International Journal of Pharmaceutics, 37 (1987) 49–64
Elsevier

IJP 01240

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

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(Received 14 November 1986)
(Accepted 21 December 1986)

Key words: Absorption–partition correlation; Colonic absorption; Membrane permeability modification; Micellar solubilization; Surfactant effects on absorption; Stagnant diffusion layer

Summary

The relationships between absorption rate constants found in rat colon and lipophilicity are investigated for a homologous series of xenobiotics (4-alkylanilines) in the presence of a non-ionic surfactant, polysorbate 80, at its CMC and at concentrations clearly supramicellar. The results are compared with those obtained in the absence of surfactant in order to substantiate the effects of this additive in the absorption of xenobiotics. At CMC, the surfactant seems to have two main effects: first, it acts as a modifier of the colonic adsorbent membrane permeability, and second, it nullifies the limiting effect on absorption which is achieved, in the absence of surfactant, by the aqueous stagnant diffusion layer adjacent to the membrane. Due to these effects a direct linear, double-logarithmic correlation is found between absorption rate constants (k_0) and partition parameters instead of the hyperbolic-type correlation which is obtained in the absence of surfactant; the more relevant consequence is a significantly enhanced absorption rate constant for highly hydrophilic and highly lipophilic compounds of the series. This behaviour can be explained by a rearranged diffusional absorption model devoid of any limiting step. At supramicellar concentrations, the solubilization of compounds decreases their absorption rate constants relative to that found at CMC, this effect being more marked as the surfactant concentration and the solute lipophilicity increase. The correlation between absorption rate constants (k_s) and partition parameters becomes bilinear, with a left branch parallel to the k_0 line. This behaviour is explained by the absorption kinetics of the free amine fraction, k_s/k_0, as well as with the aid of some partition principles. In the light of this approach a global picture on the influence of the surfactant on drug absorption is obtained whose features are discussed.

Introduction

Much is known about the effects of surfactants in drug absorption. By circumscribing the problem to dissolved or solubilized substances, it has been shown that most surfactants interact with the absorbing membranes to bring about an enhanced permeability which facilitates the penetration of some dissolved drugs; this effect becomes particularly apparent when the surfactant is below or just at its critical micelle concentration (CMC) in the drug solution, but it can be decreased and even reversed when CMC is surpassed. This latter effect is assumed to be due to solubilization of drug

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molecules in the surfactant micelles, leading to unabsorbable complexes. Apart from these effects, the reduction of the interfacial tension can provide a better contact of dissolved drugs with the absorbing membranes. These and other current topics have been excellently summarized by Florence (1981) in a rational compilative monograph.

Notwithstanding, as the same author points out, the above-mentioned effects cannot always explain, on the whole, all the available experimental results. In spite of the intensive work which has been developed, it is our impression that the possible variables for a complete study on the subject have not been exhausted. For example, the role of lipophilicity in intrinsic drug absorption from surfactant solutions at CMC and at supramicellar concentrations has not been comparatively studied.

For a comprehensive study of these correlations, the use of homologous series of compounds with increasing lipophilicity (drugs or xenobiotics in general) may constitute an excellent approach. Since the absorption–partition correlations are well established for many of such series (Ho et al., 1977; Plá-Delfina and Moreno, 1981), the study of the modifications which are produced in the presence of surfactants could lead to a more rational approach in the interpretation of their influences.

According to this purpose, in the present paper the intestinal absorption–partition correlations for a true homologous series of xenobiotics (4-alkylanilines) in the presence of surfactant at CMC and above it have been assayed and compared with those which have been found in free solution, without surfactant. Polysorbate 80, a non-ionic, was selected as a model compound due to its extensive use as absorption promoter. Absorption tests were carried out in the rat colon rather than the small intestine in order to achieve a better interpretation of the possible interaction phenomena. It has been demonstrated that the relationships between absorption and lipophilicity found in this absorption site are, in general, much simpler than those which can be obtained in the small intestine (Plá-Delfina and Moreno, 1981; Martín-Villodre et al., 1986); therefore, any modification should be, at least in theory, more easily interpreted. On the other hand, it has been reported that the effects of surfactants in absorption are more evident in the large bowel than in the small intestine (Muranishi et al., 1979). Other surfactants and absorption sites are being tested in studies yet to be reported.

Materials and Methods

Xenobiotics and surfactant

Five aromatic amines belonging to a true homologous series (4-substituted anilines) were used as xenobiotics in the experiments: aniline, 4-methylaniline, 4-ethylaniline, 4-propylaniline and 4-butylaniline. They were used as bases and were supplied as reactive grade products (Merck A.G. and Janssen Co.); their purity was checked by HPLC. Although these compounds have definite, mainly long-term, toxic properties, no effects which could affect the absorption rate were noticed during the absorption tests, in the presence or in the absence of surfactant.

Polysorbate 80 was used in the test solution, both at its CMC and clearly above it. Then, 3 series of experiences were carried out using working solutions containing the following concentrations of surfactant: (a) the CMC, which has been stated to be 0.0022% w/v (Bielsa et al., 1980); (b) a medium concentration above the CMC (1%, w/v); and (c) a higher concentration (5%, w/v). In order to ascertain the influence of polysorbate, the results obtained were compared with those found and already reported (Martín-Villodre et al., 1986) for the amines in free solution, which have been used here as unique reference points.

Colonic absorption studies

The study was developed in male Wistar rats weighing 200–280 g. Ten animals per compound were used for the experiences with surfactant at CMC, 6 animals for the series with surfactant at 1% and 5 animals for the series with surfactant at 5% solution.

The in situ rat gut preparation (Doluisio et al., 1969) was used for absorption tests, in which the following modifications were made: a segment of approximately 20 cm of gut between the proximal and the distal colon (1 cm from its junction with

the caecum and 3 cm before the anus) was cannulated and perfused with 50 ml of saline in order to remove debris and faeces and to clean the gut internal surface. The amine test solution, at 37 °C, was then introduced into the cannulated segment and, at fixed time intervals after dosing, the segment was completely emptied, a sample of 0.2–0.4 ml (depending on the amine working concentration) was removed for xenobiotic assay and the solution reintroduced in the gut. The sampling intervals were 5 min for a total of 30 min.

