Polymer Membranes for Selective Separation of Ionizing Forms of $TPPS_4$ as Drug Photodynamic Therapy

STEFANIA SIMIONESCU[1,2], SOFIA TEODORESCU[5], RODICA MARIANA ION[3,4]*, GHEORGHE NECHIFOR[1]

[1] Politechnica University of Bucharest, 1-7 Polizu Gheorghe, 011061, Bucharest, Romania

[2] National Agency of Medicines and Medical Devices, Bucharest

[3] Valahia University, Materials Dept., Aleea Sinaia, 307008, Targoviste, Romania

[4] National Research and Development Institute for Chemistry and Petrochemistry – ICECHIM, 202 Splaiul Independentei, 060021, Bucharest, Romania

[5]Multidisciplinary Scientific and Technologic Research Institute, 13 Aleea Sinaia, 307008, Targoviste, Romania

In this paper, some of the ionized forms and aggregated forms of tetrasulphonated porphyrin $TPPS_4$, have been separated by passing it through a composite polymer membranes of polysulfone type with N-methyl-pyrolidone and magnetite. The aqueous solutions of $TPPS_4$ at different pH values have been prepared, characterized and passed through the membrane, leading to the conclusion that the acidic or alkaline solutions have been transformed into neutral form with certain therapeutic properties in photodynamic therapy.

Keywords: $TPPS_4$, polysulfone membrane, ionization, aggregation

Porphyrins are a class of natural macrocyclic compounds with multiple applications in many areas such as: chemistry, photochemistry, photomedicine, conversion and storage of solar energy [1-3]. Either as free base or metal complexes, the porphyrins have spectral properties, due to their aromatic character, and to the presence of non-bonding electrons of the central nitrogen atoms, with a characteristic absorption spectrum in visible and ultraviolet range. In the last decades, the porphyrins have gained an improved interest in some therapeutic applications curing cancer tumors by so-called photodynamic therapy of cancer [4-6].

Sulfonated derivatives of porphyrins, with n sulphonated groups, where n = 1-4, have as representative 5,10,15,20-tetra-p-sulfonato-phenyl-porphyrin ($TPPS_4$) which is among the most studied phototherapeutic compounds, because possess some additional advantages as photosensitizers in photodynamic therapy cancer (in comparison with hematoporphyrin derivatives and Photofrin II); they can be synthesized as a pure drug selectively retained by the tumor tissue and not normal [7-9]. The effectiveness of these porphyrins and their tumor localization, are correlated with existing electrical charges within the molecule, and the possible structural changes occurring in *p*H variations observed at the cellular level [10]. For this reason it is necessary and important to analyze the behavior of these porphyrins solutions with different *p*H values, going the whole range of *p*H (acid-base-neutral). Since porphyrins anionic such as tetra-sulphonato-phenyl-porphyrin ($TPPS_4$) at various values of *p*H can take various forms ionizing with varied photophysical and photochemical properties, it is necessary to know the accuracy of these forms responsible for the aggregation processes of this porphyrin. For this reason, it is important to separate the ionized forms and this can be achieved by the use of the composite polymer membranes of polysulfone type.

The membrane is considered to be single-phase or a structure that is interposed between two stages or compartments which may prevent or hinder transport of material through it, or allowing the passage of only certain species of particles [11]. In this context, polysulfone membrane matrix provides an excellent support for various macromolecular compounds or enzymes. The solution found to increase the biocompatibility of polysulfone membrane consisted in obtaining a composite membrane polysulfone-N-methyl pyrrolidone (PSf-NMP) [12].

This paper presents the results provided by various spectral techniques (UV-Vis absorption spectrophotometry and microscopy) for the ionized forms of $TPPS_4$ and their separation by passing through a polysulfone membrane type with NMP and magnetite.

Experimental part

Materials and methods

For this experiment has been used: Polysulfone (Psf) (pellets, with nominal M. W. 75000; density: 1.24 g / cm^3) and 1-methyl-2-pyrrolidone (N-methylpyrrolidone) (NMP) (Merck; 99%; MW: 99.13 g / mol; density at 20 ° C: 1.03 g / mL; solubility in water: 1000 g /L at 25 ° C; boiling point: 202 ° C) and magnetite (Aldrich provenience).

2 g of magnetite, 20 (or 200) mL of NMP and 10% dissolved polysulfone have been mixed with 50 glass beads of 2 mm diameter in order to homogenise the composition. All these have been placed in a planetary mill of the type Retsch mixture. They are left for 7 h at a speed of 300 rpm. Thereafter, a quantity of polymer solution and magnetite, 5mL, is deposited on a glass substrate, Roel type to a standard thickness of 250μm. The deposited polymer film on glass is immersed in the coagulation bath (distilled water and iso-propanol, 50%) specially prepared. Membrane formation was carried out for 15 min. The appearance of membranes is shown in figure 1.

