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Partition coefficients of β-blockers in bile salt/lecithin micelles as a tool to assess the role of mixed micelles in gastrointestinal absorption

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Abstract

The objective of this study was to develop non-invasive spectroscopic methods to quantify the partition coefficients of two β-blockers, atenolol and nadolol, in aqueous solutions of bile salt micelles and to assess the effect of lecithin on the partition coefficients of amphiphilic drugs in mixed bile salt/lecithin micelles, which were used as a simple model for the naturally occurring mixed micelles in the gastrointestinal tract. The partition coefficients (K_p) at 25.0 ± 0.1°C and at 0.1 M NaCl ionic strength were determined by spectrofluorimetry and by derivative spectrophotometry, by fitting equations that relate molar extinction coefficients and relative fluorescence intensities to the partition constant K_p. Drug partition was controlled by the: (i) drug properties, with the more soluble drug in water (atenolol) exhibiting smaller values of K_p, and with both drugs interacting more extensively in the protonated form; and by (ii) the bile salt monomers, with the dihydroxylic salts producing larger values of K_p for the β-blockers, and with glycine conjugation of the bile acid increasing the values of K_p for the β-blockers. Addition of lecithin to bile salt micelles decreases the values of K_p of the β-blockers. Mixed micelles incorporate hydrophobic compounds due to their large size and the fluidity of their core, but amphiphilic drugs, for which the interactions are predominantly polar/electrostatic, are poorly incorporated in mixed micelles of bile salts/lecithin.

Keywords: Partition coefficients; Bile salts; Solubility; Mixed micelles; β-blockers

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1. Introduction

Bile salts, which are produced by the hepatic metabolism of cholesterol [1,2] and stored in the gall bladder after secretion by the liver [3,4], are amongst the most important groups of naturally occurring surfactants. They form micellar aggregates that play an important role in the physiologically vital process of gastrointestinal digestion and absorption of lipids [5–7] and of lipophilic substances [1,8]. Micelle capacity to solubilize these substances is controlled by size, rigidity and surface charge density. Bile salt micelles have low aggregation numbers, typically 2–10 and are normally smaller than micelles formed by synthetic surfactants [5,9].

Bile salts in the body are often associated with phospholipids in mixed micelles, which are larger and more fluid than simple micelles and thus, may act as better solubilizing agents for lipophilic compounds [10,11]. In fact, it has been observed that the solubility of lipophilic drugs increases when lecithin is added to a bile salt dissolution medium [12]. In the accepted model for bile salt/lecithin mixed micelles, lecithin has the polar heads on the outer surface of the micelles; but their charge is partially compensated by the hydroxylic groups of the bile salts and consequently, the net superficial charge is smaller than in bile salt micelles, a factor that may affect the solubilizing capacity of mixed micelles [13,14].

The presence in the intestinal lumen of bile salts with surface-active properties and the ability to form mixed micelles contributes to improving the solubility of many pharmaceutically important substances [15,16] and thus, facilitates their absorption. Hence, the prospective use of bile salts as adjuvants to control drug lifetime and bioavailability, and to minimise undesirable side effects [15,16].

Previous studies have demonstrated that bile salts are capable of either increasing [15] or decreasing [17–19] drug absorption from the gastrointestinal tract. These detergents can improve the bioavailability of poorly absorbable drugs by increasing the dissolution rate of formulated drugs or by facilitating the transfer of the solute across the intestinal wall. Contrastingly, charged or hydrophilic drugs that interact with the polar part of bile salt micelles may have their absorption from the gastrointestinal tract reduced, as has been found for some hydrophilic β-blockers [18–21]. Thus, the effect of bile salts on atenolol and nadolol bioavailability may have important implications for the clinical use of both bile salts and β-blockers.

In this work we have quantified the interactions of atenolol and nadolol with mixed bile salt/lecithin micelles, which were used as a simple model for the naturally occurring mixed micelles in the gastrointestinal tract. We have determined the partition coefficients between the aqueous and micellar pseudo-phases by spectrofluorimetry and by derivative spectrophotometry, and the results have shown that drug partition is controlled by the bile acid (number of hydroxyl groups, conjugation with glycine) and by the drug (hydrophilicity and acid/base properties). Furthermore, our data indicate that addition of lecithin to bile salt micelles decreases the values of the partition coefficients in the mixed micelles.

2. Experimental

2.1. Reagents and solutions

The β-blockers (atenolol and nadolol), bile salts and egg yolk lecithin were from Sigma and used as received; all other chemicals were from Merck (pro-analysi). All solutions were prepared with double-deionized water (conductivity less than 0.1 $\mu S\ cm^{-1}$). Bile acid solutions were prepared by rigorous dilution of a stock solution of known titre, which was determined by conductimetric titimetry of a solution of the bile salt (20.00 ml; ≈ 2.5 mM) with HCl 0.1 M (Merck, Titrisol), added by means of a Crison 2031 piston buret and using a Crison Micro CM 2202 conductivity meter.