In order to prepare the vehicle solutions, a volume of 990 ml of isotonic saline was buffered to pH 7.5 by adding 1.6 ml of 0.07 M H_2KPO_4 and 8.4 ml of 0.07 M HK_2PO_4 solutions, both made isotonic prior to use. The test solutions were prepared, for series which contain surfactant at CMC, by dissolving a fixed amount of amine under study in the above vehicle solution (w/v) according to its own solubility. After dissolving the test compound, the prescribed amount of polysorbate 80 was added and the pH of the solution was adjusted exactly to 7.5 by adding the necessary amount of 1 N HCl. The working concentrations were 0.02% for 4-butylaniline and 4-propylaniline and 0.05% for the remaining amines. The surfactant concentration was 0.0022% in all instances. For the two remaining series, the required amount of surfactant (to reach 1% or 5% concentration, w/v) was dissolved in the vehicle solution, the necessary amount of amine was added in order to attain a 0.10% concentration of xenobiotic and the pH was adjusted as described above.

The apparent absorption rate constants were determined for each amine solution by least-squares regression analysis of the natural logarithms of the remaining amine contents in the perfusion fluid versus time. In order to minimize the possible adsorption of solutes on the colonic mucosa (Doluisio et al., 1970), in the calculation of the absorption rate constants the initial non-perfused sample (i.e. at zero time) was not employed to calculate the regression line.

Very little reduction in volume, not exceeding 4% at 30 min, was observed, so no correction factor was applied for water reabsorption (Doluisio et al., 1969).

Partition studies

Bulk-phase partition coefficients (P) of the amines between organic solvents, chloroform and *n*-octanol, and aqueous solutions buffered to pH 7.5 and containing 0.0022% of polysorbate 80 were determined according to the classical approaches of Leo et al. (1971) and Curry and Whelpton (1983). The details of the procedure were the same as described previously (Martín-Villodre et al., 1986); the main modification was that the tubes containing the immiscible phases were centrifuged before xenobiotic assay in order to achieve a complete phase separation. No partition tests with solvents were developed at concentrations of surfactant other than CMC.

In order to determine the partition ($1/R_f - 1$) values, which are homologous of P for correlation purposes, partition TLC tests were carried out according to a previously reported technique (Plá-Delfina et al., 1980; Martín-Villodre et al., 1986) on castor oil-impregnated cellulose plates by using, as mobile phase, a mixture of acetone and phosphate buffer of pH 7.5 containing 0.0022%, 1% or 5% of polysorbate 80, in three series of parallel experiences.

Analysis of the samples

The diazotization and coupling technique (Bratton and Marshall, 1939) was used for evaluation of the amines in the sampling fluids, modified as previously described (Martín-Villodre et al., 1986). The absorbance values, measured at 540–570 nm in a Perkin-Elmer, Lambda 3, automatic spectrophotometer, were in the range of linearity previously established for each compound. The procedure is suitable for both absorption and partition samples, provided that a calibration line is developed with each determination or series of determinations.

Fitting of models to data

The absorption (k) and partition (P or $1/R_f - 1$) data were, at first, correlated by means of the hyperbolic general equation (Plá-Delfina and Moreno, 1981):

$$k = \frac{k_m \cdot P^a}{B + P^a} \qquad (1)$$

through the logarithmic-logistic linear transform recommended by Wagner (1979):

$$\text{logit } k = \log \frac{k}{k_m - k} = a \cdot \log P + b \qquad (1A)$$

In these equations, a, b and B $(= 10^{-b})$ are constants that depend on the technique, and k_m is the limiting asymptotic k value for the series, iteratively changed until the best regression is obtained.

Since these equations fit only the k values found in the absence of surfactant, other model equations have been utilized to fit the remaining series of data. One of them was:

$$k = B \cdot P^a \qquad (2)$$

through its double-logarithmic linear transform:

$$\log k = a \cdot \log P + b \qquad (2A)$$

In Eqs. 2 and 2A, a, b and B $(= 10^b)$ are also constants characteristic of the technique used. These equations have proven to be quite suitable to fit absorption rate constants found in presence of surfactant at CMC to any partition constant, as will be shown later.

In order to fit the k values obtained in the presence of surfactant above its CMC (1% and 5% solutions), the following equation was used:

$$k = \frac{B \cdot P^a}{1 + B' \cdot P^{a'}} \qquad (3)$$

or, in logarithmic form:

$$\log k = a \cdot \log P - \log (B' \cdot P^{a'} + 1) + b \qquad (3A)$$

Here, a, B $(= 10^b)$, a' and B' are constants which can be experimentally calculated, as will be shown later.

In all the above equations, when chromatographic partition constants have been used instead of partition coefficients, the term P should be substituted by the $(1/R_f - 1)$ value.

The fitting operations (except the fits to Eqn. 3, which were directly calculated) were developed in an IBM-PC computer. In order to assess the significance of the differences between the k values obtained in different conditions, the classical t-tests were applied.

Results

Absorption rate constants experimentally determined in the presence of polysorbate 80 at CMC (k_0), at 1% (k_{s_1}) and at 5% surfactant solution (k_{s_5}) are shown in Table 1. The apparent absorption rate constants were, in all cases, clearly first-order and showed excellent correlation coefficients (> 0.990) both for individual and average kinetics. Absorption rate constants found in the absence of surfactant (which have been symbolized here by k_a), already reported (Martín-Villodre et al., 1986), have also been included in Table 1 for comparative purposes.

Partition coefficients of the amines in chloroform and in *n*-octanol in the presence of surfactant at CMC (P_0) are shown in Table 2. Reversed-phase chromatographic partition constants obtained with mobile phases containing the surfactant at the three working concentrations, that is, $(1/R_f - 1)_0$, $(1/R_f - 1)_{s_1}$ and $(1/R_f - 1)_{s_5}$, are shown in Table 3. In these tables, the same partition constants found for the amines in the absence of surfactant (i.e. P and $1/R_f - 1$) have also been included (Martín-Villodre et al., 1986).

