ELSEVIER

International Journal of Pharmaceutics 173 (1998) 183–191

international journal of pharmaceutics

Skin permeation model of phenyl alcohols: comparison of experimental conditions

A. López *, V. Faus, O. Díez-Sales, M. Herráez

Department of Pharmacy and Pharmaceutical Technology, Faculty of Pharmacy, University of Valencia, Valencia, Spain

Received 9 March 1998; received in revised form 15 June 1998; accepted 3 July 1998

Abstract

This study was conducted primarily to establish the significance of the experimental conditions in the determination of permeability coefficients. In order to do this, standard in vitro skin permeation methods were used to determine the permeability coefficient (k_p) of a homologous series of phenyl alcohols, with a wide range of lipophilicity, by two different experimental conditions through rat skin; first, using solutions (at 75% saturation concentration) of the penetrants in the donor compartment and second using saturated solutions added with an excess of the penetrant. The k_p values obtained by these techniques were compared. Solubility of the phenyl alcohols in the donor phase and partition coefficients in *n*-octanol/water systems were also assessed, and the correlations between the permeability coefficients and these parameters were established. A bilinear relationship between the permeability coefficients of the penetrants and the corresponding lipophilicity index was found. The skin penetration model of phenyl alcohols was compared with another homologous series (*p*-alkylanilines) and the optimal lipophilicity value in terms of log *P* (*n*-octanol) was found to be 3.1 in both cases.

Keywords: Percutaneous absorption; Phenyl alcohols; Permeability coefficient; Partition coefficient; In vitro models

1. Introduction

In vitro methodologies have been established as a useful tool to estimate the percutaneous penetration of drugs because of the difficulty in doing in vivo human studies (Franz, 1975). For example, in vitro methodologies can be the only means of obtaining percutaneous absorption data for highly toxic compounds.

Penetration through the skin is known to depend essentially on the lipophilicity of the substances tested and for this reason the use of homologous series of compounds with a wide range of lipophilicity enables the prediction of the

* Corresponding author. Present address: Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Valencia, Avda. Vicente Andrés Estellés s/n., 46100 Burjassot, Valencia, Spain. Tel.: +34 96 3864912; e-mail: alicia.lopez@uv.es

0378-5173/98/$ - see front matter
PII S0378-5173(98)00231-2

percutaneous uptake of chemically related substances. In this way, correlations between representative penetration parameters through the membrane and parameters accounting for the lipophilicity, or other structural parameters, for homologous series of xenobiotics have been used to develop theoretical models of penetration behaviour (Guy and Hadgraft, 1989; Hadgraft, 1991; Schaefer, 1996). In turn, these models can be used for the selection of suitable candidates for transdermal or topical formulations (Flynn and Stewart, 1988).

The intrinsic ability of compounds to permeate the skin can be estimated by the determination of the permeability coefficient. However, it is well known that the value of the permeability coefficient obtained in in vitro experiments can be strongly dependent on experimental techniques and conditions (Bronaugh, 1989). For this purpose, in vitro experiments have to enable steady-state diffusion to be achieved and a number of factors need to be considered, the most important of which are that the donor vehicle must keep the membrane exposed to a constant concentration of the permeant, and that the receptor phase has to maintain sink clearance conditions for the compound. At the same time, the measurements have to be made over a sufficient period of time to ensure that steady-state conditions have been reached (Potts and Guy, 1994). All of these points must be examined even more carefully when compounds with limited water solubility are included in the experiments. If penetration data is obtained carefully, taking into account the proper variables, it seems likely that good agreement between in vivo and in vitro measurements will be found (Bronaugh, 1989).

