

Some aspects of the stratum corneum-organic solvent system

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Synopsis

A limited study of the SORPTION, DESORPTION, and DIFFUSION behavior of some ORGANIC VAPORS (benzene and toluene) in intact STRATUM CORNEUM has been carried out, and the results compared to WATER VAPOR. The effect of treating the corneum in a mixture of ORGANIC SOLVENTS (chloroform-methanol) followed by water extraction, on the water vapor sorption, desorption and diffusion characteristics has been assessed. Scanning electron microscopy (SEM), was also used to examine the structural changes in the corneum resulting from the organic solvents-water treatment.

The shape of the benzene and toluene sorption ISOTHERMS was found to be compatible with type II in the BET classification. The diffusion process of the organic vapors in the corneum is much faster than that of water vapor. An increase by 3 orders of magnitude in the value of D_i (intrinsic diffusion coefficient) has been observed as the concentration of organic vapor increases in the corneum.

Sequential treatment of GUINEA PIG corneum in organic solvent and water resulted in a marked decrease in the water vapor sorption capacity in the high humidity range. The initial portions of the water vapor sorption isotherms on the treated and intact corneum are the same (up to about 50 per cent RH). The diffusion of water vapor in the treated corneum is 10 times as fast as in the intact corneum.

INTRODUCTION

The hydration of stratum corneum has been the subject of a number of investigations (1-5). We have recently examined in detail the sorption, desorption, and diffusion behavior of water vapor in a number of keratinous materials including stratum corneum obtained from excised human skin, guinea pig and neonatal rat corneum, and human hair (6-7).

In this paper, we present the results of a limited investigation into the sorption, desorption, and diffusion characteristics of benzene and toluene vapors in human stratum corneum, and compare these to the results obtained with water vapor. We have also examined the effect of solvent damage (chloroform/methanol 2:1) on the water vapor sorption and diffusion in guinea pig stratum corneum in an attempt to identify the role of the physical structure of the corneum.

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Reviews of previous work on the permeability of skin to organic materials have been presented by Scheuplein and Blank (8) and Tregear (9). Scheuplein and Blank (8) report some data on the sorption and diffusion behavior of a series of *n*-alkanes and alcohols in stratum corneum. King and Cassie (10) studied the vapor sorption and diffusion of methanol in horn keratin and wool and of ethanol in wool.

Acetone, ether, hexane, and other common solvents were found to damage the skin, but the most effective solvents were found to have both polar and nonpolar character and the most potent of all appear to be mixed solvents like chloroform-methanol (2:1) and ether-ethanol (10:1) (1, 4, 11, 12).

In our work, we have assessed the effect of treating guinea pig corneum in the chloroform-methanol mixture in two ways: (1) comparison of the water vapor sorption and diffusion characteristics in a treated and untreated sample; and (2) examination of the treated corneum in the scanning electron microscope (SEM).