2.2. Physical measurements

Absorption electronic measurements were performed at 25.0 ± 0.1°C with a Hitachi U-2000 spectrophotometer, using thermostated quartz

cells with 1-cm optical path. Fluorimetric data was collected at 25.0 ± 0.1°C (thermostated cell) using a Shimadzu RF5001-PC steady-state fluorescence spectrometer and with a slit width of 10 nm. In all spectroscopic determinations the solvent system used as blank had the same surfactant concentration as the solutions to be measured. All potentiometric measurements were carried out with a Crison 2002 pH meter and 2031 buret controlled by a personal computer, which was also used for data manipulation [22]. The electrode assembly was made up of an Orion 900029/4 AgCl/Ag reference electrode and a Russel SWL glass electrode. System calibration was performed by the Gran method [23] in terms of hydrogen ion concentration, using a strong acid/strong base titration [HCl (1 mM)/NaOH ($\approx$ 20 mM)] with solutions whose ionic strength was adjusted to 0.1 M with NaCl. Titrations were always carried out under a nitrogen atmosphere at 25.0 ± 0.1°C in a double-walled glass cell.

2.3. Potentiometric determinations

Equilibrium constants of β-blocker/bile salt were obtained by titrating 20.00 ml of aqueous solutions of the β-blocker ($\approx$ 1 mM) and of bile salt ($\approx$ 1 mM) with HCl ($\approx$ 5 mM). All titrations were performed at 25.0 ± 0.1°C under nitrogen, and for all solutions the ionic strength was adjusted to 0.1 M with NaCl. Calculations were performed with data obtained from at least 6 independent titrations, each with more than 40 points and the experimental titration data were analysed using the computer program Superquad [24]. The errors reported in this work were calculated by the method of Albert and Serjeant [25], in which the errors are calculated as the maximum difference between the logarithm of the average of the antilogarithms of the calculated equilibrium constants ($\log K$) and their individual values.

2.4. Preparation of micelle solutions

Mixed micelles of lecithin and bile salts were prepared by dissolving a known amount of lecithin in chloroform/methanol (9:1, v/v), to which were added cholate or deoxycholate by rigorous dilution of a concentrated methanolic solution. The mixture was dried under vacuum in a rotary evaporator. The resulting film was further dried under a stream of nitrogen in the dark for at least 1 h and was then suspended in an aqueous solution of NaCl (0.1 M) using a vortex. In the resulting solutions, the bile salts were assayed by conductimetry and the total concentration of lecithin was determined by an enzymatic method, using a kit obtained from bioMérieux (Lyon, France).

2.5. Determination of critical micelle concentration

The values of the *cmc* of bile salts and of mixed micelles (bile salt/lecithin) were obtained by spectrofluorimetry (light scattering), with the signal attenuated by a Polaroid HNP′B filter. The slit width was 1.5 nm for lecithin concentrations of 750 μM, 3 nm for 500 μM and 5 nm for pure bile salt solutions; in all cases the excitation and emission wavelengths were 400 nm. Measurements were made with lecithin to which increasing amounts of bile salts were added. Final concentrations were lecithin 500 and 750 μM, cholate from 9×10^{-5} to 9×10^{-3} M, and deoxycholate from 5×10^{-6} to 5×10^{-3} M.

2.6. Solubility studies

The solubility of the neutral and protonated forms of the β-blockers was measured in water and in aqueous bile salt solutions, below and above the *cmc*. For each solution, the β-blocker was dispersed in 20 ml of each solvent and the pH adjusted to 7.0 and 10.8, respectively, by addition of aliquots of concentrated HCl or NaOH before each determination. The pH measurements and system calibration was performed by potentiometric titimetry as described previously. The saturated solutions were incubated for 60 h at 25.0 ± 0.1°C in a thermostated bath, and then filtered through filter paper (Lida, 0.45 μm), and the concentration of the β-blockers was determined by steady-state spectrofluorimetry. Emission spectra were recorded at 298 and 590 nm, respectively, using an excitation maximum of 224 nm.

The following concentrations of bile salts were

used (mM): 1.0, 2.0, 3.0, 3.5, 4.0, 5.0, 5.5, 6.0 and 6.5 for sodium deoxycholate and sodium glycodeoxycholate; 5.0, 6.0, 15, 20, 25, 30, 35, 40 and 45 for sodium glycocholate; and 1.0, 5.0, 7.0, 8.0, 9.0, 10, 15, 20, 25 and 30 for sodium cholate.

2.7. Partition coefficients determination

Aqueous solutions of the β-blocker were added to solutions of the desired micelles (either simple or mixed), and the resulting solutions were incubated in the dark and at 25.0 ± 0.1°C for 12 h. The partition coefficients of atenolol and nadolol, in bile salt and in mixed cholate or deoxycholate/lecithin micelles, were calculated from derivative spectrophotometric and from spectrofluorimetric data at pH 10.8 and 7.0.

2.8. Spectrofluorimetry

β-blocker concentrations in simple and mixed micelle solutions were determined by steady-state spectrofluorimetry, using the procedure described above. In the determinations of partition coefficients in simple micelles, the concentrations of bile salts were identical to those used in the solubility studies. Emission spectra were recorded between 250 and 650 nm using an excitation maximum of 224 nm, and a linear response was observed between 1.0×10^{-8} and 3.0×10^{-6} M for atenolol, and between 1.0×10^{-8} and 2.0×10^{-6} M for nadolol. The reported results are the average of 9 independent measurements. For mixed micelles the following concentrations of surfactants were used: (a) 750 μM lecithin, cholate from 5.0×10^{-4} to 3.0×10^{-2} M and deoxycholate from 1.0×10^{-4} to 7.0×10^{-3} M; (b) 500 μM lecithin, cholate from 1.0×10^{-3} to 3.0×10^{-2} M, and deoxycholate from 5.0×10^{-4} to 7.0×10^{-3} M.

