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An experimentally based approach for predicting skin permeability of chemicals and drugs using a membrane-coated fiber array

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Abstract

A membrane-coated fiber (MCF) array approach is proposed for predicting the percutaneous absorption of chemicals and drugs from chemical or biological mixtures. Multiple MCFs were used to determine the partition coefficients of compounds ($\log K_{MCF}$). We hypothesized that one MCF will characterize one pattern of molecular interactions and therefore the skin absorption process can be simulated by a multiple MCF array having diverse patterns of molecular interactions. Three MCFs, polydimethylsiloxane (PDMS), polyacrylate (PA) and CarboWax (Wax), were used to determine the $\log K_{MCF}$ values for a set of calibration compounds. The skin permeability log(kp) of the compounds was measured by diffusion experiments using porcine skin. The feasibility of the MCF array approach for predicting skin permeability was demonstrated with the three MCFs. A mathematical model was established by multiple linear regression analysis of the log(kp) and $\log K_{MCF}$ data set: $\log(kp) = -2.34 - 0.124 \log K_{pdms} + 1.91 \log K_{pa} - 1.17 \log K_{wax}$ ($n = 25$, $R^2 = 0.93$). The MCF array approach is an alternative animal model for skin permeability measurement. It is an experimentally based, high throughput approach that provides high prediction confidence and does not require literature data nor molecular structure information in contrast to the existing predictive models.

Keywords: Skin permeability; Predictive model; Membrane-coated fiber; Distribution coefficients; Percutaneous absorption

Introduction

Assessment of skin absorption of chemicals and drugs is important to many industrial, scientific and regulatory fields, particularly in the toxicity assessment of topical drugs, pharmaceuticals and cosmetics, the risk assessment of environmental or occupational hazards, and the development of transdermal drug delivery devices and dermatological formulations. Great efforts have been made to develop predictive models for quantitative assessment of skin absorption from physicochemical parameters. However, acquisition of the physicochemical parameters by in vivo or in vitro experimental methods has long been the bottleneck in development of prediction models (Yu and Adedoyin, 2003; Moss et al., 2002). The widely used predictive model for skin permeability has an R^2 value of 0.66 due to the large variations in the experimental data compiled from multiple literature sources (USEPA, 2004; Potts and Guy, 1992). While it is frequently difficult to assess skin absorption of individual chemicals, it is more challenging to quantitatively assess the skin absorption from chemical mixtures (Pohl et al., 1997). There are over 75,000 existing chemicals on the Toxic Substances Control Act inventory (USEPA, 1990). Each year an additional 2000 chemicals are added (De Rosa et al., 2004). It is impossible to use the existing methods to study thousands of chemicals and millions of their combinations (Cassee et al., 1998; Groton et al., 2001). In transdermal drug delivery and formulation optimization studies, methods are required for rapid determination of potential formulation effects on dermal absorption of drugs or cosmetics.

Skin is the largest organ protecting the body from harmful agents and receiving one third of the blood circulating throughout the body (Singh and Singh, 1993). Decades of research demonstrate that the stratum corneum (the outermost layer of the skin) is the primary barrier to exogenous compounds; and passive diffusion of the compounds through the lipid layer is the dominant transport mechanism (Roberts et al., 1999; Wester

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and Maibach, 1983). Therefore, attempts have been made to use synthetic membranes to mimic this passive diffusion layer. Polydimethylsiloxane membrane is the most widely used polymer membrane due to its nonporous, lipophilic nature and ready availability (Moss et al., 2002; Flynn and Yalkowsky, 1972). Efforts were also made to modify the property of the synthetic membranes to mimic the heterogeneous structure of the stratum corneum (Feldstein et al., 1998). Artificial skin was also developed to produce membranes with similar biological structures as skin, but their barrier function cannot match the performance of human skin (Asbill et al., 2000).

Addressing the challenges in mimicking the biological structures or barrier functions of the stratum corneum, efforts were made to understand the transport mechanisms and molecular interactions that govern the percutaneous absorption processes; and several types of molecular interactions were identified to be the primary factors in skin absorption: lipophilic, hydrogen bonding and π^*–electron interactions (Moss et al., 2002). We have developed a membrane-coated fiber (MCF) technique for measuring the relative strengths of the molecular interactions (the partition coefficients) with different membrane materials (Xia et al., 2003). The molecular interactions involved in skin absorption can be simulated by multiple MCF membranes; for example, polydimethylsiloxane (PDMS) for lipophilic, CarboWax (Wax) for hydrogen bonding and polyacrylate (PA) for π^*–electron interactions. In the MCF technique, a polymer membrane coated onto a fiber is used as the absorption membrane to determine the partition coefficients of chemicals from any liquid vehicle (Xia et al., 2003). The MCF technique integrates the membrane absorption and quantitative analysis into one step and fully utilizes the separation power of the automatic chromatographic instruments (GC or HPLC). It completely eliminates the emulsion problem and the other error sources associated with sample treatment and handling in liquid–liquid systems, such as in measuring $\log K_{o/w}$ values. These features allow the MCF technique to have greater sensitivity, accuracy and high throughput in measuring the partition coefficients of chemicals.

In this paper, an MCF array approach is developed to predict skin absorption of molecules from chemical mixtures or drug formulations, which is based on the quantitative measurement of the relative strength of the molecular interactions of the molecules with the membranes. We hypothesized that one MCF will characterize one pattern of molecular interactions and therefore the skin absorption process can be simulated by a multiple MCF array having diverse patterns of molecular interactions. A set of calibration compounds is used to detect the relative molecular interaction strengths of chemicals with the stratum corneum or the MCF membranes, which provide the linkage between the skin permeability, log(kp) and MCF partition coefficients ($\log K_{MCF}$). The calibration compounds are selected to cover a wide range of physicochemical diversities that will eventually determine the application range of the developed predictive model (Fuguet et al., 2002).

