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Effects of polysorbate 80 on amiodarone intestinal absorption in the rat

R.V. Martín-Algarra *, R.M. Pascual-Costa, M. Merino, V.G. Casabó

Departamento de Farmacia y Tecnología Farmacéutica, Facultad de Farmacia, Universidad de Valencia, Avda Vicente Andrés Estellés, s/n 46100 Burjassot, Valencia, Spain

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Abstract

Amiodarone is a widely used anti-arrhythmic agent which shows physico-chemical properties that are highly suitable for diffusion across lipophilic absorbing membranes, however, its low aqueous solubility could represent the rate-limiting step for absorption, making it erratic and variable. In a previous paper, the influence of an anionic surfactant (sodium lauryl sulphate) at variable supramicellar concentrations was studied. The absorption rate constants of amiodarone decreased as the surfactant concentration increased, and absorption was unusually fast at lower surfactant concentrations. The previously proposed equations for interpreting the relationships between the amiodarone absorption rate constant and sodium lauryl sulphate concentration in the intestinal lumen are extended here to the absorption of the drug in the presence of a non-ionic surfactant (polysorbate 80) at different supramicellar concentrations (from 0.4 to 80 mM). In the same way, a modification of the previously reported equations for quantifying the absorption of micellized drug through the intestinal membrane is described. This modification has been applied to the results obtained for both surfactants, but does not improve the previously postulated biophysical model.

Keywords: Amiodarone; Intestinal absorption; Sodium lauryl sulfate; Polysorbate 80; Surfactant

1. Introduction

Amiodarone hydrochloride is a widely used anti-arrhythmic agent. Its absorption from the gastrointestinal tract following oral administration is slow and variable. This may be related to the pharmacokinetics of the drug, and particularly to the effect of its low aqueous solubility, previously discussed (Martín-Algarra et al., 1994), on the absorption process.

Drugs with physicochemical characteristics similar to those of amiodarone have shown improved dissolution and absorbability in the presence of surfactants (Bates et al., 1969; Schiou et al., 1976).

In a previous paper (Martín-Algarra et al., 1994) the relationship between the amiodarone absorption rate constant and sodium lauryl sul-

* Corresponding author. Tel. (34) 6 3864912; Fax (34) 6 3864911.

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phate (ionic surfactant) concentration in the intestinal lumen was studied. It was shown that as the surfactant concentration in the perfusion fluid increased, the absorption rate constant, k_a, of amiodarone decreased. This effect was quantified by means of suitable equations, and it was concluded that in the presence of surfactant the amiodarone absorption rate constant changes because three opposite effects are in action (Amidon et al., 1982; Plá-Delfina et al., 1987; Collado et al., 1988., Bermejo et al., 1991; Fabra-Campos et al., 1994; Martín-Algarra et al., 1994). Thus, as the concentration of surfactant in luminal fluid increases, micelle solubilization leads to a progressive reduction in amiodarone k_a values. This effect is probably accompanied or followed by a direct effect of the surfactant on the membrane, which makes it, in turn, distinctly or selectively permeable to molecules (or ions) of amiodarone as the surfactant concentration increases. A third effect, characterized by a disarrangement of the stagnant aqueous layer adjacent to the membrane at its luminal side (which acts as the limiting step for absorption in the absence of surfactant), should greatly increase the k_a values of amiodarone, but this effect becomes manifest only when neither micellar solubilization nor membrane polarity enhancement is very significant, as occurs whenever the lauryl sulphate concentration is relatively low (i.e., less than 3 mM). Thus, optimal amiodarone absorption is achieved with such surfactant concentrations in perfusion fluids.

The aim of the present study was to characterize the intestinal absorption of amiodarone in the presence of increasing nonionic surfactant concentrations using previously reported equations for describing the relationship between the apparent absorption rate constants of the drug, and surfactant concentrations in the luminal fluid. We also propose a modification of the previously reported equation in order to quantify the absorption of micellized drug across the intestinal absorbing membrane.