The objective of this work was to analyse the significance of the experimental conditions in the determination of permeability coefficients, especially when they are going to be used for comparative and predictive purposes. In order to do this, standard in vitro skin permeation methods were used to determine this parameter for a homologous series of phenyl alcohols with a wide range of lipophilicity under two different experimental conditions. Under the first set of conditions, the permeant was in solution at 75% saturation concentration, periodic replacement of the donor cell compartment ensured that the concentration of permeant remained constant. In the second case, a clear excess of the permeant was used (it was in the form of a saturated solution added with an excess of the penetrant). The permeability coefficient values calculated by these techniques were compared. At the same time, the solubility of phenyl alcohols in the donor phase and their partition coefficients in *n*-octanol/water systems were also assessed. The correlations between permeability coefficients and the lipophilicity indices for this series were established and compared with series of *p*-alkylanilines.

2. Materials and methods

2.1. Permeants

Six phenyl alcohols belonging to a true homologous series were used in the diffusion experiments. They were supplied as reagent grade products (Sigma and Lancaster). Purity for all permeants was checked by high-performance liquid chromatography (HPLC).

2.2. Solubility studies

The solubility (concentration at saturation) of the permeants in a solution buffered to pH 6.2 was determined. To do so, a moderate excess of the compound was placed in a buffered solution (pH 6.2) which was then placed in a shaker bath and maintained at $37(\pm 0.5)$°C for 2 days, with gentle stirring. After filtration, a sample was taken and diluted for HPLC analysis. Ten determinations for every compound were performed.

2.3. Structural and lipophilicity indices

The number of $-CH_2-$ groups in the alkyl chain of phenyl alcohols was used as an error-free structural index. In addition, the partition coefficients in *n*-octanol/water systems were also assessed in order to compare the permeability values obtained in this work with other results. Partition coefficients were determined according to the clas-

sical approaches (Leo et al., 1971; Curry and Whelpton, 1983). Six determinations for every compound were carried out and mean values were used for correlation purposes.

2.4. In vitro diffusion experiments

All the permeation experiments were performed on Wistar rat skin (aged 20–25 days), obtained from our laboratory colony. Epidermal membranes were prepared by a heat-separation technique as previously reported (Díez-Sales et al., 1993a). Diffusion studies were done using epidermal membranes in a 6-cell battery system (Durrheim et al., 1980) with the stratum corneum towards the stirred donor compartment which contained 22 ml of penetrant solution. The skin samples were placed in the static diffusion cell in a vertical position to give an effective surface area available for diffusion of 4.52 cm^2. The receiver compartment capacity was also 22 ml and the temperature was maintained at 37($\pm$0.5)°C by immersion of the cells in a water bath. The dermal side of the skin was continuously washed with saline solution buffered to pH 7.4 and stirred by a rotating teflon-coated magnet placed inside the cell. Two series of experiments were performed:

(1) Five compounds from the phenyl alcohol homologous series were used, (2-phenyl ethanol, 3-phenyl propanol, 4-phenyl butanol, 5-phenyl pentanol and 6-phenyl hexanol). The compounds were dissolved in buffer solution (pH 6.2) at a concentration equivalent to approximately 75% of their solubility in that medium to keep constant the degree of saturation of the solution in contact with the stratum corneum (i.e. thermodynamic activity) for all the compounds. In order to ensure that the concentration of the permeants and the diffusion state were kept constant throughout the experiment the donor cell content was entirely replaced by fresh test solution every 30 min for all of the compounds tested. For this reason, assay duration was only 7 h for all of members of the series. One millilitre samples were taken from the receptor compartment every 1 h (in the case of 6-phenyl hexanol the samples were of 4 ml). The

Table 1
Partition coefficient in *n*-octanol (P) ($n=6$, mean $\pm$ S.D.) and the solubility in the donor medium (S) ($n=10$, mean $\pm$ S.D.) of phenyl alcohols and the permeability coefficient (k_p) calculated for the different series of experiments[a]

