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Fluorimetric and solubility studies of nadolol and atenolol in SDS micelles

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Abstract

The effect of sodium dodecyl sulfate (SDS) micelles on the spectrofluorimetric intensities and on the solubility of two β-blockers (atenolol and nadolol) were studied at 25.0 ± 0.1°C and $I = 0.1$ M NaCl. From the dependence of these physical properties on SDS concentration it was possible to calculate the binding constants drug-micelle, and it was found that both techniques yield similar results for the binding constants, and that are in agreement with those calculated from the effect of micelles on the apparent acidity constants of the β-blockers.

Keywords: β-blockers; SDS micelles; Spectrofluorimetry; Solubility; Binding constants

1. Introduction

Analytical methods which rely on the use of surfactants are becoming more and more numerous, since addition of surfactants provides an increase in selectivity and sensitivity [1–3]. From an analytical view point, the use of surfactants increases the solubility of organic substances in water, through shallow or deep penetration of the micelles or simply by surface adsorption [4–8], and can also catalyze specific reactions by modification of the micro-environment in which these reactions take place [1,9,10]. Surfactants at concentrations higher than the critical micelle concentration (cmc) has been extensively used in the application of spectroscospic (ultra-violet, fluorescence, phosphorescence, atomic spectroscopy), electroanalytical and separation methods to sparingly soluble analytes [11–13].

The use of micellar aggregates is also of interest in biology, as they can mimic biomembranes [14–17] and because they can be specifically modified to control the pharmacokinetic characteristics of micelle encapsulated drugs, and thus provide a pathway for controlled released formulations [18].

In this work we report the effect of SDS micelles on the fluorescence intensities and on the solubility of two β-blockers (atenolol and

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nadolol), which are then used to quantify the binding constants of the drugs to the micelles. Furthermore, both techniques were found to yield similar results for the binding constants, and in good agreement with those which have been calculated from the effect of micelles on the apparent acidity constants of the β-blockers [19].

2. Experimental

2.1. Materials

The β-blockers (atenolol and nadolol) and sodium sulfate (SDS) were from Sigma and used without further purification. All solutions were prepared with double deionized water (conductivity less than 0.1 μS cm^{-1}).

2.2. pH and spectrophotometric measurements

The automatic system used to measure pH has been described elsewhere [19], here we stress only that system calibration was performed by the Gran method [20] in terms of hydrogen ion concentration, using strong acid/strong base titrimetry.

Absorption electronic measurements used in the solubility studies were obtained at 25.0 ± 0.1°C with a Hitachi U-2000 spectrophotometer. Calibrations curves (A vs. concentration) for atenolol (at 224 and 274 nm) and for nadolol (at 224 and 276 nm) were constructed for each concentration of SDS and found to be independent on the latter quantity. The reported data are the average of four different measurements against a solution with the same concentration of SDS and at the same ionic strength, but without the drug.

2.3. Spectrofluorimetry

Steady-state fluorescence was measured at 25.0 ± 0.1°C using a Shimadzu RF5001PC fluorescence spectrometer; in all measurements a bandwidth of 10 nm was used. The excitation maximum was 224 nm and emission maxima were 298 and 590 nm. A linear dependence between fluorescence intensity and concentration was observed from 1×10^{-8} to 3×10^{-6} M for atenolol and from 1×10^{-8} to 2×10^{-6} M for nadolol. Solutions used in fluorescence were 1×10^{-6} M in the β-blocker while the SDS concentration was varied from 5×10^{-4} to 2×10^{-2} M. All experiments were performed at either pH 4.0 or 10.8, and the results were obtained from four different solutions at each pH. These values of pH were chosen so that at the lower pH the drugs are fully protonated, whereas at pH 10.8 they exist predominantly in the neutral form. The fluorescence intensity for solutions of SDS in the absence of drugs was found to be constant and used as a blank.

2.4. Solubility studies

The solubility of the neutral and protonated forms of the β-blockers was measured in water and in aqueous SDS solutions (below and above the cmc = 1.4 mM); in all solutions the ionic strength was kept constant as 0.1 M with NaCl. The following concentrations of SDS were used: 0.5, 1, 2, 3, 4, 6, 8, 10, 20 mM.

For each solution, the β-blocker was dispersed in 20 ml of each solvent and the pH adjusted by addition of concentrated HCl or NaOH. The saturated solutions were incubated for 72 h at 25.0 ± 0.1°C in a thermostated bath, and then filtrated through filter paper (Lida, 0.45 μm). The β-blocker concentration was determined by spectrophotometry and spectrofluorimetry after appropriate dilution. For each drug, the corresponding solvent system was diluted in the same way to be used as the blank.

