

# A Novel *in-Vitro* Technique for Studying Percutaneous Permeation with a Membrane-Coated Fiber and Gas Chromatography/Mass Spectrometry: Part I. Performances of the Technique and Determination of the Permeation Rates and Partition Coefficients of Chemical Mixtures

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Received July 17, 2002; accepted October 8, 2002

**Purpose.** To develop a novel *in-vitro* technique for rapid assessment of percutaneous absorption of chemical mixtures.

**Methods.** A silastic membrane was coated on to a fiber to be used as a permeation membrane. The membrane-coated fiber was immersed in the donor phase to partition the compounds into the membrane. At a given partition time, the membrane-coated fiber was transferred into a GC injector to evaporate the partitioned compounds for quantitative and qualitative analyses.

**Results.** This technique was developed and demonstrated to study the percutaneous permeation of a complex mixture consisting of 30 compounds. Each compound permeated into the membrane was identified and quantified with GC/MS. The standard deviation was less than 10% in 12 repeated permeation experiments. The partition coefficients and permeation rates in static and stirred donor solution were obtained for each compound. The partition coefficients measured by this technique were well correlated ( $R^2 = 0.93$ ) with the reported octanol/water partition coefficients.

**Conclusions.** This technique can be used to study the percutaneous permeation of chemical mixtures. No expensive radiolabeled chemicals are required. Each compound permeated into the membrane can be identified and quantified. The initial permeation rate and equilibrium time can be obtained for each compound, which could serve as characteristic parameters regarding the skin permeability of the compound.

**KEY WORDS:** *in-vitro*; percutaneous absorption; membrane-coated fiber; chemical mixtures; partition coefficient.

## INTRODUCTION

Assessment of percutaneous absorption is important to many industrial and scientific fields, particularly in the development of transdermal drug delivery devices, dermatological formulations, safety assessment of cosmetics, and risk assess-

ment of environmental or occupational hazards. Efforts have been made to develop experimental approaches to measure percutaneous absorption. To date, most of the data on percutaneous absorption have been obtained by *in vitro* diffusion chamber experiments, while *in vivo* data are commonly obtained from animal experiments via biomonitoring (1–5).

*In vitro* percutaneous absorption is generally studied with two kinds of diffusion chambers, Franz diffusion cell and flow-through diffusion cell. In these diffusion cells, the membrane is placed between two chambers, donor and receptor, and the compounds in question diffuse from the donor phase through the membrane into the receptor phase. In the Franz diffusion cell, samples are withdrawn periodically from the receptor phase and analyzed to measure the penetration flux. In the flow-through cell, the compound passing through the membrane is carried away by a receptor fluid to be collected for analysis (6).

In the diffusion experiments of environmentally relevant compounds, often only a trace amount of chemical penetrates through the membrane into the receptor phase. Trace analytical techniques are required for the assay of the chemicals, such as liquid scintillation counting (LSC), which requires the chemicals of interest to be radiolabeled, and allows only one chemical to be studied at a time. HPLC can be used for the assay of permeable chemicals, but sample treatment and enrichment usually are required (7). These obstacles make the current assessment of percutaneous absorption expensive and time consuming. Moreover, millions of chemicals and their metabolites in a variety of industrial and environmental matrices need to be screened for their percutaneous absorption; and numerous formulations need to be screened for better medicines, pharmaceuticals, and cosmetics (8–11). Therefore, developing new or improved rapid screen techniques for *in vitro* percutaneous absorption are needed, especially for studying chemical mixtures and their synergistic effects on percutaneous absorption.

In this study, a novel *in vitro* technique is proposed for rapid assessment of percutaneous permeation. An artificial membrane is coated on an inert fiber as the permeation membrane. The membrane-coated fiber (MCF) is immersed in the donor solution to partition the compounds to be studied. MCF is transferred into the injector of a gas chromatograph (GC) for analysis, eliminating the need for an additional extraction step, which adds variability to the traditional diffusion experiments. This technique is demonstrated to be applicable to the study of complex chemical mixtures and to the measurements of permeation rates and partition coefficients. The performances of the membrane coated fiber and the optimum experimental conditions are investigated. The advantages of the MCF technique and its differences from the traditional diffusion cells are discussed.

## MATERIALS AND METHODS

### Chemicals and Materials

Acetone and acetonitrile were HPLC grade (J. T. Baker). Deionized water was prepared from a Picotech Water System (Research Triangle Park, NC). A standard mixture containing 30 compounds (Table I) in acetone was purchased from AccuStandard Inc. (New Haven, CT). Solid-phase mi-

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**Table I.** Performances of the Membrane-Coated Fiber