When an MCF is exposed into a chemical mixture solution, the chemicals will partition into the membrane. Partition equilibrium will be established for all of the chemicals in the solution according to their relative strengths of molecular interactions with the membrane materials. If the partition coefficients of the chemicals ($\log K_{MCF}$) are measured by using sufficient number (n) of diverse MCFs, the skin permeability, log(kp), can be obtained:

$$\log(\mathrm{kp}) = c + a_1 \log K_{MCF1} + a_2 \log K_{MCF2} + a_3 \log K_{MCF3} + \cdots + a_n \log K_{MCFn} \quad (1)$$

where c is a regression constant and a_1, a_2, a_3 ... a_n are regression coefficients. In this study, the feasibility of the MCF array approach was demonstrated by using three MCFs (PDMS, PA and Wax) and a set of 32 calibration compounds.

Materials and methods

Chemicals and materials. Acetone (GC grade) and methanol (HPLC grade) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Deionized water was prepared from a Picotech Water System (Research Triangle Park, NC). A set of 32 calibration compounds (Table 1) having purity better than 98% were purchased from Sigma-Aldrich. Solid-phase microextraction (SPME) devices and 100-μm polydimethylsiloxane (PDMS), 85-μm polyacrylate (PA) and 50-μm carbowax/template (Wax) membrane-coated fibers were purchased from Supelco (Bellfonte, PA, USA).

Individual stock solutions with a concentration of 10.0 mg/mL in methanol were prepared for each of the neat compounds. A standard mixture in acetone containing the 32 compounds with a concentration of 100 μg/mL for each component was prepared from the individual stock solutions. A series of standard solutions in acetone were prepared from the standard mixture to be used as external calibration standards for GC/MS analysis. The calibration compounds are volatile and some of them are toxic. All of the solution preparation processes were conducted in a fume hood with gloves and goggles.

Determination of the partition coefficients. The detail procedures for measuring partition coefficients were described elsewhere (Xia et al., 2003). The partition coefficients of the calibration compounds were determined with three MCFs (100-μm PDMS, 85-μm PA and 50-μm Wax). The 100-μm PDMS fibers were conditioned at 250 °C for 30 min and 85-μm PA fiber at 300 °C for 2 h as recommended by the manufacture. The 50-μm Wax fibers were preconditioned at 220 °C for 30 min. A Combi PAL automatic sampler (CTC Analytics, Switzerland) was used to perform the partitioning experiments. The concentrations of the calibration compounds in the aqueous working solution were optimized for quantitative analysis by subdividing them into four groups with a composition of 10 ng/mL of Group 1, 100 ng/mL of Group 2, 1000 ng/mL of Group 3 and 2000 ng/mL of Group 4 compounds (Table 1). A glass vial containing 8.0 mL of the working solution was transferred into an incubator and shaken at 500 rpm for 5 min to equilibrate the sample temperature to 37 °C. A preconditioned MCF was immersed into the working solution to start the absorption experiment under constant stirring at 400 rpm and 37 °C. After a given period of time, the fiber was removed from the vial and transferred into the injector of a gas chromatograph (GC) for quantitative analysis. From the absorption profiles (absorption amount versus time), it was known that the absorption equilibrium was achieved within 2 h for all of the calibration compounds under the given experimental conditions.

Flow-through diffusion cell experiments. The skin permeability of the calibration compounds was measured by using a flow-through diffusion cell system (Bronaugh and Stewart, 1985). Porcine skin was obtained from the dorsal area of weanling female Yorkshire pigs. The skin was dermatomed to a thickness of 350 μm with a Padgett Dermatome (Kansas City, MO, USA). Each circular skin section was punched out and placed into a two-compartment Teflon flow-through diffusion cell. The skin membranes were perfused using Krebs–Ringer bicarbonate buffer spiked with dextrose and bovine serum albumin (4.5%). The temperature of the perfusate and flow-through cells was maintained at 37 °C using a Brinkman circulator (Westbury, NY, USA). The pH was maintained between 7.3 and 7.5. The flow rate of the receptor solution was 4.0 mL/h and sampled every

Table 1
Experimental measured skin permeability and partition coefficients of diverse chemicals

