Articles

Quantification of Chemical Mixture Interactions Modulating Dermal Absorption Using a Multiple Membrane Fiber Array

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Dermal exposures to chemical mixtures can potentially increase or decrease systemic bioavailability of toxicants in the mixture. Changes in dermal permeability can be attributed to changes in physicochemical interactions between the mixture, the skin, and the solute of interest. These physicochemical interactions can be described as changes in system coefficients associated with molecular descriptors described by Abraham's linear solvation energy relationship (LSER). This study evaluated the effects of chemical mixtures containing either a solvent (ethanol) or a surfactant (sodium lauryl sulfate, SLS) on solute permeability and partitioning by quantifying changes in system coefficients in skin and a three-membrane-coated fiber (MCF) system, respectively. Regression analysis demonstrated that changes in system coefficients in skin were strongly correlated ($R^2 = 0.89$–0.98) to changes in system coefficients in the three-membrane MCF array with mixtures containing either 1% SLS or 50% ethanol. The PDMS fiber appeared to play a significant role ($R^2 = 0.84$–0.85) in the MCF array in predicting changes in solute permeability, while the WAX fiber appeared to contribute less ($R^2 = 0.59$–0.77) to the array than the other two fibers. On the basis of changes in system coefficients that are part of a LSER, these experiments were able to link physicochemical interactions in the MCF with those interactions in skin when either system is exposed to 1% SLS or 50% ethanol. These experiments further demonstrated the utility of a MCF array to adequately predict changes in dermal permeability when skin is exposed to mixtures containing either a surfactant or a solvent and provide some insight into the nature of the physiochemical interactions that modulate dermal absorptions.

Introduction

Exposure to chemical mixtures is of serious concern to the public and environmental health. Skin, as the largest organ protecting the body from exogenous harmful agents, is exposed to complex chemical mixtures in most intentional applications such as topical drugs, pharmaceuticals, and cosmetics, and unintentional exposures from environmental and occupational hazards. Quantitative assessment of skin absorption from chemical mixtures remains a challenging problem. There are thousands of chemicals, drugs, and pharmaceuticals and millions of their combinations at various concentration levels and exposure vehicles. It is therefore cost prohibitive to study skin absorption using conventional experimental methodologies.

Quantitative structure–activity relationships (QSAR) are often utilized to probe the relationship between skin permeability and physicochemical parameters of chemicals. Several quantitative structure−permeability relationships (QSPRs) have been derived (*1*–*5*). Most of these developed models were based on compiled literature data for individual chemicals; however, chemical mixtures, complicated mixture effects, vehicle effects, and formulation effects were not considered in these QSPR models. Experimental data are rare in the published literature due to the complexity of chemical mixtures. Riviere and Brooks (*6*) developed a predictive model for chemical mixtures using data from various dose vehicles. A mixture factor was introduced into the predictive model to reflect the mixture effects. This work demonstrated the promise in the prediction of skin absorption from complex chemical mixtures.

Recently, we developed a membrane-coated fiber (MCF) array approach for predicting skin permeability from an aqueous solution (*7*). The MCF array is an experimentally based approach that utilizes a high-throughput MCF technique to measure the physicochemical parameters required for model development; no literature data or molecular structure information is required. 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 (*8*). The MCF technique integrates membrane uptake 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 (*9*). These features allow the MCF technique to provide greater sensitivity, accuracy, and high throughput in the quantitative assessment of the mixture effects. The MCF

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array was recently shown to adequately predict skin absorption from aqueous solutions (*7*) as well as different chemical mixtures (*10, 11*).

In this paper, the MCF array approach was further developed to predict skin permeability from varying chemical mixtures based on quantifying the specific molecular interactions present, which is a more complicated scenario in quantitative assessment of chemical mixtures. It is known that chemical exposures occur from various pharmaceutical, cosmetic, and industrial formulations and, furthermore, that dermal absorption of chemicals is significantly affected by different vehicles and formulations. Several attempts have been made to model mixture interactions, but this has often been at the level of metabolic interactions (*12, 13*) and not at the level of the absorption process. There is no methodology available for quantitative assessment of skin absorption from varying chemical mixtures. The MCF array approach is integrated with molecular descriptors that are the inherent properties of a chemical and often used in regression analyses to correlate physicochemical properties with a biological process such as chemical permeability in skin. These physicochemical properties are often termed linear solvation (free) energy relationships (LSER) that provide the link between skin permeability and some solute property such as partition coefficients (*1, 3, 4*). In our approach, changes in skin absorption from varying chemical mixtures were simulated by the changes in the MCF partition coefficients in the presence of different chemical mixtures. The focus of this paper is to examine the changes in the matrix of system coefficients from LSERs in both the skin and the MCF array and to demonstrate that there is a correlation between the skin and the MCF array. All coefficients reflect differences in the properties of the two phases between which the solutes are being transferred, whether it is between a dosing solution and skin or a dosing solution and MCF.

Material and Methods

Determination of System Coefficients. The measurement of system coefficients [*r p a b v*] were performed by (*7*) using regression analysis. In this paper, an "absorption system" is regarded as a MCF or skin exposed to either water, 50% ethanol, or 1% sodium lauryl sulfate (SLS). The values of the solute descriptors [*R* π α β *V*], given in Table 3, were obtained from the literature (*14*). System coefficients were determined for each of the three MCFs [100 μm polydimethylsilicone (PDMS), 85 μm polyacrylate (PA), and 50 μm carbowax (WAX)] in water, 50% ethanol, and 1% SLS. The 100 μm PDMS fibers were conditioned at 250 °C for 30 min, and the 85 μm PA fiber was conditioned at 300 °C for 2 h as recommended. The 50 μm WAX fibers were conditioned at 220 °C for 30 min. A CombiPal automatic sampler (CTC Analytics, Switzerland) was used to perform the partitioning experiments with the three MCFs using the MCF technique described by Xia et al. (*8*). In brief, each preconditioned MCF was immersed in the working solution containing the solute and mixture until equilibrium between the working solution and the MCF was achieved. The MCF was then removed from the working solution and transferred to the injector of a GC/MS for quantitative analysis; data from these analyses were used to calculate the partition coefficient of the solute between the MCF and the mixture.

