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Photophysical properties and interactions of xanthene dyes in aqueous micelles

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ABSTRACT

Photosensitizers (PS) photodynamic activities are regulated by their location in the biological target, which modulates their photophysical and photochemical features. In this work the PS partition for the Xanthene Dyes Fluorescein (**FSC**), Eosin Y (**EOS**), Erythrosin B (**ERY**) and Rose Bengal B (**RBB**) in biomimetic models (SDS, CTAB and Pluronic P-123 micelles) and the effects on their photophysical characteristics are evaluated. The hydrophobic and electrostatic forces that govern the PS–micelle interaction are analyzed. At physiological pH (7.25), the ability of the dianionic protolytic form of the dyes to be positioned into the micelle palisade and its micelle interaction depends not only on the hydrophobicity of the dye but also on the micellar surface charge. The Binding Constants obey exactly the same order of the Partition Coefficients for the dyes in P-123 and CTAB micelles. The Stern–Volmer treatment pointed out that dyes are located inside the micelle, especially **ERY** and **RBB**. The magnitude of the dye–micelle interaction increased from SDS, P-123 and finally CTAB micelles due to the charges between dye and micelle, and among the xanthenes, their hydrophobic characteristics. Within the micelle pseudo phase, **ERY** and **RBB** are still very efficient photosensitizers exhibiting high quantum yield of singlet oxygen, which turns them very attractive especially with P-123 polymeric system as *drug delivery systems* in photodynamic therapy.

1. Introduction

Photodynamic therapy (PDT) and photodynamic inactivation of microorganism (PDIMO) are therapeutic modalities that act against abnormal biological tissues and localized infections. The fundament of the photodynamic activity is a photosensitizer (PS) compound that can be activated by visible light in presence of molecular oxygen producing highly reactive species, which are cytotoxic [1–4].

The photosensitizers are promoted to the excited singlet state and can cross to the triplet state. At this state, the compounds show long enough lifetimes to react directly with the biological substrate or with oxygen. Reactive species are the excited reactive oxygen species (EROS) (type I photochemical reaction), and/or singlet oxygen $^1O_2$ (type II photochemical reaction). The $^1O_2$ is pointed out as the main PDT and PDIMO agent that can lead to necrosis and apoptosis of the tissues [5–7]. In consequence usual photosensitizers for PDT applications exhibit high quantum yields of singlet oxygen ($\Phi_\Delta{}^1O_2$).

Selectivity and extent of partition of PS to target tissue is an important pre-requisite to photodynamic clinical efficiency. Usually in cells the targets are lysosomes, mitochondria and plasmatic membranes [8]. In microorganism the damage is pointed out to occur mainly in the cell membrane [9,10]. The previous knowledge of the incorporation and of the cyto-localization is fundamental for successful in PDT treatments, which makes necessary the evaluation of the interaction parameters of PS toward cell membrane [11–13]. The biological cell complexity makes this interaction studies difficult, but it is possible to study these properties independently. Cell affinity depends on the hydrophobic and electrostatic characteristics of the PS and can be estimated using biomimetic systems [14–16].

The hydrophilic–lipophilic balance (HLB) of the PS is usually estimated in water/1-octanol mixture, which is considered the simplest membrane model [17]. However, more interesting studies of the penetration and specific interaction of drugs and biomimetic systems can be realized with aqueous micelles that reasonably mimetize bio-membranes and furnish useful information of organized systems [18–21]. In addition to biomimetic studies, micelles can also be used as *drug delivery systems* for medications [14,22–24]. However, ionic micelles as SDS and CTAB are not used in medical formulations due to their low drug protection and toxicity in biological fluids [25]. Very interesting formulations are obtained

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with micelles constituted by polymeric surfactants, in particular Pluronics® (or Poloxamers®). These surfactants are constituted by tri-block monomers of polyethylene oxide (PEO) and polypropylene oxide (PPO) forming (PEO)(PPO)(PEO) molecules where PPO provides the hydrophobic core while PEO portion is the water-friend counterpart. These set of polymers are non-ionic, stable, non-toxic, and biocompatible, providing large and adequate sites that solubilize several hydrophobic drugs and are less affected by the environmental changes in biological fluids [14,25–27].

The PS agents in this study are xanthene derivatives. Fluorescein (**FSC**) is an important fluorescent compound used in eyes diseases detection due to its high selectivity for ocular neo-vasculature and high fluorescent quantum yield (Φ_F) [28,29]; **FSC** does not exhibit photodynamic activity once it has low quantum yield of $^1O_2$ ($\Phi_\Delta{}^1O_2$). Despite it, some halogenated derivatives of **FSC** exhibit high production of $^1O_2$ [30] and can be evaluated as possible compounds for eye diseases treatment as age-related macular degeneration and pathologic myopia.

The chemical structures of the xanthene compounds (Fig. 1) show that the molecule presents two distinct moieties, the xanthene and benzoate rings, where the orthogonality (and rotation) between these planes depends on their substituents [31–33]. According to Nagano et al. [34] the photophysical characteristics of these dyes are determined by the xanthene part, whereas the benzoate moiety controls the excited state decay processes (by vibration and rotation energies dissipation). In the present work properties of Eosin (**EOS**), Erythrosin (**ERY**) and Rose Bengal (**RBB**) dyes (Fig. 1) that show high $\Phi_\Delta{}^1O_2$ in water at neutral and alkaline pHs [30,35,36] are investigated in aqueous micellar systems (SDS, CTAB and P-123).

