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Determination of the partition coefficients and absorption kinetic parameters of chemicals in a lipophilic membrane/water system by using a membrane-coated fiber technique

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Abstract

The absorption kinetics of chemicals in a lipophilic membrane/water system was studied with a membrane-coated fiber (MCF) technique, in which the partition coefficient, membrane diffusivity and boundary layer adjacent to the membrane were taken into account. The cumulative amount permeated into the membrane was expressed as a function of absorption time in an exponential equation. Two constants were introduced into the model. Both of them were clearly defined by the physiochemical parameters of the system and were obtained by regression of the experimental data sampled over a limited time. The partition and diffusion coefficients, as well as the thickness of the boundary layer, were calculated from the two constants. The kinetic model adequately described the absorption kinetics of the MCF technique. All of the theoretical predictions were supported by the experimental results. The measured partition coefficients correlated well with the published octanol/water partition coefficient ($R^2 = 0.91$). The thickness of the boundary layer was 5.2 μm in a solution stirred at 400 rpm. An inference of the kinetic model revealed that the contribution of the boundary layer to the absorption kinetics is significant for lipophilic chemicals by a lipophilic membrane. It suggested that the absorption rate of a very lipophilic compound could be controlled by the boundary layer even though the diffusivity of the compound in the membrane is lower than that in the solution. It was demonstrated that the MCF technique could be used to determine the partition, diffusion and permeation coefficients, as well as the thickness of the boundary layer in a lipophilic membrane/water system.

Keywords: Absorption kinetics; Membrane-coated fiber; Diffusion coefficient; Partition coefficient; Boundary layer

1. Introduction

The knowledge of the absorption kinetics of chemicals and drugs in various lipophilic membrane/water systems is of importance to pharmaceutical and toxicological studies, and occupational and environmental risk assessments. Great efforts have been made to study the absorption kinetics using a variety of in vivo and in vitro experimental techniques.

We have reported on a novel membrane-coated fiber (MCF) technique to study the absorption processes of chemicals and drugs in a polydimethylsiloxane (PDMS)/water system (Xia et al., 2003). The MCF technique is a special version of the solid-phase microextraction (SPME) technique developed by Pawliszyn and coworkers as an analytical method, in which a polymer-coated fiber is used to extract analytes from an aqueous solution, and transferred directly to the injector of a gas chromatograph for quantitative analysis (Zhang et al., 1994). For analytical applications, the extraction of analytes can be based on any extraction mechanisms including absorption, adsorption or mixed absorption and adsorption. In fact, most of the newly developed SPME fibers are based on the adsorption mechanism or mixed absorption and adsorption (Supelco, 2001; Gorecki et al., 1999). The goal of the SPME

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technique is to extract the maximum amount of analytes from the sample matrix, which is proportional to the original concentration in the sample matrix. If an absorptive membrane is coated on a fiber, such as PDMS, it can be used not only for analytical applications, but also for studying the absorption kinetics and partition equilibrium of drugs and chemicals in membrane/water systems. A number of absorptive materials can be used to make MCFs for membrane absorption kinetic studies even though they are not suitable for analytical applications. On the other hand, adsorptive membranes developed for analytical applications cannot be used for membrane absorption kinetic studies. Therefore, we call this special version a membrane-coated fiber (MCF) technique.

The MCF technique integrates the membrane permeation and quantitative analysis into one step, and fully utilizes the high separation power of gas chromatography (GC), which enables the MCF technique to have a greater sensitivity in the determination of the kinetic parameters and rapid assessment of percutaneous permeation of complex chemical mixtures (Xia et al., 2003). The utility of the MCF technique for permeation study is based on the fact that the primary barrier of human skin to many exogenous chemicals is the lipoidal stratum corneum membrane. However, the polymer membrane in the MCF technique does not have the complicated biological structure as skin. Therefore, it cannot be used to study the absorption processes where biological specific interactions and metabolical conversions are the controlling factors. If the compound of interest is not GC detectable, solvent desorption and liquid chromatography method should be used for quantitative analysis (Supelco, 2001).

In this paper, the absorption kinetics of chemicals with wide range of lipophilicity in a PDMS membrane/water system was studied by the MCF technique. A kinetic model was adapted to describe the MCF technique, in which the partition coefficient, membrane diffusivity and boundary layer adjacent to the membrane were considered. The kinetic model was evaluated by examining the compliance of the theoretical predictions with the experimental observations. Methods for obtaining the partition, diffusion and permeation coefficients, as well as the thickness of the boundary layer were established for the MCF technique.