2.9. Spectrophotometry

The partition coefficients of the β-blockers in simple and mixed micelles were also calculated from spectrophotometric data in the range 200–350 nm. In simple micelles the concentration ranges for bile salts were: cholate between 1.0×10^{-4} and 4.0×10^{-2} M; deoxycholate between 5.0×10^{-4} and 6.0×10^{-3} M. For mixed micelles, the concentrations of lecithin were 750–500 μM, and the concentrations of bile salts were identical to those used in the spectrofluorimetric studies. A linear response was observed between 3.0×10^{-5} and 1.0×10^{-4} M for both β-blockers.

3. Results and discussion

All determinations were performed at a controlled pH, which was adjusted to 7.0 and 10.8. These values were selected taking into account the acidity constants of atenolol, nadolol and of the bile salts. The pK_a values for bile salts are [26]: cholate 4.66 ± 0.01; deoxycholate 4.88 ± 0.01; glycocholate 3.64 ± 0.03, and glycodeoxycholate 3.69 ± 0.03; and for β-blockers are [22]: atenolol 9.29 ± 0.05 and nadolol 9.52 ± 0.02. Thus, at pH 7.0 the partition coefficients determined are between bile salt micelles and protonated β-blockers, whereas at pH 10.8 are between bile salt micelles and the neutral form of the β-blockers.

3.1. Values of cmc for simple and mixed micelles

These values were determined from plots of intensity of the light scattered vs. bile salt concentration, which yield two intersecting straight lines. The *cmc* is defined as the bile salt concentration at the intersection point. In this context, the *cmc* of mixed micelles represents, for each lecithin concentration, the smallest amount of bile salt that promotes the transition from vesicles to micelles; it must be stressed that this quantity is also called the micellar phase limit [27].

The obtained values for the *cmc* of simple bile salt micelles in solutions with $I = 0.1$ M NaCl (average of 8 independent measurements) are 6.0×10^{-3} M for cholate, 1.2×10^{-2} M for glycocholate, 2.5×10^{-3} M for deoxycholate, and 2.2×10^{-3} M for glycodeoxycholate. These values are similar to those found in the literature. The values (mM) for cholate range from 5.0 [28,29] to 16.0 [4], for glycocholate from 9.4 [30] to 11.9 [31], for deoxycholate from 2.0 [28,29] to 6.4 [32] and for glycodeoxycholate from 2.1 [33] to 4.3 [34].

This observation confirms the reliability of the currently described technique used in obtaining *cmc* values for simple micelles, and lends support to its extension to mixed micelles.

The cmc values for cholate/lecithin are 9.0×10^{-4} M in the presence of 750 μM lecithin, and 2.0×10^{-3} M in the presence of 500 μM lecithin; for deoxycholate/lecithin the values are 8.0×10^{-4} M, with 750 μM lecithin, and 1.2×10^{-3} M with 500 μM lecithin (all reported values are the average of 8 independent measurements). This lowering of the *cmc* values in the presence of increasing lecithin concentration, parallels what is found in the literature [5,9,11].

A comment must be made regarding the plots of light scattered intensity (I_d) vs. bile salt concentration (Fig. 1). For simple micelles the slopes of both straight lines are positive; but with those below the *cmc* being less than half the slopes after micelle aggregates are formed. Contrastingly, for lecithin solutions to which bile salt is added and for bile salt concentrations less than the *cmc* the slope is large and negative, but becomes much smaller above the *cmc*, as expected from the collapsed of the vesicular structure.

The cholate/lecithin/water phase diagram has been revised in detail recently, and it reveals that for lecithin/cholate ratios below 0.5, but above the *cmc*, both simple bile salt micelles and mixed micelles coexist in solution [35–37]. Although the phase diagram does not explicitly extend to very low lecithin concentrations, if the lecithin/cholate ratio can be extrapolated it becomes evident that the solutions used in the fluorescence and derivative spectroscopy studies are a mixture of simple and mixed micelles. Furthermore, it must be noted that similar ratios have been obtained for several other bile salts and it seems that a common global pattern exists for all bile salts, where lecithin/cholate ratios are less than 0.5–0.6, a mixture of simple and mixed micelles exists in aqueous solutions [12].

Regarding the applicability of the partition model to these latter solutions, the model assumes two pseudo-phases; in this case an aqueous and a micellar phase, but makes no assumptions regarding the composition of the micellar phase.

