A Compartment Model for the Membrane-Coated Fiber Technique Used for Determining the Absorption Parameters of Chemicals into Lipophilic Membranes

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Received January 15, 2004; accepted April 27, 2004

Purpose. The purpose of this work was to develop a compartment model for the membrane-coated fiber (MCF) technique for determining the absorption parameters of chemicals into lipophilic membranes.

Methods. A polymer membrane coated onto a section of inert fiber was used as a permeation membrane in the MCF technique. When MCFs were immersed into a donor solution, the compounds in the solution partitioned into the membrane. At a given permeation time, a fiber was removed from the solution and transferred into a gas chromatography injector for quantitative analysis. The permeation process of a given chemical from the donor phase into the membrane was described by a one-compartment model by assuming first-order kinetics.

Results. A mathematical model was obtained that describes the cumulative amount of a chemical permeated into the membrane as a function of the permeation time in an exponential equation. Two constants were introduced into the compartment model that were clearly defined by the physiochemical parameters of the system (a kinetic parameter and the equilibrium absorption amount) and were obtained by regression of the experimental data sampled over a limited time before equilibrium. The model adequately described the permeation kinetics of the MCF technique. All theoretical predictions were supported by the experimental results. The experimental data correlated well with the mathematical regression results. The partition coefficients, initial permeation rate, uptake, and elimination rate constants were calculated from the two constants.

Conclusions. The compartment model can describe the absorption kinetics of the MCF technique. The regression method based on the model is a useful tool for the determination of the partition coefficients of lipophilic compounds when it takes too long for them to reach permeation equilibrium. The kinetic parameter and the initial permeation rate are unique parameters of the MCF technique that could be used in the development of quantitative structure-activity relationship models.

KEY WORDS: absorption kinetics; lipophilic membrane; membrane-coated fiber; partition coefficient; uptake rate constant.

INTRODUCTION

A membrane-coated fiber (MCF) technique uses a polymer membrane coated onto a section of inert fiber as a permeation membrane to study membrane permeation, partition equilibrium, and intermolecular forces between the membrane and solution (1). The MCF technique was developed from a solid-phase microextraction (SPME) technique in analytical chemistry, where it is used as a stationary phase to extract analytes from sample matrixes for quantitative analysis (2). For SPME applications, analyte extraction can be based on any mechanisms including absorption and adsorption, as efficient extraction for sampling is the only requirement. In fact, most of the newly developed SPME fibers are based on the adsorption or mixed absorption and adsorption mechanisms (3). The goal of the SPME technique is to extract maximum amount of analytes from the sample matrix, which is proportional to the original concentration in the sample matrix. In contrast, the maximum amount is not required in the MCF technique. The MCF technique is designated to use the membrane where absorption is the primary mechanism, so that parameters related to membrane absorption can be studied.

A direct application of the MCF technique is to study the skin absorption of exogenous chemicals, drugs, and cosmetics. The MCF technique is a direct extension of the conventional diffusion experiment with a synthetic membrane as the skin-imitating barrier (1,4,5). In the MCF technique, a silastic membrane coated onto an inert fiber is used as the permeation membrane. It is evident that a membrane-coated fiber represents only half of the conventional diffusion cell. Therefore, it will not provide the same absorption flux information as the conventional diffusion cell experiments. Instead, it will provide some physiochemical parameters obtained from the conventional diffusion experiments (uptake and elimination rate constants) and some parameters that cannot be obtained with the conventional diffusion experiments (e.g., initial permeation rate and partition coefficient). The MCF technique integrates the percutaneous 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 (1).

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. Decades of researches have demonstrated that the primary barrier of human skin to percutaneous absorption lies within the stratum corneum, which is a thin layer (about 20 μm) composed of flat corneocytes embedded in a continuous intercellular lipid matrix. The intercellular lipid matrix is the main route for percutaneous absorption of many exogenous chemicals (6,7). *In vitro* percutaneous absorption studies using human skin provided best results for mimicking *in vivo* situations. Excised skins from different animals (e.g., pig, rat, rabbit, and snake) are routinely used for percutaneous absorption studies because of the limited availability of human skin. However, the excised human or animal skins are subjected to variability in the age, sex, anatomical site, and the general health condition of the donor. Skin-handling protocols (preparation and storage) may also introduce variability (4,5). Therefore, the excised human or animal skins may not be the ideal choice for the

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0724-8741/04/0800-1345/0

kinetic studies of percutaneous absorption, where the skin membrane is required to be uniform across an entire set of the kinetic experiments and to remain unchanged under kinetic experimental conditions (5). Some synthetic membranes have been used as skin-imitating barriers for percutaneous absorption studies, which meet the requirements for kinetic studies due to their ready availability, uniformity, chemical stability, and purity (4–6). The use of the synthetic membranes in well-defined *in vitro* experiments could provide useful kinetic information for percutaneous absorption if the stratum corneum is the primary diffusion barrier (4,5). The most commonly used synthetic membrane is polydimethylsiloxane (PDMS, silastic) membrane (5). Recently, the permeability of PDMS membrane was again demonstrated to be well correlated with that of stratum corneum (8).

