Mathematical Analysis and Optimization of a Flow-Through Diffusion Cell System

Hoo-Kyun Choi[1,2] and James T. Angello[1]

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INTRODUCTION

The Franz diffusion cell equipped with an automated flow-through system is commonly used (1–3), often with small receiver cell volumes with high flow rates. It simplifies the calculation of the total amount permeated as a function of time, since it is necessary only to measure the amount of permeant in each sample while ignoring the receiver cell content (2). However, when using a small volume receiver cell with a high flow rate, assay difficulties may arise due to excessive dilution. Besides, the large sample volume produced can be inconvenient to collect. A high flow rate may cause erratic experimental results (3). Sometimes a low flow rate is required to obtain a higher permeant concentration in the receiver cell, which in turn may make the permeant's detection easier. In this case the receiver cell concentration should be determined, since it is probably no longer negligible. It may also require an extensive data analysis, since the permeant concentration at any specific time point is not measurable. The receiver cell concentration can be continuously monitored using a spectrophotometer (4); however, this may not be a practical method for a multicell system or for compounds without UV absorption. The objectives of this paper are to model mathematically the flow-through diffusion cell and to investigate the effects that flow rate, cell volume, and extraction ratio have on the concentration of the permeant in the receiver cell and, in doing so, will enable the optimization of these parameters. The data analysis method and its associated error in estimating the total amount permeated are also described.

MATERIALS AND METHODS

Materials

Propranolol hydrochloride, glycerol formal, and *n*-heptanesulfonic acid were purchased from Sigma Chemical Co. (St. Louis, MO) and were used as received. The Silastic membrane (500-5) was purchased from Dow Corning (Midland, MI).

Methods

Diffusion Cell System

A flow-through diffusion cell system consists of a multichannel peristaltic pump (202U/AA, Watson Marlow), a fraction collector (Retriever IV, ISCO Inc.), a circulating water bath, and flow-through diffusion cells. The flow-through cells contained two side arms, which enabled conduction of receiver-cell media from a peristaltic pump to a fraction collector. The temperature was maintained at 30°C by circulating constant-temperature water through the outer jacket of the receiver cell. The surface area of the receiver cell opening was 1.77 cm^2, and the cell volumes were either 5.4 or 11.4 mL.

Procedure

Each of the components was connected via silicone rubber tubing with an internal diameter of 0.015 or 0.020 in., depending on the flow rate desired. A 50 m*M* phosphate buffer was used as the receiver cell medium. The receiver cell media were stirred by externally driven, Teflon-coated magnetic bars. The receiver cell solutions were stirred at 450 rpm, and the circulating water bath was connected. Prehydrated Silastic membranes were mounted onto each receiver cell and an O-ring and cell top were placed on the top of each membrane. These components were then clamped securely in place. The receiver cell medium reservoir was maintained at 30°C. The system was first equilibrated for 30 min, then any air bubbles that remained in the receiver cells were removed. Subsequently, 400 μL of a propranolol suspension in propylene glycol was applied on the top of each membrane. All samples were collected over 3-hr periods.

Assay

Propranolol was analyzed by an HPLC system (Shimadzu Scientific Instruments Inc., MD), consisting of a system controller (SCL-6B), a UV detector (SPD-6AV), a pump (LC-600), and an automatic injector (SIL-6B). A reversed-phase column was used (Zorbax RX, Rockland Technologies, Inc., PA). The column temperature was maintained at 35°C by a thin foil temperature controller (CH1445, SYSTEC Inc., MN). The mobile phase used consisted of 50 m*M* phosphate buffer (pH 3.0)/acetonitrile (60/40) with 0.1% heptanesulfonic acid sodium salt.

THEORY

Modeling

The flow-through diffusion cell system can be modeled schematically as shown in Fig. 1 assuming instantaneous mixing in the receiver cell.

The mass balance equation that governs the concentration change in the receiver cell is given by

$$\frac{dC(t)}{dt} = \frac{AJ(t)}{V} - \frac{F_0C(t)}{V} \quad (1)$$

where A is the diffusion area.

[1] Merck Research Laboratories, Merck & Co., Inc., Sumneytown Pike, West Point, Pennsylvania 19486.
[2] To whom correspondence should be addressed.