3. Results and discussion

3.1. Spectrofluorimetric determination of drug binding constants to SDS micelles

Analysis of spectrofluorometric data for both β-blockers in water and aqueous SDS reveals that for both drugs the fluorescence intensity increases, above the cmc, with SDS concentration, and that this increase is more pronounced at pH 4.0 than at pH 10.8. This fluorescence increase in micellar

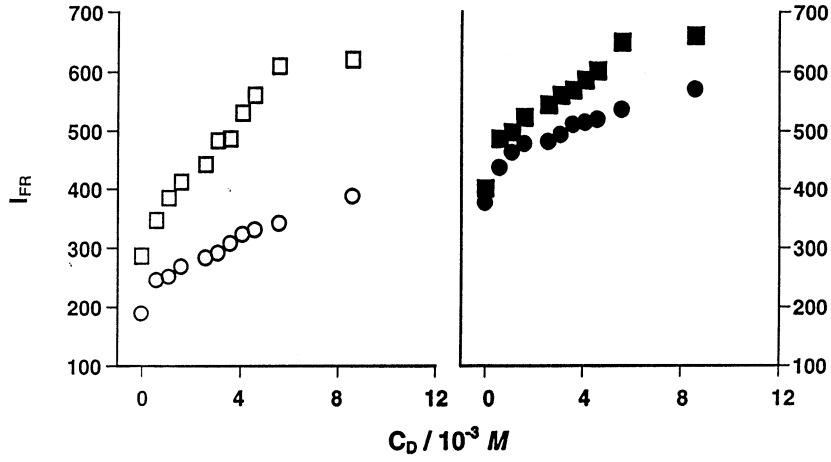

Fig. 1. Effect of micellar concentration of SDS on the fluorescence intensities of atenolol (open) and nadolol (solid) at pH 4.0 (squares) and 10.8 (circles).

media is attributed to a stabilization/protection of the excited state singlet, that hinders decay by quenching and other non-radiactive deactivation processes [10,15].

In Fig. 1 fluorescence intensity (I_{FR}) at 298 nm is plotted against C_D, the concentration of SDS in micellar form ($C_D = |SDS| - cmc$) and, both for acidic and basic solutions, it is evident a fluorescence enhancement above the cmc, that levels off at high concentrations of SDS. As the fluorescence intensity for protonated species is always higher then for neutral species, it can be inferred that protonated forms interact more strongly with the micelles than the neutral forms of the drugs.

These interactions are normally quantified by the binding constants solute/micelle, K_A^m, which are defined as

$$K_A^m = C_{MA}/C_A \times C_D \quad (1)$$

where C_A is the concentration of free drug and C_{MA} that of micelle bound drug; the values of K_A^m can be obtained from changes in fluorescence intensity caused by increased concentrations of surfactant.

As the fluorescence quantum yield, Φ, of a fluorophor in micellar media is given by

$$\Phi = (I_A + I_{MA})^{-1} (I_A\Phi_A + I_{MA}\Phi_{MA}) \quad (2)$$

in which I_A and I_{MA} are, respectively, the absorbance of free fluorophor (A) and in the presence of surfactant (MA). From $I_A/I_{MA} = \varepsilon_A C_A/\varepsilon_{MA} C_{MA}$ (ε_A is the molar extintion coefficient for free drug and ε_{MA} in the presence of surfactant), and making $\gamma = \varepsilon_{MA}/\varepsilon_A$, Eq. (2) thus becomes [21]

$$[(\Phi/\Phi_A - 1)]^{-1} = [(\Phi_{MA}/\Phi_A) - 1]^{-1}[1 + 1/(\gamma K_A^m C_D)] \quad (3)$$

Under the experimental conditions used we found (i) that the absorbance was independent of the concentration of SDS, which implies that $\varepsilon_{MA} = \varepsilon_A$, and that (ii) the micelles do not modify the extinction coefficients at the excitation wavelength, which enables Eq. (3) to be written as [22]

$$[(I/I_A) - 1]^{-1} = [I_A/(I_{MA} - I_A)] + [I_A/(I_{MA} - I_A)][1/(K_A^m)](C_D)^{-1} \quad (4)$$

where I and I_A stands for the fluorescence intensity in the presence and absence of SDS, and I_{MA} is the maximum fluorescence intensity that can be observed in SDS solutions.

A plot of $[(I/I_A) - 1]^{-1}$ against $(C_D)^{-1}$ yields a straight line for both drugs in acidic and basic media, and the ratio of intercept to the slope gives K_A^m. In Table 1 are presented the values of K_A^m obtained by this approach, as well as those obtained from solubility measurements and from the dependence of acidity constants with C_D by using the PIE model [23].

Table 1
Binding constants of neutral (K_A^m) and protonated (K_{HA+}^m) forms of β-blockers to SDS micelles

	K_A^m			K_{HA+}^m		
	Fluorescence	Solubility[a]	Changes in pK_a[b]	Fluorescence	Solubility[a]	Changes in pK_a[b]
Atenolol	112	156	134	238	255	200
Nadolol	222	196	271	426	458	464

[a] The values are the average of spectrophotometric measurements at 224 and 274 nm and of fluorescence measurements at 298 and 590 nm.

[b] Values from [19]; they were obtained by application of the PIE model [23] to apparent acidity constants determined by potentiometric and spectrophotometric methods.