In this paper, a one-compartment model is developed to describe the permeation kinetics of the membrane-coated fiber. The mathematical model was evaluated by examining the compliance of the theoretical predictions with the experimental observations. Methods for obtaining the uptake and elimination rate constants, partition coefficients, and kinetic parameters were established for the MCF technique.

THEORY

In the MCF technique, a polymer membrane is coated onto an inert fiber. When the membrane-coated fiber is immersed into a donor solution, the permeation process of a given chemical from the donor phase into the membrane can be described by a one-compartment model, assuming first-order kinetics (9):

$$\frac{dC_m}{dt} = k_1 C_d - k_2 C_m \tag{1}$$

where k_1 is the uptake rate constant (from the donor solution into the membrane), and k_2 is the elimination rate constant (from the membrane into the donor solution), C_m and C_d are the concentrations of the compound in the membrane and the donor solution, respectively.

If the permeation amount into the membrane (n) at a given time (t) can be determined, the mass balance of the compound in the donor solution and the membrane is

$$C_d = C_o - \frac{n}{V_d} \tag{2}$$

$$C_m = \frac{n}{V_m} \tag{3}$$

where C_o is the initial concentration of the compound in the donor solution, and V_d and V_m are the volumes of the donor solution and the membrane, respectively.

Substituting Eq. 2 and Eq. 3 into Eq. 1 gives

$$\frac{dn}{dt} = k_1 V_m C_o - \frac{k_1 V_m + k_2 V_d}{V_d} n \tag{4}$$

Let

$$a = \frac{k_1 V_m + k_2 V_d}{V_d} \quad \text{and} \quad b = k_1 V_m C_o \tag{5}$$

The differential equation (Eq. 4) is simplified to

$$\frac{dn}{dt} = b - an \tag{6}$$

Solving Eq. 6 with the initial condition, when $t = 0$, $n = 0$, the solution is

$$n = [1 - \exp(-at)]\frac{b}{a} \tag{7}$$

Putting the a and b expressions (Eq. 5) back into Eq. 7, the mathematical model for the MCF technique is obtained:

$$n = [1 - \exp(-at)]\frac{k_1 V_d V_m C_o}{k_1 V_m + k_2 V_d} \tag{8}$$

When permeation equilibrium is reached at infinite time ($t \rightarrow \infty$), the maximum equilibrium amount (n°) can be obtained from Eq. 8 as follows:

$$n^o = \frac{k_1 V_d V_m C_o}{k_1 V_m + k_2 V_d} \tag{9}$$

Thus, the mathematical model (Eq. 8) is simplified to:

$$n = [1 - \exp(-at)]n^o \tag{10}$$

When constants a and n° are obtained by regression of the permeation experimental data with the mathematical model (Eq. 10), the uptake rate constant (k_1) and the elimination rate constant (k_2) can be obtained by solving Eq. 5 and Eq. 9:

$$k_1 = \frac{an^o}{V_m C_o} \tag{11}$$

$$k_2 = a\left(1 - \frac{n^o}{V_d C_o}\right) \tag{12}$$

The partition coefficient of the compound between the donor solution and the membrane can be obtained from the equilibrium concentration in the membrane ($C_{me} = n^\circ/V_m$) and in the donor solution ($C_{de} = C_o - n^\circ/V_d$):

$$K = \frac{C_{me}}{C_{de}} = \frac{n^o V_d}{V_m(V_d C_o - n^o)} \tag{13}$$

MATERIALS AND METHODS

Chemicals and Materials

Acetone and hexane were of HPLC grade (J. T. Baker, Phillipsburg, NJ, USA). Deionized water was prepared from a Picotech Water System (Research Triangle Park, NC, USA). A standard mixture consisting of 30 compounds (see Fig. 5) in acetone was purchased from AccuStandard Inc. (New Haven, CT, USA). Solid-phase microextraction (SPME) devices and 100-μm polydimethylsiloxane (PDMS) coated fiber assemblies were purchased from Supelco (Bellfonte, PA, USA).

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 acetone was prepared from the standard mixture. A series of aqueous donor solutions with different initial concentrations from 0.1 to 10 ng/ml (each individual component) were prepared from the stock solution.

The concentrations in the donor solutions after the permeation experiments with the membrane-coated fibers were

measured by liquid-liquid extraction with hexane. The quantities were calibrated with standard donor solutions measured by the same liquid-liquid extraction procedures.