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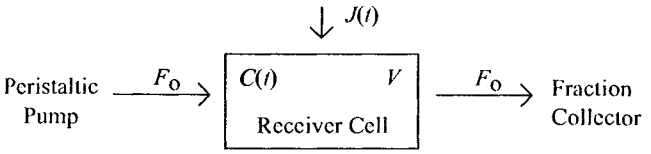

Fig. 1. Schematic diagram of the flow-through diffusion cell system. $J(t)$, input rate from donor cell; $C(t)$, concentration of the permeant in the receiver cell; V, volume of the receiver cell; F_o, constant flow rate from the peristaltic pump to the receiver cell.

The related initial condition is

$$C(t) = 0, \quad \text{when} \quad t = 0 \tag{2}$$

The area term was dropped from the following equations assuming unit diffusion area. Depending on $J(t)$, flux, the solution to Eq. (1) can be different. The simplest case is when $J(t)$ is constant; i.e., the flux is constant.

Steady-State Condition Where J(t) = J_{SS}

Equations (1) and (2) can be solved for $C(t)$ to

$$C(t) = \frac{J_{SS}}{F_o} - \frac{J_{SS}}{F_o} \exp\left(-\frac{F_o t}{V}\right) \tag{3}$$

Equation (3) describes the concentration profile of the permeant in the receiver cell as a function of time. The concentration in the receiver cell increases with time and approaches the plateau value of J_{SS}/F_o as time approaches infinity.

Non-Steady State Condition

In the case where the permeant diffuses through the isotropic monolayer and the concentration of the permeant in the donor cell remains constant; $J(t)$ can be obtained from Fick's law of diffusion and the following initial and boundary conditions:

$$J(t) = -D \frac{\partial C_m}{\partial x} \tag{4}$$

$$\frac{\partial C_m}{\partial t} = D \frac{\partial^2 C_m}{\partial x^2} \tag{5}$$

$$C_m = 0, \quad \text{where} \quad x > 0, \quad t = 0 \tag{6}$$

$$C_m = C_o, \quad \text{where} \quad x = 0 \quad \text{for all } t \tag{7}$$

$$C_m = 0, \quad \text{where} \quad x \geq h \quad \text{for all } t \tag{8}$$

where C_m is the concentration of the permeant in the membrane at time t; h, the thickness of the membrane; x, the distance from the donor solution–membrane interface; and D, the diffusion coefficient. Assuming that the diffusion coefficient is constant and the diffusion is one dimensional, Eq. (4) can be solved for C_m, and $J(t)$ can be derived. Equation (9) also can be obtained by using $C_2 = 0$ in Eq. (4.22) of Crank (5).

$$C_m = C_o\left(1 - \frac{x}{h}\right) - \frac{2C_o}{\pi} \sum_{n=1}^{\infty} \frac{1}{n} \text{Sin}\left(\frac{n\pi x}{h}\right) \exp\left(-\frac{n^2\pi^2 D}{h^2} t\right) \tag{9}$$

$$J(t) = \frac{DC_o}{h} + \frac{2DC_o}{h} \sum_{n=1}^{\infty} (-1)^n \exp\left(-\frac{n^2\pi^2 D}{h^2} t\right) \tag{10}$$

Substitute Eq. (10) into Eq. (1) and solve for $C(t)$. Then

$$C(t) = \left[-\frac{C_o D}{F_o h} - 2C_o Dh \sum_{n=1}^{\infty} (-1)^n \frac{1}{F_o h^2 - n^2\pi^2 DV}\right] \exp\left(-\frac{F_o t}{V}\right) + \frac{C_o D}{F_o h} + 2C_o Dh \sum_{n=1}^{\infty} (-1)^n \left\{\left[\exp\left(-\frac{n^2\pi^2 D}{h^2} t\right)\right] \Big/ (F_o h^2 - n^2\pi^2 DV)\right\} \tag{11}$$

$C(t)$ increases with time and approaches the plateau value of $C_o D/F_o h$ as time approaches infinity.

Release of Drug from an Ointment Base

In the same way $C(t)$ can be obtained for the case of release from an ointment base model developed by Higuchi (6).

$$C(t) = \sqrt{\frac{\pi(2C_t - C_s)C_s D}{4F_o V}} \text{Erfi} \sqrt{\frac{F_o t}{V}} \exp\left(-\frac{F_o t}{V}\right) \tag{12}$$

where C_t is the concentration of both the undissolved and the dissolved permeant in the ointment base, and C_s is the saturated concentration of the permeant in the ointment base. $C(t)$ increases with time initially, then it starts to decrease after it passes the maximum.

