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Synthesis, spectroscopic properties and photodynamic activity of Zn(II) tetraalkyltetrapyridinoporphyrazinium derivatives

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Abstract. Cationic Zn(II) tetraalkyltetrapyridinoporphyrazinium derivatives bearing N-alkyl chains of different length (ZnPc **2** R:-CH_3, ZnPc **3** R:-$(CH_2)_{11}CH_3$, ZnPc **4**, R:-$(CH_2)_{15}CH_3$) were synthesized from Zn(II) tetrapyridinoporphyrazine (ZnPc **1**). The cyclotetramerization of 3,4-pyridinedicarbonitrile with zinc(II) acetate in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) produces ZnPc **1** as mixtures of constitutional isomers with 67 % yield. The exhaustive alkylation of ZnPc **1** gives ZnPc **2**, ZnPc **3** and ZnPc **4** with 95, 91 and 90 % yields, respectively. Absorption and fluorescence spectroscopic studies of these sensitizers were analyzed in reverse micelle of *n*-heptane/sodium bis(2-ethylhexyl)sulfosuccinate (AOT)/water varying the amount of water dispersed in the reverse micelles (W_0 =[H_2O]/[AOT]). Under these conditions, solubilization of ZnPc **2-4** take places at W_0>30. Also, the spectra were analyzed at different AOT concentration keeping W_0=30 constant. These results were used to determine the binding constant (K_b) between these sensitizers and AOT reverse micelles. The values of K_b of 283, 150 and 34 were found for ZnPc **2**, **3** and **4**, respectively. The photodynamic activity was evaluated in AOT system using 9,10-dimethylanthracene (DMA). The photooxidation rate of DMA sensitized by these phthalocyanines follow the order: ZnPc **2** > ZnPc **3** ~ ZnPc **4**. Therefore the studies show that in AOT reverse micelles, the cationic ZnPc **2** is an efficient photosensitizer with potential applications in photodynamic therapy.

Introduction

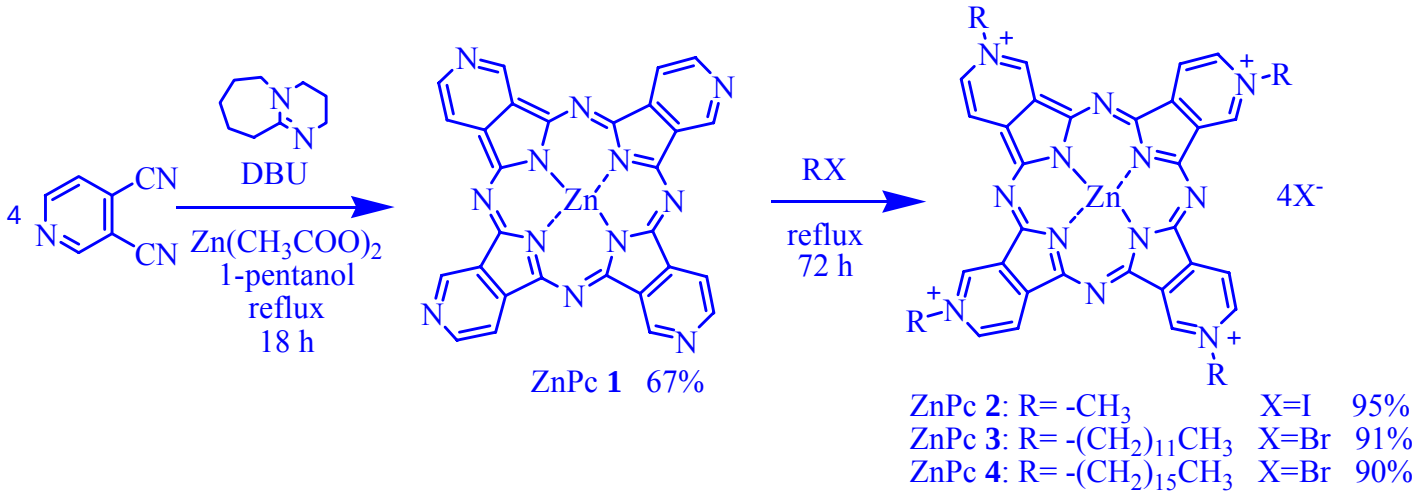
Phthalocyanines derivatives exhibit a high absorption coefficient ($\varepsilon>10^5$ $M^{-1}cm^{-1}$) in the visible region of the spectrum, mainly in the phototherapeutic window (600-800 nm) and a long lifetime of triplet excited state to produce efficiently $O_2(^1\Delta_g)$ [1]. One of more recent and promising applications of phthalocyanine in medicine is in the detection and cure of tumors. Photodynamic therapy (PDT) is an innovative treatment for several types of cancer [2]. This therapy is based on the administration of a photosensitizer, which is selectively incorporated in tumor cells. The subsequent exposure to visible light in the presence of oxygen specifically inactivates neoplastic cells. The photobiological properties of various phthalocyanine derivatives indicate that they can be very promising photosensitizers for clinical application of PDT.

