver, the concentration is finite everywhere, including $x \to \infty$.
- At the membrane–receiver interface ($x = h$), the flux leaving the membrane is equal to the flux entering the receiver.
- Across the membrane–receiver interface, the concentrations in the two regions are related by partitioning.

Letting x represent the position, and t the time, these conditions are expressed mathematically as follows:

Governing differential equations:

$$\frac{\partial C_d}{\partial t} = D\frac{\partial^2 C_d}{\partial x^2} \quad \text{donor} \tag{1}$$

$$\frac{\partial C_m}{\partial t} = D_m\frac{\partial^2 C_m}{\partial x^2} \quad \text{membrane} \tag{2}$$

$$\frac{\partial C_r}{\partial t} = D\frac{\partial^2 C_r}{\partial x^2} \quad \text{receiver} \tag{3}$$

Initial conditions:

$$C_d(x, 0) = C_0 \quad x < 0 \tag{4}$$

$$C_m(x, 0) = 0 \quad 0 < x < h \tag{5}$$

$$C_r(x, 0) = 0 \quad x > h \tag{6}$$

Boundary conditions:

$$C_m = KC_d \quad D\frac{\partial C_d}{\partial x} = D_m\frac{\partial C_m}{\partial x} \quad x = 0 \tag{7}$$

$$C_m = KC_r \quad D\frac{\partial C_r}{\partial x} = D_m\frac{\partial C_m}{\partial x} \quad x = h \tag{8}$$

$$C_d \text{ finite } x < 0 \quad C_r \text{ finite } x > h \tag{9}$$

In the permeation experiments, the primary experimentally measured quantity is the local drug concentration in the receiver in the immediate vicinity of the microdialysis probe at given times, which was obtained from PMD data. Thus, the system of equations above was solved to find an equation for the local concentration in the receiver $C_r(x, t)$. In addition, for mass balance and to facilitate other data analysis, it was necessary to have an equation for the mass of drug in the receiver as a function of time, so another goal was to find an equation for $M_r(t)$. These solutions are found below.

The above system of equations was solved using Laplace transformations, giving the local concentration in the receiver as

$$C_r(x, t) = \alpha\sum_{n=0}^{\infty}(-1)^n\beta^{2n}\text{erfc}\left[\frac{(2n+3)h + x}{2\sqrt{D_m t}} - \frac{h}{2\sqrt{Dt}}\right] \tag{10}$$

where

$$\alpha = \frac{2KC_0\sqrt{DD_m}}{\left(\sqrt{D} + K\sqrt{D_m}\right)^2} \tag{11}$$

$$\beta = \frac{\sqrt{D} - K\sqrt{D_m}}{\sqrt{D} + K\sqrt{D_m}} \tag{12}$$

and

$$\text{erfc}\, u = \frac{2}{\sqrt{\pi}}\int_u^{\infty}\exp(-w^2)dw \tag{13}$$

is the complimentary error function. The parameter α has units of concentration and β has no units.

Eq. (10) gives the concentration at a location x in the receiver as a function of time, but can be rewritten in terms of the average probe distance from the membrane x_P. Noting that the membrane/receiver interface is located at $x = h$ and the location of the probe is at $x = x_P + h$, Eq. (10) can be rewritten to give the concentration in the receiver at the probe location as

$$C_r(h + x_P, t) = \alpha\sum_{n=0}^{\infty}(-1)^n\beta^{2n}\text{erfc}\left[\frac{(n+2)h + x_P}{\sqrt{D_m t}} - \frac{h}{2\sqrt{Dt}}\right] \tag{14}$$

The rate of accumulation of drug in the receiver dM_r/dt can be calculated from Fick's First Law (Crank, 1975) evaluated at the membrane/receiver interface

$$\frac{dM_r}{dt} = -AD\left.\frac{\partial C_r}{\partial x}\right|_{x=h} \tag{15}$$

Applying this to Eq. (10) and evaluating it at $x = h$ leads to

$$\frac{dM_r}{dt} = \frac{AD\alpha}{\sqrt{\pi D_m t}}\sum_{n=0}^{\infty}(-1)^n\beta^{2n}\exp\left(-\frac{k_n^2}{t}\right) \tag{16}$$

where

$$k_n = \frac{(n+2)\,h}{\sqrt{D_m}} - \frac{h}{2\sqrt{D}} \tag{17}$$

Eq. (16) can be integrated to give the cumulative amount of drug that has entered the receiver as

$$M_r(t) = \frac{AD\alpha}{\sqrt{\pi D_m}}\sum_{n=0}^{\infty}(-1)^n\beta^{2n}\left[\sqrt{t}\exp\left(-\frac{k_n^2}{t}\right) - k_n\sqrt{\pi}\text{erfc}\left(\frac{k_n}{\sqrt{t}}\right)\right] \tag{18}$$

Since the diffusion coefficient of the drug in the donor/receiver media D is determined from separate experiments, examination of the above equation shows that the parameters K, D_m, h and x_P can be determined by fitting Eqs. (11), (12), and (14) to $C_r(x_P, t)$ vs. time

data. Subsequently, the membrane permeability of the drug P can be found as

$$P = \frac{KD_m}{h} \tag{19}$$

As noted elsewhere (Bellantone et al., 2001), even though skin is a heterogeneous membrane, since the stratum corneum is strongly rate limiting, its permeability coefficient can be modeled using Eq. (19). However, the obtained values of K, D_m, and h from regressions should be interpreted as "average" or "net" values for the entire membrane structure, rather than true thickness, partition and diffusion coefficients. Because of this, it was not possible to exactly determine the "effective" skin thickness independently. In addition, it was not possible to exactly measure the probe position because it varied along the probe. Thus, values for h and x_P were obtained by fitting.

2.3. Numerical implementation and data analysis

Parameters such as the area A and the diffusion coefficient of the drug in the gels D were determined by separate experiments and assumed to remain constant for a given setup. However, K, D_m, h and x_P are not directly measured, so the goal of the analysis was to obtain them and then allow the calculation of the membrane permeability using Eq. (19). This was done by iterating on those parameters to fit Eq. (14) to the experimental $C_r(x_P, t)$ vs. time profiles, using the Solver® function in Excel® with an imposed mass balance constraint.

