Determination of Concentration-Dependent Water Diffusivity in a Keratinous Membrane

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Received July 6, 1982, from the *Personal Care Division, The Gillette Company, Boston, MA 02106.* Accepted for publication October 22, 1982.

Abstract □ A permeation method was developed to determine water diffusivity, $D(C)$, as a function of water concentration (C) in a keratinous membrane. The method involved the determination of a series of mean diffusivities ($\overline{D}$) and mean concentrations ($\overline{C}$) in the membrane. $\overline{D}$ was obtained from $\overline{D} = F\,H(C_o - C_h)$, where F was the flux at steady state, H was the membrane thickness, and C_o and C_h were the water concentrations in the membrane at the donor and receptor sides, respectively. The difference between C_o and C_h was kept small in each experiment. Therefore, as a first approximation, $\overline{C}$ was equal to $(C_o + C_h)/2$. After successive approximations, an empirical equation was found to provide the best fit to $\overline{D}$ *versus* $\overline{C}$ and to give the best convergence between the assumed and calculated $\overline{C}$; the equation was taken as $D(C)$. $D(C)$ for water in fetal hog periderm was found to be: $D(C) = 1.0 \times 10^{-18} + 9.70 \times 10^{-9}\,C^{0.69}$.

Keyphrases □ Diffusivity—water in keratinous membranes, determination using concentration, fetal hog periderm, topical formulations □ Topical formulations—determination of water diffusivity using concentration, keratinous membranes, fetal hog periderm □ Permeability—topically applied formulations, determination of diffusivity using concentration, keratinous membranes

Passive transport of matter through a biological membrane is usually governed by Fick's law. In most cases, a constant diffusion coefficient for transported material (penetrant) can adequately describe the diffusion process. However, on some occasions, especially when the penetrant is also a solvent or plasticizer for the membrane, the diffusion coefficient could be a function of the concentration of the penetrant (1).

Permeability of a topically applied drug sometimes is influenced by the hydration state of the stratum corneum (2). Therefore, knowing the water concentration profile in the stratum corneum would be helpful in studying the percutaneous absorption of the drug. *In vivo*, water is not evenly distributed across the thickness of the tissue: a concentration gradient exists. On the dermal side of the stratum corneum, the tissue is fully hydrated, whereas on the skin surface, the water concentration is much lower and is regulated by the ambient condition. Therefore, transpiration of water through the stratum corneum is inevitably due to the concentration gradient. From the rate of transpiration and Fick's diffusion equation, one may obtain the water concentration profile in the stratum corneum and thus, the amount of water at different sites within the tissue, if the diffusivity of water in the stratum corneum is known.

Methods have been developed to determine diffusivity as a function of concentration (3). The most commonly used sorption–desorption method, devised by Crank and Park (4), is based on the mass of the membrane as the frame of reference. To obtain a concentration profile across the thickness of a membrane, one may have to use the volume of the membrane as the frame of reference. However, if the change in the volume of mixing between the penetrant and the membrane varies with the concentration, as is the case with water in the stratum corneum (5), then converting a system based on the mass to one based on volume as the reference coordinate could be very difficult, if not impossible.

A method to determine the diffusivity of water in a keratinous membrane is reported here. The method considers the concentration variations of water in the membrane as well as concentration-dependent volume change of the membrane. Fetal hog periderm was chosen as the model tissue for the keratinous membrane as it is easy to obtain in large quantities and is free from the hair usually accompanying stratum corneum.

THEORETICAL

In diffusion-controlled transpiration, if the diffusion follows Fick's laws, then the rate of transpiration (flux) could be expressed by Fick's first law:

$$F = -D\frac{dC}{dx} \qquad \text{(Eq. 1)}$$

where F is the flux (*i.e.*, the rate of transpiration of water across any place in a keratinous membrane), C is the concentration of the diffusion substance (*e.g.*, water), x is the position along the direction of diffusion (position along the thickness of the membrane), and D is the diffusivity of the penetrant. Since D may be dependent on the concentration (C), D is rewritten as $D(C)$ to illustrate the relationship between these parameters. The flux, F, at steady state may be further expressed in an integrated form of Eq. 1 (6):

$$F = \frac{1}{H}\int_{C_h}^{C_o} D(C)dC \qquad \text{(Eq. 2)}$$

where H is the thickness of the membrane and C_h and C_o are the penetrant concentrations in the membrane at the receptor and donor sides, respectively.