3.2. Binding constants of β-blockers with SDS micelles determined by distribution methods

Changes in solubility of weak acids in micellar media are caused by (i) partitioning of the neutral form of the acid in the micellar pseudo-phase or (ii) interactions with the micellar surface. To distinguish between these processes, we have determined the solubility of both drugs in (i) aqueous solution, both in acidic ($S_{w;H+}$) and alkaline ($S_{w;HO-}$) conditions, and in (ii) micellar solutions, again in acidic ($S_{m,H+}$) and alkaline ($S_{m;HO-}$) media. An increase in the ratio ($S_{m;HO-}$)/($S_{w;HO-}$) is taken to suggest an increase in partition of the neutral molecules in the micellar pseudo-phase, whereas an increase in the ratio ($S_{m;H+}$)/($S_{w;H+}$) indicates an interaction with the micellar surface [24].

The dependence of the solubility with SDS concentration is similar for both β-blockers: practically constant till it reaches the cmc, after which increases with SDS but leveling off at high SDS concentrations; furthermore, this increase in solubility was found to be more pronounced for acidic solutions.

The binding constant of any substance to a micelle K_A^m can be related with solubility through the expression $K_A^m = (S_m - S_w)/S_w C_D$ [24–26], and application of this equation to a neutral molecule (A) and to its protonated form (HA^+) yields $S_m/S_w = 1 + K_A^m C_D$ and $S_m/S_w = 1 + K_{HA+}^m C_D$, respectively [24]. These latter expressions were found to be valid only for SDS concentrations for which the solubilities change appreciable and not at high concentrations of surfactant. In Fig. 2 are presented the solubility data for atenolol and nadolol at pH 4.0 and 10.8, and in Table 2 the values of K_A^m and of K_{HA+}^m obtained from plots of S_m/S_w vs. C_D, both for solubilities measured by spectrophotometry and fluorimetry.

3.3. Comparison of the calculated binding constants

Analysis of data in Table 1 shows that the binding constants determined by different experimental methods and using different theoretical models are similar, which gives support to the models used. It is also clear that neutral species always bind to SDS micelles less strongly than the

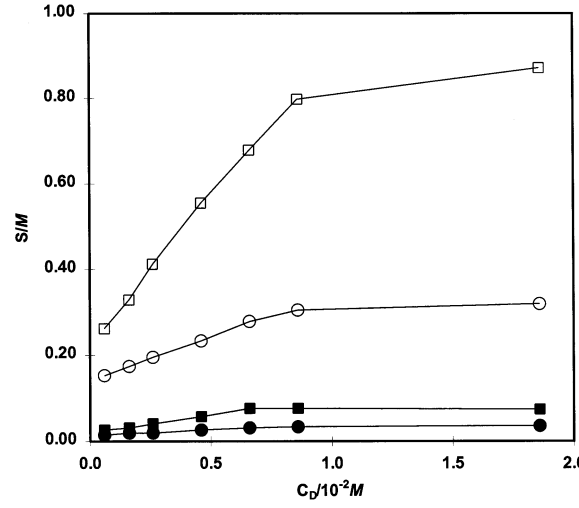

Fig. 2. Solubilization effect of micellar concentration of SDS on atenolol (open) and nadolol (solid) at pH 4.0 (squares) and 10.8 (circles).

Table 2
Slopes of plots of (S_m/S_w-1) vs. C_D for of neutral (K_A^m) and protonated (K_{HA+}^m) forms of β-blockers[a]

	Atenolol				Nadolol			
	Spectrophotometry		Spectrofluorimetry		Spectrophotometry		Spectrofluorimetry	
pH = 4.0	224 nm	254.4 (0.993)	298 nm	255.8 (0.992)	224 nm	457.8 (0.990)	298 nm	458.0 (0.991)
	274 nm	254.7 (0.993)	590 nm	255.9 (0.993)	276 nm	455.9 (0.994)	590 nm	458.2 (0.989)
pH = 10.8	224 nm	152.8 (0.988)	298 nm	160.8 (0.998)	224 nm	196.8 (0.995)	298 nm	193.7 (0.997)
	274 nm	125.4 (0.987)	590 nm	160.8 (0.998)	276 nm	196.5 (0.994)	590 nm	193.6 (0.996)

[a] The solubilites were measured by spectrophotometry at 224 and 274 nm, and by spectrofluorimerty at (emission) 298 and 590 nm. The values of the slope are the binding constants (see text); the values in parenthesis are the correlation coefficients associated with the best fit.

protonated forms, as expected from the electrostatic attraction between positively charged species and the negatively charged micelle surface. The same observation is also supported by the solubility data: as the ratio of $S_{m,H+}/S_{m;HO-}$ is always greater than 1 it can be gathered that the solubility enhancement is mainly due to the effect of micelles on the ionization of the protonated forms due to stronger interactions with the charged species. Nevertheless, hydrophobic interactions with the micellar pseudo-phase must also take place, as the solubility of the neutral molecules increases with SDS concentration.

On what concerns the differences in binding constants between atenolol and nadolol with SDS, we recall that atenolol is the more hydrophilic drug, as quantified by its higher solubility in pure water, and propose that the more extensive water solvation will hinder micelle binding.

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