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ARTICLE TYPE

Untangling interactions of a Zinc(II) complex containing a coumarin-porphyrin unit with alkaloids in water solutions: A Photophysical study

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A metal complex **1** derivative from a coumarin bearing a porphyrin unit was spectroscopically characterized and its sensing ability towards the alkaloids caffeine **2**, nicotine **3** and cotinine **4** was evaluated in these studies. This probe shows to be sensitive to the alkaloids studied, whereas the detectable amount of 2.5±0.3 μM of cotinine was determined in dam water from the Vigia Dam located in the region of Montoito village, Alentejo district, Portugal. The interaction of **1** with cotinine was also verified by MALDI-TOF-MS, whereas it was found peaks at 877.2 and 1053.3 *m/z* corresponding to the species $[\mathbf{1}H]^+$ and $[\mathbf{1}CotinineH]^+$, respectively.

Introduction

Currently, the design and synthesis of systems that are capable of sensing various alkaloids are of major interest. These natural organic compounds contain basic nitrogen atoms and are generally secondary plant metabolites with a considerable interest in medicine and society [1].

Actually due to their pharmacological effects, alkaloids are used as antibacterial (berberine), vasodilators (papaverine) [2], anaesthetics (morphine, atropine) [3], antimalarial (quinine) [4], and as stimulants (nicotine, caffeine) [5] or psychoactive (cocaine) drugs [6]. Additionally, they also have attracted interest in synthesis and catalysis [7].

Concerning the properties aforementioned, during the last years, several analytical procedures have been developed for alkaloids determination. These include high performance liquid chromatography (HPLC) [8], gas chromatography (GC) [9], capillary electrophoresis (CE) [10], and radioimmunoassay [11]. All these procedures offer good detection sensitivity, but suffer from certain disadvantages. For example, the GC, CE, and HPLC procedures exhibit low selectivity and require costly equipment, and trained operators [12].

Regarding this issue, fluorescent probes appear as a new technique in the selective detection of alkaloids [13]. Among all fluorescent chemosensors, porphyrins and analogues are good candidates, since they can be excited with light in the visible region of the spectrum, have high fluorescence quantum yields, large Stokes shift and have many different established synthetic procedures of functionalization [14]. Recently we have evaluated the sensorial response of different porphyrins [15] and conjugated porphyrins towards different anions or metal ions [16]. During this study we verified that the inclusion of the coumarin nucleus in a porphyrin framework, **1,** can enhance the fluorescence emission and furnish better solubility in aqueous medium. Following our interest, on the study of new water soluble fluorescent chemosensors, herein we present the sensorial ability of the probe **1** towards the alkaloids caffeine **2**, nicotine **3** and cotinine **4** (Figure 1).

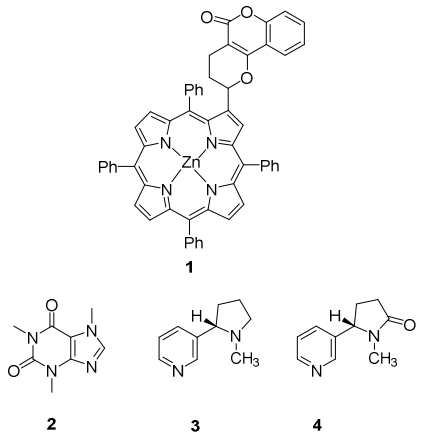

Figure 1. Structural formula of {2-(5-oxo-2,3,4,5-tetrahydro-2*H*-pyrano[3,2-*c*]chromen-2-yl)-5,10,15,20tetraphenylporphyrinato}zinc(II) **1** , Caffeine **2**, Nicotine **3**, and Cotinine **4**.

