Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 293 (2005) 171–182
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Measuring drug concentrations using pulsatile microdialysis: Theory and method development in vitro

Mohammed A. Kabir, David R. Taft, Cecil K. Joseph, Robert A. Bellantone *

Division of Pharmaceutical Sciences, Arnold and Marie Schwartz College of Pharmacy and Health Sciences, Long Island University, 75 DeKalb Avenue, Brooklyn, NY 11201, USA

Received 12 November 2004; accepted 30 December 2004

Abstract

A novel method of rapidly sampling drug concentrations, based on pulsatile microdialysis (PMD), was developed. In PMD, a dialysate fluid is pumped into a microdialysis probe, allowed to occupy the probe while at rest for some time, and then flushed at a high rate. A model that is based on a Fick's Laws was solved and tested, using methazolamide (MTZ) as the test drug in a variety of experimental setups, including time-dependent donor concentrations. Calibration plots of the donor versus sample concentrations were linear. There was excellent agreement between the calculated and experimental values of the fraction recovered obtained from the calibration plots. In a system for which the donor concentration declined in a first order manner, the data obtained using PMD and direct sampling of the donor were in nearly exact agreement with the theoretical value of $k = 0.09\,\text{min}^{-1}$. PMD was also able to collect data points quickly enough to characterize the rapid binding kinetics of MTZ by activated charcoal. It was concluded that PMD is an accurate method of sampling drug concentrations, and can obtain samples over shorter time intervals and more frequently than previously available methods.

Keywords: Microdialysis; Continuous flow; Pulsatile; In vivo; In vitro; Diffusion coefficient

1. Introduction

Microdialysis is becoming a standard technique for in vivo and in vitro analysis of drug and biochemical concentrations (Stenken et al., 2001). In this method, a short length of tubing that is highly permeable to water and small molecules (the microdialysis probe window) is connected to impermeable tubing and put in contact with donor fluids for which drug concentrations are to be sampled. A sampling solution (dialysate) is convected through the probe, and the drug passively diffuses into the dialysate from the donor medium (Rojas et al., 2000). The collected samples are analyzed for drug content, and the concentration of drug in the donor medium is then determined from that information.

In the standard microdialysis method, the dialysate is continuously perfused through the probe at a constant

* Corresponding author. Tel.: +1 718 780 4154; fax: +1 718 780 4586.
E-mail address: rbellant@liu.edu (R.A. Bellantone).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2004.12.012

flow rate. This will be referred to as continuous flow microdialysis (CFMD). At typical flow rates of 0.5–2.0 μL/min, the time required to collect samples (typically 20 μL or more) is relatively long, and the time resolution (the ability to associate a specific concentration with a specific time or a short time interval) is poor (Bolinder et al., 1993; Hildingsson et al., 1996). As a result, CFMD is not well-suited for studying systems in which the donor concentration changes rapidly, such as in vitro binding or cellular drug uptake kinetics (Iyer et al., 1999). On the other hand, the microdialysis membrane acts as a filter and eliminates the need for pre-assay separation steps (Linhares and Kissinger, 1992, 1994; Ungerstedt, 1991), during which the binding and/or uptake processes would have continued. Thus, it is apparent that a microdialysis method that is capable of good time resolution is desirable.

To attain this goal, a novel method has been developed, which will be referred to as pulsatile microdialysis (PMD). In this method, the dialysate is pumped into the probe window at a high flow rate, subsequently allowed to occupy the probe while at rest for a period referred to as the resting time, and then flushed out at the same flow rate. If this is done in a repeated fashion, it mimics the mechanics of pulsatile flow in a cylindrical tube (Womersley, 1955; Zamir, 1996). Since any portion of the dialysate only accumulates drug while it is within the probe window, the PMD method offers the advantage of sampling the donor concentration in a well-defined time interval that can also be short (due to the high surface-to-volume ratio microdialysis probes). The PMD experimental procedure can be optimized by choosing the resting time to be long enough to give measurable drug concentrations in the sample, yet short enough to provide the desired time resolution. In addition, a high flow rate is used so that the exposure time of the flowing sample is short compared to the rest time, and the sample volume can chosen to completely collect the dialysate that was at rest in the probe window while minimizing its dilution.

In this study, the PMD method was developed experimentally and mathematically modeled. The model was validated using experimental data for constant and time-varying donor concentrations, and the equations derived from the model were used for data interpretation and optimization of the experimental procedure.

2. The pulsatile microdialysis mathematical model

The relationship between concentrations of the drug in the donor fluid C_D and the dialysate sample C_S is characterized by the fractional recovery F_R. For a dialysate that is initially void of drug, this is defined as (Bouw and Hammarlund-Udenaes, 1998; Stahle, 2000; Alexander and Julie, 1999)

$$F_R = \frac{C_S}{C_D} \tag{1}$$

A number of factors influence the F_R, including the temperature (Rojas et al., 2000), dialysate flow rate (Meyerhoff et al., 1993; Kemer et al., 1993; Hashiguchi et al., 1994), probe length (Stenken et al., 1993), and the physical properties of the drug (de Lange et al., 2000), dialysate (Cussler, 1997) and membrane (Starzak, 1984). A model calculating these effects for PMD is presented below for the case of a stirred donor for which the donor concentration remains constant during the time in which an individual sample is taken.

The microdialysis setup is shown in Fig. 1, which illustrates a probe window made of a highly permeable tube of constant inner radius a, length L and volume V_W. In the most general case, microdialysis can be described in cylindrical coordinates as a transport of drug that occurs by a combination of passive diffusion in the radial direction, and convection plus passive diffusion in the axial direction. This is written mathematically as (Crank, 1975)

$$\frac{\partial C}{\partial t} = -v_z \frac{\partial C}{\partial z} + D\frac{\partial^2 C}{\partial z^2} + \frac{D}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right) \tag{2}$$

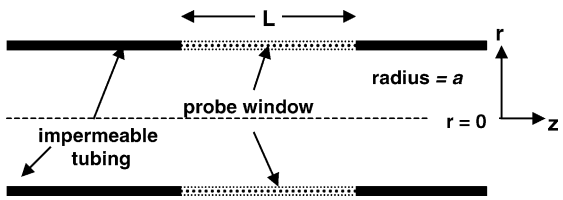

Fig. 1. Schematic diagram of microdialysis probe. The permeable tubing (probe window) is represented by the broken lines and the impermeable tubing is represented by the heavy solid lines. The length of the probe window is L and the inner radius is a. The centerline ($r = 0$) is represented by the dashed line. The thickness of the probe window, which is the difference between the outer and inner radii, is denoted by h.