Compound	$P \pm$ S.D.	$S \pm$ S.D. (mg/ml)	$k_p \pm$ S.D. ($\times 10^3$, (cm/h)			Statistical differences ($P<0.05$)		
			Series I	Series II	Series III	I–II	II–III	I–III
2-Phenyl ethanol	21.75 ± 0.86	19.17 ± 1.17	31.27 ± 2.28	114.50 ± 18.23	76.59 ± 6.90	*	*	*
3-Phenyl propanol	67.75 ± 6.38	6.68 ± 0.11	60.07 ± 3.52	130.22 ± 23.56	88.97 ± 17.94	*	*	*
4-Phenyl butanol	212.60 ± 16.66	2.60 ± 0.08	92.23 ± 8.28	157.06 ± 26.69	95.19 ± 17.65	*	*	ns
5-Phenyl pentanol	776.23 ± 54.77	1.18 ± 0.08	140.67 ± 5.49	164.61 ± 13.92	135.84 ± 10.62	*	*	ns
6-Phenyl hexanol	1960.37 ± 118.58	0.34 ± 0.01	132.97 ± 4.20	156.19 ± 8.89	138.82 ± 23.43	*	*	ns
7-Phenyl heptanol	3890.59 ± 545.87	0.08 ± 0.01	—	103.47 ± 17.32	—	—	—	—

(I) Solutions of phenyl alcohols in the donor compartment (75% of the saturation solubility) and 7 h of experiment.
(II) Saturated solutions of the permeants in the donor compartment and long duration of the experiments.
(III) Saturated solutions of phenyl alcohols in the donor compartment and 7 h of experiment ($n=4$, mean $\pm$ S.D.). Statistical analyses of permeability coefficients calculated, in the different experimental conditions, are also included.
* Significant differences.
ns, no significant differences.

Table 2
Equation and statistical parameters describing the correlations established between permeability coefficients (k_p, cm/h), calculated for the series I and II, and lipophilicity indices (number of $-CH_2-$ groups in the alkyl chain, n, partition coefficient in n-octanol, P and solubility in the donor phase) of phenyl alcohols

Model equations	Symbol	Equation and statistical parameters (values ± S.D.)		
		n	P n-octanol	Solubility
Bilinear (series I)	a	0.012 ± 0.003	$7.9 \times 10^{-3} \pm 2.6 \times 10^{-3}$	0.153 ± 0.012
	b	0.225 ± 0.035	0.47 ± 0.08	0.518 ± 0.051
	c	$6.8 \times 10^{-6} \pm 3.9 \times 10^{-5}$	$1.7 \times 10^{-5} \pm 7.5 \times 10^{-5}$	$5.1 \times 10^{-3} \pm 0.1 \times 10^{-3}$
	d	0.862 ± 0.386	1.45 ± 0.47	4.91 ± 26.54
	AIC	-46.26	-46.26	-50.09
	r	0.987	0.997	0.998
Parabolic (series I)	a	-0.055 ± 0.015	-0.211 ± 0.058	-0.326 ± 0.099
	b	0.614 ± 0.141	1.332 ± 0.304	0.155 ± 0.054
	c	-2.577 ± 0.318	-2.958 ± 0.386	-0.869 ± 0.028
	AIC	-37.74	-39.10	-35.67
	r	0.956	0.996	0.991
Hyperbolic (series I)	a	0.148 ± 0.020	0.150 ± 0.019	0.146 ± 0.021
	b	0.474 ± 0.165	0.911 ± 0.293	1.181 ± 0.463
	c	38.17 ± 39.12	9.43 ± 77.58	0.142 ± 0.156
	AIC	-34.74	-36.00	-32.68
	r	0.953	0.995	0.987
Bilinear (series II)	a	0.008 ± 0.006	0.079 ± 0.009	0.203 ± 0.022
	b	0.071 ± 0.012	0.121 ± 0.023	0.197 ± 0.039
	c	0.0002 ± 0.0003	$1.4 \times 10^{-7} \pm 4.4 \times 10^{-7}$	0.230 ± 0.126
	d	0.557 ± 0.082	1.917 ± 0.345	0.904 ± 0.116
	AIC	-53.39	-49.21	-53.39
	r	0.994	0.992	0.995
Parabolic (series II)	a	-0.029 ± 0.006	-0.132 ± 0.045	-0.129 ± 0.020
	b	0.262 ± 0.059	0.676 ± 0.227	-0.022 ± 0.016
	c	-1.386 ± 0.128	-1.648 ± 0.272	-0.788 ± 0.014
	AIC	-40.94	-36.91	-45.22
	r	0.932	0.861	0.964
Hyperbolic (series II)	a	$1.4 \times 10^{-1} \pm 1.6 \times 10^{-2}$	$1.4 \times 10^{-1} \pm 1.6 \times 10^{-2}$	$1.4 \times 10^{-1} \pm 1.7 \times 10^{-2}$
	b	$7.8 \times 10^{-1} \pm 2.304$	1.496 ± 4.726	1.808 ± 5.678
	c	9.896 ± 89.970	27.160 ± 461.012	$1.3 \times 10^{-3} \pm 2.6 \times 10^{-2}$
	AIC	-29.97	-30.07	-29.95
	r	0.475	0.459	0.450