The system coefficients [*r p a b v*] for the skin system were calculated in all three types of absorption mixtures: water, 50% ethanol, and 1% SLS. The apparent skin permeability of the 32 solutes in the same three mixtures

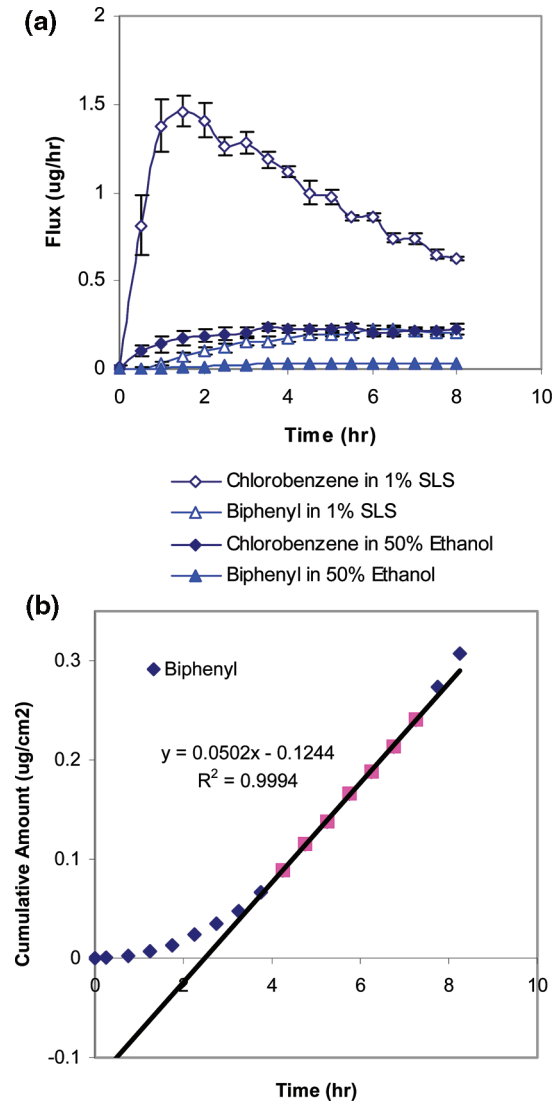

Figure 1. (a) Flux (μg/h) vs time profile for chlorobenzene and biphenyl dosed in 1% SLS or 50% ethanol in porcine skin flow-through diffusion cell system and (b) cumulative amount (μg/cm^2) vs time plot for biphenyl dosed in 50% ethanol.

was measured by using porcine skin flow-through diffusion cell system (*15*), and system coefficients were calculated by multilinear regression analyses of skin permeability to the five solute descriptors. Full details of these experiments and parameter determinations are published elsewhere (*7, 11, 16*). In brief, porcine skin sections approximately 350 μm thick were placed in a two-compartment Teflon flow-through diffusion cell. The dermal side of these skin sections was perfused with Krebs−Ringer bicarbonate buffer spiked with dextrose and bovine serum albumin (4.5%). Perfusate samples were obtained at 30 min intervals for 8 h after topical exposure, and samples were analyzed by headspaced/SPME and GC/MS methods. Data from these analyses were used to calculate the apparent permeability of the probe solutes in the various mixture scenarios described above. The apparent permeability was calculated from the ratio of the steady-state flux to solute concentration in the donor solution, and the steady-state flux (Figure 1b) was the slope of the cumulative amount vs time profile for individual solutes.

Model Development. In the MCF array approach, a chemical mixture is treated as a composition of a vehicle and other minor or trace chemicals. The vehicle is composed of the major components that dominate the physicochemical properties of

the chemical mixture, while the minor or trace components will not significantly alter the physicochemical properties. When skin or MCF membranes are exposed to the chemical mixture, the dermal absorption of compounds from the chemical mixture is governed by the relative molecular interaction strengths of the compounds with the vehicle and the stratum corneum of the skin. Our hypothesis is that these interactions, although different in each MCF membrane, may be similarly quantified in an array of MCF membranes.

In Abraham's LSER approach (*14*), the inherent molecular properties of a chemical are described by a set of effective solute descriptors [$R\ \pi\ \alpha\ \beta\ V$], while those of the absorption system (e.g., skin/mixture or MCF/mixture) are described by a corresponding set of system coefficients [$r\ p\ a\ b\ v$]. A free energy-related specific property (SP) such as chemical partitioning or chemical permeability can be correlated with the above solute descriptors of the chemical and system coefficients of the absorption system via a LSER equation (*14*):

$$\log \mathrm{SP} = c + rR + p\pi + a\alpha + b\beta + vV \qquad (1)$$

where log SP is a specific free energy-related property to be studied, such as skin permeability or the partition coefficient as described above; R is an excess molar refraction representing the molecular force of lone pair electrons; π is the effective dipolarity and polarizability of the chemical; α is the effective H-bond acidity, a summation of the acidity from all H-bonds of the chemical; β is the effective H-bond basicity, a summation of the basicity from all H-bonds of the chemical; and V is the McGowan characteristic volume that mainly represents London dispersion. The solute descriptors, [$R\ \pi\ \alpha\ \beta\ V$], are characteristic parameters of the chemical. Their values will not change when the chemical is transferred from one vehicle system to another. Each of the solute descriptors represents the relative strength of a specific type of molecular force of the chemical.

The system coefficients [$r\ p\ a\ b\ v$] are characteristic parameters of the dermal absorption or MCF partitioning system. Each of the system coefficients represents the relative strength of a specific type of molecular force interacting in the absorption system: c is a regression constant; r represents the tendency of the system to interact with chemicals through π^* and n-electron pairs; p represents the tendency of the system to interact with dipolar/polarizable chemicals; a is a measure of the H-bond basicity of the system, describing the tendency of the system to interact with the H-bond acidity of the chemical; b is a measure of the H-bond acidity of the system, describing the tendency of the system to interact with the H-bond basicity of the chemical; and v is a combination of exothermic dispersion forces that make positive contributions; it mainly measures the hydrophobicity of the system.

Changes in System Coefficients. The system coefficients will not change with minor or trace chemicals in composition or proportion. Therefore, the system coefficients for a given dermal absorption or MCF system can be detected by using a set of probe compounds with known solute descriptors. When a probe compound is added into the chemical mixture in a trace concentration not affecting the system coefficients, the apparent skin permeability (log K_p) or membrane partitioning (log K_F) of the probe compound can be measured experimentally using the conventional flow-through diffusion cell experiment and membrane-coated fiber experiments, respectively, as previously described (*7, 15*). In the Xia study (*7*), log K_F for three fibers was empirically correlated to the logK_p. The log K_p value of the compound can be scaled into the solute descriptors of the compound via the LSER eq 1, where log SP = log K_p. A LSER equation matrix is generated from all of the probe compounds. In this study, we selected 32 probe compounds (Table 1) with a wide spectrum of molecular descriptor values:

$$\log Kp^n = c + rR^n + p\pi^n + a\alpha^n + b\beta^n + vV^n \qquad (n = 1, 2, 3, \ldots 32) \quad (2)$$

where n is the number of probe compounds. The system coefficients of the absorption system [$r\ p\ a\ b\ v$] and the regression constant (c) can be obtained by multiple linear regression analysis of the LSER equation matrix (eq 2).

