# Percutaneous Absorption of Alkanoic Acids II: Application of Regular Solution Theory

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**Abstract** □ The permeability coefficient, $K_p$, of pure unbranched alkanoic acids ($C_2$-$C_7$) applied to isolated porcine skin, reached a maximum in the solubility parameter ($\delta_2$) range of 9.7–10 $cal^{1/2}/cm^{3/2}$. When these and other penetrants were delivered from a solvent vehicle, the following linear relationships could be demonstrated: (*a*) between log $K_p$ and the molar attraction constant of the penetrant [$\delta_2 v_2$ or $(-Ev)^{1/2}$] for six unbranched and six branched acids delivered from 1 M solution in *n*-heptane; (*b*) between $K_p$ and the partial molal volume difference in *n*-heptane ($\bar{v}_2 - v_2^0$) for the unbranched acids; and (*c*) between $K_p$ and ($\bar{v}_2 - v_2^0$) for propionic acid delivered from 1 M solutions in nine solvents having $\delta_1$ values in the range 7.4–12.7 $cal^{1/2}/cm^{3/2}$. Drug penetrability in a given series could be assessed from knowledge of the excess free energy of the penetrant in the delivery system used.

**Keyphrases** □ Absorption, percutaneous—alkanoic acids *in vitro*, porcine skin, permeability coefficients, application of regular solution theory □ Alkanoic acids—percutaneous absorption *in vitro*, porcine skin, permeability coefficients, application of regular solution theory □ Permeability coefficients—alkanoic acids through porcine skin, *in vitro* percutaneous absorption, application of regular solution theory

Percutaneous absorption is determined by two major processes: dissolution and diffusion. The contribution of each of these in the overall process is expressed in Fick's equation in terms of the partition coefficient and the diffusion coefficient (1). Both of these factors are affected by the molecular characteristics of the penetrant, such as solubility, size, and shape.

Solubility is dominant in skin penetration. Its importance was recognized early when it was found that compounds with both lipid and water solubilities penetrate better than substances with either high water or high lipid solubility. This led many workers to seek a correlation between skin penetrability and the partition coefficient of the penetrating compound between two reference solvents, such as ether–water (2), olive oil–water (3), benzene–water (4), and others. Later, it was recognized (3) that such partition coefficients may be misleading, since none represent the true partitioning between the penetrant and the stratum corneum.

The situation is even more complex when a vehicle is used. To ensure penetration from a vehicle, one approach utilizes the differences in thermodynamic activities of the penetrant between the vehicle and membrane phases. Ideally, such vehicles or formulations "push" the drug into the skin without causing detectable injury to the skin membrane. Intuitively, one would choose a vehicle that does not bind the incorporated drug too strongly; *i.e.*, the thermodynamic activity of the active ingredient in the vehicle should be relatively high. On the other hand, the flux is proportional to the drug concentration, and these two opposing factors lead to the conclusion that for each drug an optimal delivery system could be designed through a judicious combination of vehicle and concentration. Poulsen (5) analyzed vehicle effects for some theoretical cases in which one vehicle system, composed of two components, was used. The change in flux, related to the change in the partition coefficient and the drug concentration, was illustrated as a function of the change in the vehicle composition. The use of such an analysis in an experimental system is laborious and difficult to apply to practical problems. Thus, a more straightforward measure of vehicle effect on drug penetrability seems necessary.

Little is known about the effect of molecular size and shape on penetration through skin. An inverse relationship appears to exist between absorption rate and molecular weight (6). Small molecules penetrate more rapidly than large molecules, but within a narrow range of molecular size, there is little correlation between size and penetration rate. It has not been easy to separate the contribution of the size factor in the overall process when there is also a simultaneous change in solubility. For example, in a study of the percutaneous absorption of closely related compounds in human skin, Feldman and Maibach (7) found wide differences in penetration which could not be explained on a strict basis.