volumes withdrawn were always replaced with equal volumes of fresh receptor solution.

(2) Six compounds of the series, (2-phenyl ethanol, 3-phenyl propanol, 4-phenyl butanol, 5-phenyl pentanol, 6-phenyl hexanol and 7-phenyl heptanol), were used as saturated solution (added with an excess of the penetrant) in buffer medium at pH 6.2. Then, the effective concentration in donor compartment is, in this case, equal to the solubility value. One millilitre samples were taken from the receptor compartment every 60 min over a period of 32 h, in the case of 7-phenyl heptanol the assay duration was 56 h. The volumes withdrawn were replaced with fresh receptor solution. The receptor solution was added with polysorbate 80 at a clearly supramicellar concentration (1%, w/w) in order to provide a micellar reservoir. Consequently, sink conditions were completely fulfilled (Díez-Sales et al., 1991).

In all cases Eq. (1) was used to fit experimental data (Scheuplein, 1967).

$$Q_{(t)} = A \cdot P \cdot h \cdot C \cdot \left[D \cdot \frac{t}{h^2} - \frac{1}{6} - \frac{2}{\pi^2} \sum_{n=1}^{\infty} \frac{(-1)^n}{n^2} \cdot \exp\left(\frac{-D \cdot n^2 \cdot \pi^2 \cdot t}{h^2} \right) \right] \quad (1)$$

where $Q_{(t)}$ is the quantity of penetrant which passes through the membrane and reaches the receptor solution at a given time, t. A represents the diffusional surface area (4.52 cm^2); P, the partition coefficient of the permeant between the membrane and the donor vehicle; h, the membrane thickness; D, the diffusion coefficient of the permeant in the membrane, and C is the concentration of the penetrant in the donor solution. The terms $P \cdot h$ and D/h^2 were replaced in Eq. (1) by P_1 and P_2 respectively, and calculated through fitting the theoretical equation to individual in-vitro permeation data sets using a computerized nonlinear least-squares method (Multi) (Yamaoka et al., 1981). The permeability coefficients, k_p ($= P_1 \cdot P_2$), were calculated and used as representative permeation parameters.

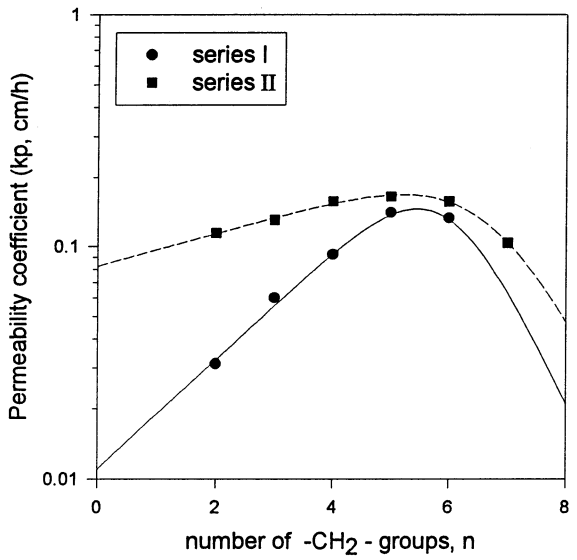

Fig. 1. Graphical plots showing the correlation between the number of $-CH_2-$ groups in the alkyl chain of phenyl alcohols, n, and permeability coefficients, k_p, calculated for the series I (●), non-saturated solutions of phenyl alcohols in the donor compartment (75% of the saturation solubility) and 7 h of experiment and for the series II (■), saturated solutions (with excess) of phenyl alcohols in the donor compartment and long duration of the experiments, according to the bilinear model.