When the vehicle or major components change in composition or proportion, the system coefficients will be altered. Therefore, the changes in the system coefficients can be used to study the skin absorption from varying chemical mixtures. If the chemical mixture has a small change in the vehicle, this change will be reflected in the system coefficients, that is, a small change will be introduced into the system coefficients [$\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v$].

Table 1. Skin Permeability of the Probe Compounds in 50% Ethanol and Their Descriptors (14)

no.	compounds	log K_p (cm/h)	R	π	α	β	V
1	toluene	−1.304	0.601	0.52	0	0.14	0.857
2	chlorobenzene	−1.735	0.718	0.65	0	0.07	0.839
3	ethylbenzene	−1.870	0.613	0.51	0	0.15	0.998
4	*p*-xylene	−1.620	0.613	0.52	0	0.16	0.998
5	bromobenzene	−1.880	0.882	0.73	0	0.09	0.891
6	propylbenzene	−2.173	0.604	0.5	0	0.15	1.139
7	4-chlorotoluene	−1.937	0.705	0.67	0	0.07	0.98
8	phenol	−2.285	0.805	0.89	0.6	0.3	0.775
9	benzonitrile	−2.076	0.742	1.11	0	0.33	0.871
10	4-florophenol	−2.723	0.67	0.97	0.63	0.23	0.793
12	iodobenzene	−2.004	1.188	0.82	0	0.12	0.975
14	acetophenone	−2.303	0.818	1.01	0	0.48	1.014
15	*m*-cresol	−2.726	0.822	0.88	0.57	0.34	0.916
16	nitrobenzene	−1.925	0.871	1.11	0	0.28	0.891
18	4-chloroanisole	−2.135	0.838	0.86	0	0.24	1.038
19	phenethyl alcohol	−2.680	0.784	0.83	0.3	0.66	1.057
20	3-methyl benzyl alcohol	−2.710	0.815	0.9	0.33	0.59	1.057
21	4-ethylphenol	−2.770	0.8	0.9	0.55	0.36	1.057
22	3,5-dimethylphenol	−2.923	0.82	0.84	0.57	0.36	1.057
25	naphthalene	−2.140	1.36	0.92	0	0.2	1.085
27	4-chloroaniline	−2.495	1.06	1.13	0.3	0.31	0.939
28	4-nitrotoluene	−2.133	0.87	1.11	0	0.28	1.032
29	4-chloroacetophen	−2.692	0.955	1.09	0	0.44	1.136
30	3-bromophenol	−3.136	1.06	1.15	0.7	0.16	0.95
31	1-methyl naphthalene	−2.480	1.344	0.9	0	0.2	1.226
32	biphenyl	−2.614	1.36	0.99	0	0.22	1.324

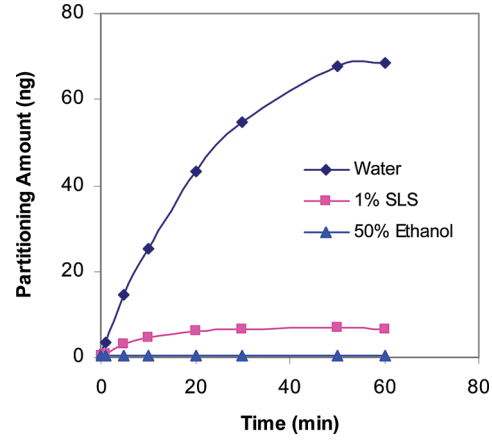

Figure 2. Solute partitioning of biphenyl into the WAX fiber in water, 1% SLS, and 50% ethanol solutions.

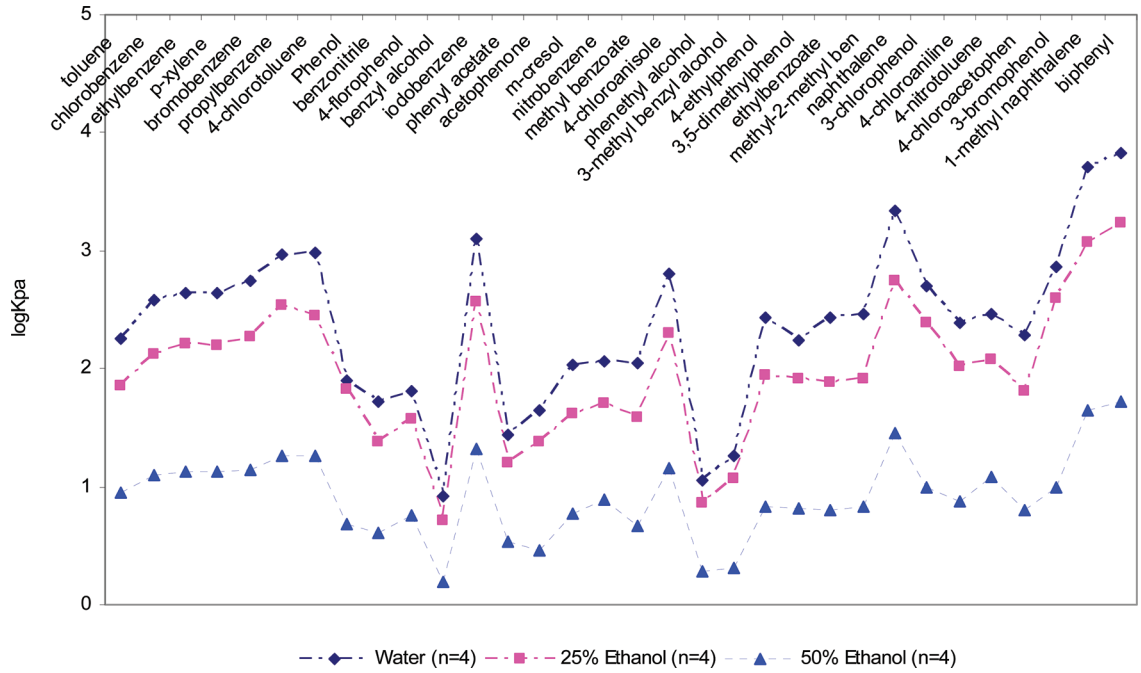

Figure 3. Distribution coefficients of the 32 probe compounds measured with PA fiber in ethanol–water solutions (the compounds were depicted in a chromatographic order).

