International Journal of Pharmaceutics, 44 (1988) 187–196
Elsevier

IJP 01500

Absorption–partition relationships for true homologous series of xenobiotics as a possible approach to study mechanisms of surfactants in absorption. II. Aromatic amines in rat small intestine

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(Received 20 August 1987)
(Accepted 5 December 1987)

Key words: Absorption–partition correlation; Intestinal absorption; Membrane permeability modification; Aqueous diffusion layer removal; Micellar solubilization; Surfactant effects on absorption

Summary

The previously proposed approaches in order to interpret the mechanisms elicited by the nonionic surfactant polysorbate 80 in the colonic absorption of 4-alkylanilines are extended here to the absorption of the same compounds in the rat small intestine, where compound behaviour seems to be complicated by the existence of a pore diffusion pathway, simultaneous with membrane absorption. Globally considered, the effects of surfactant in the intestinal absorption of anilines relative to the behaviour of these compounds in free solution are much less evident than in colon for low-molecular-weight hydrophilic compounds of the series, for which pore absorption is highly operative, but, as lipophilicity and molecular weight increase, the effects of surfactant become more and more significant, showing a close resemblance with those observed in colon. In the absence of surfactant, a bihyperbolic correlation can be established between intestinal absorption rate constants of anilines and partition constants. On this basis, the apparently complex absorption–partition correlations found in the presence of surfactant at the critical micelle concentration are explained here by assuming that pore absorption of the compounds is virtually unaffected by surfactant at this concentration, contrarily to that which occurs with membrane permeation, for which the same effects as found on bulk rate constants in colon are observed; a clearly linear and double-logarithmic correlation can be established between membrane absorption rate constants and partition constants, thus indicating that two main effects of the surfactant are elicited, i.e. the removal of the limiting effect of the aqueous diffusion layer adjacent to the membrane, and an increased membrane polarity. At 5% surfactant concentration in the perfusion fluid, clearly supramicellar, correlations between membrane intestinal absorption rate constants and partition constants become, as occurred with bulk constants in colon, bilinear, due to the multiple-phase equilibrium arising from micellar solubilization of anilines; pore absorption constants are greatly reduced at this concentration, but correlate well with partition constants through an inverse hyperbolic equation as in the former cases. Biopharmaceutical implications of these observations are briefly discussed.

Introduction

In a former paper (Plá-Delfina et al., 1987), a comprehensive mathematical approach suitable to interpret the effects of a non-ionic surfactant, polysorbate 80, on the absorption of a group of xenobiotics (4-alkylanilines) in rat colon was suggested. On the basis of colonic absorption–partition correlations found in the absence of surfactant and in the presence of this additive at its critical micelle concentration (CMC), as well as at supramicellar concentrations, and through the

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comparison and analysis of the equations derived in each case, the modifications observed in the absorption behaviour of the xenobiotics can be satisfactorily explained and even predicted in light of 3 characteristic surfactant mechanisms. These are (a) an increase in membrane polarity leading to a modified penetration of xenobiotics; (b) the removal of the limiting effect elicited by the aqueous stagnant diffusion layer adjacent to the absorbing mucosal surface, and (c) the micellar solubilization of xenobiotics, which, at suitable surfactant concentration, would completely mask the two preceding effects for practical purposes.

In the present paper, this procedure is applied to the absorption of these xenobiotics in the rat small intestine, a highly specialized absorption site in which the correlations between absorption and lipophilicity are substantially different due to the existence of an aqueous pore absorption pathway, simultaneous with membrane permeation (Plá-Delfina and Moreno, 1981; Martín-Villodre et al., 1986), thus making the interpretation of the results presumably much more complicated.

Materials and Methods

Xenobiotics and surfactants

Aniline, 4-methylaniline, 4-ethylaniline, 4-n-propylaniline and 4-n-butylaniline were used as test xenobiotics. As a model surfactant, the non-ionic polysorbate 80 was selected. Although not reported here, experiments with the cationic surfactant, dodecyl-trimethylammonium chloride, were also developed with very similar results.

