

SARIN TRANSPORT ACROSS EXCISED HUMAN SKIN III. THE EFFECT ON SOLVENT PRETREATMENT ON ADSORPTION AND DESORPTION BY KERATIN

LLOYD E. MATHESON, Jr., DALE E. WURSTER and JAMES A. OSTRENGA *

College of Pharmacy, University of Iowa, Iowa City, Iowa 52242 (U.S.A.)

(Received September 17th, 1979)

(Revised version accepted December 4th, 1979)

SUMMARY

Keratin powder was pretreated with either methyl *o*-formate, *p*-dioxane, or dimethyl sulfoxide in order to determine the effect on the adsorption and desorption of sarin. Heats of adsorption and heats of activation for adsorption and desorption were determined. A rank order correlation was observed between the heat of activation for desorption and previously reported activation energies for transport through callous membranes.

INTRODUCTION

Many factors have been shown to affect the penetration rate of various chemicals through the skin. Much of this work has been recently reviewed by Idson (1975). Very few workers, however, have considered the effects of adsorption and desorption of the penetrant by the membrane on the transport process. Wurster et al. (1960, 1961) and Dempski et al. (1963) showed that the rate of permeation through callous membranes of highly adsorbed lipid-soluble substances was slowed down significantly. Studies (Kakemi et al. 1969a, 1969b) on other types of membranes, such as gastrointestinal mucosa, have also shown that binding of drugs to tissues at the site of absorption was an important factor affecting penetration rate.

Previously, work in these laboratories (Wurster et al., 1979) has examined the permeability and adsorption characteristics of sarin and anhydrous callous tissue. Subsequent studies (Matheson et al., 1979) have examined the effect of pretreatment of callous membranes with substances such as dimethyl sulfoxide, dimethylacetamide, dimethylformamide, formamide, *p*-dioxane or methyl *o*-formate on sarin penetration

* Present address: Pharm. Chem., Palo Alto, Calif. 94301, U.S.A.

rate. The purpose of this study was to determine a possible mechanism for these solvent effects by examining the consequences of solvent pretreatment on the adsorption and desorption characteristics of sarin on keratin powder.

MATERIALS AND METHODS

Colorimetric assay

This procedure was a modification of the Schoenmann method (Gehauf et al., 1957) for sarin and was described in an earlier report (Wurster et al., 1979).

Gas chromatographic assay

The gas chromatograph¹ was equipped with a phosphorus director (Giuffrich, 1964) in order to detect sarin. Traps containing molecular sieve² were used in all gas lines. The 5 ft by 0.125 in. o.d. stainless steel column was filled with 15% STAP on 80/100 acid washed DMCS-treated solid support³. The gas flow rates were: hydrogen, 16 ml/min; nitrogen, 24 ml/min and air, 175 ml/min. Column temperature was 125°C. The injection vol. was always 3 μ l. An absolute calibration curve was established to ensure a linear response by injecting several known sarin concentrations. Each day a base value for the daily response of the gas chromatography was obtained by averaging the peak heights of 5 replicate injections of a known sarin standard. An external standardization procedure was utilized in which every 3 unknown replicate injections were bracketed by single injections of the same standard used to obtain the daily response factor. The ratio of the daily response factor to the average peak height of the external standard injections produced a detector response factor which when multiplied by the average height of the unknown sample injections corrected for changes in detector response.

Preparation of keratin powder

Anhydrous callous tissue was pulverized in a blender⁴ with the aid of dry-ice. The dried powder that passed through a standard no. 325 sieve was used for all adsorption studies and was extracted with anhydrous ether for 48 h in a soxhlet extractor followed by air-drying. Average particle size was determined at 3 porosities (0.55, 0.50, 0.46)⁵. The powder was stored in a desiccator over anhydrous calcium sulfate⁶.

Solvent pretreatment of powder

The keratin powder was shaken in the appropriate solvent for one hour at 25°C after which it was retained on a Büchner funnel and washed with 100 ml of *n*-heptane containing the pretreatment solvent. The powder was dried by means of air drawn through a column of anhydrous calcium sulfate with the aid of a vacuum pump. This continued

¹ Aerograph Hi Fi Model 600-C, Varian Aerograph, Walnut Creek, Calif. 94598, U.S.A.

² Fisher Type 5A, Fisher Scientific, Pittsburgh, Pa. 15219, U.S.A.

³ Chromosorb W, Applied Science Laboratories, State College, Pa. 16801, U.S.A.

⁴ Waring, Waring Products, Winsted, Conn. 06098, U.S.A.

⁵ Fischer Subsieve Sizer, Fisher Scientific, Pittsburgh, Pa. 15219, U.S.A.

