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Influence of drug–surfactant and skin–surfactant interactions on percutaneous absorption of two model compounds from ointment bases in vitro

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Summary

The role of either of two surfactants, sorbitan monooleate (HLB 4.3) and polyoxyethylene *n*-lauryl ether (HLB 12.8), in mass transfer of two model drugs through human epidermis from oleaginous ointments, has been investigated. The two drugs, benzocaine and 2-ethylhexyl *p*-dimethylaminobenzoate, possess different lipophilic properties. Both the thermodynamic activity coefficient changes and the apparent permeability changes produced by surfactant addition to the ointments are independently assessed, thus making it possible to separate the thermodynamic effects of the surfactants from their permeability-enhancing effects mediated through a direct action on the physicochemical properties of the biological membrane. The surfactants appear to interact with both the drugs and the skin in degrees which are dependent on the polarity of the surfactant and the drug. Although each of the surfactants interacts with human epidermis in a way and to an extent independent of the penetrant nature, nevertheless they appear to be rather specific in their action, i.e. they have shown different effects on skin permeability to drugs showing different polarity.

Introduction

Incorporation of surface-active agents into a topical product may produce adverse effects on the percutaneous absorption of a drug (Ashton et al., 1986). Indeed, these additives can influence the permeability of the skin and interact with the drug too, so modifying its thermodynamic activity. Accordingly, a satisfactory understanding of the absorption data is possible only if at least one of the two effects can be independently assessed.

Under steady-state or quasi-steady-state conditions the flux, J, per unit area of a penetrant from an external phase (vehicle) through a resistant membrane into a sink, and the permeability coefficient, P, of the membrane, are expressed by (Higuchi, 1960):

$$J = \frac{D_m}{h \cdot \gamma_m} a_v \tag{1}$$

and

$$P = \frac{J}{C_v} = \frac{D_m}{h \cdot \gamma_m} \cdot \gamma_v \tag{2}$$

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respectively, D_m and γ_m are the diffusion coefficient and activity coefficient of the penetrant in the membrane, respectively, h is the membrane thickness, and a_v and C_v are the thermodynamic activity and the concentration of the penetrant in the vehicle, respectively.

The highest thermodynamic activity of a drug is usually that of the pure drug substance; so, according to Eqn. 1, the flux of a drug across a membrane from a saturated solution is the highest achievable flux, J_{max}, and it is not influenced by the vehicle composition, provided vehicle components do not alter the properties of the membrane. As a consequence, the enhanced absorption ensuing from the addition of a surfactant to a drug suspension can be reasonably considered to be indicative of a direct action of the surfactant on the membrane, i.e. on the variables D_m, γ_m, and h. The alteration degree of membrane permeability to the penetrant can be evaluated by the value of the ratio, $R(J_{max})$, between the flux, J_{max}, from the surfactant-containing vehicle to that, J_{max}, from the reference vehicle (the vehicle without the surfactant); the ratio can be expressed, according to Eqn. 1, as:

$$R(J_{max}) = \frac{D_m/h \cdot \gamma_m}{D_m'/h' \cdot \gamma_m'} \tag{3}$$

In the case where the vehicle is not saturated, surfactant addition can influence all the variables in Eqns. 1 and 2, a_v and γ_v included. If the relative permeability is defined as the ratio, $R(P)$, between the permeability coefficient from the surfactant-containing vehicle to that from the reference vehicle, then from Eqn. 2 it follows that:

$$\frac{R(P)}{\gamma_v/\gamma_v'} = \frac{D_m/h \cdot \gamma_m}{D_m'/h' \cdot \gamma_m'} \tag{4}$$

where the apex refers, as usual, to the reference vehicle. If γ_v/γ_v' can be independently assessed, the ratio $R(P)/(\gamma_v/\gamma_v')$ permits us to verify whether membrane permeability is changing with the change in vehicle composition in the case where the thermodynamic activity of the penetrant changes at the same time. In reality, correlations between percutaneous absorption and thermodynamic activity were found only in few particular cases; for example, Kurihara-Bergstrom et al. (1986) and Barry et al. (1985) determined the thermodynamic activity of volatile penetrants in liquid vehicles by vapour pressure measurements and headspace chromatography, respectively. Various experimental methods can be used to determine the concentration of drug molecules unassociated with surfactant micelles in aqueous vehicles. However, the validity or convenience of these methods fails when they are applied to the study of drug complexation in opaque semi/solid media. In these laboratories the evaluation of the molecular interaction changes of a drug with the change in vehicle composition, was based on the determination of the "relative activity coefficient", γ_r, of the drug in the ointment (Campigli et al., 1986). The coefficient γ_r was defined as the thermodynamic activity coefficient of the drug in the ointment relative to a reference solvent:

$$\gamma_r = \gamma_v/\gamma_s \tag{5}$$

where γ_v and γ_s are the activity coefficients of the drug in the ointment and in the solvent, respectively. Then, provided γ_s is concentration independent, from Eqn. 5 it follows that the ratio γ_v/γ_v' can be calculated from measurement of the relative activity coefficient:

$$\gamma_v/\gamma_v' = \gamma_r/\gamma_r' \tag{6}$$

In a previous paper, correlations between the in vitro release of benzocaine and 2-ethylhexyl *p*-dimethylaminobenzoate (EH-PABA) from petrolatum-based ointments containing or without either of the two non-ionic surfactants, and the γ_r values of the two drugs in the vehicles, were found (Di Colo et al., 1986). The two drugs possess different lipophilic properties and while benzocaine interacted with both the surfactants, which decreased its release rate, EH-PABA interacted slightly with only the less polar of them and its release rate was not affected. The same drugs and the same surfactants, sorbitan mono-oleate (SMO) and polyoxyethylene-(n)-lauryl ether (PLE), were chosen for the present study to investigate the role of the surfactants in mass transfer through skin

from oleaginous ointments, with a view to evaluate separately the effects of the surfactant–skin and drug–surfactant interactions.

Materials and Methods

Materials

Ethyl *p*-aminobenzoate (benzocaine) (Carlo Erba SpA, Milan, Italy) was pulverized in a mortar and sieve-sized to the 40 μm range. 2-Ethylhexyl *p*-dimethylaminobenzoate (EH-PABA) (Van Dick Co., Belleville, NJ, U.S.A.), isopropyl myristate (IPM) (Givaudan, Milan, Italy) sorbitan monooleate (SMO) (Span 80, HLB 4.3, Atlas Europol, Varese, Italy), polyoxyethylene-(n)-lauryl ether (PLE) (G3707, HLB 12.8, Atlas Europol, Varese, Italy), white petrolatum (Carlo Erba SpA, Milan, Italy), paraffin wax (solidification temperature 51–53 °C) (Carlo Erba SpA, Milan, Italy), liquid petrolatum (Carlo Erba SpA, Milan, Italy), methyl alcohol (Hoechst, Italiana SpA, Milan, Italy), were used as received. Polyethylene sheetings, 10 μm thickness, were gently supplied by Solvay (Rosignano Solvay, Livorno, Italy).

The epidermal sheets were isolated from human skin, then stored and rehydrated prior to use according a procedure described in a previous paper (Campigli et al., 1988). Human skin was obtained from operation for cosmetic surgery.

Preparation of the ointments

The composition of the ointments is shown in Table 1. The solution-type ointments were prepared according to a procedure described elsewhere (Campigli et al., 1986; Di Colo et al., 1986). The suspension-type ointments were prepared by carefully levigating benzocaine into the ointments. Prior to use, the ointments were stored at 30 °C for at least 48 h.

Permeability studies

The apparatus and the method employed to follow the permeability rate of the substances under study through the membranes, were described in a previous paper (Campigli et al., 1988). The design of the permeability cells depended on whether the donor compartment contained an ointment or a liquid solution. Either a human epidermal sheet or a polyethylene sheet separated the donor from the receiving phase.

In the ointment studies, the receiving phase was an isotonic sodium chloride solution for benzocaine and a 1:1 (v/v) methanol–water solution for EH-PABA. In the experiments where the donor compartment contained a solution of EH-PABA in a methanol–water mixture, the same solvent was used for both the donor and the receiving phase.

The total amount of drug penetrating through the unit membrane surface and into the receptor was calculated and plotted as a function of time. The drug flux, J, was calculated by the slope of the linear portion of the permeation curves and expressed as the mass of drug passing across 1 cm^2 of membrane over time. A linear regression program was used to determine the equation of the line; analysis was extended to data points in the portion of plot that gave the best fit. In all cases the correlation coefficient was greater than 0.994,

TABLE 1

Composition of the ointments (%, w/w)

Components	Ointments					
	A	B	SMO-3	SMO-9	PLE-3	PLE-9
White petrolatum	60	60	58.2	54.6	58.2	54.6
Liquid petrolatum		30				
IPM	30		29.1	27.3	29.1	27.3
Paraffin wax	10	10	9.7	9.1	9.7	9.1
SMO			3.0	9.0		
PLE					3.0	9.0

in the experiments with human epidermis, and greater than 0.998 with polyethylene sheetings. The apparent permeability, P, was calculated by the ratio of the flux to the penetrant concentration in the vehicle. For comparison, the apparent permeability from a vehicle chosen as reference was taken as unity, and the relative permeability, $R(P)$, from the test vehicles was then calculated.