Cationic sensitizers have several interesting features, which make these compounds attractive photosensitizers for a variety of biological systems. The combination of hydrophobic and hydrophilic substituents in the sensitizer structure can facilitate membrane penetration and produce a better accumulation in subcellular compartments, enhancing the effective photosensitization [3]. Recently cationic phthalocyanines were also proposed for photodynamic inactivation (PDI) of bacteria in an attempt to overcome the problem of bacterial strains resistant to current antibiotics [4].

On the other hand, microheterogeneous systems such as reverse micelles are frequently used as an interesting model to mimic the water pockets that are often found in various bioaggregates such as proteins, enzymes and membranes [5,6]. Also, AOT reverse micelles form suitable and variable reaction media depending on water to surfactant ratio for the study of different types of organic and enzymatic reactions. Thus, water-soluble and water-insoluble compounds can be dissolved simultaneously in reverse micelles, which simulate a biomimetic microenvironment.

Synthesis of zinc phthalocyanines (ZnPcs)

The cationic ZnPc **2-4** were synthesized as showed in Scheme 1. First, ZnPc **1** was synthesized by the cyclotetramerization of 3,4-pyridinedicarbonitrile with Zn(II) acetate in the presence of organic base 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in *n*-pentanol [7]. After reflux for 18 h, the reaction results in the formation of the corresponding ZnPc **1** as mixtures of constitutional isomers with 67 % yield. Cationic sensitizers **2-4** were obtained treating the ZnPc **1** with alkyl halide for 72 h at reflux in N,N-dimethylformamide. The product was precipitated with cyclohexane and filtered. Them, it was re-precipitated from methanol/water and the solid washed with hexanes and dichloromethane. The exhaustive alkylation produces ZnPcs **2**, **3** and **4** with 95 %, 91 and 90 % yields, respectively. All the products were characterized by absorption, fluorescence, FAB-MS and ^{1}HNMR spectra.



Scheme 1. Synthesis of ZnPn **1-4**.

Spectroscopic studies

Absorption spectroscopy. The absorption spectra of ZnPc **2-4** were analyzed in n-heptane/AOT (0.1 M) varying the amount of water dispersed in the reverse micelles (W_0 = [H_2O]/[AOT]). The effect of changing W_0 keeping AOT concentration for ZnPc **2** is shown in Figure 1A. Similar behavior was found for ZnPc **3** and **4**. As can be observed, ZnPc **2** is not soluble in AOT system at low W_0. However, solubility of phthalocyanine takes place when the amount of dispersed water increases in the micelles. The spectra show the typical *Soret* and *Q*-bands, characteristic of zinc phthalocyanines [7]. The two close maxima in the *Q*-band region reflects that these compounds are a statistical mixture of regioisomers produced by the synthetic method [8,9]. The effect of W_0 upon ZnPc **2** solubility in AOT micelles is illustrated in Figure 1B. As can be seen, only small spectroscopic change is obtained upon $W_0 > 30$.

