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Spectroscopic analysis of aluminum chloride phthalocyanine in binary water/ethanol systems for the design of a new drug delivery system for photodynamic therapy cancer treatment

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ABSTRACT

This study evaluated the behavior of aluminum chloride phthalocyanine in a binary water/ethanol mixture using electronic absorption spectroscopy and static and time-resolved fluorescence spectroscopy. The electronic absorption spectra, resonance light scattering and fluorescence quenching of aluminum chloride phthalocyanine in water/ethanol mixtures were studied at several concentrations. The electronic absorption spectra and fluorescence quenching changed significantly at approximately 50% water (v/v). Below 50% water, the dimerization constant values were negative ($-2609.2\ M^{-1}$ and $-506.5\ M^{-1}$ at 30% and 40% of water, respectively), indicating that the formation of aggregates under these conditions is not favored. However, at 50% water, the dimerization constant value was estimated to be $559.7\ M^{-1}$, which indicates the presence of dimers. Above 60% water, the aggregation process was responsible for the balance between large complexes (such as trimers, tetramers or oligomers) formed in the medium under these conditions. The appearance of new absorption bands at 387 nm and 802 nm and their bathochromic shift relative to the monomer bands suggested that some J-type aggregates form. These results are relevant to understanding the behavior and use of aluminum chloride phthalocyanine in the design of new drug delivery systems for clinical application in photodynamic therapy as a new approach to treat skin cancer.

1. Introduction

The lack of selectivity of classical protocols and procedures in cancer treatments, such as radiotherapy and chemotherapy, is one of the many complications of using these therapies. Generally, both affect cancer cells and healthy tissue. Thus, the development of more selective treatments has emerged in recent decades as efficient and noninvasive methods [1,2]. Among the new treatments, Photodynamic Therapy (PDT) is unique. PDT is a method used to treat diseases related to abnormal growing tissue, *e.g.*, skin [3], oral mucosa [4], prostate and uterine cancers [5,6] and bacterial and fungal diseases [7]. This method consists of the topical or systemic application of a photosensitizing agent (PS) (normally a well-conjugated molecule, such as natural molecule or a commercial one) that can be incorporated into the cancer cells or tissue after activation with visible light of an appropriate wavelength *in situ*, which is generally between 600 nm and 750 nm and is known as the "therapeutic window."

In addition to the high selectivity, PDT causes minimal side effects and has been used for over forty years [8–12]. The photodynamic process has an even greater functionality for removing pollution [13] from primarily industrial waste *via* a controlled photooxidation process [14]. It can also be used to treat new non-oncological diseases, such as for healing wounds and burns to regenerate tissue *via* a process known as photobiomodulation [15].

The selection of the PS is the most important step in the above mentioned processes, and its physical chemistry behavior in all of the new drug delivery system (DDS) is designed for specific proposes. To be used in dynamic photoprocesses, the PS needs to exhibit some specific features, such as a strong absorption of visible light, a high quantum yield of singlet oxygen, a low yield of the natural photo-degradation reaction, a high affinity and selective penetration in the diseased organ or tissue, favorable pharmacokinetics, reproducibility and a high stability and low toxicity of the drug in the absence of visible light (used in photoactivation) [16].

Among these compounds, the second-generation family of phthalocyanine compounds includes aluminum chlorides (AlClPc). AlClPc have been commonly used in research since the last decade in most of the applications mentioned above [17–21] with successful results. AlClPc is defined as a tetrapyrrolic macrocycle with a central aluminum ion (Al^{3+}). In addition to the desired characteristics for photosensitization,

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AlClPc has the advantage of a high absorption at wavelengths greater than 650 nm (maximum wavelength = 672 nm in ethanol), which ensures its application in therapy against pigmented skin cancer when there is no other endogenous or exogenous photoactive molecule to compete with AlClPc in the light activation process. Melanin pigment, the most natural protection material in the skin, has a UV absorption range of up to 500 nm, which is far from the AlClPC absorption band. Nevertheless, phthalocyanine-forming compounds can interact with almost all metals, resulting in different systems with greater stability and different photophysical and photochemistry properties [22].

One of the most important properties of this compound is its high hydrophobicity, which promotes good interactions with biological systems. However, the hydrophobicity also promotes the natural process of self-aggregation in an aqueous medium, which is unfavorable to PDT.

This aggregation directly affects its photophysical and photosensitizing properties [23], increasing the deactivation of the excited state *via* non-radiative processes and reducing the photoprocessing efficacy. Therefore, dimers are considered inactive or extremely inefficient compared with the monomers in PDT [20,24–27]. Thus, AlClPc could not be directly applied to a target biological tissue, and there is a need for the development of a special DDS [9,20,23].

Based on several studies that show the high potential of the PC family of compounds, AlClPc have been used for the development of new drug formulations to treat skin cancer and other systemic cancers, such as prostate, bladder and uterine cancer, and tests have begun to determine its use in medical tissue regeneration.

The aim to this work was to precisely understand the self-aggregation process in a water/ethanol system, which has not been fully described at the molecular level until now and is useful for future designs of DDS for clinical applications. This paper describes the photophysical properties and self-aggregation of AlClPc in a water/ethanol mixture to mimic the hydrophobic–hydrophilic equilibrium that exists in a DDS. These results will contribute to understanding AlClPc behavior in cells and tissue and how this affects its photodynamic properties and mechanism of action in PDT.