2. Theory

In the MCF technique, an absorptive membrane is coated onto an inert fiber to prepare the membrane for absorption studies. When a MCF is immersed into an aqueous solution, a given chemical in the bulk solution permeates through a stagnant water layer (boundary layer) and diffuses into the membrane. At a given time, the MCF is removed from the solution and directly transferred into the injector of a gas chromatograph for quantitative analysis. When the solution is under constant stirring, the thickness of the boundary layer can be approximated to be constant. A kinetic model describing the absorption kinetics under such experimental settings has been utilized in analytical chemistry (Ai, 1997). The useful equations are cited for further development:

$$n = [1 - \exp(-at)]n^{\circ} \tag{1}$$

$$n^{\circ} = \frac{KV_{\mathrm{d}}V_{\mathrm{m}}C_0}{KV_{\mathrm{m}} + V_{\mathrm{d}}} \tag{2}$$

$$a = \frac{2AD_{\mathrm{d}}D_{\mathrm{m}}(KV_{\mathrm{m}} + V_{\mathrm{d}})}{V_{\mathrm{m}}V_{\mathrm{d}}(D_{\mathrm{d}}\delta_{\mathrm{m}} + 2KD_{\mathrm{m}}\delta_{\mathrm{d}})} \tag{3}$$

where n is the absorption amount of a chemical into the membrane at time t, n° is the maximum equilibrium amount when the absorption equilibrium is reached at infinite time ($t \to \infty$) and a is a constant representing the kinetic factor. A is the surface area of the membrane and V_{m} is the volume of the membrane. V_{d} is the solution volume with an initial concentration C_0. D_{d} and D_{m} are the diffusion coefficients of the chemical in the solution and in the membrane phase, respectively. K is the partition coefficient of the chemical between the membrane and the solution, δ_{d} is the thickness of the boundary layer and δ_{m} is the thickness of the membrane.

The contributions from the membrane and the boundary layer are revealed by rearranging the expression of constant a (Eq. (3)):

$$a = \frac{2A(K/V_{\mathrm{d}} + 1/V_{\mathrm{m}})}{\delta_{\mathrm{m}}/D_{\mathrm{m}} + 2K\delta_{\mathrm{d}}/D_{\mathrm{d}}} \tag{4}$$

In the denominator, the first item ($\delta_{\mathrm{m}}/D_{\mathrm{m}}$) is the contribution of the membrane while the second item ($2K\delta_{\mathrm{d}}/D_{\mathrm{d}}$) is the contribution of the boundary layer. When the partition coefficient (K) is large enough to satisfy a condition: $\delta_{\mathrm{m}}/D_{\mathrm{m}} \ll 2K\delta_{\mathrm{d}}/D_{\mathrm{d}}$, the contribution of the membrane can be neglected:

$$a = \frac{AD_{\mathrm{d}}(K/V_{\mathrm{d}} + 1/V_{\mathrm{m}})}{K\delta_{\mathrm{d}}} \tag{5}$$

or,

$$\delta_{\mathrm{d}} = \frac{AD_{\mathrm{d}}(K/V_{\mathrm{d}} + 1/V_{\mathrm{m}})}{aK} \tag{5a}$$

The diffusion coefficient in the membrane (D_{m}) can be calculated from the measured parameters by rearranging Eq. (3):

$$D_{\mathrm{m}} = \frac{a\delta_{\mathrm{m}}}{2A(K/V_{\mathrm{d}} + 1/V_{\mathrm{m}}) - 2aK\delta_{\mathrm{d}}/D_{\mathrm{d}}} \tag{6}$$

Once the partition coefficient (K), the diffusion coefficient (D_{m}) and the thickness of the membrane (δ_m) are known, the permeation coefficient of the membrane (k_{p}) can be calculated from its definition (Singh and Singh, 1993):

$$k_{\mathrm{p}} = \frac{KD_{\mathrm{m}}}{\delta_{\mathrm{m}}} \tag{7}$$

The calculation sequence for these parameters is given in Section 3.4.

Table 1
Partition, diffusion and permeation coefficients of selected compounds[a]

Compound	log K by three methods[c]			log $K_{o/w}$	log a		n°		log D_d	log D_m	log k_p
	1st	2nd	3rd		1/s	CV[b]	ng	CV[b]	cm^2/s	cm^2/s	cm/s
Terrazole	2.69	2.66	2.62	2.55	−3.37	16	0.25	6.28	−5.19	−7.81	−3.20
a-BHC	3.11	3.08	3.12	3.90	−3.00	7	0.80	1.56	−5.22	−6.89	−1.77
Atrazine	1.94	1.91	1.96	2.60	−3.45	21	0.056	4.74	−5.22	−7.93	−3.97
g-BHC	2.93	2.91	2.92	3.72	−2.85	10	0.51	1.72	−5.22	−6.85	−1.92
Alachlor	2.54	2.49	2.53	3.52	−3.01	18	0.20	3.28	−5.21	−7.40	−2.87
Aldrin	4.96	5.95	4.98	6.50	−4.65	5	33.1	1.40	−5.28	−8.66	−1.68
Heptachlor-E	4.31	4.32	4.32	4.98	−4.21	6	10.9	1.33	−5.28	−8.20	−1.88
p,p-DDE	5.39	5.38	5.53	6.51	−4.83	7	55.1	3.26	−5.27	−9.00	−1.47
Endrin	4.64	4.62	4.75	5.20	−4.52	19	23.7	1.84	−5.28	−8.50	−1.75
Endosulfan-S	3.63	3.64	3.63	3.66	−3.39	9	2.50	2.35	−5.29	−7.87	−2.24
p,p-DDT	5.72	5.46	6.03	6.91	−4.93	14	67.2	1.97	−5.29	−9.45	−1.42
cis-Permethrin	5.21	5.71	5.22	7.43	−4.96	3	70.5	1.66	−5.36	−9.39	−2.17

[a] The compounds were selected to representing the chromatograph of the 30 compounds studied. The rest of the compounds are chloroneb, simazine, b-BHC, d-BHC, chlorothalonil, heptachlor, dacthal, tr-chlordane, endosulfan I, *cis*-chlordane, tr-nonachlor, dieldrin, chlorobenzilate, endosulfan II, *p,p*-DDD, endrin aldehyde, methoxychlor, tr-permethrin.