In the experiments with human epidermis, each run with the vehicles to be compared was performed with sheets obtained from the same tissue section. Each run was repeated 3 or more times with new sheets obtained from either the same skin section or a different one.

The experiments were carried out at 30 °C. Blank runs demonstrated the absence of substances which interfered with the analysis.

Partition coefficient determinations

Benzocaine was partitioned between the ointments and water, while EH-PABA between the ointments and a 7 : 3 (v/v) methanol–water mixture. The apparatus and procedure used for these determinations were described in a previous paper for both benzocaine and EH-PABA (Campigli et al., 1986).

Solubility determinations

The solubility of benzocaine in normal saline was determined according to a procedure previously described for the water solubility determination of the drug (Bottari et al., 1978). The method employed to determine the solubility of EH-PABA in methanol–water mixtures was also previously described (Campigli et al., 1986). The solubilities at 30 °C of benzocaine in normal saline and of EH-PABA in 1 : 1 (v/v) methanol–water are 1.15 and 0.166 mg/ml, respectively.

Assay methods

The drug content in the solutions which had not been exposed to epidermal sheets was determined spectrophotometrically. The absorbances were recorded at 286 nm for benzocaine in normal saline, and at 316 nm for EH-PABA in methanol–water mixtures.

The drug content of samples which had been exposed to epidermal sheets was analysed by HPLC, using a 25 cm × 4.6 mm Erbasil C_{18}-10 μm column (Carlo Erba SpA, Milan, Italy), and UV detection at 286 and 316 nm for benzocaine and EH-PABA, respectively. For benzocaine, elution was carried out with a methanol/1% (v/v) phosphoric acid/diethylamine (40 : 20 : 1) mixture at a flow-rate of 1 ml/min. For EH-PABA the mobile phase consisted of a methanol/water (95 : 5) mixture, and the flow-rate was 1.5 ml/min.

Results and Discussion

The experimental conditions adopted for the permeability studies apparently fulfilled the requirements for the applicability of Eqn. 1. As shown in Figs. 1 and 2, release of benzocaine and EH-PABA from ointment A was zero-order only when a membrane separated the donor from the receiving phase. This should prove that diffusion through the membranes provided the rate-limiting step. As for the requirements for the applicability of Eqn. 6, there is evidence of the concentration independence of γ_s for benzocaine up to 80% saturation in water (the solvent used for the parti-

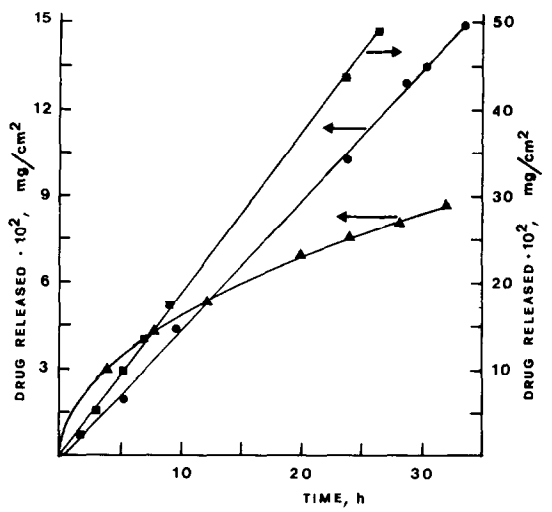

Fig. 1. Typical release curves of benzocaine from suspension-type (■) and solution-type (●, ▲) ointment A. ■, through a polyethylene membrane (C_v = 10%, w/w); ●, through human epidermis (C_v = 4.38 mg/ml); ▲, with no membrane separating the donor from the receiving phase (C_v = 1.75 mg/ml).