Pk #	Compounds	Reproducibility <sup>c</sup>		Initial permeation rate (ng/min)			log K <sup>b</sup>	log Ko/w <sup>a</sup>
		Amount (ng)	STDEV (%)	Static	Stir	Stir/static		
1	Terrazole	29.45	5.70	0.568	eq	n/a	2.75	2.55
2	Chloroneb	24.68	4.88	0.768	eq	n/a	1.59	1.93
3	a-BHC	28.79	5.72	0.897	eq	n/a	4.04	3.9
4	Simazine	1.86	2.30	0.001	eq	n/a	2.20	2.18
5	Atrazine	0.55	9.94	0.004	eq	n/a	2.74	2.6
6	b-BHC	6.50	3.55	0.387	eq	n/a	3.70	3.8
7	g-BHC	23.24	3.36	0.823	eq	n/a	3.87	4.14
8	d-BHC	13.06	3.20	0.570	eq	n/a	3.51	3.7
9	Chlorothalonil	10.04	2.26	0.169	eq	n/a	3.02	2.9
10	Heptachlor	3.92	7.26	0.050	1.43	28.56	5.13	5.44
11	Alachlor	9.47	5.87	0.431	eq	n/a	2.40	2.9
12	Aldrin	2.06	6.70	0.074	1.05	14.06	5.08	5.68
13	Dacthal	29.04	4.33	0.891	7.74	8.68	3.95	4.87
14	Heptachlor Epoxide	19.90	4.81	0.552	7.95	14.41	4.64	4.6
15	tr-Chlordane	6.89	4.29	0.095	2.31	24.29	5.53	6
16	Endosulfan I	18.76	7.85	0.552	7.14	12.95	3.60	3.55
17	cis-Chlordane	7.23	8.87	0.125	2.65	21.18	5.53	5.9
18	tr-Nonachlor	5.09	5.63	0.061	1.71	28.27	5.66	5.8
19	Dieldrin	18.30	2.67	0.438	5.70	13.01	4.92	5.2
20	p,p-DDE	3.89	8.13	0.012	1.14	94.55	5.44	5.9
21	Endrin	8.01	6.79	0.099	3.16	31.85	4.54	5.2
22	Endosulfan II	22.83	5.69	0.734	7.08	9.65	4.05	3.62
23	Chlorobenzilate	22.67	7.03	0.628	6.21	9.89	4.41	4.58
24	p,p-DDD	8.21	7.45	0.195	2.97	15.23	5.15	6.02
25	Endrin Aldehyde	12.90	3.91	0.603	3.35	5.55	4.03	4.9
26	Endosulfan Sulfate	21.54	3.52	0.721	5.55	7.71	3.37	3.66
27	p,p-DDT	7.92	6.25	0.055	1.12	20.51	6.21	6.2
28	Methoxychlor	14.33	7.62	0.203	3.66	18.01	4.97	5.08
29	cis-Permethrin	15.28	2.34	0.066	0.96	14.52	4.97	6.1
30	tr-Permethrin	3.24	6.29	0.010	0.20	21.11	5.21	6

Note: Initial permeation rates were measured with 100- $\mu$ m PDMS membrane coated fibers, statically or stirred at 400 rpm, in 150-ml 100-ng/ml donor solutions.

<sup>a</sup> Published octanol/water partition coefficients (12,13). eq: Equilibrium was reached within 30 min.

<sup>b</sup> Partition coefficients were calculated with the maximum permeation amounts obtained from the permeation time profiles (5 min–72 h) measured with a 100- $\mu$ m PDMS membrane coated fiber ( $V_m = 0.612 \mu$ l) in 150-ml 1.00-ng/ml donor solutions stirred at 400 rpm.

<sup>c</sup> Permeation experiments were repeated under identical conditions ( $n = 12$ ) with one 100- $\mu$ m PDMS membrane coated fiber immersed in a fresh 25-ml 100-ng/ml donor solution for 5 min each time.

croextraction (SPME) devices and 100- $\mu$ m polydimethylsiloxane (PDMS) coated fiber assemblies were purchased from Supelco (Bellfonte, PA).

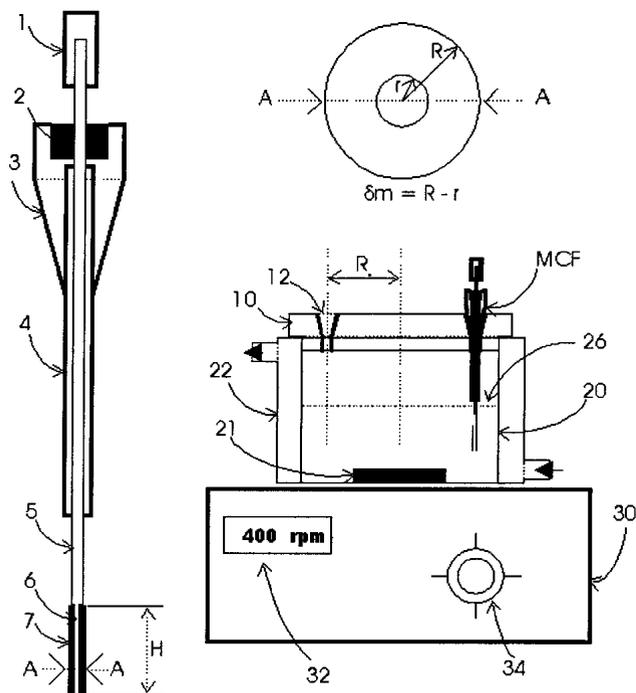
A series of standard solutions in acetone were prepared from the standard mixture to be used as external calibration standards for quantitative analysis. A stock solution of 20  $\mu$ g/ml of individual component in acetonitrile was prepared from the standard mixture. Two aqueous donor solutions with concentrations of 1.00 and 100 ng/ml (each individual component) were prepared from the stock solution.