#	Compound name [a]	Exp. log(kp)		$\log K_{pdms/w}$ (n=12)		$\log K_{pa/w}$ (n=8)		$\log K_{wax/w}$ (n=10)		log(kp) [b]
		cm/h	CV	Average	CV	Average	CV	Average	CV	Cm/h
1	Toluene[2]	−1.215	3.69	2.590	0.56	2.250	2.55	2.306	1.35	−1.51
2	Chlorobenzene[1]	−0.998	4.52	2.654	0.48	2.584	2.58	2.687	1.89	−1.56
3	Ethylbenzene[1]	−1.269	5.25	3.017	0.61	2.531	2.74	2.775	1.13	−1.32
4	*p*-Xylene[1]	−1.043	5.34	3.024	0.60	2.576	2.7	2.820	1.13	−1.32
5	Bromobenzene[1]	−0.801	5.06	2.743	0.45	2.767	3.44	2.916	0.58	−1.71
6	Propylbenzene[1]	−1.135	4.84	3.448	0.73	2.781	2.33	3.168	1.22	−1.04
7	4-Chlorotoluene[1]	−1.036	9.69	3.105	0.49	2.764	1.86	3.128	0.82	−1.31
8	Phenol[3]	−1.546	3.30	0.124	7.39	1.641	4.92	2.052	0.39	−2.36
9	Benzonitrile[2]	−1.064	2.91	1.253	0.65	1.823	3.36	1.710	0.48	−2.35
10	4-Florophenol[3]	−1.724	2.91	0.107	8.69	1.649	5.07	2.135	0.31	−2.26
11	Benzyl alcohol[4]	–	–	–	–	1.042	3.65	1.278	0.98	−2.68
12	Iodobenzene[1]	−0.765	5.59	2.974	0.37	3.154	2.64	3.342	0.50	−1.80
13	Phenyl acetate[3]	–	–	0.878	3.37	0.335	3.9	–	–	−2.58
14	Acetophenone[3]	−1.134	3.78	1.352	0.58	1.704	3.87	1.727	0.33	−2.43
15	*m*-Cresol[3]	−1.622	2.81	0.352	3.44	1.821	4.98	2.208	0.38	−2.11
16	Nitrobenzene[2]	−0.849	4.43	1.507	0.48	2.163	3.9	2.166	0.36	−2.27
17	Methyl benzoate[2]	–	–	1.882	0.32	2.015	4.06	1.561	1.73	−2.16
18	4-Chloroanisole[2]	−0.733	4.66	2.646	0.25	2.959	2.7	3.077	0.25	−1.76
19	Phenethyl alcohol[4]	−1.915	2.31	0.441	4.11	1.179	4.29	1.472	0.34	−2.59
20	3-Methyl benzyl alcohol[4]	−1.781	2.63	0.364	8.15	1.448	4.52	1.698	0.29	−2.43
21	4-Ethylphenol[3]	−1.342	3.92	0.887	0.75	2.224	4.24	2.685	0.20	−1.78
22	3,5-Dimethylphenol[3]	−1.483	3.90	0.850	0.71	2.135	4.13	2.643	0.31	−1.93
23	Ethylbenzoate[2]	–	–	2.400	0.19	2.206	3.08	1.887	1.70	−1.90
24	Methyl 2-methyl benzoate[2]	–	–	2.410	0.19	2.536	4.74	2.729	0.23	−1.83
25	Naphthalene[1]	−0.642	7.49	3.089	0.17	3.332	3.8	3.670	0.34	−1.34
26	3-Chlorophenol[3]	–	–	0.517	3.25	2.291	5.92	2.886	3.19	−1.87
27	4-Chloroaniline[3]	−0.983	4.38	1.347	0.93	2.370	6.81	2.562	0.35	−2.31
28	4-Nitrotoluene[2]	−0.747	5.81	1.973	0.25	2.581	3.27	2.737	0.24	−2.00
29	4-Chloroacetophenone[2]	−0.953	4.62	2.034	0.19	2.399	4.3	2.564	0.20	−2.13
30	3-Bromophenol[3]	−1.350	4.15	0.640	2.72	–	–	3.164	0.24	−2.03
31	1-Methylnaphthalene[1]	−0.829	6.07	3.512	0.18	3.631	4.44	4.308	0.68	−1.04
32	Biphenyl[1]	−0.925	7.81	3.674	0.19	3.885	1.87	4.626	1.28	−1.04

[a] The compounds were listed in a chromatographic order (Fig. 1). The superscripted number at the end of the compound name indicates the subgroup into which this compound was placed. The subgroups were made to optimize for quantitative analysis. The blanks (–) in the table indicated the values were not obtained for the reasons given in the results section.

[b] Skin permeability was estimated from a refined Potts and Guy model in USEPA Supplemental Guidance for Dermal Risk Assessment (USEPA, 2004).

30 min for the first 4 h and every 1 h for the rest of the 8 h diffusion experiments. The concentrations of the calibration compounds in the collected samples were analyzed by a headspace/SPME and GC/MS method.

Headspace/SPME GC/MS analyses. A Combi PAL automatic sampler was used to perform the headspace/SPME analysis. The sample vials containing 1.00 mL of the receptor solutions sampled at different time-points were transferred into an incubator and shaken at 500 rpm for 10 min to equilibrate the sample temperature to 60 °C. A preconditioned PDMS/DVB fiber was inserted into the headspace of the sample vial. The chemicals in the gas phase equilibrating with those in the sample phase were absorbed into the stationary phase of the fiber. The headspace absorption was held statically at 60 °C for 20 min; then the fiber was removed from the sample vial and injected into the injector of GC/MS for quantitative analysis. The quantitative analysis was calibrated with a set of external calibration solutions. The external calibration solutions were prepared by stepwise dilution of the dosing solutions with the receptor solution, which ensured that the matrix of the calibration solutions was similar to those of the collected receptor solutions in headspace/SPME analysis.

Quantitative and qualitative analyses of the chemicals were performed with a Varian GC/MS 4000 equipped with ion trap mass selective detector. A Combi PAL automatic sampler was used for liquid injection, fiber absorption and headspace experiments. The injection port was maintained at 280 °C for using PDMS and PA fibers, 250 °C for Wax fibers and 270 °C for PDMS/DVB fibers. These temperatures were selected for optimal thermal desorption and lifetime of the fibers. 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 40 °C and held for 1 min, ramped at 20 °C/min to 60 °C and 3 °C/min to 97 °C, held at 97 °C for 3.5 min, then ramped at 20 °C/min to 200 °C and 40 °C/min to 250 °C and finally held at 250 °C for 5 min. An electronic pressure control was used to maintain a carrier gas flow of 1.00 mL/min helium.

