Fluorescence studies of the drug Piroxicam in reverse micelles of AOT and microemulsions of Triton X-100

Abstract The microenvironment of the polar core of reverse micelles of the anionic surfactant AOT in isooctane and the microemulsions of the non ionic surfactant Triton X-100 in cyclohexane/hexanol were studied by electronic absorption and steady-state fluorescence spectroscopy incorporating an antiinflammatory nonsteroidal drug Piroxicam. Two acid-base equilibria of this probe in water ($pK_a = 3.1$ and $pK_a = 4.8$) are strongly and differently affected by the nature of the two interfaces and inner pools of each microheterogeneous system.

In AOT, fluorescence quantum yields vary up to $w_0 = 10$ reflecting changes in the microviscosity sensed by the probe and intramicellar pH gradients, reaching a nearly constant value up to $w_0 = 40$. In Triton X-100 the interface is polar and protic and interacts with the probe as a Lewis base. Three different spectroscopic species were detected: one attached to the interface at $w_0 = 0$, the second one with structured water up to $w_0 = 8$ and a third one in free water at large $w_0$ values ($w_0 = 16$), with increasing microviscosity values.

Fluorescence anisotropy studies with other probes in the same micellar systems are in good agreement with the patterns of microviscosity found in both systems.

Key words Reverse micelles – microemulsions – fluorescence – Piroxicam

Introduction

Microheterogeneous systems such as micelles and microemulsions have been considered as model systems which provide a suitable microvicinity for inclusion of macromolecules such as proteins, polynucleotides and drugs. Unique interfacial properties confer a great potential interest to micellar aggregates in various fields, for example, paints, pharmaceutical drugs and enhanced oil recovery [1, 2].

Reverse micelles and microemulsions formed in organic solvents incorporate a pool of water which has properties clearly distinct from those of bulk water [3, 4]. Various techniques have been applied in the study of reverse micelles, e.g., light-scattering and N.M.R. which make use of intrinsic properties of the system. On the other hand, there are methods which are based on the fluorescence of a probe molecule incorporated in the system. The latter enable the correlation of photophysical data with some parameters associated with the structure of the aggregate and therefore have been used extensively [5].

Several drugs are interesting in this context. Piroxicam, Prx, (4-hydroxy-2-methyl-N-pyridil-2H-1,2 benzothiazine-3-carboxamide-1,1 dioxide) (Fig. 1) is a non steroidal antiinflammatory drug which induces photosensitive action. Photophysical studies of this molecule exhibit excited state intramolecular proton transfer (ESIPT) in aprotic solvents [6]. This effect is perturbed in protic solvents through the formation of an anion and/or an open conformer in the ground state.
Fluorescence of Piroxicam in reverse micelles

In this paper, the absorption and emission properties of Pfx incorporated in an anionic reverse micelle, sodium 1,4-bis(2-ethylhexyl)sulfosuccinate, (AOT) and a nonionic microemulsion of (iso-octylphenoxy-poly(oxyethylene) glycol, Triton X-100, are compared and applied to extract information on the micropolarity and microviscosity of the respective interfaces and inner pools.

**Experimental**

**Reagents**

Pfx was a generous gift from “Laboratórios Medinfar” and Acridine Orange was obtained from Sigma. Triton X-100 (Trx) was purchased from Riedel-de-Haan and used as supplied, AOT was from Sigma and used without purification as well. All solvents used were spectroscopic grade, except for 1-hexanol. Buffer solutions were made up using recommended procedures [7].

**Apparatus**

Absorption spectra were recorded with a Jasco V-560 UV/VIS spectrophotometer. Steady-state emission spectra and fluorescence anisotropy measurements were recorded with a Perkin–Elmer LS 50 B spectrofluorimeter. All data were stored in a computer. The instrumental response at each wavelength was corrected by means of a curve obtained using the appropriate fluorescence standards (until 400 nm) together with the one provided with the apparatus. The fluorescence quantum yields of Pfx were measured using quinine sulphate (QS) in 1 N H2SO4 solution ($\phi_f \approx 0.546$) as standard. The integration of the corrected spectra was made over the emission wavelength range and corrections for changes in the respective refractive index were carried out [8].

**Sample preparation**

Microemulsions of Trx were prepared by adding 1-hexanol in cyclohexane, in order to have a solution of 0.2 M in Trx, 3:2 (w/v) ratio of Trx/Hexanol and $w_0 = [H_2O]/[Surf] = 0$. Buffered water was then added to vary $w_0$. The incorporation of Pfx was achieved by adding a small amount of Pfx in acetone, bubbling a stream of N2 to remove the acetone, the final concentration of Pfx being $5 \times 10^{-6}$ M. AOT micelles were prepared by dissolving it in isoctane and then adding the buffer with continuous shaking. The buffer concentrations used were 50 mM and 25 mM respectively in Trx and AOT.

**Results and discussion**

**Pfx in aqueous solutions**

The absorption of Pfx in aqueous solutions is strongly pH dependent, reflecting the equilibrium of two forms, neutral ($\lambda_{max} = 335$ nm) and anionic ($\lambda_{max} = 360$ nm) with an isobestic point at $\lambda = 348$ nm. The emission of the neutral form is much stronger than the anionic one. On the basis of the variation of the absorption spectra with the pH, a value of $pK_a = 3.1$, for the formation of the monoanion was obtained in good agreement with $pK_a = 3.3$ previously reported [6]. At higher pHs another species is formed, possibly a di-anion which is also in equilibrium with the neutral form, the $pK_a \approx 4.8$.

The photophysical parameters obtained for the neutral form are nearly identical to those found for the closed conformer in aprotic solvents [6]. However, an open conformer would be more likely to exist in aqueous media due to hydrogen bonding solute-solvent.

**Pfx in AOT reverse micelles**

The absorption and emission of Pfx in reverse micelles of AOT in isoctane were studied as a function of the water content ($w_0$) and at different pHs (Fig. 2). In the absence of water, $w_0 = 0$, the absorption spectra is similar to that obtained in the apolar solvent, showing that no association exists between this probe and the surfactant. The increase of $w_0$ leads to a batochromic shift at a given pH = 3.8 with the same isobestic point at $\lambda = 348$ nm showing the same equilibrium neutral form $\Leftrightarrow$ monoanion with the neutral form being dominant at smaller $w_0$ values. The same equilibrium persists at pH = 6.8, whereas for higher pHs (pH = 10.2) even at $w_0 = 2.5$ only the di-anion form exists with the maximum shifted to