The interpretation of these results may illustrate the importance of each surfactant action on the absorption process of the drug, and establish, if possible, the conditions which would lead to optimal amiodarone absorbability.

2. Materials and methods

2.1. Analytical procedure

Intestinal samples were assayed as described previously (Martín-Algarra et al., 1994).

The accuracy and precision of the method were validated using only three conditions, corresponding to 0.4, 4.0 and 80 mM concentrations of polysorbate 80. Four amiodarone concentrations (10, 40, 60 and 80 μg/ml), covering the calibration range of the analytical method, were assessed.

Accuracy was evaluated by calculating the relative error, which was always less than 5%. Precision was evaluated by calculating the variation coefficient, which was in all cases not higher than 7.09%. These results are completely suitable for this kind of experiment (Karnes and March, 1993).

2.2. Absorption studies

Male Wistar rats weighing 200–300 g, fasted for 20 h and anesthetized 1 h before the experiment by intraperitoneal injection of ethylurethane (25% w/v) were used. The in situ rat gut technique using the whole small intestine, adapted as previously described (Casabó et al., 1987; Martín-Algarra et al., 1994), was applied.

The absorption experiments were carried out using eight polysorbate 80 solutions (Tween® 80, Fluka Chemika) (0.4, 0.8, 2, 4, 8, 20, 40 and 80 mM in NaCl 0.78%) containing amiodarone hydrochloride (75 μg/ml) (Sigma Chemicals). The final osmolarity was 300 ± 10 mOsm. The pH of the solution to be perfused was adjusted to 7.0 using NaOH or 0.1 N HCl. All solutions were clearly supramicellar. Absorption and water reabsorption tests were performed exactly as previously reported (Martín-Algarra et al., 1994).

2.3. Fitting of models to data

To establish a relationship between the actual amiodarone absorption rate constant, k_a, and the surfactant concentration in the intestinal lumen, previously described equations, expressed here as Eq. 1 and 2 (Martín-Algarra et al., 1994), and a

modification of these equations (Eq. 3) were used. The derivation of Eq. 1 and 2 was given previously (Martín-Algarra et al., 1994) and that of Eq. 3 is given in the Appendix.

$$\text{model 1} \quad k_a = \frac{k_{af}}{1 + \frac{V_m}{V_a} \cdot P_i} \tag{1}$$

$$\text{model 2} \quad k_a = \frac{k_{ao} \cdot 10^{\left(\frac{V_m}{V_a} \cdot a\right)}}{1 + \frac{V_m}{V_a} \cdot P_i} \tag{2}$$

$$\text{model 3} \quad k_a = \frac{k_{af}}{1 + \frac{V_m}{V_a} \cdot P_i} + \frac{k_{am} \cdot \frac{V_m}{V_a}}{1 + \frac{V_m}{V_a} \cdot P_i} \tag{3}$$

where k_{af} represents the absorption rate constant for the free amount of drug; k_{ao} is the absorption rate constant which should apply in the presence of the surfactant at its critical micelle concentration, CMC (i.e., the absorption rate constant of the drug in the absence of surfactant micelles); k_{am}, in Eq. 3, represents the absorption rate constant for the micelle-solubilized drug; P_i denotes the in vivo partition coefficient of the amiodarone between the micelles and the aqueous phase; a, in Eq. 2, is a constant which depends on the experimental technique used; V_m is the micellar volume and V_a the aqueous volume, therefore, the ratio V_m/V_a can be expressed as the concentration of the surfactant in the perfusion fluid. Although some variation in V_a exists due to water reabsorption, this variation is in practice irrelevant within the 30 min interval. Therefore, the k_a values become truly representative of the influence of each initial surfactant concentration on absorption.