Fig. 1. Composite Membrane Psf/ NMP/ magnetite

* email: rodica_ion2000@yahoo.co.uk; Tel: 021 3163094

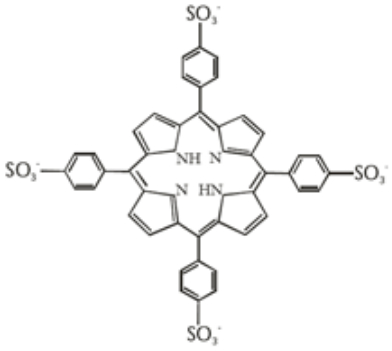


Fig. 2. Structure of 5,10,15,20-tetra-p-sulphonato- phenyl porphyrin ($TPPS_4$)

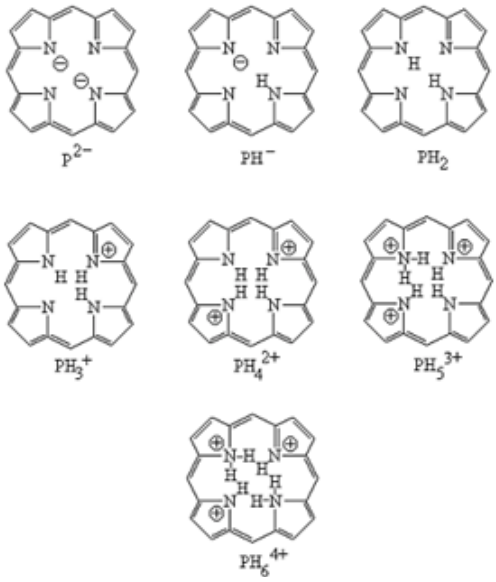


Fig. 3. Ionized forms of $TPPS_4$

5,10,15,20-tetrakis-p-sulfonato-phenyl-poprfirina ($TPPS_4$) figure 2 was prepared in the laboratory by synthetic methods known in the literature and purified by repeated washings with methanol mixture chloroform [13] .

Analytical equipment used

Ultraviolet absorption spectra and visible were obtained with a Specord M400 Jena Carl Zeies microprocessor and double beam.

For optical microscopy was used a NOVEX microscope equipped with a Leica EC3 room with magnifications 40x to 600x in the area.

Results and discussions

Porphyrins are amphoteric molecules who can add one or two protons at the central nitrogen atoms of free base (PH_2) forming monocationic (PH_3^+) or dication (PH_4^{2+}) species, and are able to lose two protons from pyrrole generating monoanion (PH^-) or the dianion (P^{2-}) forms [15-17].

Anionic porphyrins, such as $TPPS_4$ are considered to be more basic than other porphyrins and have a great tendency to aggregate [18]. They can adopt various forms at different *p*H values, as follows: at *p*H = 7, $TPPS_4$ shows an *aetio* structure of absorption band, characteristic for the meso-substituted porphyrins, i.e. a distinct Soret band in the 410-440 nm region and four bands of decreasing intensity in the region 500-700 nm) [19, 20]. As is known, $TPPS_4$ supports a gradual protonation at *p*H 6, which may lead to monocation form at *p*H 3.5 and to a dication form at *p*H = 1 [20,21]. At *p*H values between 6.5 and 3.5, the spectrum absorption, supports a Soret band splited into two bands: 412nm and 434nm. On lowering the *p*H below 3.5, the Soret band at 412nm gradually disappears, so at *p*H < 1.5 in the absorption spectrum there will be only one Soret band placed at 434nm, which is caracteristic to the specific form of the dication. By changing the *p*H values from 7 to 1, in the 490-700 nm spectral area will appear significant changes, as follows: a new band at 490 nm appears, and the band from 519 nm will gradually disappears with decreasing *p*H values, specific to monocation. At *p*H = 3.5, will increase in intensity the bands from 644 nm and those from 713 nm, simultaneously with a hipsochromic shift; in the alkaline range of *p*H values, the 469 nm band gradually disappears as the *p*H increase. The band at 713 nm, gradually disappears, and at *p*H = 10, it will record only the neutral form of such absorption peaks specific to porphyrin free base forms.