The mass balance constraint was required because there were four numerical degrees of freedom (D_m, K, h and x_P), and many combinations resulted in acceptable fits. In addition, the particular final iterated parameter values were very sensitive to initial estimates. Because the calculated mass vs. time in the receiver and the final iterated values of these parameters were strongly interdependent, and because the final value of the mass in the receiver could be experimentally determined, the mass balance constraint was imposed to reduce the numerical degrees of freedom to three. This was accomplished by experimentally determining the mass in the receiver immediately after the last PMD sample was taken, and requiring that the calculated mass in the receiver at the time of the last PMD sample equal the experimentally obtained value. Thus, at the end of the experiment, all of the donor gel was collected and the mass of drug remaining in it was determined. The final mass of drug in the receiver was estimated as the difference between the amounts of drug in the donor at the beginning and the end of the experiment. Neglecting the amount of the drug in the membrane gives the approximation

$$M_r(\text{end of expt}) = M_d(\text{start of expt}) - M_d(\text{end of expt}) \tag{20}$$

The mass balance constraint was imposed on the regressions by requiring that values of M_r at the end of the experiment calculated using Eqs. (18) and (17) be equal to the experimental values estimated using Eq. (20). When this was done, very good fits were obtained and the final parameter values were relatively insensitive to the initial estimates. In addition, all parameters were checked to make sure they were physically reasonable (especially h and x_P, which could be estimated but not measured).

As noted in Section 2.2, the donor and receiver are modeled as semi-infinite regions, while both are finite in the actual experiment. This approximation simplifies the mathematical analysis but can introduce errors at longer times, when depletion of the donor or the effects of the finite size of the region begin to cause deviations from the model. However, with respect to data obtained at early enough times, these effects may be neglected. Thus, it was necessary to estimate a maximum time for which data could be used. This was done by assuming that two conditions must be satisfied.

- Experimental data taken at times later than one or two times $L^2/2D$ should be excluded, where L is the smaller than the donor and receiver region thicknesses. At times longer than this, the back edge of the region begins to physically affect the diffusion behavior.
- The maximum amount of drug lost from the donor is less than ~35–40% of the total initially loaded into the donor. This expresses limitations on the allowed degree of depletion of drug from the donor, and is similar to conditions expressed elsewhere (Higuchi, 1962).

In this work, these limitations did not affect the ability to collect sufficient data to obtain membrane permeability, which did not exceed 24 h in all cases.

It is important to note that the time required to collect a PMD sample was approximately 35 min in the permeation experiments, and the PMD sample concentration was treated as though it occurred at the time corresponding to the midpoint of the time interval during which the sample was collected. As described in Section 3.4, each PMD sample in the permeation experiments consisted of 34 combined pulses of 1.5 μL each, so the concentration of the complete PMD sample was the average of the collected pulses. Since the local receiver concentration changed relatively slowly in the permeation experiments, the local receiver concentration changed in an approximately linear manner with respect to time during the collection of a PMD sample. This allowed the approximation that the average sample concentration be considered to occur at the midpoint of the sampling interval.

3. Materials and methods

3.1. Materials

Doxepin HCl was obtained from Sigma–Aldrich (St. Louis, MO). Chlomipramine HCl, white bees wax, Spectra/Por RC Hollow Fiber Bundles (molecular weight cutoff 18 kDa), and Spectra/Por cellulose membrane (molecular weight cutoff 1000) were obtained from Spectrum Laboratories (New Brunswick, NJ). Human cadaver skin (male, back) was obtained from the New York Firefighter's skin bank (New York Hospital Cornell Medical Center). Acetonitrile (HPLC grade), HPLC water, and acetic acid were obtained from VWR (West Chester, PA). Nylon membrane filters (0.45 μm, 47 mm) were obtained from Pall Corporation (Ann Arbor, MI). Methocel K15M Premium was obtained from the Dow Chemical Company (Midland, MI). Tygon Microbore tubing was obtained from Norton Performance Plastics (Akron, OH). Tygon Long Flex Life Pump Tubing was obtained from Saint-Gobain Performance Plastics (Strongsville, OH). Polyimide tubing was obtained from MicroLumen, Inc. (Tampa, FL). Gas-Tight syringes (2.5 mL and 5 mL) and microsyringes (500 μL) were obtained from Hamilton Company (Reno, Nevada).

3.2. Modified Franz diffusion cell setup

Franz and modified Franz diffusion cells are probably the most commonly used set-ups for in vitro transdermal studies. In this study, modified diffusion cells were used (Crown Glass Company, Somerville, NJ). These cells have two chambers, one containing the active agent in the donor medium, and the other containing a receiver medium, separated by a membrane. For the modified Franz cells used in this study, the donor compartment had a capacity of up to 3 mL and the receiver was cylindrical with volume 13.1 mL. The diffusional area of the membrane separating the compartments is 1.76 cm^2 (1.5 cm diameter). The receiver is enclosed in a water-jacket system for temperature control.

To successfully adapt PMD for use with modified Franz diffusion cells, a number of issues were addressed. Factors such as the probe placement and length, mechanical stability, and PMD parameters such as resting times, flush rates, number of pulses, and sample volume were evaluated and adjusted where necessary. When PMD was used for sampling the receiver, the probe was placed just underneath the membrane on the receiver side and ran diametrically across the diffusional cross area. Instead of using an O-ring and pinch clamp to hold the setup together, white melted wax was used to keep the donor compartment in place and avoid leaks of the drug solution. The melted wax was dropped around the membrane and the donor cell, and allowed to cool.

3.3. Gel and solution preparation

The gel solutions were prepared by hot/cold techniques. Methocel powder was mixed thoroughly using 1/3 of the required total amount of water as hot water (80–90 °C). The total amount of water was determined to be 40 mL. The mixture was agitated until all particles were melted and a consistent dispersion was obtained. The remainder of the water was added as cold water while it was still agitating. The solution was cooled down to less than 30 °C. Agitation was then continued for 20 min. The drug powder was added to the final gel solution while it was agitated.

3.4. Pulsatile microdialysis (PMD)

PMD was used to determine concentrations of drugs in the unstirred receiver. It is a recently developed variation of microdialysis, in which a fluid (dialysate) is pumped into a microdialysis probe (a small, highly permeable tube) at a high flow rate, then stopped and allowed to remain at rest in the probe for a given resting time, and subsequently flushed out at a high flow rate for collection (Kabir et al., 2005). While the dialysate is in the probe, it can accumulate drug molecules because they cross the wall of the probe by diffusion, and the amount accumulated is proportional to the concentration in the medium outside the probe.

PMD probes were made using a tubular dialysis membrane with a nominal inner radius of 100 μm and molecular weight cutoff of 18 kDa, made from reconstituted cellulose (Spectra/Por RC Hollow Fiber Bundles). The dialysis membrane tubing was connected to impermeable polyimide tubing and put in contact with the media for which drug concentrations were to be sampled. Two segments of polyimide tubes with an outer radius of 83 μm were connected to both sides of the microdialysis probe and glued using instant cyanoacrylate glue. One of the segments was connected to a 15 cm Tygon tube that was connected to a syringe pump and used as an inlet. The other segment served as the outlet for sampling. The permeable part of the probe, referred to as the probe window, was 1 cm in length with a volume of 0.73 μL.