Analysis of Data

To calculate the diffusion parameters, the total amount of permeant is typically plotted as a function of time. When the flow rate is high relative to the receiver cell volume, the cumulative amount of permeant penetrated can be calculated by adding up the amount of permeant in each sample. The amount of permeant in the diffusion apparatus is not added in, since this amount is negligible. However, if the permeation rate is slow, samples can be produced which are below the limit of quantitation. In such a case, accumulation of the permeant in the receiver cell is desirable, and accordingly, a slower flow rate of the receiver cell medium would be necessary. However, in these circumstances the amount of permeant in the receiver cell may not be negligible, especially as the volume of the receiver cell increases. When the amount of the permeant remaining in the receiver cell is ignored, an underestimation of the initial permeation rate may result.

To calculate the cumulative amount permeated, a measurement of the permeant's concentration at specific time points is required. Due to the nature of the flow-through diffusion cell system, measuring the concentration of the permeant in the receiver cell medium at a specific time point is not possible unless the concentration change is continuously monitored. However, what can be measured is the permeant's average concentration over the time period used to collect the sample. An equation that describes the average

concentration of the permeant in the receiver cell which can be derived from the following general equation:

$$\overline{C(t)} = \frac{1}{\tau}\int_{t}^{t+\tau} C(t)dt \tag{13}$$

where $\overline{C(t)}$ is the average concentration of the permeant between time t and time $t + \tau$ and τ is the time interval. Applying Eq. (13) to Eqs. (3), (11), and (12) yields the following equations for the average concentration, $\overline{C(t)}$, of the permeant in the receiver cell for the time period between t and $t + \tau$.

$$\overline{C(t)} = \frac{J_{ss}}{F_o} + \frac{J_{ss}V}{\tau F_o^2}\left[\exp\left(\frac{F_o t}{V}\right) - \exp\left(\frac{F_o(t+\tau)}{V}\right)\right] \tag{14}$$

$$\overline{C(t)} = \frac{1}{\tau}\left[\frac{C_o DV}{F_o^2 h} + \frac{2C_o DhV}{F_o}\sum_{n=1}^{\infty}(-1)^n \frac{1}{F_o h^2 - n^2\pi^2 DV}\right]\left[\exp\left(-\frac{F_o(t+\tau)}{V}\right) - \exp\left(-\frac{F_o t}{V}\right)\right] + \frac{C_o D}{F_o h} - \frac{2C_o h^3}{\pi^2\tau}\times\sum_{n=1}^{\infty}(-1)^n \frac{1}{n^2 F_o h^2 - n^4\pi^2 DV}\left(\exp\left(-\frac{n^2\pi^2 D(t+\tau)}{h^2}\right) - \exp\left(\frac{n^2\pi^2 Dt}{h^2}\right)\right) \tag{15}$$

$$\overline{C(t)} = \frac{1}{\tau}\left[\frac{\sqrt{(2C_t - C_s)C_s D(t+\tau)}}{F_o} - \frac{\sqrt{(2C_t - C_s)C_s Dt}}{F_o} + \sqrt{\frac{(2C_t - C_s)C_s DV\pi}{4F_o^3}}\times\left\{\mathrm{Erfi}\left(\sqrt{\frac{F_o t}{V}}\right)\exp\left(-\frac{F_o t}{V}\right) - \mathrm{Erfi}\left(\sqrt{\frac{F_o(t+\tau)}{V}}\right)\exp\left(-\frac{F_o(t+\tau)}{V}\right)\right\}\right] \tag{16}$$

Since Eqs. (14)–(16) do not show when the concentration reached the average concentration, the total amount permeated cannot be calculated accurately. However, if the flux is relatively constant between time t and time $t + \tau$, the following equation can be used to estimate the total amount permeated as a function of time.