⁶ Drierite, W.A. Hammond Drierite, Xenia, Ohio 45385, U.S.A.

until the powder was dried to a constant weight, which was checked using a thermogravimetric analyzer⁷.

Adsorption isotherms

A weighed quantity of solvent-treated, powdered keratin and 5 ml of a known concentration of sarin in *n*-heptane containing the pretreatment solvent (50 ml to 950 ml of *n*-heptane) were shaken⁸ for 3 h at 25°C. After equilibration, at least three 1 ml samples were removed and analyzed colorimetrically. At the higher sarin concentrations three 1 : 25 dilutions were made and three 1 ml samples were analyzed from each flask.

Desorption isotherms

The amount of sarin adsorbed per gram of keratin powder was determined at 25, 35 and 45°C for the *p*-dioxane and methyl *o*-formate systems by immersing a known weight (~2 g) of solvent-treated keratin in 10 ml of a known concentration (~44 mg/l) of sarin in solvent modified *n*-heptane (50 ml of either *p*-dioxane or methyl *o*-formate to 950 ml *n*-heptane) as described earlier. The adsorbate was filtered with the aid of a slight positive pressure through a fritted glass disc built into the adsorption-desorption flask. The adsorbate solution was removed as completely as possible with air pressure. Desorption began by resuspending the powder with 10 ml of the appropriate fresh solvent system at the temperature of the adsorption experiment. Shaking was stopped at suitable times for 1 min prior to removing about 100 µl of solution. Samples were kept in gas chromatographic sample storage flasks in a freezer and were allowed to return to room temperature before injecting 3 µl samples into the gas chromatograph.

The desorption of sarin from dimethyl sulfoxide-treated keratin powder was studied at 20, 25 and 35°C. Changes in the experimental procedure were necessitated by the presence of dimethylsulfoxide in the system, which caused the phosphorus detector to lose sensitivity and behave non-linearly. Consequently, the colorimetric procedure was utilized. Adsorption and desorption proceeded as just described except that 25 ml of solvent was used and 1 ml samples were withdrawn for analysis.

Treatment of desorption data

Data were submitted to a non-linear least-squares estimation and fitted to a first order kinetic model (Patrick et al., 1961; Alexander et al., 1949).

RESULTS AND DISCUSSION

In order to study the effect of the adsorption of sarin by the keratin on the diffusion process, both adsorption and desorption were studied. It was assumed that the solvent and adsorbate do not compete for the same adsorption sites. Since adsorption may be considered as a reversible equilibrium process,

$$K = \frac{k_a}{k_d} = \frac{C_{(\text{powder})}}{C_{(\text{bulk})}} \quad (1)$$

where *K* is the equilibrium constant; *k_a* and *k_d* are the specific rate constants for adsorp-

⁷ Dupont Model 950 Thermogravimetric Analyzer, Dupont, Wilmington, Dela. 19898, U.S.A.

⁸ Incubator Shaker, Eberbach, Ann Arbor, Mich. 48106, U.S.A.

tion and desorption, respectively, $C_{(\text{powder})}$ is the mg/ml adsorbed and $C_{(\text{bulk})}$ is the mg/ml remaining in the bulk solution after equilibration. At temperatures T_1 and T_2 , for equal weights of adsorbent and equal vols. of bulk solution we have

$$\frac{K_2}{K_1} = \frac{X_2}{X_1} \quad (2)$$

where K_1 and K_2 are the equilibrium constants and X_1 and X_2 are the amounts adsorbed at temperatures T_1 and T_2 . Then from the integrated form of the van't Hoff equation

$$\log \frac{X_2}{X_1} = \frac{\Delta H^0}{2.303R} \frac{(T_2 - T_1)}{(T_1 T_2)} \quad (3)$$

and the difference in the amount of adsorbate adsorbed at two temperatures, it is possible to calculate the heat of adsorption for a relatively small temperature range. In addition,

$$k_a = Ae^{-H_a^\ddagger/RT} \quad (4)$$

$$k_d = Ae^{-H_d^\ddagger/RT} \quad (5)$$

where H_a^\ddagger is the heat of activation for adsorption, H_d^\ddagger is the heat of activation for desorption, A is the frequency factor and the remaining terms are as defined earlier. Then,

$$K = \frac{k_a}{k_d} = \frac{e^{-(H_a^\ddagger - H_d^\ddagger)}}{RT} = e^{-\Delta H/RT} \quad (6)$$

thus

$$\Delta H_a = H_a^\ddagger - H_d^\ddagger \quad (7)$$

When desorption is first order and assuming that upon desorption, the adsorbate does not return to the adsorbent surface and that the volume of desorbing solvent remains constant, it can be shown that (Alexander et al., 1949)

$$\log \frac{X_e}{X_e - X} = \frac{k_d T}{2.303} \quad (8)$$

where X_e is the equilibrium amount of adsorbate in solution and X is the amount of solution at time, t . The determination of k_d at various temperatures then permits calculation of H_d^\ddagger .