Absorption studies

Male Wistar rats weighing 200–280 g were used (5 animals per compound and series). The in situ rat gut technique using the whole small intestine (Doluisio et al., 1969), adapted as previously described (Martín-Villodre et al., 1986) was performed. The preparation and characteristics of the perfusing solutions were the same as in colon experiences (Plá-Delfina et al., 1987) except that xenobiotic solutions were adjusted to pH = 6.2 in order to preserve the natural environmental conditions and to obtain realistic absorption rates (Doluisio et al., 1969).

Two series of absorption experiences were developed: in the presence of the surfactant at its CMC (0.0022% at the working pH; Bielsa et al., 1980) in the perfusing solution (k_0 values), and in the presence of surfactant at 5%, w/v, a clearly supramicellar concentration (k_s values). The results obtained were compared with the absorption rate constants found for the xenobiotics in free solution, that is, in the absence of surfactant (k_a values), previously reported (Martín-Villodre et al., 1986).

A significant reduction in volume of the perfused solutions was observed at the end of the sampling period (25 min), so that a correction for water reabsorption became necessary in order to accurately describe the absorption kinetics. In order to achieve this, a method based on the direct measure, at fixed times, of the remaining volumes of the test solutions perfused independently in selected animals was utilized (Martín-Villodre et al., 1986). The corrected remaining concentrations, A, were then used to calculate the first-order absorption rate constants by regression analysis of the ln A values vs time; the zero-time sample was not utilized for regression, in order to minimize the contribution of membrane adsorption effects (Doluisio et al., 1970).

Partition constants

Since through colon experiences, it was observed that the partition constants give, essentially, the same values, as correlation with absorption rate constants is concerned, independent of the presence or absence of surfactant in the working partition system (Plá-Delfina et al., 1987), the previously reported partition coefficients of the selected amines between chloroform (P_1) or *n*-octanol (P_2) and an aqueous phosphate buffer of pH = 6.2, as well as chromatographic partition constants ($1/R_f - 1$) found on cellulose plates impregnated with castor oil by using mixtures of aqueous phosphate buffers of pH = 6.2 and acetone (60 : 40, v/v) as mobile phases (Martín-Villodre et al., 1986) were selected as reference partition constants for correlation with absorption rate constants found in each condition (k_a, k_o or k_s).

Analysis of the samples

The intestinal samples were analyzed for amine content using the diazotation and coupling technique previously described for colon experiences (Plá-Delfina et al., 1987).

Fitting of models to data

In order to fit absorption–partition data found in the absence of surfactant (i.e. k_a, P data) the general bihyperbolic equation found for aniline series was utilized (Martín-Villodre et al., 1986):

$$k_a = k_1 + k_2 = \frac{k_m \cdot P^a}{B + P^a} + \frac{k_p \cdot B'}{B' + P^{a'}} \quad (1)$$

in which, k_m and k_p are the limiting asymptotic values of k_1 and k_2 (the membrane and aqueous pore diffusion rate constants of the solutes, respectively), which are characteristic of the aniline series, and k_a represents the experimentally found absorption rate constant for each compound, already corrected for reabsorption. The terms a, B, a' and B' are constants for the technique, easily calculated by non-linear computer regression (Plá-Delfina and Moreno, 1981; Martín-Villodre et al., 1986).

In order to correlate absorption–partition data for series developed in the presence of surfactant at its CMC in the perfusion fluid (i.e. k_o, P data), it was assumed that the pore absorption rate constant, k_{o2}, is the same as k_2 in equation 1, whereas the membrane absorption rate constant, k_{o1}, was calculated, as was k_1 in colon tests (Plá-Delfina et al., 1987), from the expression:

$$k_{o1} = C \cdot P^d \quad (2)$$

through its double-logarithmic linear transform:

$$\log k_{o1} = d \cdot \log P + c \quad (3)$$

with d and C (10^c) being constants for the technique utilized. Accordingly, the global value of k_o was assumed to be:

$$k_o = k_{o1} + k_{o2} = C \cdot P^d + \frac{k_p \cdot B'}{B' + P^{a'}} \quad (4)$$

Since the resulting fits were excellent, no attempts were made to modify equation 4, which was assumed to be entirely reliable for correlation purposes.