Absorption rate constants were correlated with partition parameters found in the same conditions by applying the corresponding equations. Thus k_a values were correlated with P and $1/R_f - 1$ values through Eqn. 1, as shown in Table 4; k_0 with P_0 and $(1/R_f - 1)_0$ through Eqn. 2, as indicated in Table 5; k_{s_1} with $(1/R_f - 1)_{s_1}$ and k_{s_5} with $(1/R_f - 1)_{s_5}$ through Eqn. 3, as shown in Tables 6 and 7, respectively. In addition, and as indicated in the above tables, all the absorption rate constants experimentally determined were also correlated with partition constants P and $1/R_f - 1$ obtained in the absence of surfactant since these values are often the only partition parameters which are determined in the usual tests, and they can be considered as quite general reference points. Two of the correlations obtained have been graphically outlined, as representative of the general behaviour, in Figs. 1 and 2.

TABLE 1

Absorption rate constants of the amines in rat colon (± S.D.)

No.		Concentration of polysorbate in the perfusion fluid (w/v)			
		0% [a]	0.0022% (CMC)	1%	5%
1	Aniline	2.766 ± 0.19	3.311 ± 0.40	3.010 ± 0.31	2.210 ± 0.29
2	4-Methylaniline	3.759 ± 0.44	3.759 ± 0.31	2.855 ± 0.41	1.745 ± 0.20
3	4-Ethylaniline	4.213 ± 0.45	4.286 ± 0.41	2.648 ± 0.33	1.443 ± 0.04
4	4-Propylaniline	4.356 ± 0.30	4.840 ± 0.32	2.221 ± 0.29	1.072 ± 0.12
5	4-Butylaniline	4.558 ± 0.37	5.566 ± 0.28	1.694 ± 0.12	0.793 ± 0.07
Number of animals		5	10	6	5

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

TABLE 2

Bulk-phase partition coefficients of the amines in chloroform and n-octanol (± S.D.)

No.	Tested amines	Chloroform		*n*-Octanol	
		No surfactant [a]	Surfactant at CMC (0.0022%)	No surfactant [a]	Surfactant at CMC (0.0022%)
1	Aniline	23.96 ± 3.15	23.50 ± 1.01	9.62 ± 0.37	8.04 ± 0.15
2	4-Methylaniline	83.70 ± 3.05	73.62 ± 2.97	24.60 ± 0.96	29.02 ± 0.78
3	4-Ethylaniline	191.08 ± 7.19	182.66 ± 8.42	92.37 ± 6.56	70.19 ± 2.21
4	4-Propylaniline	981.40 ± 67.88	982.57 ± 51.37	253.49 ± 11.10	285.03 ± 26.07
5	4-Butylaniline	2350.74 ± 99.10	2128.62 ± 111.74	1110.36 ± 29.36	778.08 ± 26.44

[a] From Martin-Villodre et al. (1986).

TABLE 3

Chromatographic partition constants of the amines: $1/R_f - 1$ values (± S.D.)

No.	Tested amines	Concentration of polysorbate 80 in the mobile phase			
		0% [a]	0.0022% (CMC)	1%	5%
1	Aniline	0.646 ± 0.02	0.642 ± 0.07	0.508 ± 0.02	0.336 ± 0.02
2	4-Methylaniline	1.205 ± 0.11	1.144 ± 0.06	1.117 ± 0.02	0.870 ± 0.04
3	4-Ethylaniline	1.854 ± 0.14	1.901 ± 0.08	1.641 ± 0.03	1.300 ± 0.09
4	4-Propylaniline	3.888 ± 0.39	3.725 ± 0.20	3.632 ± 0.06	2.278 ± 0.17
5	4-Butylaniline	7.710 ± 0.38	7.234 ± 0.03	6.507 ± 0.40	3.341 ± 0.12

[a] From Martin-Vollodre et al. (1986).

TABLE 4

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

Correlation between k_a and	Eqn. parameters				Eqn. number
	k_m (h^{-1})	a	B	r [a]	
P (chloroform)	4.534	0.938	12.585	0.996	4
P (*n*-octanol)	4.470	1.168	8.598	0.995	5
$1/R_f - 1$	4.537	1.857	0.285	0.997	6

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

TABLE 5

Parameters describing the linear, double-logarithmic correlation between absorption rate constants found in the presence of surfactant at CMC (k_0) and partition constants

Correlation between k_0 and	Eqn. parameters				Eqn. number
	a	b	B	r [a]	
P (chloroform)	0.1098	0.3690	2.339	0.994	7
P (*n*-octanol)	0.1087	0.4186	2.622	0.998	8
$(1/R_f) - 1$	0.2093	0.5630	3.656	0.997	9
P_o (chloroform) [b]	0.1099	0.3717	2.353	0.993	10
P_o (*n*-octanol) [b]	0.1125	0.4164	2.608	0.997	11
$[(1/R_f) - 1]_o$ [b]	0.2138	0.5641	3.665	0.999	12

[a] Between experimental and model-predicted k values.

[b] Values obtained in the presence of surfactant at CMC in the aqueous phase.

TABLE 6

Parameters describing the bilinear correlation between absorption rate constants found in the presence of polysorbate 1% (k_{s_1}) and partition constants

Correlation between k_{s_1} and	Equation parameters [a]					Eqn. number
	a	B	a'	B'	r [b]	
P (chloroform)	0.1098	2.339	0.6449	0.0160	0.982	13
P (n-octanol)	0.1087	2.622	0.6327	0.0320	0.983	14
$(1/R_f) - 1$	0.2093	3.656	1.2222	0.2211	0.983	15
$[(1/R_f) - 1]_{s_1}$ [c]	0.2038	3.774	1.2009	0.2643	0.985	16

[a] Values a and B calculated through Eqn. 2 (see Table 5). Values a' and B' calculated through Eqn. 33 (see Table 10).
[b] Between experimental and model-predicted k values.
[c] Value determined in the presence of polysorbate (1% in the aqueous phase).