Here C is the concentration of the dialysate inside the probe at a given position and time, D the diffusion coefficient of the drug in the dialysate, and v_z is the axial velocity, which in general is a function of r but is typically (i.e., for CFMD) held constant with respect to time. On the right hand side of Eq. (2), the first term represents the effects of convection, while the second and third terms represent the contribution the axial and radial diffusion, respectively.

For the case of PMD, Eq. (2) can be simplified as follows.

- The exposure time of the sample is chosen to be short enough to neglect axial diffusion. From the theory of separation of variables, for a tube of radius a, the relaxation time characteristic of the approach to equilibrium for diffusion in the radial direction is $\sim a^2/D$ (Carslaw and Jaeger, 1985). From random walk theory, the average distance traveled by diffusing molecules during a time interval t is $\sim\sqrt{Dt}$ (Reichl, 1980). When the exposure time is comparable to the relaxation time, the average axial distance traveled due to diffusion is $\sim a$. Since $a \ll L$ for microdialysis probes, axial diffusion will have a negligible effect on the mass balance in the sample.
- While the dialysate is stationary in the probe, $v_z = 0$ and the convection term can be ignored. Even when the dialysate is being flushed, the exposure time for the flowing sample is short enough so the axial gradient does not have time to develop, and the $v_z(\partial C/\partial z)$ may be neglected.

From the above considerations, Eq. (2) reduces to

$$\frac{\partial C}{\partial t} = \frac{D}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C}{\partial r}\right) \tag{3}$$

which requires one initial condition and two boundary conditions for its complete solution. The initial condition is that the dialysate is initially void of drug when it enters the probe window. The boundary conditions are obtained from the following considerations:

- The donor concentration C_D is constant during each sampling period.
- The drug concentration is finite everywhere in the microdialysis probe.
- The dialysis tube wall is very thin and highly permeable, so pseudo steady state in the wall is established quickly. Thus, the flux of drug from the donor into the dialysate is proportional to the concentration difference across the wall of the probe window. The proportionality factor is the permeability P of the probe window, and is assumed to remain constant. For the probes used here, it can be assumed that the drug does not partition into the probe material, and thus permeates the probe window wall exclusively through pores. If the donor and receiver media are similar, the partition coefficients between the pore medium and the donor or dialysate may be taken as unity. Thus, denoting the probe window porosity, thickness (difference between the outer and inner radii) and tortuosity by ε, h and τ, respectively, the permeability of the window is given by

$$P = \frac{\varepsilon D}{\tau h} \tag{4}$$

Thus, the initial and boundary conditions are written mathematically as follows:

$$\begin{aligned} &\text{Initial condition :} \quad C(r,0) = 0, \quad t = 0 \\ &\text{Boundary conditions :} \\ &-D\frac{\partial C}{\partial r} = P(C_D - C), \quad r = a \\ &C(0,t) = \text{finite}, \quad r = 0 \end{aligned} \tag{5}$$

Using the separation of variables method, Eqs. (3) and (5) can be solved to give the concentration in a volume element of dialysate at a given radius as

$$C(r,t) = C_D\left[1 - 2\sum_{n=1}^{\infty}\frac{\lambda J_0(\beta_n r/a)}{(\beta_n^2+\lambda^2)J_0(\beta_n)} \times \exp\left(-\frac{\beta_n^2 Dt}{a^2}\right)\right] \tag{6}$$

Here t is the length of time that a given volume element of dialysate was in the probe window (the exposure time). J_0 and J_1 are the zero-order and first-order Bessel function of the first kind, respectively (Carslaw and Jaeger, 1985; Ozisik, 1989), and the β_n are the roots of the equation

$$\beta_n J_1(\beta_n) - \lambda J_0(\beta_n) = 0 \tag{7}$$

where

$$\lambda = \frac{aP}{D} = \frac{a\varepsilon}{\tau h} \tag{8}$$

Values of β_n have been tabulated for various values of λ and n in the literature (Crank, 1975), and

can also be calculated from Eq. (7) using the nonlinear solvers included with spreadsheets such as EXCEL®. The second equality in Eq. (8), which was obtained using Eq. (4), shows that λ depends on properties of the probe window, but not properties of the drug or solvent.

The total amount of drug collected by the dialysate in the probe window after a given exposure time is found by integrating the concentration over the volume of the sample. Since the axial dependence is neglected in the mass balance, the mass in a sample of volume V with an exposure time t can be found from

$$M = \frac{V}{\pi a^2}\int_0^a 2\pi r C(r,t)\,\mathrm{d}r \tag{9}$$

Not all parts of a collected dialysate sample will be exposed to the donor for the same length of time, and two portions must be considered. One portion of the sample (referred to as the continuous portion) flows through the probe window without resting. The other portion (referred to as the pulsed portion) is pumped into the window, allowed to remain at rest for a resting time t_R, and then pumped out. For the continuous portion, the dialysate exposure time is simply the transit time t_Q required for an element of fluid to move through the probe window. The exposure time t_P for the pulsed portion is the sum of the resting and transit times. These are given by

$$t_Q = \frac{V_W}{Q}$$

$$t_P = t_R + t_Q$$

where Q is the volume flow rate of the flushing. Thus, for a sample of volume V_S, the pulsed portion has a volume V_W and accumulates a mass M_P during a total exposure time of t_P, while the continuous portion has a volume $V_S - V_W$ and accumulates a mass M_Q during an exposure time of t_Q. M_P can be found by setting $t = t_P$, in Eq. (6), performing the integration in Eq. (9), and multiplying by the length of the probe window $V_W/\pi a^2$. M_Q can be found by setting $t = t_Q$ in Eq. (6), performing the integration in Eq. (9), and multiplying by a length $(V_S - V_W)/\pi a^2$. The total mass of drug M in a collected sample is given by

$$M = M_P + M_Q \tag{10}$$

where

$$M_P = V_W C_D\left[1 - \sum_{n=1}^{\infty}\delta_n \exp(-\gamma_n t_P)\right] \tag{11}$$

$$M_Q = (V_S - V_W)C_D\left[1 - \sum_{n=1}^{\infty}\delta_n \exp(-\gamma_n t_Q)\right] \tag{12}$$