Among all alkaloids, caffeine **2** and nicotine **3** are the most attractive targets because of their important pharmacological effects. Caffeine **2** is a natural constituent of tea, coffee, guarana paste and cacao [17]. This alkaloid is also a component of pharmacological preparations including analgesics (where

caffeine acts as an adjuvant) and remedies for weight loss [18]. Nicotine **3** is a dinitrogen alkaloid present in tobacco [19]; its excessive consumption can lead to a great variety of negative physiological effects, like pulmonary cancer and cardiovascular diseases [20]. However, it is also known that this alkaloid has some therapeutic effects in neurodegenerative diseases, like those of Alzheimer and Parkinson [21]. Concerning **3**, Han et al. [22] reported a "turn on" system based on 2-(4-pyridyl)imino nitroxide (A) and *meso*-tetraphenylporphyrinatozinc(II) (B) stable complex, in which the addition of nicotine displaced the compound (A) leading to an increased in the fluorescence signal. Cotinine **4** is the principal metabolite of Nicotine. Regarding this aspect, Deviprasad [23], designed a porphyrin fluorescent chemosensor for selective detection of nicotine and cotinine in solution. Their binding mechanism involves the axial coordination of pyridine unit of nicotine into the zinc ion porphyrin cavity. Additionally, Noworyta et al. [24] published two zinc porphyrins, the 5-(2-phenoxyacetamide)-10,15,20-tris(triphenylamino)porphyrinatozinc(II) and 5-(2,5-phenylenebis(oxy)diacetamide)-10,15,20-tris(triphenylamino) porphyrinatozinc(II) with receptor sites able to recognise selectively nicotine and cotinine. The alkaloids analytes were determined at the concentration level of 0.1 mM.

Experimental Section

Chemicals. Caffeine, (-)-Nicotine and (-)-Cotinine were purchased from Sigma-Aldrich. All these chemicals were used without further purification. The solvents were obtained from Panreac and Riedel-de Haen and used as received.

*Spectrophotometric and spectrofluorimetric measurements of conjugate **1**.* Absorption spectra were recorded on JASCO V-650 spectrophotometer and fluorescence emissions were performed on a Spectrofluorimeter HORIBA-JOBIN-YVON Fluoromax 4. The linearity of the fluorescence emission vs. concentration was checked in the concentration range used (10^{-4} to 10^{-6} M). A correction for the absorbed light was performed in the case of absorbance (A) at the excitation wavelength higher than 0.2 [25]. The spectroscopic characterizations and titrations were performed by appropriate dilution of the stock solution (*ca*. 10^{-3} M) up to 10^{-5} to 10^{-6} M of complex **1** in absolute ethanol.

Titrations of complex **1** were carried out by the addition of microliter amounts of standard solutions of alkaloids in EtOH:H_2O (50:50). All the measurements were performed at 298 K.

The detection limit was determined according with procedure reported in supporting information.

The unknown amount detectable for the analytes in real samples, dam water, was measured by a standard addition method [26].

MALDI-TOF-MS titrations. The MALDI-MS analyses have been performed with a MALDI-TOF-TOF-MS model Ultraflex II Bruker, Germany, equipped with a nitrogen laser, from the BIOSCOPE group. Each spectrum represents accumulations of 5 × 50 laser shots. The reflection mode was used. The ion source and flight tube pressure were less than 1.80×10^{-7} and 5.60×10^{-8} Torr, respectively. The MALDI mass spectra of the soluble samples (1 mg mL^{-1}) were recorded using the conventional sample preparation method for MALDI-MS. 1 µL with 1, 2 and 10 equivalents of the cotinine alkaloid was put on the sample holder on which the complex **1** had been previously spotted. The sample holder was inserted in the ion source. Chemical reaction between probe **1** and the alkaloid occurred in the holder and complexed species were produced. The samples were prepared in ethanol.

Synthesis of organic ligands. Conjugate **1** was synthesized according to the procedure described within the literature [16], [27]. The molecular integrity of the complex **1** was established from FAB mass, elemental analysis and ^{1}H NMR studies.

Results and Discussion

The photophysical characterization of complex **1** in ethanol was performed at 298 K and the data are gathered in table 1.