The present work deals with the application of regular so-

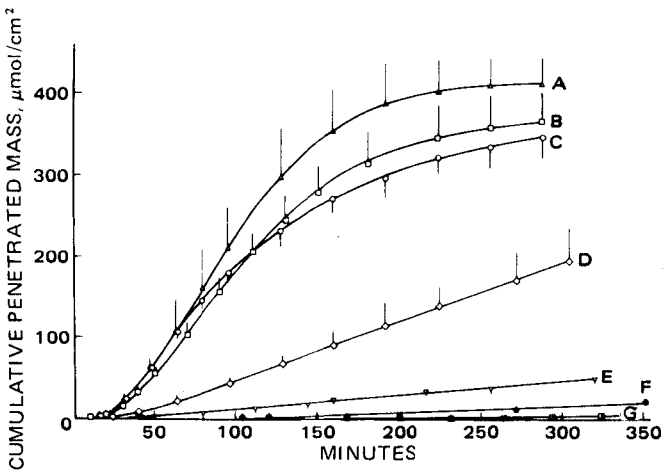


**Figure 1**—*Penetration of pure straight-chain alkanoic acids (430 μmol) through porcine skin at 37°C, determined by titrimetry. Number of carbon atoms: (A) 3; (B) 4; (C) 2; (D) 5; (E) 6; (F) 7; (G) 8.*

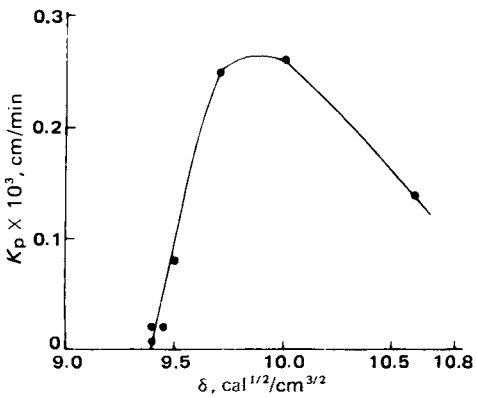


**Figure 2**—*Relationship between the permeability coefficient and the solubility parameter of pure straight-chain alkanoic acids.*



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**Table I—Penetration of Pure Straight-Chain Alkanoic Acids Through Porcine Skin at 37°C [a]**

| Carbon Atoms in Molecule | Flux at Steady Rate ($J_s$) μmol/cm²/min | Permeability Coefficient ($K_p \times 10^3$) cm/min | Solubility[b] Parameter ($\delta$) cal$^{1/2}$/cm$^{3/2}$ |
|---|---|---|---|
| 2 | 2.46 | 0.14 | 10.6 |
| 3 | 3.27 | 0.26 | 10.0 |
| 4 | 2.68 | 0.25 | 9.7 |
| 5 | 0.74 | 0.08 | 9.5 |
| 6 | 0.19 | 0.02 | 9.5 |
| 7 | 0.15 | 0.02 | 9.4 |
| 8 | 0.04 | 0.006 | 9.4 |

[a] Determined by the titrimetric procedure. [b] From Ref. 14.

lution theory (8) to the percutaneous absorption of alkanoic acids as model penetrants. This theory provides for the contribution of both solubility and size in the dissolution process.

## THEORETICAL

**Solubility and Molal Volume**—Fick's equation was originally formulated using penetrant concentrations within the membrane:

$$J_s = \frac{D_m \Delta C_m}{X} \qquad \text{(Eq. 1)}$$

where the subscript m refers to the membrane. In the extreme case where the solubility of the penetrant in the membrane is nil, $\Delta C_m = 0$, and the flux ($J_s$) must vanish. The concentrations within the membrane can be related to the concentrations in the donor and receptor phases using the partition coefficient term. Fick's law is then expressed in its more familiar form; hence, knowledge of $D_m$ and $K_m$ should allow the prediction of drug penetrability, but these two constants are usually not accessible.

Scheuplein and Blank (3) studied the penetrability of straight-chain alcohols, members of an homologous series. They found that homologues from methanol to octanol had almost the same diffusion coefficient. Therefore, any change in the permeability coefficient could be related directly to the change in the partition coefficient or the solubility of a particular homologue. In view of this, one expects to find for members of an homologous series a correlation between the permeability coefficient $K_p$ and $K_m$ or its equivalent in terms of regular solution. A necessary oversimplification is the consideration of the admittedly complex structure of the skin as an homogenous phase. Such an approach would be justified if the analysis of skin penetration data could be simplified, or at least correlated with parameters predicted from regular solution theory. In this theory, the heat of mixing $\Delta H^M$ was given by Hildebrand *et al.* (9) as:

$$\Delta H^M = (X_1 v_1 + X_2 v_2)(\delta_1 - \delta_2)^2 \phi_1 \phi_2 \qquad \text{(Eq. 2)}$$

where $X$ is the mole fraction, $\phi$ is the volume fraction, $v$ is the molal volume,