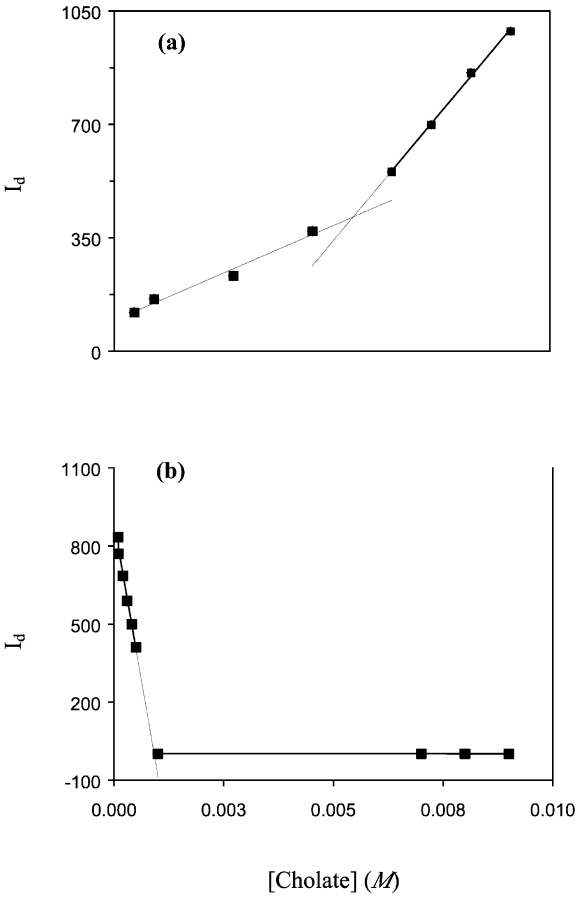

Fig. 1. Light scattered intensity (I_d) as a function of sodium cholate concentration in simple (a) and mixed (b) micelle solutions.

In the foregoing discussion the mixtures of simple and mixed micelles are treated as a single pseudo-phase.

3.2. Solubility of β-blockers in bile salt micelles

Drug behaviour in trihydroxy bile salts (cholate and glycocholate) was found to be different from that observed in dihydroxy bile salts (deoxycholate and glycodeoxycholate) and the experimentally obtained solubilities of the β-blockers are included in Table 1. For the former, β-blocker solubility is independent of bile salt concentration

Table 1
Solubility (mM) of atenolol and nadolol in cholate, glycocholate, deoxycholate and glycodeoxycholate solutions, below and above the *cmc*, at 25.0 ± 0.1°C, $I = 0.10$ M; pH 7.0 and 10.8[a]

		Cholate		Glycocholate		Deoxycholate		Glycodeoxycholate	
		pH = 7.0	pH = 10.8	pH7.0	pH = 10.8	pH = 7.0	pH = 10.8	pH = 7.0	pH = 10.8
Atenolol									
Below the *cmc*	Without[b]	252 ± 2	137 ± 1	252 ± 1	137 ± 1	252 ± 2	137 ± 1	252 ± 2	137 ± 1
	1 mM[c]					380 ± 1	199 ± 1	530 ± 5	263 ± 2
	2 mM[c]					601 ± 1	280 ± 1	754 ± 6	349 ± 1
Above the *cmc*		686 ± 5	321 ± 1	851 ± 4	387 ± 2	848 ± 9	427 ± 3	919 ± 5	452 ± 1
Nadolol									
Below the *cmc*	Without[b]	18.6 ± 0.1	13.6 ± 0.2	18.6 ± 0.1	13.6 ± 0.2	18.6 ± 0.2	13.6 ± 0.1	18.6 ± 0.1	13.6 ± 0.1
	1 mM[c]					32.0 ± 0.1	24.5 ± 0.1	56.2 ± 0.2	29.0 ± 0.1
	2 mM[c]					41.2 ± 0.1	27.8 ± 0.2	62.5 ± 0.5	34.2 ± 0.1
Above the *cmc*		52.5 ± 0.5	27.8 ± 0.1	62.7 ± 0.3	35.9 ± 0.1	59.4 ± 0.8	33.6 ± 0.5	74.6 ± 0.4	39.6 ± 0.2

[a] The reported values are the average of measurements at 298 and 590 nm; the values below the *cmc* are the average for three solutions with different concentrations, whereas above the *cmc* are the average of five different solutions.
[b] In the absence of bile salt.
[c] Bile salt concentration.

up to the *cmc*, where it increases dramatically and remains approximately constant thereafter.

On the other hand, in solutions of deoxycholate and glycodeoxycholate at concentrations below the *cmc*, β-blocker solubility increases with an increase in concentration of bile salt, due to formation of drug/dihydroxy bile salt (1:1) complexes. This equilibrium was quantified potentiometrically [22–25] and the following values for $\log K$ were obtained: 2.51 ± 0.05 for deoxycholate/atenolol; 3.74 ± 0.04 for deoxycholate/nadolol; 2.86 ± 0.05 for glycodeoxycholate/atenolol and 4.02 ± 0.05 for glycodeoxycholate/nadolol. Above the *cmc*, drug solubility remains approximately constant, similar to what was found for trihydroxy bile salts. However, with the concentrations used in fluorescence and derivative spectrophotometry, for which the ratio β-blocker/bile salt is less than 1:1000, the presence of drug does not affect (within experimental error) the values obtained for the *cmc* of bile salts.

Our data also show that the drugs are more soluble in the protonated form (pH 7.0) and that atenolol is more soluble both in water and in micelle solutions, than nadolol, probably as a result of the higher hydrophilicity of the former. The solubility of atenolol and nadolol in bile salt solutions depend on the latter conjugation; they are always more soluble in glycocholate or in glycodeoxycholate, thus, implying that glycine conjugation increases drug solubility.