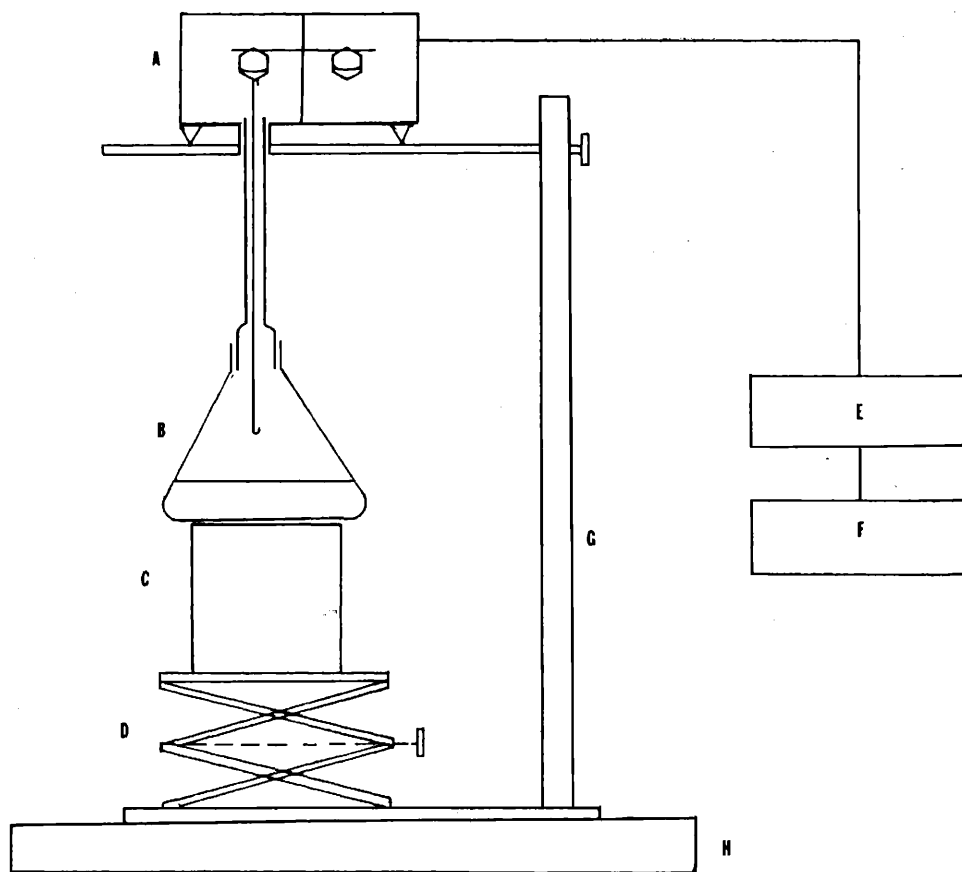


Figure 1. Gravimetric technique for vapor sorption and desorption

EXPERIMENTAL

The gravimetric technique used to study benzene and toluene vapor sorption or desorption is shown in Fig. 1. The method employs a recording microbalance and stirred organic solutions to generate the required relative vapor pressure. The corneum sample was hung on a very thin glass rod, which was suspended from the microbalance terminal (A) into a 500 ml flask containing the organic vapor system (B). The distance between the sample and the organic solution was maintained at about 1 in. To eliminate draughts, the glass rod was shielded by a glass tube, the upper end of which fitted tightly into the base of the microbalance, while the lower end was connected to a ground glass joint. The flask was placed on a small magnetic stirrer (C), which gently rotated a small magnetic bar placed in the solution. The magnetic stirrer was placed on a regular lab jack (D), which could be conveniently raised or lowered to allow quick change of flasks containing solutions of varying vapor pressures. The microbalance was placed on a specially constructed base which was secured to a stand (G). The whole set-up was placed on antivibration base (H) in a constant temperature and humidity room (23°C, 55 per cent RH). In Fig. 1, E and F depict the balance control of a Beckman*-microbalance L-600 and a chart recorder, respectively. This method was also used to examine the sorption, desorption, and diffusion of water vapor in keratins using saturated salt solutions. The salt solutions used to generate the required relative humidities at 23°C are as follows:

Lithium chloride	11.5 per cent
Potassium acetate	21.5 per cent
Sodium iodide	38.0 per cent
Sodium dichromate	54.0 per cent
Copper chloride	68.0 per cent
Ammonium chloride	78.0 per cent
Potassium chromate	87.0 per cent
Ammonium dihydrogen phosphate	93.0 per cent

A flask containing drierite provided zero humidity. The partial pressure P of the organic solvents was controlled by using solutions of benzene or toluene in hexadecane, the latter being essentially nonvolatile. According to Raoult's law, assuming ideality

$$P = P^0 X$$

where P^0 is the vapor pressure of pure benzene or toluene and X is the mole fraction of benzene or toluene in the hexadecane.

The sorption experiments were carried out in the relative vapor pressure range from about 0.3 to 0.94. Lower relative vapor pressure conditions were employed in a number of cases. The stratum corneum samples were initially dried over Drierite to obtain a base weight. The experimental approach was as follows: after drying out the corneum, it was exposed to the organic vapor atmosphere of a given relative vapor

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pressure and the kinetics of vapor sorption was monitored continuously until equilibrium was achieved. At this point, desorption was started by replacing the organic solvent system with activated charcoal or Drierite. Both drying systems were found to be equally effective. Experiments were carried out at 23 and 32°C. Measurements at the higher temperature were carried out using the technique described in (6).

Stratum corneum was prepared according to the procedure suggested by Kligman and Christopher (13) and described in detail in (14). The treatment of the stratum corneum in organic solvents was conducted as follows: a piece of guinea pig stratum corneum was placed between 2 small pieces of saran gauze held in a specially constructed Teflon®* frame. The frame was placed in a beaker containing the chloroform-methanol mixture (2:1), maintained at 40°C, for 30 min with gentle agitation. This was followed by immersing the frame in a beaker containing distilled water maintained at the same temperature for another 30 min. The corneum was then taken out and dried at room temperature before storing it in the refrigerator. Although, no quantitative estimate was made, it was observed that the above described treatment brought about a substantial decrease in the dry weight of the corneum (~ 30 to 50). The treated corneum was examined for its water vapor sorption and diffusion properties, which were compared to data on intact (untreated) guinea pig corneum, obtained earlier.

In order to acquire some information on possible structural changes in the corneum samples, a preliminary SEM examination was conducted. The sample preparation for the SEM was as follows. Pieces of the stratum corneum were mounted on stubs using transfer tape. The stubs were then placed into a vacuum evaporator and coated with 5 nm of carbon followed by 30 nm of 80:20 gold/palladium from two angles.

DISCUSSION

Quantitative analysis of equilibrium sorption isotherms of water vapor in keratin has been described in detail (6). Briefly, a number of theories and equations were employed, including the BET and D'Arcy-Watt equations, the Flory-Huggins polymer solution theory, and Zimm's clustering function. In general, the water vapor sorption isotherms on excised skin and hair were found to fit the D'Arcy-Watt eq. (16).

$$W = \sum_{i=0}^l \frac{A_i B_i (P/P_0)}{1 + B_i (P/P_0)} + C(P/P_0) + \frac{DE(P/P_0)}{1 - E(P/P_0)} \quad (1)$$

where W is the weight of sorbate adsorbed by 1 g of sorbent; $A_i = mn_i/N$ is the number of primary sites of type i , multiplied by the molecular weight of sorbate and divided by Avogadro's number N ; B_i is a constant which is a measure of the attraction of the sites for the sorbate; l is the number of different types of sorption sites for primary adsorption described by a Langmuir isotherm; C is a constant for the linear ap-

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proximation to Langmuir adsorption on specific sites; and D and E are constants describing secondary adsorption processes. P/P_0 is the relative vapor pressure (relative humidity) of the sorbate.

The results of the analysis indicate that in the low relative humidity range (0 to 30 per cent RH) sorption of water vapor occurs on reactive sites. Multilayer formation, accompanied by extensive clustering, occurs at the high relative humidity end of the sorption isotherm. Clustering was evaluated according to Zimm's Eq. (17):

$$\frac{G_{AA}}{V_A} = -\phi_B \left[\frac{\partial(a_A/\phi_A)}{\partial a_A} \right] PT^{-1} \quad (2)$$

where G_{AA} is the cluster integral for water molecules; and V_A and a_A denote, respectively, the partial molar volume and the activity (relative humidity) of water. ϕ_A and ϕ_B are the volume fractions of components water and keratin.

Examination of the sorption/desorption isotherms showed that hysteresis is not a general phenomena in keratins, and was observed in a number of samples only. In general, the sorption isotherm was shown to exhibit the equilibrium properties required for thermodynamic treatment.