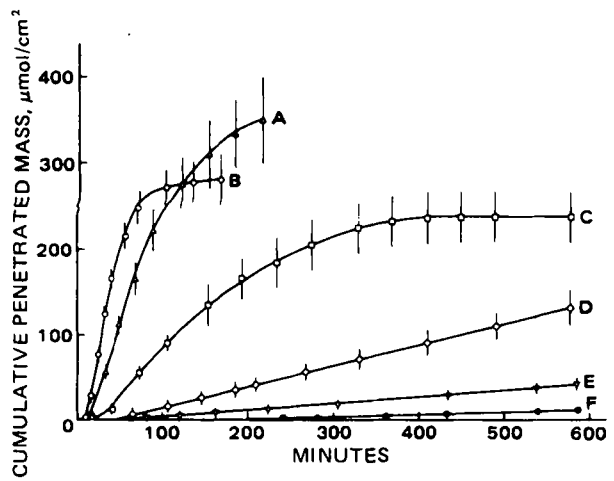


**Figure 3**—*Penetration of straight-chain alkanoic acids (300 μL of 1 M solutions in* n*-heptane) through porcine skin at 37°C, determined by titrimetry. Number of carbon atoms: (A) 3; (B) 2; (C) 4; (D) 5; E (6); (F) 7.*

**Table II—Penetration of Straight-Chain Alkanoic Acids in Solution Through Porcine Skin at 37°C [a]**

| Carbon Atoms in Molecule | Permeability Coefficient $K_p \times 10^3$, cm/min | Permeability Coefficient log $K_p$ | Difference in Partial Molal Volume at 25°C $\bar{v}_2 - v_2^0$, mL/mol | $\delta_2 v_2 = (-Ev)^{1/2}$, kcal-L$^{1/2}$/mol |
|---|---|---|---|---|
| 2 | 5.86 | −2.23 | 5.57 | 0.61 |
| 3 | 3.00 | −2.52 | 2.90 | 0.75 |
| 4 | 1.00 | −3.00 | 1.94 | 0.89 |
| 5 | 0.25 | −3.60 | 0.84 | 1.04 |
| 6 | 0.08 | −4.10 | 0.72 | 1.19 |
| 7 | 0.03 | −4.52 | 0.05 | 1.35 |

[a] Determined by the titrimetric procedure using 300 μL of 1 M solutions in *n*-heptane.

and subscripts 1 and 2 relate to the solute and solvent, respectively[1]. The Gibbs free energy change in the mixing process is given by:

$$\Delta F^M = \Delta H^M - T\Delta S^M \qquad \text{(Eq. 3)}$$

The smaller $\Delta F^M$ the more compatible the elements being mixed. This means that the best mixing conditions will be achieved when $\delta_2$ approaches $\delta_1$. If we consider the stratum corneum membrane as a solvent having an average solubility parameter $\delta_1$, different molecules will dissolve and penetrate through this medium in accordance with their specific solubility parameter, $\delta_2$.

Davis (10) expressed the relationship between solubility parameter and partition coefficient, and later Srebrenik and Cohen (11) developed a more refined expression that takes into account partial molal volume changes. The partition coefficient was given by:

$$\ln K_m = \frac{-2\delta_1\delta_2}{RT} v_2 + \frac{\delta_1^2}{RT}\bar{v}_{21} - \frac{\bar{v}_{21}}{v_1} - \left(-2\frac{\delta_3\delta_2}{RT} v_2 + \frac{\delta_3^2}{RT}\bar{v}_{23} - \frac{\bar{v}_{23}}{v_3}\right) \qquad \text{(Eq. 4)}$$

where $K_m$ is the partition coefficient, subscript 2 refers to solute which partitions between two phases (subscripts 1 and 3), and $\bar{v}_{21}$ and $\bar{v}_{23}$ are the partial molal volumes of solute 2 in phase 1 and phase 3, respectively. The application of Eq. 4 to skin transport analysis is not straightforward, but nevertheless it could be used with approximation, as will be shown later. A dissolution process is accompanied by a change in the molal volume of the solute. The degree of this change depends on the similarity between the solute-solvent pair; for low solute concentrations it is given by:

$$\frac{\bar{v}_2 - v_2^0}{v_2^0} = \frac{(\delta_1 - \delta_2)^2}{(\partial E/\partial v)_T} \qquad \text{(Eq. 5)}$$