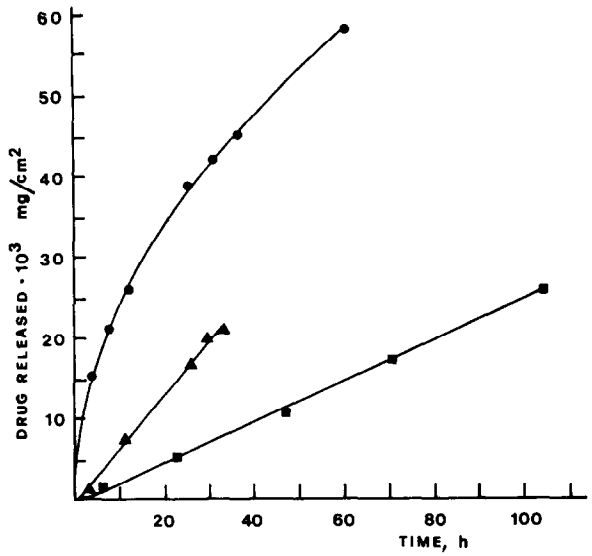

Fig. 2. Typical release curves of EH-PABA from ointment A. ■, through human epidermis ($C_v = 8.75$ mg/ml); ▲, through a polyethylene membrane ($C_v = 8.75$ mg/ml); ●, with no membrane separating the donor from the receiving phase ($C_v = 1.75$ mg/ml).

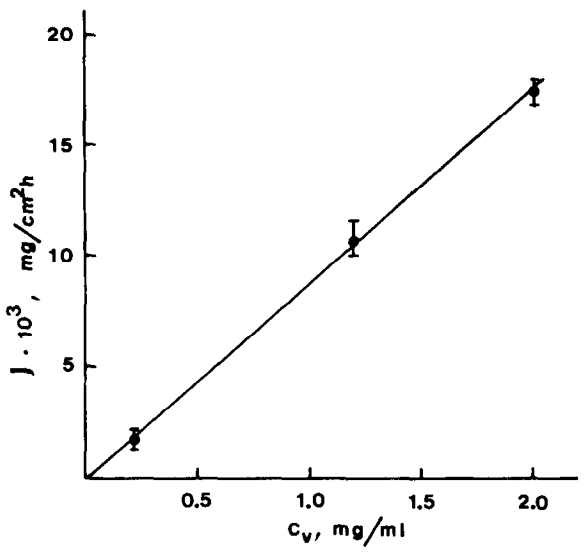

Fig. 3. Relationship between the steady-state flux, J, of EH-PABA through polyethylene membrane from a 7:3 (v/v) methanol–water solution and donor phase concentration, C_v. Bars represent the range.

TABLE 2

Permeation of benzocaine through human epidermis and polyethylene membranes. Relationship between maximum flux ($R(J_{max})$), permeability ($R(P)$) and activity coefficient (γ_v/γ_v') data

Membrane	Ointment	Drug conc. (mg/ml)	$J \times 10^3$ (SD) ($mg \cdot cm^{-2} \cdot h^{-1}$)	$R(J_{max})$		$R(P)$		γ_v/γ_v'	$R(P)/(\gamma_v/\gamma_v')$
				Mean (S.D.)	n [a]	Mean (S.D.)	n [a]		
Epidermis	A	4.38	5.21 (0.79) [b]			1 [c]		1 [c]	1 [c]
		10% (w/w) [d]		1 [c]					
	B	10%(w/w) [d]		0.95 (0.17)	3				
	PLE-3	1.75				0.82 (0.12)	4	0.37 [e]	2.22
		6.13				0.71 (0.16)	4	0.33	2.15
	PLE-9	4.38				0.43 (0.02)	3	0.18	2.39
		10% (w/w) [d]		2.33 (0.33)	4				
	SMO-3	1.75				0.80 (0.16)	4	0.71 [e]	1.13
		6.13				0.75 (0.19)	4	0.62	1.21
	SMO-9	4.38				0.72 (0.17)	4	0.59	1.22
		10% (w/w) [d]		1.32 (0.15)	4				
Polyethylene	A	10% (w/w) [d]		1 [c]					
	PLE-9	10% (w/w) [d]		1.06 (0.07)	3				
	SMO-9	10% (w/w) [d]		0.99 (0.09)	3				

[a] Number of determinations.
[b] Mean value ($n = 7$).
[c] Reference.
[d] Suspension-type ointment.
[e] Datum from Campigli et al. (1986).

tion coefficient determinations of this drug) (Bottari et al., 1977). For EH-PABA, γ_s should be concentration independent at least up to 60% saturation in a 7 : 3 (v/v) methanol–water mixture (the solvent used for the partition coefficient determinations of this drug). Indeed, steady-state flux of the drug from the methanolic solution through a polyethylene membrane was proportional to drug concentration in the donor solution over the range 0.2–2.0 mg/ml (Fig. 3). Moreover, since γ_r values for both benzocaine and EH-PABA in ointment A proved to be concentration independent over the concentration ranges 0.87–5.56 and 1.75–8.75 mg/ml, respectively, this ointment, containing either 4.38 mg/ml of benzocaine or 8.75 mg/ml of EH-PABA, was chosen as reference ointment.