3.3. Partition coefficients of drugs in micelles

The partition coefficients are given by [38]:

$$K_p = \frac{([C_m]/\ [C_t])/\ [S_m]}{([C_w]/\ [C_t])/\ [W]} \quad (1)$$

where $[C_t]$ is the total concentration of β-blocker (C); $[C_m]/[C_t]$ is the mole fraction of β-blocker in the micelle (m); $[C_w]/[C_t]$ is the mole fraction of β-blocker in water (w); and $[S_m] = [S_t] - cmc$, with S standing for the surfactant (bile salt); obviously, $[C_t] = [C_m] + [C_w]$. Fitting modified versions of Eq. (1) (see below) to the experimental data yields the partition coefficient, and two methods were used for this fitting; non-linear regression (using the non-linear subroutines of DeltaGraph 4.0), and the double reciprocal plot. This approach avoids the need to physically separate the aqueous and micellar phases, as it monitors a continuous variation of a physical property

of the bulk solution, and such methodology precludes alterations of the equilibria in solution.

The observed relative intensities of fluorescence (F), or absorbance (A) are the sum of the relative intensities of fluorescence (or absorbencies) due to free and micelle bound forms of the drug, and thus:

$$F_i = F_w + F_m = f_w[C_w] + f_m[C_m] \quad (2a)$$

$$A_i = A_w + A_m = \varepsilon_w[C_w] + \varepsilon_m[C_m] \quad (2b)$$

In this expression, ε is the molar absorptivity and $f = \phi \times \varepsilon$, where ϕ is quantum yield; the subscript i refers to a solution with a specific concentration of bile salt, and the subscripts w and m to free and micelle bound drugs, respectively.

From $[C_t] = [C_m] + [C_w]$, and letting $f_d = f_m - f_w = \phi_m\varepsilon_m - \phi_w\varepsilon_w$ and $\varepsilon_d = \varepsilon_m - \varepsilon_w$, Eq. (2a) and Eq. (2b) become, respectively:

$$F_i = f_w[C_t] + f_d[C_m] \quad (3a)$$

$$A_i = \varepsilon_w[C_t] + \varepsilon_d[C_m] \quad (3b)$$

From Eq. (1), Eq. (3a) and Eq. (3b) it is possible to derive Eqs. (4a) and (4b)

$$F_i = F_w + \frac{f_d[C_t]K_p[S_m]}{1 + K_p[S_m]} \quad (4a)$$

$$A_i = A_w + \frac{\varepsilon_d[C_t]K_p[S_m]}{1 + K_p[S_m]} \quad (4b)$$

which allow for the calculation of partition coefficients for the protonated and neutral forms of atenolol and nadolol by fitting these equations to the experimental data using non-linear regression, as the adjustable parameters are f_d (or ε_d) and K_p.

These equations can be linearized to yield:

$$(F_w - F_i)^{-1} = (F_w - F_m)^{-1} + \frac{1}{(F_w - F_m)K_p}[S_m]^{-1} \quad (5a)$$

$$(A_w - A_i)^{-1} = (A_w - A_m)^{-1} + \frac{1}{(A_w - A_m)K_p}[S_m]^{-1} \quad (5b)$$

Preliminary values for the partition coefficients can be obtained from these latter equations using the double reciprocal method, in which from a plot of $(F_w - F_i)^{-1}$ vs. $[S_m]^{-1}$ or $(A_w - A_i)^{-1}$ vs. $[S_m]^{-1}$, the intercepts are respectively $(F_w - F_m)^{-1}$ or $(A_w - A_m)^{-1}$ and yield F_m or A_m, and the slope allows for the determination of K_p [39]. This approach provides a method to visually test the applicability of the model and the quality of the experimental data.

3.4. Bile salt micelles. Partition coefficients of atenolol and nadolol

Fluorescence. For any of the bile salts studied at concentrations higher than the *cmc*, the maxima of emission spectra of both β-blockers do not change with the concentration of bile salt, but the fluorescence relative intensity (F_i) decreases as the concentration of the bile salt increases. Fig. 2 depicts this behaviour for cholate solutions.

However, below the *cmc* the trihydroxy bile acids behave differently from the dihydroxy bile acids. The values of F_i for the former are constant and equal to those observed in the absence of bile salt, a situation that is not observed for deoxycholate and glycodeoxycholate, for which the values of F_i increase with bile salt concentration until the *cmc* is reached. For the latter surfactants, the value of F_w used in Eq. (2a), Eq. (3a) Eq. (4a) was not that observed in the absence of bile salt, but the value at the *cmc*; this approach compensates for any changes in the molar extinction coefficients due to formation of (1:1) complexes of the drug/bile salt monomer, and assures that additional changes in ε above the *cmc* can be attributed to effects arising from incorporation/aggregation of the drug into micelles.

The partition coefficients of the β-blockers between the aqueous and the micelle pseudophases were calculated from double reciprocal method; the value of F_m and A_m was calculated

from a plot of $(F_w - F_i)^{-1}$ vs. $[S_m]^{-1}$or $(A_w - A_i)^{-1}$ vs. $[S_m]^{-1}$, respectively, in which the values of $(F_w - F_i)$ and $(A_w - A_i)$ are the average of 9 readings. In Fig. 3 such a plot is depicted for both β-blockers at pH 7.0 and 10.8 in cholate solutions.

The experimental data can be fitted by Eq. (4a) with correlation coefficients greater than 0.99, and the values for the partition coefficients are similar to those obtained from the double reciprocal plot. In Fig. 4a the experimental values of the fluorescence relative intensity of nadolol are plotted as a function of $[S_m]$, for sodium cholate solutions at pH 7.0; and superimposed is the curve obtained from Eq. (4a) that best describes the experimental data.