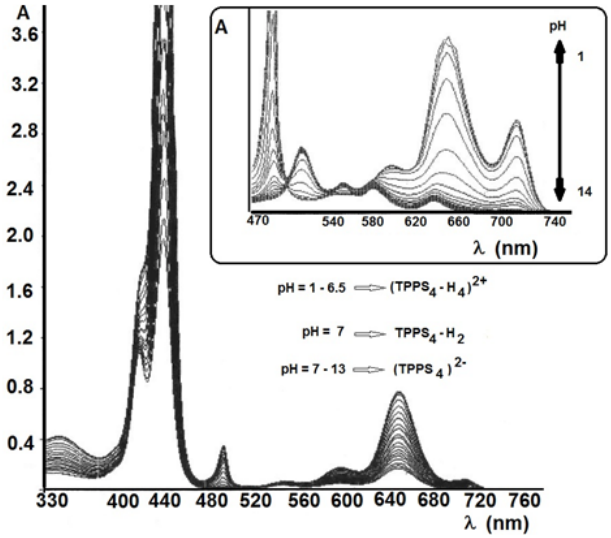


Fig. 4. Absorption spectra of the various ionized forms of $TPPS_4$

Taking into account the above results, by passing a solution of $TPPS_4$ c = 1.5 x10^{-5} M through a Psf / NMP / Fe_3O_4 membrane, a neutralizing effect of *p*H values from the porphyrin solution will occur and the absorption in the visible spectrum reveals a comeback spectrum of the acid