### Membrane-Coated Fiber and Experimental Setup

The membrane-coated fibers were modified from the PDMS coated fibers as shown in Fig. 1 (US Patent pending). It is comprised of a piercing needle attached to a needle base. A sealing septum is inserted in the needle base. The needle base has a tapered end for positioning during permeation studies. A fiber attachment tubing slides inside of the piercing needle through the sealing septum. The top end of the fiber attachment tubing is attached to a holding tip. A chemically

inert fiber is attached to the lower end of the fiber attachment tubing. One section of the inert fiber (fused-silica) is coated with PDMS membrane of 100- $\mu$ m in thickness and 1.00 cm in length. When the membrane-coated fiber is not in use, the fiber is withdrawn into the inside of the piercing needle to protect the membrane from damage. To conduct the permeation experiment, the membrane-coated fiber is pressed out of the piercing needle by pressing the holding tip while holding the needle base. The membrane-coated fiber is exposed into the donor solution and fixed in position by the tapered end of the needle base.

The permeation container comprises a special needle holding cap and a solution container with a water jacket. The needle holding cap has 8 holes drilled in a specific shape to fit the tapered end of the needle base. All of the holes are on the same radius, which is the radius of the membrane-coated fiber to the vertical centerline of the container ( $R_c$ ) as shown in Fig.1. The needle holding cap is fitted precisely into the solution container to control the radius ( $R_c$ ). The permeation container sits on a magnetic stirrer, which has a precise speed control and a tachometer display. A magnetic stir bar is



**Fig. 1.** Membrane-Coated Fiber and Experimental Setup 1: Holding tip, 2: Sealing septum, 3: Needle base, 4: Piercing needle, 5: Fiber attachment tubing, 6: Inert fiber, 7: PDMS membrane,  $r$ : Radius of the inert fiber,  $R$ : Radius of the membrane,  $\delta m$ : Thickness of the membrane,  $R_c$ : The hole radius on the cap, 10: Needle holding cap, 12: MCF positioning holes, 20: Solution container, 21: Stirring bar, 22: Water jacket, 26: Donor solution, 30: Magnetic Stirrer, 32: Tachometer, 34: Stirring speed control.

placed in the permeation container to stir the donor solution. The water jacket maintains the solution at constant temperature.

### MCF Desorption Conditions and Reproducibility

A PDMS membrane coated fiber was preconditioned at 280°C for 10 min under a stream of helium. The preconditioned fiber was immersed in a donor solution consisting of the 30 compounds while stirring at 400 rpm for 30 min. The fiber was transferred into a GC injector to desorb the partitioned compounds for quantitative analysis. The injector was set at different temperatures to identify the optimum desorption temperature. At a given temperature, the fiber was held inside the injector for different periods of times to determine the optimum desorption time. The optimum desorption conditions were chosen to guarantee that all of the compounds to be studied were completely desorbed. After the desorption of the partitioned compounds at the optimum desorption conditions, the fiber was re-injected into the injector to detect any membrane carryovers.

One preconditioned MCF was immersed in a 25-ml 100 ng/ml aqueous donor solution under stirring at 400 rpm for 5 min and analyzed under the optimum desorption conditions. The same MCF was reused to repeat the permeation experiments with a fresh donor solution under identical conditions to test the reproducibility of the MCF technique. Six preconditioned MCFs were used to repeat the permeation experiments to check the variability between different fibers.

### MCF Permeation in Static and Stirred Donor Solutions

A given volume of the donor solution was transferred into the permeation container. The donor solution was stirred with the magnetic stirrer at 400 rpm for 30 min to equilibrate the solution temperature to 37°C. The magnetic stirrer then was turned off to perform the static permeation experiments. The preconditioned fibers were placed on the needle holding cap. To start the permeation experiment, the needle holding cap with the fibers was placed quickly into the permeation container to immerse the membrane coating into the donor solution. At a given period of time, one fiber was removed to end its permeation. The permeation time was defined as the time when the membrane was immersed in the donor solution. The permeation amount was obtained by transferring the fiber directly into the GC injector for quantitative analysis.

The procedures to perform the stirred permeation were the same as the static permeation experiments described above, except that the donor solution was constantly stirred throughout the permeation experiments. The stirring speed was set constant (e.g., 400 rpm) to measure the permeation amount at different permeation times to obtain the permeation time profiles.