The fitting process was performed by means of nonlinear weighted least-squares regression, using the Marquardt algorithm, in a IBM-PC computer. In order to check the goodness of the fits and select the best equation and model, correlation coefficients between estimated and experimental values, and the Akaike information criterion (AIC) were used (Imbimbo et al., 1991). As a

Table 2
Average absorption rate constants and initial amounts of amiodarone (k_a and $A_o \pm s$) found at different starting polysorbate 80 concentrations

Polysorbate 80 concentration (mM)	Absorption rate constant $k_a \pm s$ (h^{-1})	Amiodarone amount at intercept $A_o \pm s$ (μg)
0.4	2.46 ± 0.16	680 ± 8
0.8	2.20 ± 0.23	608 ± 7
2	2.22 ± 0.27	680 ± 4
4	2.05 ± 0.42	653 ± 10
8	1.71 ± 0.13	642 ± 9
20	1.22 ± 0.11	650 ± 6
40	1.02 ± 0.26	600 ± 7
80	1.00 ± 0.21	652 ± 3

Table 1
Remaining volumes at the sampling times, number of animals used and water reabsorption kinetic parameters

Polysorbate 80 concentration (mM)	Remaining mean volume (± SE) at each sampling time (ml)			n at each sampling time		
	10	20	30	n_{10}	n_{20}	n_{30}
0.4	9.95 ± 0.44	9.04 ± 0.25	8.54 ± 0.43	4	3	3
0.8	9.61 ± 0.41	9.34 ± 0.32	7.75 ± 0.35	4	4	3
2	10.28 ± 0.64	8.68 ± 0.40	7.70 ± 0.33	3	3	4
4	9.74 ± 0.50	9.35 ± 0.48	8.09 ± 0.13	4	4	3
8	9.77 ± 0.16	9.46 ± 0.24	8.15 ± 0.37	5	5	4
20	9.98 ± 0.16	9.22 ± 0.41	8.49 ± 0.24	3	3	3
40	9.45 ± 0.40	9.44 ± 0.34	7.79 ± 0.69	3	3	3
80	9.77 ± 0.30	8.88 ± 0.15	8.19 ± 0.384	3	3	3
Mean value	9.81 ± 0.13	9.20 ± 0.11	8.09 ± 0.13	83		
$V_o \pm$ SE (ml)		$k_o \pm$ SE (ml/min)		P		
10.742 ± 0.189		−0.0854 ± 0.009		0.0001		

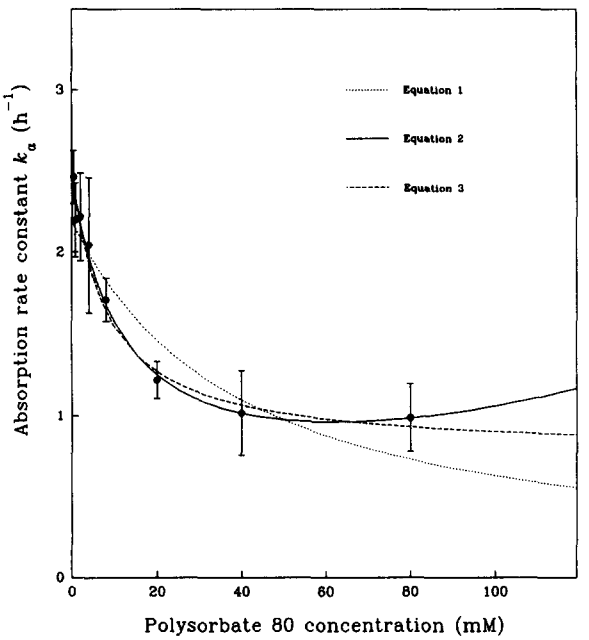

Fig. 1. Graphical plot representing the fits of Eq. 1–3 to Polysorbate 80 data.

complementary criterion, the weighted sum of squares found for each fit and standard deviations of parameter values were also calculated.

3. Results

3.1. Water reabsorption tests

In Table 1, the remaining volumes (mean value ± SE) at each sampling time, obtained from each experimental solution, and the number of animals per set are shown. Water reabsorption kinetic parameters, and the statistical significance of the regression are also given.