The dialysate was pumped into and out of the probe using a HARVARD Model PHD 2000 programmable pump (Harvard Apparatus, Holliston, MA). Each pulse was pumped at a flow rate of 100 μL/min, and the dialysate resting time was 1 min. After discarding the first pulse, 34 pulses of volume 1.5 μL each were collected and combined, for a total sample volume of ~50 μL. For PMD samples taken during permeation experiments, the concentration in the PMD sample was plotted at the time corresponding to the midpoint of the sampling interval during which the pulses were collected.

3.5. Chemical analysis

The drug used in this study was Doxepin HCl, which is a weak base with $pK_a = 9.0$, MW = 316 g/mol, and a melting point of 185–190 °C. Doxepin HCl salt is a white, crystalline solid that is free soluble in water, and with an apparent partition coefficient of $\log P = 2.37$ in octanol/phosphate buffer (pH 7.4) systems. The HPLC assay was a modification of a previously reported method (Queiroz Costa et al., 1995). Chromatography was carried out using a reverse phase analytical column LiChrosfer® 60 R-P select B column (5 μm). The wavelength of detection of Doxepin HCl was 225 nm. The isocratic mobile phase was consisted of acetonitrile and 0.25 N acetate buffer (pH 5.5) in a 60/40 ratio, respectively. All solvents used were HPLC grade. The drug Clomipramine HCl was used as internal standard. The mobile phase flow rate was 1.3 mL/min, and the approximate retention times were 5.9 min for Doxepin and 8.5 min for Clomipramine. Samples of 20 μL volume were injected directly into the system using an auto sampler. The method was validated and the coefficient of variation was less than 2%. The HPLC standard curve was linear ($R^2 = 0.999$), and the limit of quantitation was 0.6 μg.

4. Experimental

In what follows, unless otherwise specified, all gels consisted of 0.5% Methocel in water, and all solutions were deionized water (pH ~ 6). In all cases, the membranes and receivers were initially void of the drug. Unless otherwise noted, the donor volumes were 3 mL and the receiver volumes were 13.1 mL, and all studies were performed with a receiver temperature of 37 °C.

4.1. In vitro probe calibration for PMD

The calibration of the microdialysis probes was an important step because sampling from the receiver medium was done using PMD. In order to measure the local concentration of the medium outside the probe, it must be related to the concentration in the PMD sample. This is done using the fraction recovery (F_R) of the microdialysis, which for setups in which the dialysate is initially void of drug is given by

$$F_R = \frac{C_S}{C_G} \tag{21}$$

For a given probe and PMD parameters, F_R is determined by performing a probe calibration, which was done here by a modification of previous methods (Chen et al., 2002). Probe calibrations were performed using an unstirred gel-like (Methocel 0.5%) solution as an outside medium, with various uniform concentrations of the drug (from 5 to 80 μg/mL). For each drug concentration, samples of ~50 μL were collected by PMD, in which the 34 pulses of 1.5 μL were combined, with a 1 min resting time and 100 μL/min flush rate. The collected pulses were combined to form one sample, which was then assayed by HPLC. The procedure was repeated 3 times at each concentration. A calibration curve was constructed by plotting the PMD sample concentration vs. the concentration in the medium outside the probe, and the slope was taken as the F_R.

4.2. Determination of diffusion coefficient in the gel

The diffusion coefficient of Doxepin HCl in 0.5% Methocel® was determined using modified Franz diffusion cells. The donor was a 0.5% Methocel gel that contained an initial Doxepin HCl concentration of $C_0 = 1$ mg/mL and the receiver was a stirred solution of deionized water that was maintained at 37 °C. A highly permeable cellulose membrane (molecular weight cut-off 1000 and 60 μm nominal thickness) was used to separate the donor and receiver compartments, so transport of the drug in the gel to the membrane was rate limiting. The receiver was stirred by a magnetic stirrer. Direct samples of 100 μL were taken every hour for 8 h, using a syringe and then filtered. Assays were done using 50 μL of the drug

samples and 10 μL of the internal standard, which were injected into HPLC to analyze for drug content.

4.3. Producing coated cellulose membranes

Cellulose membranes were coated with Surelease® (commercially available 25% ethyl cellulose in water dispersion). The coating was done by depositing a layer of 2 mL of Surelease® directly onto the membrane using a 1 mL plastic pipette, and allowing it to dry for several hours. The final coating solution and volume were determined by trial and error, with the goal of reducing the membrane permeability enough so permeation was membrane controlled in the presence of an unstirred gel donor, but high enough so a pseudo-steady state model could be used in the presence of stirred liquid donor/receiver setups (as described in Section 5.3).

4.4. Permeation studies with coated cellulose membranes – stirred solutions for both donor and receiver

Side-by-side cells were used. The donor was a stirred solution of 1 mg/mL Doxepin HCl in deionized water, the receiver was a stirred solution of deionized water, and the membrane was coated cellulose. Both the donor and receiver were stirred with magnetic stirring bars, and both were maintained at 37 °C. Direct samples of 100 μL, obtained using a syringe and then filtering, were taken every hour for at least 6 h. Assays were done using 50 μL of the drug samples and 10 μL of the internal standard, which were injected into HPLC to analyze for drug content.

4.5. Permeation studies with coated cellulose membranes – unstirred gels for both donor and receiver

Modified Franz diffusion cells were used. The donor was an unstirred 0.5% Methocel gel initially loaded with of 1 mg/mL Doxepin HCl, the receiver was an unstirred 0.5% Methocel gel, and the membrane was coated cellulose. The temperature of the receiver was maintained at 37 °C. PMD was used to periodically sample the local receiver concentration, using 1 cm probes, 1 min resting time, and a flush rate of 100 μL/min flush rates. The probes were placed in the receiver as close as possible to the membrane, and running as closely as possible across the diameter of the membrane. After discarding the first pulse, 34 pulses were combined to form the sample for HPLC analysis. Assays were done using 50 μL of the drug samples and 10 μL of the internal standard, which were injected into HPLC to analyze for drug content. The PMD sample concentration was converted to the local concentration in the receiver by Eq. (21).

4.6. In vitro permeation experiments using human cadaver skin – unstirred donor gel and stirred receiver solution

The donor was an unstirred 0.5% Methocel gel initially loaded with of 10 mg/mL Doxepin HCl; the receiver was a stirred solution of deionized water, and the membrane was human cadaver skin. The temperature of the receiver was maintained at 37 °C. At appropriate times, receiver sampling was done concurrently using both direct and PMD sampling. Direct sampling was done using a syringe to withdraw 100 μL of receiver, which was then filtered. PMD sampling was done by combining 34 pulses, using 1 cm probes, 1 min time, and a flush rate of 100 μL/min. HPLC assays were done using 50 μL of the drug samples (direct or PMD) and 10 μL of the internal standard, which were injected into HPLC to analyze for drug content. The PMD sample concentration was converted to the local concentration in the receiver by Eq. (21).

