Complex Combinations of Some Transition Metals with New Unsymmetrical Porphirins

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Porphyrinic complexes are applied as effective photosensitizers in diagnosis and therapy of cancer due to preferential affinity for malignant tissues and their favorable photophysical characteristics. In this study, the synthesis and spectral evaluation of new unsymmetrical porphyrinic complexes, Zn(II)-5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)porphyrin and Cu(II)-5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)porphyrin, is described. The spectral properties and aggregation ability of new porphyrinic complexes in different solvent (ethanol, isopropyl alcohol, dichloromethane, dimethylformamide, dimethyl sulfoxide) were evaluated. The obtained results have shown small changes of the spectral characteristics and the absence of molecular associations in studied systems.

Keywords: unsymmetrical metalloporphyrins, microwave synthesis, spectral studies

The biological and biomedical importance of the porphyrinic compounds have made them attractive target of study for many years. They are used in treatment of various diseases such as cancer therapy, treatment of psoriasis, viral and bacterial infections [1-19]. The therapeutic effect of porphyrins originates from their ability to preferentially accumulate in the diseased tissue and to photogenerate singlet oxygen or free radicals, species responsible for the cell inactivation. These reactive species can be produced through the interaction of porphyrinic compound with visible light and in the presence of molecular oxygen.

Due of their luminescent properties, porphyrinic compounds are also used as fluorescent markers in detection of various cancer type [1-6, 20].

Although many metalloporphyrins have demonstrated versatility for both diagnosis and therapy, their poor intracellular localization justify further researches concerning increasing their tumor selectivity thereby increasing their ability to delineate tumor cells and protect healthy cells during photodynamic therapy.

Taking into account the fact that nature substituents at the periphery of the tetrapyrrole macrocycles and the type of the metallic ion are responsible for localization at the cellular level [21, 22], in our research we synthesized metalloporphyrins with a slight amphoteric character which favours a better transfer at the cellular level, both in polar (the external environment of the cell) and nonpolar (the lipidic double layer of the cellular membrane) media [23-29].

Another factor with direct influence on biomedical efficiency of the porphyrinic compounds is their purity degree, which is highly dependent on the route of the approached synthesis.

As part of our ongoing research in the development of novel synthetic routes to the synthesis of new asymmetrical porphyrinic compounds by use of green chemistry techniques, in this paper we describe, comparatively with the classical method, a convenient route for the direct synthesis of two unsymmetrical metalloporphyrins starting from substituted benzaldehydes, pyrrole, metallic salts and under solvent free conditions. Thus, Zn(II)-5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)porphyrin (Zn(II)TMAPOHo) and Cu(II)-5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)porphyrin (Cu(II)TMAPOHo) (fig. 1) were obtained under environmental and reproducible laboratory conditions, in a short time and with good yields.

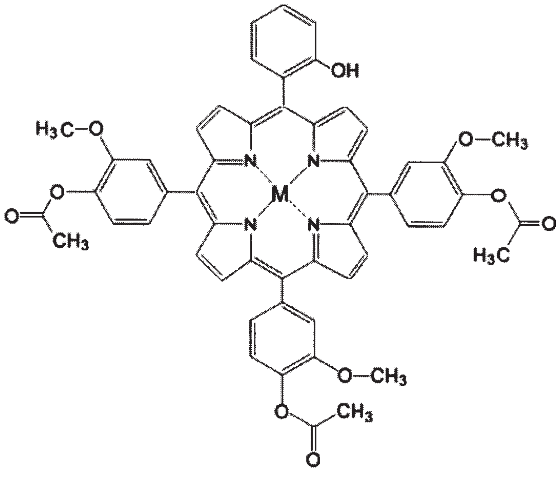

Fig. 1. General structures of M(II)-5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)porphyrin, M=Cu(II), Zn(II)

Experimental part

Materials and methods

Commercially available chemicals and solvents were used as received from Sigma-Aldrich and Merck. For the microwave assisted synthesis a domestic microwave oven with power and temperature controlled microwave oven was used. IR spectra were recorded with a FT-IR Bruker

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Tensor 27 spectrometer, in the 4,000–500 cm^{-1} spectral range. The UV-Vis spectra of the mesoporphyrinic complexes were recorded with a Lambda 35 Perkin-Elmer spectrophotometer using a 10 mm path length quartz cell, in single beam mode. Fluorescence spectra were recorded with a Jasco FP 6500 spectrofluorimeter. The metalloporphyrin solutions in different solvents (ethanol, isopropyl alcohol, dimethylformamide, dichloromethane, dimethyl sulfoxide) were freshly prepared in the spectrally pure solvents at the concentration 2.5 . 10^{-6}M and kept in dark until the measurement to prevent photodegradation.