Data analysis. The partition coefficient of a calibration compound in a given absorption system ($\log K_{MCF}$) was calculated from the equilibrium absorption amount ($n°$) by the definition of the partition coefficient (Xia et al., 2003):

$$K_{MCF} = \frac{C_{pe}}{C_{me}} = \frac{n° V_m}{V_p (V_m C_o - n°)} \quad (2)$$

where C_o is the initial concentration of the compound in the working solution; V_m is the volume of the working solution; V_p is the volume of the MCF membrane (V_p=0.612 μL for 100-μm PDMS, 0.520 μL for 85-μm PA and 0.330 μL for 50-μm Wax); C_{pe} is the equilibrium concentration in the membrane ($C_{pe}=n°/V_p$) and C_{me} is the equilibrium concentration in the working solution ($C_{me}=C_o-n°/V_m$).

The permeability coefficient of a chemical through the skin membrane is calculated via the following equation;

$$k_p = \frac{J_{ss}}{AC_d} \quad (3)$$

where J_{ss} is the steady-state flux (μg/h), A is the dose area (0.64 cm^2) and C_d is the concentration of the chemical in the donor solution (μg/cm^3). The resulting unit for permeability (kp) is cm/h. The steady-state flux was the slope of the accumulation absorption amounts at different time-points (Addicks et al., 1987).

The compounds having a complete set of log(kp) and logK_{MCF} values were used for statistical analysis (Table 1). The multiple linear regression analysis was performed by using SAS Analyst from SAS Institute Inc (Cary, NC, USA).

Results

MCF absorption from aqueous mixtures

Fig. 1 shows a GC/MS spectrum acquired in scan mode using a PDMS fiber. All of the 32 compounds in the chemical mixture were detected in the spectrum and identified by matching the fingerprint spectra (e.g., 1A in Fig. 1) to that in the MS database. The quantity of a given compound partitioned into the membrane was determined by its peak area against the calibration standard under the same GC/MS conditions.

Partition coefficients by the MCF technique

The MCF technique was used to determine the equilibrium absorption amounts ($n°$) of the chemicals in the membrane. The partition coefficient for a given chemical in a specific MCF was obtained from $n°$ and the initial concentration (C_o) (Eq. (2)). Three sets of partition coefficients were measured with three MCFs (PDMS, PA and Wax) for the 32 calibration compounds (Table 1). The coefficient of variations for multiple measurements was less than 8.69% for PDMS ($n=12$), 6.81% for PA ($n=8$) and 3.19% for Wax ($n=10$). The partition coefficient of benzyl alcohol was not measured due to the extremely low absorption amount by the PDMS MCF under the experimental conditions. The absorption amounts of 3-bromophenol and phenyl acetate were too low to be measured accurately by using PA and Wax MCFs, respectively. Therefore, their corresponding partition coefficients were not obtained.

Skin permeability by diffusion experiments

The permeability coefficients obtained for the calibration compounds were listed in Table 1. The receptor solution was formulated to mimic the microvascular circulation of the skin, composed of 4.5% bovine serum albumin in a Krebs–Ringer bicarbonate buffer with dextrose (0.12%). The quantitative analysis of phenyl acetate, 3-chlorophenol and benzyl alcohol was interfered by the impurities of the albumin-containing media. The benzoate compounds (methyl, ethyl and methyl 2-methyl) were metabolized under the experimental conditions. The log(kp) values were not obtained for these compounds. The initial 32 compounds were reduced to 25 that have a complete set of data for permeability and partition coefficients. This data set was used for the following regression analysis.

Correlation of log(kp) with logK_{MCF} combinations

The permeability coefficients of the calibration compounds were correlated with the partition coefficients of the compounds measured with individual MCFs (Fig. 2). The correlation coefficients (R^2) of the experimental log(kp) with the log$K_{pdms/w}$ and log$K_{wax/w}$ were 0.56 and 0.39, respectively. PA fiber provided a better correlation with an R^2 of 0.61. These results revealed that individual MCFs have limited performance in the skin absorption prediction.

Fig. 3 depicts the correlations of the experimental log(kp) values with the predicted log(kp) values by two MCF combinations and the corresponding partial regression plots. The correlation with the log(kp) values predicted by PDMS and Wax fibers yielded an R^2 value of 0.56, indicating that the contribution of the Wax fiber was insignificant in the PDMS and Wax combination. The significance level of the Wax fiber was under 0.05 in the stepwise regression analysis. The correlation with the log(kp) predicted by Wax and PA fibers yielded an R^2 value of 0.90, which was significantly better than the individual

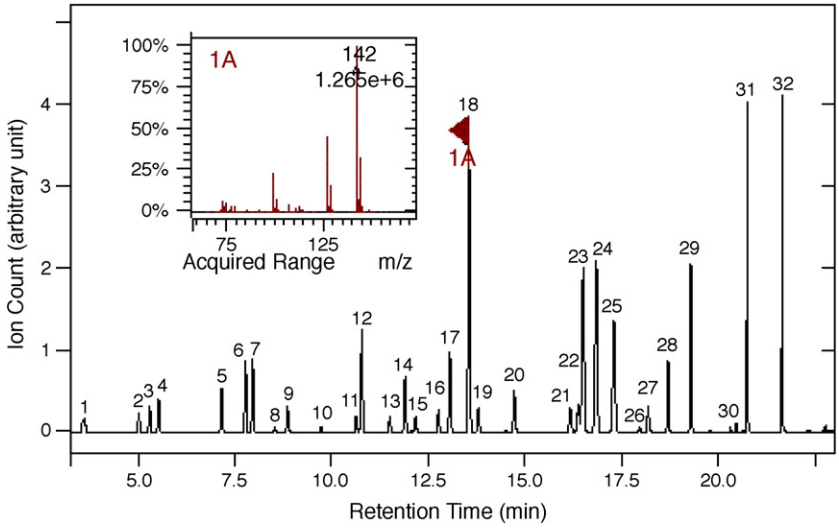

Fig. 1. GC/MS spectra acquired with a 100-μm PDMS MCF. A 100-μm PDMS MCF was immersed in 8-mL working solution containing 32 compounds for 30 min, and then the fiber was directly injected into the injection port. The spectrum was acquired in scan mode; the compounds were identified with Varian WorkStation software and MS database and listed in Table 1.