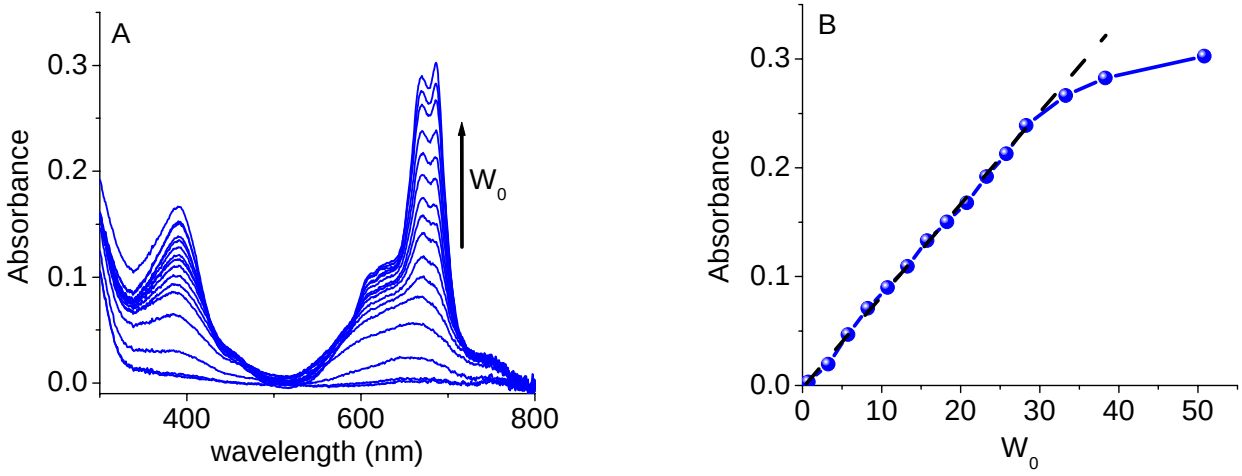


Figure 1. (A) Absorption spectra of ZnPc **2** in n-heptane/AOT (0.1 M) at different W_0; (B) variation of absorbance at 686 nm with W_0, [ZnPc **2**] = 2.7 μM.

Fluorescence spectroscopy. The steady-state fluorescence emission spectra of ZnPc **2-4** were studied in n-heptane/AOT (0.1 M) varying W_0. As showed above for absorption spectroscopy, the fluorescence intensity increase with the amount of water dispersed in the reverse micelles. Representative results are observed in Figure 2A for ZnPc **2**. The emission spectra show a typical shape for phthalocyanines with a peak at ~ 692 nm [7,8]. As can be observed in Figure 2B, only small changes in the intensity are found upon $W_0 > 30$.

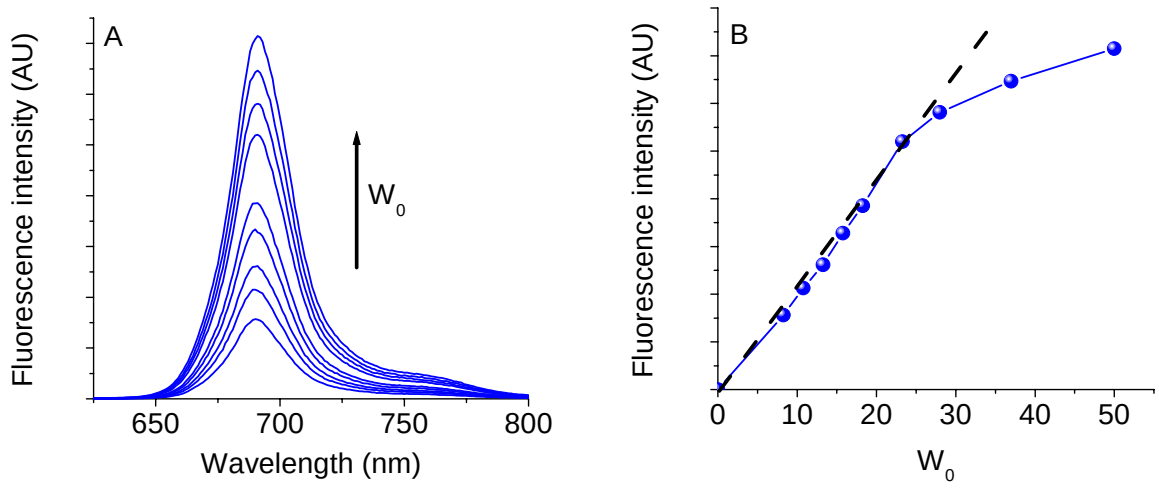


Figure 2. (A) Fluorescence emission spectra of ZnPc **2** in n-heptane/AOT (0.1 M) at different W_0 (λ_{exc}=615 nm); (B) variation of fluorescence intensity at 692 nm with W_0, [ZnPc **2**] = 2.7 μM.