### GC/MS Analyses

Quantitative and qualitative analyses were performed on an HP 5890 II gas chromatograph coupled with a HP 5970B mass selective detector. An HP 7675 automatic sampler was used to inject 4  $\mu$ l of the calibration standard solution, while the membrane-coated fibers were injected manually. The injector was maintained at 280°C for sample vaporization and thermal desorption. Separation was performed on a 30 m  $\times$  0.25 mm (i.d.)  $\times$  0.25  $\mu$ m (df) Rtx-5MS capillary column (Restek Corp., Bellefonte, PA). The column oven was programmed as follows: held at the initial temperature 100°C for 1 min., ramped at 15 °C/min to 150°C, 1°C/min to 220°C and 3°C/min to 280°C, and held for 5 min. An electronic pressure control was used to maintain a carrier gas flow of 1.00 ml/min helium.

The chemicals permeated into the membrane were qualitatively analyzed in scan-mode. The identification of each compound in the complex mixture was accomplished by using an HP ChemStation software and matching its fingerprint spectra with an HP MS database. For quantitative analysis, the selected ion monitoring (SIM) mode was used, 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 abundance produced by the compound.

### Data Analyses

When the permeation equilibrium is established, the maximum permeation amount ( $n^0$ ) of a given compound can be obtained from its permeation time profile. This equilibrium permeation amount can be used to calculate the partition coefficient ( $K$ ) of the permeant between the silastic membrane and aqueous donor phase:

$$K = \frac{C_{me}}{C_{de}} = \frac{n^0 V_d}{V_m (V_d C_o - n^0)} \quad (1)$$

where  $C_o$  is the initial concentration of the given compound in the donor solution,  $V_d$  is the volume of the donor solution,  $V_m$  is the volume of the membrane,  $C_{me}$  is the equilibrium concentration in the membrane ( $C_{me} = n^\circ/V_m$ ) and  $C_{de}$  is the equilibrium concentration in the donor solution ( $C_{de} = C_o - n^\circ/V_d$ ).

The initial permeation rate was obtained by linear regression of the initial linear section of the permeation amount (ng) vs. time (min) profile. The initial permeation rate (ng/ml) was the slope obtained by the linear regression. Since the initial permeation rate depends on the surface area of the membrane, MCFs with permeation variability less than 10% were used for the initial permeation rate measurements.

## RESULTS

### Optimum Desorption Conditions

Quantitative desorption of the chemicals from the membrane is required in the MCF technique. The main parameters affecting the thermal desorption are the injector temperature, the desorption time, and the flow rate of the carrier gas. While the desorption time was kept at 5 min and the flow rate of the carrier gas was 1.00 ml/min, the maximum desorption amounts of all 30 compounds occurred when the injector temperature reached 280°C. While the injector temperature was 280°C and the flow rate was 1.00 ml/min, the thermal desorption was completed within 30 seconds. To incorporate the thermal desorption and the fiber precondition into one step, the optimum desorption conditions were chosen as 280°C for 5 min under a helium flow of 1.00 ml/min. After desorption at the optimum conditions, the fiber was re-injected into the injector at 280°C for 5 min. No chemical residues were detected in the second injection, where the instrumental detection limits of the 30 compounds were in the range of 0.015–0.03 ng. This indicated the membrane carryover from one experiment to next was negligible under the optimum desorption conditions. Thus, these optimum desorp-

tion conditions were used throughout the experiments and the fibers were reused directly for next permeation experiment without reconditioning.

### Permeation of Chemical Mixtures

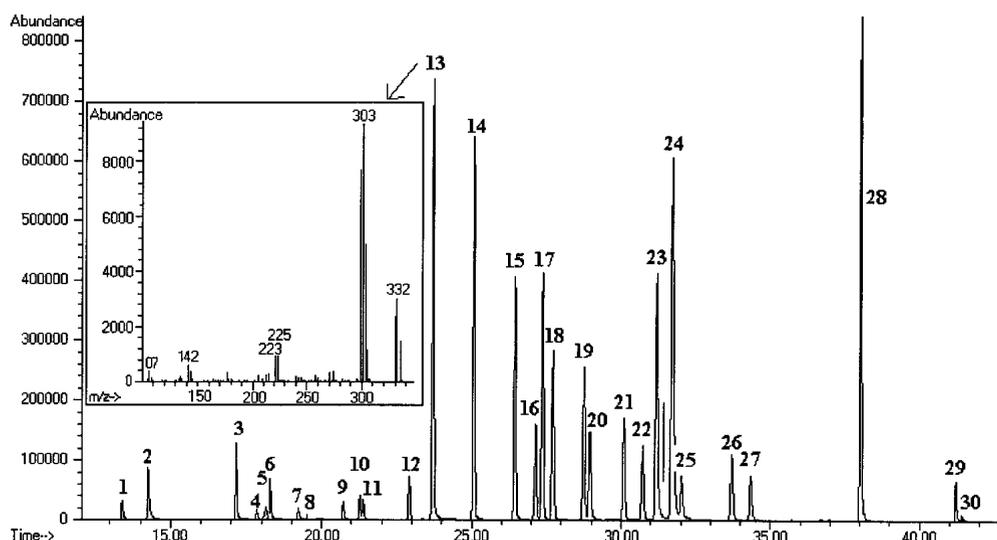
A membrane-coated fiber was immersed in a donor solution consisting of the 30 compounds for 30 min. The compounds partitioned into the membrane were thermally desorbed into the GC injector. A GC/MS spectrum was acquired in scan mode (Fig. 2). A chemical desorbed from the membrane was detected as a peak in the GC/MS spectrum. All of the 30 compounds containing in the solution were observed in the spectrum. Each compound was identified by matching its fingerprint spectra to that in the MS database and quantified by its peak area in the spectra against the calibration standard acquired under the same GC/MS conditions.