Synthesis of porphyrinic complexes

The synthesis reactions of porphyrinic complexes was performed in a first variant by refluxing in the dichlormethane solution of a equimolar ratios of the 5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)–21,23-H porphine [32]and metalic salts, in the presence of basic catalyst 2,6-dimethylpyridine, at 60°C for 1h. The presence of the metalloporphyrin in the reaction mixture was monitored by UV–Vis spectroscopy and thin layer chromatography. After cooling the reaction mixture was purified by column chromatography using dichloromethane/diethyl ether 25:1 (v/v) as eluent and silica gel (100–200 mesh size) as stationary phase. The solutions of the porphyrinic complexes were concentrated by simple distillation. The violet crystals were dried at about 100°C for 12 h.

With a view to establish the reaction parameters that would provide obtaining the best qualitative and quantitative results, synthesis of new complexes was made by microwave irradiation under solvent free condition. For this purpose, 2-hydroxybenzaldehyde (0.01 mol), 4-acetoxy-3-methoxybenzaldehyde (0.03 mol), pyrrole (0.04 mol), anhydrous metallic salts (0.01 mol zinc acetate respectively 0.01 mol copper chloride) and 2–3 g of silica gel 60 (200–500μm, 35–70 mesh) was irradiated in a microwave oven at 500W for 10 min in the presence of 2,6-dimethylpyridine as catalyst. The complexation reaction was monitored by UV–Vis spectroscopy and for this purpose extraction of samples was performed after every 2 min of irradiation. Also, absence of chlorins (reduced forms of the porphyrins) was confirmed by monitoring the Q bands in the visible spectra recorded for extracted samples. After cooling, the reaction product was dissolved in dichloromethane/diethyl ether 25:1 (v/v) and then filtered. The solvent was removed under vacuum and the solid product was chromatographically separated on silica gel (100–200 mesh size) with dichloromethane/diethyl ether as eluent. The obtained yields were 32% for Zn(II)TMAPOHo and 35% for Cu(II)TMAPOHo. The synthesis reactions have been repeated several times with identical results, then the complexes were characterized by spectral analysis and obtaining data for porphyrins with the same metallic ion but synthesized by the two different methods described above, were identical.

Results and discussions

In order to establish the reaction parameters that favor obtaining the best quantitative and qualitative results, in this study synthesis of new complexes was made both the classical pathway and by microwave irradiation under solvent free condition.

Obtaining of metalloporphyrins by classical method involves refluxing in dichloromethane solution of equimolar ratios of the porphyrinic ligands and metal salts in the presence of a basic catalyst. Although complexation reaction is carried out with good yield, this method requires in a first step synthesis of the ligand by condensation of pyrrole and substituted benzaldehydes, in reaction condition (acidic medium and high temperature) which favours forming of chlorins (reduced forms of the porphyrins) as secondary products, therefore purification of porphyrinic compounds requires difficult chromatographic separations with decreasing total yield of reaction. The comparative analysis on both approached method in obtaining Zn(II)TMAPOHo and Cu(II)TMAPOHo revealed for microwave irradiation synthesis several advantages such as: shorter reaction times, good reaction yields, a smaller number of isomers in final reaction mixture compared with the classical procedure, absence chlorins in the final product. Furthermore microwave irradiation under solvent free condition is an ecological method for synthesis with a low environmental impact.

Infrared spectra

The infrared spectral assignments corresponding to the synthesized complexes are presented in table 1. The large band recorded at about 3,410 cm^{-1} can be assigned to the O-H stretching vibration of the –OH functional group from the structure of the copper and zinc complexes. The medium band observed in the higher wave number region (~2,922 cm^{-1}) can be assigned to C–H vibrations of the phenyl groups. The medium band which was identified at ~2,852 cm^{-1} confirms the presence of $-O-CH_3$ group in synthesized structures. Another medium IR bands are identified in the spectral range of 1,760–1,762 cm^{-1} and can be assigned to C=O stretching vibration. Also, for both