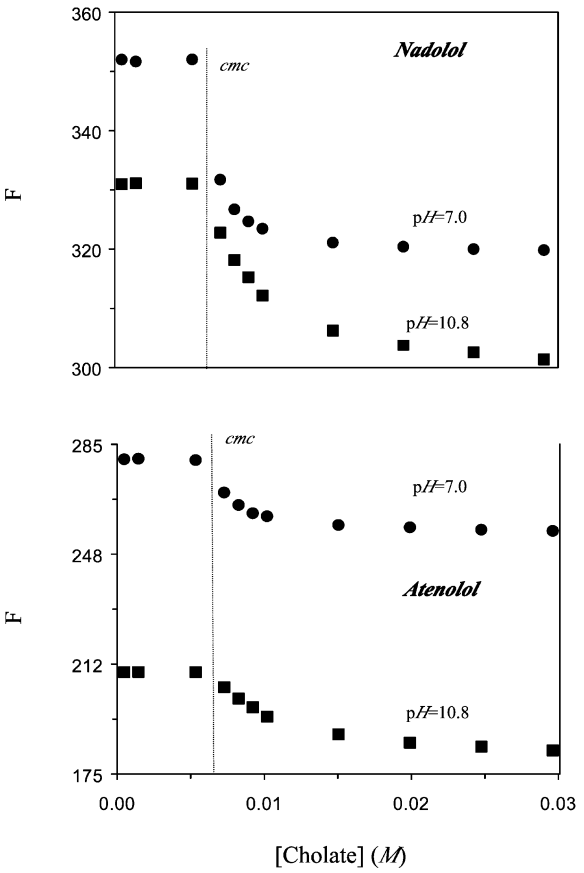

Fig. 2. Fluorescence relative intensity (F_i) of nadolol and atenolol in cholate solutions; $\lambda_{emission}$ 298 nm and at pH 7.0 and 10.8.

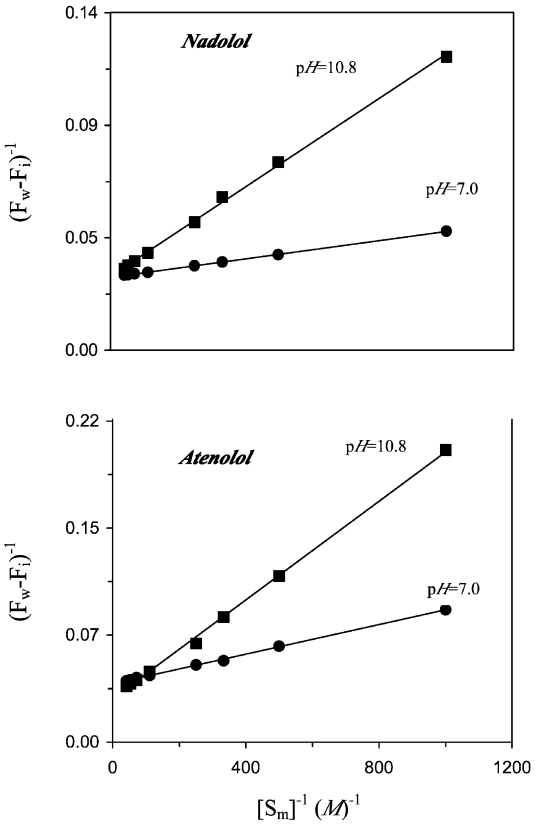

Fig. 3. Double reciprocal plots for the determination of F_m and K_p of nadolol and atenolol in cholate solutions; $\lambda_{emission}$ 298 nm and pH 7.0 and 10.8.

3.4.1. Spectrophotometry

The values of K_p for atenolol and nadolol in cholate and deoxycholate solutions were also calculated from derivative spectrophotometry (first and second derivative); this approach eliminates problems arising from scattering caused by the presence of surfactants [38]. An approach identical to that used for fluorescence was used to obtain the values of K_p, and in Fig. 4b is depicted the fitting to atenolol in cholate solutions at pH 10.8.

3.4.2. Factors that affect K_p

The values of K_p for atenolol and nadolol obtained by fluorescence and by spectrophotometry are presented in Table 2, and it must be