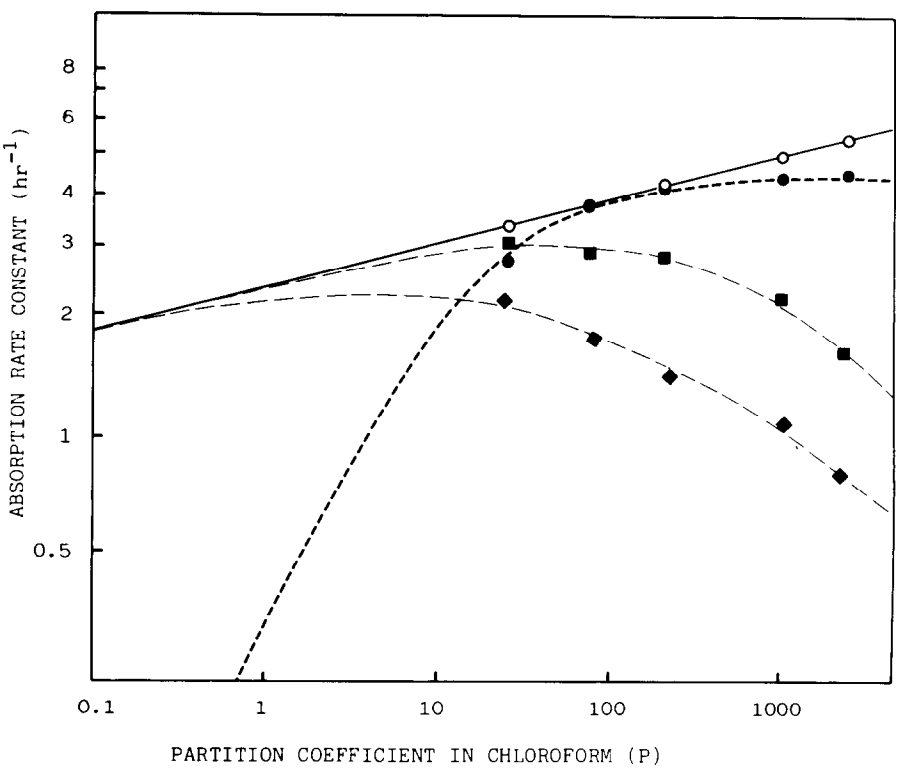


Fig. 1. Plots of the absorption rate constants experimentally found for the selected amines versus their partition coefficients between chloroform and an isotonic aqueous buffer (pH = 7.5) without surfactant. Absorption rate constants were determined in the absence of surfactant (●- - - - -●, k_a), in the presence of polysorbate at CMC (○———○, k_0), in the presence of 1% polysorbate (■— — —■, k_{s_1}) and in the presence of 5% polysorbate solutions (◆— — —◆, k_{s_5}). Accordingly, the regression lines are graphical representations of Eqns. 4, 7, 13 and 17, respectively.

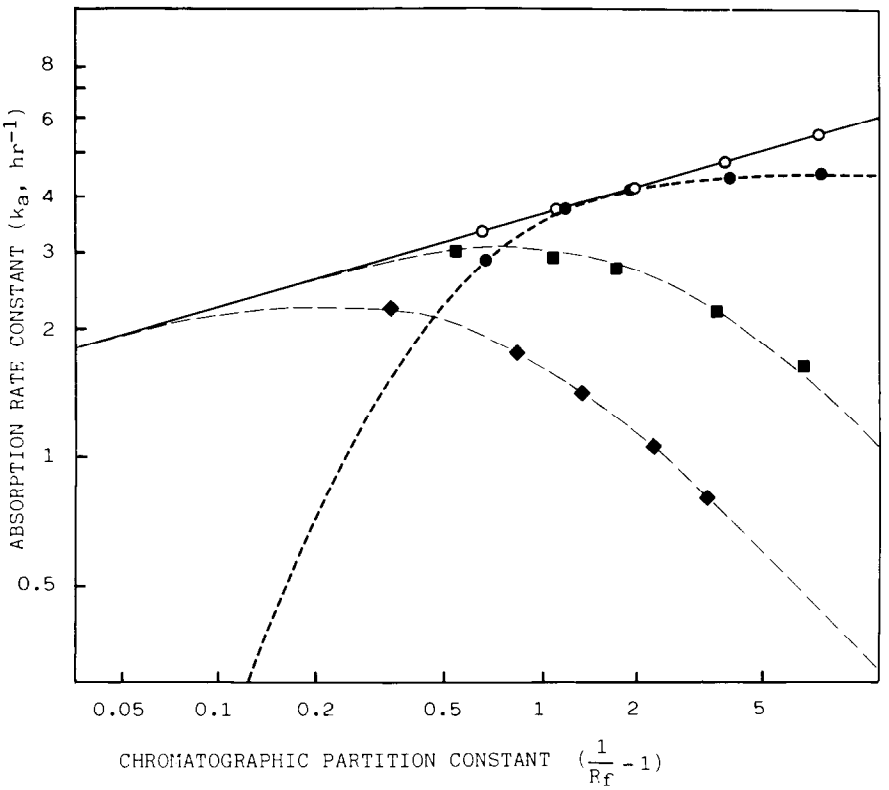


Fig. 2. Plot of the absorption rate constants of the tested amines versus chromatographic partition constants experimentally found in the same conditions (i.e. without surfactant, with surfactant at CMC, in 1% and 5% solutions of surfactant). The symbols are the same as in Fig. 1. The regression lines have been calculated through Eqns. 6, 12, 16 and 20, respectively.

TABLE 7

Parameters describing the bilinear-type correlation between absorption rate constants found in the presence of polysorbate 5% (k_{s_5}) and partition constants

Correlation between k_{s_5} and	Eqn. parameters [a]					Eqn. number
	a	B	a'	B'	r [b]	
P (chloroform)	0.1098	2.339	0.5203	0.1080	0.987	17
P (n-octanol)	0.1087	2.622	0.5105	0.1893	0.982	18
$(1/R_f) - 1$	0.2093	3.656	0.9866	0.8991	0.981	19
$[(1/R_f) - 1]_{s_5}$ [c]	0.2242	4.093	1.0820	1.5023	0.999	20

[a] Values a and B calculated through Eqn. 2 (see Table 5). Values a' and B' calculated through Eqn. 33 (see Table 10).
[b] Between experimental and model-predicted k values.
[c] Value determined in the presence of polysorbate (5% in the aqueous phase).