3.2. Absorption kinetic parameters

The absorption rate constants, k_a, and amounts of amiodarone at the intercept, A_o, calculated according to first-order kinetics (mean value ± s, eight animals per set), are listed in Table 2.

Significant differences between k_a values were found when the ANOVA test was applied ($F = 47.58$; $P \leq 0.0001$).

3.3. Correlations between absorption rate constant and polysorbate 80 concentrations

In Table 3, the parameter values, with standard deviation, correlation coefficient, weighted sum of squares and AIC value found for fitting Eq. 1–3 to the data are given. In Fig. 1, graphs are plotted according to the fitting equations.

4. Discussion

4.1. Water reabsorption studies

Water reabsorption was considerable (> 20% at 30 min) but independent of the working surfactant concentration ($F > 0.398$; $P \leq 0.906$, through a covariance test). Therefore, the remaining concentrations of amiodarone in the luminal samples

Table 3
Polysorbate 80: parameter values obtained after fitting the selected equations to data (statistical figures found for each fit are also shown)

Fitting equation	Parameters	Parameter values ± s	AIC	WSS	$r >$
1	k_{af} (h^{-1})	2.2 ± 0.2	−11.92	0.137	0.950
	P_i (mM^{-1})	0.025 ± 0.007			
2	k_{ao} (h^{-1})	2.5 ± 0.06	−33.63	0.007	0.993
	P_i (mM^{-1})	0.08 ± 0.01			
	a (mM^{-1})	0.0058 ± 0.0005			
3	k_{af} (h^{-1})	2.5 ± 0.1	−26.47	0.017	0.989
	P_i (mM^{-1})	0.12 ± 0.03			
	k_{am} (h^{-1})	0.77 ± 0.08			

were corrected according to the global parameters shown in Table 1.

4.2. Amiodarone absorption in the presence of surfactant

As pointed out elsewhere (Martín-Algarra et al., 1994), according to allometric calculations, the selected dose of amiodarone was 0.75 mg, dissolved in 10 ml of the perfusion fluids. Polysorbate 80 concentrations in the latter ranged from 0.4 mM (which provides the minimal amount of surfactant leading to amiodarone solubilization) to 80 mM (which seemed to provide the maximum amount of surfactant which produces no observable anatomical damage in the small intestine).

The pattern of amiodarone absorption was clearly a first-order kinetic process and was characterized by passive diffusion across the intestinal lipoidal membrane. Since amiodarone has a high molecular mass (681.8 Da), diffusion through the aqueous pores does not have to be taken into account (Plá-Delfina and Moreno, 1981; Martín-Algarra, 1992).

Table 2 demonstrates that the apparent absorption rate constants of amiodarone decrease as the surfactant concentration increases; the differences were statistically significant on the ANOVA test. This is probably no more than a consequence of the multiple effects of the surfactant on absorption, with an outstanding predominance of micellar solubilization (Plá-Delfina et al., 1987) as pointed out above. Correlation between the absorption rate constants of amiodarone and the surfactant concentration in perfusion fluids could aid in quantifying each of these effects by means of the equations proposed.

4.3. Fitting biophysical absorption models to data

Models 1 and 2 (Eq. 1 and 2, respectively) were used in previous assays carried out with 75 $\mu g/ml$ of amiodarone in presence of increasing sodium lauryl sulphate concentrations (2.6 to 104 mM). The decrease in the absorption rate constant value as the surfactant concentration increases was much better described when Eq. 2 was used (see Table 4) (Martín-Algarra et al., 1994).