[b] Relative coefficient variation (%) in regression ($n = 10$).

[c] In 1st method, K was calculated with Eq. (2) by using the maximum absorption amounts after 41 h absorption in a solution of 75 ml 1.00 ng/ml. In 2nd method, K was calculated from its definition by using the measured and membrane concentrations after 41 h absorption in a solution of 75 ml 1.00 ng/ml. In the 3rd method, K was calculated with Eq. (2) when n° was obtained from the regression. The permeability coefficients were calculated with the partition coefficients obtained by the 3rd method.

3. Experimental procedures

3.1. Chemicals and materials

Acetone, hexane and acetonitrile were of HPLC grade from J.T. Baker (Phillipsburg, NJ). A standard mixture consisting of 30 compounds (referring to Table 1) in acetone was purchased from AccuStandard Inc. (New Haven, CT). Solid-phase microextraction (SPME) devices and 100 μm polydimethylsiloxane (PDMS)-coated fiber assemblies were purchased from Supelco (Bellfonte, PA).

A series of standard solutions in acetone were prepared from the standard mixture to be used as external calibration standards for quantitative analysis. A stock solution of 20 μg/ml of individual component in acetonitrile was prepared from the standard mixture. A series of aqueous solutions with different initial concentrations from 0.1 to 8 ng/ml (each individual component) were prepared from the stock solution. The concentrations of the aqueous solutions were under the solubility limits of all the compounds in the permeation experiments except for one point (8 ng/ml) in studying the dependence of the absorption amount on the initial concentration as detailed below. No solubility effect was observed as shown in Fig. 1.

The concentrations in the donor solution after the permeation experiments were measured by liquid–liquid extraction with hexane. The quantities were calibrated with standard donor solutions measured by the same liquid–liquid extraction procedures.

3.2. Absorption with the membrane-coated fiber

The experimental setup of the MCF technique and the procedures to conduct the absorption experiments have been described in detail previously (Xia et al., 2003). A given volume of the solution was transferred into an absorption container. The solution was stirred constantly with a magnetic stirrer at 400 rpm under constant temperature (25 °C) controlled by a circulating water bath. A membrane-coated fiber was immersed into the solution to partition the permeants of interest into the membrane. At a given absorption time, the membrane-coated fiber was removed and transferred into a GC injector for quantitative analysis.

To obtain the absorption time profiles, the absorption amounts were measured at a series of absorption times while keeping the solution volume and initial concentration con-

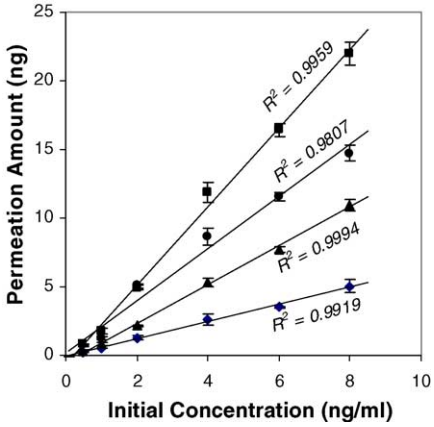

Fig. 1. Predicted linear relationship of absorption amounts vs. initial concentrations. Terrazole (♦), dacthal (■), chlorobenzilate (▲), *cis*-chlordane (●). The membrane-coated fiber was 100 μm PDMS. The initial concentrations of the solutions were from 0.1 to 8 ng/ml (individual component), and the absorption time and stirring speed were kept as constants at 12 min and 400 rpm, respectively. The error bars represented the standard deviations of three repeated experiments.

stant at 75 ml and 1.00 ng/ml, respectively. A fresh solution was used for each time point. To study the dependence of the absorption amount on the initial concentration, the absorption amounts from solutions with different initial concentrations (0.1–8 ng/ml) were measured with the MCF technique while the solution volume and the absorption time were maintained constant at 75 ml and 12 min, respectively.

Each PDMS MCF was preconditioned at 280 °C for 30 min in order to obtain a stable membrane material (manufacture recommended). When MCF was injected into the injection port at 280 °C to thermally desorb the partitioned chemicals for quantitative analysis, it was held for 5 min serving as a preconditioning for the next absorption experiment. MCF was discarded if the change in absorption amount from a calibration solution was observed (>5%) or it was reused in more than 100 absorption experiments.

3.3. GC/MS analysis

Quantitative and qualitative analyses were performed on a HP 5890 II gas chromatograph coupled with a HP 5970B mass selective detector. A HP 7673 automatic sampler was used to inject 4 μl of the calibration standard solution, while the membrane-coated fiber was injected manually. The injection port was maintained at 280 °C for sample vaporization and thermal desorption. The analytical conditions were improved to reduce analytical time and increase analytical sensitivity. Separation was performed on a 30 m × 0.25 mm (i.d.) × 0.25 μm (df) HP-5MS capillary column (Agilent, Palo Alto, CA). The column oven was programmed as follows: the initial temperature was 100 °C and held for 0.5 min, ramped at 20 °C/min to 200 °C and 8 °C/min to 280 °C, and held at 280 °C for 5 min. An electronic pressure control was used to maintain a carrier gas flow of 1.00 ml/min helium. The selected ion monitoring (SIM) mode was used for quantitative analysis, in which the 30 compounds were grouped according to their retention times and two or three character ions were monitored for each compound depending on the ion intensity produced by the compound.