A background review of the mathematics of diffusion, and of the application of the principles developed by Crank and coworkers to the problem of water vapor diffusion in swelling keratins (for example, stratum corneum and human hair), has been given in (7). Determination of the diffusion coefficient is based on accurate measurements of the kinetics of vapor sorption or desorption in a small sheet of stratum corneum according to the following equation:

$$\frac{M_t}{M_\infty} = 1 - \sum_{n=0}^{\infty} \frac{8}{(2n+1)^2 \pi^2} e^{-D(2n+1)^2 \pi^2 t/4l^2} \quad (3)$$

where M_t denotes the total amount of diffusing substance which has entered the sheet at time t , and M_∞ the corresponding quantity after infinite time. Equation (3) is the exact solution of Fick's basic diffusion equation, assuming a constant D , for the boundary conditions

$$\begin{aligned} C &= C_0 & 0 < X < l & & t = 0 \\ C &= C_1 & X = 0 \text{ and } X = l & & t > 0 \end{aligned}$$

where C_0 is the initial concentration of water in the sheet when the surfaces ($X = 0$ and $X = l$) are exposed to a constant concentration C_1 of vapor. The boundary conditions describe sorption or desorption depending on the values of C_0 and C_1 .

A simple method based on eq. (3) for the determination of a mean value \bar{D} (when D is not a constant) has been suggested by Crank (18). The value of t/l^2 for which $M_t/M_\infty =$

$1/2$ (designated as $(t/l^2)_{1/2}$ or simply half-time) is determined experimentally according to the relationship

$$\bar{D} = \frac{0.04919}{(t/l^2)_{1/2}} \quad (4)$$

More accurate values for \bar{D} are obtained when the average of sorption \bar{D}_s and desorption \bar{D}_d is taken. Our findings indicate that the diffusion properties of the water vapor-stratum corneum system can be characterized by a concentration-dependent diffusion coefficient. A detailed analysis of the behavior of the diffusion coefficient as a function of the water content in stratum corneum shows that in highly swelling samples, a maximum is observed in the relationship between the mean diffusion coefficient (\bar{D}) and water content. If the mean diffusion coefficient is corrected for swelling (intrinsic diffusion coefficient [D_i])

$$D_i = \frac{\bar{D}}{(1 - V)^3} \quad (5)$$

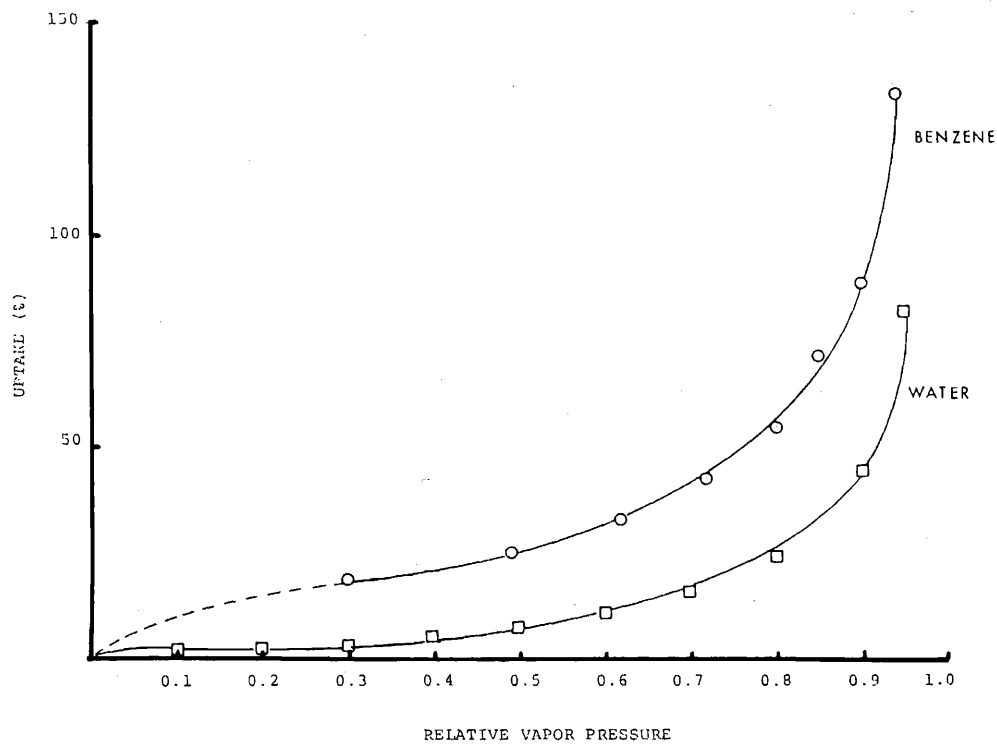


Figure 2. Sorption isotherms of benzene and water vapor on female stratum corneum, age 20, at 23°C