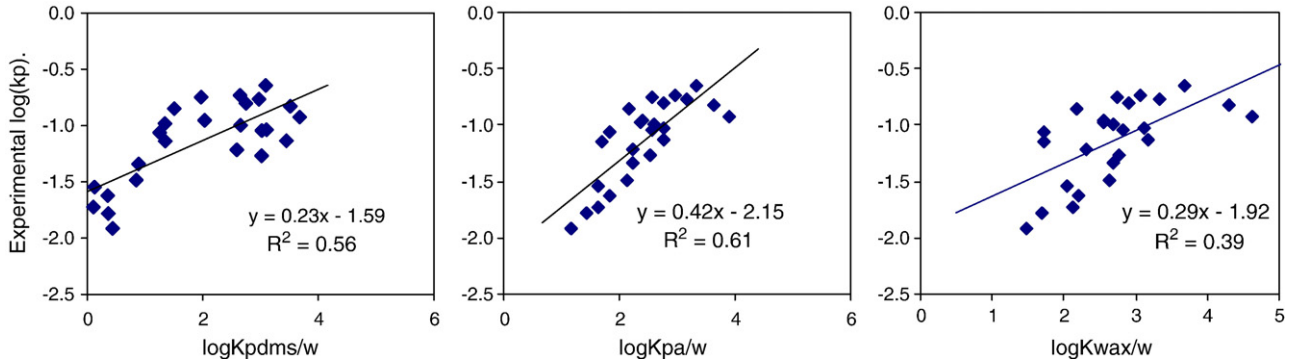

Fig. 2. Correlation of skin permeability with partition coefficients by individual MCFs. The skin permeability log(kp) of the calibration compounds was measured by traditional diffusion experiments. The partition coefficients of the compounds in aqueous solutions were determined with three MCFs (PDMS, PA and Wax).

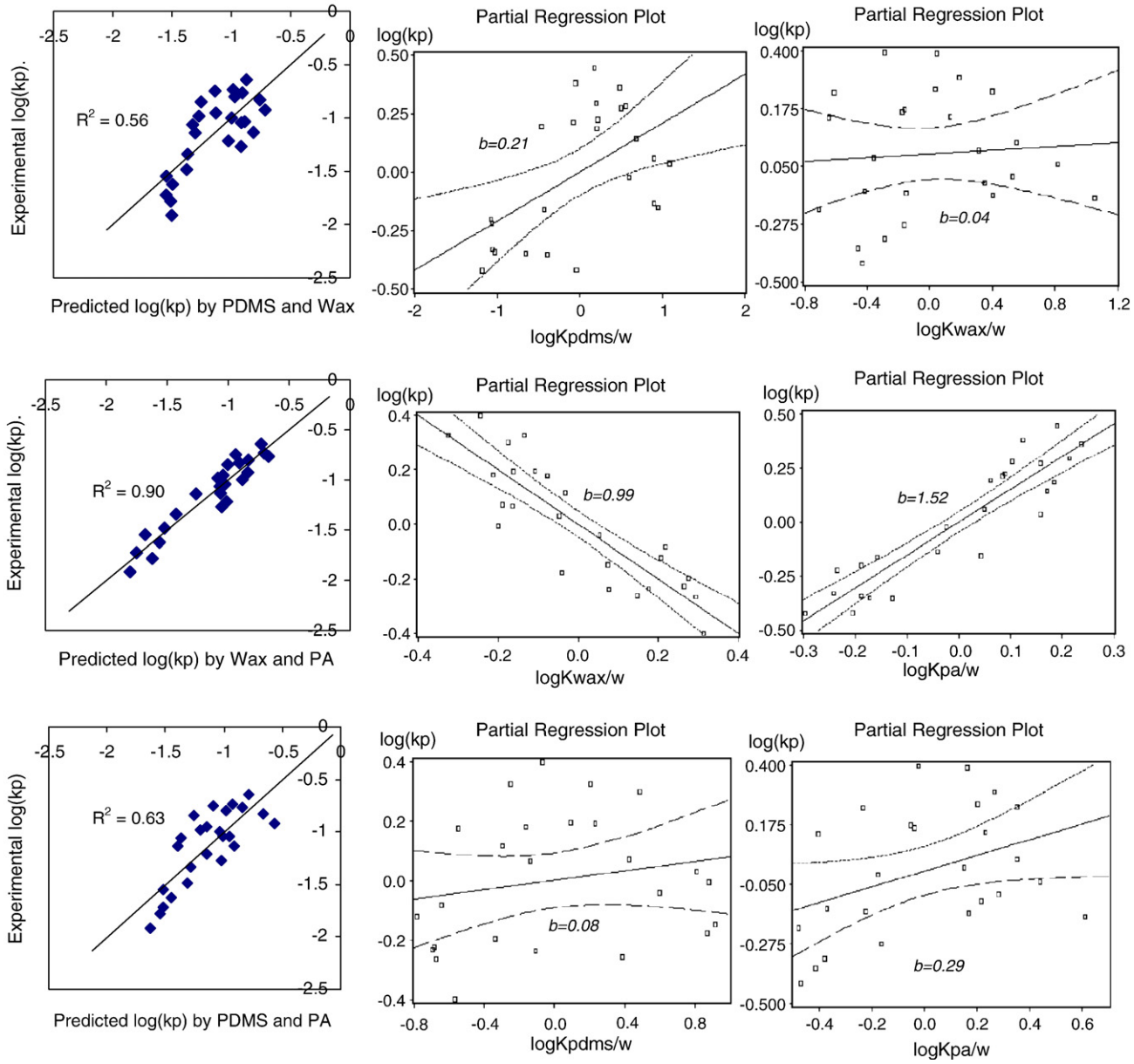

Fig. 3. Correlation of skin permeability with two MCF combinations. The partial regression plots were depicted for a corresponding correlation. The dashed lines indicated the 95% confidence levels.