The adsorption isotherm for sarin on methyl *o*-formate-treated keratin appears to be type IV which is characterized as having capillaries. The appearance of the curve is also similar to isotherms obtained by Bartell et al. (1952) describing the preferential capillary adsorption of water in which the upswing of the curve is explained as the formation of a water-rich phase in the capillaries. A similar phenomenon might occur in the sarin system with the formation of a sarin-rich phase. A second explanation of the upswing might be formation of multimolecular layers which were more easily formed once the monolayer was established.

The adsorption isotherm for sarin on *p*-dioxane-pretreated powder appears to be type I; however, it might also demonstrate evidence of capillaries if it had been feasible to carry out the isotherm to higher sarin concentrations.

An adsorption isotherm for sarin on dimethyl sulfoxide-treated powder was difficult to obtain using a batch process technique since the concentration of sarin in the adsorbate solution after equilibration was greater than the initial concentration. This suggests preferential adsorption of the dimethyl sulfoxide by the keratin powder.

The kinetics of desorption of sarin from either methyl *o*-formate- or *p*-dioxane-pretreated keratin powder were followed at 25, 35 and 45°C and from dimethyl sulfoxide-treated keratin at 20, 25 and 35°C. A representative desorption isotherm for the methyl *o*-formate system at 25°C is shown in Fig. 1. Raw desorption data were corrected for unadsorbed sarin remaining in the system after adsorption by immediate withdrawal of a sample after resuspending the absorbent. Computer values were obtained using a non-linear least-squares estimation.

Desorption rate constants were obtained from apparent first order plots as shown in Fig. 2 using computer estimates of the equilibrium amount of sarin desorbed, in order to avoid the long amount of time needed to achieve equilibrium. The desorption rate constants are shown in Table 1. The heat of activation for desorption (H_d^\ddagger) was estimated from a plot of $\log k_d$ vs the reciprocal of the absolute temperature.

The amount of sarine adsorbed in the methyl *o*-formate and *p*-dioxane systems at the 3 temperatures are shown in Table 2. As stated previously, the amount of sarin adsorbed on the dimethyl sulfoxide-treated keratin was not obtained.

The thermodynamic data for the desorption systems are given in Table 3. While there

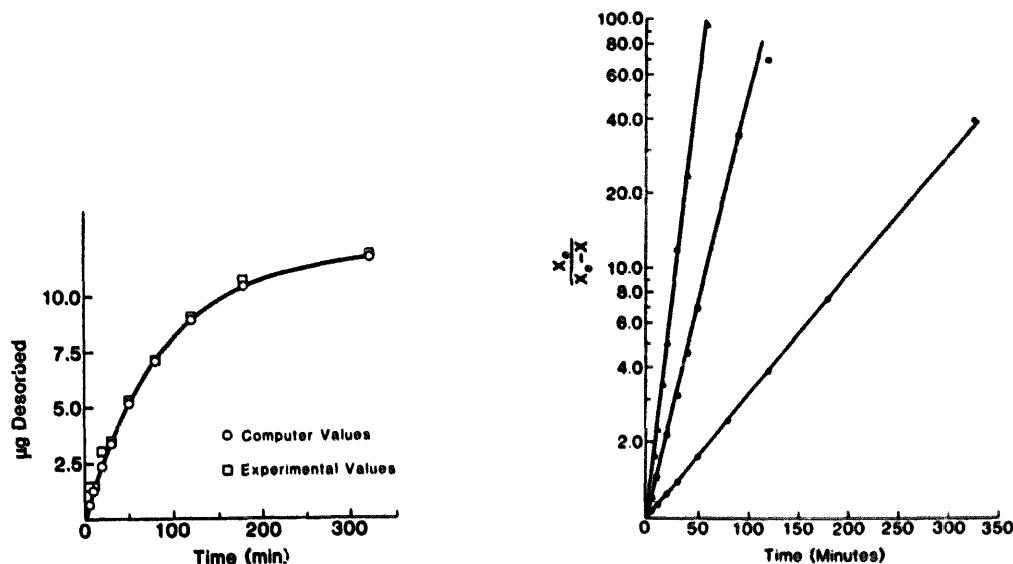


Fig. 1. Desorption isotherm for sarin from methyl *o*-formate-conditioned keratin powder at 25°C.

Fig. 2. Apparent first order plots for the desorption of sarin from methyl *o*-formate-conditioned keratin powder at 25°C, ●; 35°C, ■; and 45°C, ▲.