2. Materials and methods

The AlClPc (85% purity) was purchased from Sigma-Aldrich and used without further purification. Ultrapure water (Milli-Q) was used in all of the experiments, and anhydrous ethanol was purchased from J.T. Becker® as analytical grade. The stock solution was prepared at 0.7 mmol L^{-1} in anhydrous ethanol and was sealed and stored in a refrigerator at 4 (±2) °C. The electronic absorption spectra were obtained using a UV/Visible Ultrospec 2100 pro spectrophotometer from 300 to 800 nm. For the fluorescence measurements, a Hitachi F-4500 spectrofluorometer with a fixed excitation wavelength of 610 nm and an emission from 650 to 800 nm was used. Resonance Light Scattering (RLS) was performed with the same spectrofluorometer using the synchronous mode with $\Delta\lambda = 0$ nm from 300 to 800 nm.

The fluorescence lifetimes were measured using a Microtime 200 (PicoQuant) based on the method of Time-Correlated Single Photon Counting (TCSPC) in the unique Time-Tagged Time Resolved (TTTr) measurement mode. A picosecond diode laser with an excitation wavelength of 640 nm and a repetition rate of 80 MHz was used as the excitation source. The experimental decay fit was performed using SymPhoTime® software. The electronic absorption and fluorescence measurements were conducted with a 1-cm path length quartz cell. All experiments were performed at 28 (±1) °C, but the fluorescence lifetimes were performed at 24 (±2) °C.

2.1. Molar absorption coefficient of AlClPc in ethanol

The molar absorption coefficient (ε) of AlClPc in ethanol was determined *via* spectrophotometric titration in anhydrous ethanol to produce different concentrations of AlClPc from 7.4 cmol L^{-1} to 6.5 μmol L^{-1}. From the stock solution of AlClPc in ethanol, aliquots were transferred to an optical cuvette containing 2.00 mL of anhydrous ethanol, and after each addition, the respective spectra were measured.

2.2. Effect of water on AlClPc aggregation in the water/ethanol mixture

To assess the physio-chemical behavior of AlClPc in the homogeneous medium, we initially prepared various water/ethanol mixtures with water percentages from 0 to 100%. AlClPc aliquots of an AlClPc stock in ethanol were added to the solutions to achieve a concentration of 3.0 μmol L^{-1}. After preparation, the solutions were homogenized in an ultrasonic bath at 60 Hz power for 5 min, followed by the electronic absorption and static/time-resolved fluorescence measurements after 24 h in the dark.

2.3. Static and time-resolved fluorescence quenching

We studied the static and time-resolved fluorescence quenching of AlClPc in ethanol using water molecules. The AlClPc concentration was 30 nmol L^{-1} (absorption below 0.1) to avoid an inner filter effect [28,29]. The data were treated with Eqs. (1) and (2), respectively,

$$F_0/F = 1 + K_{sv}[Q] \quad (1)$$

$$\tau_0/\tau = 1 + k_q \tau_0 [Q], \quad (2)$$

where F_0 and F are the fluorescence intensities in the absence and in the presence of the suppressor, respectively, [Q] is the water concentration, τ_0 and τ are the fluorescence lifetimes in the absence and presence of water, respectively, and k_q is the constant rate for the collisional quenching process.

2.4. AlClPc dimerization constant in the water/ethanol mixtures

The dimerization constants (K_d) of AlClPc in the water/ethanol mixtures were evaluated using electronic absorption spectroscopy. AlClPc solutions were prepared at concentrations of 1.5, 2.5, 3.5, 4.5, 5.5, and 6.5 μmol L^{-1} in a water/ethanol mixture of 30, 50, 60 and 70% water. The absorption intensity values at 350 nm as a function of AlClPc concentration were adjusted using Eq. (3)

$$A_M = (1 + 8K_d[Pc]^{0.5} - 1\varepsilon_M/(4K_d), \quad (3)$$

where A_M is the absorbance of the monomer, K_d is the dimerization constant, [P_c] is the concentration of aluminum chloride phthalocyanine and ε_M is the molar absorptivity of the monomer [30].

3. Results

The AlClPc in ethanol had two main electronic absorption bands that are related to its monomeric form (Fig. 1A, continuous line). The more intense band with a maximum at 672 nm (band Q_I) is attributed to the electronic transition from the ground state to the first excited electronic state (S0 → S1), whereas the band at approximately 350 nm, known as the B band (or Soret band), is related to the electronic transition from the ground state to the second excited electronic state (S0 → S2) [31,32].

Furthermore, two bands of lower intensity at approximately 607 nm (Q_{III}) and 642 nm (Q_{II}) are related to vibronic transitions from $S_0 \rightarrow S_1$ excitation [28,31]. The maximum absorption values of the Q_I band exhibited Lambert–Beer law deviations along the X axis (Fig. 1B) above 1.5 μmol L^{-1} (A = 0.41). It is well known that both the self-assembly of phthalocyanines and the inner filter effect can cause a deviation in the absorption spectrum [31,28].