3.4. Data analyses

The two constants (a and n°) in the kinetic model (Eq. (1)) were obtained by regression of the absorption amount (n) at series of time points (t) using non-linear regression software, WinNonlin (Pharsight Corp., Mountain View, CA). The partition coefficient of a given compound between the solution and the membrane was calculated with Eq. (2) from the equilibrium amount (n°), where the membrane volume (V_m) was 0.612 μl, the solution volume (V_d) was 75 ml and the initial concentration was 1.00 ng/ml (each individual component).

The thickness of the boundary layer, the diffusion coefficients in the membrane (D_m) and in the solution (D_d), the membrane permeability (k_p) and partition coefficient (K) were obtained as follows:

(a) The diffusion coefficient in the aqueous solution (D_d) was estimated with a published method (Hayduk and Laudie, 1974):

$$D_d = \frac{13.26 \times 10^{-5}}{\mu^{1.4} Va^{0.589}} \quad (8)$$

where D_d is the diffusion coefficient of a given compound in the solution (cm^2/s), μ is the viscosity of the aqueous solution ($\mu = 0.8937$ cP at 25 °C), and V_a is the molar volume of the compound (cm^3/mol). The molar volumes of the compounds were from a published reference database (Mackay et al., 1999).

(b) The thickness of the boundary layer (δ_d) was estimated by an approaching method, i.e., δ_d value was calculated for each compound with Eq. (5a) from the obtained parameters (a and K) and the estimated D_d value. The thickness (δ_m), surface area (A) and volume (V_m) of the membrane-coated fiber were 100 μm, 0.094 cm^2 and 0.612 μl, respectively. The calculated $\log(\delta_d)$ versus $\log K$ is plotted. When the partition coefficient (K) is high enough to satisfy the condition: $\delta_m/D_m \ll 2K\delta_d/D_d$, the thickness of the boundary layer is approached.

(c) The diffusion coefficient of a given compound in the membrane (D_m) was calculated from the measured parameters with Eq. (6). The permeation coefficient (k_p) of the membrane was calculated with Eq. (7).

(d) The partition coefficients between the donor phase and the membrane were measured by three methods with the MCF technique. The first method was to measure the permeation amount into the membrane until the permeation equilibrium was reached. The partition coefficient K was calculated from the initial concentration (C_0) and the measured maximum permeation amount (n°) using Eq. (2). The second method was to measure the equilibrium concentrations in the membrane ($C_{me} = n^\circ/V_m$) and in the donor solution (C_{de}). The equilibrium concentration in the donor solution was determined by the liquid–liquid extraction with hexane. The partition coefficient was calculated from its definition ($k = C_{me}/C_{de}$). The third method was to use the mathematical model to obtain the equilibrium amount (n°) by regression of the permeation data sampled before equilibrium. The partition coefficient was calculated from the initial concentration (C_0) and the equilibrium permeation amount (n°) using Eq. (2).

4. Results

4.1. Absorption kinetic model

In addition to the two basic partition coefficient and membrane diffusivity factors, a boundary layer adjacent to the membrane was considered in the kinetic model. The cumulative amount partitioned into the membrane (n) is expressed

as a function of the absorption time (t) in an exponential equation (Eq. (1)). Two constants, a and n°, were introduced into the theoretical model. Both of the constants were clearly defined by the physiochemical parameters of the absorption system (Eqs. (2) and (3)). Constant n° is the equilibrium absorption amount (Eq. (2)), while constant a is a kinetic factor representing the contributions from the diffusion coefficients in the membrane and the boundary layer, as well as their geometry parameters (Eq. (3)).

4.2. Linear relationship between n and C_0

The absorption amount (n) is related to the initial concentration (C_0) of the solution as predicted by the theoretical model (Eqs. (1) and (2)). To study this relationship, the absorption amounts from a series of solutions with different initial concentrations were measured while the absorption time was held constant. The relationships of the absorption amounts with different initial concentrations are shown in Fig. 1 for terrazole, dacthal, chlorobenzilate and *cis*-chlordane. It was observed that these relationships were linear and crossing the zero intersection. In fact, this type of linear relationship was observed for all of the 30 compounds in the solutions (results not shown).

4.3. Static and stirred absorption time profiles

The absorption profiles of heptachlor epoxide and methoxychlor from static and stirred solutions are shown in Fig. 2. The absorption profiles from static solutions were linear in the entire experimental time (ca. 1000 min). When the solution was stirred at 400 rpm, the absorption amounts of the two compounds were much higher than those in static solution. The absorption equilibrium of the two compounds was reached within 400 min.