The constants γ_n are δ_n are given by

$$\gamma_n = \frac{\beta_n^2 D}{a^2} \tag{13}$$

$$\delta_n = \frac{4\lambda^2}{\beta_n^2(\beta_n^2 + \lambda^2)} \tag{14}$$

where

$$\sum_{n=1}^{\infty}\delta_n = 1 \tag{15}$$

The fractional recovery in the sample can be expressed in terms of the mass in the sample and the sample volume as $F_R = M/V_S C_D$. Similarly, the fractional recoveries of the pulsed (F_{RP}) and continuous (F_{RQ}) portions of the sample are defined as

$$F_{RP} = \frac{M_P}{V_W C_D} = 1 - \sum_{n=1}^{\infty}\delta_n \exp(-\gamma_n t_P) \tag{16}$$

$$F_{RQ} = \frac{M_Q}{(V_S - V_W)C_D} = 1 - \sum_{n=1}^{\infty}\delta_n \exp(-\gamma_n t_Q) \tag{17}$$

The total mass in the sample can be written as

$$M = V_W C_D F_{RP} + (V_S - V_W)C_D F_{RQ}. \tag{18}$$

Since M is the proportional to the donor concentration C_D, linear calibration plots relating C_D and C_S can be constructed according to Eq. (1) as

$$F_R = \frac{V_W}{V_S}(F_{RP} - F_{RQ}) + F_{RQ} \tag{19}$$

For a constant flush rate, F_{RQ} corresponds to the fractional recovery for CFMD, as can be seen from Eqs. (16), (17) and (19) when $t_P = t_0$. Since F_{RQ} can be measured experimentally, it is possible to obtain F_{RP}

as a function of the exposure time t_P (the experimental verification is presented in Section 5).

The approach to equilibrium is characterized in the above equations by the exponential transient terms in the infinite series. For all values of λ and n, both the δ_n and exponential terms are between zero and one, and both tend toward zero with increasing n or time of exposure. Using nominal values for the dialysis probes used in this study ($a = 100 - 125\ \mu$, $h = 8 - 12\ \mu$, $\varepsilon < 0.1$) and a typical tortuosity value ($\tau > 1.5 - 2$) in Eq. (8) shows that $0 < \lambda < 1$. Numerical calculations show that for $\lambda < 0.5$ (which is usually the case), $\delta_2/\delta_1 < 0.005$ and the $n > 1$ terms can always be neglected (less than 0.1% error). For $0.5 < \lambda < 1$, the $n > 2$ terms can be always be neglected, and the $n > 1$ terms can be neglected when $Dt_P > 5 \times 10^{-6}\ \text{cm}^2$. Since $D > 10^{-6}\ \text{cm}^2/\text{s}$ at room temperature or above for most drugs in aqueous vehicles in the absence of agents that significantly enhance the viscosity (such as surfactants or polymers), it is usually possible to neglect the $n > 1$ terms when the exposure time exceeds ~5 s, so that Eq. (16) can be rewritten as

$$\ln(1 - F_{RP}) = \ln \delta_1 - \gamma_1 t_P \tag{20}$$

In theory, plots of $\ln(1 - F_{RP})$ versus t_P can be used to determine γ_1 and δ_1 (which, in turn, can be used in Eqs. (7) and (14) to find the more fundamental parameter λ). In practice, this is true for finding γ_1 because small experimental errors will minimally affect the slope. However, for the systems studied here, the intercept is typically close to zero because δ_1 is close to 1, so experimental errors can result in significant relative errors in the intercept. This is important because, in this range, small differences in the value of δ_1 will result in relatively large differences in the corresponding value of λ. Thus, if the value for λ is needed, other methods that measure it directly may be preferred. These will be presented in a follow-up paper.

3. Materials and methods

The PMD method was tested using methazolamide (MTZ) as the test drug in a variety of in vitro experiments. The details of the experimental procedures are given below, and the results are discussed in the following section.

3.1. Microdialysis

The microdialysis probes were constructed in our laboratory. Hollow tubes made of reconstituted cellulose (Spectrum Laboratory, Brunswick, NJ), with a molecular cut-off of 18 kDa, were used as the dialysis membrane for the probe windows. The inner radius of the dialysis membrane tube was 100 μ and the thickness of the membrane wall was 8 μ (nominal values). Two segments of polyimide tubing (MicroLumen, Tampa, FL), with an outer radius of 83 μ, were connected to the both sides of the dialysis probe window and glued together using cyanoacrylate glue, leaving a probe window of the desired length (typically 5−10 cm). One segment (7 cm length) was then glued to a 15 cm Tygon tube with an inner diameter of ~100 μm, which was connected to a syringe pump and served as inlet tubing. The other segment (10 cm length) was used to collect the samples. After construction, all probes were measured to exactly determine the length of the window.

A HARVARD Model PHD2000 programmable syringe pump (Harvard Apparatus, Holliston, MA) was used to pump 0.005N NaOH dialysate through the probe. A jacked beaker with a magnetic stirrer was used as the in vitro donor compartment. Different concentrations of MTZ (Sigma Chemical, St. Louis, MO) in 0.005N NaOH (50 mL volume) were used as the donor solutions. The probe window was immersed in the donor medium and held stationary with a clamp. The MTZ solution was stirred continuously, so the concentration at the fluid-membrane boundary could be taken as equaling that of the bulk donor fluid. All studies were performed at 37 °C. The dialysate was collected from the outer tubing in 0.25 mL pre-chilled plastic micro-centrifuge tubes, and the samples were immediately analyzed using HPLC. Preliminary studies found no evidence of non-specific binding or adsorption of MTZ to the sampling tubes.