In order to correlate absorption-partition data for series developed in the presence of 5% surfactant in perfusion fluid (i.e. k_s, P data), the reasoning previously established by Plá-Delfina et al. (1987) was used with the following modifications: (a) the membrane absorption rate constants, k_{s1}, were assumed to be governed, at this supramicellar concentration, by the bilinear-type equation deduced from colon experiences:

$$k_{s1} = \frac{C \cdot P^d}{1 + E \cdot P^f} \quad (5)$$

and (b) the pore absorption rate constant at this concentration, k_{s2}, was calculated from the expression, also derived from colon tests:

$$k_{s2} = \frac{k_p \cdot B'}{(1 + E \cdot P^f)(B' + P^{a'})} \quad (6)$$

In Eqns. 5 and 6, a' and B', as well as C and d, have the same meaning as in Eqns. 1 and 2, respectively, whereas f and E are related constants derived through the procedure which is described later. Accordingly, the global value of k_s will be:

$$k_s = k_{s1} + k_{s2} = \frac{C \cdot P^d}{1 + E \cdot P^f} + \frac{k_p \cdot B'}{(1 + E \cdot P^f)(B' + P^{a'})} \quad (7)$$

an equation which fits very well the experimental data found in the presence of polysorbate 80 at 5% concentration in the perfusion fluids. In all the above equations, when chromatographic partition constants have been used instead of partition coefficients, the P term should be substituted by $(1/R_f) - 1$.

The fitting operations, when necessary, were developed in an IBM-PC computer. In order to appreciate the goodness of fits, the correlation coefficients between experimental and model-pre-

dicted absorption rate constants were calculated in all cases.

Results

Absorption rate constants found in the presence of surfactant at CMC and at 5% in the perfusion solutions are shown in Table 1 (k_o and k_s values, respectively, clearly first-order absorption rate constants in all cases). Previously determined absorption rate constants found for anilines in the absence of surfactant (Martín-Villodre et al., 1986) have been also included in the table, as reference points (k_a values). Partition coefficients, P, in chloroform (P_1) and in *n*-octanol (P_2), as well as chromatographic partition constants ($(1/R_f) - 1$) reported for anilines in the absence of surfactant (Martín-Villodre et al., 1986) have also been included in Table 1.

Each set of absorption rate constants was correlated with each one of partition constants through Eqns. 1, 4 and 7, respectively. Equation parameters are given in Tables 2–4. The correlations found by using P_1 values as partition constants have been graphically outlined in Fig. 1, as representative of the general behaviour of the tested compounds.

Decomposition of the absorption rate constants experimentally found (k_a, k_o and k_s) into their membrane and pore components, according to the preceding equations, is shown in Tables 5–7. In

TABLE 1

Absorption rate constants found for the tested compounds in different conditions and partition coefficients used for correlation

Tested compounds	No surfactant	Non-ionic CMC	Non-ionic 5%	Chloroform partition coefficient	n-Octanol partition coefficient	Chromatographic partition constant
	k_a (h^{-1}) [a]	k_o (h^{-1})	k_s (h^{-1})	(P_1) [a]	(P_2) [a]	$((1/R_f) - 1)$ [a]
Aniline	5.645	6.334	5.159	26.36	9.56	0.339
	(0.49)	(0.43)	(0.57)	(0.82)	(0.27)	(0.02)
4-Methylaniline	6.410	7.095	4.639	79.42	22.49	0.725
	(0.37)	(0.78)	(0.33)	(1.55)	(0.18)	(0.07)
4-Ethylaniline	7.375	8.015	4.245	189.89	86.52	1.531
	(0.31)	(0.51)	(0.31)	(3.61)	(6.20)	(0.11)
4-Propylaniline	7.917	8.201	3.024	752.28	248.68	3.433
	(0.22)	(0.34)	(0.27)	(50.9)	(5.71)	(0.41)
4-Butylaniline	6.590	9.597	2.118	1640.12	852.52	7.298
	(0.30)	(1.12)	(0.19)	(34.4)	(53.9)	(0.33)

Figures in brackets are the S.D.S.