Permeation with the Membrane-Coated Fiber

The experimental setup of the MCF technique and the procedures to conduct the permeation experiments have previously been described in detail (1). A given volume of the donor solution was transferred into the permeation container. The donor solution was stirred constantly with a magnetic stirrer at 400 rpm under ambient temperature (25°C). A membrane-coated fiber was immersed into the donor solution to partition the permeants of interest into the membrane. At a given permeation time, the membrane-coated fiber was removed and transferred into a GC injector for quantitative analysis.

To obtain the permeation time profiles, the permeation amounts were measured at different permeation times from 5 min to 48 h, while the donor volume and initial concentration were kept constant at 75 ml and 1.00 ng/ml, respectively. A fresh donor solution was used for each time point.

To study the dependence of the permeation amount on the initial donor concentration, the permeation amounts were measured from donor solutions with different initial concentrations from 0.1 to 10 ng/ml, while the donor volume and the permeation time were kept constant at 10 ml and 12 min, respectively.

GC/MS Analysis

The quantitative analysis was performed on an HP 5890 II gas chromatograph coupled with an HP 5970B mass selective detector (Hewlett-Packard, Palo Alto, CA, USA). An HP 7673 automatic sampler was used to inject 4 μl of the calibration standard solution, whereas the membrane-coated fibers were 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, USA). 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 2 or 3 character ions were monitored for each compound depending on the ion intensity produced by the compound.

Data Analyses

The experimental data were regressed with the proposed mathematical model (Eq. 10) by using nonlinear regression software (WinNonlin, Pharsight Corp., Mountain View, CA, USA). The two constants in the mathematical model vary in several orders of magnitude for different compounds. It is difficult to estimate the starting values for regression. To overcome this difficulty, the experimental maximum permeation amounts (n^{max}) were used as the first estimated values for the equilibrium permeation amounts. The permeation amounts (n) were converted to n/n^{max}, and the regression of n/n^{max} vs. permeation time t was performed with equation $n/n^{\max} = 1 - \exp(-at)$, derived from Eq. 10, to obtain the first estimated values for constant a. The first estimated values of the two constants were used to perform the final regression with the mathematical model Eq. 10 to obtain the values of the two constants.

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 and the measured maximum permeation amount (n°) using Eq. 13. The second method was to measure the equilibrium concentrations in the membrane (C_{me}) and in the donor solution (C_{de}) and to calculate the partition coefficient from its definition ($K = C_{me}/C_{de}$). The equilibrium concentration in the donor phase was measured by liquid-liquid extraction with hexane. The third method was to use the mathematical model to obtain the equilibrium amount (n°) by regression of the permeation data sampled over a limited period of time. The partition coefficient was calculated with Eq. 13 and the equilibrium permeation amount (n°).

When the constant a and the equilibrium permeation amount (n°) were obtained for a given compound, the uptake and elimination rate constants, k_1 and k_2, were calculated with Eq. 11 and Eq. 12. The membrane volume of the 100-μm PDMS MCF was 0.612 μl.

RESULTS

One-Compartment Model

A one-compartment model was used to describe the permeation kinetics of the MCF technique. The cumulative amount of a compound partitioned into the membrane (n) can be expressed as a function of the permeation time (t) in an exponential equation (Eq. 10). Two constants, a and n° introduced into the mathematical model, were clearly defined by the physiochemical parameters of the permeation system (Eq. 5 and Eq. 9). These two constants can be obtained by regression of the permeation experimental data with the mathematical model (Eq. 10). The uptake and elimination rate constants, k_1 and k_2, can be calculated from the two constants with Eq. 11 and Eq. 12. The partition coefficient can also be calculated from the equilibrium permeation amount with Eq. 13.

The normalized permeation time profiles ($n/n^\circ - t$) predicted with the theoretical model are shown in Fig. 1. It is observed that for a given compound the value of constant a determines the shape of the permeation time profile. The permeation will reach equilibrium faster if a compound has a higher a value.

Linear Relationship between n and C_o

The permeation amount (n) is related to the initial concentration (C_o) of the donor solution as predicted by the theoretical model (Eq. 8). To study this relationship, the permeation amounts from a series of donor solutions with different initial concentrations were measured while the permeation time was held constant. Figure 2 shows the relationships

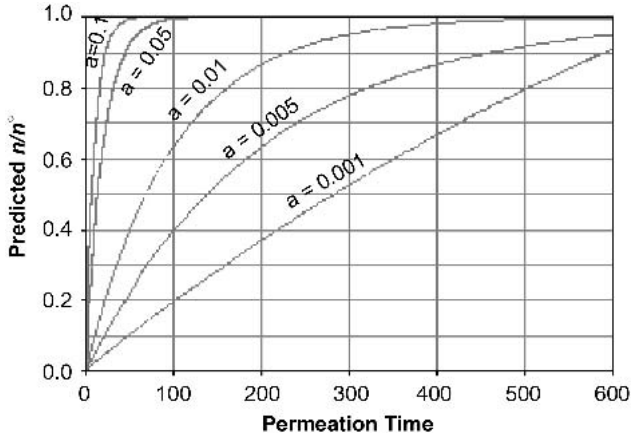

Fig. 1. Prediction of the permeation time profiles with different *a* values. Numbers on curves are constant *a* values.

for terrazole, dacthal, and chlorobenzilate when the permeation time was 12 min. It was observed that these relationships were linear and crossed the zero intersection. In fact, this linear relationship was observed for all of the 30 compounds in the donor solutions.