3.2. Chemical analyses

MTZ was analyzed by isocratic reversed-phase HPLC with UV detection (9). The HPLC system consisted of a Shimadzu LC-10AD constant flow pump and SPD-10VA ultraviolet detector (Shimadzu, Piscataway, NJ), and a column. Output from the detector was processed on a personal computer using TC4

Turbochrom HPLC software (Perkin-Elmer, Shelton, CT). Separation was accomplished with a μBondapak 300 mm × 3.9 mm C18 column (Waters, Milford, MA). The mobile phase consisted of a 20:80 acetonitrile:sodium acetate buffer (0.05 M, pH 4.0). The mobile phase flow rate was 1.5 mL/min and the detection wavelength was 290 nm. Samples (20 μL) were injected directly into the system using an auto sampler. The method was validated in buffer and the coefficient of variation of the method was less than 2%. The HPLC standard curve was linear and followed the equation $A = 12.320C$ ($R^2 = 0.999$), where A is the area under the absorbance peak and C is the MTZ concentration in ng/mL. The limit of quantitation was 25 ng/mL. All solvents used were HPLC grade.

4. Experimental

4.1. CFMD (continuous flow microdialysis)

A dialysate of 0.005N NaOH was perfused through microdialysis probes with different continuous flow rates (Q = 3, 5, 7, 10, 15, 20, 30, 40, 50 and 60 μL/min). The probes were immersed in stirred MTZ solutions of known concentration. For each flow rate, samples were collected into 250 μL microtubes and analyzed by HPLC.

4.2. PMD (pulsatile microdialysis)

A dialysate of 0.005N NaOH was convected into microdialysis probes, allowed to sit inside the probe for various resting times, and then flushed out. The sample volumes were chosen to exceed the total of the probe window plus the outer tubing volumes. Samples were collected and immediately analyzed by HPLC. Studies were performed varying the resting times, flush volumes, flush rates and probe lengths as follows:

- resting time t_R: 5, 7, 10, 15, 20, 25, 30, 40, 50 and 60 s;
- sample volume V_S: 5, 7 and 10 μL;
- flush rate Q: 50, 60, 80, 100, 150 and 200 μL/min;
- nominal probe window length L: 5 and 10 cm;
- nominal probe window volume V_W: 1.57 and 3.14 μL.

In all cases except the charcoal studies (see below), the pump was programmed to repeat the above procedure seven times, with the first two collections being discarded and the last five combined to form the sample. For the charcoal studies, the procedure was done only once due to the rapid changes in the donor concentration. All experiments were done in triplicate.

4.3. In vitro probe calibration

PMD was performed for each probe window. The length of the window was measured, and it was subsequently immersed in MTZ solutions of various known concentrations. A sample volume of V_S = 5 μL and flush rate of Q = 100 μL/min were selected. Samples were collected for each donor concentration and immediately analyzed by HPLC. Calibration studies were performed for various values of t_R (10 and 15 s) and probe window lengths (nominally 5 and 10 cm, corresponding to window volumes of ∼1.57 and 3.14 μL, respectively). All experiments were done in triplicate, and the calibration plots were constructed by plotting C_S versus C_D according to Eq. (1).

In addition to the initial probe calibration, subsequent "three-point" probe calibrations were done when appropriate to verify that the original calibration curves were still valid. These were done by repeating the above calibration procedure using three donor concentrations over the donor concentration range of interest and comparing the results with the original calibration curves.

4.4. In vitro first-order drug uptake simulation experiment

The MTZ concentration (initially 54 μg/mL) in 50 mL of the donor solution was reduced over time by adding 4.5 mL/min fresh 0.005N NaOH to the donor while simultaneously withdrawing 4.5 mL/min of the mixed donor solution, both in a continuous manner. For this setup, the MTZ concentration in the donor solution followed a first-order decline, with a theoretical rate constant of $k = 0.09\ \text{min}^{-1}$ and half-life of 7.7 min. PMD was performed using a 10 cm probe with a flush volume of 5 μL, flush rate of 100 μL/min, and resting time of 10 s. Samples (25 μL) were collected at the same times (0, 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 50 and 60 min) from both the donor solution and the dialysate, and immediately analyzed using HPLC. Three point

probe calibrations were performed before each experiment.

4.5. Charcoal MTZ adsorption kinetics experiment

A charcoal suspension was prepared at least 24 h before use by adding 0.6 g of activated charcoal (Sigma Chemical, St. Louis, MO) to 100 mL of 0.6% Dextran Solution (Birkmeier et al., 1995). Subsequently, 2.0 mL of that charcoal suspension was quickly mixed into 48 mL of stirred MTZ solution (200 μg/mL in 0.005N NaOH), which was taken as the start time ($t = 0$) for the binding experiment. Samples were collected during 10 second intervals, and the start times for the sampling intervals were 0, 0.2, 0.4, 0.7, 1, 2, 5, 10 and 30 min. The flush rate was 100 μL/min, the rest time was 8 s, and the sample volume was 5 μL. The samples were immediately analyzed using HPLC. The study was done at 37 °C, and a three-point probe calibration was performed before and after each experiment. Direct sampling of the donor was also done at various times for comparison with the PMD data. The direct donor samples were immediately filtered to remove the activated charcoal and analyzed using HPLC.

5. Results and discussion

Experiments were done to evaluate: (i) the sensitivity of the PMD technique; (ii) the variability of measurements made with PMD; (iii) the properties of the concentration calibration plots; (iv) the ability of the method to measure rapidly varying donor concentrations. The results are discussed below.

5.1. Sensitivity of PMD compared to CFMD

The sensitivity of the PMD method was compared to that of the CFMD method by comparing the F_R as a function of the average flow rate, which was taken as Q for the CFMD and $\langle Q \rangle$ for the PMD, where

$$\langle Q \rangle = \frac{V_S}{t_S} \tag{21}$$

Here t_S is the time required to collect the entire sample, and is given by

$$t_S = t_R + \frac{V_S}{Q} \tag{22}$$

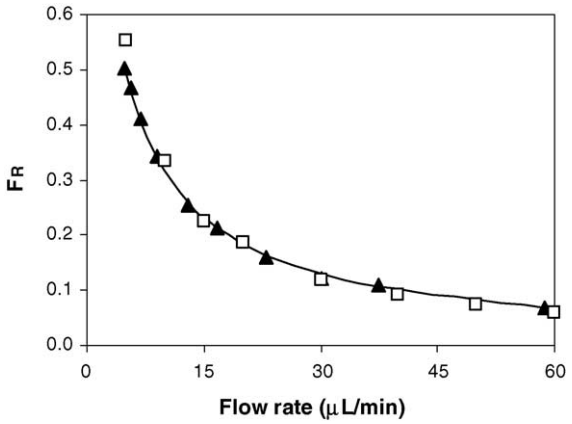

Fig. 2. Comparison of F_R for PMD and CFMD at various flow rates. The filled triangles represent PMD data, the open squares represent CFMD data, and the line represents the theoretical fit of the PMD. For PMD, an "average" flow rate ⟨Q⟩ was used, as given by Eq. (21), which was varied by changing the resting time.

