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Determination of the photodynamic activity of porphyrins: Potential photosensitizers for treatment of age-related macular degeneration

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Age-related macular degeneration (AMD) is the leading cause of irreversible visual loss in people between 65 and 74 years. Recently, the photodynamic therapy (PDT) is used as experimental treatment for exudative AMD. In a PDT process, a reaction takes place when a photosensitiser (PS), light of appropriated wavelength, and oxygen are present at the same time. In general, the PS are porphyrins and related systems. The aim of this study was to evaluate the photodynamic activity (PA) of the benzoporphyrin (BPH_2), protophorphyrin-IX (Proto), tetrakis(*p*-hydroxyphenyl)porphyrin ($THPPH_2$) and tetrakis(2-hydroxy-5-nitrophenyl)porphyrin ($T2H5NPPH_2$). The PA is related to quantum yields (φ_Δ) for the singlet oxygen ($^1O_2$) production. Uric acid, a known singlet oxygen scavenger, is utilised as a chemical dosimeter in the PDT. When the uric acid (UA) and PS solution is irradiated with laser light, the UA band absorbance at 293 nm decreases as a rapid evaluation of relative PA of the PS.

Key words: *porphyrins, photodynamic activity, age-related macular degeneration*

1. Introduction

The photodynamic therapy (PDT) is one of the more promising new modalities currently being explored for use in the control and treatment of tumors. PDT is based on the use of a light-sensitive molecule, photosensitizer (PS). Porphyrins, phtalocyanines and chlorins, represent a major class of PS agents useful in an array of medical areas, including oncology, cardiology, ophthalmology, dermatology, immunology, gynecology and urology [1, 2].

Age-related macular degeneration (AMD) is the leading cause of irreversible visual loss. Laser photocoagulation of choroidial neovascular membranes (CNVMs) in AMD is currently the only well-studied and widely accepted treatment modality. But,

the basic premise of PDT is that, through the use of an intravascular compound that causes vascular occlusion by a photochemical reaction, as occurs with laser photocoagulation. Two treatment strategies can be envisioned. First, vessels may be occluded through a purely photodynamic mechanism. Second, photosensitizers may be used to enhance laser photocoagulation ablation of choroidal neovascular membranes thus decreasing rates of persistence, decreasing the thermal energy necessary to achieve occlusion [3].

The red absorption maxima of porphyrins and related compounds allow the activating light to penetrate deeper into tissue. It has been shown that oxygen plays a fundamental role in photochemistry of the many photosensitising drugs used in PDT. When the PS is irradiated in the presence of oxygen, the energy transfer from the excited triplet state of a PS to molecular oxygen leads to generation of singlet oxygen ($^1O_2$), the (excited) singlet state of molecular oxygen from otherwise benign precursors ($^3O_2$). This seems to be the first step in the photodynamic action of the most PS used in PDT. Then $^1O_2$ reacts with cellular targets leading to cell death.

Optimizing the PDT and photodetection of early cancer in a clinical context involves the variation of several parameters, such as the drug dose, light dose, light dose rate, time delay between drug injection and light application, wavelength of excitation of the dye, as well as the chemical purity and stability of the PS and their photodynamic activity [4].

Uric acid, a known singlet oxygen scavenger, is utilised as a chemical dosimeter for the determination of photodynamic activity in the PDT. The PA is related to quantum yields (φ_Δ) for the singlet oxygen ($^1O_2$) production. When the acid uric (UA) and PS solution is irradiated with laser light, the UA band absorbance at 293 nm decreases as a rapid evaluation of relative PA of the photosensitizer. Based on UA test it is possible to define a photodynamic activity scale, which may be a tool comparing the PA of different photosensitizers or irradiation conditions a proposal for a photodynamic activity scale based on the uric acid test. Fischer investigated the photodynamic activity and used the following relation [5]:

$$PA = \frac{\Delta A_{UA} \cdot 10^5}{E_0 t A_{PS\,\lambda_{\text{irr}}}} \tag{1}$$

where PA is the photodynamic activity ($m^2/(W \cdot s)$), ΔA_{UA} – UA absorbance decrease at 293 nm in UA and PS solution after irradiation, E_0 – power density of laser (W/m^2), t – irradiation time (s), $A_{PS\,\lambda_{\text{irr}}}$ – absorbance of PS in UA and PS solution after irradiation [5].

In this work, we determined the PA of the benzoporphyrin (BPH_2), protophorphyrin-IX (Proto), tetrakis(p-hydroxiphenyl)porphyrin ($THPPH_2$), and tetrakis(2-hydroxy-5-nitrophenyl)porphyrin ($T2H5NPPH_2$) by modified Fischer's expression using the uric acid (UA) test [4, 5]. Irradiations were performed at 683 nm using an MMD-Optics Laser with the power of 50 mW.

