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KRAMERS-KRONIG ANALYSIS OF 5,10,1,20-TETRA-P–SULFONATO-PHENYL-PORPHYRIN (TSPP) AS PHOTOSENSITIZER FOR PHOTODYNAMIC THERAPY

M. A. Calin*, R. M. Ion[a], N. Herascu

National Institute of Research and Development for Optoelectronics INOE 2000, Bucharest, Romania
[a]National Institute of Research and Development for Chemistry and Petrochemistry – ICECHIM, Splaiul Independentei 202, Bucharest, Romania

Photodynamic therapy is a local treatment modality of cancer, based on the administration of photosensitizer, which is selectively retained al tumoral level, and which after the optical radiations exposure lead to the tumoral tissue destruction. The application of the photodynamic therapy in clinical practice presents many problems. The present study aims to correlate the refraction indices of the tumoural tissue with refractive indices of photosensitizer solutions and optical radiation distribution at tumoral level. There were used diffuse reflection spectrophotometry and molecular absorption spectrophotometry for the characterization of the optical properties of certain photosensitizer and the Kramers-Kronig analysis for the determination of the refraction indices of the solutions.

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1. Introduction

Photodynamic therapy (PDT) is a new treatment modality of cancer, age related macular degeneration, psoriasis and other chronic diseases [1].

PDT consists in the administration of photosensitizer, which selectively accumulates in the target cells, followed by local optical radiation exposure. Photochemical reactions lead to cells damage via production of singlet oxygen.

The efficacy of PDT depends on several factors such as:

- *photosensitizer:* chemical purity, physico-chemical properties, dose, preference accumulation in target cells and rapid clearance from health cells, capability to generate the cytotoxic species usually singlet oxygen, optical absorption in the therapeutical window, etc. [2];
- *optical radiation:* light source (continuous or pulsed emission), radiation parameters (wavelength, energy, pulse duration, pulses frequency, irradiation time, etc.), optical transmission through tissue and absorption by photosensitizer, light distribution in tissue, etc. [3,4].

Till now, from cellular level to clinical trial, all these factors were studied and the results have lead to the elaboration of treatment protocols for some hyperproliferative diseases.

The researches continue in this domain, aiming to the increased efficacy of photodynamic therapy. In this respect several solutions were proposed: the usage of new photosensitizer with optimal properties, the improvement of the mode of light delivery into tissue, the combination of photodynamic therapy with ionizing radiation, hyperthermia, etc. [5].

* Corresponding author: micalin@inoe.inoe.ro

Starting from these new research directions, in the present study, we have drawn the attention on the analysis and matching of the refractive indices of the tumoural tissue and photosensitizer aiming the improvement of the light delivery in the tumoural tissue.

2. Materials and methods

2.1 Materials

The photosensitizer used in this study was (5,10,1,20-tetra-p–sulfonato-phenyl-porphyrin) (TSPP). Their molecular structures are depicted in figure 1.

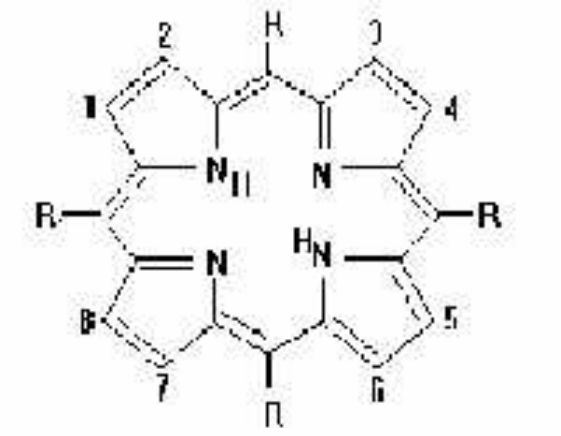


Fig. 1. Molecular structures of TSPP (5,10,1,20-tetra-p –sulfonato-phenyl-porphyrin).