Discussion

The behaviour in the absence of surfactant

It has been shown (Dietschy et al., 1971; Wilson and Dietschy, 1974) that the entire surface of the intestinal absorbent membrane is coated by a relatively unstirred aqueous boundary layer whose features are physicochemically distinct from those

of the well-stirred intraluminal fluid contents. Some authors have established that diffusion across this aqueous layer acts as the rate-determining step for absorption of lipophilic compounds (Suzuki et al., 1970; Ho et al., 1977; Winne, 1978; Higuchi et al., 1981). In the former report, Suzuki and coworkers came to this conclusion through the analysis of the absorption/partition data found for some xenobiotics belonging to homologous series of increasing lipophilicity; they found a direct hyperbolic correlation between intestinal absorption rate parameters and partition coefficients, as predicted from the biophysical absorption model previously established by the authors. In light of later reports, this type of correlation seems to be especially suitable when the absorption experiences have been developed in non-specialized absorption sites of the digestive tract, such as the stomach or the large bowel; in the small intestine, this relationship is found only when the molecular size of the solutes makes the intestinal pore route unavailable for diffusion (Plá-Delfina and Moreno, 1981; Martín-Villodre et al., 1986).

Wagner (1979), by assuming that the rate-limiting step for absorption could not lie in the stagnant water layer but rather in the serosal side, in the vicinity of the capillary plasma, developed an extraction theory, which leads to similar conclusions, with the advantage of having a biophysical absorption model which can be treated in a simpler and much more functional way (i.e. through the logarithmic-logistic linear transform, already described as Eqn. 1A); this treatment can be recommended as general for this type of function and has been utilized in the present study for fitting the colonic absorption–partition data found in the absence of surfactant. Wagner, like the preceding authors, found a direct hyperbolic correlation between absorption rate constants and partition parameters.

Although, as will be shown later, the anatomical location of the limiting aqueous barrier is a point of crucial importance, this feature is mathematically irrelevant in order to characterize the correlation between k_a and P (or $1/R_f - 1$), which, in both cases, can be represented by Eqn. 1, in which, k_m is the diffusion rate constant across the limiting aqueous barrier (stagnant water or serose layers) and can be identified as the maximum k_a value the members of a given series will attain as the lipophilicity increases towards infinity (i.e. the asymptote of the corresponding hyperbola), whereas a and B are constants depending on the experimental partition technique, which are necessary for extrapolating partition constants found in vitro to those which prevail in vivo, between membranes and luminal fluids.

In our experimental conditions, when the data found in the absence of surfactant are considered, the already outlined provisions are perfectly fulfilled. For the 5 selected amines, the correlation obtained between k_a and P or its related chromatographic constant $(1/R_f - 1)$ is truly hyperbolic in nature, so that, according to the partition system used, Eqns. 4, 5 or 6 are obtained, as shown in Table 4 and in Figs. 1 and 2 (dotted lines). This is no more than a confirmation of other observations (Wagner, 1979; Plá-Delfina et al., 1980; Martín-Villodre et al., 1986). In our case, however, it will constitute an excellent reference point in order to interpret the results obtained in the presence of surfactant.

Tests developed in the presence of surfactant at CMC

Computer iteration evidence shows that, when the logits of k_0 values are fitted against the logarithms of P, P_0 or the related chromatographic constants through Eqn. 1A the term P^a becomes negligible with respect to B, so that Eqn. 1 reduces to:

$$k_0 = \frac{k_m}{B} \cdot P^a \qquad (2B)$$

or, in logarithmic form:

$$\log k_0 = a \cdot \log P + \log \frac{k_m}{B} \qquad (2C)$$

equations which are analogous to Eqns. 2 and 2A, respectively. That is, a linear, double-logarithmic correlation between k_0 and partition constants is substantiated, as indicated in Table 5 (Eqns. 7–12). This would mean that, if some limiting factor exists for colonic absorption, it is so large that it can be neglected. In Figs. 1 and 2, these straight

fits have been drawn as continuous lines.

In order to explain this experimental evidence, a diffusional biophysical model type approach, similar to others which have been postulated (Suzuki et al., 1970) but without the consideration of the aqueous diffusion layer adjacent to the membrane, can be used. A derivation of this model is shown in the Appendix. As can be observed, this approach leads to Eqn. 2 and can be considered as the limit of the hyperbolic equation when the limiting step is cancelled.

The obvious conclusion is that, in the absence of surfactant, the aqueous stagnant layer effectively serves as the rate-limiting factor for absorption of the lipophilic solutes, but, in the presence of surfactant at CMC, this limiting step is lacking. This is, probably, the main reason for the enhanced absorption rate constants found for the highly lipophilic compounds of the series (4-butylaniline and even 4-propylaniline), for which the diffusion in the aqueous stagnant layer will stabilize, in the absence of surfactant, their absorption rate constants, k_a, to an asymptotic k_m value. In Figs. 1 and 2 it can be observed that these amines are situated practically on the asymptote when they are tested without surfactant, whereas, in the presence of it at CMC, their corresponding k_0 values increase parallel to their lipophilicity, an increase which becomes more evident when higher compounds are considered (as occurs, for example, with 4-cyclohexylaniline, for which, the linearity between absorption and partition data is perfectly maintained and which increment in the absorption rate constant relative to that found in the absence of surfactant is as high as 1.65 h^{-1}).

The mentioned effect on the diffusion layer could be expected in view of old (Riegelman and Crowell, 1958) and also recent (Sakai et al., 1986) observations about the cleansing action and the removal of surface membrane coating materials by surfactants. Moreover, Amidon et al. (1982) reported also, through in vitro experiments, that surfactant micelles can reduce the resistance of the aqueous surface layers developed on artificial lipophilic membranes. But we think that the implications of this phenomenon on absorption become more evident through the analysis of the experimental data afforded here.

For the remaining compounds of the series, that do not reach the k_m value when tested in the absence of surfactant (and, therefore, which are not clearly limited by the aqueous stagnant layer in normal conditions), a different picture is seen. The more hydrophilic compound (aniline) shows a slight but significant increase in absorption constant when tested in the presence of surfactant at CMC (Table 8); it is to be expected that this increase will be more evident for series including compounds with partition coefficients lower than that of aniline (see Figs. 1 and 2, left hand side of the graphs). Such an increase does not appear for the two medium-lipophilicity compounds of the tested series (4-methylaniline and 4-ethylaniline), which keep their values apparently unmodified in presence or in absence of surfactant (i.e. $k_a \approx k_0$).