The changes in the system coefficients can be obtained by subtraction of the system coefficients of the chemical mixture from those after the change:

$$\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v = [r\ p\ a\ b\ v]_x - [r\ p\ a\ b\ v]_o$$
$$= [r_x - r_o\ p_x - p_o\ a_x - a_o\ b_x - b_o\ v_x - v_o] \quad (3)$$

where $[r\ p\ a\ b\ v]_o$ are the system coefficients of the original chemical mixture; $[r\ p\ a\ b\ v]_x$ are the system coefficients after the change of a major component in the chemical mixture, and $[\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v]$ are the changes of the system coefficients. In this study, we modified the skin and MCF systems by adding a solvent (50% ethanol) or a surfactant, 1% SLS.

Correlation of the Changes in System Coefficients. The MCF array approach for predicting the apparent skin permeability is based on the fact that both permeability coefficient ($\log K_p$) and MCF partition coefficients ($\log K_F$) are free energy-related quantities, which are linearly additive from the contribution components (eq 1). The changes in the system coefficients of the skin permeability for a given vehicle change in skin, $[\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v]_{skin}$, will be reflected in alteration of system coefficients of multiple MCFs $[\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v]_{F1}$, $[\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v]_{F2}$, ...$[\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v]_{Fm}$, where m is the number of diverse MCFs.

It is hypothesized that a quantitative correlation exists between the changes in system coefficients of skin permeability and those of MCF partition coefficients under varying chemical mixtures (eq 4)

$$\begin{pmatrix}\Delta r\\ \Delta p\\ \Delta a\\ \Delta b\\ \Delta v\end{pmatrix}_{skin} = a_0 + a_1\begin{pmatrix}\Delta r\\ \Delta p\\ \Delta a\\ \Delta b\\ \Delta v\end{pmatrix}_{F1} + a_2\begin{pmatrix}\Delta r\\ \Delta p\\ \Delta a\\ \Delta b\\ \Delta v\end{pmatrix}_{F2} + \ldots + a_m\begin{pmatrix}\Delta r\\ \Delta p\\ \Delta a\\ \Delta b\\ \Delta v\end{pmatrix}_{Fm} \quad (4)$$

The molecular interaction properties of the two systems are described by a set of system coefficients [$r\ p\ a\ b\ v$]. In this paper, we will use difference of system coefficients (DSC) technique to study the DSC of skin. In this study, three diverse fibers were PDMS, PA, and WAX. The DSC was calculated by subtracting the coefficients of each fiber absorbed in water from either 50% SLS or ethanol. We denote the DSC by $[\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v]$ for these three diverse fibers to access the DSC for skin. Each Δ value represents the difference of each system coefficient for three fibers when partitioned from water and when partitioned from either 50% ethanol–water or 1% SLS−water.

Multiple Regression Analysis. The multiple regression techniques give the coefficients a_1, a_2, and a_3 with intercept a_0. We use stepwise regression with all three independent variables. The independent variables are the array $[\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v]$ for the DSC (mixture − water) for WAX, PDMS, and PA, respectively. The dependent variable is $[\Delta r\ \Delta p\ \Delta a\ \Delta b\ \Delta v]$, the DSC (mixture − water) for apparent skin permeability. We can express this in the form of eq 4 as

$$\begin{pmatrix}\Delta r\\ \Delta p\\ \Delta a\\ \Delta b\\ \Delta v\end{pmatrix}_{skin} = a_0 + a_1\begin{pmatrix}\Delta r\\ \Delta p\\ \Delta a\\ \Delta b\\ \Delta v\end{pmatrix}_{WAX} + a_2\begin{pmatrix}\Delta r\\ \Delta p\\ \Delta a\\ \Delta b\\ \Delta v\end{pmatrix}_{PDMS} + a_3\begin{pmatrix}\Delta r\\ \Delta p\\ \Delta a\\ \Delta b\\ \Delta v\end{pmatrix}_{PA}$$

The above matrix equation can be written in form $y = a_0 + a_1x_1 + a_2x_2 + a_3x_3$ where x_1, x_2, and x_3 are the difference of mixture coefficients of either a mixture of 50% ethanol or a mixture of 1% SLS for WAX, PDMS, and PA, respectively. For simplicity in understanding, we will interchangeably use the terms WAX, PDMS, and PA for x_1, x_2, and x_3. First, we performed the statistical analysis of the data when each fiber is absorbed in water and 1% SLS. We used two set of coefficients $[r\ p\ a\ b\ v]$ values for 1% SLS and water mixtures. Stepwise regression is a technique for choosing the variables, that is, terms, to include in a multiple regression model. For each term on the y-axis, the plot describes the regression coefficients as a point with horizontal bars indicating confidence intervals. Blue points represent the terms that are in the model, while red points indicate terms that are not currently in the model. The R square value is one minus the ratio of the error sum of squares to the

Table 2. Difference of System Coefficients (Δ = Mixture − Water) for Corresponding Molecular Descriptors Following Skin or MCF Exposure to (a) 1% SLS or (b) 50% Ethanol[a]

	Δ*r*	Δ*p*	Δ*a*	Δ*b*	Δ*v*
(a) system coefficients Δ values for 1% SLS solution					
skin observed	0.412	0.955	0.149	−0.955	1.978
skin predicted	0.338	0.855	0.120	−0.922	2.044
WAX observed	0.786	0.011	0.590	−1.293	1.163
WAX predicted	0.941	0.255	0.768	−0.898	1.275
PDMS	0.399	−0.152	−0.127	−1.218	1.743
PDMS predicted	0.520	0.405	0.229	−0.890	1.977
PA observed	0.412	−0.336	−0.229	−0.739	1.525
PA predicted	0.768	−0.017	0.094	−0.441	1.937
(b) system coefficients Δ values for 50% ethanol solution					
skin observed	0.170	1.629	0.503	−1.476	2.647
skin predicted	0.325	0.905	0.451	−1.198	2.789
WAX observed	0.616	−0.052	0.056	−2.300	1.978
WAX predicted	1.187	0.596	0.691	−1.394	2.393
PDMS observed	0.049	0.317	−0.881	−1.698	2.213
PDMS predicted	0.742	1.004	−0.104	−0.991	2.853
PA observed	0.214	0.018	0.022	−1.740	1.814
PA predicted	0.863	0.640	0.645	−1.360	2.684

[a] The coefficients for mixtures and water were derived from regression analysis of 32 solutes in skin and MCF array (*7*).

total sum of squares. *R* square indicates that the model accounts for a certain percentage of the variability in the observations and the adjusted *R* square—the *R* square statistic adjusted for the residual degrees of freedom—and the RMSE denotes root mean squared error of the current model. These analyses were performed with Matlab (The MathWorks, Inc., Novi, MI) by using the packages stepwise, regstat, and polytool.