H_2TSPP (Fig. 1) has been prepared according to the procedure:
5 g of 5,10,15,20- tetra-phenyl-porphyrin (TPP) was dissolved by stirring for aprox. 6 hours at room temperature, then poured over 180 g of crushed ice. After ice melting, the product was isolated by centrifugation and washed twice with 100 ml of water. The product was dissolved in aprox. 250 ml of water keeping the pH above 10 with addition of 10N NaOH, and the pH then adjusted to 8 with HCl. The solution was filtered and then applied to a C-18 silica column. The column was eluted with a mixture of methanol and water, increasing progressively the methanol content. The final fractions were collected and tested on HPLC, selecting only the H_2TS4PP fraction. The preparation and purification procedure is in a good agreement with the literature data [7,8].

The solutions were freshly prepared before each experiment and during all the experiments the solutions were protected from the solar light avoiding the photodegradation processes which could appear.

The values of pH were realized with tampon solutions with analytic purity.

2.2 Measurement of spectral properties

Optical reflectance spectra in the wavelength range (400 – 1000) nm were obtained with AvaSpec spectrophotometer. This portable spectrophotometer is equipped with a tungsten halogen lamp, CCD detector array (2048 pixel) and a reflection probe tip FCR-7IR200-2 with one illuminating fiber in center surrounded by six fibers which collect the light reflected from the sample (Fig 2).

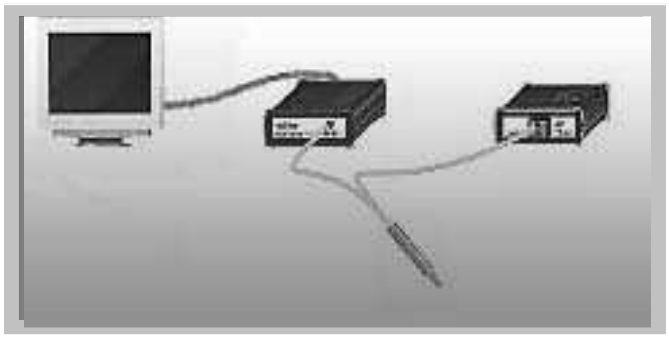


Fig. 2. Experimental set-up used to measure the reflectance of the TSPP solution.

2.3 Kramers-Kronig analysis

The optical properties of the TSPP solutions (refractive index and absorption coefficient) indirect were determined by the Kramers-Kronig analysis of the reflectance spectra acquired with AvaSpec System.

Kramers-Kronig relations decompose mathematic the complex reflectance spectra R(ω) in two different spectra [8]:

- refractive index spectra n(ω)

$$n(\omega) = \frac{1 - R(\omega)}{1 + R(\omega) - 2\sqrt{R(\omega)}\cos\varphi(\omega)} \tag{1}$$

- absorption coefficient spectra k(ω)

$$k(\omega) = \frac{-2\sqrt{R(\omega)}\cos\varphi(\omega)}{1 + R(\omega) - 2\sqrt{R(\omega)}\cos\varphi(\omega)} \tag{2}$$

where:

- R (ω) represent the reflectance spectra on the complete spectral domain of (0 - ∞);
- φ (ω) phase function defined thus:
-

$$\varphi(\omega) = \frac{1}{2\pi}\int_0^{\infty} \ln\left|\frac{\omega' - \omega}{\omega' + \omega}\right| \frac{d\ln R(\omega')}{d\omega'} d\omega' \tag{3}$$

To determine the refractive index n(ω) and the absorption coefficient of the TSPP solutions using this method we follow the next steps:

1. We acquired the reflectance spectra of the TSPP solutions in the spectral domain of (400 – 1000) nm equivalent with (10000 – 25000) cm^{-1}.
2. We extrapolated the measured data in the spectral domain of ($\nu_m = 0$) cm^{-1} and ($\nu_M \rightarrow \infty$) cm^{-1}, aiming to obtain the reflectance spectra on the complete spectral domain of (0-∞) cm^{-1} using certain extrapolation function, which describes the optical behavior in these ranges.
3. We calculated the phase function φ(ω) from the extrapolated reflectance spectra.
4. We determined the optical constants n(ω) and k(ω) from R(ω) and φ(ω).