This behaviour can only be explained by assuming that, as has been often reported (Davis et al., 1970; Gibaldi and Feldman, 1970; Kreutler

TABLE 8

Statistical comparison of the absorption rate constants obtained in different conditions

No.	Tested amines	Statistical significance of the difference in k [a]					
		k_a/k_0	k_a/k_{s_1}	k_a/k_{s_5}	k_0/k_{s_1}	k_0/k_{s_5}	k_{s_1}/k_{s_5}
1	Aniline	*	–	**	–	***	**
2	4-Methylaniline	–	**	***	***	***	***
3	4-Ethylaniline	–	**	***	***	***	***
4	4-Propylaniline	*	***	***	***	***	***
5	4-Butylaniline	***	***	***	***	***	***
Degrees of freedom		13	9	8	14	13	9

[a] – = not significant; * = $p < 0.02$; ** = $p < 0.01$; *** = $p < 0.001$.

and Davis, 1971; Kwalafallah et al., 1975), a significant modification of the membrane permeability is produced, leading to higher polarity. This modification would result in an increase in penetration rate for the more hydrophilic compounds (only aniline in our case) but would not apparently change that of the medium-lipophilicity components of the series due, probably, to an interaction between this permeability effect and the removal of the limiting step already considered. For the highly lipophilic compounds, the absorption values will increase due to the net predominance of this latter effect on permeability modification.

Therefore, the global picture found for absorption in the presence of surfactant at CMC relative to that found in free solution cannot be interpreted without the consideration of the interaction of the two described effects: (a) an increase of membrane polarity leading to a higher penetration rate for hydrophilic compounds; and (b) the disappearance of the aqueous unstirred layer limiting step leading to an increase in absorption rate for lipophilic components of the series.

Tests developed in the presence of surfactant above its CMC

The apparent absorption rate constants found for the tested amines in the presence of polysorbate at supramicellar concentration (1% and 5%) show a manifest tendency to decrease in comparison with those obtained in the presence of the surfactant at CMC or in free solution without surfactant, with the possible exception of aniline (Table 1). It can be assumed that the presence of micelles leads to solubilization of the amines and that the solubilized, bound fraction is not directly available for absorption, as has been already reported (Gibaldi and Feldman, 1970; Gouda et al., 1975). As the absorption of the free fraction proceeds, a corresponding amount of bound amine is allowed to leave the micellar phase and to pass into free solution in order to maintain the dynamic equilibrium. The "absorption rate constants" found in the presence of such surfactant concentrations should, therefore, be considered as hybrid values since they are referred to the total amine contents (free + bound) analytically determined in the intestinal samples.

The modification of membrane permeability, as well as the removal of the aqueous layer limiting step are effects which, undoubtedly, must also be produced by the surfactant molecules above CMC. But they are apparently masked by solubilization at the working polysorbate concentrations, so that the net result is the above mentioned decrease — sometimes dramatic — in the apparent absorption rate constants, k_s (Table 1). Aniline, the most hydrophilic component of the tested series, shows the lowest degree of solubilization, so that the k_s value found in the presence of surfactant (k_{s_1}) is only slightly smaller than that found in the experiences with surfactant at CMC; the difference is not statistically significant. But in the remaining instances a significant reduction in k_s values is found relative to k_0 or k_a. The extent of this reduction seems to increase with the surfactant concentration (a greater number of micelles per volume unit in the perfusing solution) as well as with lipophilicity of the solutes (a stronger affinity of the amines to the micellar phase as their lipophilicity increases, leading to a more complete solubilization).

The interaction of the multiple variables which are responsible for this behaviour should necessarily result in a complex correlation between k_s values and in vitro partition constants. However, in light of the results obtained at CMC, on the basis of the absorption kinetics of the free amine fraction and through the consideration of some partition principles, it is possible to reach a tentative interpretation of such correlations.

Let us assume that, at these high surfactant concentrations, the capacity for micellar solubilization of the amines in 0.1% solution is far from its saturation. Since first-order absorption kinetics has been observed in the absorption tests in all cases, we may write:

$$\frac{dA_t}{dt} = -k_s \cdot A_t \qquad (21)$$

where A_t represents the total amount of amine in the working solution, as measured in the intestinal samples, and k_s is the apparent, experimentally determined absorption rate constant. On the other hand, it can be assumed that the time course of

the solubilized and free amounts of the amine in the luminal fluid during the absorption process will be, respectively:

$$\frac{dA_m}{dt} = -k_1 \cdot A_m + k_2 \cdot A_f \quad (22)$$

$$\frac{dA_f}{dt} = k_1 \cdot A_m - k_2 \cdot A_f - k_0 \cdot A_f \quad (23)$$

where A_m and A_f are the amounts of solubilized and free amine, k_1 and k_2 are the rate constants governing the equilibrium between these two forms in the luminal medium, and k_0 is the absorption rate constant which performs when the xenobiotic is 100% free (i.e. the value found in the presence of surfactant at CMC since, theoretically, the same amount of surfactant molecules should exist at CMC and at supramicellar surfactant concentrations). The time course of the total amine present in the colonic lumen can also be described in terms of the sum of the variations of free and solubilized amine forms (Eqns. 22 and 23), so that, after simplification, we have:

$$\frac{dA_t}{dt} = \frac{dA_m}{dt} + \frac{dA_f}{dt} = -k_0 \cdot A_f \quad (24)$$

and, since Eqns. 21 and 24 describe the same process, it may be written:

$$k_s \cdot A_t = k_0 \cdot A_f \quad (25)$$

If the process is considered in terms of fractions, F, rather than amounts, A (i.e. $A_t = 1 = F_m + Ff$), the free fraction of amine in presence should be:

$$F_f = \frac{k_s}{k_0} \quad (26)$$

That is, the free fraction of the xenobiotic in the working solution can be calculated as the ratio of its k_s value and the k_0 value obtained from a solution containing the surfactant at CMC. Obviously, the solubilized, bound fraction should be:

$$F_m = 1 - \frac{k_s}{k_0} \quad (27)$$

The F_f values for the tested amines at the two selected working concentrations of polysorbate are shown in Table 9.