fibers (Fig. 2). The PA MCF made positive contribution to log (kp), while the Wax MCF made negative contribution (Fig. 3). The correlation with the log(kp) values predicted by PDMS and PA fibers yielded an R^2 value of 0.63, which was not significantly improved compared to the PA fiber alone (Fig. 2). The significance level of the PDMS fiber was under 0.05 in the stepwise regression analysis.

Predictive model of the MCF array approach

Fig. 4 shows the correlation of experimental log(kp) with those predicted by the three MCFs. Good linear correlation was obtained with R^2 of 0.93 and $C(p)$ of 4.0. All of the explanatory variables ($\log K_{MCF}$ values measured with three MCFs) were statistically significant in the model ($p=0.05$). The F-value was 93 at the probability less than 0.0001. The predictive model with the three MCFs was established as follows:

$$\log(\text{kp}) = -2.34 - 0.124\log K_{\text{pdms/w}} + 1.91\log K_{\text{pa/w}} - 1.17\log K_{\text{wax/w}} (n = 25, R^2 = 0.93, F = 93 \text{ and } C(p) = 4.00) \quad (4)$$

The partial regression plots of the three fiber correlation were shown in Fig. 4. The dashed lines indicated the 95% confidence levels of the partial regressions. The slopes of the partial regression plots were equal to the regression coefficients, representing the contribution of the specific MCFs. The slopes of the $\log K_{\text{pdms/w}}$, $\log K_{\text{pa/w}}$ and $\log K_{\text{wax/w}}$ were −0.124, 1.91 and −1.17, respectively. The contribution of the PA MCF was strong and proportion to the skin permeability; the contribution of the Wax MCF was strong but inverse proportion to the log(kp) values. The correlation of the log(kp) values with $\log K_{\text{pdms/w}}$ was weak and provided less contribution to the predictive model.

The residual plot versus predicted skin permeability by the three MCFs is shown in Fig. 5. The randomly scattered data indicated that the multiple linear regression model (Eq. (4)) was an adequate model for skin permeability prediction. The experimental data were consistent without particular outliers.

Discussion

MCF array approach versus empirical approach

Potts and Guy's predictive model, the classic example of empirical approach, has been widely used for skin permeability estimation (Potts and Guy, 1992). It was adapted by the US Environmental Protection Agency and refined for skin permeability estimation in the final version of Supplementary Guidance for Dermal Risk Assessment (USEPA, 2004):

$$\log(\text{kp}) = -2.80 + 0.66\log K_{\text{o/w}} - 0.0056\ \text{MW} (R^2 = 0.66) \quad (5)$$

This empirical model was based on a human skin permeability data set compiled by Flynn from 15 literature sources over last