Results

Table 1 lists the apparent permeability of the probe solutes in 50% ethanol as well as molecular descriptors for these solutes. The compounds are listed in a chromatographic order, and the descriptor values were obtained from Abraham's solute descriptors (*14*). The presence of 1% SLS or 50% ethanol significantly reduced the apparent permeability in skin and solute partitioning in the MCF array. Figure 1a depicts the flux profile for two of the 32 solutes evaluated in the porcine skin diffusion cell system. Figure 2 depicts the membrane uptake kinetics of biphenyl by the WAX fiber from water, 1% SLS, and 50% ethanol, and Figure 3 depicts the significant differences in partitioning of the solutes into the PA fiber at various ethanol concentrations. Table 2 summarizes the observed and predicted Δ effects of 1% SLS or 50% ethanol on apparent solute permeability in skin and solute partitioning into the PDMS fiber.

Regression analysis demonstrated that changes in apparent dermal permeability in the presence of 50% ethanol or 1% SLS were strongly correlated to changes in partitioning in the MCF array of three fibers. *R* square values ranged from 0.59 to 0.98 when changes in both systems were compared in either ethanol or SLS mixtures (Table 3). The best correlations were observed when the full MCF array was included in the regression analysis.

In Figure 4, all of the dependent terms are in the regression model describing effects of 1% SLS, and the regression coefficients x_1, x_2, and x_3 are −0.931, 3.81, and −2.66, respectively, with the intercept $a_0 = 0.548$. Thus, the regression equation is $y = 0.548 - 0.931x_1 + 3.81x_2 - 2.66x_3$ with x_1, x_2, and x_3 representing Δ values for WAX, PDMS, and PA, respectively. The *R* square is 0.9445, indicating that the model accounts for over 94% of the variability in the observations and the adjusted *R* square—The *R* square statistic adjusted for the residual degrees of freedom is 0.92.

Table 3. *R* Square Values for Full MCF Array and for Stepwise Regression for Pairs of MCFs and Individual MCFs Following Correlation Analyses between System Coefficients for Skin and MCF Following Exposure to 1% SLS or 50% Ethanol for 32 Solutes

	R square	RMSE
1% SLS		
full MCF array	0.984	0.275
pair of MCFs		
PA + PDMS	0.896	0.499
PA + WAX	0.738	0.793
PDMS + WAX	0.850	0.598
individual MCF		
PA	0.717	0.673
WAX	0.597	0.803
PDMS	0.841	0.504
50% ETOH		
fill three MCF	0.894	1.014
pair of MCFs		
PA + PDMS	0.873	0.783
PA + WAX	0.887	0.740
PDMS + WAX	0.859	0.826
individual MCF		
PA	0.846	0.705
WAX	0.772	0.858
PDMSt	0.848	0.700

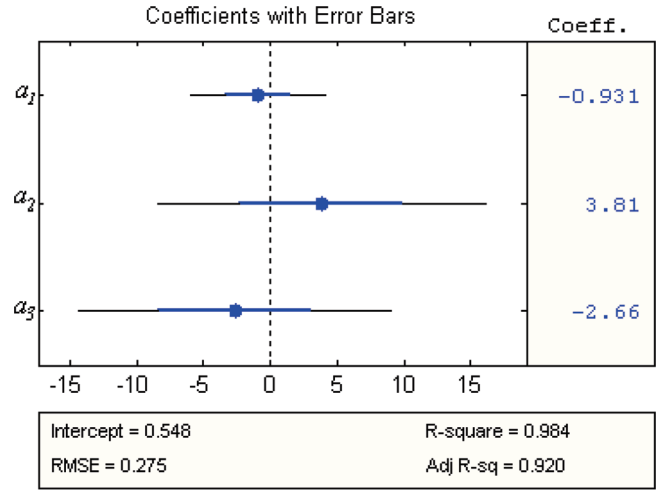

Figure 4. Regression coefficients (a_1, a_2, and a_3) with corresponding 95% confidence limits derived from the correlation of Δ system coefficients for skin with Δ system coefficients for full MCF array. Both skin and MCF arrays were exposed to 1% SLS.

When the same regression analysis was performed with two terms (i.e., two fibers), there were significant changes to the *R* square values. We also determined what happens when the regression coefficient a_1 for the WAX fiber is removed, while in the second and third case, a_2 for PDMS and a_3 for PA are removed, respectively, in a stepwise regression. It should be noted that that when PDMS is included in the regression, then the *R* square is greater than 0.85 and RMSE is less than 0.56, and when it is removed, the *R* square is less than 0.74 and RMSE is more than 0.78 (Figure 5). This demonstrates that PDMS (x_2) may be the most important variable in the MCF array for mixture evaluation. When the regression analysis was performed with only one MCF at a time, the best value of *R* square in this case was 0.84 (Figure 6), and this is also for PDMS; the smallest *R* square value was 0.59 for the WAX fiber (Figure 7). This again confirms that PDMS is an important variable for model analysis for mixtures containing 1% SLS. Figure 8a demonstrates the strong relationship (*R* squared = 0.984) between the five observed changes in system coefficients [Δ*r* Δ*p* Δ*a* Δ*b* Δ*v*] and those predicted Δ values from the

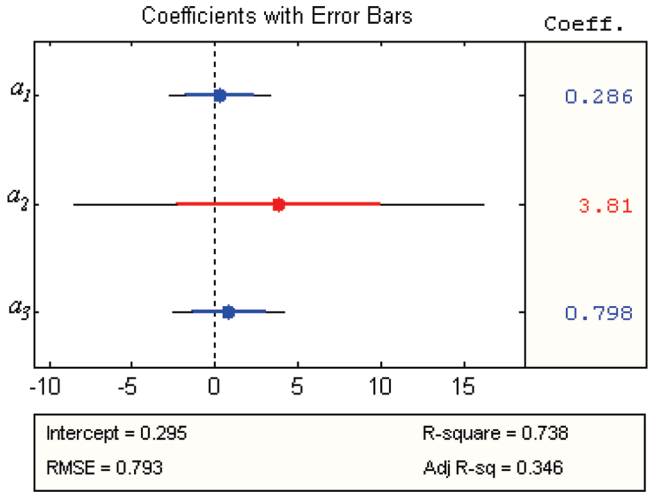

Figure 5. Regression coefficients (a_1, a_2, and a_3) with corresponding 95% confidence limits derived from the correlation of Δ system coefficients for skin with Δ system coefficients with PDMS fiber removed from the MCF array. Both skin and MCF arrays were to 1% SLS.