MCF Permeation Profiles

The permeation profiles determined by the MCF technique carry the basic information of the permeation processes. Figure 3 shows the permeation profiles of aldrin, tr-chlordane, endosulfan sulfate, and p,p-DDT. The permeation profile of aldrin was a representative profile under the experimental conditions, which consisted of an initial linear section, a middle transition section, and a flat equilibrium section. Endosulfan sulfate reached equilibrium in about 50 min (referring to the insert of Fig. 3), aldrin reached equilibrium in about 1500 min, whereas tr-chlordane and p,p-DDT did not reach equilibrium in the experimental time period. From the initial linear section, an initial uptake rate was obtained for the compound (the insert of Fig. 3). The initial uptake rate was not obtained for endosulfan sulfate because equilibrium was reached. If the experimental data were sampled earlier, the linear section could be obtained for endosulfan sulfate.

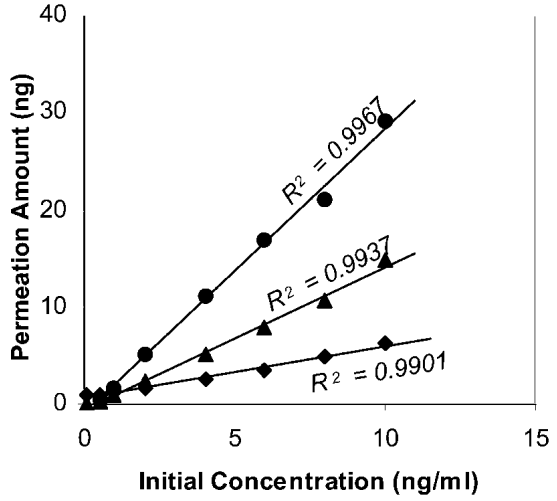

Fig. 2. Predicted linear relationship of permeation amount vs. initial concentrations. The scattered points were experimental values for terrazole (♦), dacthal (●), and chlorobenzilate (▲). The solid lines were their corresponding linear regressions. The membrane-coated fiber was 100 μm PDMS, the initial concentrations of the donor solutions were from 0.1 to 10 ng/ml (each individual component), and the permeation time and stirring speed were kept as constants at 12 min and 400 rpm, respectively.

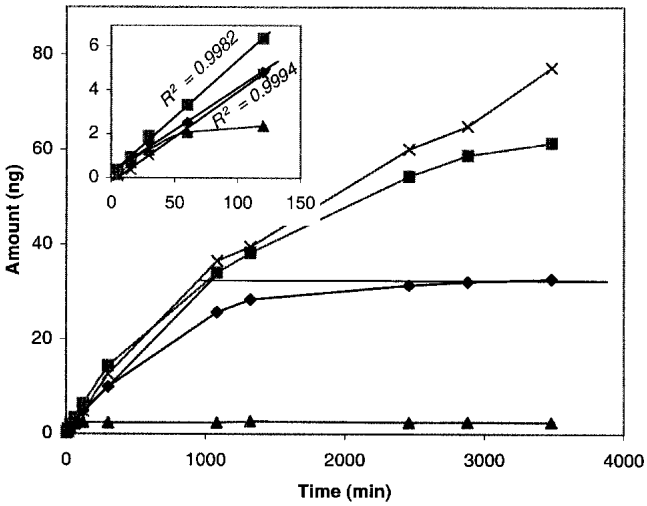

Fig. 3. MCF permeation profiles. The profiles of endosulfan sulfate (▲), aldrin (♦), tr-chlordane (■), and p,p-DDT (x) were measured by 100-μm PDMS MCFs in donor solutions of 75 ml 1.00 ng/ml (individual component). The insert was an expansion of the initial period of time.

Regression of the Experimental Data

The two constants, *a* and *n*°, can be obtained by regression of the permeation experimental data using the theoretical model (Eq. 10). For each compound, the regression was performed with 10 permeation data points (the permeation amounts vs. permeation times) sampled from 5 min to 48 h. A 100-μm PDMS fiber was used for the permeation experiment with a donor solution of 75-ml 1.00 ng/ml (individual component). Figure 4 shows the regression results for a-BHC, heptachlor epoxide, endosulfan I, and methoxychlor. The experimental data fitted well with the mathematical model over the entire range of permeation profiles. The two constants were obtained for all of the 30 compounds in the donor solution, and those selected to represent the entire chromatograph of the 30 compounds are listed in Table I.