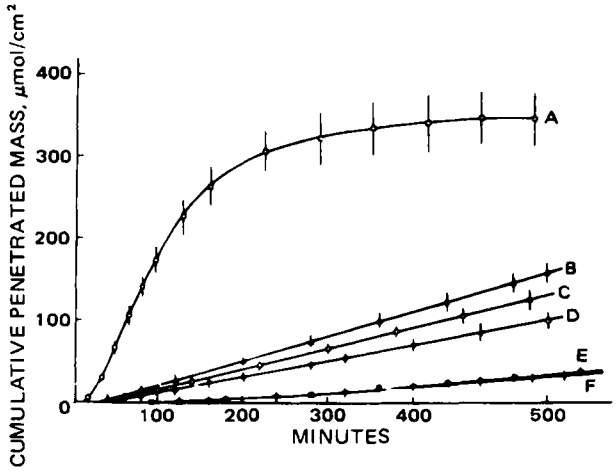


**Figure 4**—*Penetration of branched alkanoic acids (300 μL of 1 M solutions in* n*-heptane) through porcine skin at 37°C, determined by titrimetry. Key: (A) Cyclopropane carboxylic; (B) 3-methylbutyric; (C) 2-methylbutyric; (D) pivalic; (E) 3,3-dimethylbutyric; (F) 2-ethylbutyric, superimposed on (E).*

[1] For the sake of consistency with previous publications and throughout this article, the subscript 2 refers only to solute or penetrant, subscripts 1 or 3 refer to solvents or biophase.

**Table III—Penetration of Branched-Chain Alkanoic Acids in Solution Through Porcine Skin at 37°C [a]**

| Acid | Permeability Coefficient $K_p \times 10^3$, cm/min | Permeability Coefficient log $K_p$ | Solubility[b] Parameter ($\delta$), cal$^{1/2}$/cm$^{3/2}$ | $\delta_2 v_2 = (-Ev)^{1/2}$, kcal·L$^{1/2}$/mol |
|---|---|---|---|---|
| Butyric | 1.00 | −3.00 | 9.7 | 0.89 |
| Cyclopropane-carboxylic | 2.28 | −2.64 | 9.5 | 0.76 |
| 3-Methylbutyric | 0.31 | −3.51 | 9.6 | 1.06 |
| 2-Methylbutyric | 0.26 | −3.59 | 9.3 | 1.02 |
| Pivalic | 0.20 | −3.70 | 10.3 | 1.13 |
| 3,3-Dimethylbutyric | 0.08 | −4.10 | 9.3 | 1.19 |
| 2-Ethylbutyric | 0.07 | −4.15 | 9.5 | 1.20 |

[a] Determined by the titrimetric procedure using 300 μL of 1 M solutions in *n*-heptane. [b] From Ref. 14.

For solutions obeying regular solution theory, the solute excess free energy is directly proportional to the change in the solute partial molal volume (12):

$$\Delta F_2^{ex} = (\bar{v}_2 - v_2^0)(\partial E/\partial v)_T \qquad \text{(Eq. 6)}$$

The excess free energy of the solute in the donor phase is an important parameter that may be considered as the driving force for the penetration process through the skin membrane. Since the value of the excess free energy is higher, the flux (and hence the permeability coefficient) is increased. In principle, knowledge of $\delta_2$ and $v_2^0$ of a particular homologue in a given series should allow the prediction of its relative penetrability through a common membrane, in this case the stratum corneum.

## EXPERIMENTAL

The experimental system used has been described in detail in the previous paper (1). All skin penetration experiments were done on excised porcine skin maintained at 37.0°C or 25.0°C on either side of the membrane. Pure penetrants were applied to the donor side in a constant mass. Solutions of penetrants in *n*-heptane (1 M) were applied in a constant volume. The total penetrated mass over time was determined either by automatic titration of acid appearing in the perfusate or by scintillation counting of the carbon-14 radioisotope label added to the penetrant (1).

The effect on the skin of the pure solvent as vehicle was studied by simulating penetration experiments in diffusion cells with no penetrant added. The skin was removed after 5 and 20 h, and the condition and thickness of the stratum corneum were examined using the paraffin and frozen-section techniques (13). No significant changes were observed from untreated skin.