Table 1. Photophysical data of complex **1** in absolute ethanol at 298 K. The values correspond to single measurements. The fluorescence quantum yield value corresponds to an average of three independent experiments (n=3).

Compds.	λ_{max}(nm) : log ε	λ_{em} (nm)	Stoke's shift (nm)	Φ
1	427: 5.23; 555: 3.95; 590 3.21	599,647	9	0.020 ±0.005 (n = 3)

Figure 2 shows the absorption (only Q band region), excitation and emission spectra, in absolute ethanol of complex **1.**

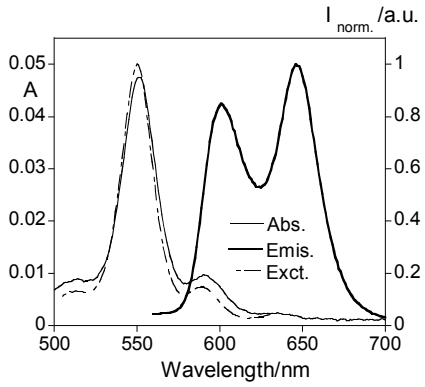

Figure 2. Absorption, normalized emission and excitation spectra of porphyrin **1** in absolute ethanol, ([**1**] = 5.0×10^{-6} M, λ_{exc}= 555 nm, λ_{emiss} = 647 nm).

The absorption spectrum of the metalloporphyrin conjugate **1**, showed two weak Q bands at *ca*. 555 and 590 nm and the perfect match between the absorption and the excitation spectra rules out the presence of any emissive impurity. The fluorescence emission spectrum of zinc porphyrin **1** presents two bands centered at 599

and 647 nm, which are characteristic of metalloporphyrin derivatives.

The sensing ability of derivative **1** towards the alkaloids caffeine **2**, nicotine **3** and cotinine **4** was investigated by titration of the complex, dissolved in absolute ethanol, with the addition of small amounts of the alkaloids **2**, **3** and **4** dissolved in ethanol:water (50:50). The experiments, monitored by UV/Vis and fluorescence emission spectroscopy at room temperature, showed with all alkaloids spectral changes in the absorption and emission spectra. Concerning the interaction of probe **1** with alkaloid **2**, the absorption spectra show a small red-shift of the Soret band from 426 nm to 429 nm ($\Delta\lambda$ = 3 nm), that it is accompanied by a decrease in its intensity at 426 nm, and an increase at 429 nm.

A similar behavior was observed in the Q-band region, whereas a red-shift from 555 nm to 573 nm ($\Delta\lambda$ = 18 nm) is accompanied by a decrease in the intensity at 555 nm and an increase of the new band at 573 nm (see Fig. 3A). A new band also appears at 600 nm.

Considering the emission spectrum of **1** (Fig. 3B), the addition of caffeine was responsible for a red-shift from 599 to 608 nm and from 647 to 659 nm followed by an enhancement in the emission intensity of *ca.* 40% at 608 nm and a quenching, *ca.* 10%, at 659 nm.

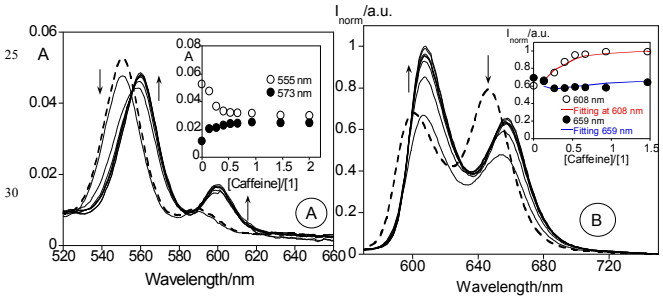

Figure 3. Spectrophotometric (A) and spectrofluorimetric (B) titrations of complex **1** with the addition of caffeine in EtOH:H_2O (50:50). The insets represent the absorption (A) and the emission intensity (B) as a function of [caffeine]/[**1**] at 555 nm and 573 nm for (A), and 608 nm and 659 nm for (B); the solid lines represent the fit to obtained using HypSpec. ([**1**] = 5 $\times 10^{-6}$M, λ_{exc}= 555 nm, T=298 K).