Uptake and Elimination Rate Constants

The uptake constant k_1 and the elimination rate constant k_2 were calculated from the two regression constants (*a* and *n*°) with Eq. 11 and Eq. 12 (Table I). The uptake rate constants (k_1) of all the compounds studied were larger than the elimination rate constants (k_2). The range of the uptake rate constants was from 1.96 (atrazine) to 141 (dieldrin) for the selected compounds, whereas the range of the elimination rate constants was from 0.00019 (tr-chlordane) to 0.0598 (atrazine) (Table I). The initial permeation rates of the selected

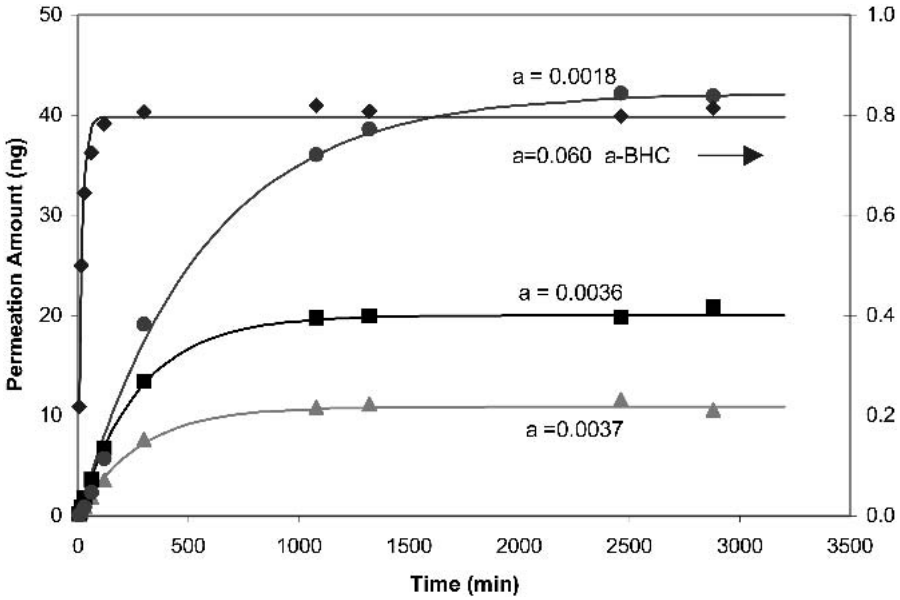

Fig. 4. Experimental permeation time profiles and their regressions. The scattered points were experimental values for a-BHC (♦), methoxychlor (●), endosulfan I (■), and heptachlor epoxide (▲) measured by 100-μm PDMS MCFs in donor solutions of 75 ml 1.00 ng/ml (individual component). The curve lines were their corresponding regressions with the mathematical model. The *a* values obtained by regression were placed next to their regression lines. Right y-axis is for a-BHC, whereas left y-axis is for other compounds.

compounds are also listed in Table I, which were calculated from the regression constants (*a* and n°) using an equation,

$$\frac{dn}{dt}\Big/_{t\to 0} = an^o$$

derived by the first differentiation of Eq. 10 and set time approached to zero. The initial permeation rates were not significantly different for the selected compounds.

Partition Coefficients Determined by the MCF Technique

Another direct application of the MCF technique is to determine the membrane/media partition coefficients. The equilibration times were predetermined in the equilibration methods, which were in a range from few minutes to 41 h for different compounds. The maximum permeation amount was obtained at equilibrium for each compound. The maximum permeation amount was used to calculate the partition coefficient with Eq. 13 in the first equilibrium method. The equilibrium concentrations in the membrane and donor solution were determined and used to calculate the partition coefficient in the second equilibrium method. The partition coefficients obtained by the two equilibrium methods are given in Table II.

The partition coefficients were also calculated from the equilibrium permeation amounts (n°) obtained by the regres-

Table I. Regression Results of the Selected Compounds*

	a		n°		an°†		
Compound	1/min	CV‡	ng	CV‡	ng/min	k_1	k_2
Terrazole	0.025	16	0.25	6.2	0.0064	10.4	0.0253
a-BHC	0.060	7	0.80	1.6	0.048	78.7	0.0598
Atrazine	0.021	21	0.056	4.7	0.0012	1.96	0.0213
Heptachlor	0.0012	29	35	3.3	0.044	72.6	0.00067
Dacthal	0.012	10	2.4	2.3	0.028	47.2	0.0115
Heptachlor Ep	0.037	6	11	1.3	0.040	65.4	0.00314
tr-Chlordane	0.0012	2	47	0.7	0.057	93.8	0.00045
tr-Nonachlor	0.00074	8	55	3.6	0.041	67.3	0.00019
p,p-DDE	0.00089	7	55	3.2	0.049	80.4	0.00024
Dieldrin	0.0024	6	35	1.3	0.086	141	0.00124
p,p-DDD	0.0014	8	59	1.6	0.081	133	0.00028
Methoxychlor	0.0018	10	42	3.0	0.074	122	0.00077
cis-Permethrin	0.00066	3	70	1.7	0.046	46.6	0.00028