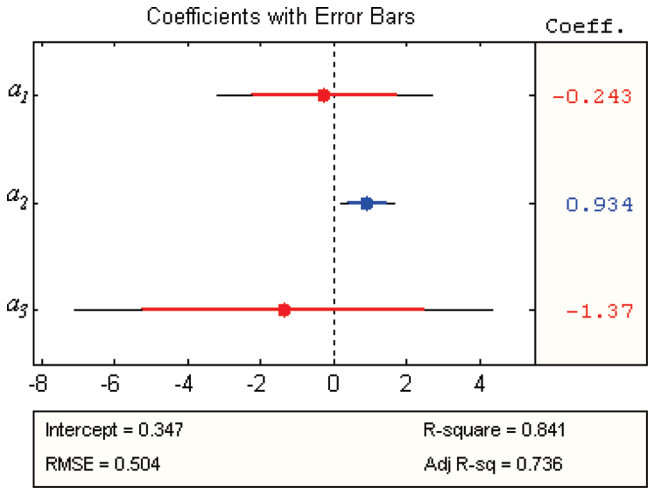

Figure 6. Regression coefficients (a_1, a_2, and a_3) with corresponding 95% confidence limits derived from the correlation of Δ system coefficients for skin with Δ system coefficients with WAX and PA fibers removed from the MCF array. Both skin and MCF arrays were exposed to 1% SLS.

polynomial model used in the previously described matrix (eq 4). The residual analysis as depicted in Figure 9a demonstrates an almost excellent spread for the full MCF array.

The regression equation for ethanol mixtures is $y = 0.619 - 1.076x_1 + 0.288x_2 + 2.128x_3$ with x_1, x_2, and x_3 representing Δ values for WAX, PDMS, and PA fibers, respectively. The correlation between changes in apparent skin permeability and changes in MCF partitioning was strongest (R squared = 0.89) for the full MCF array of three fibers (Figure 10). Removal of only PDMS from this array did not significantly decrease the correlation (R squared = 0.887); however, removal of both PDMS and PA resulted in a signficant decrease (R squared = 0.772). In general, the correlations were not signficantly affected when a MCF was included or removed from the MCF array exposed to ethanol mixtures. This was not the case with SLS mixtures previously described. Figure 8b demonstrates the strong relationship (R squared = 0.894) between the observed changes in system coefficients [Δr Δp Δa Δb Δv] and those predicted Δ values for mixtures containing 50% ethanol. The residual analysis as depicted in Figure 9b demonstrates a good spread for the full MCF array and several individual fibers.

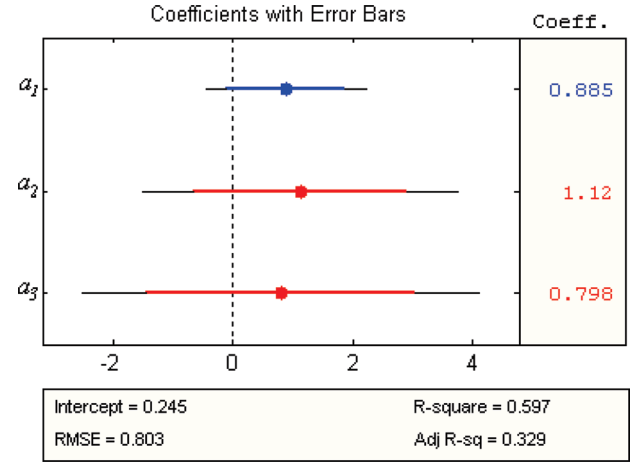

Figure 7. Regression coefficients (a_1, a_2, and a_3) with corresponding 95% confidence limits derived from the correlation of Δ system coefficients for skin with Δ system coefficients with PDMS and PA fibers removed from the MCF array. Both skin and MCF arrays were exposed to 1% SLS.

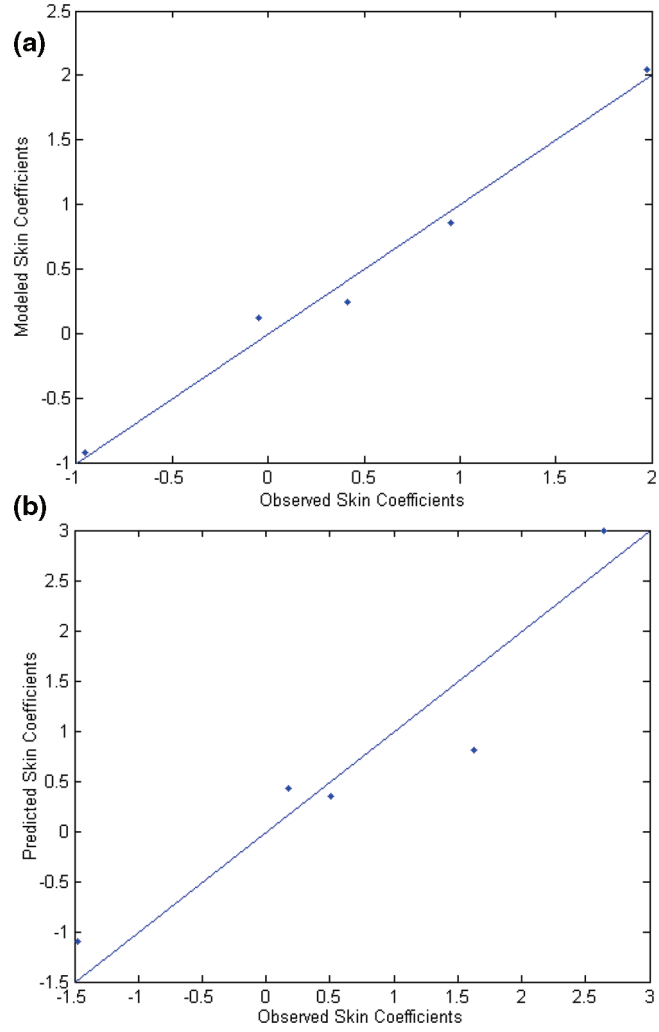

Figure 8. Predicted vs observed Δ system coefficients for skin for the full MCF array exposed to (a) 1% SLS and (b) 50% ethanol.