Binding constants of phthalocyanines to n-heptane/AOT reverse micelles. When the absorption spectra of ZnPc **2-4** were studied varying AOT concentration, an increase in the intensity of the *Soret* and *Q*-bands was observed as the [AOT] increases. Typical results are shown in Figure 3A for ZnPc **3**. This effect can be attributed to the interaction between the phthalocyanine and the micelle [6]. The strength of the association between phthalocyanine and AOT was determined through the binding constant, $K_b=[ZnPc_b]/[ZnPc_f][AOT]$ (where the terms $[ZnPc_b]$ and $[ZnPc_f]$ refer to the concentration of bound and free phthalocyanine, respectively, and [AOT] is the total surfactant concentration). Thus, the spectral changes were analyzed using the Ketelaar´s Equation 1 [6]:

$$\frac{1}{A - A_{Hp}} = \frac{1}{(\varepsilon_b - \varepsilon_{Hp})[ZnPc]_0} + \frac{1}{(\varepsilon_b - \varepsilon_{Hp})[ZnPc]_0 K_b[AOT]} \qquad (1)$$

where $[ZnPc]_0$ is the total concentration of the porphyrin, A is the absorbance at different [AOT], A_{Hp} is the absorbance in *n*-heptane, ε_b and ε_{Hp} are the molar absorptivity for the phthalocyanine bound to the interface and in the organic medium, respectively. Plotting the left-hand side term of Equation 1 vs. 1/[AOT], the value of K_b can be calculated from the slope and the intercept as it is shown in Figure 3B for ZnPc **3**. The values of K_b are shown in Table 1. Similar behavior was also obtained from fluorescence emission studies.

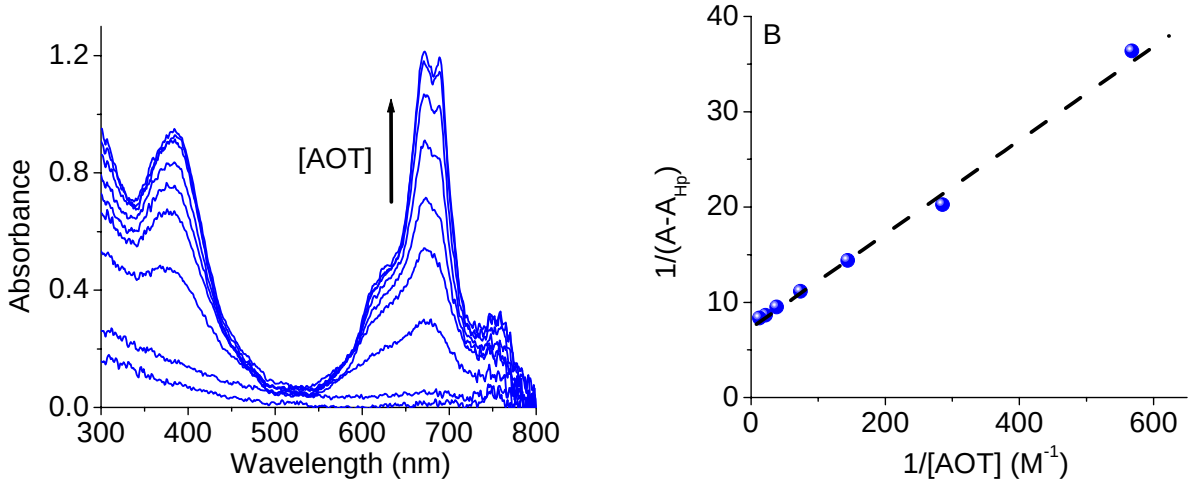


Figure 3. (A) Absorption spectra of ZnPc **3** in n-heptane/AOT/W_0=30 at different AOT concentration; (B) variation of $1/(A-A_{Hp})$ vs AOT concentration for ZnPc **3**. Dotted line: linear regression fit by Equation 1, λ_{max}= 674 nm.