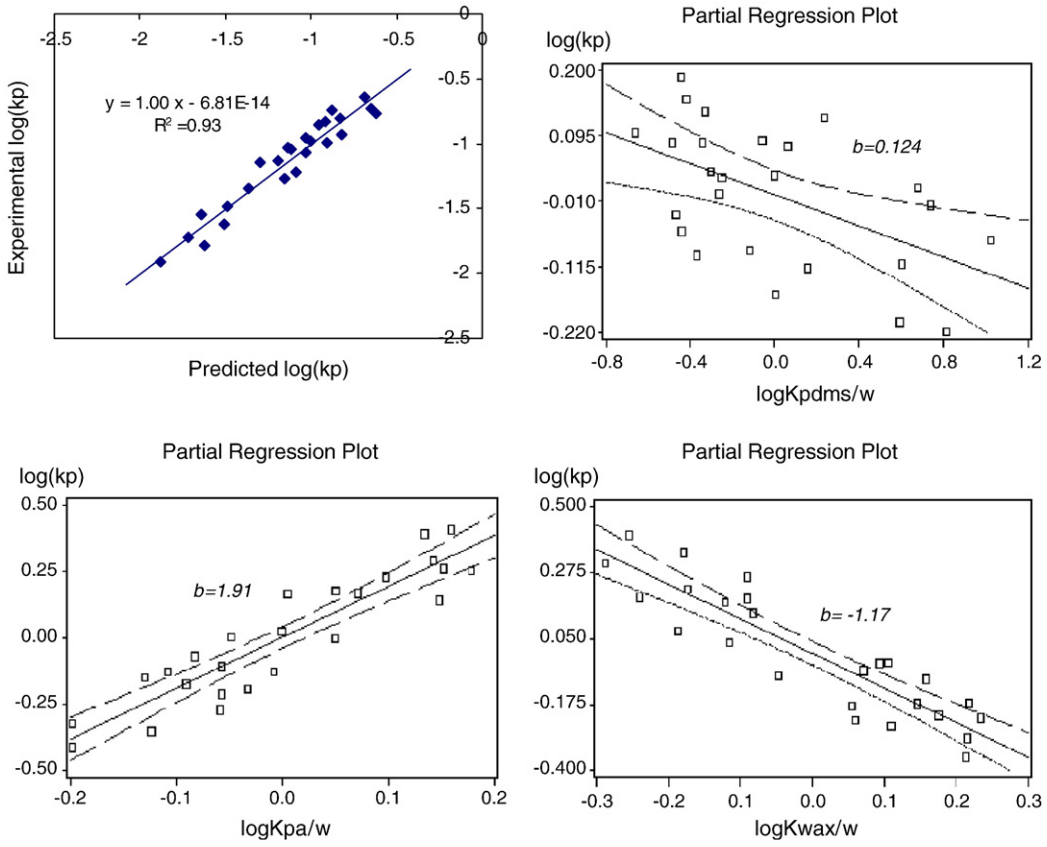

Fig. 4. Correlation of the skin permeability with three MCFs and the partial regression plots. The dashed lines in the partial regression plots indicated the 95% confidence levels.

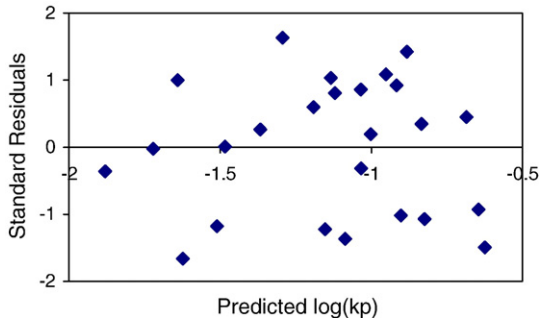

Fig. 5. Residual plot of the 3-MCF regression analysis.

three decades (Flynn, 1990). The statistical fit to Eq. (5) is comparatively poor. However, it was realized that up to 30% variability in the experimental data was to be expected, so that an R^2 of approximately 66% was as good a fit as was achievable.

The skin permeability estimated from Eq. (5) for the calibration compounds was listed in the last column of Table 1. For permeability unit consistency with the present work (cm/h), the refined EPA model (Eq. (5)) was used for the calculation instead of the original Potts and Guy model (cm/s). The values predicted from Eq. (4) are consistent with the estimated log(kp) values by Eq. (5) (Table 1) considering the large discrepancies in log(kp) values in the literature (Moss et al., 2002; Flynn, 1990).

The MCF array approach is an experimentally based, high throughput approach. It provided a high statistical confidence ($R^2=0.93$, Eq. (4)) over the empirical model ($R^2=0.66$, Eq. (5)), which was composed of tremendous efforts by many researches over the last three decades. The difficulties in measurements of skin permeability using conventional methods were discussed in the literature. To develop predictive models for skin penetration, all the data should be measured using the same protocol, with skin from the same animal. Large experimental errors are expected by using different skin and protocols due to the complicated biological and physical factors in the experiments. It is virtually unrealistic to obtain a large, chemically heterogeneous database of skin permeability values (Moss et al., 2002). Therefore, the proposed MCF array approach could be a useful tool for further development to overcome the unmet challenges.

Significance of the partition coefficients

The significance of octanol/water partition coefficient ($\log K_{o/w}$) in modeling biological processes has been well documented in the literature. Other solvent/water partition coefficients were also used in the predictive model developments (Liu et al., 2003; Abraham et al., 1999). However, it is difficult to determine the water/solvent partition coefficients for new compounds in high throughput systems since the traditional liquid–liquid extraction involves tedious manual operation, complicated sample handling and emulsion problems.

We have developed a novel membrane-coated fiber technique to overcome these difficulties. The MCF technique is a special version of the solid-phase microextraction (SPME) method developed in analytical chemistry (Arthur and Pawliszyn, 1990). For analytical applications, the goal is to extract the analytes in proportion to the original concentration in the sample solution. Therefore, any uptake mechanisms can be employed, absorption, adsorption or mixed absorption and adsorption. In fact, most of the newly developed SPME fibers are based on adsorption or mixed adsorption and absorption mechanisms (Supelco, 2001). For the determination of membrane/solvent partition coefficients, only absorption membranes can be used; porous adsorption membrane or mixed adsorption/absorption membranes cannot be used since these mechanisms do not reflect the molecular interactions in the skin absorption processes. We are developing novel membrane materials specifically for optimizing the performance of the MCF array approach, which may not be suitable for analytical purposes. The MCF technique integrates the membrane absorption and quantitative analysis into one step and fully utilizes the separation power of the automatic chromatographic instruments (GC, HPLC). It completely eliminates the emulsion problem and the other error sources associated with sample treatment and handling in water/solvent systems. These features enable the MCF technique to have greater sensitivity, accuracy and high throughput in the determination of the membrane/solvent partition coefficients (Xia et al., 2003, 2005a).

Modeling skin absorption by multiple MCF array

The stratum corneum is a heterogeneous membrane with complex biological structures (Monteiro-Riviere, 2006). Because of this complexity, a single synthetic membrane cannot mimic its biological structure or its performance. For percutaneous absorption in the stratum corneum, transport is governed by the relative strength of several molecular interactions including lipophilicity, hydrogen bonding, and π^*–electron interactions (Moss et al., 2002). The MCF technique can be to measure the partition coefficients of chemicals, which are quantitative measure of the molecular interactions between the solution and membrane phases (Xia et al., 2003). A single MCF can be used to characterize one pattern of molecular interactions that govern percutaneous absorption. To cover the range of molecular interactions involved in skin absorption, multiple MCFs could be selected properly to form multiple dimensions of the molecular interaction vectors. The present study demonstrated the feasibility of the MCF array to characterize the molecular interactions and to develop a model to predict the permeability constant for diverse chemicals. The novelty of the MCF array approach is to study the relative strengths of the molecular interactions in skin absorption instead of mimicking the biological structure or barrier function of the stratum corneum.