The studies performed with nicotine, showed that upon its addition to conjugate **1** the spectral changes were similar to the ones described for caffeine (data not shown).

Considering the addition of cotinine, the spectral changes observed in the absorption spectra of **1** were similar to the ones visualized for caffeine and nicotine. However, in the emission spectra a quenching in the emission intensity was detected. In the emission spectra of **1** (Fig. 4A), the titration with cotinine was responsible for a decrease in the emission intensity of *ca.* 40% at 598 nm and also at 646 nm (*ca.* 60%), followed by a red-shift from 599 to 606 nm and from 646 to 656 nm.

The association constants for the interaction of alkaloids **2**, **3** and **4** with probe **1** were determined using the HypSpec [28] program and values of Log $K_{assCaffeine}$= 5.38 ± 0.03 (n =3), Log $K_{ass.Nicotine}$= 5.87 ± 0.02 (n=3) and Log $K_{ass.Cotinine}$= 5.57 ± 0.01 (n =3) with a stoichiometry of 1:1 were obtained. A stoichiometry of one ligand per alkaloid was postulated, and was further confirmed by Job's plot method (see figure SI1) [29]. Nevertheless, the association constants for the three alkaloids are in the same order and with the same stoichiometry suggesting that the interaction between the alkaloids and probe **1** is of the same type. Most likely, the interaction involves the coordination of purine (**2**) or pyridine (**3** or **4**) nitrogen atoms and the zinc(II) metal ion in the porphyrin core as it is suggested in figure 5C.

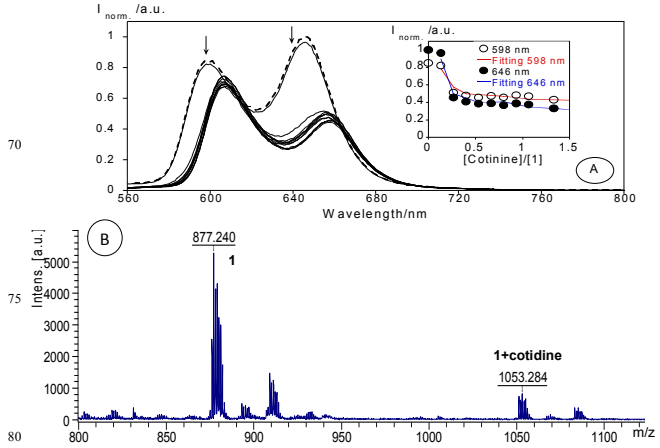

Figure 4. (A) Spectrofluorimetric titration of probe **1** upon addition of the alkaloid cotinine in absolute ethanol at room temperature. The inset represents the emission intensity (A) as a function of [caffeine]/[**1**] at 598 and 646 nm; the solid lines represent the fit to obtained using HypSpec. ([**1**] = 5 $\times 10^{-6}$M, λ_{exc}= 555 nm, T=298 K). (B) MALDI-TOF-MS spectra of complex **1** and **1**+cotinine, without matrix.

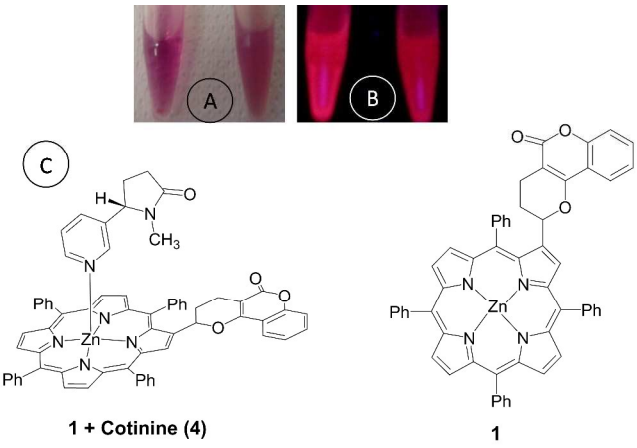

Figure 5. (A) and (B) Naked eye picture and under a UV lamp (λ_{exc} = 365 nm) of **1** (left) and **1**+cotinine (right). (C) Hypothetical coordination structure of the cotinine as an example of the alkaloids studied and probe **1**.