2. Experimental

Materials: Uric acid (UA) was purchased from Merck. Dimethylformamide anhydrous (DMF) and ethanol were purchased from Mallinckrodt. Benzoporphyrin (BPH_2), protophorphyrin-IX (Proto) and tetrakis(*p*-hydroxyphenyl)porphyrin ($THPPH_2$) were obtained from MidCentury. The precursor tetrakis(2-hydroxy-5-nitrophenyl)porphyrin ($T2H5NPPH_2$) was synthesized in our laboratory [6] using Adler Longo method. All reagents were used without further purification.

Uric acid test [5]: The samples were irradiated in a quartz cell with solutions containing only PS (10^{-5} mol·dm^{-3}) and solutions containing UA (10^{-3} mol·dm^{-3}) and a PS (10^{-5} mol·dm^{-3}). The irradiations were made at 25 °C for 1 hour, in air-saturated solution. Before and during the irradiation, absorbance spectra were registered each 3 min with a HP 8543 spectrophotometer. The UA procedure was used to investigate the UA absorbance decrease with different irradiation wavelengths in the wavelength range of each PS (BPH_2, Proto, $THPPH_2$ and $T2H5NPPH_2$).

Photodynamic activity scale (PA): Based on UA test it is possible to define photodynamic activity scale of different photosensitizers. The data obtained for PS could be mathematically determined by the modified Fischer's expression (2) All laser light is focused into the sample solution.

$$PA = \frac{\Delta A_{UA} \cdot 10^5}{WtA_{PS\,\lambda_{\mathrm{irr}}}} \tag{2}$$

where: PA is the photodynamic activity (1/(mW·s)), ΔA_{UA} – UA absorbance decrease at 293 nm in UA and PS solution after irradiation, W – laser power (mW), t – irradiation time (s), $A_{PS\,\lambda_{\mathrm{irr}}}$ – absorbance of PS in UA and PS solution after irradiation.

3. Results and discussion

The samples were irradiated in a quartz cell with solutions containing only PS (10^{-5} mol·dm^{-3}) and solutions containing UA (10^{-3} mol·dm^{-3}) and PS (10^{-5} mol·dm^{-3}). The PS absorbance did not decrease in the absence of uric acid. The UA has two absorption maxima in the UV/Vis range of the spectrum – 231 and 293 nm (Fig. 1). The parameters (irradiation wavelength, laser power, irradiation time and PS concentration) remained constant.

The absorbance spectrum of the porphyrins containing UA: $T2H5NPPH_2$, $THPPH_2$, Proto, and BPH_2, before and after laser irradiation, are shown in Figs. 2–5.

After laser irradiation at 683 nm, the photodegradation of uric acid was observed by changing the concentration of the solutions containing the PS. The PA values were determined by the Fischer's expression modification (2**)** and are shown in the table.