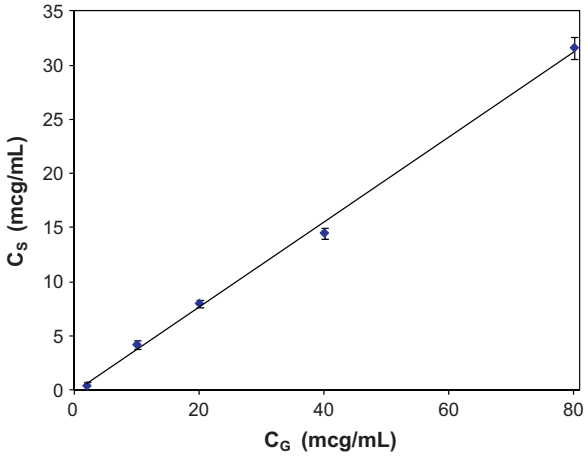

Fig. 3. PMD probe calibration curve in 0.5% Methocel gel. The probe was 1 cm in length. The data fit the line $C_S = 0.386C_G$ for 1 min resting time.

4.7. In vitro permeation experiments using human cadaver skin – unstirred gels for both donor and receiver

The donor was Doxepin HCl in unstirred Methocel® 0.5% gel, with a uniform initial concentration of $C_0 = 10$ mg/mL. The receiver was an unstirred Methocel 0.5% gel that was maintained at 37 °C. PMD was used to sample the local receiver concentration at appropriate times, using a 1 cm probe, a one-minute resting time, and a flush rate of 100 μL/min flush rates. After discarding the first pulse, 34 pulses were combined to form the sample for HPLC analysis. Assays were done using 50 μL of the drug samples and 10 μL of the internal standard, which were injected into HPLC to analyze for drug content. The PMD sample concentration was converted to the local concentration in the receiver by Eq. (21).

5. Results

5.1. PMD probe calibration in 0.5% Methocel gel

The microdialysis probe was calibrated to relate the local concentrations of drug in the gel and the concentration in the PMD samples, according to Eq. (21). The calibrations were done by plotting the concentration in the sample collected using PMD (C_S) vs. the concentration in the external gel medium (C_G). It was assumed that the relation between C_S and C_G did not change during the calibration experiment. As shown in Fig. 3, the plots were linear ($R^2 > 0.99$). In addition, the PMD sampling was reproducible with relative standard deviations less than 10% at all drug concentrations and less than 3% at concentrations above 10 μg/mL. Using Eq. (21), the value was taken as F_R, which was 0.386 in this study.

5.2. Diffusion coefficient of Doxepin HCl in 0.5% Methocel gel

As shown in Eq. (18), the accumulation of drug in the receiver in this model depends in part on the diffusion coefficient D of the drug in the donor/receiver gels, which was done using the procedure described in Section 4.1. The donor was an unstirred gel containing 0.1% Doxepin HCl® 0.5% and the receiver was a stirred solution of deionized water with a cellulose membrane separating the receiver and the donor compartments. After very early times, the rate at which the drug entered the membrane was limited by release from the gel, which resulted in plots of the mass in the receiver vs. the square root of time for becoming linear. (It should be noted that the plot would deviate from being linear after ~40% of the drug has been released from the gel donor.) This is shown in Fig. 4. Thus,

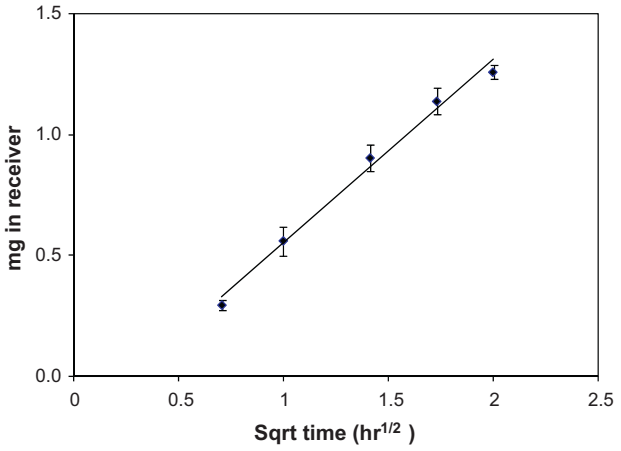

Fig. 4. Plot to determine the diffusion coefficient of Doxepin HCl in Methocel 0.5% gel.

for highly permeable membranes, the release can be modeled as a modified square root of time plot, given by

$$M = 2AC_0\sqrt{\frac{Dt}{\pi}} + \text{intercept} \tag{22}$$

where A is the diffusional area of the membrane and C_0 is the initial uniform concentration of drug in the donor, and M is the mass of the drug in the receiver. Using the slope of the linear portion of the M vs. $t^{1/2}$ profile and Eq. (22) leads to

$$D = \left(\pi \times \frac{\text{slope}^2}{4A^2C_0^2}\right) \tag{23}$$

For the Doxepin HCl in Methocel 0.5% systems, the diffusion coefficient in the gel was determined to be 0.14 cm^2/h.

5.3. Permeation studies with coated cellulose membranes – stirred solutions for both donor and receiver

In this setup, the donor and receiver were both stirred solutions, and samples were taken directly from the receiver using a gas tight syringe. Initially, an amount of drug M_0 was loaded into the donor, and the receiver was void of drug. The membrane permeability was calculated using a pseudo-steady state method as follows. Denoting the donor and receiver volumes as V_d and V_r, respectively, the rate of appearance of drug in the receiver dM_r/dt is given by

$$\frac{dM_r}{dt} = AP(C_d - C_r) = AP\left(\frac{M_d}{V_d} - \frac{M_r}{V_r}\right) \tag{24}$$

where M_d and M_r are the amounts of drug in the donor and receiver at any time. Mass balance can be expressed as $M_d + M_r = M_0$, where accumulation of drug in the coated cellulose is neglected. Eq. (24) can be solved to give

$$\ln\left(1 - \frac{C_r}{C_{Eq}}\right) = -kt \tag{25}$$

where C_{Eq} is the final concentration in the receiver at infinite time, given by

$$C_{Eq} = \frac{M_0}{V_d + V_r} \tag{26}$$

and

$$k = \frac{A(V_d + V_r)}{V_d V_r} \tag{27}$$

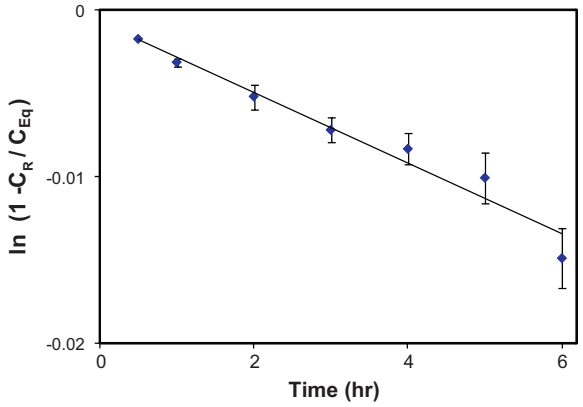

Fig. 5. Plot of $\ln(1 - C_R/C_{Eq})$ vs. time from setup using coated cellulose and stirred donor and receiver.