In an attempt to determine whether the MCF array was better correlated to solute solubility than apparent permeability, multiple regression analysis was used to correlate the MCF array (three MCF fibers) with chemical solubility in (i) water and (ii) 50% ethanol. The solubility data for these solutes in water

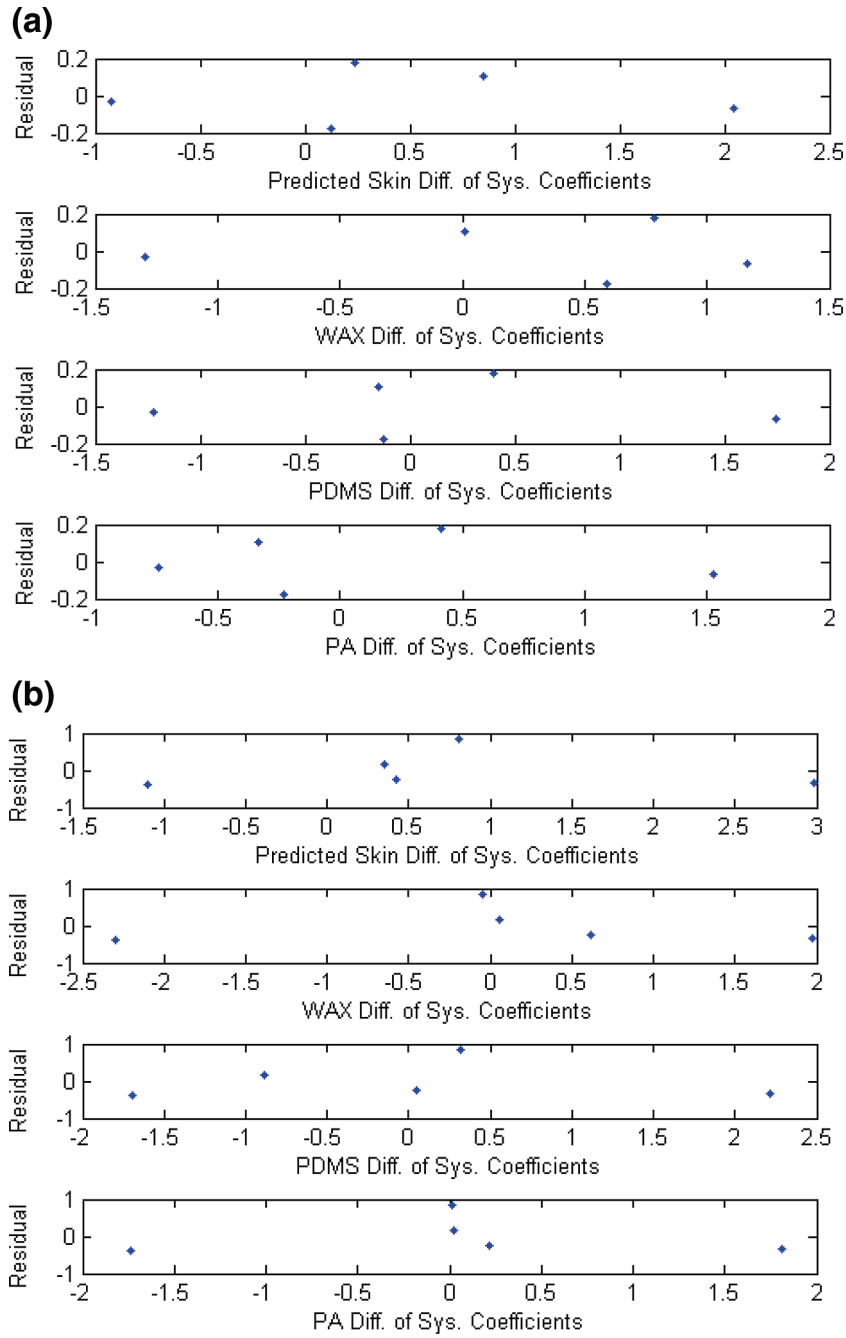

Figure 9. Predicted Δ system coefficients for skin vs the residuals from the correlation of the predicted Δ system coefficients with the observed Δ system coefficients for the full MCF array, WAX, PDMS, or PA fibers exposed to (a) 1% SLS and (b) 50% ethanol.

or 50% ethanol were obtained from the Absolve database (ADME Boxes, Pharma Algorithms, Toronto, Canada); this database does not provide the solubility data for 1% SLS; therefore, the correlation between solubility in 1% SLS and the MCF array was not evaluated. Our correlation analyses determined that the MCF array better predicted apparent permeability ($R^2 = 0.93$) than solubility ($R^2 = 0.54$) in 50% ethanol. The correlations were almost equivalent ($R^2 = 0.93$ and 0.94) in 100% water.

Discussion

Dermal exposure to pharmaceuticals or toxicants can result in significant changes in dermal permeability when the skin is simultaneously exposed to chemical additives that can modulate how much of the toxicant penetrates into the skin and/or becomes systemically absorbed. This study demonstrated that a MCF array of three physicochemically diverse fibers can accurately predict changes in dermal permeability. Our novel approach utilized several well-accepted technologies (MCF) in analytical chemistry as well as physicochemical properties defined by differences in system coefficients or DSCs [Δr Δp Δa Δb Δv] in a LSER that captured these mixture interactions independently in both the skin and the MCF array. We used the Abraham LSER model as it is generally accepted as a represenation and robust QSAR approach. Multilinear regression was able to statistically analyze these matrices of DSCs to determine the significant contributions of individual MCFs or combinations of MCFs to accuately reflect mixture-induced permeability changes in the skin.