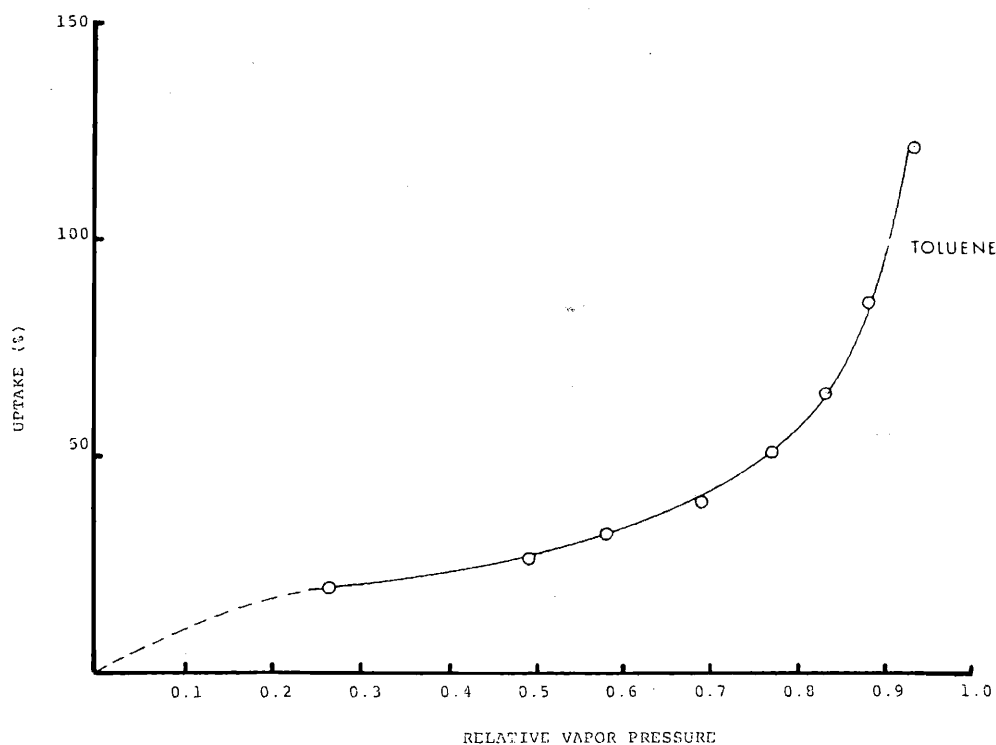


Figure 3. Sorption isotherm of toluene vapor on female stratum corneum, age 20, at 23°C

where V is the volume fraction of water, it was found that the diffusion coefficient usually shows a continuous increase with increase in water content. It was thought of interest to extend our investigations to some organic vapor systems to improve our understanding of the physical and chemical factors associated with sorptive and barrier properties of the stratum corneum.

The benzene and toluene vapor sorption isotherms of female stratum corneum (age 20), are shown in Figs. 2 and 3. The uncertainty in the uptake values at the high end of the isotherms is quite high (± 15 per cent at the highest relative vapor pressure); nevertheless, it is clear that the sorption isotherms are of type II according to the BET classification. Similar sorption isotherms were obtained for other human corneum samples and the results generally indicate a higher sorption capacity for the organic vapor. However, if the uptake values are expressed in moles rather than the conventional weight increase in grams per 100 gm corneum, it was found that the organic vapor (benzene, toluene) sorption values were generally higher than those obtained for water up to about 0.5 relative vapor pressure then progressively fell below the water uptake values. These preliminary findings seem to indicate that there are more binding sites available for benzene or toluene in the corneum samples examined in the vapor range where the sorption process is presumed to follow a Langmuir model. At higher relative vapor pressure, formation of multilayers and clusters of sorbate molecules may occur, hence no binding between the sorbate molecules and the substrate. These data seem to

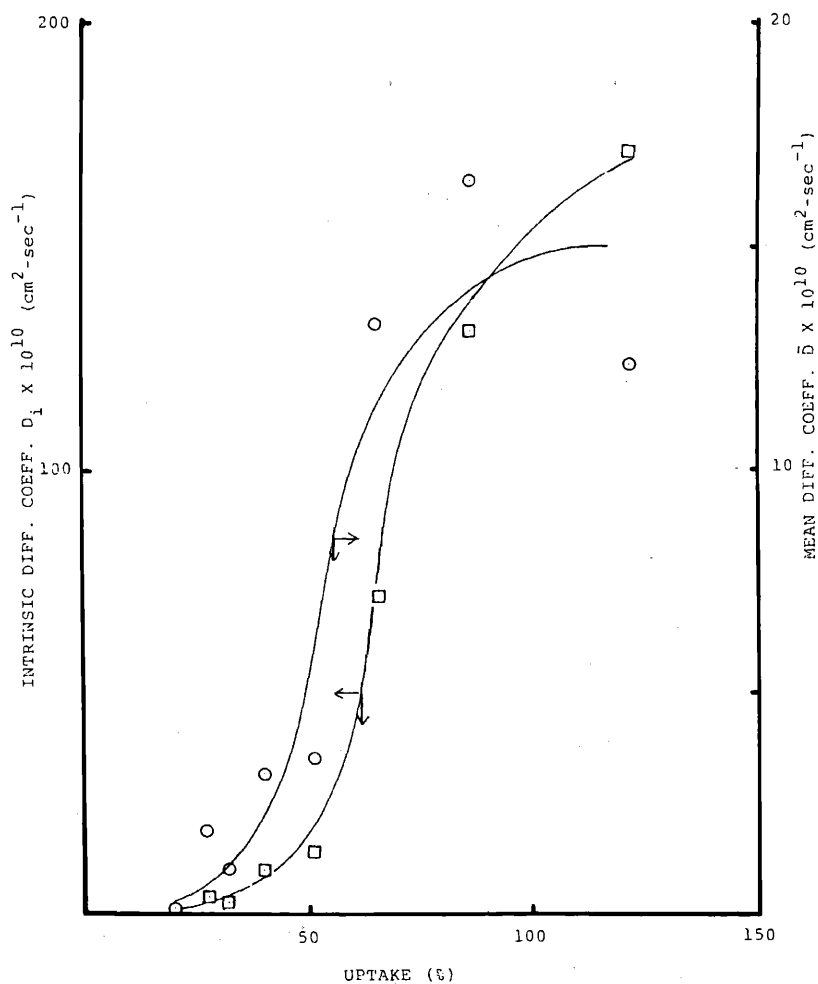


Figure 4. (\square) intrinsic (D_i); and (\circ) mean (\bar{D}) diffusion coefficient of toluene vapor in stratum corneum (female, age 20) as a function of concentration, at 23°C