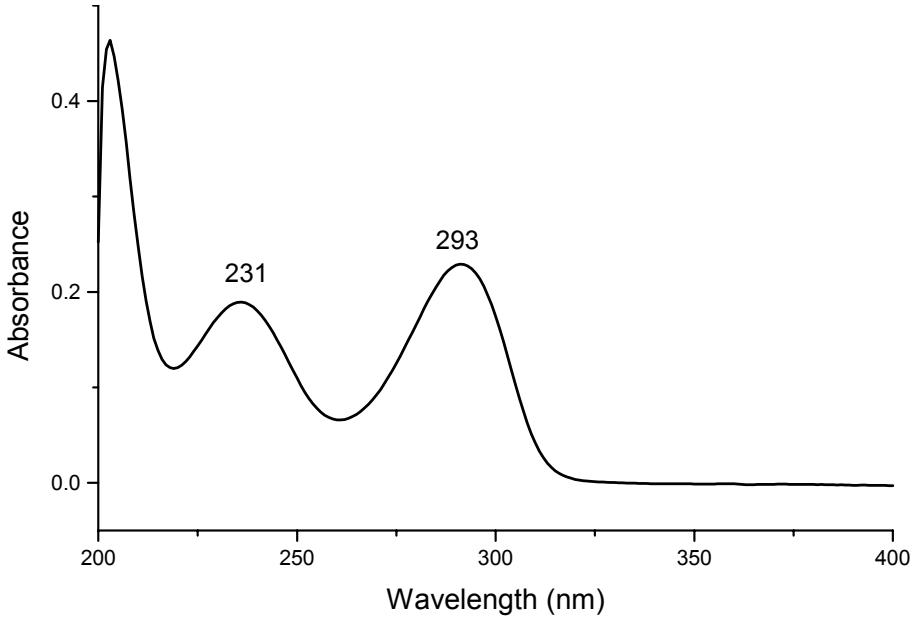

Fig. 1. Absorption spectrum of UA in ethanol

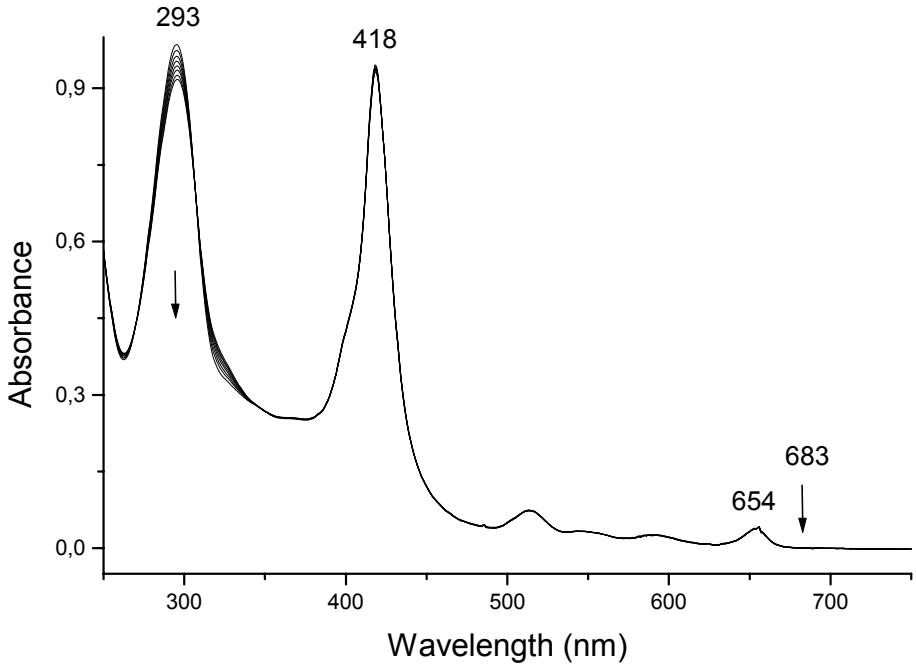

Fig. 2. Absorption spectrum of UA and T2H5NPPH$_2$ in ethanol

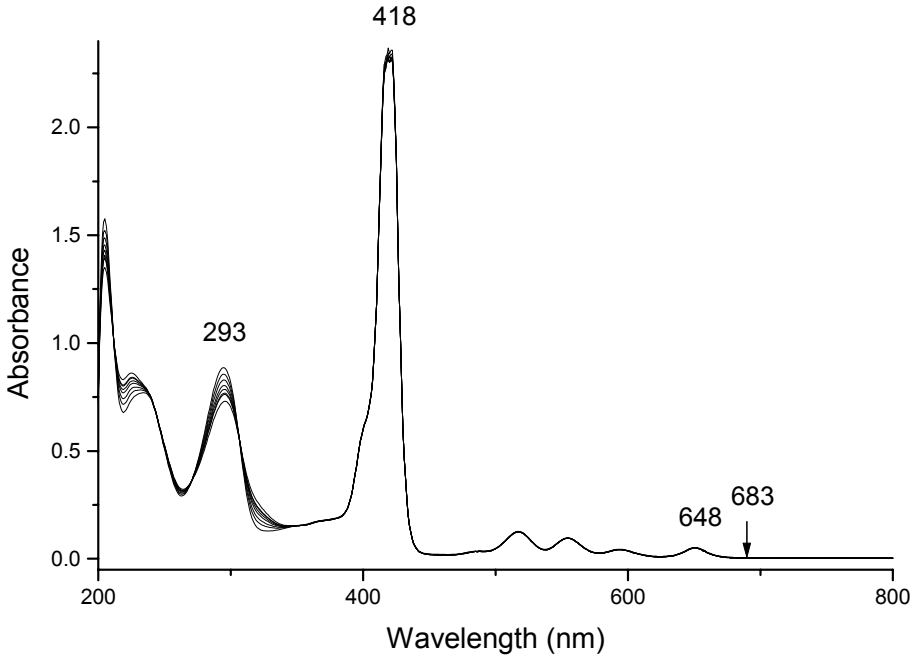

Fig. 3. Absorption spectrum of UA and $THPPH_2$ in ethanol

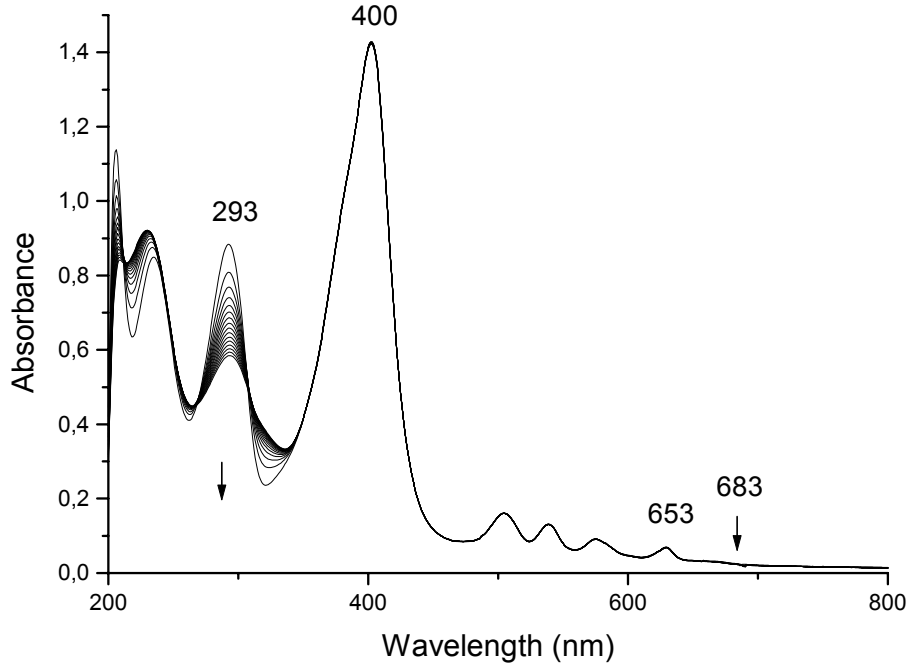

Fig. 4. Absorption spectrum of UA and Proto in ethanol

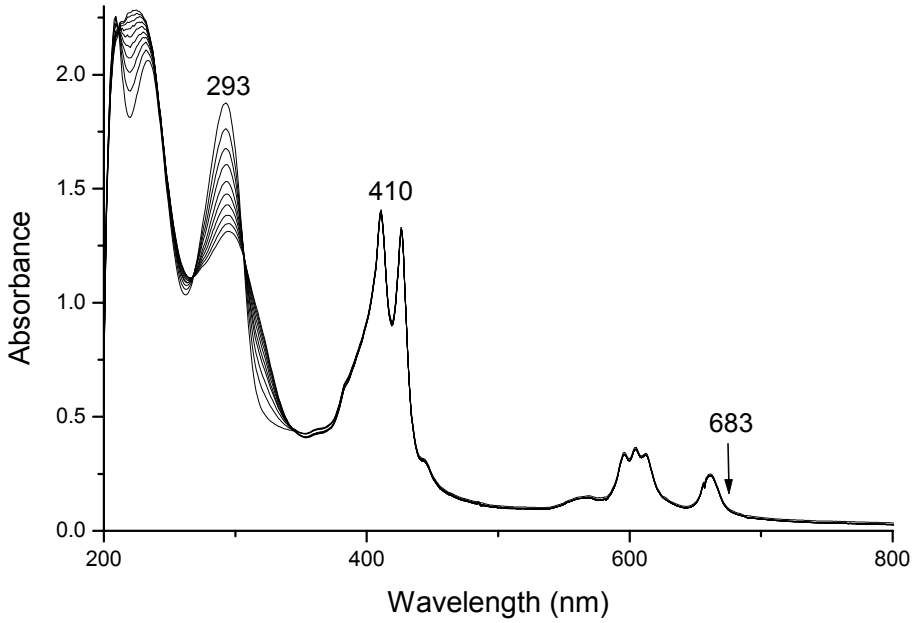

Fig. 5. Absorption spectrum of UA and BPH_2 in ethanol

Table. PA values determined by the Fischer's modification expression to $T_2H_5NPPH_2$, $THPPH_2$, Proto and BPH_2 in ethanol

Porphyrine	ΔA_{AU}	t/s	$A_{PS\lambda_{irr}}$	PA
T2H5NPPH$_2$	0.070	4200	$2{\cdot}99{\cdot}10^{-4}$	111
THPPH$_2$	0.159	4200	$1{\cdot}03{\cdot}10^{-3}$	74
Proto	0.279	4200	$2{\cdot}24{\cdot}10^{-3}$	59
BPH$_2$	0.672	4200	$1{\cdot}10{\cdot}10^{-2}$	29

4. Conclusion

The uric acid test is a suitable tool for a rapid and simple relative determination of the photodynamic activity of the photosentizers: T2H5NPPH$_2$, THPPH$_2$ and Proto in ethanol solutions. It presents a good stability and high values of PA even with a low absorption coefficient at 683 nm. Based on the UA test, it was possible to define a photodynamic activity scale, and compare the relative PA of the different photosentizers:

T2H5NPPH$_2$ > THPPH$_2$ > Proto > BPH$_2$

Because the reproducibility of the UA test depends on reproducibility of the environmental and irradiation conditions and reproducibility of PS properties [5]: The presence of the NO_2 and OH groups bounded to the phenyl is responsible for the high stability of the $T2H5NPPH_2$.

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