As in the case of sodium lauryl sulphate, Eq. 2 fits the k_a values obtained in the presence of polysorbate 80 much better than Eq. 1 (see Table 3). It is therefore clear that the amiodarone absorption rate constant decreases mainly due to micellar solubilization, however, other effects should also be considered, as clarified by model 2. However, the authors considered another possible explanation for this phenomenon:

Model 3: In models 1 and 2 it was assumed that the micelle-solubilized drug fraction is not available for absorption. Since, however, it has been postulated that micelles – with the incorporated drug – are capable of close contact with membrane boundaries (Amidon et al., 1982), one can imagine that some type of partitioning of the

Table 4
Sodium lauryl sulphate: parameter values obtained after fitting the selected equations to data (statistical figures found for each fit are also shown)

Fitting equation	Parameters	Parameter values ± s	AIC	WSS	$r >$
1 [a]	k_{af} (h^{-1})	2.1 ± 0.6	−1.826	0.378	0.864
	P_i (mM^{-1})	0.028 ± 0.02			
2 [a]	k_{ao} (h^{-1})	4.2 ± 0.6	−18.94	0.016	0.996
	P_i (mM^{-1})	0.22 ± 0.05			
	a (mM^{-1})	0.0069 ± 0.0006			
3	k_{af} (h^{-1})	9.3 ± 8.9	−15.86	0.026	0.990
	P (mM^{-1})	1.11 ± 1.45			
	k_{am} (h^{-1})	0.7433 ± 0.0748			

[a] From Martín-Algarra et al. (1994).

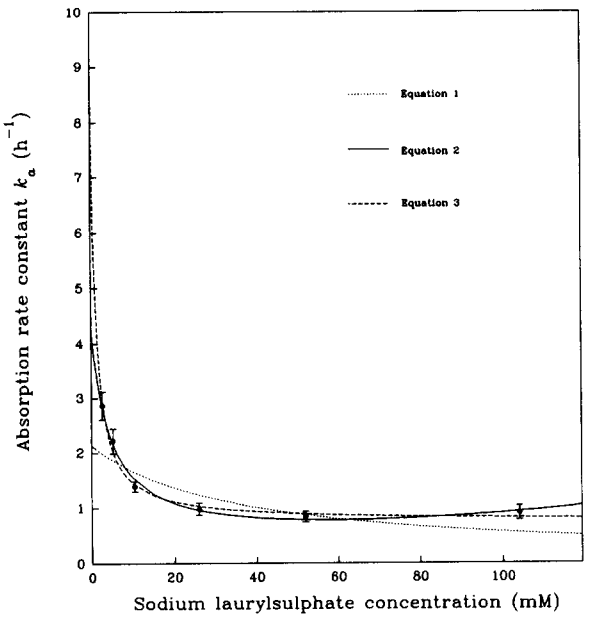

Fig. 2. Graphical plot representing the fits of Eq. 1–3 to sodium lauryl sulphate data.

drug between the micelles and the membrane could exist, without any intervention of the aqueous media. This would mean that there could be an independent absorption pathway, and that it should be quantified by Eq. 3. However, as can be seen in Table 3, statistical figures clearly show that this model does not improve the results given by model 2, i.e., AIC and WSS values are lower and the correlation coefficient higher for model 2. Moreover, parameter values showed considerably lower deviations. On the other hand, when the selected equations were applied to previous tests carried out with lauryl sulphate, similar values were obtained (see Table 4).

The excellent fit provided by Eq. 2, as compared with the two remaining ones, can be graphically observed in Fig. 1 and 2. The absorption rate constant of amiodarone progressively decreases as micellar solubilization progresses, but is not completely nullified, for a significant modification of permeability coexists. As a result of this, a residual absorption is maintained or even slightly enhanced at extremely high concentrations of surfactant, an effect which Eq. 2 perfectly predicts and quantifies.

Thus, it should be concluded that the absorption of micellized drug is probably negligible, and Eq. 2 is the best one for interpreting the influence of surfactants on absorption, at least for ionized lipophilic drugs such as amiodarone.

In conclusion, more detailed studies using homologous series of drugs with a large interval of lipophilicity, varying degrees of ionization and working concentrations, in the presence of several types of surfactants and surfactant concentrations, should be carried out in order to confirm definitely and to extrapolate these criteria to the generality of drugs.