3. Results and discussion

The reflectance spectra of TSPP solutions are shown in Fig. 3.

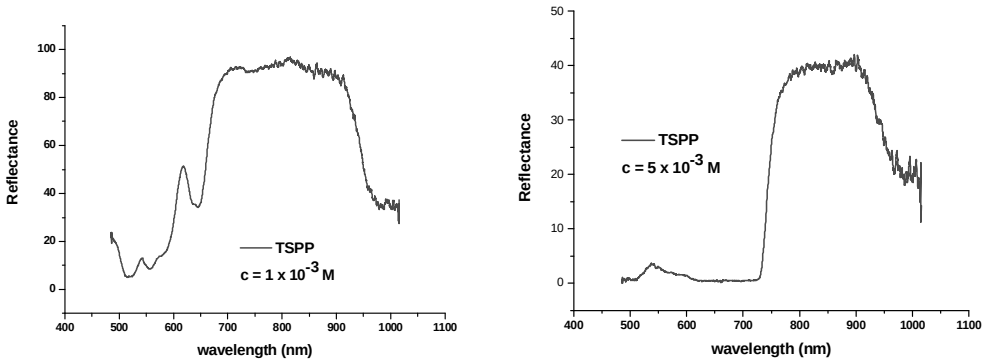


Fig. 3. Reflectance spectra of TSPP solutions, a) $c = 1 \times 10^{-4}$M; b) $c = 5 \times 10^{-3}$ M

Fig. 3 shows that TSPP solutions have an reflectance maximum at λ_1 = 864.1 nm and another reflectance maxima at λ_2 = 541.82 nm, λ_3 = 618.3 nm. At these wavelengths the laser radiations are not efficient for the activation of the photosensitizer. The reflection of these laser radiations can be used for the determination of the degree of accumulation of the photosensitizer at the tumoural level and the optimal time for laser irradiation with appropriate wavelength.

The Kramers - Kronig analysis of these reflectance spectra has lead to the absorption spectra (Fig. 4) and the refractive index variation with wavelength (Fig. 5).

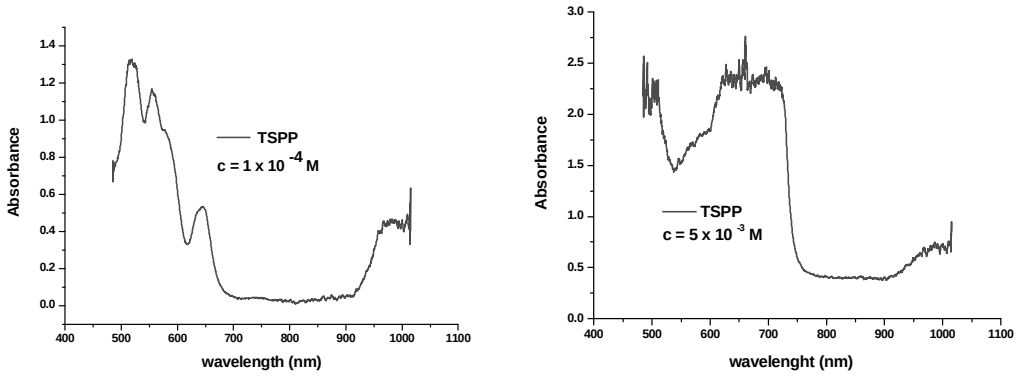
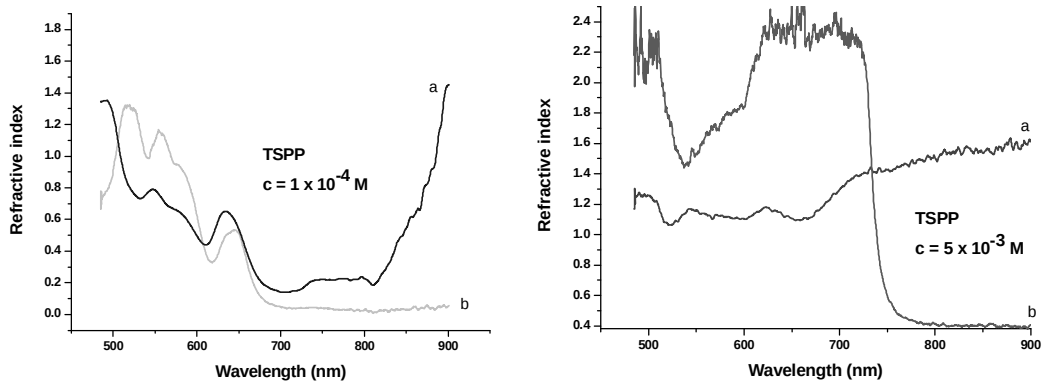