Table 1

CHARACTERISTIC IR VIBRATIONS OF THE PORPHYRINIC COMPLEXES

Characteristic vibration	**Wavenumber of the IR band (cm^{-1})**	
	Zn(II)TMAPOHo	**Cu(II)TMAPOHo**
$\nu_{O\text{-}H}$	3410 *m*	3401 *m*
$\nu_{C\text{-}H}$	2922 *m*	2922 *m*
$\nu_{C\text{-}H\ \text{from -O-CH3}}$	2853 *m*	2852 *m*
$\nu_{C=O}$	1761 *m*	1762 *m*
$\nu_{C\text{-}N}$	1595 *m*	1594 *m*
$\nu_{C=N}$	1501 *m*	1504 *m*
$\nu_{C\text{-}H\ \text{pyrrole}}$	1458 *m*	1459 *m*
$\nu_{C\text{-}O}$	1194 *s*	1196 *s*
$\delta_{C\text{-}H}$	1070 *m*	1115 *m*
$\gamma_{C\text{-}C}$	1000 *w*	1006 *w*
$\gamma_{C\text{-}N\ \text{pyrrole}}$	798 *m*	801 *m*
$\gamma_{C\text{-}H}$	719 *m*	722 *m*

The intensities of the signals are described as weak *(w)*, medium *(m)*, strong *(s)*, complexes signal at ~1,196 cm^{-1} corresponds to the C-O bond vibrations. In the infrared spectra of the Zn(II)TMAPOHo and Cu(II)TMAPOHo a band corresponding to C=N stretching vibration at 1501–1504 cm^{-1} and another at ~1595 cm^{-1} corresponding to C–N vibrations was highlighted.

Absorption and emission spectra

Absorption and fluorescence spectroscopic studies aimed on one part to the confirmation of the structure of new complexes and on the other part to the evaluating of their spectral characteristics with a view to their use in diagnosis and therapy of cancer. Also, in order to better understand the possible localization of the porphyrinic complexes at the cellular level, the studies performed in this paper have included assessment of their spectral characteristics in environments with different polarities. Absorption spectra of Zn(II)TCMPOHo and Cu(II)TCMPOHo were studied in ethanol, isopropyl alcohol, dimethyl sulfoxide, dimethyl formamide, dichloromethane for solutions containing 2.5 . 10^{-6} M of the porphyrinic complex. Absorption spectral data of two complexes are listed in table 2.

In all solvents, at a 2.5 . 10^{-6} M concentration, the obtained molecular spectra are typical of metalloporphyrins [23-30] and do not reveal associations between metalloporphyrin molecules. These display a higher intensity band in the spectral range of 415–430 nm (Soret band) and either bands of lower intensity in the visible region betwen 539–602 nm (Q bands).

The general analysis of spectral characteristics of the two porphyrinic complexes (table 2) confirmed that these new structures shows maximum absorption in the spectral range required of a good photosensitizer [1-5] and show that the main differences are determined by the solvent polarity and type of the metallic ion. Looking at the influence polarity of the solvent, in molecular absorption spectra of the both complexes are observed smaller blue shifts of the absorption maxima by increasing environmental polarity. These shifts of the spectral bands to a smaller wavelength can be assigned physical interaction which appear between polar substituent of the porphyrinic ring and solvent molecule. The comparative analysis of the spectral data obtaining for the copper and zinc complexes proves that electronic structure of the metallic ion has a direct influence on the absorption characteristics. For the same solvent, in the Zn(II)TCMPOHo spectra the absorption peaks are located at higher wavelengths than for Cu(II)TCMPOHo. In agreement with Gouterman's theory [31] this spectral behaviour is due to the stronger conjugation effects between the Cu(II) orbitals and the π electrons of the porphyrinic ring compared to the zinc porphyrins.

Under the experimental conditions used fluorescence signals were registred only for Zn(II)TMAPOHo(Cu(II) TMAPOHo is not emissive). The emission spectra for Zn(II)-5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl) porphyrin in organic solvents with different polarities, at room temperature, were recorded at λex=420 nm.

The obtained fluorescence data showed the maximum peak at 605-608 nm and another maxim at 651-659 nm, depending on solvent (fig. 3, table 2).

Similar to absorption behavior, Zn(II)TMAPOHo shows smaller blue shifts of the emission maxima by increasing environmental polarity as a consequence of hydrogen bonding that appear between the polar solvent molecules and the porphyrinic substituents.