The coordination of cotinine to probe **1** was also confirmed by MALDI-TOF-MS (figure 4B) where it was found peaks with *m/z* at 877.2 and 1053.3 corresponding to the species $[\mathbf{1}H]^+$ and $[\mathbf{1}CotinineH]^+$, respectively.

In order to evaluate the potential of probe **1** for caffeine and cotinine, the minimal detectable amount (LOD) was determined in etanol:water (50:50), whereas values of 0.021±0.002 at 567 nm, and of 1.05±0.04 at 646 nm were determined for absorption and emission, respectively. Then, the minimal detectable amount of cotinine was 1.0±0.1 µM (absorption) and of 0.7±0.1 µM (emission). Concerning the caffeine the minimal detectable

amount was of 1.7±0.1 μM (absorption) and 1.0±0.1 μM (emission).

In addition, being cotinine the principal metabolite of nicotine and appearing as an important contaminant in water, the ability of probe **1** to detect cotinine was tested in several Portuguese dam samples. The dam water comes from the Vigia Dam located in the region of Montoito village, Alentejo district, Portugal. This experiment was carried out by the standard addition method (see SI). The detectable amount obtained by emission spectroscopy for cotinine in dam water was 2.5 ±0.3 μM.

Comparing the present results to those of the literature, the probes published by Noworyta et al. [24] (0.1 mM) were able to recognise selectively nicotine and cotinine, but the minimal detectable amount was much higher than the one obtained in our system. Moreover, we proved that our system can work with dam water samples, detecting small amounts of the cotinine alkaloid, as well.

Conclusions

The complex porphyrin-coumarin (**1**) reveals to be a potential probe towards, the alkaloids caffeine, nicotine and cotinine, with a stoichiometry of one ligand *per* alkaloid. Probe **1** is able to detect the amount of 2.5 ±0.3 μM in dam water. Value lower than the ones reported until now, thereby increasing the possibilities of application of probe **1**. Additionally, this conjugate was able to detect cotinine by MALDI-TOF-MS spectrometry.

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Untangling interactions of a Zinc(II) complex containing a coumarin-porphyrin unit with alkaloids in water solutions: A Photophysical study

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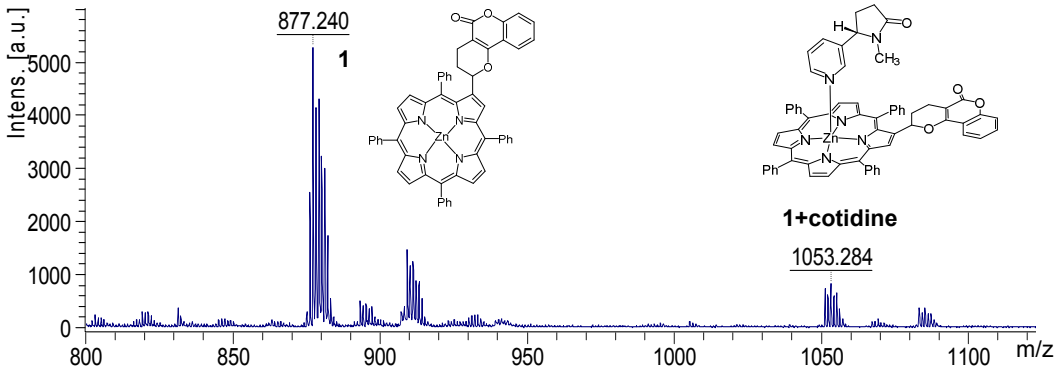
Graphical Abstract

Contents List

A metal complex **1** derivative from a coumarin bearing a porphyrin unit is sensitive to the alkaloids caffeine **2**, nicotine **3** and cotinine **4** in solution and/or in gas phase.