Fig. 4. The absorption spectra of TSPP solutions, a) $c = 1 \times 10^{-4}$M; b) $c = 5 \times 10^{-3}$ M



a b

Fig. 5. Refractive index variation with wavelength of TSPP solutions, a) $c = 5 \times 10^{-3}$ M; b) $c = 1 \times 10^{-4}$M.

Fig. 4 shown that TSPP solutions present some absorption maxima at λ_1 = 510 nm, λ_2 = 554 nm, λ_3 = 581 nm, and λ_5 = 644 nm, which correspond with the data from literature [9, 10] and validate the Kramers – Kronig analysis of reflectance spectra.
The variation of the refractive index with the wavelength (Fig. 5) depends on the concentration of the TSPP solutions. In the proximity of an absorption maximum of TSPP solutions, it could be observed that the refractive index decrease. The refractive indices of TSPP solutions at the maximum absorbance wavelength are presented in Table 1.

Table 1. The refractive indices of the TSPP solutions.

Concentration of TSPP solution	λ (nm)	n
$c_1 = 1 \times 10^{-4}$ M	510	0.915
	554	0.8116
	581	0.6443
	644	0.6112
$c_2 = 5 \times 10^{-3}$ M	510	1.1967
	554	1.1342
	581	1.1155
	644	1.1216

At λ = 632 nm, used in photodynamic therapy with TSPP as photosensitizer, the values of 0.6229 and 1.1209 of the TSPP refractive index have been determinated for two concentrations $c_1 = 1 \times 10^{-4}$ M and respectivelly $c_2 = 5 \times 10^{-3}$ M of solutions. At the same wavelength, a typical value for the refractive index of the normal tissue is 1.4 [11] and for tumoural tissue is 1.5 [12].

The addition of the photosensitizer to the tumoural tissue changes those refractive index depending on the concentration of photosensitizer. In our study, by adding to the tumour tissue TSPP solution with two concentration, we have obtained refractive index of the 2.525 and 1.888 for the two concentrations $c_1 = 1 \times 10^{-4}$ M and respectivelly $c_2 = 5 \times 10^{-3}$ M. These values of refractive index are more different from those of surrounding tissue and thus the losses of light energy by diffuse reflection will be small in tissue. As the difference between the refractive indices of tumour and surrounding tissue increases, the losses of light energy by reflection are smaller and the transport of the light through the tumour is efficient. We could increase this difference by administration of a suitable concentration of the photosensitizer solution.

4. Conclusions

Refractive index is one of the key parameter for the efficacy of photodynamic therapy.

Diffuse reflectance spectroscopy and Kramers-Kronig analysis are two tools for the non-invasive and rapid determination of the refractive index both tissue and photosensitizer.

Knowledge of the refractive index of the tumour and photosensitizer is a very important feature of the fundamental understanding of the laser-tissue interaction and a better correlation of these two refractive indices can lead to an optimal photodynamic treatment.

Acknowledgements

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