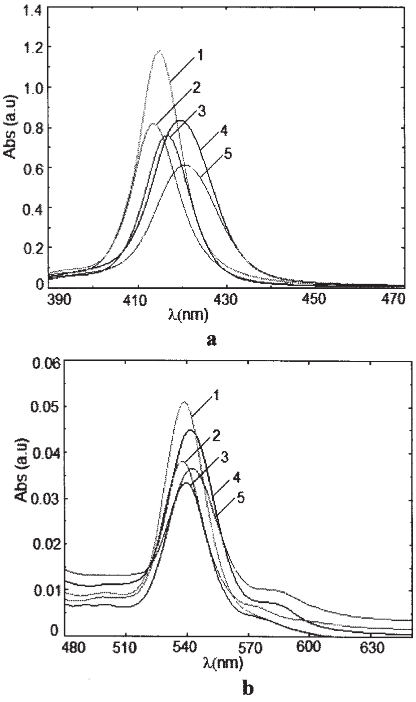

Fig. 2. Absorption spectra of Cu(II)TMAPOHo in different solvents: 1-isopropyl alcohol, 2-ethanol, 3- dichloromethane, 4- dimethyl formamide, 5-dimethyl sulfoxide. (a-Soret band; b-Q bands)

Table 2
Absorption and emission data of the porphyrinic complexes in various solvents (c=2.5×10^{-6} M, λ_{ex}= 420 nm)

Solvent	**Absorption** λ_{max}(nm) [lgε (L mol^{-1} cm^{-1})]			**Emission** λ_{max}(nm)	
	Soret band B	Q_y (0,0)	Q_x(1,0)	Q(0,0)	Q(0,1)
Zn(II)-5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)porphyrin					
EtOH	425 [5.72]	558 [4.28]	598 [3.87]	605	654
Iso-PrOH	424 [5.72]	557 [4.27]	597 [3.83]	605	651
DCM	422 [5.67]	549 [4.30]	587 [3.61]	602	651
DMSO	430 [5.65]	561 [4.22]	602 [3.92]	608	655
DMF	429 [5.75]	561 [4.33]	601 [4.04]	605	656
Cu(II)-5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl)porphyrin					
EtOH	415 [5.49]	543 [4.12]	-	-	-
Iso-PrOH	415 [5.67]	539 [4.31]	-	-	-
DCM	417 [5.60]	540 [4.35]	-	-	-
DMSO	418 [5.46]	540 [4.21]	-	-	-
DMF	420 [5.52]	542 [4.25]	-	-	-

EtOH=ethanol, *iso*-PrOH=isopropyl alcohol, DCM=dichloromethane, DMF=dimethylformamide, DMSO=dimethyl sulfoxide

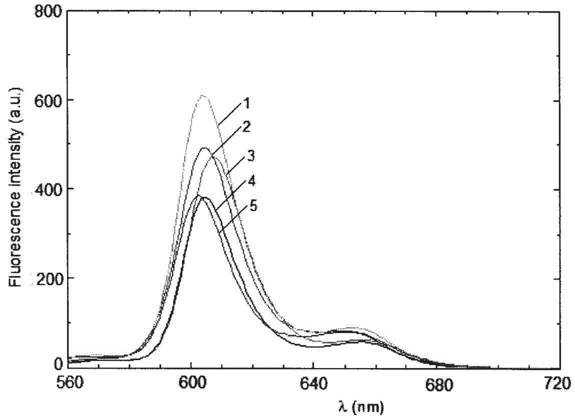

Fig. 3. Fluorescence spectra of Zn(II)TMAPOHo in different solvents: 1- ethanol, 2- isopropyl alcohol, 3- dimethyl sulfoxide, 4- dimethyl formamide, 5- dichloromethane

Conclusions

In this study we have reported the synthesis and spectral evaluation of the new unsymmetrical metalloporphyrins. Synthesis of Zn(II)-5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl) porphyrin and Cu(II)-5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl) porphyrin was made both the classical method as well as by microwave irradiation technique. The complexes were characterized by spectral analysis and obtaining data for porphyrins with the same metallic ion but synthesized by the two different methods described above, were identical. Zn(II)-5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl) porphyrin and Cu(II)-5-(2-hydroxyphenyl)-10,15,20–tris-(4-acetoxy-3-methoxyphenyl) porphyrin shows absorption maximum in the visible region of the molecular spectrum with a Soret band in the spectral range 415–430 nm and Q bands between 539–602nm, spectral maximum which respect the requirements for a good photosensitizer. Analysis of the spectral behaviour of the synthetised complexes in polar and nonpolar solvent revealed small changes of the absorption and emission with increasing solvent polarity.

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