$$M_n = V\overline{C_n(t)} + \frac{S_n}{2} + \sum_{i=1}^{n-1} S_i \tag{17}$$

where S_n is the amount of permeant in the nth sample collected between time $(n - 1)\tau$ and time $n\tau$; $\overline{C_n(t)}$, the average concentration of permeant in the nth sample; and M_n, the total amount permeated by time $n\tau - (\tau/2)$. Equation (17) estimates the total amount permeated as a function of time assuming that the average concentration is achieved at the mid point between time t and time $t + \tau$. Only one-half of the amount of permeant in the nth sample was added in Eq. (17), the average concentration of permeant in the nth sample achieved at time $n\tau - (\tau/2)$. The extent of the error in estimation depends on various parameters of the diffusion study. It is larger in the beginning and will be reduced as the receiver cell concentration reaches its steady-state value. Typically, the error is less than 5% and it can be reduced by taking more frequent samples.

RESULTS AND DISCUSSION

The Effects of Flow Rate and Receiver Cell Volume

One of the important parameters that control the receiver cell concentration of the permeant is the flow rate of receiver cell medium from the peristaltic pump. Our discussion focuses on case 2, since it is the most commonly observed case in the experiment. Figure 2 shows a computer simulation of the receiver cell concentration of the permeant using Eq. (11). In this case, the receiver cell volume was fixed at 5 mL and the flow rates were varied from 0.1 to 2 mL/hr; the effect of high flow rates is discussed later. As Eq. (11) indicates, the steady-state concentration is inversely proportional to the flow rate. In addition, these concentration differences increase with time until steady state is reached. The flow rate must be minimized to reach significant receiver cell concentrations, especially when the limit of detection is high. However, the flow rate must be maintained above a certain level depending on the accuracy of the pump, sample volume size limits, etc.

When flow rate is very high relative to the cell volume, as shown in Fig. 3, the amount of the permeant found in the receiver cell becomes negligible as discussed previously. In this case, the receiver cell concentration is relatively low and reaches steady state very quickly due to the high extraction ratio (F_o/V) of the receiver cell content. The total amount permeated with time can be easily calculated by simply adding the amount of the permeant in each sample. This may be

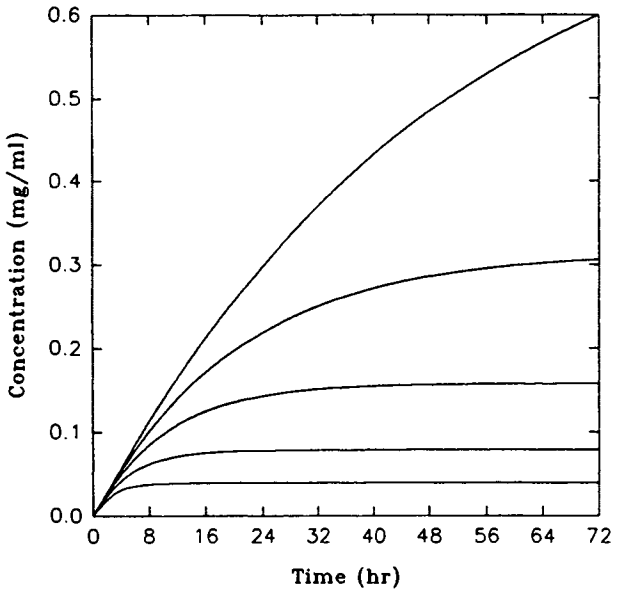

Fig. 2. Effect of flow rate on the concentration profile of the permeant in the receiver cell. The receiver cell volume is fixed at 5 mL. Diffusion coefficient, membrane thickness, and donor cell concentration are assumed to be 0.0001 cm^2/hr, 0.0127 cm, and 10 mg/mL, respectively. Moving from the top curve downward, the flow rates are 0.1, 0.25, 0.5, 1.0, and 2.0 mL/hr, respectively.