### Reproducibility of the MCF Technique

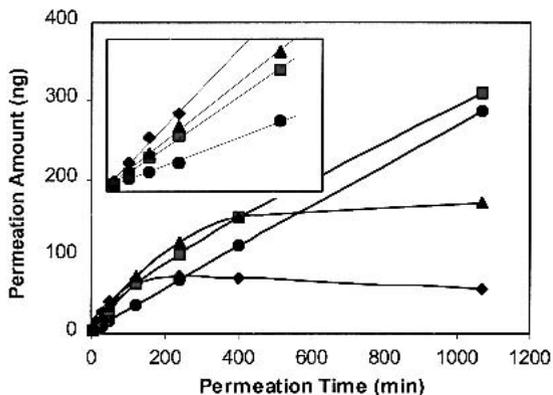
To assess the reproducibility of the MCF technique, a series of permeation experiments under identical experimental conditions were conducted. The average quantities of 12 repeated experiments are listed in Table I. The relative standard errors were less than 10% for all 30 compounds containing in the donor solution. The variability between different fibers was less than 10% ( $n = 6$ ).

### MCF Permeation in Static Donor Solution

The static permeation amount-time profiles for four compounds are shown in Fig. 3. These four compounds were chosen according to their retention time to represent the whole chromatographic spectra. In fact, this type of permeation profiles was typical for all 30 components contained in the donor solution. From Fig. 3, it is seen that a-BHC reaches permeation equilibrium in about 240 min; chlorobenzilate in about 400 min; while heptachlor epoxide and methoxychlor can not reach equilibrium in 1,000 min.



**Fig. 2.** GC/MS Spectra Acquired with the Membrane-Coated Fiber A 100- $\mu$ m membrane coated fiber was immersed in a 150-ml 100-ng/ml donor solution for 30 min, and transferred into the GC injector to desorb the partitioned compounds. The spectrum was acquired in scan mode. The chemicals identified with HP ChemStation software and MS databases were listed in Table I.

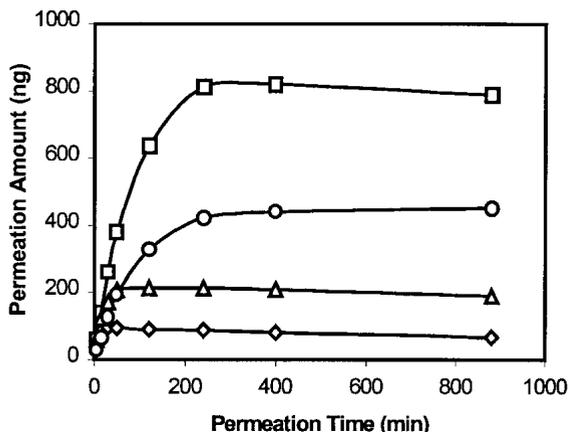


**Fig. 3.** Static Permeation Profiles with Membrane-Coated Fiber The permeation experiments were performed statically with a 100- $\mu$ m PDMS membrane coated fiber immersed in a 150-ml solution consisting of 30 components with an individual concentration of 100 ng/ml; a-BHC ( $\diamond$ ), Heptachlor epoxide ( $\blacksquare$ ), Chlorobenzilate ( $\blacktriangle$ ), and Methoxychlor ( $\bullet$ ).

In Fig. 3, the permeation profile of a-BHC is a representative permeation profile with the membrane-coated fiber, which comprises an initial linear section, a transition section and a flat equilibrium section. The initial linear section reveals that the permeation amount increases linearly with the permeation time, i.e., the permeation rate (the slope of the initial linear section) is constant over the initial period. The initial permeation rates of the four compounds are depicted in the inserted section in Fig.3. The initial permeation rates determined for all 30 compounds in a static donor solution are listed in Table I.