It can be defined an "internal" partition coefficient of the solute between the micellar and aqueous phases in a surfactant solution (Collett and Koo, 1975; Tomida et al., 1978), P_i:

$$P_i = \frac{C_m}{C_a} = \frac{A_m/V_m}{A_a/V_a} = \frac{A_m \cdot V_a}{A_a \cdot V_m} = \frac{A_m \cdot V_a}{A_f \cdot V_m} \quad (28)$$

where C_m and C_a are the concentrations of the solute in the micelles and in the free solution, respectively, and V_m and V_a are the respective volumes of the phases; the amount in free solution, A_a, is, obviously, equal to the free amount, A_f. Making V_a/V_m constant for a given series of experiences (K), we have:

$$P_i = K\frac{A_m}{A_f}$$

so that:

$$\frac{P_i}{K} = P_a = \frac{A_m}{A_f} \quad (29)$$

where P_a represents the "apparent" internal partition coefficient of the solute between the micellar and aqueous phases, the only parameter which can be calculated since the volume-phase ratio is not known. By considering the partitioning process in

TABLE 9

Calculated free fraction of amines (F_f) and internal apparent partition coefficients (P_a) between micellar and aqueous phases

No.	Tested amines	F_f [a]		P_a [b]	
		(1%)	(5%)	(1%)	(5%)
1	Aniline	0.909	0.667	0.100	0.499
2	4-Methylaniline	0.759	0.464	0.317	1.155
3	4-Ethylaniline	0.618	0.337	0.618	1.967
4	4-Propylaniline	0.459	0.221	1.179	3.525
5	4-Butylaniline	0.304	0.142	2.289	6.042

[a] Calculated from Eqn. 26.
[b] Calculated from Eqn. 30.

terms of fractions:

$$P_a = \frac{F_m}{F_f} \tag{30}$$

and, by substituting the F_m and F_f values according to Eqns. 26 and 27, the equivalence of k_s in terms of P_a can be obtained:

$$k_s = \frac{k_0}{1 + P_a} \tag{31}$$

Then, by expressing k_0 in terms of in vitro P values according to the corresponding equation (Eqn. 2), we have:

$$k_s = \frac{B \cdot P^a}{1 + P_a} \tag{32}$$

and, since P_a values for amines, calculated through Eqn. 30 (Table 9), can also be correlated with those of P (Barry and El-Eini, 1976; Tomida et al., 1978), we can also express the former in function of the latter. The correlations found between P and P_a should have the form:

$$P_a = B' \cdot P^{a'} \tag{33}$$

and are shown in Table 10, according to the partition systems used. If we substitute P_a in Eqn. 32, we have the final expression of the correlation between k_s and the partition parameters determined in vitro:

$$k_s = \frac{B \cdot P^a}{1 + B' \cdot P^{a'}} \tag{34}$$

or, in logarithmic form:

$$\log k_s = a \cdot \log P - \log (B' \cdot P^a + 1) + b \tag{35}$$

(where $B = 10^b$). That is, a bilinear type equation is found, which could be explained by considering the existence of a multiple-phase equilibrium of the solute in the micellar phase, the aqueous luminal solution, the lipoidal membrane and the plasma water. This bilinear behaviour, which has been claimed as a general phenomenon in absorption processes (Kubinyi, 1976, 1979), seems, therefore, to appear as a consequence of the micellar solubilization, a first step which does not exist in the absence of surfactant, in which case a direct hyperbolic correlation between absorption and partition data should be found, as demonstrated in the preceding paragraphs. This observation does not mean that bilinear equations could not be of general use. On the contrary, it is the opinion of the authors that, as Kubinyi (1976, 1979) points out, they should satisfy the processes in which multiple-phase equilibria are implicated much better than the empirical parabolic equations currently employed (i.e. most structure–activity relationships).

Since all the parameters in Eqns. 34 and 35 can be calculated from the experimental data found through Eqns. 2 and 33 (see Tables 6 and 7), the lines representing the correlation between k_s and P or the related parameter $1/R_f - 1$ can be drawn (Figs. 1 and 2, discontinuous lines) for the tested solutes according to the partition system used. Note that the left branch of the bilinear curves runs together with the k_0 line; this is to be expected from Eqns. 31 and 33 since, as lipophilicity decreases:

$$\lim_{P \to 0} k_s \frac{k_0}{1 + B' \cdot P^{a'}} = k_0$$

so that, apart from the above considerations, it can be also stated that this bilinear type of corre-

TABLE 10

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

Partition constants which are correlated	Parameter values [a]			
	a'	B'	r	r [b]
P_a (1%)/P (chloroform)	0.6449	0.0160	0.986	0.990
P_a (5%)/P (chloroform)	0.5203	0.1080	0.992	0.996
P_a (1%)/P (*n*-octanol)	0.6327	0.0320	0.982	0.998
P_a (5%)/P (*n*-octanol)	0.5105	0.1893	0.988	0.993
P_a (1%)/$(1/R_f)-1$	1.2222	0.2211	0.984	0.994
P_a (5%)/$(1/R_f)-1$	0.9866	0.8991	0.990	0.995
P_a (1%)/$[(1/R_f)-1]_{s_1}$	1.2009	0.2643	0.991	0.991
P_a (5%)/$[(1/R_f)-1]_{s_5}$	1.0820	1.5023	0.996	0.998

[a] From Eqn. 33.

[b] Correlation coefficient without aniline, which shows the so-called "first-term deviation" (Leo et al., 1971).

lation should be necessarily associated with the disappearance of the aqueous stagnant layer limiting step. Collett et al. (1978) estimated with good approximation, the in vivo gastric absorption rate for three acidic compounds of medium partition coefficient from in vitro data (Collett and Koo, 1975) without the consideration of the behaviour of the solutes at CMC. Based on absorption rate values found with and without a previous treatment of the stomach with polysorbate 20, the authors point out also that no modification of the membrane permeability exists after treatment with the surfactant. This could be due to the intrinsic lipophilicity values of the compounds tested, for which the permeability modification and the removal of the limiting step could have been balanced, so that no apparent differences in absorption rates at CMC and in free solution could be found (see the values for 4-methylaniline and 4-ethylaniline at 0% surfactant and at CMC in Table 1). Previously to any extrapolation, the knowledge of the behaviour of the solutes in the presence of surfactant at CMC is, in our opinion, necessary.

All the above considerations should be applied, strictly speaking, only to unionized compounds, as is the case for the tested amines at the pH of the working solution (about 99.9% unionized), but could be easily extended to partially ionized compounds by introducing a factor for the unionized fraction.