The modulating effects of SLS and ETOH on solute permeability and solute partitioning into the stratum corneum or a

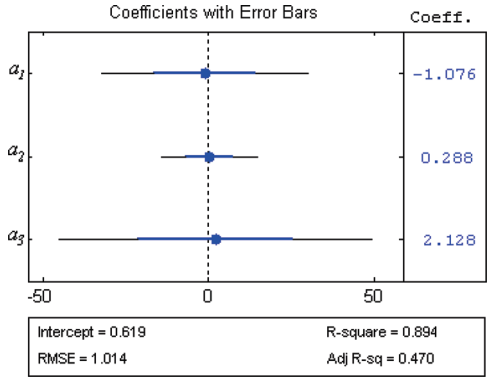

Figure 10. Regression coefficients (a_1, a_2, and a_3) with corresponding 95% confidence limits derived from the correlation of Δ system coefficients for skin with Δ system coefficients for the full MCF array. Both skin and MCF arrays were exposed to 50% ethanol.

MCF array have been demonstrated independently by our laboratory (*7, 17, 18*). We also have previously shown that MCF partition coefficients could predict skin permeability for aqueous solution and mixture exposures (*7, 11*). This is the first study to link the quantitative changes in skin permeability with quantitative changes in solute partitioning into a MCF array on the basis of changes in a matrix of molecular descriptores. The use of five solvatochromatic parameters described in the LSER allowed for this linkage to be made between physicochemical interactions in skin and an inert membrane system. These data support the theory that PA, PDMS, and WAX possess functional groups that are collectively influenced in the same proportion to similar or related functional groups in skin when exposed to SLS or ethanol.

These three MCFs among other commercial fibers have been routinely used in last 15 years in analytical chemistry laboratories in solid-phase microextraction (SPME) of pesticdes, drugs, and other chemicals (*19, 20*). These fibers are simply polymers with different functional groups. In our MCF array, the PDMS, PA, and WAX fibers can theoretically be representative of the more nonpolar, moderately polar, and polar components of the epidermis, respectively, as has been recently described (*16*). The PDMS fiber surface often has silonol (SiOH) groups, which renders the PDMS surface hydrophobic and at the same time resists adsorption of hydrophobic and negatively charged species. The PA and WAX fibers are more polar than the PDMS fiber, and these polymers consist of COH groups and COOH functional groups. Functional groups in the MCF array provide a substrate for interactions with solutes through hydrogen bonding, π^*, and *n*-electron pairs and other interactions describe in our five-parameter LSER. The primary sorption mechanism of interaction between the solutes and these fibers has been a source of argument, but it is widely accepted to be more by "absorption" than adsorption (*21*). This permeation process by solutes into fibers can be described by a one-compartment model, which was characterized earlier (*22*). As with single solute exposures, one can assume that the process is a first order kinetics across all three fibers and even in the presence of SLS and ethanol mixtures evaluated in this study.

The presence of surfactants and organic solvent is known to increase skin permebility of organic solutes. However, our experiments found that 1% SLS or 50% ethanol signficantly descreased solute permeability into skin and solute partitioning into all three fibers when compared to 100% water solutions (*11, 23*). These observations are not unusual, as the presence of an organic solvent or surfactants can cause the lipophilic solute to be retained in the liquid phase of the dosing solution and less likely to partition into the skin or MCF than if the dosing solution was 100% water (*17, 18*). Our experiments utilized 1% SLS, which is significantly greater than its critical micelle concentration (CMC) of 0.25%, and this could have resulted in retention of solutes within micelles in the dosing solution, making solutes less available for dermal absorption. This is especially true for some very volatile chemicals like chlorobenzene (Figure 1) mixed with surfactants, as rapid evaporation from the skin surface and dosing solution can result in decreased absorption with time.

The full three MCF array was necessary to describe and quantify these changes in skin permeability for mixtures containing 1% SLS. PDMS appeared to be the most important fiber in predicting mixture effects from SLS mixtures. For example, when PDMS was not included in the regression analysis, the correlation coefficient (R^2) decreased from 0.98 to 0.74 and the presence of PDMS alone in the regression provided the best fit ($R^2 = 0.84$) as compared to regression analysis with other individual fibers. This observation is not surprising as PDMS is more physicochemically similar to the stratum corneum layer of the epidermis, which is the first epidermal layer exposed to the test chemicals.

In contrast to the 1% SLS mixtures described above, the performances of the MCF array, any pair of fibers, only PA, and only PDMS were similar in predicting the changes in solute permeability in the presence of 50% ethanol ($R^2 = 0.85$–0.89). Although PDMS and PA are physicochemically distinct substrates (nonpolar vs polar), it is possible that there is significant/equal ethanol uptake into these fiber as per skin and that changes in paritioning into PDMS or PA in the presence of 50% ethanol are equal and reflective of changes in the skin permeability. Alcohols are known to form hydrogen bonds with oxygen atoms in the PDMS fiber (*24*), which probably accounts for most of the solvent−membrane interactions observed in our study. However, the mixture effects in either of these MCF systems do not appear to be additive as suggested by the stepwise regression when PA + PDMS was assessed. These correlations may not be applicable at lower solvent concentrations, which is the more likely scenario for environmental and occupational exposures (*25*). As with 1% SLS, the WAX fiber alone was a poor predictor for permeability changes in 50% ethanol. The latter is not surprising as WAX is an extremely polar substate as compared to PDMS and PA and therefore by itself is very difficult to capture physicochemical changes in the epidermis, which is predominantly lipid in nature.

Our analyses demonstrated that solubility plays a significant role in the permeation process, as there were equivalent strong correlations between skin permeability and MCF array and solubility and the MCF array in water. However, our analysis further demonstrated that when ethanol was present (50% ethanol mixtures), the MCF array is superior at predicting skin permeability than solubility. It is therefore possible that solute solubility may not be the only or primary determinant of skin permeability for chemical mixtures. As the focus of this study was to demonstrate mixture effects on permeability, our analyses suggest that the MCF array may be a useful approach to assess mixture effects on skin permeability.

In conclusion, this study demonstrated the utility of a three MCF array to accuately predict changes in the matrix of LSER descriptors for skin permeability in ethanol or SLS mixtures. The use of human skin in the permeability studies would have been an optimal approach and may have improved the correlations. However, porcine skin is anatomically and physiologically similar to human skin (*26*); therefore, it's safe to assume that

mixture effects described in this paper are comparable to human skin exposure to similar mixtures. The three MCF array appears to be more important for predicting these changes with SLS mixtures, while the PDMS or PA may be satisfactory for predicting changes in skin permeability for 50% ethanol mixtures. Further studies are focused on whether this pattern is consistent at tertiary mixture level (e.g., SLS + ethanol mixtures) and at different mixture concentrations. The MCF technique, however, has the advantages of quickly making these assessments and in provided much needed estimates of how a chemical mixture can significantly alter the dermal bioavailability of hazardous chemicals.

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