Spectral Evaluation of Some Unsymmetrical Mesoporphyrinic Compounds in Interaction with Micellar Media

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The spectral behaviour and aggregation ability of new unsymmetrical mesoporphyrinic compounds in interaction with micellar media were evaluated by UV-Vis and fluorescence spectroscopy. Study was carried out in order to understand the mechanism of interaction of new porphyrins with nanostructures such as micelles, considered to be simplified cell membrane model. Also, the obtained results will allow continuation of studies regarding the pharmaceutical formulations of these new synthesized porphyrins. The porphyrinic solutions were prepared using tert-octylphenoxypolyethoxyethanol (Triton X-100), methanol, cyclohexane and polyethylene glycol 300. The experimental data have shown small changes of the spectral characteristics and the absence of molecular associations in interaction porphyrinic compounds with Triton X-100 micellar solution.

**Keywords:** unsymmetrical porphyrinic compounds, Triton X-100, spectroscopy

Porphyrins represent a class of macrocyclic compounds with important roles in many biological processes and with numerous biomedical applications which include photodiagnosis and photodynamic therapy of malignant tumors, photoinactivation of viruses and bacteria [1-11].

The broad spectrum of biomedical applications of porphyrins is due to their photophysical profile described by a series of characteristics such as: absorption coefficients in the spectral range 600–680nm, emissions in the spectral range 600-800nm, large Stokes shift, high fluorescence lifetime, great ability for the generation of singlet oxygen [4, 5].

Also, it is known that biomedical efficiency of the porphyrinic structures depends on their localization at the cellular level, directly influenced by their architectural profile, respectively by the hydrophobic and hydrophilic groups placed in various proportions and positions in the peripheral area of the porphyrin macrocycle [12-14].

Therefore, in our researches we aimed obtaining porphyrinic compounds with appropriate photophysical properties and with various degrees of hydrophobic/hydrophilic substitutions that allow them a good localization at the cellular target [15-21].

However, due to their large π conjugate systems, the porphyrinic compounds have a disadvantage that can form molecular aggregates in biological medium with decrease ability for intracellular localization and reduce the efficiency to form reactive oxygen species.

For this reason, before the pharmaceutical formulation of the same tetrapyrrolic structure, is required a rigorous evaluation of their spectral behaviour and aggregation ability in membrane mimetic environments.

Among the most simple models of biological membranes are included micelles because they are an spherical or spheroidal shape with a significant flexibility and thermodynamic stability. In addition, the presence of polar groups and hydrophobic chains in their structure allows assessing affinity of a porphyrinic molecules to cell membrane [22-28].

In order to better understand a possible localization at the cellular level, in this study a series of new unsymmetrical porphyrins, 5-(2-hydroxyphenyl)-10,15,20-tris-(4-acetoxy-3-methoxyphenyl)-21,23-H porphine (TMAPOHo), 5-(3-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)-21,23-H porphine (TMAPOHm), Zn(II)-5-(2-hydroxyphenyl)-10,15,20-tris-(4-acetoxy-3-methoxyphenyl)porphyrin (Zn(II)TMAPOHo), Cu(II)-5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)porphyrin (Cu(II)TMAPOHo), Zn(II)-5-(3-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)porphyrin (Zn(II)TMAPOHm), Cu(II)-5-(3-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)porphyrin (Cu(II)TMAPOHm), were spectrally evaluated by UV-Vis and fluorescence spectroscopy.

**Fig. 1. Molecular structures of the studied porphyrins**

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Experimental part
Materials and methods

The porphyrinic compounds used in this study were synthesized as previously described [29, 30]. Poly (oxyethylene) tert-octyphenyl ether (Triton X-100, purity > 99%), methanol (HPLC gradient grade), cyclohexane (analytical grade), dichloromethane (analytical grade) and polyethylene glycol 300 were bought from Sigma and used without supplementary preparation before. Water was double distilled and deionized before use.

Molecular absorption spectra were recorded on a Lambda 35 Perkin-Elmer UV-Vis spectrophotometer in 10 mm path length quartz cells, in single beam mode. Fluorescence spectra were recorded on a steady-state Jasco FP 6500 spectrofluorimeter in 10 mm path length quartz cells.

The solutions used were prepared by repeated dilution to obtain a final 2.5x10^{-6}M concentration of each compound in the solvents.

The 0.24 mM TX-100 in water (w/w) direct micelles and 0.66 M TX-100 in cyclohexane (w/w) reverse micelles loaded with 2.5x10^{-6}M porphyrinic complexes were prepared according to the procedure described in reference [31]. Appropriate volumes of complexes in dichloromethane solutions were evaporated to dryness at room temperature on the bottom of a test tube. Aliquots of 3 mL of appropriate concentrations of Triton X-100 in water and cyclohexane were added, and then the tubes were mildly vortex mixed for 5 min, capped and then left still overnight to ensure the solubilization and diffusion of the metalloporphyrins into the micelles. The final concentration of each porphyrinic complex in micellar media was set at 2.5x10^{-6}M; the solutions were kept in dark to prevent photodegradation before the measurements, which were performed 24 h after preparation.