2. Materials and methods

2.1. Materials

All solvents employed were analytical grade and were used without further purification. Fluorescein (**FSC**, Carlo Erba), Eosin Y (**EOS**, Reagen), Erythrosin B (**ERY**, Vetec) and Rose Bengal B (**RBB**, Nuclear) were analyzed and identified by ^{1}H NMR. SDS, CTAB and P-123 were purchased from Sigma–Aldrich and the solutions were prepared by weight of the previously dried materials in desiccators under vacuum for 24 h. The experiments were conducted in a UV–Vis spectrophotometer Cary-50 apparatus or in a fluorescence spectrofluorometer Cary-Eclipse. All experiments were conducted at 30.0 °C in aqueous solutions with the pH 7.25 controlled by buffer (McIlvaine, [Na_2HPO_4] = [citric acid] = 7.5×10^{-3} mol L^{-1}) and the ionic strength controlled by NaCl addition (0.10 mol L^{-1}). For absorbance experiments the dyes concentrations were 5.00×10^{-6} mol L^{-1} and for fluorescence measurements were 5.0×10^{-7} mol L^{-1} (absorbance lower than 0.05 to avoid internal filter problems).

2.2. Methods

2.2.1. Partition coefficient (K_p)

To a biphasic mixture containing octanol/water at 50% (v/v) was added the xanthenes dyes (3.0×10^{-6} mol L^{-1}), and after intense stirring and 48 h rest in the dark, the xanthene concentrations in both phases was evaluated by UV–Vis. The partition coefficient of the octanol phase (K_p) was calculated by

$$K_P = \frac{[PS]_{oct}}{[PS]_{water}} \quad (1)$$

where $[PS]_{oct}$ and $[PS]_{water}$ are the molar concentrations of dyes in octanol and water, respectively.

Table 1
Φ_F values of xanthene dyes in water used as standard, pH 9.2 [38].

Dye	Φ_F	λ_{exc} (nm)
FSC	0.920	470
EOS	0.200	493
ERY	0.020	508
RBB	0.018	515

2.2.2. Binding constant (K_b)

The binding constants (K_b) of xanthenes with micelles were evaluated by the fluorescence emission spectra of the dyes (5.0×10^{-7} mol L^{-1}). Aliquots of surfactant were added using concentrated stock solutions of surfactants directly to a cuvette containing xanthenes in water. The experimental data were theoretically fitted using Eq. (2) [37].

$$F = F_f + \frac{(F_0 - F_f)}{(1/K_b([S] - CMC)^N) + 1} \quad (2)$$

where F, fluorescence emission intensity of xanthenes; F_f, fluorescence of the bounded PS to the surfactant; F_0, fluorescence in the absence of surfactant; [S], surfactant concentration; and N, number of surfactant by PS molecules.

2.2.3. Stern–Volmer suppression constant (K_{SV})

Fluorescence quenching experiments were conducted with titration of xanthenes solutions (5.0×10^{-7} mol L^{-1}) in water and in the presence of micellar systems using iodide ion as a water-soluble quencher. The NaI stock solutions were 1.00 mol L^{-1} for SDS and P-123, and 0.10 mol L^{-1} for CTAB systems. The K_{SV} were calculated from Eq. (3).

$$\frac{F_0}{F} = 1 + K_{SV}[I^-] \quad (3)$$

where F_0 and F, fluorescence intensities in the absence and presence of the suppressor, respectively, and [I^-], iodide concentration.

2.2.4. Fluorescence quantum yield (Φ_F)

Φ_F values were obtained in water and in aqueous micellar SDS, CTAB and P-123 solutions by use of Eq. (4). The standard compounds used were the xanthenes in pure water (Table 1).

$$\phi_F = \frac{Abs_{Std} F_D n_{Std}^2}{Abs_D F_{Std} n_D^2} \cdot \phi_{Std} \quad (4)$$

where Abs, absorbance intensity; F, fluorescence spectrum area; and n, refraction index. The subscripts in the symbols refer to the standard (Std) and to the dye (D). The concentration of the surfactants was kept fixed and above their CMC.

2.2.5. Quantum yield of the singlet oxygen ($\Phi_\Delta{}^1O_2$) of the dyes

$\Phi_\Delta{}^1O_2$ were determined from the phosphorescence intensity decays at 1270 nm. Data were recorded with a time-resolved NIR fluorometer (Edinburgh Analytical Instruments) equipped with Nd:YAG LASER (Continuum Surelite III) λ_{exc} = 532 nm (pulse ~ 30 ns). The emitted light passed through a silicon and an interference filter and a monochromator before detection with a NIR Photomultiplier (Hamamatsu Co. R5509). The singlet oxygen lifetime was determined by applying first-order exponential fitting to the curve of the phosphorescence decay. In all the experiments the absorbance at 532 nm was kept around 0.3 (determined in a Shimadzu spectrophotometer UV-240 PC).

2.2.6. Resonance light scattering (RLS)

Spectrofluorometer operating in synchronous mode ($\lambda_{exc} = \lambda_{emis}$) was used to characterize the formation of