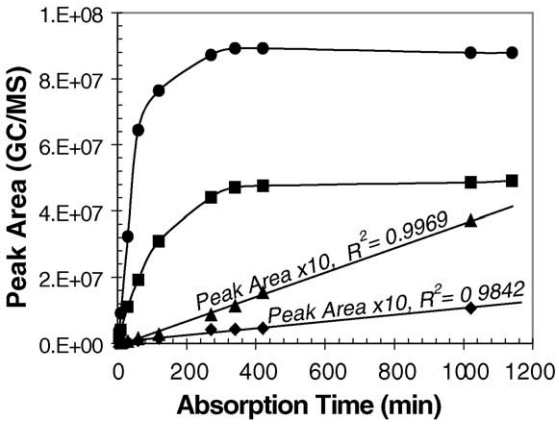

Fig. 2. Absorption time profiles in static and stirred solutions. Absorption profiles of heptachlor epoxide (♦) and methoxychlor (▲) in static solutions. Absorption profiles of heptachlor epoxide (■) and methoxychlor (●) in solutions stirred at 400 rpm. A fresh solution (75 ml) was used for each time point. The initial concentration of the solutions was 1.00 ng/ml (individual component).

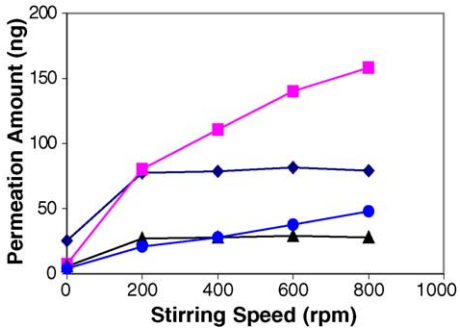

Fig. 3. Effect of stirring speed on absorption rate. Absorption amounts measured in 2 min for a-BHC (♦), dacthal (●), chlorobenzilate (▲) and methoxychlor (■) from an aqueous solution of 5.00 ng/ml (individual component) with a 100 μm PDMS fiber.

4.4. Effect of the stirring speed on absorption

The absorption amount in 2 min was measured at different stirring speeds, which represents the average absorption rate within 2 min. The effect of the stirring speed on the absorption amounts of a-BHC, dacthal, chlorobenzilate and methoxychlor is shown in Fig. 3. The absorption rates were increased significantly for all of the compounds when the stirring speed increased from static to 200 rpm. After 200 rpm, the effects of the stirring rate were different for different compounds. The absorption amount within 2 min was unchanged for a-BHC, increased slightly for chlorobenzilate and increased linearly for dacthal and methoxychlor. This is because the absorption of a-BHC and chlorobenzilate reached absorption equilibrium within 2 min under stirring higher than 200 rpm. The absorption rates of dacthal and methoxychlor increased with the stirring speed since the absorption was occurring before their absorption equilibrium.

4.5. Regression of the experimental data for a, n° and K

The two constants, a and n°, were obtained by regression of the absorption experimental data with the theoretical model (Eq. (1)). Fig. 4 shows the regression results for dacthal, tr-nonachlor, aldrin and g-BHC. The experimental data were fitted well with the theoretical model over the entire range of absorption time. Constant n° determined the maximum absorption amount, while constant a determined the shape of the absorption profiles. The absorption will reach equilibrium faster if a compound has a higher a value. The two constants obtained for 12 selected compounds in the solution were listed in Table 1. These compounds were selected to represent the 30 compounds in a GC/MS spectrum.

The partition coefficient (K) was calculated from constant n° with Eq. (2). The partition coefficients calculated for the selected compounds were listed in Table 1. The partition coefficients obtained by the equilibrium method were also given in Table 1. Fig. 5 shows a correlation of the measured PDMS/water partition coefficients by the MCF technique with a set of published octanol/water partition coefficients

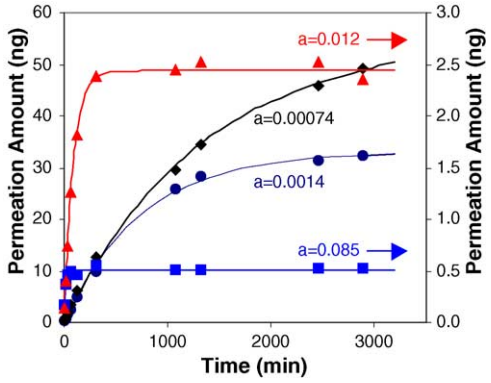

Fig. 4. Regressions of the experimental absorption time profiles. Solid curves are regressions of the kinetic model. Scattered data are experimental results of dacthal (▲), tr-nonachlor (♦), aldrin (●) and g-BHC (■). A fresh solution (75 ml) was used for each time point. The initial concentration of the solutions was 1.00 ng/ml (individual component).

(Montgomery, 1997). The measured PDMS/water partition coefficients correlated well ($R^2 = 0.91$) with the octanol/water partition coefficients.

4.6. Thickness of the boundary layer

The thickness of the boundary layer was obtained by an approaching method. The estimation of the δ_d value was calculated for each compound with Eq. (5a) from the estimated D_d value (Eq. (8)). A plot of the calculated $\log(\delta_d)$ versus $\log K$ is shown in Fig. 6. When the partition coefficients were high enough to satisfy the condition, $\delta_m/D_m \ll 2K\delta_d/D_d$, the thickness of the boundary layer was approached. The thickness of the boundary layer was estimated to be 5.2 μm ($\log \delta_d = -3.28$) when the solution was stirred at 400 rpm.