In the PMD experiments $\langle Q \rangle$ was changed by varying the resting time, while keeping the sample volume and flush rate constant ($V_S = 5$ μL and $Q = 100$ μL/min). Fig. 2 shows that, at similar average flow rates, the PMD and CFMD data displayed similar values of F_R, which decreased with increasing flow rates for both methods due to shorter exposure times.

The minimum measurable donor concentration was estimated by dividing the assay limit of quantitation by the F_R. In this study, the HPLC limit for MTZ was ∼25 ng/mL. For a 10 s t_R and 10 cm probe window, $F_R \sim 0.2$ and the minimum measurable C_D was ∼125 ng/mL. For the same system and a 30 s t_R, $F_R \sim 0.6$ and the minimum measurable C_D was ∼40 ng/mL. Thus, the sensitivity of the PMD method can be effectively adjusted by varying the resting time.

5.2. Variability and reproducibility

All of the calibration plots were reproducible and showed low variability. For the same resting time and solutions, the R.S.D. in the sample concentration between experiments was typically in the range of 0.5–1.5%. Comparisons of F_R values obtained from individual calibration plots (not using average of triplicates) showed similar R.S.D. values and did not exceeded 1.5% in this study. Similar results were seen with other drugs (data not shown).

The low variability of the experiments is likely due to two factors. First, the programmable pump setup minimizes the human variation. Second, by collecting five smaller samples to make one larger one (as described in the experimental section), small variations between the individual samples will tend to average out when they are combined to form the larger final sample.

5.3. Concentration calibration plots

To correlate the PMD sample and donor concentrations in vitro for a given probe, calibration plots of C_S versus C_D were constructed. It was observed that calibration plots were linear ($R^2 > 0.99$) in all cases, and the fraction recovered F_R was taken as the slope of the plot, in accordance with Eq. (1).

PMD data for a typical probe is shown in Fig. 3. Increasing the resting time from 10 to 15 s at the same flush rate increased the F_R from 0.221 to 0.294. As the resting time was increased at a constant flush rate, $F_{RP} \rightarrow 1$ while F_{RQ} did not change, and the F_R increased towards an upper limit of $F_R = V_W/V_S + [(1 - V_W)/V_S]F_{RQ}$, in accordance with Eq. (19). This is illustrated in Fig. 4.

The F_R decreased with increasing sample volume and increased with the volume of the probe window in a manner that was also consistent with Eq. (19). In all

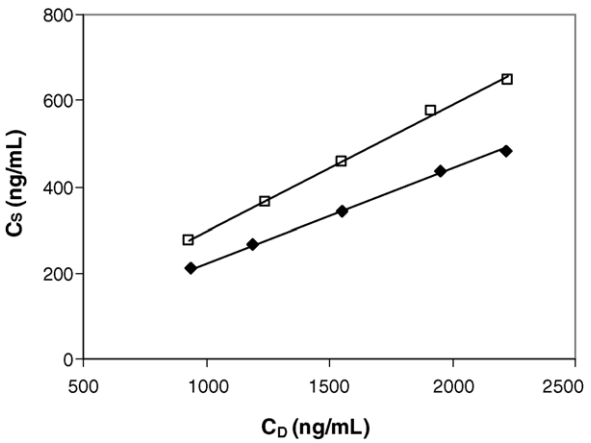

Fig. 3. PMD probe calibration curves for two different resting times. The diamonds represent PMD data using $t_R = 10$ s, and the open squares represent PMD data using $t_R = 15$ s. The data fit the line $C_S = 0.221C_D$ ($F_R = 0.221$) for 10 s resting times, and $C_S = 0.294C_D$ ($F_R = 0.294$) for 15 s resting times.

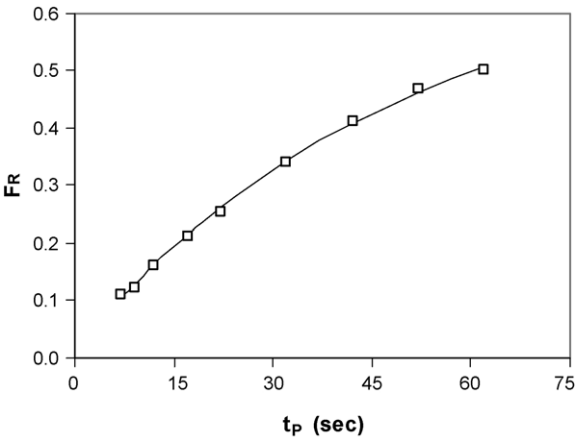

Fig. 4. F_R vs. exposure time t_P for methazolamide in an aqueous buffer. The open squares represent the experimental data, and the line represents the theoretical curve. The F_R approaches an upper limit of ~0.7 for this PMD setup.

cases (data not shown), plots of F_R versus $1/V_S$ using PMD data were linear, and the intercepts (F_{RQ}) were in agreement with the F_R obtained from CFMD experiments (for which the CFMD flow rate was the same as the PMD flush rate Q). This is to be expected because the intercept occurs when $1/V_S \rightarrow 0$, which physically corresponds to CFMD. Values of F_{RQ} calculated using Eq. (17) were also in agreement. Thus, CFMD data were used experimentally and Eq. (17) was used in certain data analysis to compute the F_{RQ}.

It can be seen that the F_R depends on the resting time, flow rate, and the ratio of V_W/V_S. In addition, it is also dependent on the temperature due to changes in the diffusion coefficient. Thus, probes must be specifically calibrated using PMD parameters (resting time, flush rate, sample volume, etc.) that will match those under which subsequent PMD measurements are made. Since there can also be some variation between probes, each one must be calibrated individually even if the same PMD parameters are used.