Partial molal volumes of penetrants ($\bar{v}_2$) at high dilution in a given solvent were determined by a previously described densitometric procedure (14). All

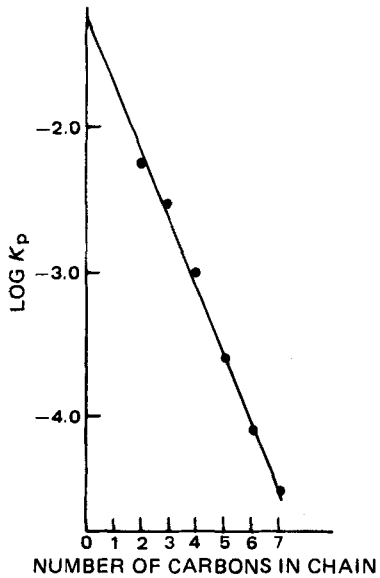


**Figure 5**—*Penetration of straight-chain alkanoic acids (1 M in* n*-heptane) through porcine skin at 37°C. Permeability coefficient as a function of the number of carbons in the chain; log* $K_p = -1.17 - 0.48n$ *(r = 1.00).*

**Table IV—Vehicle Effect on the Penetration of Propionic Acid from 1 M Solutions Through Porcine Skin at 25°C [a]**

| Solvent | Permeability Coefficient $K_p \times 10^3$, cm/min | Permeability Coefficient log $K_p$ | Solubility[b] Parameter of Solvent ($\delta_1$), cal$^{1/2}$/cm$^{3/2}$ | Partial Molal Volume Change ($\bar{v}_2 - v_2^0$), mL/mol |
|---|---|---|---|---|
| Heptane | 1.25 | −2.90 | 7.4 | 2.90 |
| Cyclohexane | 1.67 | −2.78 | 8.2 | 4.84 |
| Carbon Tetrachloride | 0.92 | −3.04 | 8.6 | 1.83 |
| Toluene | 0.88 | −3.06 | 8.9 | 0.83 |
| Chlorobenzene | 0.80 | −3.10 | 9.5 | 0.49 |
| *o*-Dichloroben-zene | 0.69 | −3.16 | 10.0 | 0.41 |
| Bromonaph-thalene | 0.41 | −3.39 | 10.6 | 0.02 |
| Nitroethane | 0.10 | −4.00 | 11.1 | −0.82 |
| Acetonitrile | 0.006 | −5.22 | 11.9 | −1.47 |
| Nitromethane | 0.007 | −5.15 | 12.7 | −1.15 |

[a] Determined by the radioisotope procedure. [b] Taken from Ref 17.

chemicals and solvents were of the highest analytical grade available commercially.

## RESULTS AND DISCUSSION

**Pure Straight-Chain Alkanoic Acids**—The donor phase consisted of 430 μmol of each tested compound. The acids may be classified into two main groups according to the rate of penetration (Fig. 1; Table I). The faster penetrants were the acetic–butyric acids; the slower ones were hexanoic–octanoic acids; valeric acid had an intermediate penetration rate. The slower penetrants showed a steady penetration rate over the entire experimental period, whereas the more rapid penetrants showed a characteristic curve (1) with a lag phase region, a steady-state region, and a decay region. The latter could be due to the decline in penetrant mass at the donor side or its dilution with water through back-diffusion. Propionic acid, rather than acetic acid, had the highest rate of penetration. A plot of the permeability coefficients of these acids as a function of their solubility parameter (Fig. 2), shows a maximum at $\delta_2 = 9.7 - 10.0$ cal$^{1/2}$/cm$^{3/2}$. This range could be taken to represent the solubility parameter of the stratum corneum, but with the reservation that the lower acids could considerably alter this parameter with increasing applied mass, as observed earlier for butyric acid (1).

**Straight and Branched-Chain Alkanoic Acids**—*n-Heptane Solution Experiments*—The problem encountered in the use of the pure penetrants could be minimized by studying penetration rates from dilute solutions in a relatively "inert" solvent, in this case *n*-heptane. The penetration of the straight-chain alkanoic acids, delivered from 300 μL of 1 M solutions in this solvent, is shown in Fig. 3. From the penetration curves thus obtained, the permeability coefficients were calculated and tabulated with the corresponding values of the partial molal volume differences, ($\bar{v}_2 - v_2^0$ determined at 25°C) and the values of the square root of the volume–cohesive energy product or $\delta_2 v_2^0$ (Table II). The value ($\bar{v}_2 - v_2^0$) is proportional to excess free energy $\Delta F_2^{ex}$ at infinite dilution in *n*-heptane (Eq. 6); $\delta_2 v_2^0$ is the molar attraction constant of the penetrant, irrespective of vehicle used. On theoretical grounds (Eq. 4), there should exist a correlation between $K_m$ (and hence $K_p$) and each of these parameters. The penetration data of some branched alkanoic acids are given separately (Fig. 4, Table III). Cyclopropane carboxylic acid was the fastest penetrant, whereas the isomeric 3,3-dimethylbutyric and 2-ethylbutyric acids were the slowest, both showing a similar penetration profile.