Results and discussions
UV-Vis spectroscopy

It is well known that profile of the UV-Vis spectra of porphyrinic compounds is influenced by pH value and environment polarity, their ability to aggregate as well as by the nature of the metallic ion in the case of metalloporphyrins [32, 33].

The analysis of the UV-Vis spectral data confirm the presence of the porphyrinic structures in all studied systems (organic solvents and Triton-X 100 micelles) and an spectral profile of Q and Soret bands typical to the monomeric forms.

Thus, the absorption spectra of the free base porphyrins showed the typical Soret band at 417–427 nm and four Q bands between 513 and 650 nm (tables 1), which are located in the phototherapeutic window [34].

Zinc and copper porphyrinic complexes used in this study display in their molecular absorption spectra one Soret band with maximum situated in the spectral range 415–435 nm and one or two Q bands situated between 538-604 nm (tables 1). For illustration, in figure 2 is presented the absorption spectra of Cu(II)TMAPOHo in micellar solutions and reference solvents.

From the experimental data we could observe that, for metalloporphyrins the main differences in the spectral band position are due to the type of the metallic ion and less to the environmental polarity. Therefore, for zinc porphyrins, the absorption bands are bathochromically shifted (Soret bands ~10-16 nm and Q bands ~20 nm) compared to the corresponding copper porphyrins having the same ligand, in the same solvent. In agreement with Gouterman’s theory, these spectral differences are the results of conjugation effects that appear between the metallic ion electrons and the \( \pi \) electrons of the tetrpyrrolyl ring, effects more intense at copper porphyrins compared with zinc porphyrins [35, 36].

For the experimental determinations, the organic solvents were chosen according to their relative polarity, as defined by Reichard (cyclohexane - 0.0006, methanol - 0.762, polyethylene glycol - 0.790) [37]. Polyethylene glycol (PEG) has been chosen as reference because is frequently used in a variety of pharmaceutical formulation and has comparable structure with polar chains of the TX-100 molecules.

In the direct micelles TX-100 in water, for all studied porphyrins, have been recorded the bathochromic shifts of the absorption maxima compared with the PEG 300 and methanol. These spectral changes suggest a localization of the free bases porphyrins and metalloporphyrins in the polar region of the direct micelles, in the peripheral area of the polyethyleneoxide chains.

The obtained results are in agreement with literature data, which show that the transfer of porphyrins between two environments with different polarities alters their electronic state resulting in bathochromic shifts of their absorption bands [38-41].

In TX-100 in cyclohexane reverse micelles, the absorption maxima corresponding studied porphyrins are situated in the same spectral regions as PEG 300 (table 1), indicating a localization of the porphyrinic molecules in an environment that has a similar polarity to that of PEG 300, respectively inside the micelles at the oxyethylene chains level, closer to the interface between the polyethylene oxide chains and the tert-octyl-phenyl etheric group.

Therefore, it can be considered that the porphyrinic molecules are included in the interior of the micelles and they are not simply adsorbed at the periphery of the micellar surfaces.
The spectral profile of the emission bands, indicate that these porphyrins are mainly dissolved as monomer in the micellar systems and reference solvents, confirming the results obtained by the UV-Vis analysis.

In the direct micelles, Triton X-100/water, the maximum of the emission bands of the porphyrins and zincporphyrins are situated in the same spectral regions as PEG 300 and methanol, suggesting a localisation of the compounds in a polar area of the micelle.

In the reverse micellar systems, Triton X-100/cyclohexane, the fluorescence data registered for TMAPOHo, TMAPOHm and their zinc complexes confirm

**Fluorescence spectroscopy**

The fluorescence emission spectra of the free bases porphyrins and their corresponding Zn(II) complexes were performed in the same solvents and micellar systems (c=2.5x10⁻⁶ M, λ_ex = 420 nm) as those used in UV-Vis spectral analysis.

The fluorescence spectral data show for porphyrinic compounds two bands located in the spectral region of 602–660 nm and reveal very smaller shifts of the emission maxims by changing the environmental polarity (table 2, fig. 3).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>MeOH</th>
<th>Chx</th>
<th>PEG 300</th>
<th>TX/water</th>
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<tr>
<td>λ_max (nm)</td>
<td>607</td>
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In the reverse micellar systems, Triton X-100/ cyclohexane, the fluorescence data registered for TMAPOHo, TMAPOHm and their zinc complexes confirm
their localization inside the micelles around of the polyethyleneoxide chains.

Conclusions

In this study the spectral behaviour and aggregation ability of new unsymmetrical porphyrinic compounds in interaction with TX-100/water and TX-100/cyclohexane micellar systems were evaluated by UV-Vis and fluorescence spectroscopy.

On the basis of the obtained results, we can conclude that the interaction unsymmetrical porphyrins with micelles, affects weakly the absorption and emission characteristics which are responsible for photodynamic activity. Also, the experimental data confirm that the porphyrinic molecules are included in the interior of the micelles, in the area of the polyethyleneoxide chains; they are not simply adsorbed at the periphery of the micellar surfaces.

The obtained results by this study are important for the understanding of the spectral behaviour of porphyrins in interaction with cellular membrane and will allow continuation of studies regarding to the pharmaceutical formulations of these new synthesized porphyrins.

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References