5.4. Estimating the diffusion coefficient of a drug in the dialysate

Fig. 4 also compares experimental and. simulated PMD data for F_R versus exposure time, which was used to estimate the diffusion coefficient of MTZ in an aqueous buffer at 37 °C. This was done by performing nonlinear regressions using Eqs. (16), (17) and (19) to

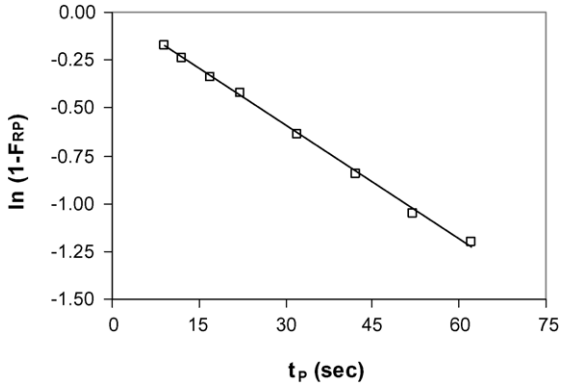

Fig. 5. Ln $(1 - F_{RP})$ vs. exposure time t_P for methazolamide in an aqueous buffer. The open squares represent the experimental data, and the line represents the linear regression.

obtain the parameters γ_1 and λ. From the fitted value of λ, the β_n, γ_n and δ_n ($n \leq 3$) were obtained using Eqs. (7), (13), and (14), and the diffusion coefficient was calculated as $D = \gamma_1 a^2/{\beta_1}^2$. The probe window volume was $V_W = 3.5\,\mu L$ and the sample volume was $V_S = 5\,\mu L$. The values of F_{RP} were calculated from Eq. (19), using a value of F_{RQ} that was obtained from CFMD data for a flow rate of 100 μL/min (the same flush rate used in the PMD). Exposure times of more than 7 s were used, and Eq. (20) was assumed to hold, as supported by the linearity of the $\ln(1 - F_{RP})$ versus t_P shown in Fig. 5. A value of $\gamma_1 = 0.02\,s^{-1}$ was obtained from the slope of the line, and was used as the initial guess in the iterations.

Satisfactory numerical convergence was obtained for a number of final values of λ, which could not be determined a priori for the probes. However, it was possible to estimate upper and lower limits for λ from Eq. (8), and these were used as initial estimates in the nonlinear regressions. For each limit, a tortuosity estimate of $\tau = 2$ was used, porosity range was estimated as between 0.004 and 0.008 (based on conversation with the probe manufacturer). The lower λ estimate resulted in values of $\lambda = 0.166$, $\gamma_1 = 0.0197\,s^{-1}$ and $D = 6.5 \times 10^{-6}\,cm^2/s$. The upper λ estimate resulted in values of $\lambda = 0.336$, $\gamma_1 = 0.0196\,s^{-1}$ and $D = 3.3 \times 10^{-6}\,cm^2/s$ (R.S.D. < 1% in all cases). This range of D is consistent with values found in the literature ($\sim 4 \times 10^{-6}\,cm^2/s$) (a method to determine of λ is being developed and will be presented in a follow-up paper).

5.5. In vitro first-order MTZ uptake simulation

This experiment was done to assess the ability of the method to measure changes in the donor concentration that are slow compared to the sampling time t_S. A first order uptake of MTZ was simulated by continuously adding 0.05N NaOH to a donor solution of MTZ in NaOH at a rate of $q = 4.5$ mL/min, and simultaneously removing the stirred solution at the same rate, to maintain a donor solution volume of $V = 50$ mL. The theoretical MTZ donor concentration in the donor is given by $C_D = C_0 \exp(-kt)$, where $k = q/V$. For this system, the initial donor concentration was $C_0 = 54\,\mu g/mL$ and the calculated rate constant was $k = 0.090\,min^{-1}$ (half life of 7.7 min). Plots of $\ln C_S$ versus t (from PMD with $t_P = 10$ s) and $\ln C_D$ versus t (from the direct donor sampling) are shown in Fig. 6. The two lines are nearly parallel and in excellent agreement with the theoretical data, yielding rate constants of $k = 0.089\,min^{-1}$ for the PMD and $k = 0.088\,min^{-1}$ for the direct donor sampling. The intercepts for PMD and direct sampling are 9.150 and 10.887, respectively. Taking the log of Eq. (1) shows that $\ln C_S - \ln C_D = \ln F_R$, so the difference between the intercepts should equal $\ln F_R$. Taking the antilog of the difference in the intercepts gives a value of $F_R = 0.176$, which is in agreement with the value of

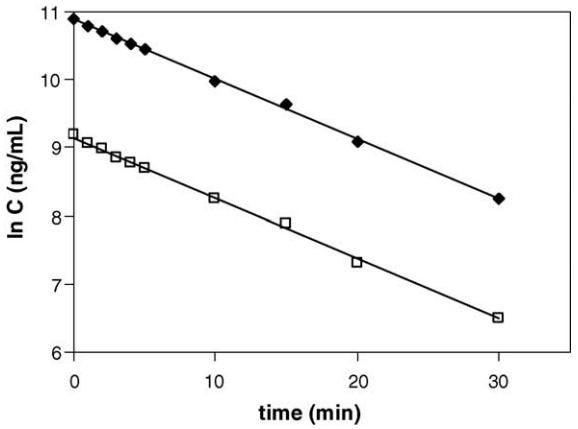

Fig. 6. log concentration vs. time for simulated first order drug uptake of MTZ. The experiment mimicked a first order rate constant of $k = 0.090\,min^{-1}$. The solid diamonds represent direct sampling of the donor solution (slope = $-0.088\,min^{-1}$) and the open squares represent PMD data (slope = $-0.089\,min^{-1}$). From the difference in the intercepts, $F_R = 0.176$, which is in agreement with $F_R = 0.170$ obtained from the probe calibration.

$F_R = 0.170$ that was calculated from the calibration plot for this probe using Eq. (1).

5.6. Charcoal MTZ adsorption kinetics study

This experiment was done to assess the ability of PMD to sample rapidly changing donor concentrations. An activated charcoal suspension was added to a stirred donor solution containing MTZ, and the concentrations were simultaneously sampled using PMD and direct donor sampling.