The mean diffusivity $\overline{D}$, at the mean concentration $\overline{C}$, is defined as:

$$\overline{D} = \left[\int_{C_h}^{C_o} D(C)dC\right] \Big/ (C_o - C_h) \qquad \text{(Eq. 3)}$$

and by substituting Eq. 2 into Eq. 3, $\overline{D}$ becomes:

$$\overline{D} = FH/C_o - C_h) \qquad \text{(Eq. 4)}$$

$\overline{D}$ may be readily calculated from Eq. 4 using the measurements of F, H, C_o, and C_h. Furthermore, if the difference between C_o and C_h is small, then the mean concentration, $\overline{C}$, can be expressed as:

$$\overline{C} = (C_o + C_h)/2 \qquad \text{(Eq. 5)}$$

Therefore, as a first approximation to determine the empirical equation for $D(C)$, a series of permeation experiments in various concentration ranges can be conducted by keeping the difference of C_o and C_h small in each experiment. From the experimental results, a series of $\overline{D}$ and $\overline{C}$ can be calculated. Plotting $\overline{D}$ *versus* $\overline{C}$, an empirical equation for $D(C)$ which gives the best fit to the experimental data can be determined.

This equation is then inserted into Eq. 1, which can be integrated to give C as a function of x (or *vice versa*). The curve of C *versus* x, *i.e.*, the water distribution profile across the thickness of the membrane, is then integrated to obtain the area under the curve. This area, divided by the membrane thickness (H), yields the mean concentration ($\overline{C}$) of water in the membrane. These calculated values of $\overline{C}$ are then compared with the values of $\overline{C}$ used to obtain $D(C)$. If the assumed and calculated values

0022-3549/83/1200-1421$01.00/0

of $\overline{C}$ are not equal, then the assumed values are adjusted to obtain a new empirical equation for $D(C)$. The adjusted values are again compared with the calculated values. This process is repeated until the two sets of numbers are equal or converge. If more than one functional form for $D(C)$ is obtained from the first approximation, the functional form which results in the best convergence and provides the best fit to the experimental data will prevail in the iteration and is the best equation for $D(C)$.

In the previous calculation, the thickness of the membrane, H, is a known parameter. Its value lies between H_h and H_o (the membrane thickness when the water concentrations in the membrane are C_h and C_o, respectively). Although the relationship between H and C is not known, it may be assumed that:

$$H = (H_o + H_h)/2 \qquad \text{(Eq. 6)}$$

because the difference between C_o and C_h is small.

EXPERIMENTAL

Tissue Preparation—Fetal hog skin was obtained from a commercial source[1]. The periderm was prepared using a procedure reported for human stratum corneum (7). A whole fetal hog skin was immersed in a 60° water bath for ~1 min. The skin was then taken out of the bath, and the periderm was carefully peeled from the tissue.

Determination of Rate of Water Loss (F)—Measurement of the rate of water loss, *i.e.*, the flux (F), through the periderm was carried out using diffusion cells. The cell was half filled with a saturated salt solution to produce a constant relative humidity inside the cell and, hence, a constant water concentration (C_o) at the donor side of the membrane. The cell was placed in a desiccator filled with another saturated salt solution to produce a lower relative humidity outside of the cell and therefore, a constant water concentration (C_h) at the receptor side of the membrane. The desiccator was placed in a constant temperature room at 21°. The rate of water loss at steady state was obtained gravimetrically when the rate became constant. The experiments were carried out in small relative humidity ranges of 0–12%, 12–23%, 23–33%, 33–44%, 44–57%, 57–75%, 75–85%, 75–100%, and 85–100%. The salts used were phosphorous pentoxide, lithium chloride, potassium acetate, magnesium chloride, potassium carbonate, potassium bromide, sodium chloride, and potassium chloride for relative humidities of 0, 12, 23, 33, 44, 57, 75, and 85%, respectively.