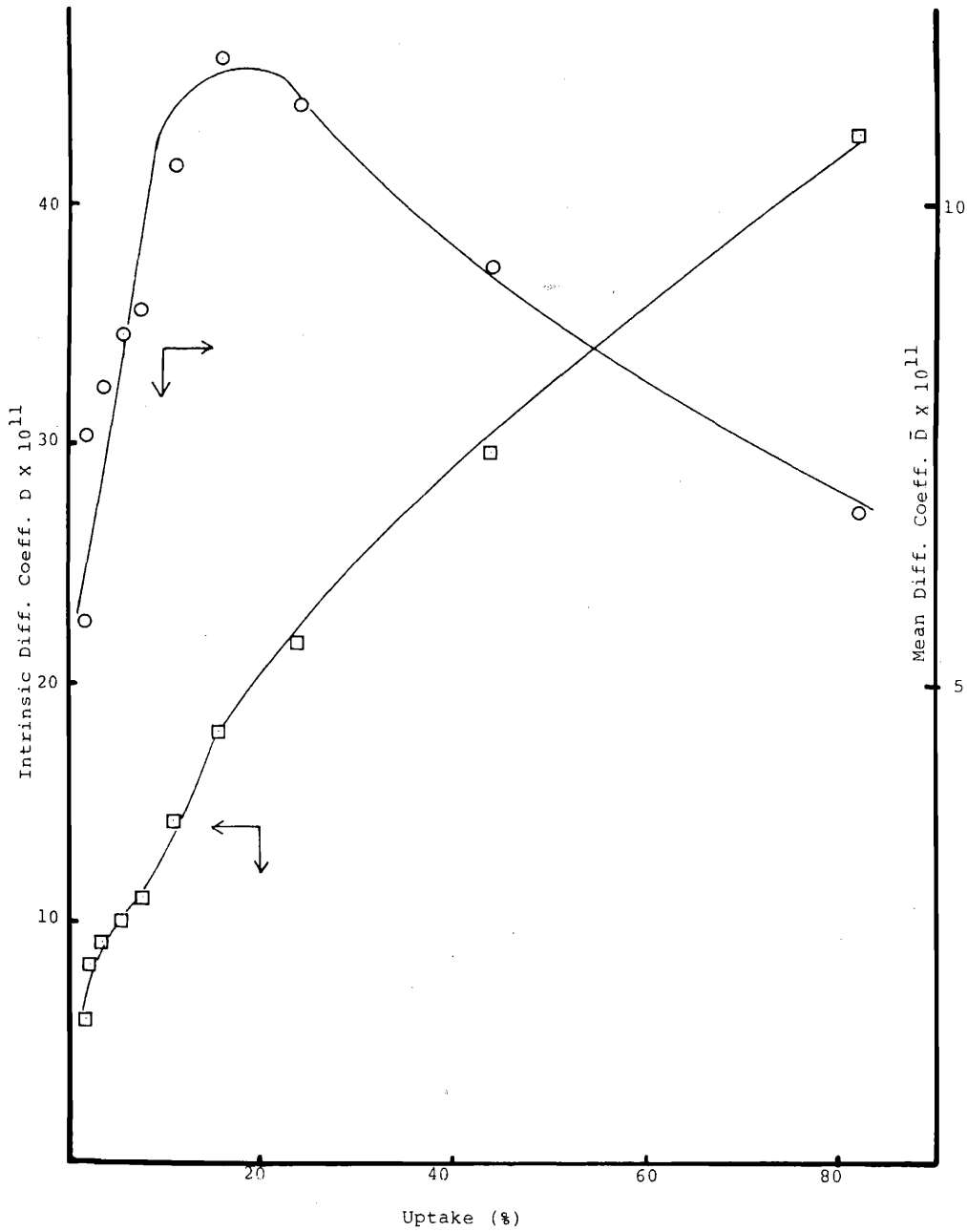


Figure 5. (□) intrinsic (D); and (○) mean (\bar{D}) diffusion coefficient of water vapor in stratum corneum (female, age 20) as a function of concentration at 32°C