[a] Taken from Martín-Villodre et al. (1986).

TABLE 2

Parameters of the bihyperbolic equations which correlate absorption rate constants (k_a) found in the absence of surfactant and partition constants

Correlation between k_a and:	Equation parameters [a]						r^b	Equation number
	k_m (h^{-1})	a	B	k_p (h^{-1})	a'	B'		
P_1	5.980	1.250	$1.22 \cdot 10^3$	5.400	2.129	$2.29 \cdot 10^6$	0.998	8
P_2	6.323	1.319	$1.15 \cdot 10^3$	5.830	2.413	$2.68 \cdot 10^6$	0.958	9
$(1/R_f) - 1$	5.807	2.134	3.149	5.563	2.267	24.120	0.991	10

[a] Through Eqn. 1, from Martín-Villodre et al. (1986).

[b] Between experimental and model-predicted k_a values.

TABLE 3

Parameters describing the correlation between absorption rate constants found in the presence of surfactant at CMC (k_o) and partition constants

Correlation between k_o and:	Equation parameters [a]					r [b]	Equation number
	C	d	k_p (h^{-1})	a'	B'		
P_1	0.182	0.509	5.400	2.129	$2.29 \cdot 10^6$	0.964	11
P_2	0.163	0.580	5.830	2.413	$2.68 \cdot 10^6$	0.956	12
$(1/R_f) - 1$	1.966	0.756	5.563	2.267	24.12	0.987	13

[a] Values k_p, a' and B' are the same as in Table 2. Values C and d have been calculated from Eqn. 3 (see Table 8).
[b] Between experimental and model-predicted k_o values; flatness of the curve makes these values indicative of a good fit.

TABLE 4

Parameters describing the correlation between absorption rate constants found in the presence of surfactant at 5% in the perfusion fluid (k_s) and partition constants

Correlation between k_s and:	Equation parameters [a]							r [b]	Equation number
	C	d	E	f	k_p (h^{-1})	a'	B'		
P_1	0.182	0.509	0.031	0.630	5.400	2.129	$2.29 \cdot 10^6$	0.998	14
P_2	0.163	0.580	0.071	0.581	5.830	2.413	$2.68 \cdot 10^6$	0.993	15
$(1/R_f) - 1$	1.966	0.756	0.622	0.865	5.563	2.267	24.12	0.995	16

[a] Values k_p, a' and B' are the same as in Table 1. Values C and d are the same as in Table 3. Values E and f have been calculated from Eqn. 23 (see Table 10).
[b] Between experimental and model-predicted k_s values.

TABLE 5

Predicted membrane (subindex 1) and pore (subindex 2) diffusion rate constants found by decomposition of the calculated global k_a, k_o and k_s values into their respective components

Tested compounds	No surfactant		Non-ionic, CMC		Non-ionic, 5%	
	k_1	k_2	k_{o1}	k_{o2}	k_{s1}	k_{s2}
Aniline	0.280	5.397	0.937	5.397	0.766	4.393
4-Methylaniline	0.976	5.374	1.721	5.374	1.124	3.515
4-Ethylaniline	2.194	5.237	2.778	5.237	1.469	2.776
4-Propylaniline	4.569	3.413	4.788	3.413	1.765	1.259
4-Butylaniline	5.356	1.330	8.267	1.330	1.824	0.294

Data were obtained from the correlation with chloroform partition coefficients (P_1). Absorption rate constants are expressed in reciprocal time (h^{-1}).

TABLE 6

Same as Table 5 but calculated from n-octanol partition coefficients (P_2)

Tested compounds	No surfactant		Non-ionic, CMC		Non-ionic, 5%	
	k_1	k_2	k_{o1}	k_{o2}	k_{s1}	k_{s2}
Aniline	0.106	5.829	0.505	5.829	0.414	4.745
4-Methylaniline	0.316	5.826	1.269	5.826	0.829	3.810
4-Ethylaniline	1.500	5.729	2.286	5.729	1.209	3.036
4-Propylaniline	3.515	4.757	3.443	4.757	1.268	1.756
4-Butylaniline	5.464	1.078	8.519	1.078	1.880	0.238