Volume of the Membrane—Volumes of the membranes at various relative humidities were determined using air comparison pycnometry (8).

Determination of Water Concentration—Dry periderm was equilibrated in a desiccator at a constant humidity maintained by a saturated salt solution. After each tissue reached a constant weight, the weights of the tissues were taken and, from the weight gain and the volume of each tissue, the concentrations of water in the membranes at various relative humidities were determined.

Determination of Membrane Thickness—The thickness of the membrane at various humidities was obtained from the weight per area and the density of the tissue at each humidity. To do this, a sheet of periderm was placed on a sheet of aluminum foil. This was done by floating the tissue in water and carefully raising the aluminum foil beneath the tissue to bring the tissue out of the water. The tissue was free of wrinkles and could expand and contract freely. The tissue with the aluminum foil was then dried and put in a desiccator with a constant relative humidity. The desiccator and a microbalance were placed in a humidity chamber having the same relative humidity as the desiccator. After the tissue was equilibrated, a piece of the tissue–aluminum foil was cut and its weight was determined with the balance. The tissue was then removed from the aluminum foil, and the weight of the aluminum foil was determined. From the weight difference, the weight of the tissue was obtained; from the weight of the aluminum foil alone and a predetermined weight–area correlation curve for the aluminum, the area of the tissue was determined. Thus, the weight per unit area of periderm at each humidity could be determined.

RESULTS AND DISCUSSION

The rates of water loss (F) at various experimental conditions are listed in Table I. Table II shows the water concentration and the membrane thickness at the various relative humidities of interest. Large standard deviations were found in the rate of water loss and in the thickness measurements. These deviations are probably due to the large variation of the tissue sample used. The water concentration data are fairly consistent. Because each fetal hog provided <0.2 g of the dry periderm, at least 10 animals were required for each volume determination. Inherent variations in the tissues were probably balanced by the large sample size to produce the fairly consistent data in the water concentrations. The mean water diffusivityes, $\overline{D}$, obtained here (4×10^{-10} to 3.2×10^{-9} cm²/sec) are in the range of that for human stratum corneum (9).

From the plot of $\overline{D}$ *versus* $\overline{C}$, four functional forms can be found to provide a reasonable fit to the experimental data (Fig. 1). The four functional forms are $D(C) = D_o - Ae^{-BC}$, $D(C) = D_o + AC^B$, $D(C) = D_o + Ae^{BC}$, and $D(C) = D_o + AC/(1 + BC)$, where D_o, A, and B are constants.

Table I—Rate of Water Loss Through Fetal Hog Periderm at 21° and Various Relative Humidities

Experimental Condition (% RH_h − % RH_o)[a]	Rate of Water Loss, g/cm² sec × 10^8
0–12	0.95 ± 0.17 (6)[b]
12–23	1.69 ± 0.83 (7)
23–33	2.36 ± 1.08 (7)
33–44	3.88 ± 1.92 (9)
44–57	6.98 ± 1.92 (9)
57–75	10.73 ± 4.87 (11)
75–85	8.64 ± 0.55 (6)
75–100	20.54 ± 6.02 (6)
85–100	11.89 ± 4.43 (11)

[a] % RH_h and % RH_o were the relative humidity conditions under which C_h and C_o were determined. [b] Number of samples in parentheses.

Table II—Water Concentration and Thickness of Fetal Hog Periderm at Various Relative Humidities

Relative Humidity, %	Water Concentration, g/cm³	Membrane Thickness, cm × 10^4
0	0	10.1 ± 3.31
12	0.024	10.3 ± 4.92
23	0.043	10.5 ± 1.50
33	0.059	10.6 ± 3.31
44	0.084	10.9 ± 2.40
57	0.116	11.4 ± 2.32
75	0.173	11.8 ± 4.54
85	0.223	16.5 ± 5.95
100	0.290	24.3 ± 9.86