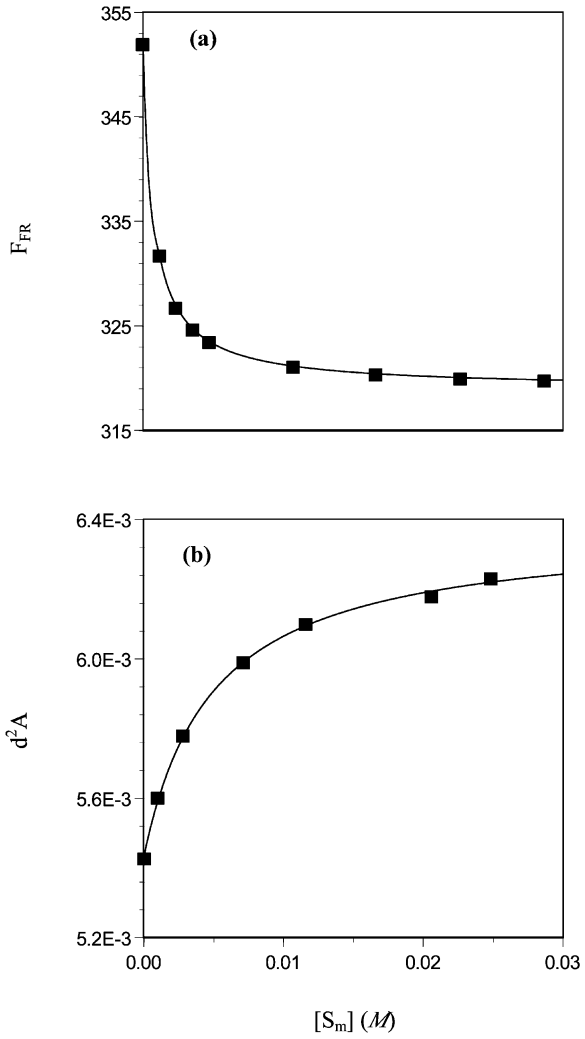

Fig. 4. Plots as a function of [S_m] for sodium cholate of: (a) fluorescence relative intensity (F_i) of nadolol, $\lambda_{emission}$ 298 nm and at pH 7.0; and (b) second derivative spectrophotometric values (d^2A), at 240 nm for atenolol at pH 10.8. The lines represent the best fit of Eq. (4a) and Eq. (4b) obtained by non-linear regression (see text).

pointed out that the values obtained by the two techniques are in good agreement.

Regarding factors that influence the values of K_p, the data show that for the same bile salt, the values of K_p for nadolol are always higher than for atenolol, and that, for both drugs the values of K_p increase in more acidic solutions. The first observation is a direct consequence of the higher hydrophobicity of nadolol, as can be gathered from its molar solubility in water, which is nine times smaller than atenolol. The latter observation is related to the predominant β-blocker form in solution. At pH 7.0 both drugs exist predominantly (> 90%) in the protonated form and thus, the interaction with the deprotonated carboxylate of the side chains of the bile salt micelles will be mainly electrostatic (opposite charges) and would then explain the large partition coefficients observed at pH 7.0. At the higher pH (10.8), the drugs will be present mainly in the neutral form, thus, precluding charge attraction by the carboxylate side chains of the micelles.

A more interesting result is that both drugs interact with any of the bile salts studied, although to a much larger extent with the dihydroxy bile salts. This result clarifies statements in the literature claiming that β-blockers do not interact with trihydroxy bile salts [21]; they do interact but to a much less extent than with the dihydroxy bile salts.

The effect of conjugation of the bile salts with glycine on the values of K_p parallel, as expected, the behaviour of the solubility of β-blockers; conjugation increases both K_p and the solubility.

3.5. Mixed bile salt / lecithin micelles. Partition coefficients of atenolol and nadolol

The partition coefficients were obtained only for mixed micelles of cholate and deoxycholate with lecithin, and for two concentrations of phospholipid, either 500 or 750 μM. The values of K_p were determined by the methods used for bile salt micelles, and in Table 3 are presented the values for atenolol and nadolol obtained by fluorescence and by spectrophotometry in the mixed micelles. Once more, the values obtained by fluorescence and by derivative spectrophotometry were found to be in good agreement.

3.6. Factors that affect the values of K_p

The most striking result is the decrease in the partition coefficients of atenolol and nadolol on going to mixed micelles. This situation must be contrasted with what is observed for cholesterol

Table 2
Partition coefficients of atenolol and nadolol in bile salt micelles composed of sodium cholate (C), sodium glycocholate (GC), sodium deoxycholate (DC) and sodium glycodeoxycholate (GDC) obtained by spectrofluorimetry[a] and spectrophotometry[b] at 25.0 ± 0.1°C, $I = 0.1$ M; pH 7.0 and 10.8

		Non-linear regression				Double reciprocal plot			
		Spectrofluorimetry		Spectrophotometry		Spectrofluorimetry		Spectrophotometry	
		7.0	10.8	7.0	10.8	7.0	10.8	7.0	10.8
Atenolol	C	795 ± 15	189 ± 15	797 ± 30	205 ± 20	793 ± 32	192 ± 10	795 ± 28	196 ± 11
	GC	921 ± 10	306 ± 10	–	–	938 ± 16	309 ± 17	–	–
	DC	1040 ± 60	239 ± 10	1120 ± 30	280 ± 45	1074 ± 43	244 ± 11	1134 ± 31	255 ± 15
	GDC	1300 ± 30	367 ± 20	–	–	1296 ± 62	375 ± 23	–	–
Nadolol	C	1595 ± 20	313 ± 10	1573 ± 60	355 ± 50	1597 ± 40	319 ± 10	1583 ± 38	328 ± 20
	GC	1779 ± 50	536 ± 20	–	–	1790 ± 60	550 ± 30	–	–
	DC	2110 ± 78	521 ± 26	1952 ± 100	524 ± 15	2130 ± 90	526 ± 25	2080 ± 90	540 ± 16
	GDC	2693 ± 25	671 ± 33	–	–	2710 ± 40	676 ± 32	–	–

[a] The fluorimetric results are the average of at least two independent experiments each with values obtained at $\lambda_{emission}$ 298, 309, 580 and 590 nm, respectively.
[b] The spectrophotometric results are the average of at least two independent experiments each with results obtained by first and second derivative spectrophotometry at 236, 240, 250 and 280 nm, respectively.

in mixed micelles bile salt/lecithin, for which the partition coefficients of cholesterol increase significantly with lecithin content [40,41]. This increase has been attributed to the larger size of the mixed micelles upon incorporation of lecithin, that could more easily incorporate cholesterol molecules inside the micelle core [40,41].