TABLE 7

Same as Table 5 but calculated from chromatographic partition constants $((1/R_f) - 1)$

Tested Compounds	No surfactant		Non-ionic, CMC		Non-ionic, 5%	
	k_1	k_2	k_{o1}	k_{o2}	k_{s1}	k_{s2}
Aniline	0.178	5.543	0.791	5.543	0.647	4.512
4-Methylaniline	0.800	5.454	1.641	5.454	1.072	3.567
4-Ethylaniline	2.560	5.017	2.998	5.017	1.586	2.659
4-Propylaniline	4.735	3.313	4.888	3.313	1.802	1.222
4-Butylaniline	5.556	1.170	8.427	1.170	1.859	0.259

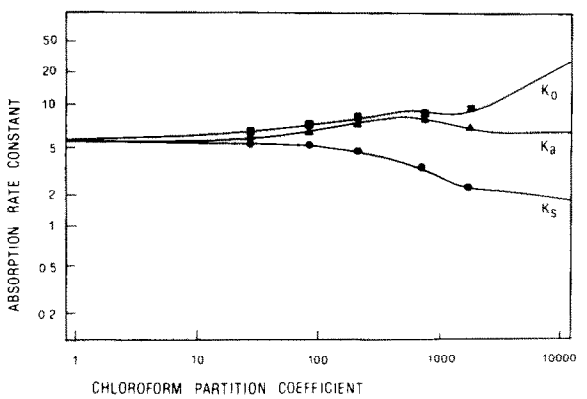

Fig. 1. Plots of the global absorption rate constants found for the tested amines vs. their chloroform partition coefficients. Absorption rate constants were determined in free solution (k_a, Eqn. 8), in the presence of polysorbate 80 at CMC (k_o, Eqn. 11) or in the presence of 5% polysorbate 80 (k_s, Eqn. 14).

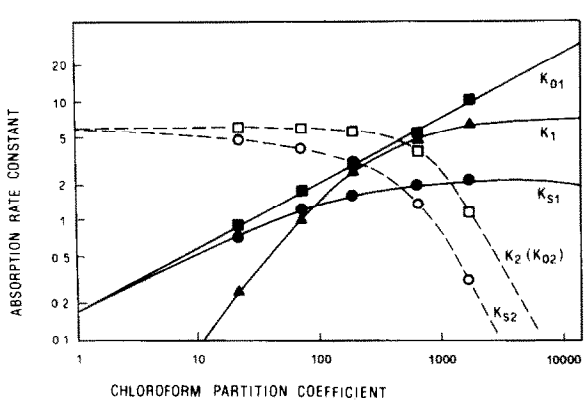

Fig. 2. Decomposition of the curves shown in Fig. 1 into their lipoidal membrane (subindexes 1, continuous lines) and aqueous pore (subindexes 2, dotted lines) components, according to the assumptions made in the text.

Fig. 2, a representative plot of the decomposition of the curves into these two kinetic components is shown.

Discussion

The behaviour in the absence of surfactant

In a former report (Martín-Villodre et al., 1986), it was demonstrated that, for this aniline series, absorption–partition correlations are clearly bihyperbolic in nature, fitting very well to Eqn. 1 (more specifically, to Eqns. 8–10, according to the partition system used). These equations are perfectly functional when applied to the compounds tested, which are true homologous components of the series, as shown in Table 2; the existence of an aqueous diffusion layer adjacent to the mucosal absorbent surface (Ho et al., 1977) limits the membrane absorption rate constants, k_1, to an asymptotic k_m value ranging from 5.8 to 6.3 h^{-1}, whereas the pore absorption rate constants, k_2, are limited by the molecular size of the basal structure of the series (Plá-Delfina and Moreno, 1981) to a maximum k_p value ranging from 5.4 to 5.8 h^{-1}. Consequently, the global absorption rate constants, k_a, when plotted vs partition constants, P_1, P_2 or $(1/R_f) - 1$, will give, according to Eqn. 1, the final bihyperbolic correlations given in Table 2. In Fig. 1, the correlation between k_a and P_1 (Eqn. 8) has been graphically reproduced as representative of this behaviour (k_a line).