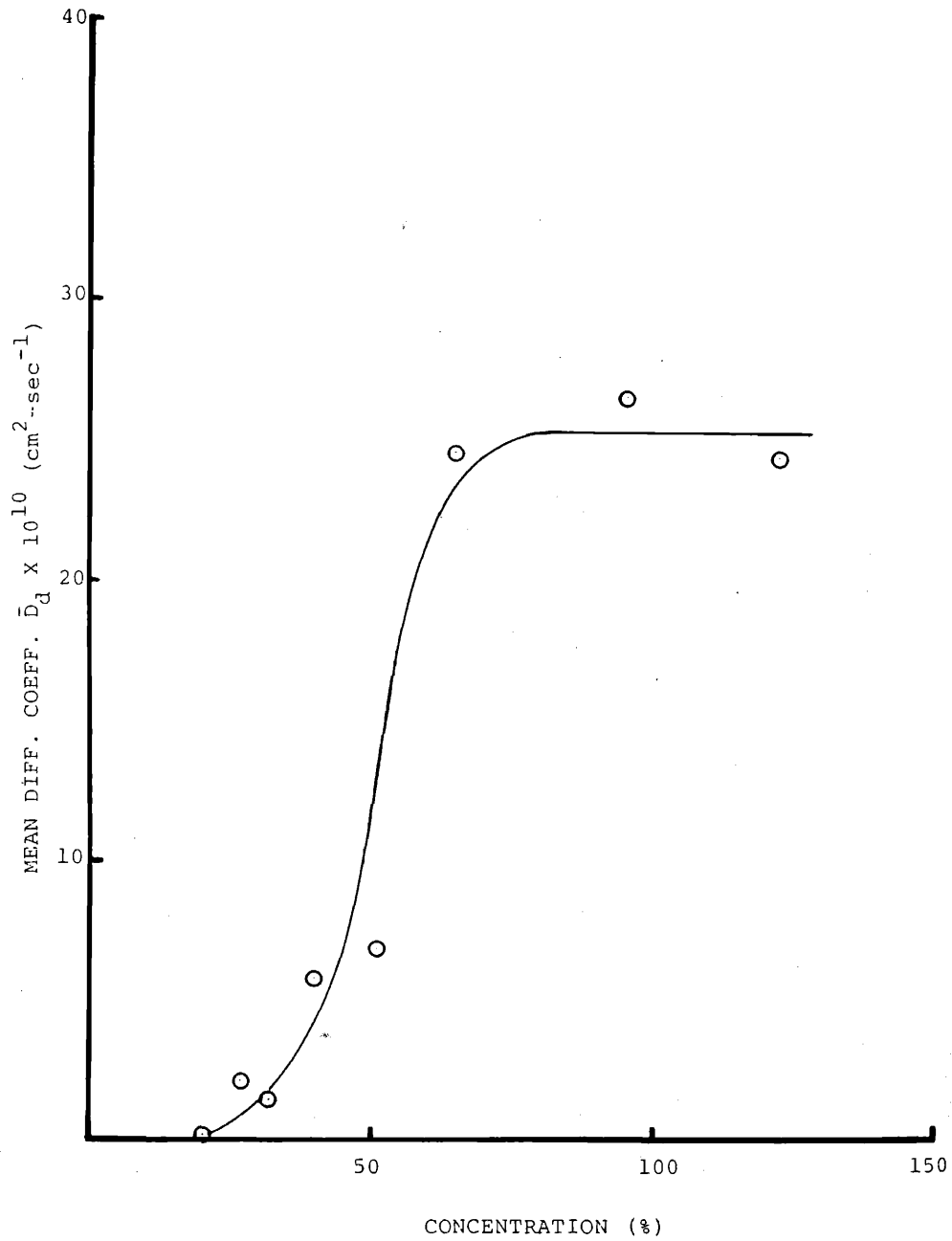


Figure 6. Mean desorption diffusion coefficient (\bar{D}_d) of toluene vapor in stratum corneum (female, age 20) as a function of concentration, at 23°C

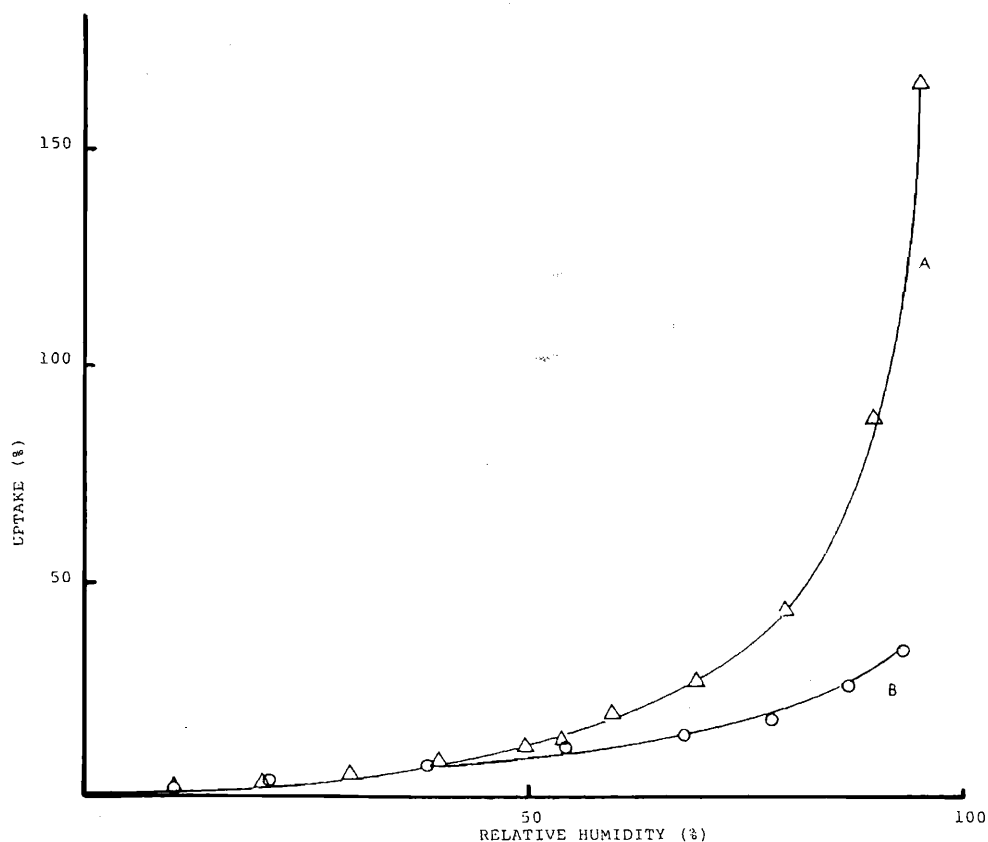


Figure 7. Sorption isotherm of water vapor in untreated (curve A) and organic solvent-water treated (curve B) stratum corneum (guinea pig), at 32°C

confirm our earlier conclusions, based on the wetting behavior of human stratum corneum and hoof keratin, that these keratinous materials are, on balance, hydrophobic in nature (15).

The diffusion process of toluene vapor in the stratum corneum is also quite interesting. The graphs of the intrinsic and mean diffusion coefficients as a function of concentration are shown in Fig. 4. It can be seen that, as the concentration of toluene in the stratum corneum increases, the diffusion coefficient (D_i or \bar{D}) increases by three orders of magnitude in the range of relative vapor pressure examined. In the case of water vapor sorption, the observed increase of D with C was one order of magnitude at most, as shown in Fig. 5. Starting from the high concentration end, the desorption process is marked by a constant mean diffusion coefficient \bar{D}_d as shown in Fig. 6; this is followed by a sharp decrease in the value of \bar{D}_d . The constancy in the \bar{D}_d values can be interpreted as reflecting the evaporation process of the "free" toluene in the corneum, and the sharp decrease in the diffusion coefficient marks the start of desorption from high energy binding sites.