From Fig. 5, the correlation between log $K_p$ and the number of carbon atoms in the molecule of unbranched acids is very good ($r = 1.00$). A similar result was obtained by Scheuplein and Blank (3) for normal alcohols in aqueous solution.

In view of the linear relationship between the number of carbon atoms in the molecule and log $K_p$ on the one hand, and the number of carbon atoms and the square root of the volume–cohesive energy product $(-Ev)^{1/2}$ or $\delta_2 v_2^0$ (14) on the other, one expects to find a linear relationship between $(-Ev)^{1/2}$ and log $K_p$. Such a plot is shown in Fig. 6 where $K_p$ had been derived from data at 37°C, but $(-Ev)^{1/2}$ from data at 25°C. The linearity of the plot should not be affected by this difference in temperature since it can be demonstrated (15) that in related compounds with an almost common thermal expansion coefficient:

$$(\delta v)_T = (\delta v)_{T^0} \cdot \text{constant} \qquad \text{(Eq. 7)}$$

Remarkably, the branched-chain homologues follow the same relationship. The fit to a straight line [$\log K_p = -0.16 - 3.26(-Ev)^{1/2}$] is very good ($r =$

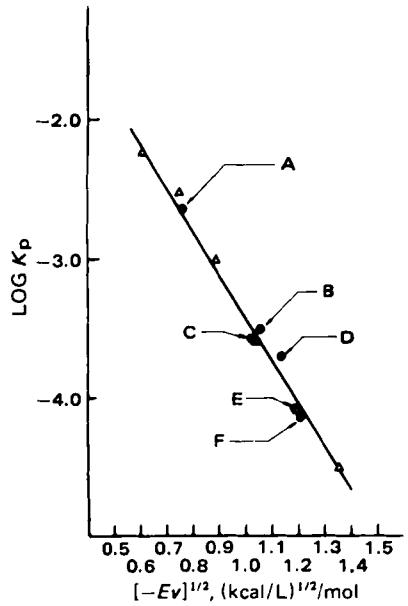


**Figure 6**—*Penetration of alkanoic acids through porcine skin at 37°C. Permeability coefficient as a function of the square root of the volume cohesive energy product: log* $K_p = -0.16 - 3.26(-Ev)^{1/2}$, (r = 0.99). *See Fig. 4 for key; Δ refers to straight-chain acids.*

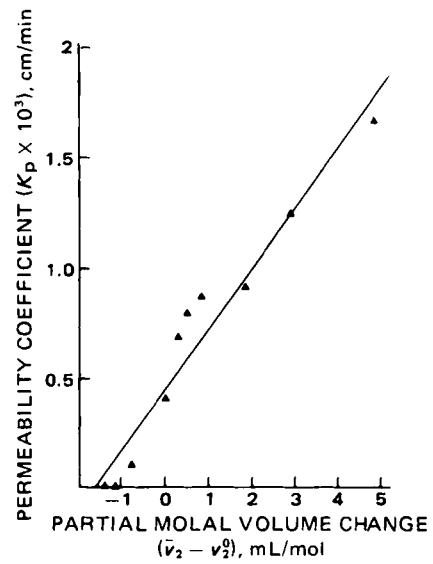


**Figure 7**—*Penetration of propionic acid from 1 M solutions through porcine skin at 25°C showing vehicle effect.* $K_p = 4.57 \times 10^{-4} + 2.75 \times 10^{-4}(\bar{v}_2 - v_2^0)$.

0.99). This relationship is important because it enables one to evaluate the permeability coefficient of an untested acid from its solubility parameter ($\delta_2$) and molal volume ($v_2^0$). In this context, there is some analogy between the present findings and the demonstration by Ostrenga (16) of the relationship between the molal attraction constant and the relative degree of drug-receptor interaction for several different groups of structurally related compounds.

From Fig. 6 and Eq. 4 one can derive the following empirical relationship (see the *Appendix*):

$$\ln K_p \simeq \frac{-2(\delta_1 - \delta_3)}{RT}\delta_2 v_2^0 + b \qquad \text{(Eq. 8)}$$

where $\delta_1 = \delta_m$, the solubility parameter of the stratum corneum; $\delta_3 = 7.4$, the solubility parameter of *n*-heptane; and *b* (constant) = −0.16. From the slope (−3.26), temperature (310 K), and any given value of $\delta_2 v_2^0$, it can be shown that $\delta_1 \simeq 9.7$. This value is compatible with the rapid penetration rates found for pure propionic and butyric acids (Figs. 1 and 2), which have comparable $\delta_2$ values.