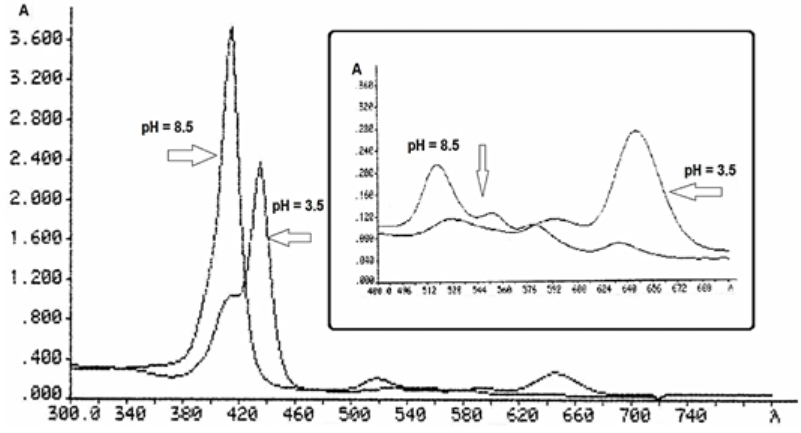


Fig. 5. Transformation of TPPS4 from *p*H 8.5 to *p*H 3.5

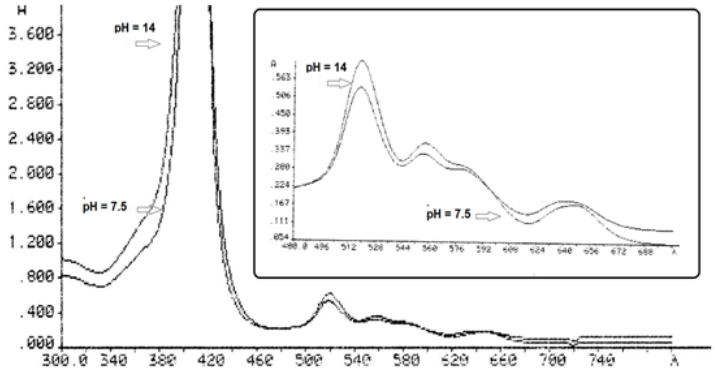


Fig. 6. Conversion of $TPPS_4$ from *p*H 14 to *p*H 7.5

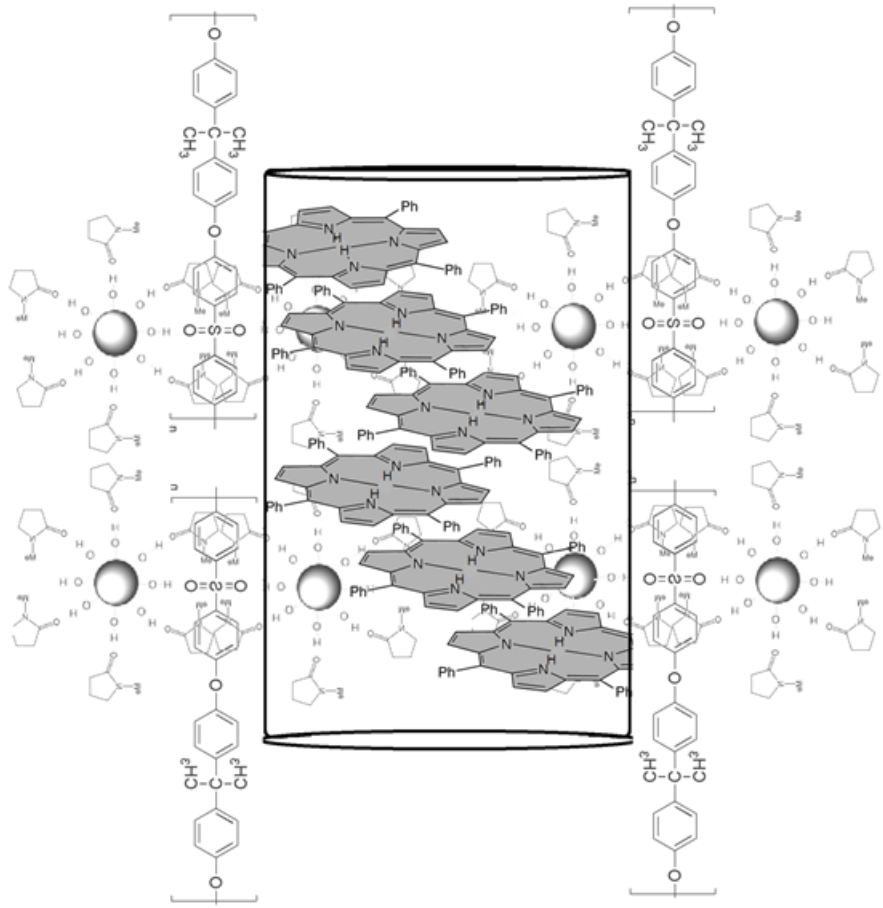
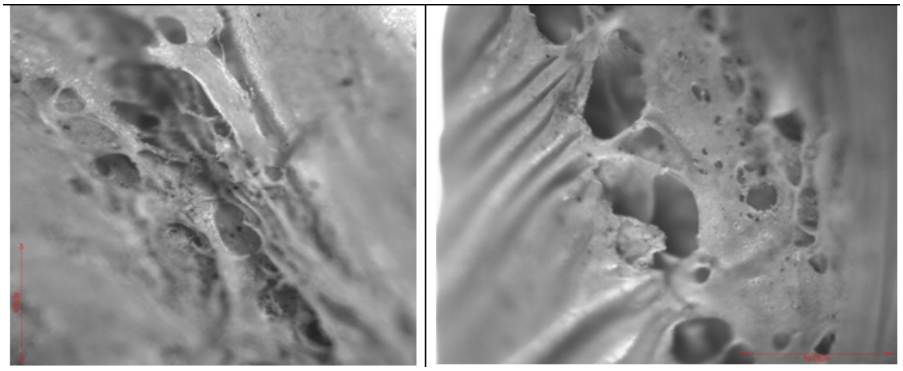


Fig. 7. Schematic diagram of membrane pores encapsulation $TPPS_4$ in Psf / NMP / Fe_3O_4



pH2 pH8

Fig. 8. The microscopic aspect of the pores of the membrane Psf / NMP / Fe_3O_4 after passing $TPPS_4$ at different *p*H values

or alkaline to neutral range. Also in the experiment can be seen as the color of the solution changes from green (characteristic to acid *p*H) in purple (specific to neutral species) figures 5.6.

The probable mechanism consists of electrostatic interactions and of hydrogen bonds between different groups OH or oxygen compounds participating in this type of membrane (O = S = O from Psf, OH groups from magnetite and C = O from NMP) and porphyrin that at acidic *p*Hs is in a J-aggregated form, figure 7.

At acidic *p*H because of ionized species and the existence of porphyrin aggregate (aggregated J), the membrane pores will be blocked without a clear breakdown. As the *p*H value increases pores are progressively increase in sizes, such as *p*H 8 is observed wide pores with fringes aspects, figure 8.

For argue the microscopy observations, the porosity measurements are performed according to the method of literature [22]. The porosity expressed in percentage varies according to the *p*H value for the whole range (acid-neutral-alkaline), table 1.

Table 1
THE POROSITY MEMBRANES DEPENDING ON THE *p*H

Nr.crt	pH	Porosity (%)
1	-	1.82
2	2	1.98
3	3.5	2.04
4	5.5	2.36
5	8.5	4.25
6	11.5	5.67

Conclusions

In this paper the identification and separation of different ionizing forms of TPPS4 have been analyzed, through some analytical techniques: UV-Vis, microscopy and porosimetry.

Was very important that by introducing the membranology, was possible to separate some of the ionizing and aggregated of this porphyrin and to transform them into neutral ones.

It was used a new membrane type (Psf/NMP/Fe_3O_4) which wasn't used up to now for such purposes.

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