2.5. Analytical procedure

The concentration in the samples was determined by HPLC using a Perkin-Elmer liquid chromatograph which included an Isocratic Series 10 pump, a LC sample processor ISS 200 model automatic injector, a LC 95 UV detector, set at 254 nm (in the case of 7-phenyl heptanol at 204 nm) and a 1020 model integrator. An analytical Novapack C-18 column was employed. The mobile phases were composed of mixtures of acetonitrile and phosphate buffer solution (pH 6.2) in variable proportions, depending on the phenyl alcohol assayed, and were delivered at a flow rate of 1 ml/min at ambient temperature.

Calibration curves covering the entire range of concentrations assayed for the compounds were prepared in triplicate. The accuracy of the method was evaluated by calculating the relative error, which was always less than 12.5%, and precision was evaluated by calculating the variation coefficient, which was lower than 9.9% and is considered acceptable (Karnes and March, 1993).

2.6. Fitting of models to data

Since there is a perfect homology in the tested series, initially kp values and the number of $-CH_2-$ groups in the alkyl chain, n, were correlated through the three classical model equations: bilinear and parabolic (as representative of the probabilistic approaches) and hyperbolic (as representative of the compartmental approaches). This was merely a theoretical approach. In order to place the experiments within a more practical context, in a second step, kp values were correlated with partition coefficients in n-octanol. The solubility values of the phenyl alcohols were also used as lipophilicity indices. The fitting operations were done with the aid of the nonlinear least-squares regression program, Multi:

$$\text{Bilinear} \qquad k_p = \frac{a \cdot K^b}{1 + c \cdot K^d} \quad (2)$$

Parabolic $$k_p = 10^{(a \cdot K^2 + b \cdot K + c)} \quad (3)$$

Hyperbolic $$k_p = \frac{a \cdot K^b}{c \cdot K^b} \quad (4)$$

In these equations, a, b, c and d are constants which can be calculated experimentally and are different for each equation.

2.7. Statistical analysis

ANOVA was used prior to the Tukey test. The statistical criteria for assessing the quality of the fits were the Akaike information criterion, AIC (Akaike, 1976) and the correlation coefficient between experimentally observed and model predicted k_p values.

3. Results and discussion

Accumulated amounts as a function of time in the receptor compartment for the two series of tests (non saturated and saturated solutions) were determined and permeability coefficients, k_p, calculated for each compound; these are shown in Table 1. Values are the means of $n = 4$ determinations.

In the first series (I), the k_p of phenyl alcohols was determined using a solution of each penetrant at 75% of its solubility in the donor compartment. As previously indicated, the duration of the experiments was 7 h. Samples of the donor content, which was replaced every half hour, were analysed. In all of the experiments, less than 10% of the donor phase was transported. The concentration of the compound in the receptor compartment at the end of the experiments (7 h) was always less than 10% of the saturation solubility of the permeants in the medium. Therefore, requirements for zero-order flux were maintained in both cell compartments (Barry, 1983). However, the short duration of experiments (7 h), due to the necessary replacement of donor content, meant that it was not possible to ensure that a steady-state had been reached (Potts and Guy, 1994). In order to analyse this aspect a new series of experiments were carried out (series II) using an excess of penetrants in the donor phase. This procedure does not need donor cell replacements and permits us to increase the time of the experiments. As the accumulated amounts in the receptor compartment at the end of experiments increase as their duration increases, polysorbate 80 at supramicellar concentration was added to guarantee sink conditions (Díez-Sales et al., 1991). However the presence of undissolved amounts of the penetrants in contact with the stratum corneum could modify the diffusional process through the membrane. Therefore to examine this possibility we have calculated the k_p values of the compounds from the amounts penetrated, as a function of time, if the experiments with saturated solutions (added with an excess of the penetrant) were finished in 7 h. These are also shown in Table 1 and are identified as series III.