The pore and membrane absorption rate constants found for each compound in the absence of surfactant, k_2 and k_1, respectively, are given in Tables 5–7. These values will be unique reference

points in order to interpret the results indicated below.

Tests developed in the presence of surfactant at CMC

In order to fit these absorption–partition data, it was assumed, in principle, that surfactant does not appreciably interfere with the pore penetration process of solutes, i.e. that k_2 and k_{o2} are virtually identical. On this basis, the differences $k_o - k_2$ were calculated, and the resulting values (assumed to be equivalent to the membrane absorption rate constants of the solutes in the presence of surfactant at CMC, k_{o1}) were fitted to Eqn. 3, previously deduced from colon experiences (Plá-Delfina et al., 1987); it should be remembered that, for colonic absorption, only the membrane route is intended to be operative due to the virtual absence of pores with a diameter large enough to allow xenobiotics to penetrate via aqueous diffusion pathways (Plá-Delfina and Moreno, 1981).

Equation parameters are shown in Table 8. In view of the good fits found, the above assumptions were assumed to be reliable in order to describe membrane absorption–partition correlations from the available data.

Correlations between global absorption rate constants values, $k_o(k_{o1} + k_{o2})$, and partition constants are shown in Table 3. Fig. 1 reproduces the graphical plot of k_o vs P_1; the shape of the line, apparently very complex in nature, can be easily explained in light of the preceding reasonings.

TABLE 8

Linear, double-logarithmic correlations found between membrane absorption rate constants (k_{o1}) and partition constants. Note the goodness of two of the fits

Correlated constants	Equation parameters [a]		r	Equation number
	C	d		
k_{o1} vs P_1	0.182	0.509	0.997	17
k_{o1} vs P_2	0.163	0.580	0.987	18
k_{o1} vs $((1/R_f)-1)$	1.966	0.756	0.996	19

[a] From Eqn. 3.

In Tables 5–7, the membrane and pore absorption rate constants (k_{o1} and k_{o2}) predicted in the presence of surfactant at its CMC are given. As can be seen from Fig. 2, the plot of the calculated membrane absorption constants, k_{o1}, vs chloroform partition coefficients, P_1, is essentially similar to that found between k_1 and P_1 values in colon tests (Plá-Delfina et al., 1987). This has been thought to be confirmative of the two main effects the surfactant can develop on the absorbing membrane at CMC: the removal of the limiting effect of the aqueous diffusion layer (which enhances absorption of highly lipophilic compounds), and the increase in membrane polarity (by virtue of which, the slope of the correlation straight line is less than expected, so that absorption of highly hydrophilic compounds of the series is increased relative to that found in free solution); the k_{o1} values calculated for intermediate lipophilicity compounds are virtually unchanged relative to k_1, as occurred in colon tests (Plá-Delfina et al., 1987). When P_2 or $(1/R_f) - 1$ are used for correlation instead of P_1, similar results are obtained.

It should be pointed out, however, that the small absolute values predicted for intestinal membrane absorption rate constants (k_{o1} as well as k_1), specially for the lower elements of the series, relative to that found in colon tests (Plá-Delfina et al., 1987) deserves attention. Such effect can not be attributed to ionization effects leading to a reduced solute lipophilicity from pH 7.5 to pH 6.2, as evidenced from partition constants, which are essentially similar at both pH values. If the assumptions made here are correct, it might indicate that some interdependence between membrane and pore absorption processes could exist. But it could also be associated with some deficiencies in fitting the data for the lower elements of the tested series, attributable to the absence of highly lipophilic, low-molecular-weight compounds (aniline is, indeed, a very lipophilic first element). If such hypothetical compounds could have been tested, the resulting fits would have provided lower k_p and proportionally higher k_1 and k_{o1} values, so that a flatter slope of the straight line relating k_{o1} and partition constants would have been obtained. Since the number of compounds tested here is, by far, insufficient to

develop an effective statistical fitting analysis, this point is intended to be investigated in our laboratory division through the use of a new series including 9 truly homologous compounds, the lower ones being more hydrophilic than aniline at the working pH.

