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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