* The compounds were selected to represent the chromatograph of the 30 compounds studied.
† The initial permeation rate (an°) was calculated from constants *a* and n°.
‡ CV was the relative coefficient variation of the regression (n = 10).

Table II. Partition Coefficients of the Selected Compounds

Compound	Log *K* by MCF technique				Log $K_{o/w}$
	First	Second	Third	Mean	
Terrazole	2.69	2.66	2.62	2.65	2.55
a-BHC	3.11	3.08	3.12	3.10	3.90
Atrazine	1.94	1.91	1.96	1.94	2.60
Heptachlor	5.20	5.66	5.03	5.29	5.44
Dacthal	3.57	3.55	3.61	3.57	4.87
Heptachlor Ep	4.31	4.32	4.32	4.32	4.60
tr-Chlordane	5.54	5.38	5.32	5.41	6.00
tr-Nonachlor	5.66	5.46	5.54	5.56	5.80
p,p-DDE	5.39	5.38	5.53	5.43	5.90
Dieldrin	5.08	4.90	5.04	5.01	5.20
p,p-DDD	5.51	5.17	5.67	5.45	6.02
Methoxychlor	5.01	4.92	5.20	5.04	5.08
cis-Permethrin	5.21	5.71	5.22	5.38	6.10

In the first method, *K* was calculated with Eq. 13 by using the maximum permeation amounts (n°) after 41-h permeation in a donor solution of 75 ml 1.00 ng/ml. In the second method, *K* was calculated from its definition by using the measured donor and membrane concentrations after 41-h permeation in a donor solution of 75 ml 1.00 ng/ml. In the third method, *K* was calculated with Eq. 13 when n° was obtained by the regression. Log $K_{o/w}$ was compiled from published data (10,11).

sion method (Table II). The advantage of the regression method is that the permeation data can be sampled before equilibrium. The octanol/water partition coefficients of the selected compounds are also listed in Table II. The partition coefficients measured by the three methods with the MCF technique were consistent and showed satisfactory agreement with the published octanol/water partition coefficients (10,11). Figure 5 shows five sets of partition coefficients of the 30 compounds contained in the donor solution. The x-axis represents the compounds in the chromatograph sequence under the given GC/MS conditions. Howard series and Mackay series were log $K_{o/w}$ values compiled from reference handbooks (10–12). *K*-1st, *K*-2nd, and *K*-3rd series were obtained with the first, second, and third methods using the MCF technique. The linear correlation coefficients (R^2) of Howard, K-1st, K-2nd, and K-3rd series with Mackay series were 0.88, 079, 0.79, and 0.80, respectively.

DISCUSSION

Predictions of the Mathematical Model

The one-compartment model was used to describe the permeation process of the MCF technique by assuming first-order kinetics. To evaluate the model, the predictions by the mathematical model are examined for compliance with the experimental observations.

When the permeation equilibrium is reached, $dC_m/dt = 0$, Eq. 1 becomes $k_1C_{de} - k_2C_{me} = 0$, or

$$K = \frac{C_{me}}{C_{de}} = \frac{k_1}{k_2} \tag{14}$$

Substituting Eq. 14 into Eq. 9, the relationship of the partition coefficient (*K*) with the equilibrium permeation amount (n°) was obtained from the theoretical derivation, which is exactly the same expression as Eq. 13, the definition of the partition