4.7. Membrane diffusion and permeation coefficients

The diffusion coefficient of a given compound in the solution was estimated (Eq. (8)) with the published method (Hayduk and Laudie, 1974). The diffusion coefficient of the compound in the membrane (D_m) was calculated with Eq. (6) from the measured parameters (a and K) and the estimated D_d value (Table 1).

The membrane permeation coefficient was not a directly measurable quantity in the MCF technique. It was calculated by its definition (Eq. (7)) from the partition coefficient (K) and diffusion coefficient (D_m) directly measured by the MCF technique (Table 1).

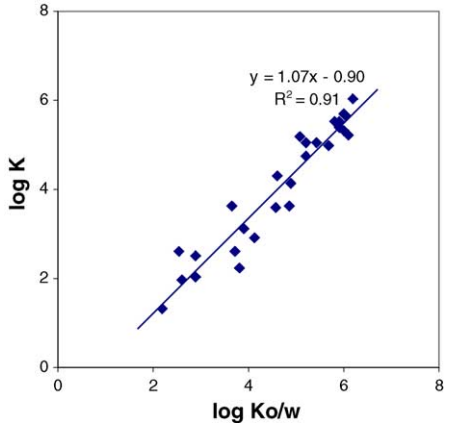

Fig. 5. Correlation of $\log K$ measured by MCF Technique with $\log K_{o/w}$.

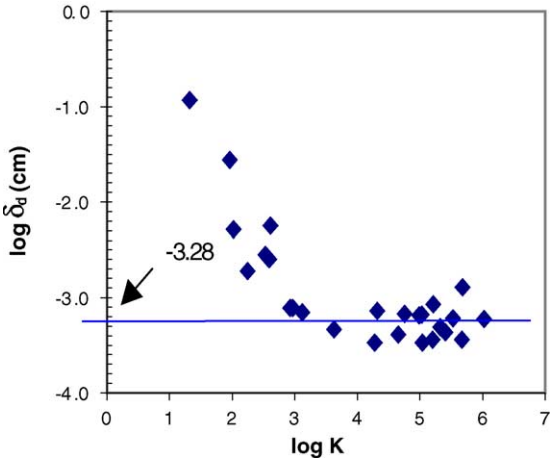

Fig. 6. Thickness of the boundary layer.

5. Discussion

5.1. Evaluation of the kinetic model

The kinetic model originally developed for analytical chemistry application (Ai, 1997) was adapted to describe the absorption kinetics of the MCF technique. To evaluate the model, the predictions by the theoretical model are examined for compliance with the experimental observations.

The partition coefficient obtained by the model is expressed in Eq. (2), which is derived from the theoretical model by letting the absorption time approach infinity ($t \to \infty$). It is exactly the same expression as that derived from the definition of the partition coefficient (Xia et al., 2003). This suggests that the theoretical derivation and assumptions made are adequate, and the model accurately represents the kinetics of the equilibrium status.

The theoretical model (Eq. (1)) describes the dependency of the cumulative amount of a given compound permeated into the membrane (n) on the absorption time (t). By taking the first derivative of Eq. (1), the absorption rate at any absorption time can be obtained as $\frac{dn}{dt} = an^{\circ} \exp(-at)$. It is predicted that the initial absorption rate is constant, $\left.\frac{dn}{dt}\right|_{t\to 0} = an^{\circ}$; and the equilibrium absorption rate is zero, $\left.\frac{dn}{dt}\right|_{t\to\infty} = 0$. These predictions are in agreement with the experimental observations reported in our earlier paper (Xia et al., 2003). The initial absorption rates for all compounds

studied were constant in the initial absorption section. The absorption time profile of each compound consisted of the initial linear section, a transition section and a flat equilibrium section, where the absorption rate was zero.

If the absorption time is kept as constant (t'), the expression, $\left[1 - \exp\left(-at'\right)\right] \frac{KV_dV_m}{KV_m+V_d}$, will become a constant (Eqs. (1) and (2)). Thus, it is predicted that the absorption amount (n) will be linearly related to its initial concentration in the solution (C_0), and it is also predicted that the linear relationships will cross the zero intersection. These predictions were supported by the determination of the absorption amounts from a series of solutions with different initial concentrations, while keeping the absorption time constant (Fig. 1). All of the linear relationships crossed the zero intersection as predicted by the model.

The regression of the experimental data with the theoretical model (Eq. (1)) was shown in Fig. 4. It is observed that the experimental data are well fitted with the theoretical model over the entire range of absorption time. This suggests that the developed theoretical model well represents the transport kinetics of the MCF technique.

5.2. Constants introduced into the kinetic model

The two parametric constants, a and n°, introduced into the theoretical model were clearly defined with the physiochemical parameters of the absorption system. Constant n° is the equilibrium absorption amount, as it was derived from Eq. (1) by setting the absorption time to infinity. It is a measure of the thermodynamic factor of the system in the kinetic model. Thus, constant n° can be used to calculate the partition coefficient K (Eq. (1)).

Constant a is a measure of the kinetic factor of the system in the kinetic model. It governs the kinetic characteristics of the system, and determines the shape of the absorption time profiles (Fig. 4). A compound having a larger value of constant a will reach the absorption equilibrium faster. Constant a is defined in Eq. (1) by the geometric parameters of the MCF technique (A, δ_m, V_m and V_d) and the physiochemical parameters of the absorption system (D_d, D_m, δ_d and K). In the MCF technique, the contributions of the physiochemical parameters to constant a were studied by keeping the geometric parameters constant throughout the experiments.