**MCF Permeation in Stirred Donor Solution**

When the donor solution was stirred, the permeation rate was significantly increased and the equilibration time was considerably reduced. Fig. 4 shows the permeation profiles for the same four compounds, while the donor solution was



**Fig. 4.** Stirred Permeation Profiles with Membrane-Coated Fiber The permeation profiles were obtained with a 100- $\mu$ m PDM membrane coated fiber immersed in a 150-ml donor solution consisting of 30 components with an individual concentration of 100 ng/ml stirred constantly at 400 rpm throughout the permeation experiments; a-BHC ( $\diamond$ ), Heptachlor epoxide ( $\square$ ), Chlorobenzilate ( $\Delta$ ), and Methoxychlor ( $\circ$ ).

stirred at 400 rpm during the permeation experiments. It is seen that a-BHC reaches equilibrium within 5 min, considerably faster than the static permeation. Chlorobenzilate reaches equilibrium within 10 min; heptachlor epoxide and methoxychlor reach equilibrium in about 400 min.

The initial permeation rates in stirred donor solution can be obtained from the initial linear sections. Fig. 5 shows the initial linear sections of dieldrin, p,p-DDE, endrin, p,p-DDD and p,p-DDT. Chlorobenzilate and endosulfan sulfate show curved lines in this period because their permeation profiles are in the transition section under these experimental conditions. The observation time should be reduced to obtain the linear section for chlorobenzilate or endosulfan sulfate. The initial permeation rates of all 30 compounds in stirred donor solution are listed in Table I.

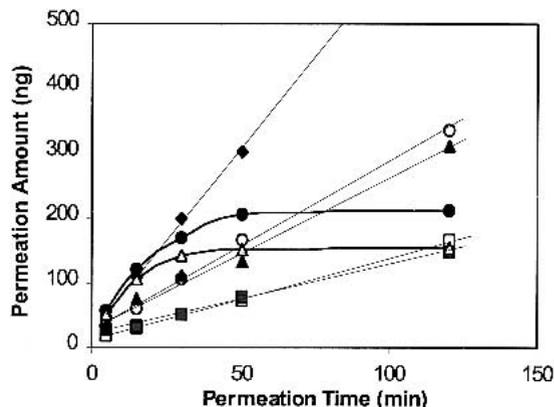
**Partition Coefficients Measured by the MCF Technique**

The partition coefficients ( $K$ ) calculated with Eq. (1) for all 30 compounds are listed in Table I. The octanol/water partition coefficients ( $K_{o/w}$ ) from published databases for the 30 compounds are also listed in Table I (12,13). The correlation between  $\log K$  and  $\log K_{o/w}$  is depicted in Fig. 6. It is observed that the partition coefficients ( $\log K$ ) measured by the MCF technique are correlated well to the published octanol/water partition coefficients over a range of  $\log K_{o/w}$  from 2 to 6. When  $\log K_{o/w} < 4$ , the measured  $\log K$  values are close to the published  $\log K_{o/w}$  values. However, when  $\log K_{o/w} > 4$ , larger discrepancies are noted.

**DISCUSSIONS**

**Comparison of the MCF Technique with Traditional Diffusion Cells**

Artificial membranes have been used widely in *in vitro* percutaneous absorption studies (14–18). It is used as a flat membrane in traditional diffusion cells (6,16). In the MCF technique, the artificial membrane is coated on a fiber. For percutaneous permeation experiments, the membrane-coated



**Fig. 5.** Stirred Initial Permeation with Membrane-Coated Fiber The permeation experiments were performed with a 100- $\mu$ m PDM membrane coated fiber immersed in a 150-ml donor solution consisting of 30 components with an individual concentration of 100 ng/ml stirred constantly at 400 rpm throughout the permeation experiments; Dieldrin ( $\diamond$ ), p,p-DDE ( $\blacksquare$ ), Endrin ( $\blacktriangle$ ), Chlorobenzilate ( $\bullet$ ), p,p-DDD ( $\circ$ ), Endosulfan sulfate ( $\Delta$ ), and p,p-DDT ( $\square$ ).

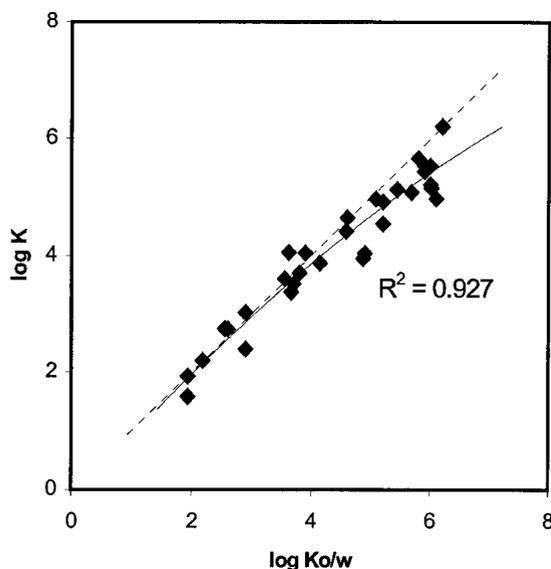


Fig. 6. Estimated  $\log K$  plotted against reported  $\log K_{o/w}$ .

fiber is immersed in the donor solution to partition the permeants from the donor phase into the membrane (Fig. 1). At a given permeation time, the membrane-coated fiber is removed from the donor solution and transferred directly into the GC injector to evaporate the partitioned permeants into the capillary column for quantitative and qualitative analyses.