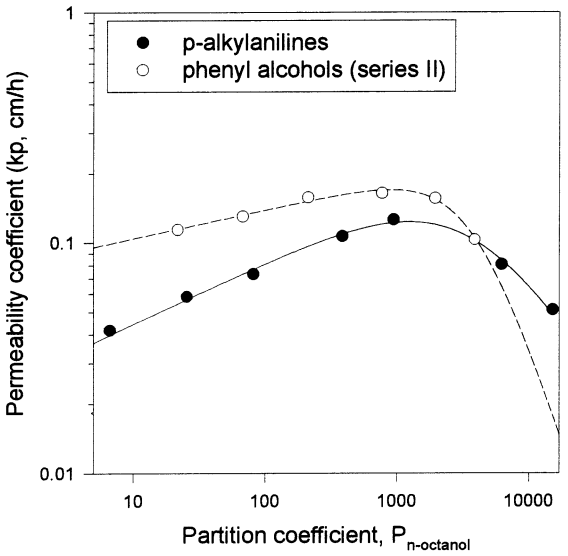

Fig. 2. Graphical plots showing the correlation between partition coefficients in n-octanol, P, and permeability coefficients, k_p, calculated for the series II (○) saturated solutions of the phenyl alcohols in the donor compartment and long duration of the experiments and the kp for the p-alkylanilines (●) through Wistar rat epidermis (Díez-Sales et al., 1993b), according to the bilinear model.

The statistical analysis (Table 1) shows that when we compare the k_p values calculated for 7 h and those obtained for experiments of long duration from experiments carried out with donor saturated solutions (series II and III), there are significant differences for all of the penetrants.

Therefore, 7 h was apparently not enough time to achieve steady-state diffusion and the permeability coefficients obtained were underestimates of the true values.

On the other hand, if we compare the k_p values calculated from the two different experimental conditions (non saturated and saturated solutions in the donor compartment) but considering only 7 h of experiments (series I and III), we can see that any statistical differences are dependent on the lipophilicity of the penetrants. In fact for the more lipophilic compounds (4-phenyl butanol, 5-phenyl pentanol and 6-phenyl hexanol) no statistical differences were found. Then, we can say that, although the measurements were made over too short a period to reach steady-state diffusion, the evolution to this situation, for these penetrants, is made in a similar way in the two experimental conditions compared. However, for the more hydrophilic compounds of the series (2-phenyl ethanol and 3-phenyl propanol) there are statistical differences between the k_p calculated with the two experimental conditions. It could be explained considering that the excess of the penetrant in contact with the membrane could produce a self-enhancing effect on its penetration. In fact, these compounds (2-phenyl ethanol and 3-phenyl propanol) have been previously demonstrated as percutaneous penetration enhancers of 5-fluorouracil (López et al., 1997), thus confirming their enhancer effect.

It is now clear that there is a functional dependence of the steady-state permeability coefficient upon lipophilicity. Correlations between k_p and lipophilicity constants were done, in order to determine how the differences observed in permeability coefficients derived from the different experimental conditions used could be modified. The data for the lipophilicity parameters used for this purpose (solubility, S, and the partition coefficients, P) are also included in Table 1. The relationships obtained between these parameters and the number of $-CH_2-$ groups in the alkyl chain, n, are linear and have excellent correlation coefficients.

$$\log S_n = n \cdot 0.46(\pm 0.02) - 2.24(\pm 0.11) \quad (r > 0.995) \tag{5}$$

$$\log P_n = n \cdot 0.49(\pm 0.10) + 0.46(\pm 0.02) \quad (r > 0.996) \tag{6}$$

Equation and statistical parameters of the correlations between epidermal permeability coefficients calculated for the series of experiments I and II and the lipophilicity indices are shown in Table 2. Based upon statistical parameters obtained from the fits, the bilinear model was found to be the model of choice for both series of experiments.