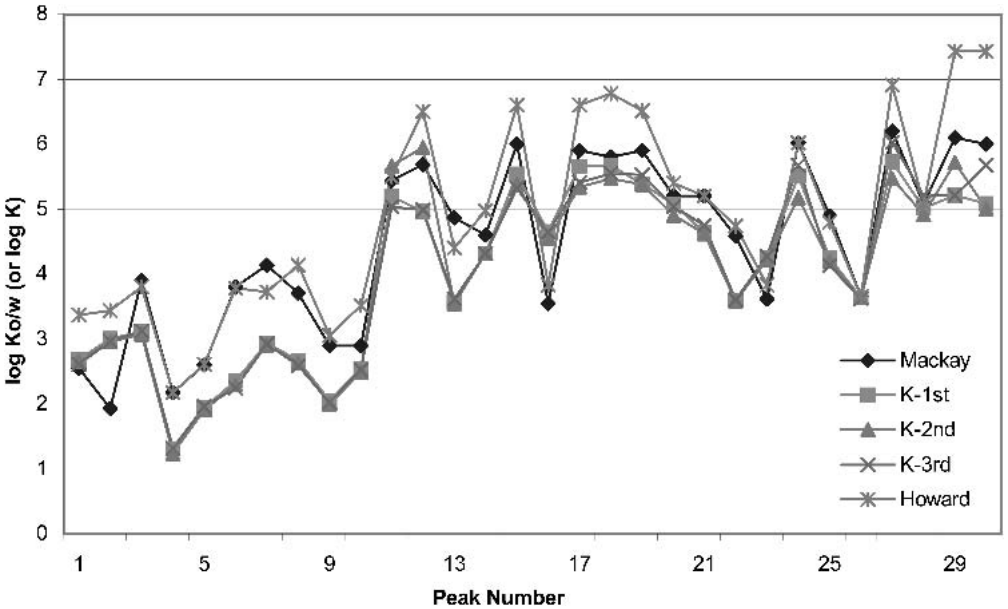

Fig. 5. Comparison of the measured log *K* and published log $K_{o/w}$. Mackay series were log $K_{o/w}$ values from the Mackay *et al.* and the Montgomery edited reference handbooks (10,11). Howard series were log $K_{o/w}$ values from the Howard *et al.* edited reference handbook (12). *K*-1st, *K*-2nd, and *K*-3rd were measured log *K* values by the MCF technique. Each tick mark of the x-axis represents one compound and numbered in the chromatograph sequence 1, terrazole; 2, chloroneb; 3, a-BHC; 4, simazine; 5, atrazine; 6, b-BHC; 7, g-BHC; 8, d-BHC; 9, chlorothalonil; 10, alachlor; 11, heptachlor; 12, aldrin; 13, dacthal; 14, heptachlor epoxide; 15, tr-chlordane; 16, endosulfan I; 17, cis-chlordane; 18, tr-nonachlor; 19, p,p-DDE; 20, dieldrin; 21, endrin; 22, chlorobenzilate; 23, endosulfan II; 24, p,p-DDD; 25, endrin aldehyde; 26, endosulfan sulfate; 27, p,p-DDT; 28, methoxychlor; 29, cis-permethrin; and 30, tr-permethrin.

coefficient. This suggests that the theoretical derivation of the model and the assumption of first-order kinetics were adequate, and the one-compartment model described the kinetics of the equilibrium state.

The theoretical model, Eq. 10, describes the dependency of the cumulative amount of a given compound to permeat into the membrane (n) on the permeation time (t). By taking the first differentiation of Eq. 10, the permeation rate at any permeation time can be obtained

$$\frac{dn}{dt} = an^o \exp(-at).$$

It is predicted that the initial permeation rate is constant,

$$\frac{dn}{dt}\Big/_{t\to 0} = an^o$$

and the equilibrium permeation rate is zero,

$$\frac{dn}{dt}\Big/_{t\to \infty} = 0$$

These predictions are supported by the experimental observations (Fig. 3). The initial permeation rate can be obtained for each compound as we reported previously (1). The permeation time profile of each compound consisted of three sections: the initial linear section, a transition section, and a flat equilibrium section where the permeation rate was zero.

If the permeation time is kept constant (t'), the expression of Eq. 8,

$$[1 - \exp(-at')]\frac{KV_dV_m}{KV_m + V_d}$$

becomes a constant. Thus, it is predicted that the permeation amount (n) will be linearly related to its initial concentration in the donor solution (C_o), and it is also predicted that the linear relationships will cross the zero intersection. These predictions were supported by the determination of the permeation amounts from a series of donor solutions with different initial concentrations while keeping the permeation time constant (Fig. 2). All of the linear relationships crossed the zero intersection as predicted by the model.

The regression of the permeation data with the theoretical model (Eq. 10) is shown in Fig. 4. It was observed that the experimental data fitted well with the theoretical model over the entire range of permeation profiles. This suggests that the one-compartment model well represents the transport kinetics of the MCF technique.

Constants Introduced into the Mathematical Model

The two parametric constants, a and n°, introduced into the theoretical model were clearly defined by the physiochemical parameters of the permeation system. Constant n° is the equilibrium permeation amount, as it was derived from Eq. 8 by setting the permeation time to infinity. It is a measure of the thermodynamic factor of the system in the mathematical model. Thus, constant n° can be used to calculate the partition coefficient K (Eq. 13).

Constant a is primarily a measure of the kinetic factor of the system in the mathematical model. It governs the kinetic characteristics of the system and determines the shape of the permeation time profiles. A compound having a larger value of constant a will reach the permeation equilibrium faster.