In an alternative approach, a plot of $K_p$ at 37°C as a function of the net molal expansion ($\bar{v}_2 - v_2^0$), at infinite dilution in *n*-heptane at 25°C (Table II) gave a straight line (not shown) with an acceptable correlation coefficient ($r = 0.97$) for this type of experiment. This means that permeability could be related to excess free energy (Eq. 6). As this last function becomes larger for a given solvent-solute system the permeability coefficient of the penetrant through the skin should increase.

*Vehicle Effect*—The vehicle effect was studied for 1 M propionic acid solutions in the solvents listed in Table IV. The penetration experiments with *n*-heptane through bromonaphthalene were done in the titration assembly system, whereas those with nitroethane through nitromethane were done in the automatic radioisotope sampling system. Experiments with *n*-heptane were also carried out in the last system as a cross-check.

If regular solution behavior were to hold throughout the whole range of solvents used, Eqs. 5 and 6 predict a parabolic relationship between $K_p$ and $\delta_1$, with a minimum in penetration rate for the solvent having the closest solubility parameter to that of propionic acid. However, the experimental data show a steady decrease in $K_p$ as one moves from *n*-heptane to nitromethane (a monotonous increase in the solubility parameter). The reason for this phenomenon might be due to the breakdown of regular solution behavior beyond a certain polarity ($\delta_1 > 10$).

Irrespective of this apparent discrepancy it is still possible to analyze the vehicle effect by use of the excess free energy approach. Figure 7 is a plot of the permeability coefficient $K_p$ as a function of the partial molal volume change; the fit, over this wide range, is very good ($r = 0.97$). This function also accounts for the higher permeability coefficient of the penetrant in cyclohexane ($\delta_1 = 8.2$) relative to *n*-heptane ($\delta_1 = 7.4$). Negative values of $\bar{v}_2 - v_2^0$ for nitroethane, acetonitrile, and nitromethane are also well correlated with the change in the permeability coefficient. The excess free energy is a measure of the force driving the penetrant molecule from its solution in a given vehicle. The determination of the partial molal volume of the penetrant in a given solvent affords an excellent means for the assessment of its delivery into the skin.

## APPENDIX

The following applies to penetrants that are members of an homologous series delivered from a common solvent vehicle into a common skin sample. Under these conditions, $\delta_1$, $\delta_3$, $v_1$, and $v_3$ become constant and Eq. 4 can be written:

$$\ln K_m = \frac{-2(\delta_1 - \delta_3)}{RT}\delta_2 v_2^0 + \bar{v}_{21}a_1 - \bar{v}_{23}a_2 \qquad \text{(Eq. 9)}$$

where $a_1$ and $a_2$ are constants.

If the differences between the partial molal volumes of the solute in each of the three phases considered is negligible, then:

$$v_2^0 \simeq \bar{v}_{21} \simeq \bar{v}_{23} \qquad \text{(Eq. 10)}$$

For an homologous series:

$$v_2^0 = a_3 + a_4 \cdot n \qquad \text{(Eq. 11)}$$

where $a_3$ and $a_4$ are constants and $n = 0,1,2,3\ldots$ Eq. 9 now becomes:

$$\ln K_m = \frac{-2(\delta_1 - \delta_3)}{RT}\delta_2 v_2^0 + (a_1 - a_2)(a_3 + a_4 \cdot n) \qquad \text{(Eq. 12)}$$

For low *n* values $a_3 > a_4 \cdot n$, and the expression $(a_1 - a_2)(a_3 + a_4 \cdot n)$ is practically constant. Even at $n = 5$ (equivalent to heptanoic acid), the change in the value $(a_1 - a_2)(a_3 + a_4 \cdot n)$ is still small compared with the value of $-2(\delta_1 - \delta_3)/RT\ \delta_2 v_2^0$, so that:

$$\ln K_m \simeq \frac{-2(\delta_1 - \delta_3)}{RT}\delta_2 v_2^0 + a_5 \qquad \text{(Eq. 13)}$$

where $a_5$ is a constant.