Tests developed in the presence of surfactant above CMC

As correlations found in the presence of surfactant at 5% concentration are concerned, the interpretation becomes even more complicated. Due to micellar solubilization of the compounds, the pore absorption process should be necessarily modified, as is membrane permeation, relative to that found in free solution or in the presence of surfactant at CMC, since only the free amine fraction is available to both absorbing routes.

The pore absorption rate constants, k_{s2}, can be calculated on the basis of the consideration of: (1) the free amine fraction, F_f, assumed to be equivalent to k_s/k_o, as in colon tests (Plá-Delfina et al., 1987) and shown in Table 9; and (2) the pore absorption rate constants at CMC, k_{o2}. Since only the free fraction of amines is available for pore diffusion, the absorption rate constant through pores in the presence of surfactant at 5% in the perfusion solution, k_{s2}, will be:

$$k_{s2} = F_f \cdot k_{o2} \tag{20}$$

F_f values can also be expressed as a function of the apparent "internal" partition coefficients, P_a,

TABLE 9

Calculated free fraction of amines (F_f) and internal apparent partition coefficients (P_a) between micellar and aqueous phases, from absorption rate constants found in the presence of surfactant at 5% in the perfusion solution

Tested compounds	Free fraction of amine (F_f) [a]	Internal partition coefficient (P_a) [b]
Aniline	0.8145	0.228
4-Methylaniline	0.6538	0.529
4-Ethylaniline	0.5296	0.888
4-Propylaniline	0.3687	1.712
4-Butylaniline	0.2207	3.531

[a] Calculated from the ratio (k_s/k_o) (Plá-Delfina et al., 1987).
[b] Calculated from Eqn. 21.

TABLE 10

Correlation between the calculated internal partition coefficients (P_a) and partition coefficients and chromatographic partition constants experimentally found

Partition constants correlated	Equation parameters [a]		r
	E	f	
P_a vs P_1	0.031	0.630	0.995
P_a vs P_2	0.071	0.581	0.993
P_a vs $((1/R_f)-1)$	0.622	0.865	0.998

[a] From Eqn. 23.

between the micellar and aqueous phases (Plá-Delfina et al., 1987), as:

$$F_f = \frac{1}{1+P_a}; \quad P_a = \frac{1}{F_f} - 1 \tag{21}$$

Therefore, P_a values can be easily calculated; they are shown in Table 9. The equivalence of k_{s2} in terms of P_a can be found by combining Eqns. 20 and 21, as:

$$k_{s2} = \frac{1}{1+P_a} k_{o2} \tag{22}$$

and, since P_a is a potential function of the in vitro partition constants (P_1, P_2 or $(1/R_f)-1$), it may be written as:

$$P_a = E \cdot P^f \tag{23}$$

with P being the particular partition constant experimentally determined. E and f values, calculated by regression, are shown in Table 10. By substituting, in Eqn. 22, k_{o2} and P_a by their equivalences, deduced from Eqn. 4 (second addend) and from Eqn. 23, respectively, the mathematical expression for the absorption rate constants through pores from a 5% surfactant solution, k_{s2} is obtained:

$$k_{s2} = \frac{1}{1+E \cdot P^f} \frac{k_p \cdot B'}{B' + P^{a'}} \tag{24}$$

formerly stated as Eqn. 6, which is here justified. That is, an inverse monohyperbolic equation is found, in which the asymptote runs together with

the k_{o2} or k_2 line, as can be seen from Fig. 2 (k_{s2} values); this was to be expected, since for highly hydrophilic compounds, micellar solubilization should be negligible. The k_{s2} values found, shown in Tables 5–7, were correlated with partition constants; the correlation coefficients found, as good as 0.999 for P_1, 0.996 for P_2 and 0.999 for the chromatographic partition constants, make this equation highly functional.