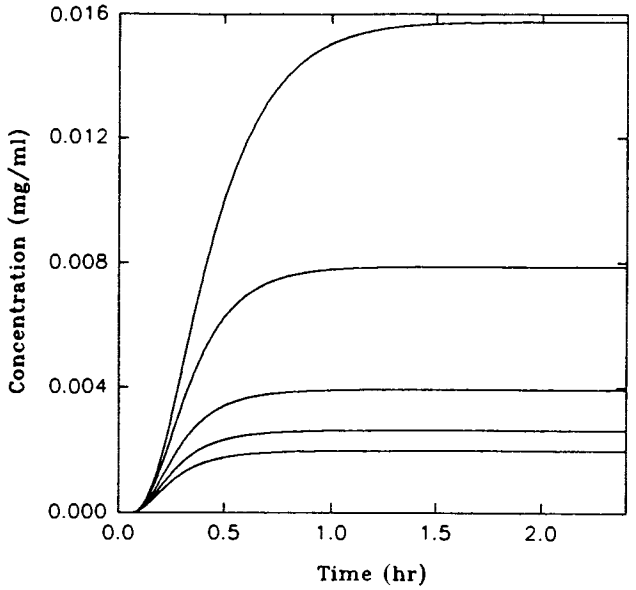

Fig. 3. The effect of flow rate on the concentration profile of the permeant in the receiver cell. The receiver cell volume is fixed as 1 mL. The same parameters as in Fig. 2 are used. Moving from the top curve downward, the flow rates are 5, 10, 20, 30, and 40 mL/hr, respectively.

a convenient way of calculating the total amount permeated with time. However, calculating the total amount permeated by this method may not be appropriate when the detection limit is high or with low steady-state concentrations if the permeation rate is very slow or the flow rate is extremely high.

Figure 4 shows the effect of cell volume on the concentration of the permeant in the receiver cell when the flow rate is fixed at 0.5 mL/hr. In this case, the steady-state receiver cell concentration does not change with changing receiver cell volume. However, the lower the flow rate, the higher the receiver cell concentration until steady state is reached. The concentration differences are larger in the earlier phase, then they start to converge toward the steady-state concentration. This indicates that a smaller cell volume is preferred when a higher concentration is desired in the receiver cell, which in turn will prevent a detection limit problem in the earlier phase.

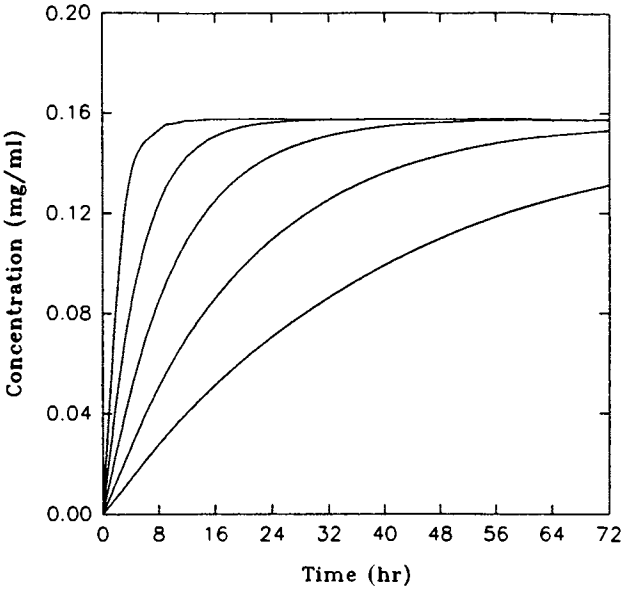

Fig. 4. Effect of cell volume on the concentration profile of the permeant in the receiver cell. The flow rate is fixed at 0.5 mL/hr. The same parameters as in Fig. 2 are used. From the top curve, the receiver cell volumes are 1.0, 2.5, 5, 10, and 20 mL, respectively.

To find how the model for case 2 predicts receiver cell concentrations, the permeation of propranolol across Silastic membranes was measured. Figure 5 shows the concentration of propranolol in the receiver cell as a function of time. Solid and dashed lines show the theoretical concentration profile of propranolol. The diffusion coefficient of propranolol was estimated as $7.13 \pm 0.99 \times 10^{-5}$ cm^2/hr using the lag time, and C_o was calculated to be 2.68 ± 0.06 mg/mL from the average concentration of the last three points using the limit value of Eq. (11); it was assumed that the receiver cell concentration had reached its steady-state level, C_oD/F_oh, at this time. In one of the four groups, the one with a larger cell volume and a slower flow rate, the receiver cell concentration did not reach steady state, and it was not used in the estimation of C_o. The diffusion coefficient and C_o are the average of 16 and 12 measurements, respectively. The actual measurements closely match the theoretical line in Fig. 5. When the total amounts of propranolol permeated from each group were plotted as a function of time, their profiles matched closely regardless of their respective cell volumes and flow rates. The total amount permeated should