In an homologous series, $D_m$ is almost constant, therefore:

$$\ln K_p \simeq \ln K_m + \text{constant} \qquad \text{(Eq. 14)}$$

or

$$\ln K_p \simeq \frac{-2(\delta_1 - \delta_3)}{RT}\delta_2 v_2^0 + \text{constant} \qquad \text{(Eq. 8)}$$

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# Quantitative Analysis of Trimethobenzamide Hydrochloride by Ion-Pair Column Chromatography and Semiquantitative Analysis of 3,4,5-Trimethoxybenzoic Acid by Thin-Layer Chromatography

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**Abstract** □ An ion-pair column chromatographic/UV spectrophotometric method for assaying trimethobenzamide hydrochloride in capsules and injections is presented, as well as a method for the detection of 3,4,5-trimethoxybenzoic acid in trimethobenzamide hydrochloride bulk drug and dosage forms. Results obtained by the USP XX, Pharmacopeial Forum, and ion-pair column assay procedures are compared, and results of a collaborative study of the proposed assay and impurity detection methods are presented.

**Keyphrases** □ Trimethobenzamide hydrochloride—quantitative analysis by ion-pair column chromatography, semiquantitative analysis of 3,4,5-trimethoxybenzoic acid □ 3,4,5-Trimethoxybenzoic acid—semiquantitative analysis by thin-layer chromatography, quantitative analysis of trimethobenzamide hydrochloride □ Ion-pair column chromatography—quantitative analysis of trimethobenzamide hydrochloride, semiquantitative analysis of 3,4,5-trimethoxybenzoic acid by thin-layer chromatography

Monographs (1, 2) for trimethobenzamide hydrochloride (I) in capsules and injections have several shortcomings. The USP (1) assay method for the capsules involves direct dilution in 0.1 M HCl and UV spectrometric comparison with the USP reference standard. This procedure does not separate possible impurities, such as 3,4,5-trimethoxybenzoic acid (II), or interfering excipients. The *Pharmacopeial Forum* (PF) assay for injection preparations (2) also involves a UV assay which has several shortcomings.

This paper describes an ion-pair column chromatographic assay procedure, in which I is quantitatively removed from an aqueous acidic chloride column with a chlorinated organic solvent. Ether is used to remove phenolic ingredients and breakdown products prior to the elution of I.

Also, a TLC procedure is reported for the detection of II in amounts as low as 0.25% of the weight of I. Compound II is both a synthetic precursor and a breakdown product of I and could be encountered as a contaminant in drug preparations.

**Table I—Recovery Data for I using the Proposed Ion-Pair, USP, and PF Injection Assay Methods**

| | Column Ion Pair | USP Injection | PF Injection |
|---|---|---|---|
| I, mg | 8 | 200 | 200 |
| Number of assays | 10 | 10 | 10 |
| Mean amount recovered, % | 99.4 | 96.6 | 97.6 |
| Range, % | 98.3–101.0 | 95.4–98.1 | 96.1–98.4 |
| *SD* | 0.81 | 0.91 | 0.68 |
| *CV*, % | 0.82 | 0.94 | 0.70 |

## EXPERIMENTAL

**Reagents**—Trimethobenzamide hydrochloride USP reference standard[1] was dried at 105°C for 4 h prior to use. Methylene chloride[2], pentane[2], and ether[2] were commercial distilled-in-glass grade. Compounds I[3] and II[3], chromatographic diatomaceous earth[4], glass wool, and the other reagents were used as received.

**Apparatus**—An ultrasonic bath, chromatographic tubes[5], a tamping rod[5], commercial TLC plates coated with a 250-μm layer of silica gel with a fluorescent indicator, a suitable TLC developing chamber, and a recording UV spectrophotometer were used.

**Standard Preparation**—Approximately 10 mg of trimethobenzamide hydrochloride USP reference standard was accurately weighed and transferred to a 100-mL volumetric flask. Methylene chloride (70 mL) was added, and the mixture was sonicated. The resulting solution was diluted to volume with methylene chloride. A 20-μg/mL solution was obtained by diluting quantitatively and stepwise with methylene chloride.

**Chromatographic Column**—A pledget of fine glass wool was packed in the base of a chromatographic column. A flexible spatula was used to mix 1 g of chromatographic diatomaceous earth with 500-μL of 1 M HCl in a 50-mL beaker. The mixture was transferred to a column and tamped.

**Capsule Assay Preparation**—The contents of ≥20 capsules were transferred to a tared container, and the average weight/capsule was determined. The

[1] USP Reference Standards; U.S. Pharmacopeial Convention, Rockville, Md.
[2] Burdick and Jackson Laboratories, Muskegon, Mich.
[3] Hoffmann-LaRoche, Inc., Nutley, N.J.
[4] Celite; Johns Manville Corp, New York, N.Y.
[5] *AOAC Book of Methods 13th Ed.*, 37.002(a) and (b) (3).



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