Permeability data and activity coefficient variations of benzocaine are shown in Table 2 as a function of both ointment composition and drug concentration. First, the drug flux from ointment A was measured through 7 different epidermal sheets obtained from the same skin section; the value of the standard deviation shown in Table 2 for the flux value of 5.21×10^{-3} mg · cm^{-2} · h^{-1} is expected for experiments with biological membranes and confirms the reliability of the experimental data. Second, in order to find out if IPM influences the skin permeability, the suspension-type ointments A and B, where B is a wholly hydrocarbon vehicle, were compared; the $R(J_{max})$ value of 0.95($\pm$0.167) reported in Table 2 for ointment B appears to indicate that IPM does not alter the skin permeability.

When the $R(P)$ values calculated for the permeation of benzocaine through human epidermis from the solution-type ointments PLE-3, PLE-9, SMO-3, and SMO-9 were examined, all of them resulted in less than 1.0 (Table 2). Inspection of the γ_v/γ_v' data presented in Table 2 shows that the drug activity coefficient was also clearly lowered when ointment A was loaded with either of the two surfactants. Moreover, both $R(P)$ and γ_v/γ_v' values show a trend to decline with an increase in either drug or surfactant concentration; nevertheless each $R(P)$ value was higher than the corresponding γ_v/γ_v' value. It is evident that the drug-surfactant interactions do not entirely account for the apparent variations of skin permeability. Very probably the skin–surfactant interactions play a part in determining the observed changes; according to Eqn. 4, in an attempt to appraise the contribution of these interactions to the permeability changes, the ratio $R(P)/(\gamma_v/\gamma_v')$ was computed for each ointment. As shown in Table 2, the $R(P)/(\gamma_v/\gamma_v')$ values were higher than 2.0 for PLE-containing ointments, and a little higher than 1.0 for SMO – containing ointments; furthermore, each set of values appears to be independent (within the boundaries of expected experimental variability) of both drug and surfactant concentration. These data were compared with the $R(J_{max})$ values, assessed independently from the suspension-type ointments PLE-9 and SMO-9 (Table 2). Both the $R(J_{max})$ value of 2.33 or that of 1.32, observed for the suspension-type ointments, are in a satisfactory agreement with the $R(P)/(\gamma_v/\gamma_v')$ data calculated for the corresponding solution-type ointments. When considering, according to Eqns. 3 and 4, that both $R(J_{max})$ and $R(P)/(\gamma_v/\gamma_v')$ provide a gauge of the degree of alteration of skin permeability, the conclusion to be drawn is that both the surfactants enhance the permeability of human epidermis as well as lower the drug activity; the extent of these effects depends on the surfactant nature while it appears independent of both drug and surfactant concentration. Permeability experiments through a polyethylene membrane, carried out with the suspension-type ointments A, SMO-9 and PLE-9, gave $R(J_{max})$ values which were not significantly different from 1.0 (Table 2). According to Eqn. 3, results like these are expected just when the additive does not alter the membrane, so that the flux is only dependent on the thermodynamic activity of the penetrant.

In vitro permeability studies of such compounds as EH-PABA, which are essentially hydrophobic, encounter difficulties which can be attributed to permeant insolubility in the aqueous receptor fluid; since these compounds do not readily partition from the skin into the receptor, their permeability usually appears much lower than expected. To facilitate the partition of EH-PABA into the receiving phase, a 1 : 1 (v/v) methanol–water mixture, wherein the drug solubility is 0.166 mg/ml, was tentatively substituted for normal

TABLE 3

Influence of receptor-fluid composition on benzocaine permeation through human epidermis from ointment A. Comparison of permeability of benzocaine and EH-PABA

Drug	Drug conc. (mg/ml)	Receiving phase	$R(P)$		$R(P)$	
			Mean (S.D.)	n [a]	Mean (S.D.)	n [a]
Benzocaine	4.38	NaCl 0.9%	1 [b]		42.4 (3.2)	4
		Methanol–water (1 : 1, v/v)	1.01 (0.15)	5		
EH-PABA	8.75	Methanol–water (1 : 1, v/v)			1 [b]	

[a] Number of determinations.
[b] Reference.

saline. Permeability experiments with benzocaine substantiated this choice since, as shown in Table 3, the use of the methanolic solution as a receptor fluid resulted in skin permeability data similar to those obtained with normal saline. As can be observed, the permeability of benzocaine from ointment A and into the methanolic receptor was many times greater than that of EH-PABA. This result was not unexpected; indeed, while benzocaine is moderately soluble in the ointments under study (Campigli et al., 1986; Nannipieri et al., 1981), EH-PABA is fully miscible with them (Campigli, et al., 1986). Hence, benzocaine does partition into stratum corneum better than EH-PABA does.