From the slope of a plot of the left hand side of Eq. (25) vs. time, the permeability of the membrane can be determined form Eq. (27) as

$$P = \frac{kV_dV_r}{A(V_d + V_r)} \tag{28}$$

The procedure was repeated using three membranes, each time with an initial donor Doxepin HCl concentration of 1 mg/mL. The permeability values were determined from Eq. (28) for each case, and it was found that $P = 0.0079 \pm 0.0009$ cm/h. Results are shown in Fig. 5.

5.4. Permeation studies with coated cellulose membranes – unstirred gels for both donor and receiver

In this setup, experiments were done using coated cellulose membranes and unstirred donor/receiver gels of 0.5% Methocel. The donor initially contained 1 mg/mL of Doxepin HCl. The receiver was sampled by PMD and the values for local receiver concentrations were obtained from the PMD sample concentrations using $F_R = 0.386$ in Eq. (21). The permeability values were calculated from a four-parameter non-linear regression subject to a mass balance constraint, as described in Section 2.3. Experimentally determined concentrations in the receiver near the probe and the associated fits are shown in Fig. 6, and the total mass in the receiver calculated at the same time points are shown in Fig. 7. The fits were acceptable

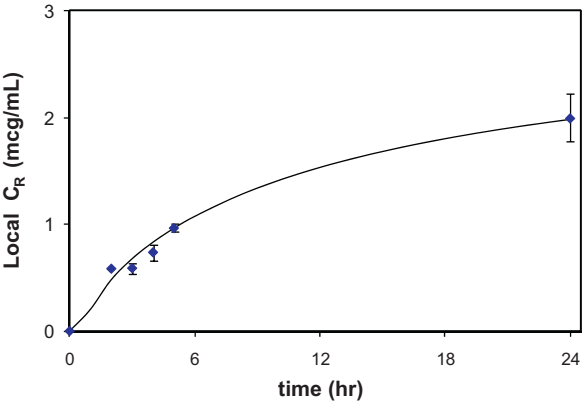

Fig. 6. Concentration in the receiver near PMD probe vs. time from setup using coated cellulose membrane and unstirred donor/receiver. The diamonds represent local concentration in the receiver obtained from PMD data and Eq. (21), and the line represents the fitted concentration in the receiver at x_P vs. t calculated from Eq. (14). The donor and receiver were unstirred Methocel 0.5% gel.

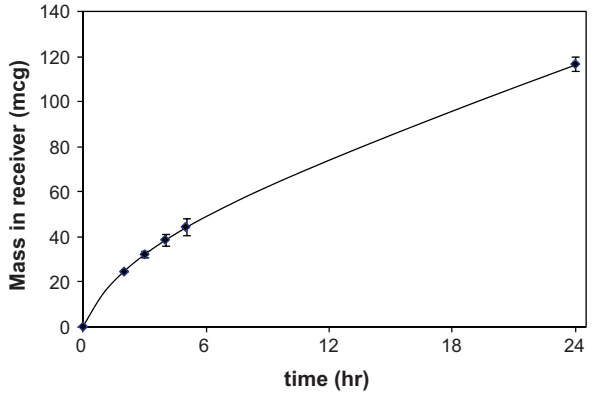

Fig. 7. Mass in receiver vs. time from setup using coated cellulose membrane and unstirred donor/receiver. The diamonds represent calculated mass at each experimental time point and the line represents mass calculated from Eq. (18). The donor and receiver were unstirred Methocel 0.5% gel.

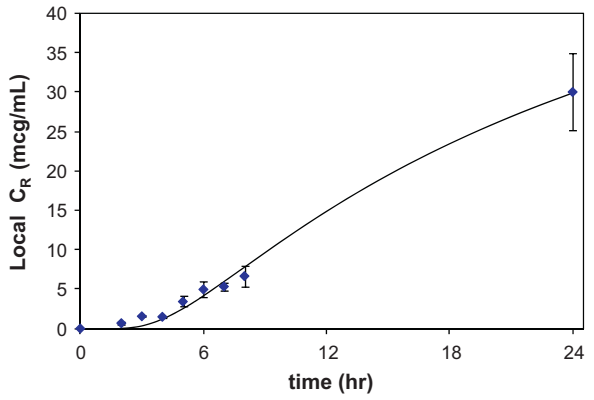

Fig. 9. Local concentration in the receiver near PMD probe vs. time from setup using human cadaver skin and unstirred donor/receiver. The diamonds represent local concentration in the receiver obtained from PMD data and Eq. (21), and the line represents the fitted concentration in the receiver at x_P vs. t calculated from Eq. (14). The donor and receiver were unstirred Methocel 0.5% gel.

in all cases ($R^2 > 0.99$). Taking the average of three experiments, the membrane permeability was found to be $P = 0.0036 \pm 0.0004$ cm/h.

5.5. In vitro permeation experiments using human cadaver skin – unstirred donor gel and stirred receiver solution

In this setup, the donor was an unstirred gel containing Doxepin HCl 10 mg/mL in 0.5% Methocel gel, and the receiver was stirred deionized water. Since the receiver was stirred, the concentration was uniform and determined in two ways – by direct sampling using a gas tight syringe, and by PMD sampling and Eq. (21). The mass in the receiver was the concentration multiplied by the receiver volume. As shown in Fig. 8, the results obtained using direct sampling and PMD were similar, indicating that the setup did not disrupt the PMD probes. Subsequently, the permeabilities were calculated from mass vs. time profiles during the first 24 h using a previously reported method (Bellantone et al., 2001), as summarized below.

For an unstirred donor and stirred receiver held under sink conditions (the concentration in the receiver was low enough to satisfy that requirement), an expression giving the cumulative amount of drug in the receiver at early times was obtained and given by

$$M = a\left[\sqrt{t}\exp\left(-\frac{\beta^2}{t}\right) - b\sqrt{\pi}\,\mathrm{erfc}\left(\frac{\beta}{\sqrt{t}}\right)\right] \tag{29}$$

$$a = \frac{4AKC_0\sqrt{D_d D_m}}{\sqrt{\pi(\sqrt{D_d} + K\sqrt{D_m})}} \tag{30}$$

$$b = \frac{h}{2\sqrt{D_m}} \tag{31}$$

Values for a and b can be determined by nonlinear regression analysis using Eq. (29), and the membrane permeability is given by

$$P = \frac{a\sqrt{\pi D_d}}{2b(4AC_0\sqrt{D_d} - a\sqrt{\pi})} \tag{32}$$

It was seen that the permeabilities obtained from the same experiments but from direct vs. PMD sampling were in agreement, with $P = 0.0137 \pm 0.0016$ cm/h using direct sampling and $P = 0.0155 \pm 0.0012$ cm/h using PMD sampling. (Because of the small sample size, it was not possible to say that the two values were statistically equivalent.) The fits were acceptable in all cases ($R^2 > 0.99$).