To account for the behaviour of atenolol and nadolol we note that these drugs are amphiphilic [42] and appreciably soluble in water (Table 1) and thus, it is reasonable to expect that they are not fully incorporated in the hydrophobic core of simple bile salt micelles, in contrast with what is found for cholesterol. The observation that the protonated form is more soluble than the neutral form of the drug (Table 1) not only supports the above statement, but also suggests that the drug–bile salt micelle interactions, notwithstanding the recognition that the lipophilic part of the drug must be incorporated in the micelle core, have a large polar/electrostatic component, as has been found for the interactions of the same drugs with the anionic surfactant SDS [22,43]. Addition of lecithin led to the formation of mixed micelles with larger hydrophobic cores but with less net superficial charge density [12,14]. Also, as the interaction of atenolol and nadolol with the micelles is expected to have an important polar/electrostatic component, the values of K_p should decrease as observed.

We point further that the reduction in drug K_p on going to bile salt/lecithin mixed micelles increases with the amount of lecithin, and that the reduction is more pronounced at pH 7.0 and for the trihydroxy bile salts. The reduction in the values of K_p are similar for both drugs. Also the values (Table 3) for mixed micelles relatively to those of simple micelles, are ≈ 80–85% (pH 7.0) and ≈ 55–60% (pH 10.8) for cholate; whereas for deoxycholate the values are ≈ 50–55% (pH 7.0) and ≈ 30–35% (pH 10.8).

The pH dependence is related to drug deprotonation and to the consequent weaker polar/electrostatic interactions of the neutral drugs. The dependence with the number of hydroxyl groups can be explained by invoking the accepted model for mixed bile salt/lecithin micelles [13] in which the hydroxyl groups interact with the lecithin choline; more hydroxylic groups compensate more charge and thus, reduce more extensively the net superficial charge density. Again, this observation corroborates the importance of polar/electrostatic interactions of the drug with bile salt micelles.

Table 3
Partition coefficients of atenolol and nadolol in mixed sodium cholate and deoxycholate/lecithin micelles obtained by fluorimetry[a] and spectrophotometry[b] at 25.0 ± 0.1°C, $I = 0.10$ M and for two concentration of lecithin

		Lecithin 500 μM				Lecithin 750 μM			
		Non-linear regression		Double reciprocal plot		Non-linear regression		Double reciprocal plot	
		Fluorimetry	Spectro-photometry	Fluorimetry	Spectro-photometry	Fluorimetry	Spectro-photometry	Fluorimetry	Spectro-photometry
Cholate									
Atenolol	pH = 7.0	216 ± 12	228 ± 10	219 ± 6	225 ± 7	112 ± 5	118 ± 5	114 ± 3	116 ± 3
	pH = 10.8	104 ± 11	101 ± 10	108 ± 3	105 ± 4	78 ± 10	76 ± 5	76 ± 3	75 ± 4
Nadolol	pH = 7.0	563 ± 10	575 ± 15	570 ± 11	573 ± 14	295 ± 13	310 ± 6	300 ± 7	303 ± 6
	pH = 10.8	205 ± 20	210 ± 15	221 ± 8	227 ± 10	172 ± 19	186 ± 12	188 ± 10	190 ± 9
Deoxycholate									
Atenolol	pH = 7.0	776 ± 8	805 ± 24	784 ± 10	790 ± 20	476 ± 10	489 ± 11	481 ± 18	490 ± 15
	pH = 10.8	212 ± 9	208 ± 10	204 ± 6	209 ± 10	173 ± 5	174 ± 14	173 ± 7	175 ± 6
Nadolol	pH = 7.0	1443 ± 22	1460 ± 50	1440 ± 15	1473 ± 46	963 ± 25	975 ± 10	974 ± 13	986 ± 20
	pH = 10.8	429 ± 11	454 ± 20	433 ± 10	467 ± 15	375 ± 10	341 ± 8	374 ± 7	378 ± 15

[a]The fluorimetric results are the average of at least two independent experiments each with values obtained at $\lambda_{emission}$ 298, 309, 580 and 590 nm, respectively.
[b]The spectrophotometric results are the average of at least two independent experiments each with results obtained by first and second derivative spectrophotometry at 236, 240, 250 and 280 nm, respectively.

As a final commentary it can be said that mixed bile salt/lecithin micelles incorporate hydrophobic compounds due to their large size and the fluidity of their core [12]. But that amphiphilic drugs, for which the interactions are predominantly polar/electrostatic, are not completely incorporated in mixed micelles of bile acids/lecithin. These results may be relevant to the bioavailability of β-blockers indicating a mechanism whereby their absorption from the gastrointestinal tract is decreased. Indeed, several in vivo studies [18–20,44] have shown that atenolol and nadolol absorption is reduced by bile salts, which was attributed to formation of a 'stable' association complex with bile salt micelles. In this work, we were able not only to quantify the effect of lecithin on the extension of this interaction, but also show that drug/micelle interactions are controlled by the chemical nature of the bile salts and of the pharmaceutical compound.

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