As expected, the free drug concentration in the donor dropped rapidly over the first minute, and declined more slowly after that. Fig. 7 shows experimental data obtained using PMD (solid circles) during the first 5 min of the experiment (the start time $t = 0$ min was taken as the time when the activated charcoal was added). The time required to take each sample was $t_S \sim 11$ s, and four data points (in addition to the initial data point) were obtained during the first minute. The measured concentrations were plotted at times corresponding to the midpoint of the sampling interval (taken as 5 s after the start of each sample). The free MTZ concentration decreased from an initial concentration of ~195 to 21 μg/mL after the first minute, 8.6 μg/mL after ~2 min, and 6.7 μg/mL after ~5 min. Direct donor sampling during the first minute was not reproducible and could not be compared with the PMD data. However, after the first minute, the PMD and direct donor sampling data were in good agreement (not shown).

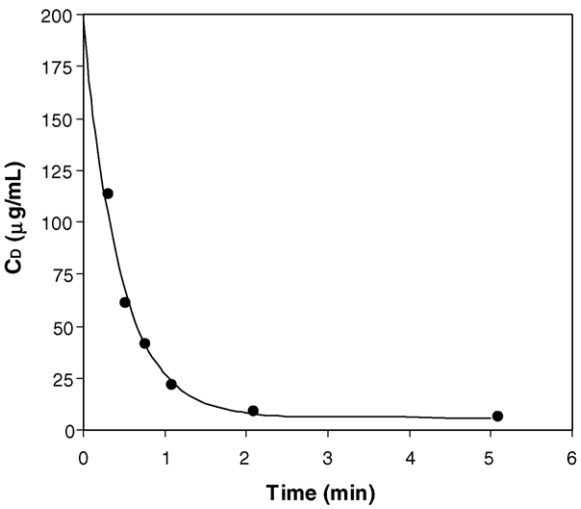

Fig. 7. Adsorption of MTZ by activated charcoal: free concentration vs. time data obtained using PMD. The temperature was 37 °C. The total exposure time was $t_P = 10$ s and the time required to take each sample was $t_S = 11$ s. The data points are plotted at the midpoint of the sampling interval. The solid circles represent experimental data obtained using PMD, and line represents the fitted curve obtained using Eq. (23).

Since the concentration appeared to approach an equilibrium value, a simple binding/release model was used to test the PMD data. (We do not claim that this model is ultimately the correct one for this system. However, it is probably a reasonable one to use for checking the feasibility and consistency of the data.) In this model, it was assumed that the rate at which free drug binds to the charcoal is proportional to the concentration of free drug C_F and the concentration of empty adsorption sites E, and the rate of release of bound drug is proportional concentration of bound drug C_B. Thus, the rate of change of free drug in the donor is given by

$$\frac{dC_F}{dt} = -k_f E C_F + k_r C_B$$

where k_f and k_r are the binding and release rate constants, respectively. If the number of binding sites on the charcoal is assumed to be large, then E can be taken as approximately constant, and this equation can be solved to give

$$C_F = C_{eq} + (C_0 - C_{eq}) \exp[-(k_f E + k_r)t] \qquad (23)$$

where C_0 and C_{eq} are the initial and equilibrium free drug concentrations in the solution, respectively. The experimental data shown in Fig. 7 was used in a nonlinear regression of Eq. (23), and values were found of $C_0 = 201$ μg/mL, $C_{eq} = 5.97$ μg/mL, $k_f E = 2.19$ min^{-1}, and $k_r = 0.067$ min^{-1}. For comparison, the values predicted by Eq. (23) are also shown in Fig. 7 (the solid line), and fit the experimental data well ($R^2 > 0.99$).

In these experiments, the donor concentration dropped rapidly enough so that the assumption of a constant donor concentration during the sampling time did not hold for the first minute or so. As a result, two issues must be considered—the ability of the probe to detect the changes, and the time at which the sample concentration should be plotted.

- The microdialysis probes must be able to respond quickly to changes in the donor concentration. The response time of the probe can be estimated as the time it takes to establish a pseudo steady state within the probe wall, which is $\sim 0.5h^2/D$. For the probes used here and values of $D > 10^{-6}$ cm^2/s (typical in aqueous media), the response time of the probe is $\sim$0.5 s or less. Thus, if the change in the donor concentration is small during the response time, the pseudo steady state boundary condition given in Eq. (5) at the probe wall will still be applicable.
- If the sample time intervals t_S are short enough, the donor concentration drops in a nearly linear fashion within a given sample interval and the average donor concentration will occur at the midpoint of the sampling interval.

From Fig. 7, it can be seen that the change in the concentration during any time in was nearly linear over any 10 s time interval (corresponding to the t_S used in this study). Further, the fractional change in concentration over a fraction of a second was small. Thus, the donor concentration was obtained from the sample concentrations using the calibrated F_R and plotted at the midpoint of the sample interval. The excellent agreement between the experimental and predicted data suggests that PMD can be used to characterize systems displaying rapid concentration changes. A more complete treatment, based on an optimized experimental setup and extension of the mathematical model, will be presented in a follow-up paper.

6. Conclusion

A novel method for monitoring drug concentrations was developed, based on the concept of pulsatile microdialysis (PMD). The method was modeled mathematically, and the experimental data was well described by the model. The sensitivity of the PMD method is comparable to that of conventional microdialysis methods, as is evidenced by its F_R values and the limits of quantitation. The interval of time required for each sample can be short, after which the samples are removed from contact with the donor by being pumped out of the probe window. Thus, the amount of drug in the sample does not change after the sample is taken, and the measurement provides an accurate "snapshot" of the system at the time of sampling. For experiments in which the drug concentration changes rapidly over time, such as certain in vitro cellular uptake or binding studies, this can be an important advantage over other, more traditional methods of concentration measurement that require pre-assay sample cleanup or preparation.

Acknowledgments

This paper was abstracted from the Ph.D. Dissertation of Mohammed A. Kabir, in partial fulfillment of the Ph.D. requirements of the Department of Pharmaceutics and Industrial Pharmacy, Arnold and Marie Schwartz College of Pharmacy & Health Sciences, Long Island University, Brooklyn, NY. The authors would like to express their gratitude to the Division of Pharmaceutics and Industrial Pharmacy, Arnold & Marie Schwartz College of Pharmacy and Health Sciences, Long Island University, for providing facilities and financial support.