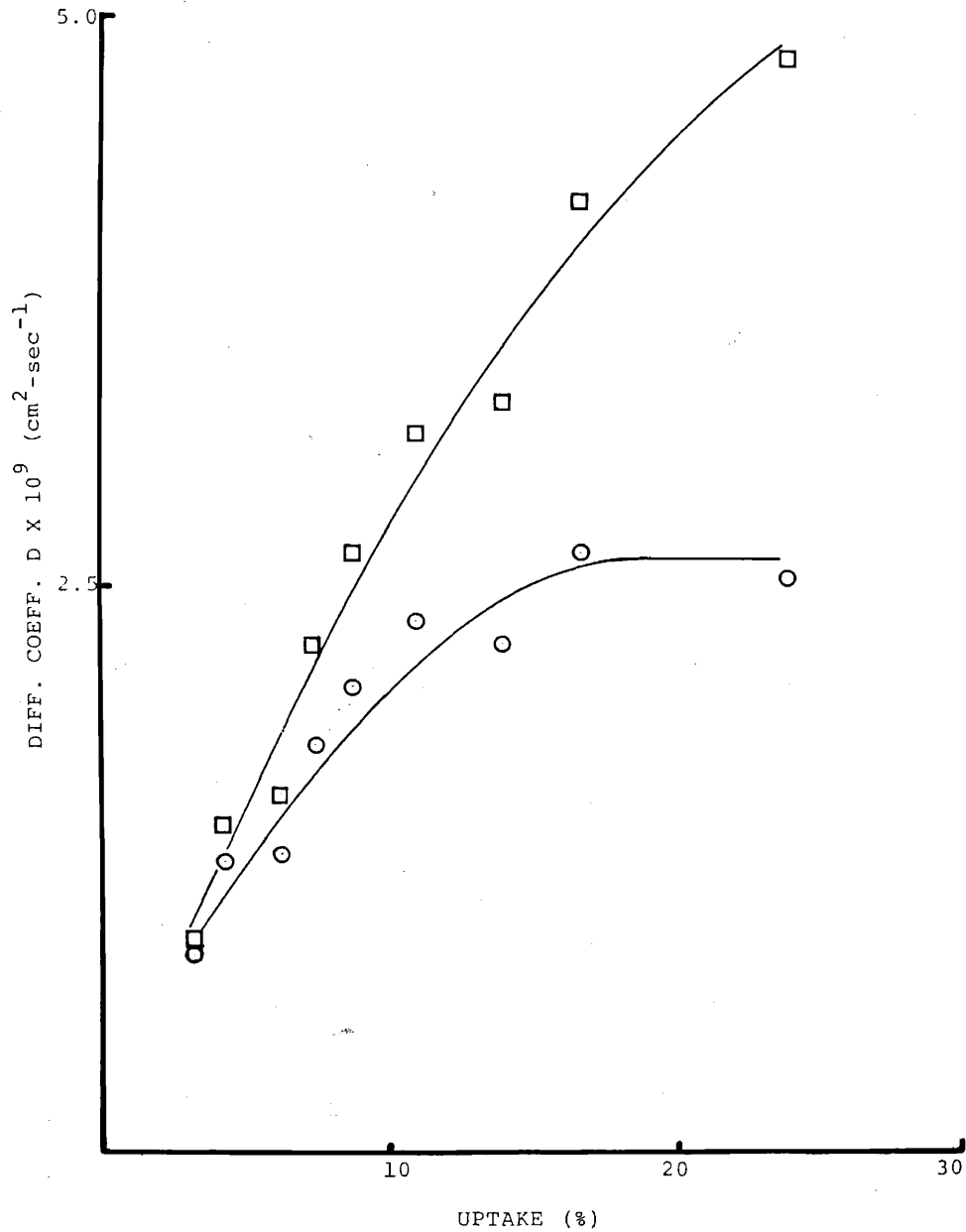


Figure 8. (□) intrinsic and (○) mean diffusion coefficient of water vapor in organic solvent-water treated guinea pig corneum, at 32°C