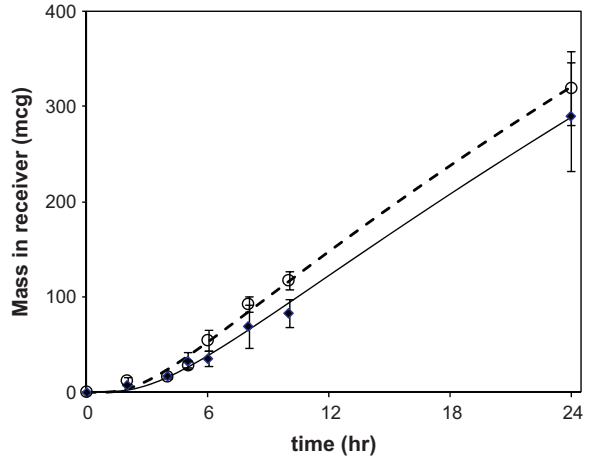

Fig. 8. Mass in receiver vs. time from setup using human cadaver skin, unstirred donor gel and stirred liquid receiver. The solid diamonds and open circles represent experimental data obtained using direct sampling and PMD, respectively. The solid and dashed lines represent profiles calculated from Eq. (29) for the direct sampling and PMD data, respectively.

5.6. In vitro permeation experiments using human cadaver skin – unstirred gels for both donor and received

In this setup, experiments were done using human cadaver skin membranes and unstirred donor/receiver gels of 0.5% Methocel. The donor initially contained 10 mg/mL Doxepin HCl. The receiver was sampled by PMD and the values for local receiver concentrations were obtained using $F_R = 0.386$ in Eq. (21). Values of P were calculated from a four-parameter non-linear regression subject to a mass balance constraint. Experimentally determined concentrations in the receiver near the probe and the associated fits are shown in Fig. 9, and the total mass in the receiver calculated at the same time points are shown in Fig. 10. The fits were acceptable in all cases ($R^2 > 0.99$). Taking the average of three experiments, the permeability was found to be $P = 0.0026 \pm 0.0001$ cm/h.

A comparison of this result with those of the gel donor, stirred receiver skin experiments, indicates that the calculated permeabilities were consistent but not identical. Comparing the results above with those of the stirred receiver sampling using PMD

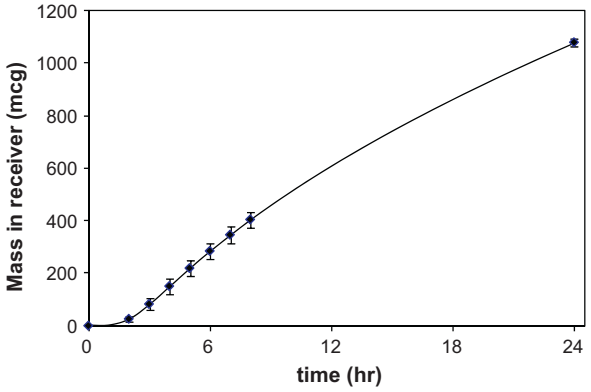

Fig. 10. Mass in receiver vs. time from setup using human cadaver skin and unstirred donor/receiver. The diamonds represent calculated mass at each experimental time point and the line represents mass calculated from Eq. (18). The donor and receiver were unstirred Methocel 0.5% gel.

(P = 0.0155 cm/h), it was seen that the permeability coefficient obtained using the gel donor/receiver setup was approximately 1/6 the value determined when the skin was in contact with a liquid receiver. This is presumed to result from the different osmotic environment that occurred in the presence of the liquid receiver compared to the gel receiver, and reflects perhaps less hydration and/or skin breakdown.

6. Discussion

The motivation for this study was to develop a method for determining permeability values of drugs through skin from in vitro experiments in which the skin is subjected to environments that more closely mimic in vivo conditions. Ultimately, this would involve three steps – developing an appropriate experimental method, formulating the associated mathematical model to evaluate the experimental data, and determination of the best donor/receiver media, which may also be a function of the drug properties. In this paper, the first two steps are considered.

The new method uses an unstirred donor and receiver in a modified Franz cell setup. In the method development, the receiver media for the new method consisted of aqueous gels of 0.5% Methocel. Sampling of the receiver was done using pulsatile microdialysis (PMD), which gave local receiver concentrations. The associated mathematical model allowed the membrane permeability to be determined from the PMD data, plus independently determined values for the membrane area and diffusion coefficient of the drug in the donor and receiver gels.

Microdialysis and in particular pulsatile microdialysis (PMD) are well suited for sampling unstirred media, and have found wide application for in vivo sampling. This is because the membranes used act as size filters, so only dissolved drug that is not bound or complexed can cross into the dialysate (Brunner and Langer, 2006; Sato and Kim, 1984). Thus, the method preferentially reflects the free or diffusible drug concentration in the medium surrounding the probe. In addition, it does not further discriminate between types of drug other than on the basis of the molecular weight. Because of this property, the mathematical model presented in this work is applicable to drug molecules of all types, as long as they are not too large or bound to larger structures.

6.1. Model and numerical evaluation of the equations

The experimental process was described by writing Fick's second law in three regions along with three initial and six boundary conditions. The diffusion model and resulting equations were fully transient, so there was no use of any steady state approximations in the membrane. This had two important implications. First, there was no need to be concerned about whether diffusion through the membrane or gel was rate limiting. Instead, the diffusion model accounts for the effects of the diffusion coefficient in the donor and receiver media as well as through the membrane, so even data obtained at early times could be used, as long as the receiver concentrations could be accurately assayed. This is reflected, for instance, in Eqs. (17) and (18), which required the value of the diffusion coefficient in the gels D for evaluation. The second implication is that the concentrations in the donor and receiver very close to the membrane interfaces were properly taken into account, and not approximated as the average concentrations in each region. This was important because the local concentration in the media near the membrane interface can differ significantly from the average concentration in the region and introduce errors into the permeability coefficient calculation (Bellantone et al., 2001).

The PMD probe was placed in the receiver just beneath the membrane, and was aligned to run as closely as possible across the membrane diameter. This placement and alignment served to minimize variations in x_P. In addition, running the probe across the diameter would be expected to reduce effects of any local membrane variations by sampling as much of the membrane as possible without curving the probe. This would be expected to be reduced further because of lateral diffusion of the drug in the receiver gel. In this study, the diffusion coefficient of the Doxepin HCl in the receiver gel was relatively high (D = 0.14 cm^2/h), so the timeframe for local lateral diffusion was expected to be shorter than for delivery of drug across the membrane into the receiver. Thus, lateral diffusion would be expected to allow surrounding areas of the membrane to also be included in the PMD data. However, as with all permeation studies, the membrane-to-membrane deviations must be examined after the experiments to determine if the number of replicates is sufficient.