When the membrane-coated fiber is compared with the traditional diffusion cells, it is evident that the membrane-coated fiber represents only a half compartment of the traditional diffusion cell (i.e., only the permeation processes from the donor phase into the membrane phase are involved; no penetration from the membrane to the receptor phase is involved). Thus, the permeation processes and the penetration processes can be studied separately. In this study the experiment results reported are restricted to the permeation processes. If the membrane-coated fiber is pre-loaded with given amounts of chemicals, then inserted into a receptor solution, the penetration processes from the membrane eliminating into the receptor phase could be studied.

In the MCF technique, the membrane must meet three requirements: (a) having similar permeation properties as skin (17); (b) having high thermal stability for desorption of the compounds into a GC injector without damage to the membrane itself and (c) quantitative desorption of the partitioned compounds. Several materials can be used for this purpose, such as, polydimethylsiloxane (PDMS), polyacrylate and PDMS-Carbonsil (14–18). Silastic (PDMS) membrane was used in this work to prepare the membrane-coated fiber, since this membrane has been thoroughly evaluated for percutaneous absorption in traditional diffusion experiments (14–17). The PDMS membrane also has high thermal stability as required by the membrane coated fiber.

One membrane-coated fiber can be reused and undergoes hundred degrees of cooling-heating cycles. One obvious concern is if the membrane and its permeability are changed during these cycles. This can be assessed by observing the permeability change when one MCF is reused to repeat the permeation experiments under identical experimental conditions. Table I shows a series of 12 repeated permeation experiments with one MCF under identical experimental con-

ditions. The relative standard errors are less than 10% for all 30 compounds containing in the donor solution. This demonstrates that the MCF technique has high reproducibility, and also indicates that the membrane and its permeability have not changed during the cooling-heating cycles.

#### Advantages of the MCF Technique

One potential advantage of the MCF technique is its capability to study the percutaneous permeation of complex mixtures. Fig. 2 shows a GC/MS spectrum acquired with the MCF technique. All of the 30 compounds containing in the donor solution can be studied simultaneously. The chemicals partitioned into the membrane were desorbed in the injector and detected as peaks in the GC/MS spectra. Each compound was identified by its fingerprint spectra matching its standard spectra in MS database and quantified by its peak area in the spectra against the calibration standard acquired under the same GC/MS conditions. This capability of the MCF technique is particularly useful in studying chemical mixtures and the synergistic effect of multiple components and their combinations on percutaneous permeation. It is known that humans are more likely to be exposed to chemical mixtures than to a single chemical under most environmental and occupational conditions. Most of the topical medications and cosmetics are chemical mixtures (8–11). This technique is comparable with the recent developed cassette dosing technique, in which several drugs are administered simultaneously to a single animal for rapid drug discovery screening (19).

In the experimental procedures, MCFs were not washed before injection into the GC injector since the viscosity and concentration of the aqueous donor solutions were low (e.g., <0.89 centipoise and <100 ng/ml, respectively). Washing the fiber before injection did not alter the detected mass (results not shown). It was observed that the water surface tension keeps the solution off the membrane when the fiber is removed smoothly from the donor solution. However, fiber washing is necessary for higher concentration or higher viscous donor solutions. Otherwise, the fiber membrane and the GC column could be contaminated. With proper fiber cleaning before GC injection, viscous matrices other than water (cream or ointment formulations) could be studied with this technique by modifying the apparatus in Fig. 1 to reduce the sample volume and changing the agitation method.

The MCF technique offers several other advantages over the traditional methods: (a) It characterizes only one half of the compartments of the traditional diffusion cells, which allows for the permeation processes and the penetration processes to be studied separately. (b) The experimental conditions are easily manipulated to study the kinetic processes and the effects of boundary layer. (c) The composition of the donor phase can be changed to study the vehicle effect, composition effect. (c) Expensive radiolabeled chemicals are not required. (e) MCF is directly transferred into the injector for quantitative analysis; no complicated sample handling and treatments are required. This technique is simple, rapid and reliable in quantitative analysis. These features are useful for rapid screening the percutaneous toxicity of complex mixtures in industrial and environmental matrixes, and screening for better formulations for medicines, pharmaceuticals and cosmetics.

It should be understood that the approach of the MCF

technique to study the percutaneous permeation is not to mimic the *in vitro* and *in vivo* experiments, but to measure some of the physiochemical parameters of the permeation system normally obtained in the *in vitro* and *in vivo* experiments, and to measure the kinetic parameters that usually can not be obtained with the traditional diffusion experiments. The MCF technique can be used directly to measure the relative permeation parameters in the formulation improvements for cosmetic and pharmaceutical industries. However, the physiochemical parameters measured by the MCF technique only represent the artificial membrane system, calibration is necessary if the results are used to predict the *in vitro* and *in vivo* physiochemical parameters in skin. This limitation is shared by all surrogate membrane approaches. The calibration method could be similar to the calibration of  $\log K_{o/w}$  measured by the HPLC method, where a series of standard compounds with known  $\log K_{o/w}$  values was used to calibrate the measured values (20).