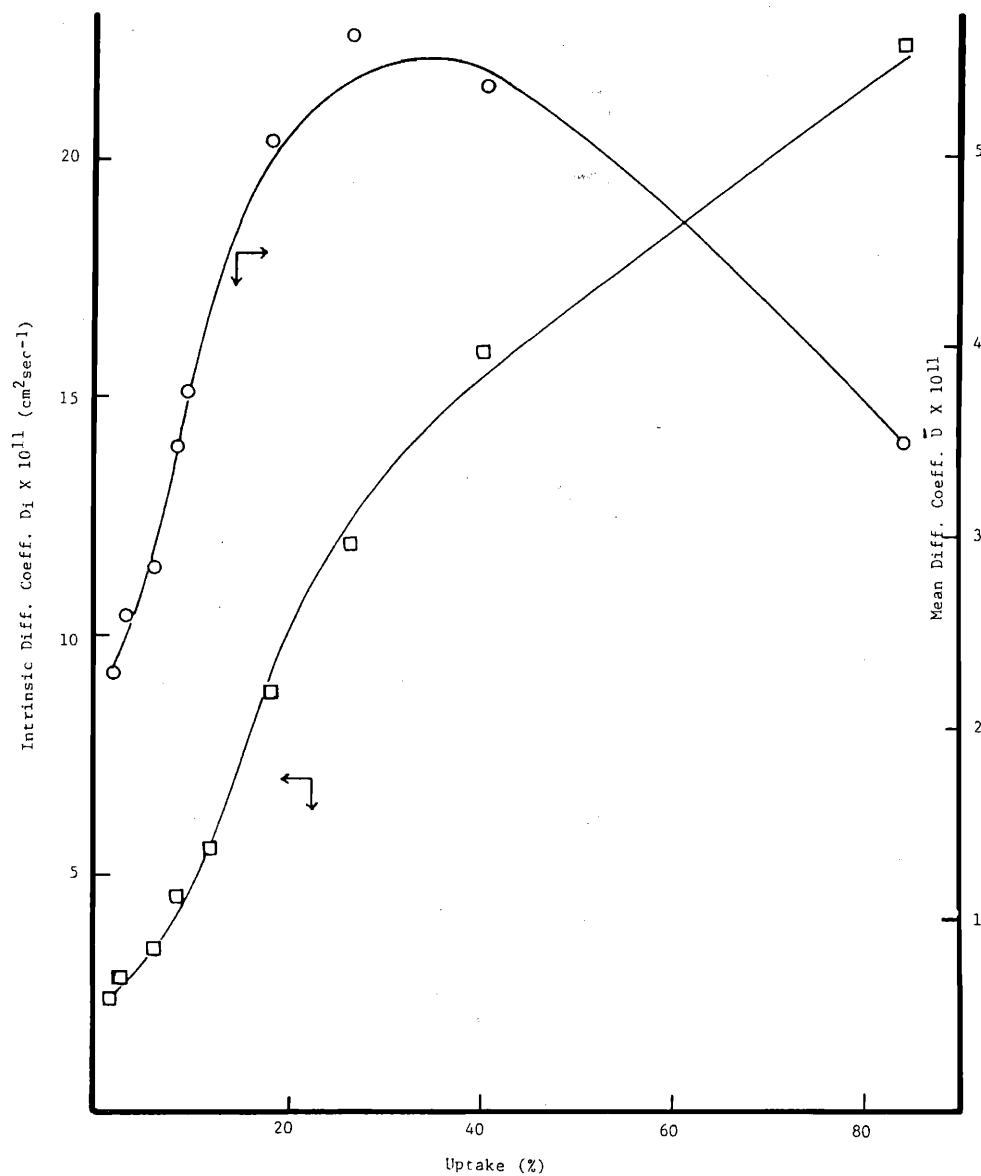


Figure 9. D_1 and \bar{D} of water vapor in untreated guinea pig corneum at 32°C

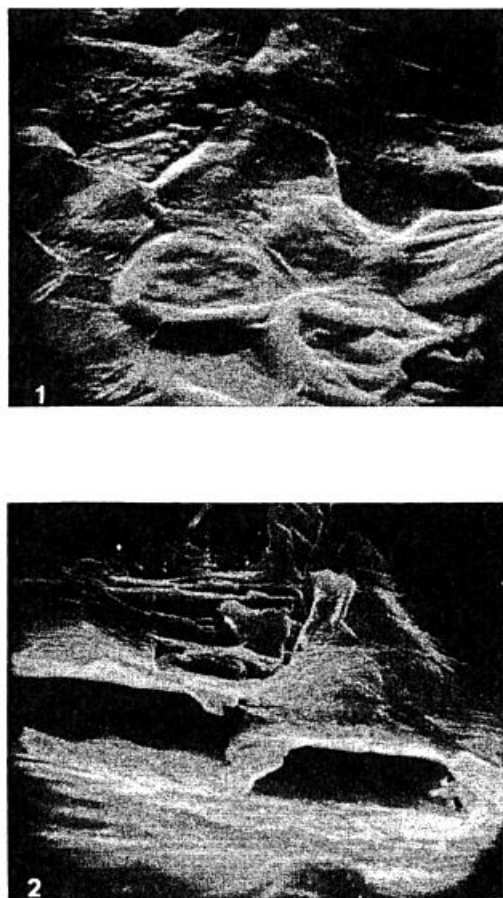


Figure 10. (1) Untreated guinea pig corneum, 200 \times , (2) guinea pig corneum treated in 2:1 chloroform-methanol mixture, followed by extraction in distilled water, 200 \times

The effect on the water vapor sorption isotherm of treating guinea pig corneum in a 2:1 chloroform-methanol mixture, followed by extraction in distilled water, is shown in Fig. 7. Curve A depicts the water vapor sorption isotherm for intact (untreated) corneum, and curve B depicts the effect of the organic solvents-water treatment. Similar results for this system have previously been reported by Singer and Vinson (4). It is seen that the initial portions of the isotherms up to about 50 per cent RH almost coincide, but the two curves markedly diverge beyond 70 per cent RH. A total number of 4 water vapor sorption-desorption isotherms were obtained on different corneum pieces from the same guinea pig sample, treated in the organic solvents and water. The reproducibility of the data points on the isotherms was within 1 per cent of the mean. The substantial decrease in the water vapor sorptive capacity of the treated guinea pig corneum in the higher humidity range indicates a marked change in the corneum structure which affects primarily the formation of multilayers. The extraction of some materials and, perhaps, disruption of molecular bonds could conceivably result in a more open

matrix structure and, hence, decrease the possibility of multilayer formation. It is also expected that the diffusion of water vapor molecules would be faster in such a modified structure. This is evidenced by the diffusion coefficient-concentration relationship, shown in Fig. 8 as the intrinsic diffusion coefficient D_i and mean diffusion coefficient \bar{D} against percentage water vapor uptake. An average increase of an order of magnitude in the value of D is observed as a result of the solvent treatment. Compare Fig. 9 which shows data for the untreated corneum. The effect of treatment was also assessed with the help of SEM. Figure 10 (1) and (2) provide a comparison between intact (untreated) and treated guinea pig corneum. The effects of the organic solvent treatment are quite apparent in (2), as judged by the extent of cleavage and disruption of the cellular matrix.

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