Fig. 2 shows that an individual MCF has limited power in simulating the skin permeability. This was in the similar situation as $\log K_{o/w}$ in skin permeability estimation. The correlation coefficient (R^2) was only 0.24 when the skin permeability from Flynn data set correlated with the $\log K_{o/w}$ values alone (Flynn, 1990). This low correlation also was due to the data variations compiled from 15 laboratories using different experimental protocols.

A multiple MCF combination provided higher correlation with the skin permeability if the molecular interaction vectors of different MCFs were matched as in the case of PA and Wax (Fig. 3). However, if the molecular interaction vectors were not matched or collinear, poor correlation was observed as in the cases of PDMS and Wax or PDMS and PA combinations (Fig. 3). When three MCFs were used to predict the skin permeability, excellent correlation was achieved (Fig. 4). These data suggested that the MCF array approach can be used to simulate the skin absorption by proper selection of the multiple MCFs. The selection of these three MCF membranes in this study was bounded on commercial availability. It is anticipated that different membranes could be matched to improve the prediction performance.

Potential application for studying the vehicle and formulation effects

A major advantage of this technique is that the MCF partition coefficients may be determined in different vehicles or formulations. In skin absorption, the chemicals of interest are usually minor components (e.g., in ppm concentrations). The changes of the minor components in composition and concentration do not affect the regression coefficients [c, a_1, a_2, a_3, ... a_n] in Eq. (1). However, if the vehicle or the major components of a formulation change in proportion or compositions, the regression coefficients will be altered significantly. Therefore, the MCF array approach can be deployed for two types of applications: the first type of application is to predict the skin permeability of the minor components while keeping the vehicle and major components constants. The second type of application is to measure the changes of the regression coefficients with a set of calibration compounds to study the vehicle and formulation effects. To our knowledge, the second type of application is the only experimental approach for quantitative determination of the vehicle and formulation effects.

In this paper, the first type of application was presented to establish and validate the MCF array approach for skin permeability prediction. The feasibility of the second type of application for studying solvent effects and surfactant effects has been described elsewhere (Xia et al., 2005b). The MCF array approach was demonstrated to be sensitive to detect the changes in regression coefficients. We have also shown that such vehicle interactions can be detected by analyzing solvochromic parameter effects on skin permeability using classic QSAR approach (Riviere and Brooks, 2005). An important use of the MCF array would be to characterize a specific formulation by the three MCFs. The effect of formulation changes on altered skin permeability could then be easily assessed by changes in the regression coefficients.

Chemical mixtures

Assessment of skin absorption from chemical mixture is challenging due to lack of data for chemical mixtures. Most of the physicochemical parameters in the literature are measured for individual chemicals (Pohl et al., 1997). In the present work, the MCF or skin absorption was conducted in chemical mixtures. The physicochemical parameters were determined for all of the compounds in the mixture simultaneously. This not only increased the experimental throughput but also reduced the experimental variation from batch-to-batch experiments using individual chemicals. Measuring the physicochemical parameters with the present approach, only trace concentrations were used due to the high sensitivity of the modern analytical instrumental methods, such as GC/MS, HPLC. In analytical chemistry, unit fugacity constants are used at such low concentrations. However, if the chemical reaction, association or evaporation occurs, the actual concentrations of the chemicals must be measured for obtaining the log(kp) and logK_{MCF} values.

MCF technique has a concentration factor up to 100 times so that high accuracy can be achieved in trace analysis. This advantage is inherited from the original solid-phase microextraction (SPME) method (Arthur and Pawliszyn, 1990; Zhang et al., 1994). The feasibility of using low concentrations reduces the concentration effects of multiple components among themselves, so that many chemicals can be studied simultaneously (Fig. 1). When multiple compounds were measured in a single run, it not only provides the high throughput, but also provides the high consistency in measuring the relative strengths of the molecular interactions for a series of compounds. It eliminates the errors introduced from batch-to-batch experiments for single chemicals in traditional diffusion experiments. It also allows skin permeability to be assessed at environmentally relevant low exposure concentrations.

Applicability and limitation of the MCF approach

The MCF array approach is used to simulate the biological processes where passive diffusion is the rate limiting transport mechanism. It is well known that passive diffusion is the rate limiting process in skin absorption of many organic compounds (Roberts et al., 1999; Wester and Maibach, 1983). Passive diffusion plays an important role in many biological transport processes, such as cell membrane, intestinal mucosa and stratum corneum membranes. In some biological processes, passive diffusion and active transport are coexisting. If the passive diffusion can be quantitatively assessed by the MCF approach, the quantitative prediction can be made by the summary of the two contribution components.

The purpose of this study is to demonstrate the feasibility of the MCF array approach and the model developing process. The established predictive model can be used to predict skin permeability for chemicals having similar physiochemical properties to the calibration compounds. Larger deviations will be produced if the chemicals are significantly different from the calibration compounds. Therefore, a set of calibration compounds should be selected to cover the range of physicochemical properties of interest for specific applications.

Conclusion

We have established the MCF array approach that can be used to predict skin permeability via the established model. The

MCF array approach is an animal alternative model, in which animal skin is required only for model development; animal skin is not required in the application of the established model to predict the skin permeability for the chemicals of interest. This approach considerably reduces the animal usage and removes the cost prohibition in large scale assessment of chemical absorptions or formulation optimizations. It could be potentially used for industrial, scientific and regulatory applications in quantitative assessment of percutaneous absorption of chemicals and drugs.

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