The kinetic parameter a is a unique parameter determined by the MCF technique. It is strictly dependent on the experimental conditions. For example, stirring the solution significantly increased the permeation rate and reduced the equilibration time, consequently, increased the a value, whereas the equilibrium amount (n°) and the partition coefficient (log K) were not affected (1). This is a crucial difference of the kinetic parameter (a) from thermodynamic parameter (n° or log K). The kinetic parameter a is also related with the equilibrium permeation amount (n°), that is, the higher the n°, the smaller the a value (Fig. 4). For a compound at given experimental conditions, the kinetic parameter a is a constant over the permeation profile. For the given experimental conditions, different compounds have different a values. If the hydrodynamic condition changes, the kinetic parameter a will change, whereas the thermodynamic parameters, n° or log K, will not change. We have demonstrated that stirring the donor solution significantly changed the permeation rates of all the compounds studied (1). Thus, the kinetic parameter a could be a better parameter to represent the permeation characteristics of a given compound than n° or log K under changing hydrodynamic experimental conditions. It can be used in the development of quantitative structure-activity relationship (QSAR) models. It carries the kinetic information and the thermodynamic information of the compound in changing experimental conditions. This kinetic information might provide important insight into chemical interactions in the fields, which modulate rate of compound uptake into biological membranes. For example, a kinetic parameter (a) term could be added to the widely used skin permeation QSAR model developed by Potts and Guy (13) to reduce the prediction variability from different experimental conditions. This is one of the advantages of the MCF technique in QSAR studies.

Uptake and Elimination Rate Constants, Initial Permeation Rates

The uptake and elimination rate constants were two basic parameters in the one-compartment model. The values of the uptake rate constants of the selected compounds were higher than those of the elimination rate constants. This revealed the tendency of the lipophilic compounds to permeat into a lipophilic membrane. It is interesting to note that the changes of both the uptake and elimination rate constants of the selected compounds were two orders of magnitude (Table I). The general trend was that a compound having high k_1 value would have small k_2 value. This is consistent with the permeation tendency that it was easy for the lipophilic compounds to permeat into the lipophilic membrane, whereas it was difficult to leave the membrane. The opposite trends of the uptake and elimination rate constants led to the high partition coefficients for the lipophilic compounds (Eq. 14).

The initial permeation rate is a unique parameter determined by the MCF technique. It cannot be detected in the conventional diffusion cell experiments where the flux is determined after the initial permeation time. This initial permeation rate could be the maximum permeation rate of a given compound under the experimental condition. This information is valuable for many practical applications in threshold estimation. From Eq. 11, it is known that the initial permeation rate (an°) is concentration dependent ($an^\circ = k_1V_mC_o$),

whereas the uptake rate constant (k_1) is not concentration dependent (Eq. 1).

Partition Coefficients

The partition equilibrium must be reached if the first or second method (equilibration methods) is used to measure the partition coefficients. This is often the case for compounds having lower partition coefficients. For hydrophobic compounds (e.g., log $K > 4$), it might take too long (e.g., days or weeks) to reach their permeation equilibrium (1). Therefore, it is difficult to use the equilibration methods to obtain the partition coefficients for hydrophobic compounds. Sometimes, the log K values might be underestimated when the permeation equilibrium is not reached. With the current model, the equilibrium amount (n°) can be obtained by the regression of the permeation data sampled over a limited period of time before equilibrium. This provides a convenient method for rapid assessment of the percutaneous permeation of hydrophobic compounds.

The partition coefficients obtained by the three methods were very consistent among themselves. This shows the reliability of the values measured by the MCF technique. The log K values measured by the MCF technique correlated well with the published octanol/water partition coefficients (Fig. 5) regarding the large variances in published reference values (10–12). In the MCF technique, all of the partition coefficients for the 30 compounds were measured simultaneously and generated from one set of experimental data. If the donor concentration and equilibrium conditions are properly selected, these partition coefficients should be more consistent in reflecting their relative lipophilicity than those compiled from different literature sources. For practical application, the differences between the log K values measured by the MCF technique and the log $K_{o/w}$ values could be calibrated with a surrogate method as used in a HPLC method (14).

CONCLUSIONS

The one-compartment model adequately described the permeation kinetics of the MCF technique. All of the predictions of the mathematical model in the initial linear section, middle transition section, and flat equilibrium section were supported by the experimental observations. The cumulative amount permeated into the membrane was a function of the permeation 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. The uptake and elimination rate constants and the partition coefficients were calculated from these two constants. The kinetic parameter a and the initial permeation rate are unique parameters determined by the MCF technique, which could be used in the development of QSAR models.

ACKNOWLEDGMENTS

This research was supported by NIOSH grants R01-OH 03669 and 07555. Part of this work was presented at the 41st Annual Meeting of the Society of Toxicology in Nashville, Tennessee (2002).

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