A mathematical simplification resulted by designing the experiments to use the same gels in the donor and receiver. Other mathematical simplifications occurred by considering the donor and receivers to be semi-infinite, which placed two restrictions on the experimental data that should be used in the mathematical analyses using Eq. (18) – data obtained at times greater than $1–2 \times L^2/2D$ should not be used, where L is the smaller of the donor and receiver depth, and data should not be used for times corresponding to masses in the receiver exceeding ~35–40% of the mass originally loaded into the donor. However, these were not unduly restrictive in practice, and allowed for collection of sufficient data to support numerical analyses of the data. For the coated cellulose and human cadaver skin studies, experimental data obtained up to 24 h satisfied these criteria for use in the analyses, which were sufficient to determine the associated membrane permeabilities.

Eqs. (14) and (18) were developed for the calculations of local concentration in the receiver and the total mass in the receiver as functions of time. For a given permeation experiment, comparisons of the local concentration vs. mass in the receiver profiles (e.g., Figs. 6 and 7 for coated cellulose, or Figs. 9 and 10 for human cadaver skin) show that the profiles show that the mass in the receiver is not proportional to the local concentration determined using PMD. This reflects the fact that the receivers were not stirred and was one of the reasons the diffusion model presented was needed for data evaluations and permeability determinations.

Despite the complexity of the system, there were only three numerical degrees of freedom in the data analyses. The final equations depended on only four parameters that could not be independently determined – the membrane thickness h, the gel-membrane partition coefficient K, the diffusion coefficient of the drug in the membrane D_m, and the distance of the microdialysis probe from the membrane x_P – plus one mass balance constraint.

The values for h, K, D_m and x_P were obtained by nonlinear regressions, fitting Eq. (14) to local receiver concentration vs. time profiles. However, the combinations were not unique and acceptable fits were obtained with many combinations, some of which included physically unrealistic parameters. In addition, the final iterated parameter values were strongly dependent on the initial estimates.

The mass balance constraint was imposed to address this problem, and required that the final mass of the drug in the receiver calculated using Eq. (18) match the experimentally determined value. This reduced the numerical degrees of freedom in the regressions from four to three, but still provided for acceptable fits in all cases. In addition, the final iterated parameter values became relatively independent of the initial estimates, and tended to converge to a single set of roots.

The success of the fits with only three degrees of freedom supports the mathematical model. In addition, the final parameter values were examined and found to be physically reasonable. For instance, values for h and x_P were obtained by regressions because they could not be measured accurately enough, but they could be estimated for comparisons. It is also important to note that some parameters were averaged or net valued. For instance, the distance of the probe from the membrane might not be exactly constant over its position, so x_P would represent an "average" value.

It was also assumed that the membranes could be considered as homogeneous. As discussed elsewhere (Bellantone et al., 2001), even though skin is a heterogeneous structure, it can be approximated as homogenous because permeation tends to be limited by a single layer (the stratum corneum). In the case of skin, which is a heterogeneous structure, the final iterated value h would not be expected to be the thickness of the skin, but instead an effective thickness that is closer to that of the rate limiting part (such as the hydrated stratum corneum in the case of skin). Analogous considerations would apply to K and D_m. However, because the experimental data represents the combined effect through the permeability, the combination of values given in Eq. (19) should still provide an accurate value for P. This led to a set of experiments in which membrane permeabilities obtained using the new method were compared with those obtained using other experimental setups and mathematical analyses.

It should be noted that even if the membrane permeabilities were similar, the profiles obtained characterizing the permeation experiments can show differences. For instance, the permeabilities were fairly similar for coated cellulose and human cadaver skin, with calculated values of 0.0036 cm/h and 0.0026 cm/h, respectively. However, comparison of Figs. 6 and 9, which display the local receiver concentration vs. time profiles obtained from the coated cellulose and human cadaver skin setups with unstirred receiver media, showed visual differences, specifically with respect to the times at which the inflection points occurred. For coated cellulose experiments, the inflection points occurred at ~1–1.5 h, while they occurred at ~6 h for the human cadaver skin experiments. This difference is because the membrane permeability depends on partitioning, the diffusion coefficient of the drug in the membrane, and the membrane thickness (K, D_m and h) as given by Eq. (19). Numerical tests of Eq. (14) showed that the time of the inflection point for the local receiver concentration vs. time plots were particularly dependent on D_m and h, occurring earlier for increased values of D_m and later for increased values of h. Thus, as a hypothetical example, if the values of h and D_m both doubled (assuming the same K), the permeability value would stay the same according to Eq. (19), but the inflection point would occur at an earlier time.

Heuristic arguments can be made that diffusion through the coated cellulose should be different from diffusion through skin, even if the values of P are relatively close. For instance, diffusion through coated cellulose is likely through aqueous filled pores, while through skin it is through various layers such as the stratum corneum. Since diffusion coefficients through water are higher than through skin layers, the fitted value of D_m is likely to be higher for coated cellulose than for skin. Thus, it would be anticipated that the inflection point in Fig. 6 would occur at an earlier time than in Fig. 9. Arguments based on the membrane thickness might also be made. However, care should be taken not interpret the individual values of K, D_m and h too literally, especially with regard to skin data, because of the approximation as a homogeneous membrane.

One of the most important applications of the method presented in this paper would be to account for the effects of different receiver media when calculating membrane permeabilities. In general, the receiver media to be chosen would likely reduce the permeation of the drug through the membrane, in part due to less hydration in the case of skin and in part because using unstirred donor and receiver media would reduce the concentration difference between the donor and receiver in the immediate vicinity of the membrane.

Ideally, the receiver medium should be chosen with the following in mind. (1) It should mimic the physiological osmotic environment. (2) It should be inert with respect to the drug (e.g., does not complex). (3) The diffusion coefficient of the drug in the receiver medium should be high enough to allow the drug to diffuse away from the membrane to mimic physiological removal of the drug from the membrane area. In addition, it should allow lateral diffusion just beneath the membrane to occur. Diffusion coefficients that are too small may not mimic physiological removal of the drug after it has penetrated the skin, and may affect the PMD sensitivity.

The anticipated effects of changing the receiving medium would be to alter the osmotic environment to which membrane is exposed, which in turn would change the hydration effects and physical behavior, which would be reflected in a different permeability coefficient value. However, the mass of drug accumulated in the receiver depends on factors beside the membrane permeability, such as the partitioning behavior and the drug diffusion coefficients in the donor and receiver. However, if these donor/receiver parameters are characterized, reflected in the parameters K and D, then the change in membrane permeability can be properly evaluated. are known.