Plots relating permeability coefficients, for series I and II, and the alkyl chain $-CH_2-$ groups, according to the bilinear model are reproduced in Fig. 1. We can see graphically that by increasing the time of the experiments, the permeability values obtained for all the compounds increase. This increase is higher for the more hydrophilic compounds of the series. If we compare the plots of series I and II we can observe the different slope of the ascending branch of the bilinear curve, which can be attributed to the self-enhancing effect on penetration for 2-phenyl ethanol and 3-phenyl propanol. However, in spite of differences related to experimental conditions, it is important to note that the optimal lipophilicity value for penetration deduced from the fits is the same independently of the series (Fig. 1).

Additionally, in order to compare the penetration behaviour model of phenyl alcohols assayed in the present work (series II) with those obtained with the homologous series of *p*-alkylanilines through the same membrane (Díez-Sales et al., 1993b), *n*-octanol partition coefficients were used as the lipophilicity index because they show a high correlation with the number n of $-CH_2-$ groups in the aliphatic chain of the homologous series (Fig. 2). As can be seen, the bilinear model established for the phenyl alcohols assayed is clearly similar to that obtained with the *p*-alkylaniline homologous series (Díez-Sales et al., 1993a,b, 1996). Because a bilinear correlation implies the existence of an optimum of lipophilicity for permeation, the compounds which have a lipophilicity higher or lower than this optimum value will diffuse with more difficulty through the membrane. The optimal lipophilicity values are the same in both series of compounds, in terms of $\log P$ (*n*-octanol), it would be 3.10. Similar con-

clusions can be deduced from some results reported in the literature with other homologous series and membranes (Durrheim et al., 1980; Roberts et al., 1977; Díez-Sales et al., 1993a) and with a number of different compounds with a wide range of lipophilicity values (Borrás-Blasco et al., 1997). It has been argued that the lack of further increase in permeability coefficients at high values of $\log P_{oct}$ (>3) could reflect retention of the compounds in the stratum corneum or a change in the penetration rate controlling step for these compounds (i.e. partitioning of the penetrant from the lipophilic stratum corneum into the viable epidermis or its uptake in the aqueous receptor phase) (Guy and Hadgraft, 1989; Hadgraft, 1992).

In conclusion, this work points out, once again, the importance of the experimental conditions in the determination of permeability coefficients. At the same time, the results of this work, add new experimental data about the optimal lipophilicity value for skin penetration that is similar to those obtained previously, $\log P_{oct} \cong 3$, which could be considered of use to improve the design of drugs intended for percutaneous administration.

Acknowledgements

The present work is part of a research project (PM 92-0149) carried out with a grant from the DGICYT (Minister of Education and Science) of Spain.

References

Akaike, H., 1976. An information criterion (AIC). Math. Sci. 14, 5–9.

Barry, B.W., 1983. Methods for studing percutaneous absorption. In: Barry, B.W. (Ed.), Dermatological Formulations. Marcel Dekker, New York, pp. 234–295.

Borrás-Blasco, J., López, A., Morant, M.J., Díez-Sales, O., Herráez-Domínguez, M., 1997. Influence of sodium lauryl sulphate on the in vitro percutaneous absorption of compounds with different lipophilicity. Eur. J. Pharm. 5, 15–22.

Bronaugh, R.L., 1989. Determination of percutaneous absorption by in vitro techniques. In: Bronaugh, R.L., Maibach H.I., (Eds.), Percutaneous Absorption. Mechanism, Methodology, Drug Delivery. Marcel Dekker, New York, pp. 239–258.

Curry, S.H., Whelpton, R., 1983. Manual of Laboratory Pharmacokinetics. Wiley, New York, pp. 39–54.