4.4. Practical implications

From these results and previously reported data (Martín-Algarra et al., 1994), the absolute value found for the absorption rate constant, k_a, of amiodarone in the presence of rather low concentrations of surfactant in the luminal fluid should be emphasized. For polysorbate, $k_a = 2.46$ h^{-1}, and for lauryl sulphate $k_a = 2.86$ h^{-1} (Martín-Algarra et al., 1994). These values indicate an absorption half-life of about 15 min, which could account for virtually complete absorbability, provided that the proper surfactant concentration is present at the absorption site.

Therefore, the previously reported conclusions about the convenience of designing more reliable forms of amiodarone, containing a suitable dose of surfactant as a solid dispersion or similar preparation, have been entirely confirmed here. Polysorbate preparations are preferable, since the adverse effects of this surfactant are much smaller than those of lauryl sulphate.

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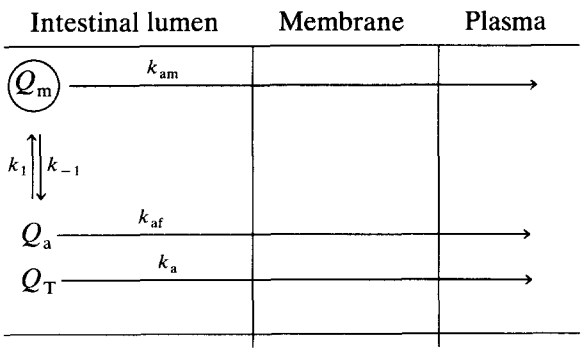
Appendix 1

Model 3

This model is consistent with the principles leading to model 1 (Martín-Algarra et al., 1994), except that some of the solubilized drug is assumed to be absorbed via a direct interaction between the micelles and the membrane, i.e., the drug is released at the interface of the micelle and absorbed through a direct membrane/micelle partitioning process, without the intervention of aqueous media (Amidon et al., 1982). The model can be represented as:



The rate of change of the total amount of drug in the intestinal luminal fluid can be expressed as:

$$\frac{dQ_T}{dt} = \frac{dQ_a}{dt} + \frac{dQ_m}{dt} \tag{1A}$$

where Q_T is the total amount of drug, Q_m the amount of drug solubilized into surfactant micelles, and Q_a the amount of drug dissolved in the aqueous medium. On the other hand, since drug absorption is an apparent first-order kinetic process, it can also be said that:

$$k_a \cdot Q_T = k_{af} \cdot Q_a + k_{am} \cdot Q_m \tag{2A}$$

where k_a represents the apparent absorption rate constant experimentally found at a given SMC of surfactant, k_{af} denotes the intrinsic absorption rate constant for the free (i.e., dissolved) amount of drug and k_{am} represents the absorption rate constant for the micelle solubilized drug.

On the other hand, an internal intrinsic partition coefficient, P_i, between the micelles and the aqueous luminal medium may be defined (Plá-Delfina et al., 1987) as:

$$P_i = \frac{\dfrac{Q_m}{V_m}}{\dfrac{Q_a}{V_a}} = \frac{Q_m \cdot V_a}{Q_a \cdot V_m} \tag{3A}$$

where V_m and V_a are the micellar and aqueous volumes, respectively, for a given experiment.