6.2. Comparisons of the membrane permeability obtained using other methods

In order to assess the new method, comparisons of permeabilities obtained using the new method and older methods were made for two systems. In the first case, coated cellulose membranes were used, and the membrane permeabilities were obtained using the new method and an established setup in which the donor and receiver were both stirred solutions. Coated cellulose membranes were used because they were not expected to show significant hydration or breakdown in the presence of liquid donor/receiver solutions. Thus, similar permeabilities should be obtained from both experimental setups. In fact, this was the case, with permeability values of $P = 0.0079 \pm 0.0009$ cm/h obtained using stirred liquid donor/receiver and direct sampling, and $P = 0.0036 \pm 0.0004$ cm/h obtained using the unstirred gel donor/receiver and PMD sampling. Since the two permeabilities were obtained using different experiments and different mathematical analyses, they would not be expected to agree exactly, in part because the presence of the gel might further affect pores in the membrane. Given that consideration, however, the rough agreement supports the new experimental procedure and the associated mathematical analysis.

In a second set of comparisons, the permeability of Doxepin HCl in human cadaver skin was assessed. One experimental setup was based on a previously reported method (Bellantone et al.,

2002), using an unstirred 0.5% Methocel gel as the donor and a stirred solution as the receiver. Receiver sampling was done using direct sampling and PMD concurrently, and the permeabilities were determined using data collected over 24 h. It was seen that the values of P determined using direct sampling and PMD data were nearly identical, indicating that microdialysis probes can be used successfully in the modified Franz diffusion cell apparatus when sealed with wax. (When pinch clamps were used to hold the donor and receiver cells together, the mechanical stresses on the microdialysis probes caused inaccurate results that were not acceptably reproducible.)

The permeability of Doxepin HCl in human cadaver skin was also determined using the new method, employing an unstirred donor and receiver, and using PMD for receiver sampling. The permeability was calculated using PMD data collected over 24 h, and compared to the value obtained in the previous method. The permeabilities were consistent but significantly different, with $P = 0.0155 \pm 0.0012$ cm/h from the gel donor with liquid receiver data and $P = 0.0026 \pm 0.0001$ cm/h from the gel donor and gel receiver data. Given the difference in the receiver media, this difference seems reasonable and explainable on the basis of excess skin hydration. This supports the notion that measuring skin permeabilities using liquid donors and/or receivers can lead to significant errors in permeability values. In addition, it highlights the need for follow-up work that would evaluate the effects of changing the donor and receiver media.

7. Conclusion

Exposing membranes such as skin to liquid aqueous media during in vitro permeation experiments can alter the membrane properties and associated permeabilities. This provided the motivation to develop a alternative experimental method for measuring the permeability of drugs through membranes. The new method uses unstirred donor and receiver media, and sampling in the receiver is done using pulsatile microdialysis (PMD). The method was mathematically modeled and equations were obtained for data analysis to provide permeability values. The method was used to obtain permeabilities of Doxepin HCl through artificial membranes and human cadaver skin, and gave results that were consistent with values found using other methods. Thus, the results supported the ability of the method and model to determine permeability coefficients and changes in membrane permeabilities resulting from changing donor and receiver media.

While the method is mathematically robust, there are some limitations that should be kept in mind. Most important are interactions between the drug being evaluated and the receiver medium. Since interactions such as complexation with the receiver would change the probe calibration results, receiver media should always be evaluated for this and avoided if interactions are detected. In addition, if the membrane is damaged or not uniform, the probe may not adequately characterize the average drug permeability due to its small diameter. Thus, data must be carefully examined, and additional replicates might be considered if variations in the data are too large.

Finally, the method developed and presented in this paper is the first step toward the ultimate goal of developing experiments that more closely mimic in vivo conditions, and which might provide better in vitro–in vivo correlations. Follow-up work is needed to evaluate the best choice of donor and receiver media, which would provide the most physiologically relevant environment for skin during permeation experiments. In addition, the properties of the drug are not evaluated in this context (lipophilic vs. hydrophilic, weak acid/base, etc.). Ultimately, it is likely that such evaluations involving a number of media and a range of drug physicochemical properties will be needed before the best choice of medium can be made for a given drug type for the development of IVIVC's. However, this is an issue that is separate from the development of the experimental method and model, and will be the subject of a future report.

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References

Bellantone, R., Nicolettos, N., Plakogiannis, F., 2001. Faster determination of the membrane permeability without using the lag time method. Int. J. Pharm. 248, 81–92.

Bronaugh, R.L., Maibach, H.I., 1991. In vitro Percutaneous Absorption: Principles, Fundamentals, and Applications. CRC Press, Florida.

Brunner, M., Langer, O., 2006. Microdialysis versus other techniques for the clinical assessment of in vivo tissue drug distribution. AAPS J. 8 (2), Article 30 (http://www.aapsj.org).

Chen, K.C., Hoistad, M., Kehr, J., Fuxe, K., Nicholson, C., 2002. Theory relating in vitro and in vivo microdialysis with one or two probes. J. Neurochem. 81, 108–121.

Crank, J., 1975. The Mathematics of Diffusion, 2nd ed. Oxford, New York.

Higuchi, W.I., 1962. Analysis of data on the medicament release from ointments. J. Pharm. Sci. 51, 802–804.

Holbrook, K.A., Wolff, K., 1993. The Structure and Development of Skin. McGraw-Hill, New York.

Kabir, M.A., Taft, D.R., Joseph, C.K., Bellantone, R.A., 2005. Measuring drug concentrations using pulsatile microdialysis: theory and method development in vitro. Int. J. Pharm. 293, 171–182.

Mazzo, D.J., Fong, E.K.F., Biffar, S.E., 1986. A comparison of test methods for determining in vitro drug release from transdermal delivery dosage forms. J. Pharm. Biomed. Anal. 4, 601–607.

Nicolettos, N., 1998. Transdermal Delivery of Certain Tricyclic Antidepressants: In vitro/in vivo Evaluations, Ph.D. Dissertation, Long Island University, Brooklyn, NY.

Queiroz Costa, R.H., Lanchote, V.L., Bonato, P.S., de Carvalho, D., 1995. Simultaneous HPLC analysis of tricyclic antidepressant and metabolites in plasma samples. Pharm. Acta Helv. 70, 181–186.

Sato, S., Kim, S.W., 1984. Macromolecular diffusion through polymer membranes. Int. J. Pharm. 22, 229–255.

Schaefer, H., Redelmeier, T., 1996. Skin Barrier: Principles of Percutaneous Absorption. Karger, Basel.

Van Hal, D.A., Jeremiasse, E., Junginger, H.E., Spies, F., Bouwstra, J.A., 1996. Structure of fully hydrated human stratum corneum: a freeze-fracture electron microscopy. J. Invest. Dermatol. 106, 89–95.