Permeability data for EH-PABA through both human epidermis and polyethylene membranes are shown in Table 4. Obviously, all the ointments were solution-type. From the table, it can be observed that surfactant addition can enhance, decrease or have no effect on apparent drug permeability through human epidermis; the extent and the direction of the effect depend on both surfactant nature and drug concentration. In particular, the skin permeability shows a significant increase for 8.75 mg/ml of the drug in ointment SMO-9 ($R(P) = 1.30$), and a plain decrease for 69.6 mg/ml of the drug in ointments A and PLE-9 ($R(P)$ is 0.51 and 0.77, respectively). The γ_v/γ_v' data reported in Table 4 show that EH-PABA, up

TABLE 4

Permeation of EH-PABA through human epidermis and polyethylene membranes. Relationship between permeability (R(P)) and activity coefficient (γ_v/γ_v') data

Membrane	Ointment	Drug conc. (mg/ml)	$J \times 10^5$ (SD) ($mg \cdot cm^{-2} \cdot h^{-1}$)	$R(P)$		γ_v/γ_v'	$R(P)/(\gamma_v/\gamma_v')$
				Mean (S.D.)	n [a]		
Epidermis	A	8.75	24.7 (0.78) [b]	1 [c]		1 [c]	1 [c]
		69.60		0.51 (0.09)	4	0.57	0.89
	SMO-9	8.75		1.30 (0.10)	3	0.84	1.55
		69.60		0.98 (0.23)	4	0.56	1.75
	PLE-9	8.75		1.17 (0.12)	3	0.98	1.19
		69.60		0.77 (0.19)	4	0.61	1.26
Polyethylene	A	1.75		1 [c]		1 [c]	1 [c]
	SMO-9	1.75		0.88 (0.07)		0.84	1.04
	PLE-9	1.75		0.92 (0.05)		0.98	0.94

[a] Number of determinations.
[b] Mean value ($n = 4$).
[c] Reference.

to 8.75 mg/ml, does not interact with PLE while it does slightly interact with SMO, thus corroborating previous findings (Campigli et al., 1986); moreover, the activity coefficient of EH-PABA undergoes a clear decrease (about 40% in each of the ointments under study) when the drug concentration was increased up to 69.6 mg/ml. Since the same drop in the γ_v/γ_v' value was found for both ointment A and the surfactant-containing ointments, it very likely is wholly due to self-association phenomena of the drug. However, whatever the cause of such a drop, once again in the present investigation the activity coefficient variations do not fully account for the permeability changes. Indeed, from the data in Table 4, it appears that only the $R(P)/(\gamma_v/\gamma_v')$ ratios for ointments A and PLE-9 are very close to unity. In contrast, for ointment SMO-9 the $R(P)$ and γ_v/γ_v' values disagreed; once more the disagreement can be interpreted as being due to the contribution to the variations of the apparent permeability of the skin from the absorption-enhancing effect of the additive, mediated through a direct action on the biological membrane. As for the results of the experiments with the polyethylene membranes, they were consistent with the findings previously gained with benzocaine. Indeed, they confirmed, as can be seen from the $R(P)/(\gamma_v/\gamma_v')$ values reported in Table 4, that when the surfactants do not alter the barrier permeability, the permeation rate is entirely dependent on the thermodynamic activity of the penetrant.

In summary, a fairly simple method has been presented for the assessment of the permeability-enhancing effect of additives incorporated in semisolid vehicles, mediated through a direct action on the physicochemical properties of the biological membranes, and partially or totally balanced by drug–additive interactions, which reduce the drug activity in the vehicles. With regard to the additives, they appear to interact with both the drugs and the skin in degrees which are dependent on the polarity of the surfactants and the drugs. Although each of them very probably interacts with human epidermis in a way and to an extent independent of the penetrant nature, nevertheless they appear to be rather specific in their action, i.e. they showed different effects on skin permeability to drugs showing different polarity.

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