Biopharmaceutical implications

An inspection of Figs. 1 and 2 shows that, in the range of low partition coefficients (i.e. highly hydrophilic compounds, left hand side of the graphs), the absorption rate constants obtained in the presence of surfactant will increase — sometimes dramatically — in relation to those found in free solution and, to a certain extent, independent of the surfactant concentration in the solution since micellar solubilization is practically inoperative. This could explain the impressive increments in absorption which have been observed for some hydrophilic drugs in the presence of surfactants, even at concentrations much above their CMC (Davis et al., 1970; Kreutler and Davis, 1971; Muranishi et al., 1979).

On the opposite side, in the range of high partition coefficients (highly lipophilic compounds, right hand side of the graphs), the surfactant at CMC does actually increase the absorption rate constant in comparison with that found in free solution, whereas at surfactant concentrations above CMC, absorption rate constants are greatly reduced. Consequently, when a highly lipophilic drug is administered orally together with a large amount of surfactant (i.e. in solubilized form), it may have in vivo a strongly decreased absorption (Levy et al., 1966; Gibaldi and Feldman, 1970; Gouda et al., 1975). But, if the dilution with the gastrointestinal fluid contents reduces the actual surfactant concentration in the lumen to a value near or below its CMC, the absorption rate for the drug could even be increased in relation to that which the drug shows in free solution without surfactant. Some contradictory results found for lipophilic drugs could, therefore, be explained if this dilution effect is considered.

Finally, it can be observed that, within the range of medium partition coefficients (central side of the graphs), the incidence of the surfactant in the absorption rate constants of the solutes becomes less evident; this could explain the lack of influence of surfactants on the absorption of some drugs showing these partition characteristics in vivo.

It is the opinion of the authors that, if the global picture shown in the graphs is representative of a general situation, then the criteria outlined here can help to illustrate the complex effects of surfactants in absorption and to gain a better knowledge about the net results of the interaction between the three main mechanisms the surfactants can develop: the modification of membrane permeability, the removal of the aqueous stagnant layer limiting step (two effects which are produced at any surfactant concentration) and the micellar solubilization of the solutes when the surfactant concentration exceeds the CMC.

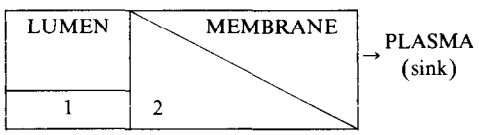
Acknowledgements

The present work is part of an investigative project (No. 1563/82) developed with a grant from the "Comisión Asesora de Investigación

Científica y Técnica (CAICYT)", of the Ministry of Education and Science of Spain. We are indebted to Dr. Joaquín Moreno Dalmau, to Mrs. Matilde Merino Sanjuán and to Mrs. M[a] Carmen García Carbonell for their most helpful contribution to the arrangement of this paper.

Appendix

Diffusional treatment of an absorption system without the consideration of the limiting aqueous stagnant water layer adjacent to the membrane.



The flux in the membrane can be described, according to the Fick's law, by the following equation:

$$-\frac{dQ_2}{dt} = \frac{D_2 \cdot S}{L_2}(C_{2.1} - 0) \quad (1)$$

in which, Q_2 represents the amount of xenobiotic in the membrane phase, D_2 the diffusion coefficient of the latter, S the surface area at the interface lumen/membrane, L_2 the membrane thickness and $C_{2.1}$ the xenobiotic concentration in membrane at the interface membrane/lumen; the xenobiotic concentration at the interface membrane/plasma is assumed to be zero since plasma is considered to be a perfect sink.

It is assumed that the absorption rate (or the disappearance rate of the xenobiotic from the lumen) equals rate flux in the membrane, which is the slower step. Therefore, being Q_1 the amount of the xenobiotic in the luminal phase:

$$-\frac{dQ_1}{dt} = -\frac{dQ_2}{dt} \quad (2)$$

By substituting Eqn. 1 into Eqn. 2, we have:

$$-\frac{dQ_1}{dt} = \frac{D_2 \cdot S}{L_2} C_{2.1} \quad (3)$$

Since the partition coefficient in vivo (P_i) is:

$$P_i = \frac{C_{2.1}}{C_{1.2}} \quad (4)$$

or:

$$C_{2.1} = P_i \cdot C_{1.2} \quad (5)$$

$C_{1.2}$ being the concentration of the xenobiotic in the luminal phase, by substituting Eqn. 5 into Eqn. 3:

$$-\frac{dQ_1}{dt} = \frac{D_2 \cdot S}{L_2} P_i \cdot C_{1.2} \quad (6)$$

Dividing by V_1, the volume of the luminal phase, we have:

$$-\frac{dC_{1.2}}{dt} = \frac{D_2 \cdot S}{V_1 \cdot L_2} P_i \cdot C_{1.2} \quad (7)$$

Since for first-order absorption, the following equation should apply:

$$-\frac{dC_{1.2}}{dt} = k_a \cdot C_{1.2} \quad (8)$$

the value of k_a should be, according to Eqns. 7 and 8:

$$k_a = \frac{D_2 \cdot S}{V_1 \cdot L_2} P_i \quad (9)$$

equation in which, for the same xenobiotic, the same absorption site and a given standard technique, D_2, S, V_1 and L_2 are constants. Therefore:

$$k_a = K \cdot P_i \quad (10)$$

According to current partition criteria, the in vivo partition coefficient, P_i, is related to the in vitro partition coefficient, P, through a linear double-logarithmic equation, provided that suitable solvents or chromatographic systems are being utilized. Therefore:

$$\log P_i = a \cdot \log P + b \quad (11)$$

or, in non-logarithmic form:

$$P_i = 10^b \cdot P^a \quad (12)$$

in which, a and b are constants depending on the in vitro partition technique utilized. By substituting the value of P_i from Eqn. 10 into Eqn. 12, we have:

$$k_a = (K \cdot 10^b) \cdot P^a \quad (13)$$

or, in logarithmic form:

$$\log k_a = a \cdot \log P + \log (K \cdot 10^b) \quad (14)$$

That is, a linear, double-logarithmic relationship should exist between k_a and P when no limiting factor is considered for absorption. Eqn. 14 is equivalent, at all effects, to Eqn. 2A (see text).

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