Díez-Sales, O., Copoví, A., Casabó, V.G., Herráez, M., 1991. A modelistic approach showing the importance of the stagnant aqueous layers in in vitro diffusion studies, and in vitro/in vivo correlations. Int. J. Pharm. 77, 1–11.

Díez-Sales, O., Perez-Sayas, E., Martín-Villodre, A., Herráez-Domínguez, M., 1993a. The prediction of percutaneous absorption: I. Influence of the dermis on in vitro permeation models. Int. J. Pharm. 100, 1–7.

Díez-Sales, O., Ruiz Caldés, M.J., López Castellano, A.C., Maiques Llácer, F.J., Herráez Domínguez, M., 1993b. A comparative in vitro study of permeability of basic and non ionic homologous series of compounds through biological membranes. In: Brain, K.R., James, V.J., Walters, K.A. (Eds.), Prediction Percutaneous Penetration, vol. 3B. STS Publishing, Cardiff, pp. 68–75.

Díez-Sales, O., Watkinson, A.C., Herráez-Domínguez, M., Javaloyes, C., Hadgraft, J., 1996. A mechanistic investigation of the in vitro human skin permeation enhancing effect of Azone. Int. J. Pharm. 129, 33–40.

Durrheim, H., Flynn, G.L., Higuchi, W.I., Behl, C.R., 1980. Permeation of hairless mouse skin. I. Experimental methods and comparison with human epidermal permeation by alkanols. J. Pharm. Sci. 69, 781–786.

Flynn, G.L., Stewart, B., 1988. Percutaneous drug penetration: choosing candidates for transdermal development. Drug Dev. Res. 13, 169–185.

Franz, T.J., 1975. Percutaneous absorption. On the relevance of in vitro data. J. Invest. Dermatol. 64, 190–195.

Guy, R.H., Hadgraft, J., 1989. Structure-activity correlations in percutaneous absorption. In: Bronaugh, R.L., Maibach, H.I. (Eds.), Percutaneous Absorption. Mechanism, Methodology, Drug Delivery. Marcel Dekker, New York, pp. 95–119.

Hadgraft, J., 1991. Structure activity relationships and percutaneous absorption. J. Control. Release 15, 221–226.

Hadgraft, J., 1992. Pharmacokinetic modeling of penetration through the skin. Acta Pharm. Nord. 4, 118–119.

Karnes, H.T., March, C., 1993. Precision, accuracy, and data acceptance criteria in biopharmaceutical analysis. Pharm. Res. 10, 1420–1426.

Leo, A., Hansch, C., Elkins, D., 1971. Partition coefficients and their uses. Chem. Rev. 71, 525–553.

López, A., Pellett, M.A., Llinares, F., Díez-Sales, O., Herráez, M., Hadgraft, J., 1997. The enhancer effect of several phenyl alcohols on percutaneous penetration of 5-fluorouracil. Pharm. Res. 14, 681–685.

Potts, R.O., Guy, R.H., 1994. Drug transport across the skin and the attainment of steady-state flux. Proc. Int. Symp. Control. Release Bioact. Mater. 21, 162–163.

Roberts, M.S., Anderson, R.A., Swarbrick, J., 1977. Permeability of human epidermis to phenolic compounds. J. Pharm. Pharmacol. 29, 677–683.

Schaefer, H., 1996. Relationship between the structure of compounds and their diffusion across membranes. In: Schaefer, H., Redelmeir, T.E. (Eds.), Skin Barrier Principles of Percutaneous Absorption. Karger, Basel, pp. 86–177.

Scheuplein, R.J., 1967. Mechanism of percutaneous absorption. II. Transient diffusion and the relative importance of various routes of skin penetration. J. Invest. Dermatol. 48, 79–88.

Yamaoka, K., Tanigawara, Y., Nakagawa, T., Uno, T., 1981. A pharmacokinetic analysis program (multi) for microcomputer. J. Pharm. Dyn. 4, 879–885.