Table 1. Binding constants (K_b), kinetic parameters (k_{obs}) and quantum yield of $O_2(^1\Delta_g)$ production (Φ_Δ) of phthalocyanines in n-heptane/AOT (0.1 M)/W_0=30)

Phthalocyanine	K_b (M^{-1})	k_{obs}^{DMA} (s^{-1})	Φ_Δ[a]
ZnPc **2**	283±10	(8.3±0.4) x 10^{-4}	0.55±0.05
ZnPc **3**	150±6	(6.3±0.4) x 10^{-4}	0.42±0.40
ZnPc **4**	34±4	(6.2±0.4) x 10^{-4}	0.41±0.40

[a]Ref. 10 Φ_Δ (TTAP) = 0.73

Photodynamic activity

Photooxidation of 9,10-dimethylanthracene (DMA). The aerobic irradiations with monochromatic light (λ=674 nm) of photosensitizers in n-heptane/AOT(0.1 M)/W_0=30 were performed in the presence of 9,10-dimethylanthracene (DMA). This substrate quenches $O_2(^1\Delta_g)$ by exclusively chemical reaction [3]. Therefore, it was used in this work to evaluate the ability of the sensitizers to produce $O_2(^1\Delta_g)$. A time-dependent decrease in the DMA concentration was observed by following a decrease in its absorbance. From first-order kinetic plots the values of the observed rate constant (k_{obs}^{DMA}) were calculate for DMA (Table 1). The quantum yield of $O_2(^1\Delta_g)$ production (Φ_Δ) was calculated by direct comparison of the k_{obs}^{DMA} values using 5,10,15,20-tetrakis(4-trimethylamoniumphenyl)porphyrin (TTAP) as reference, k_{obs}^{DMA}=(1.1±0.1)x10^{-3} s^{-1}, Φ_Δ (TTAP) = 0.73 [10] (Table 1). Under these

conditions, the photodynamic efficiency for these phthalocyanines follow the order: ZnPc **2** > ZnPc **3** ~ ZnPc **4**.

Conclusions

Tetracationic ZnPc **2-4** were conveniently synthesized by alkylation of ZnPc **1**, which was obtained from the condensation of phthalonitrile derivative in presence of DBU and Zn(II) acetate.

The spectroscopic absorption and fluorescence studies indicate that the solubility of ZnPc **2-4** in AOT reverse micelles increases with the amount of water dispersed in the micellar system. Thus, at low W_0 these ZnPcs show a broadening band at ~650 nm, indicating that they are mainly aggregated, as it is typical for many phthalocyanine derivatives [6,7]. However, a sharp absorption band was obtained in AOT micelles at $W_0 > 30$. Under these conditions, sensitizers **2-4** are mainly solubilized as monomers in the micellar pseudophase indicating that there is not aggregation of these ZnPcs in the systems. Thus, AOT micelles at $W_0 = 30$ was used as biomimetic medium to evaluate the photodynamic activity of these sensitizers.

The interaction between the sensitizers and micellar systems are also evidenced varying AOT concentration at $W_0 = 30$. The values of binding constants (K_b) diminish with the increase in the length of hydrocarbon chains. Thus, lower values were found for ZnPc **3** and **4**, which present a higher lipophilic character with respect to ZnPc **2**.

As can be observed in Table 1, a higher efficiency in the $O_2(^1\Delta_g)$ production was found for ZnPc **2** with respect to ZnPc **3** and **4** in AOT micelles. However, the values of Φ_Δ can significantly change in a different medium, diminishing when the sensitizer is partially aggregated. Also, the biological microenvironment of the sensitizer can induce important modifications in the photophysics of the porphyrin established in solution [11]. In consequence, there are limitations to predict photodynamic efficiencies of sensitizers in biological systems on the basic of photophysical investigations in solution.

These studies show that the cationic ZnPc **2** presents interesting properties as photosensitizer with potential applications in photodynamic therapy treatment. Further *in vitro* studies concerning the photoinactivation of microorganism are presently in progress in our laboratory.

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