By definition, we have:

$$Q_T = Q_a + Q_m \tag{4A}$$

Combining Eq. 3A and 4A, we have:

$$Q_a = \frac{Q_T}{1 + \dfrac{V_m}{V_a} \cdot P_i} \tag{5A}$$

and combining Eq. 4A and 5A, we have:

$$Q_m = Q_T - \frac{Q_T}{1 + \dfrac{V_m}{V_a} \cdot P_i} \tag{6A}$$

Substituting the values of Q_a and Q_m from Eq. 2A, Eq. 3 in the text is obtained:

$$k_a = \frac{k_{af}}{1 + \dfrac{V_m}{V_a} \cdot P_i} + \frac{k_{am} \cdot \dfrac{V_m}{V_a}}{1 + \dfrac{V_m}{V_a} \cdot P_i} = k_1 + k_2 \tag{3}$$

In other words, two pathways leading to drug absorption are considered with their corresponding rate constants: k_1 is that of the free drug (i.e., the amount dissolved in the luminal fluid), and k_2, that of the micelle-solubilized drug. Thus, the global absorption rate constant will decrease from k_{af} to k_{am} as the micellar volume increases.

References

Amidon, G.E., Higuchi, W.I. and Ho, N.F.H., Theoretical and experimental studies of transport of micelle-solubilized solutes. *J. Pharm. Sci.*, 71 (1982) 77–84.

Bates, R.T., Gibaldi, M., and Kaning, J.L., Rate of dissolution of griseofulvin and hexestrol in bile salt solutions. *Nature*, 210 (1969) 1368.

Bermejo, M.V., Pérez-Varona, A.T., Segura-Bono, M.J., Martín-Villodre, A., Plá-Delfina, J.M. and Garrigues, T.M., Compared effects of synthetic and natural bile acid surfactants on xenobiotic absorption: I. Studies with polysorbate and taurocholate in rat colon. *Int. J. Pharm.*, 69 (1991) 221–231.

Casabó, V.G., Núñez-Benito, E., Martínez-Coscollá., A., Miralles-Loyola, E., Martín-Villodre, A. and Plá-Delfina, J.M., Studies on the reliability of a bihyperbolic functional absorption model: II. Phenylalkylamines. *J. Pharmacokinet. Biopharm.*, 15 (1987) 633–643.

Collado, E.F., Fabra-Campos, S., Peris-Ribera, J.E., Casabó, V.G., Martín-Villodre, A. and Plá-Delfina, J.M., 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. *Int. J. Pharm.*, 44 (1988) 187–196.

Fabra-Campos, S., Climen, E., Sanchís-Cortés, A. and Plá-Delfina, J.M., Compared effects of synthetic and natural bile acid surfactants on xenobiotic absorption: I. Studies with polysorbate and taurocholate in rat colon. *Int. J. Pharm.*, 109 (1994) 197–207.

Imbimbo, B.P., Martinelli, P., Rocchetti, M., Ferrari, G., Bassotti, G. and Imbimbo, E., Efficiency of different criteria for selecting pharmacokinetics multiexponential equations. *Biopharm. Drug Dispos.*, 12 (1991) 139–147.

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

Martín-Algarra, R.V., Influencia de los tensiactivos en la absorción gastrointestinal de la Amiodarona: Laurilsulfato sódico y Polisorbato 80. Tesis Doctoral, Valencia (1992).

Martín-Algarra, R.V., Pascual-Costa, R.M., Merino, M. and Casabó, V.G., Effects of surfactants on amiodarone intestinal absorption: I. Sodium lauryl sulphate. *Pharm. Res.*, 11 (1994) 1042–1047.

Plá-Delfina, J.M. and Moreno, M., Intestinal absorption-partition relationships. A tentative functional non-linear model. *J. Pharmacokinet. Biopharm.*, 9 (1981) 191–215.

Plá-Delfina, J.M., Pérez-Buendía, M.D., Casabó, V.G., Peris-Ribera, J.E. and Martín- Villodre, A., Absorption-partition relationships for true homologous series of compounds as a possible approach to study mechanisms of surfactants in absorption: I. Aromatic amines in rat colon. *Int. J. Pharm.*, 37 (1987) 49–64.

Schiou, W.L., Chen, S.J. and Athanikar, N., Enhancement of dissolution rates of poorly water-soluble drugs by crystallization in aqueous surfactant solutions: sulfathiazole, prednisone and chroramphenicol. *J. Pharm. Sci.*, 65 (1976) 1702–1704.