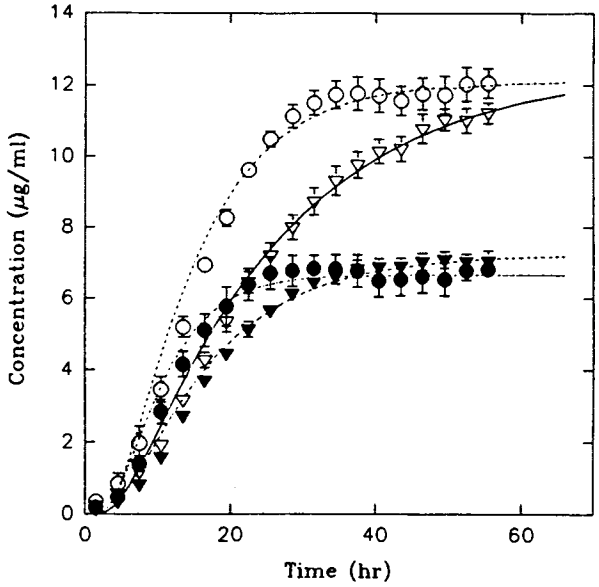

Fig. 5. The theoretical and the measured concentration profile of propranolol in the receiver cell. Diffusion coefficient and membrane concentration (C_o) of propranolol were assumed to be 7.13×10^{-5} cm^2/hr and 2.68 mg/mL for theoretical concentration simulation, respectively. The thickness of the Silastic membrane is 0.0508 cm. Each point represents the measured value ± SD. (--○--) Cell volume of 5.4 mL and flow rate of 0.55 mL/hr; (—▽—) cell volume of 11.4 mL and flow rate of 0.53 mL/hr; (--●--) cell volume of 5.4 mL and flow rate of 1.0 mL/hr; (--▼--) cell volume of 11.4 mL and flow rate of 0.92 mL/hr.

not depend on the cell volume or flow rate as long as a sink condition is maintained and the receiver cells are properly mixed. In the literature, flow rate effects on the permeation of compounds were noted (2,3). These effects may in fact be due to different degrees of mixing and/or different concentration gradients resulting from different extraction ratios.

Constant Extraction Ratio with Varying Cell Volume and Flow Rate

The ratio of receiver cell concentrations between any two cells, when the extraction ratio (F_o/V) is fixed, can be derived from Eq. (11), assuming that all parameters are fixed except cell volume and flow rate.

$$\frac{C(t,F_o = F_{o,1})}{C(t,F_o = F_{o,2})} = \frac{F_{o,2}}{F_{o,1}} \tag{18}$$

The ratio of any two receiver cell permeant concentrations is inversely proportional to the ratio of their respective flow rates at all times. Therefore, the time required to reach steady-state concentrations in the receiver cell will be the same in all groups. The time required to reach steady state will be increased as the extraction ratio decreases, as shown in Figs. 2 and 4. This can also be seen in the experimental results: In Fig. 5, where the group having the smallest extraction ratio (open triangle) has a receiver cell concentration that is still increasing at the end of the experiment.

CONCLUSIONS

The flow rate and cell volume affect the temporal permeant concentration in the receiver cell significantly; however, they may not affect the overall permeation profile as long as proper mixing and sink conditions are maintained. The error caused by such a data reduction method can be analyzed as well as minimized by optimizing the experimental parameters, including flow rate, cell volume, and sampling interval, using the model developed. The model can be used to find out what the approximate concentration of the permeant in the receiver cell will be before initiating an experiment. Based on this estimated concentration profile, the ideal experimental parameters can be targeted more easily. The alternative, is a trial-and-error method for optimizing these parameters, which may require the researcher to carry out several additional preliminary experiments. Furthermore, the data from these preliminary studies may not be usable if the experimental parameters were not appropriate for the concentration profile obtained. Thus, put succinctly, this models' primary use is to act as a tool, which enables the researcher to minimize his/her time and effort spent on determining a diffusion systems ideal parameters. This model is especially useful when working with a large receiver cell volume and a low flow rate, since, in such a case, the limit of detection is often in doubt. In addition, this model enables the researcher to estimate the membrane concentration of the permeant when the diffusion coefficient is known, and vice versa.

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