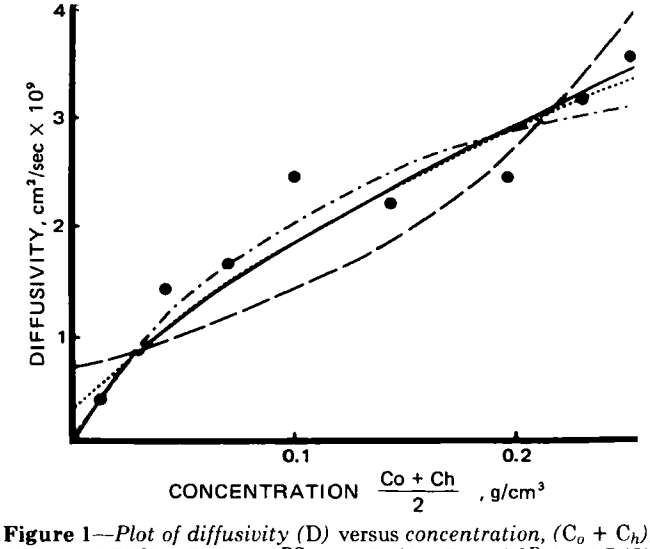

Figure 1—*Plot of diffusivity* (D) versus *concentration*, $(C_o + C_h)/2$. *Key:* (·····) $D(C) = D_o - Ae^{-BC}$; (——) $D(C) = D_o + AC^B$; (- - -) $D(C) = D_o + Ae^{BC}$; (-·-·-) $D(C) = D_o + AC/(1 + BC)$.

[1] Texas Biological Inc., Dallas, Tex.

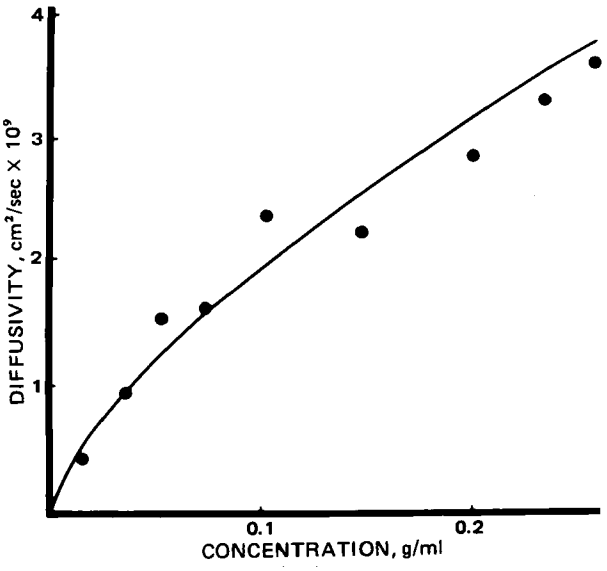

Figure 2—*Comparison of experimental data and the diffusivity equation. Key: (●) experimental data; (—)* $D(C) = 1.0 \times 10^{-18} + 9.70 \times 10^{-9} C^{0.69}$.

A computer was used to carry out the successive iterations. In the calculation, the equations were linearized. The value of D_o was determined by trial and error; values for B and A were obtained from the slope and the intercept of the linearized equation. In the iteration, the concentrations used were restricted between C_h and C_o. At the end of the iteration, $D(C) = D_o + AC^B$ was found to give the best convergence of the assumed and calculated mean concentrations and to provide the best fit to the experimental data (Fig. 2). The final equation for $D(C)$ is:

$$D(C) = 1.0 \times 10^{-18} + 9.70 \times 10^{-9} C^{0.69} \quad \text{(Eq. 7)}$$

The large standard deviation associated with the measured thickness, H (Table II), raised concern about the appropriateness of the approximation for H. Therefore, the iteration process was altered. The mean water concentration $\overline{C}$, was fixed as $(C_o + C_h)/2$ while the assumed values for H were adjusted in the iteration until they converged with the calculated values. The resulting equation is shown below:

$$D(C) = 1.0 \times 10^{-18} + 10.0 \times 10^{-9} C^{0.72} \quad \text{(Eq. 8)}$$

Comparison of Eq. 8 with Eq. 7 shows that they are not significantly different. Therefore, Eq. 7 is a reasonable representation for the diffusivity of water in fetal hog periderm.

The method developed here provides an alternative way to obtain the diffusivity as a function of the penetrant concentration. With the appropriate experimental designs and the data analyses mentioned herein, this method can circumvent some of the difficulties associated with the other methods of obtaining diffusivity.

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