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Determination of the detection (LOD) limit. Ten different measurements of a solution containing the selected probe were collected, without addition of any alkaloid. For these values, the LOD was determined by the formula:

$Y_{dl} = y_{blank} + 3std$ where y_{dl} = signal detection limit and std =standard deviation

Additionally to a solution containing complex **1,** small amounts of the alkaloids were added in order to determine the minimal detectable amount out of the LOD value.

Determination of the amount detectable in real samples by the Standard Addition Method. In the standard addition method, known quantities of the alkaloid are added to a solution of dam water containing the complex **1**. This method requires a linear response to analyte. A linear regression is ploted, and when y=0, the value of the unknown concentration of the alkaloid is obtained.

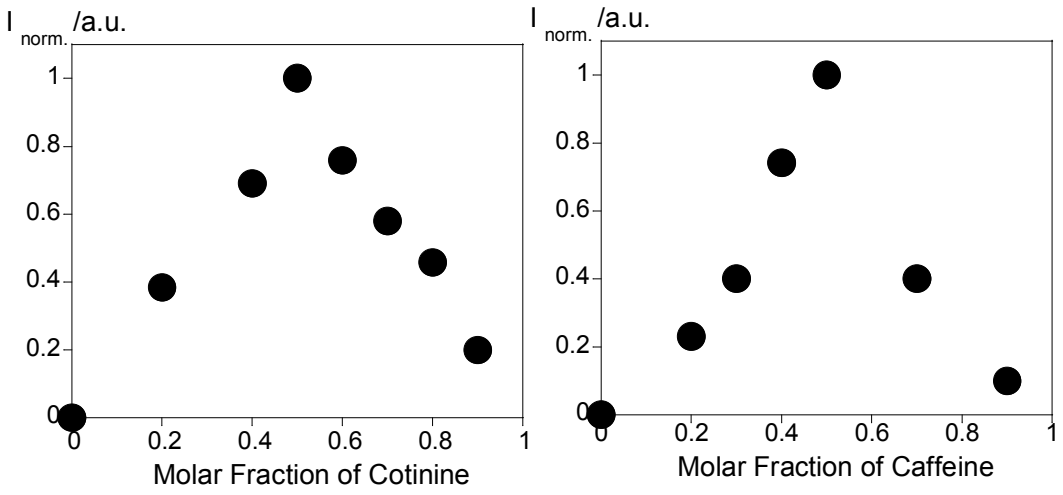

Figure SI1 – Job's plot of complex **1** upon addition of cotinine and caffeine in EtOH:H_2O (50:50), (λ_{exc} = 608 nm).

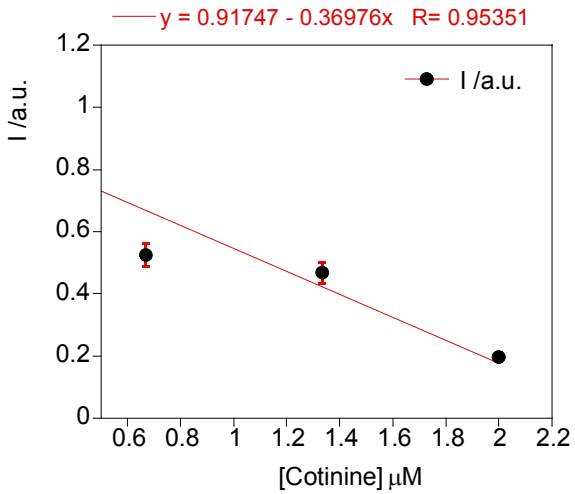

Figure SI2 – Standard addition method by emission of complex **1** upon the increasing addition of cotinine in dam water from the Vigia Dam located in the region of Montoito village, Alentejo district, Portugal ([**1**] = 5 ×10^{-6}M, λ_{exc}= 555 nm, T=298 K). Relative standard deviation (RSD) of the values was below 15 %, n=3.