From a similar reasoning, the membrane absorption rate constants, k_{s1}, can be expressed as:

$$k_{s1} = F_f \cdot k_{o1} \quad (25)$$

or, merely, they can be calculated from the differences $k_s - k_{s2}$ with the same results. These constants, shown in Tables 5–7 (k_{s1} values), were correlated with partition constants through the bilinear-type equation previously established through colon tests (Plá-Delfina et al., 1987), equation 5, which, in double logarithmic form, can be written as follows:

$$\log k_{s1} = d \cdot \log P - \log(1 + E \cdot P^f) - \log C \quad (26)$$

where all parameters have been characterized. In view of the fits obtained ($r = 0.999$ for P_1, 0.968 for P_2 and 0.981 for the chromatographic constants), the resulting curves were assumed to be reliable and representative of the membrane absorption behaviour of the tested amines from a 5% surfactant solution. As occurred in colon tests, the left branch of the curves runs together with the k_{o1} line, thus indicating a virtual absence of micellar solubilization (Fig. 2, k_{s1} values).

Global effects and biopharmaceutical implications

Summarizing, as intestinal absorption of amines is concerned, absorption through membrane is affected by the surfactant to the same quality and extent as colonic absorption by itself, whereas absorption through aqueous pores seems to be hardly affected. In general, as can be seen in Fig. 1 (which can be taken as representative of the general behaviour), the effects of the surfactant at its critical micelle concentration, which should lead to an increased absorption (k_o) relative to that found in free solution (k_a) in non-specific absorption sites (Plá-Delfina et al., 1987), are seen here only for highly lipophilic compounds; for hydrophilic elements, the influence of surfactant is irrelevant due to pore absorption, which completely masks its characteristic effects on the membrane polarity (left-hand side of the plot; see also Table 11, k_a/k_o column).

TABLE 11

Statistical comparison of the absorption rate constants found in different conditions

Tested compounds	Statistical significance of the difference in k [a]		
	k_a/k_o	k_a/k_s	k_o/k_s
Aniline	–	–	**
4-Methylaniline	–	***	***
4-Ethylaniline	–	***	***
4-Propylaniline	–	***	***
4-Butylaniline	** [b]	***	** [b]

[a] According to classical t-test. The symbols are: –, not significant; *, $P < 0.02$; **, $P < 0.01$; ***, $P < 0.001$.
[b] According to the Mann–Whitney U-test.

On the contrary, the solubilization phenomena arising when the surfactant is clearly above CMC, leading, in non-specifical absorption sites (Plá-Delfina et al., 1987), to a reduced absorption (k_s) relative to that found both in free solution (k_a) and in the presence of surfactant at CMC (k_o), are clearly observed and become more and more evident as the solute lipophilicity increases (Fig. 1, right-hand side of the graph; Table 11, k_a/k_s and k_o/k_s columns).

The practical implications of these observations are obvious: as absorption in the small intestine is concerned, the role of the surfactant at CMC, leading to an increased rate bioavailability, for drugs which are well-absorbed through pores, will be considerably reduced as compared with colon, stomach and other non-specialized absorption sites. Solubilization effects, leading to a reduced absorption, will be, on the contrary, almost as operative as in these non-specific absorption sites.

It can be predicted that, for series including elements above 200–250 in molecular weight, for which the pore route is not available, the differences in the presence of surfactant at CMC relative to the behaviour in free solution would be

much greater since the bihyperbolic model will collapse to monohyperbolic (Plá-Delfina and Moreno, 1981); the picture would be, then, similar to that obtained for k_1 and k_{o1} values shown in Fig. 2, as occurs in non-specialized absorption sites such as stomach or colon. In fact, the promotion of absorption due to surfactants has been evidenced mainly for highly hydrophilic and/or high-molecular-weight compounds, such as penicillins, cephalosporins (Kreutler and Davis, 1971), phenolsulfonphtalein (Malik et al., 1975; Kwalafallah et al., 1975), pentobarbital (Gouda et al., 1975), aminoglycosides (Muranishi et al., 1979), sulfamethoxazole (Bielsa et al., 1980), thioridazine (Florence, 1981) or sodium iodide (Riegelman and Crowell, 1958).

Acknowledgments

The present work is part of an investigative project developed with a grant from the "Dirección General de Investigación Científica y Técnica (PB86-580), of the Ministry of Education and Science of Spain.

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