From Eq. (1), it is noticed that constant a is affected by the thickness of the boundary layer (δ_d). Reducing the thickness of the boundary layer by stirring the solution will increase the a value and consequently reduce the equilibration time (Fig. 2) and increase absorption rate (Fig. 3).

5.3. Partition and permeation coefficients

The fundamental biological process is characterized by the membrane partition coefficient, which represents the equilibrium permeant distribution between the aqueous phase and the membrane. The partition coefficient is demonstrated to be the main factor determining skin permeability (Potts and Guy, 1992). Measurements of drug or chemical membrane partitioning have been the subject of many studies over the last several decades. However, it is still challenging to determine the membrane partition coefficients for very lipophilic compounds. One of the difficulties is to reach equilibrium. Another difficulty is the quantitation of the very low concentration in aqueous solutions. The MCF technique provides a novel and simple approach in determination of the membrane partition coefficient. It is particularly useful in determination of the membrane partition coefficients for lipophilic compounds. A series of the absorption data at different time intervals are sampled before the equilibrium, then absorption data are regressed with the present kinetic model to obtain the equilibrium absorption amount (n°). The membrane partition coefficient can be calculated with Eq. (2). The partition coefficients obtained with the present regression method for the selected compounds are listed in Table 1. The partition coefficients obtained with the equilibrium methods and octanol/water partition coefficients (Montgomery, 1997) are also given in Table 1. The partition coefficients obtained with the regression method were comparable with those obtained with the equilibrium method. It is observed that the measured PDMS/water partition coefficients correlated well with the published octanol/water partition coefficients for the 30 compounds (Fig. 5). The correlation coefficient (R^2) was 0.91. Therefore, the MCF technique might be a useful tool for determining the octanol/water partition coefficients for lipophilic compounds.

The permeation coefficient of the membrane cannot be directly measured with the MCF technique since the receptor phase does not exist. It can be calculated from its definition (Eq. (7)) with the quantities directly measured by the MCF technique. The calculated permeation coefficients for the selected compounds are given in Table 1. The membrane permeability (k_p) calculated from its definition should bear the same biological meaning as that obtained by the conventional diffusion cell experiments if same kinetic factors were considered. These permeation coefficients are expected to be higher than those obtained from conventional diffusion experiments. In conventional diffusion experiments using Franz static diffusion cells or Bronaugh flow-through cells, the solution is static (Addicks et al., 1987; Bronaugh and Stewart, 1985). The thickness of the boundary layer in static aqueous solution was as high as 1966 μm (Hidalgo et al., 1991). The diffusion resistance of the boundary layer has not been separated from the membrane diffusion resistance in the conventional diffusion experiments. Regarding to the significant effects of the boundary layer on the absorption rate (Figs. 2 and 3), the permeation coefficients of lipophilic chemicals in a lipophilic membrane maybe under estimated if the boundary layer involvement is not considered. Unfortunately, we cannot find available absorption data for these compounds in the published literature for comparison. Further works should be directed to more hydrophilic drugs having permeability data either in vitro or in vivo from the literature.

5.4. Effect of the stirring speed

To study the stirring effect on absorption rate, short absorption time period before equilibrium is required. After equilibrium, stirring speed does not show effects on the absorption rate. From the absorption time profiles in stirred and static solutions (Fig. 2), it is seen that the absorption rate in stirred solution was much higher that in static solution. The absorption rate increased with the stirring speed (Fig. 3). Traditional basic kinetic models cannot be used to explain this significant effect of the stirring speed on the absorption rate, where the boundary layer is not considered. The magnetic stirring neither changes the properties of the membrane, nor the chemical properties of the solution. The only parameter of the absorption system that could be changed by magnetic stirring is the thickness of the boundary layer. The thickness of the boundary layer is inversely proportional to constant a (Eq. (3)). When the stirring speed is increased, the thickness of the boundary layer is reduced. Therefore, constant a is increased, and consequently the absorption rate is increased and the equilibration time is reduced.

5.5. Diffusion coefficients in the membrane and in the solution

In traditional percutaneous absorption kinetics, the diffusion coefficients in the membrane are calculated from the absorption data. The concentration of the bulk solution was used for the membrane diffusivity calculation without taking account of the possible boundary layer effects (Potts and Guy, 1992; Moss et al., 2002; Flynn and Yalkowsky, 1972). In fact, the boundary layers adjacent to the membrane in the donor or receptor solution exist even under magnetic stirring (Hidalgo et al., 1991). The stagnant water layer adjacent to the membrane was estimated to be 5.2 μm under stirring at 400 rpm by the MCF technique (Fig. 6). Large errors may impose on the determination of the membrane diffusivity from absorption data if the boundary layer is not considered (Grassi and Colombo, 1999).