### Equilibration Times

The equilibration time of a given compound can be obtained from its permeation time profile. The times for different compounds to reach equilibrium are considerably different (Fig. 3). This equilibration time could be a characteristic parameter to describe the permeability differences between different compounds. For less hydrophobic compounds, it takes minutes or hours to reach equilibrium. For highly hydrophobic compounds (e.g.,  $\log K_{o/w} > 4$ ), it might take days or weeks to reach equilibrium in static solution. This observation indicates that the partition equilibrium of hydrophobic compounds between the donor phase and the membrane may not be reached in the traditional diffusion experiments.

When the donor solution was stirred, the equilibration time was considerably reduced (Fig. 3 and Fig. 4). For example, the equilibration times of a-BHC and chlorobenzilate in static donor solution are about 40 times longer than in the stirred donor solution. This phenomenon has not been observed with traditional diffusion cells. In this paper, it is suggested that stirring the donor solution increased the permeation rate and consequently reduced the equilibration times.

### Initial Permeation Rates

The permeation rates for all compounds are constant in the initial permeation timeframe. The initial permeation rate measured by the MCF technique is the same initial permeation rate existing in the traditional diffusion cells. However, this initial permeation rate can not be observed with the traditional diffusion cells because traditional diffusion experiments measure the penetration flux, which is the transport process after the initial permeation time. The initial permeation rate could be an important parameter to reflect how fast the chemical could permeate into the membrane.

When the donor solution was stirred, the permeation rates were significantly increased. From Table I, it is noticed that the permeation rates increased several folds when the donor solution was stirred. This high initial permeation rate in the stirred donor solution has not been well evaluated in the traditional diffusion experiments, where the permeation flux is measured after the initial permeation timeframe. In this paper, it is suggested that stirring the donor solution

reduced the thickness of the boundary layer in the vicinity of the membrane and consequently increased the initial permeation rate (17,21–23). From the permeation time profile (Fig. 3 to Fig. 5), it is observed that the initial permeation rate for a given compound is the maximum permeation rate of the compound into the membrane. This initial permeation rate is correlated to the uptake rate of the compound in the traditional diffusion experiments (23). This initial permeation rate has toxicological significance as it is the limiting step in determining the utility of many decontamination procedures.

### Correlation between $\log K_{o/w}$ and $\log K$

The partition coefficients measured by the MCF technique ( $K$ ) and the octanol/water partition coefficients ( $K_{o/w}$ ) describe the same hydrophobicity of a given compound. For instance, a more hydrophobic compound has a higher  $K_{o/w}$  value and consequently a higher concentration in the octanol phase. In the MCF technique, the same mechanism occurs (i.e., a more hydrophobic compound will have higher concentration in the membrane and consequently gives a higher  $K$  value). Thus, a correlation between  $\log K_{o/w}$  and  $\log K$  exists, with which the  $K_{o/w}$  value could be predicted from the  $K$  value measured by the MCF technique.

It is observed that the partition coefficients ( $\log K$ ) measured by the MCF technique are well correlated to the published octanol/water coefficients ( $R^2 = 0.93$ ) over a range of  $\log K_{o/w}$  from 2 to 6. When  $\log K_{o/w} < 4$ , the measured  $\log K$  values are close to the published  $\log K_{o/w}$  values. When  $\log K_{o/w} > 4$ , however, larger discrepancies are noted (Fig. 6). This phenomenon has been realized in analytical chemistry, when solid-phase microextraction method was used to measure the partition coefficients (24–26). For application purpose, these discrepancies could be calibrated with a series of standard compounds with known  $\log K_{o/w}$  values (20).

### CONCLUSIONS

The MCF technique can be used to study percutaneous permeation, in which the MCF membrane and its permeability are not changed during repeated uses, and no carryover exists under the optimum desorption conditions. The percutaneous permeation of a complex mixture containing as many as 30 compounds can be studied. No expensive radiolabeled chemicals are required. Each compound permeated into the membrane can be identified and quantified.

The MCF technique can be used to measure some kinetic parameters that usually can not be obtained with the traditional diffusion experiments. The initial permeation rate and equilibrium time can be obtained for each compound, which could serve as characteristic parameters regarding the percutaneous permeability of the compound. The partition coefficients measured by the MCF technique are well correlated with the reported octanol/water partition coefficients.

Future research should be directed toward defining the limitation inherent to using artificial membranes to model human stratum corneum. However, many steps in the dermal absorption processes are dependent on relative rate and extents of formulation interactions compared to membrane absorption processes, which can be studied using the MCF technique.

## 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.

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