TABLE 1
DESORPTION RATE CONSTANT DATA

Temperature	Desorption rate constant (min ⁻¹)		Desorption rate constant (min ⁻¹)	
	Methyl o-formate	p-Dioxane	Temperature	Dimethyl sulfoxide
25	0.011	0.009	20	0.028
35	0.048	0.013	25	0.021
45	0.082	0.034	35	0.051

TABLE 2
ADSORPTION DATA

Temperature (°C)	μg adsorbed/g	
	Methyl o-formate	p-Dioxane
25	9.3	10
35	6.8	8
45	6.0	7.5

TABLE 3
THERMODYNAMIC DATA FOR DESORPTION SYSTEMS

Solvent treatment	H _a [#]	H _d [#]	ΔH _a
Methyl o-formate	14.7	18.9	-4.2
p-Dioxane	10.0	12.7	-2.7
Dimethyl sulfoxide	-	8.4	-

TABLE 4
CORRELATION OF ACTIVATION ENERGY FOR TRANSPORT WITH ENERGY OF ACTIVATION FOR DESORPTION

Solvent treatment	Transport activation energy (Kcal mol ⁻¹)	Energy of activation for desorption (Kcal mol ⁻¹)
Methyl o-formate	22.4	18.9
p-Dioxane	20.4	12.7
Dimethyl sulfoxide	9.1	8.4

is a certain amount of scatter in the experimentally difficult dimethyl sulfoxide system the trend to the lower H_d^\ddagger value is evident. The heat of activation for adsorption (H_a^\ddagger) is estimated from Eqn. 7.

A comparison of the activation energy for transport reported in an earlier study (Matheson et al., 1979) with the heat of activation for desorption is shown in Table 4. The rank order correlation existing between these parameters suggests that a possible mechanism of action for the enhancement of penetration through skin by dimethyl sulfoxide may be that it aids in some way the desorption of substances bound to the skin protein.

REFERENCES

Alexander, P. and Hudson, R.F., The kinetics of the heterogeneous reaction between potassium permanganate and wool. *J. Phys. Colloid Chem.*, 53 (1949) 733–752.

Bartell, F.E. and Donahue, D.J., Preferential capillary adsorption of water from solutions of alcohols by silica gel. *J. Phys. Chem.*, 56 (1952) 665–670.

Dembski, R.E., Wurster, D.E. and Becker, S.L., Permeability of excised human keratin to lipid-soluble substances II. *J. Pharm. Sci.*, 52 (1963) 165–166.

Gehauf, B., Epstein, J., Wilson, G.B., Witten, B., Sass, S., Bauer, V.E. and Rueggeberg, W.H.C., Reaction for colorimetric estimation of some phosphorus compounds. *J. Anal. Chem.*, 29 (1957) 278–281.

Giuffrich, L., A flame ionization detector highly selective and sensitive to phosphorous – a sodium thermionic detector. *J.A.O.A.C.*, 47 (1964) 293–300.

Idson, B., Percutaneous absorption. *J. Pharm. Sci.*, 64 (1975) 901–924.

Kakemi, K., Arita, T., Hori, R., Konishi, R. and Nishimura, K., Absorption and excretion of drugs. XXXIII. The correlation between the absorption of barbituric acid derivatives from the rat small intestine and their binding to the mucosa. *Chem. Pharm. Bull.*, 17 (1969a) 248–254.

Kakemi, K., Arita, T., Hori, R., Konishi, R., Nishimura, K., Matsui, H. and Nishimura, T., Absorption and excretion of drugs. XXXIV. An aspect of the mechanism of drug absorption from the intestinal tract in rats. *Chem. Pharm. Bull.*, 17 (1969b) 255–261.

Matheson, L.E., Jr., Wurster, D.E. and Ostrenga, J.A., Sarin transport across excised human skin II. The effect of solvent pretreatment on permeability. *J. Pharm. Sci.*, 68 (1979) 1410–1413.

Patrick, R.L. and Payne, G.O., Jr., The rate of desorption of stearic acid from planar surfaces – a new technique. *J. Colloid Sci.*, 16 (1961) 93–100.

Wurster, D.E. and Dembski, R.E., Adsorption of lipid-soluble substances by human keratin. *J. Pharm. Assoc., Sci. Ed.*, 49 (1960) 305–307.

Wurster, D.E. and Dembski, R.E., Permeability of excised human keratin to lipid-soluble substances. *J. Pharm. Sci.*, 50 (1961) 588–591.

Wurster, D.E., Ostrenga, J.A. and Matheson, L.E., Jr., Sarin transport across excised human skin I: permeability and adsorption characteristics. *J. Pharm. Sci.*, 68 (1979) 1406–1409.