In the present kinetic model, the boundary layer is accounted for in the absorption kinetics. The membrane diffusion coefficients can be calculated from the absorption data (Eq. (6)). However, the thickness of the boundary layer and the diffusion coefficients are both involved in Eq. (6). This is because the measured absorption data included both of the contributions (resistances) from the membrane and the boundary layer. To our knowledge, reference diffusion coefficients for the 30 compounds are not available in the published literature. Therefore, an estimation method was used to calculate the diffusion coefficients in the aqueous solution, which offered a prediction average deviation less than 5% for all data compiled by Hayduk and Laudie (1974). If the diffusion coefficients (D_d) can be measured directly, the estimation accuracy of the boundary layer thickness will be increased.

From Table 1, it is noticed that the membrane diffusion coefficients are much lower than those in aqueous solution. This is consistent with the Stokes–Einstein's theory that the membrane has higher viscosity comparing to aqueous solution, therefore it has lower diffusion coefficients (Tucker and Nelken, 1990). There are no reference diffusion coefficients for these compounds found in the literature. However, these diffusion coefficient values are quite consistent with the diffusion coefficients of nine small solutes in stratum corneum, where the smallest solute is octanol with MW = 130 and $\log D = -6.4$, and the largest solute is aldosterone with MW = 360 and $\log D = -8.7$ (Mitragotri, 2000).

5.6. Significant effect of the boundary layer

The contributions of the membrane and the boundary layer to the absorption kinetics (constant a) are described in Eq. (4). It indicates that the boundary layer could have significant effect on the absorption kinetics for chemicals with high partition coefficients. When the $\log K$ value of a chemical is high enough to satisfy the condition, $\delta_m/D_m \ll 2K\delta_d/D_d$, the absorption kinetics is transiting from the membrane controlled diffusion to the boundary layer controlled diffusion. Therefore, the boundary layer could control the absorption kinetics of the hydrophobic chemicals even though their diffusion coefficients in the solution are larger than those in the membrane. This inference is worth of consideration in many other absorption experiments, such as, percutaneous absorption where the lipophilic membrane is assumed to be the dominant diffusion barrier and the boundary layer is neglected (Potts and Guy, 1992; Moss et al., 2002).

This is a useful conclusion even though the manifestation of the boundary layer is not known under normal in vivo conditions. (a) It provides a direction on how to increase or decrease the percutaneous absorption rates in transdermal drug delivery, risk assessment and optimize topical formulations in pharmaceutical, cosmetic and nutrition absorption. (b) It provides a direction for the design of absorption experiments and how to interpret the experimental data. (c) It also provides a theoretical direction on development of drug, pharmaceutical and cosmetic formulations. For example, the absorption rate of a compound could be increased by reducing the boundary layer via reduction in the viscosity of the formulation or application of a mechanical or ultrasound vibration.

It is difficult to understand the significant effect of the boundary layer. Traditionally, the membrane is assumed to be the main absorption barrier in percutaneous absorption (Moss et al., 2002). As more and more evidences directed to the contribution of the boundary layer, the boundary layer is at most treated with equal importance as the membrane (Hidalgo et al., 1991). The present kinetic model reveals that the contribution of the boundary layer could become the dominant resistant layer in percutaneous absorption of chemicals, particularly for lipophilic chemicals by lipophilic membranes. If one closely examine the absorption processes, it is not difficult to understand this theoretical inference. For a given

compound permeated from the bulk solution into the membrane, the average traveling distance in the solution is much larger than that in the membrane since the concentration in the membrane could be K times higher than that in the solution ($K = C_{me}/C_{de}$). Therefore, the diffusion resistance by the boundary layer could be much higher than the membrane for hydrophobic compounds.

The significant effect of the boundary layer does not contradict most of the traditional diffusion experimental results. In the traditional diffusion experiments, most of the chemicals and drugs studied have low octanol/water partition coefficients ($\log K_{o/w} < 4$). The contribution from the boundary layers was not significant. For hydrophobic compounds ($\log K_{o/w} > 4$), organic solvents were often used as vehicles to increase their solubility. Traditional diffusion studies were seldom done in aqueous solution with chemicals of $\log K_{o/w} > 4$ because of their sparing water solubility. The organic solvents reduced the vehicle/membrane partition coefficients close to unity. For example, $\log K_{o/w}$ for dodecane is 6.1, while its jet fuel/stratum corneum partition coefficient was only 0.97 (Baynes et al., 2000). Therefore, the permeability measured by traditional diffusion experiments would not deviate considerably when the boundary layer was considered. However, when the uptake of hydrophobic compounds from aqueous solutions are considered, such as, the uptake of hydrophobic drug from blood, or the uptake of toxic chemicals from water; the boundary layer cannot be neglected. The boundary layer effect could be the reason why the lipophilic compounds were found to be outliers in QSAR study in percutaneous absorption assessment (USEPA, 2001). Finally, when traditional diffusion studies are employed to compare vehicle or treatment effects on a compound's permeability through a model membrane, the existence of the boundary layer can be statistically treated as a constant effect.

6. Conclusions

The kinetic model was adequately described the absorption kinetics of the MCF technique; in which the cumulative amount permeated into the membrane was a function of the absorption time in an exponential equation. The two constants introduced into the model, clearly defined with the physiochemical parameters of the system, can be obtained by regression of the experimental data sampled over a limited time. All of the predictions of the kinetic model in the initial linear section, middle transition section and flat equilibrium section were supported by the experimental observations. The diffusion, permeation and partition coefficients were calculated from the two constants.

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