References

Alexander, N.K., Julie, A.S., 1999. Enhanced microdialysis extraction efficiency of ibuprofen in vitro by facilitated transport with β-cyclodextrin. Anal. Chem. 71, 1257–1264.

Birkmeier, J., Yang, D.C., Yuan, J., Stolzenbach, J., 1995. Determination of protein binding by in vitro charcoal adsorption. J. Pharma. BioPharm. 23, 41–55.

Bolinder, J., Ungerstedt, U., Arner, P., 1993. Long-term continuous glucose monitoring with microdialysis in ambulatory insulin-dependent diabetic patients. Lancet 342, 1082–1085.

Bouw, M.R., Hammarlund-Udenaes, M., 1998. A modified equilibrium dialysis technique for measuring plasma proteins binding: experimental evaluation with diazepam and nortriptyline. Pharm. Res. 15, 1643–1646.

Carslaw, H.S., Jaeger, J.C., 1985. Conduction of Heat in Solids. Clarendon Press, Oxford.

Crank, J., 1975. The Mathematics of Diffusion. Clarendon Press, Oxford.

Cussler, E.L., 1997. Diffusion. Cambridge Press, USA.

de Lange, C.M. Elizabeth, de Boer, A.G., Douwe, D. Breimer, 2000. Methodological issue in microdialysis sampling for pharmacokinetic studies. Adv. Drug Deliv. Rev. 45, 125–148.

Hashiguchi, Y., Sakakida, M., Nishida, K., Uemura, T., Kajiwara, K.L., Shichir, M., 1994. Development of a miniaturized glucose monitoring system by combining a needle-type glucose sensor with microdialysis sampling method. Long-term subcutaneous tissue glucose monitoring in ambulatory diabetic patients. Diabetes Care 17, 387–396.

Hildingsson, U., Sellden, H., Ungerstedt, U., Marcus, C., 1996. Microdialysis for metabolic monitoring in neonates after surgery. Acta. Paed. 85, 589–594.

Iyer, G.R., Bellantone, R.A., Taft, D.R., 1999. In vitro characterization of the erythrocyte distribution of methazolamide: a model of erythrocyte transport and binding kinetics. J. Pharmacokin. Biopharm. 27, 45–66.

Kemer, W., Kiwit, M., Linke, B., Keck, F.S., Zier, H., Pfeiffer, E.F., 1993. The Function of a hydrogen peroxide-detecting electroenzymatic glucose electrode is markedly impaired in human subcutaneous tissue and plasma. Biosens. Bioelectron. 8, 473–482.

Linhares, M.C., Kissinger, P.T., 1992. In vivo sampling using loop microdialysis probe coupled to a liquid chromatography. J. Chromatogr. 578, 157–163.

Linhares, M.C., Kissinger, P.T., 1994. Pharmacokinetics Studies Using Microdialysis Probe in Subcutaneous Tissue: Effects of The Co-administration of Ethanol and Acetaminophen. J. Pharm. Biomed. Anal. 12, 1619–1627.

Meyerhoff, C., Bischof, F., Mennel, F.J., Stemberg, F., Pfeiffer, E.F., 1993. On line continuous monitoring of blood lactate in men by a wearable device based upon an enzymatic amperometric lactate sensor. Biosens. Bioelectron. 8, 409–414.

Ozisik, M.N., 1989. Boundary Value Problems of Heat Conduction. Dover Publication, New York.

Reichl, L.E., 1980. A Modern Course in Statistical Physics. University of Texas Press, Austin, TX.

Rojas, C., Nagaraja, N.V., Derendorf, H., 2000. In vitro recovery of triamcinolone acetonide in microdialysis. Pharmaz 55, 659–662.

Stahle, L., 2000. On mathematical models of microdialysis: geometry, steady-state models. recovery and probe radius. Adv. Drug Deliv. Rev. 45, 149–167.

Starzak, M.E., 1984. The Physical Chemistry of Membranes. Academic Press, New York.

Stenken, J.A., Topp, E.M., Southard, M.Z., Lunte, C.E., 1993. Anal. Chem. 65, 2324–2328.

Stenken, J.A., Holunga, D.M., Decker, S.A., Sun, L., 2001. Experimental and theoretical microdialysis studies of in situ metabolism. Anal. Biochem. 290, 314–323.

Ungerstedt, U.J., 1991. Microdialysis—principles and applications for studies in animals and man. J. Intern. Med. 230, 365–373.

Womersley, J.R., 1955. Oscillatory motion of a viscous liquid in a thin-walled elastic tube-I: the linear approximation for long waves. Philos. Mag. 46, 199–221.

Zamir, M., 1996. Mechanics of pulsatile flow in a tube, comments. Theor. Biol. 4, 31–61.

Glossary of terms

a: inner radius of the microdialysis probe window
β_n: roots of Eq. (7)
CFMD: continuous flow microdialysis
C_D: concentration in the donor solution
C_S: average concentration in a collected dialysate sample
D: diffusion coefficient of the drug in the dialysate
δ_n: defined by Eq. (14)
F_R: fractional recovery for a sample C_S/C_D
F_{RQ}: fractional recovery of the continuous portion of the PMD sample
F_{RP}: fractional recovery of the pulsed portion of the PMD sample
γ_n: defined by Eq. (13)
h: thickness of the wall of the probe window (outer minus inner radius)
L: length of the microdialysis probe (cm)
λ: defined by Eq. (8)
M: total amount of drug in the collected dialysate sample
M_Q: amount of drug in the sample portion that did not rest in the probe window
M_P: amount of drug in the sample portion that rested in the probe window
PMD: pulsatile microdialysis
Q: flush rate (μL/min)
t_P: exposure time for the pulsed portion of the dialysate sample ($t_R + t_Q$)
t_Q: transit time for the continuous portion of the dialysate sample = V_W/Q
t_R: resting time for dialysate in the probe window
t_S: duration of sampling time interval defined by Eq. (22)
τ: tortuosity of pores in the probe window wall
V_W: volume of the probe window (equals the volume of dialysate allowed to rest)
V_S: volume of one dialysate sample