

MULTIFUNCTIONAL PORPHYRINIC MATERIALS ENCAPSULATED INTO MACRONETS WITH PHOTOCHEMOTHERAPEUTIC APPLICATIONS

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Abstract

Supramolecular chemistry is expected to keep a high developing advanced of molecular devices based on multifunctional materials. Porphyrins and their analogues should play a significant role as a consequence of their catalytic, electrocatalytic, photochemical and photoelectrochemical properties. Such molecular materials contain a high porosity with large cavities and galleries that can be functionalized yielding to a desired chirality and structure. The functionalization implies inserting into macrocyclic cavity, followed by auto-assembling as columnar aggregates. The obtained cavities are used as host for different molecular guests. H and J-aggregates of some porphyrins are based on the intermolecular interactions of 3-5 Kcal/mol per porphyrin face. The columnar structure formed by porphyrins has a length of 5 to 27 porphyrin unities. In this paper we focused on our own strategy based on coordination chemistry for the design and build-up of supermolecules and supramolecular structures constituted by a porphyrin (TSPP) and a new and revolutionary method for stabilizing porphyrins (as organic part), by their incapsulation into supports with controlled porosity as macronets (as anorganic parts), obtaining some hybrids materials. Included are also their properties and potential applications.

Key words: porphyrins, macronets, photochemotherapy

1. INTRODUCTION

Nanotechnology and nanomaterials fuel advanced conceptual approaches in many areas of medical science and engineering. Scientists world-wide are continuing to discover unique properties of everyday materials at the sub micrometer scale [1,2]. This size domain is better known as nano- (a billionth) meter domain.

Now, biomedical nanotechnology, in which bioengineers construct nanoparticles combining polymeric and biological materials, is moving to the forefront of this rapidly advancing field of science. The integration of nanotechnology with biology and medicine will result in major medical advances leading to the development of multifunctional systems and smart drugs that will target only the diseased site in the body.

Nanoparticles are defined as colloidal particles with a size below 1 μm . Because of the potential applications in such diverse fields as optics, electronics, and catalysis, nanoparticle research has necessarily been interdisciplinary research. In the life sciences, nanomaterials are widely used in separation technologies, histological studies, clinical diagnostic assays, and drug delivery systems (DDSs). In the DDS applications, nanoparticles have several merits, such as ease of purification and sterilization, drug targeting possibilities, and a sustained release action. In recent years, an increasing number of researchers have considered the possibility of using nanoparticles in photodynamic therapy (PDT). PDT is based on the concept that photosensitizer (PS) molecules can be preferentially localized in tumor tissues upon systemic administration. Reactive oxygen species, such as singlet oxygen ($^1\text{O}_2$), or free radicals are the main cytotoxic substances, which can irreversibly damage the treated tissues. It

is generally accepted that singlet oxygen is the primary cytotoxic agent responsible for photobiological activity [3,4]

These novel properties of common materials observable only at the nano-scale dimensions have already found their first commercial applications. For example, nanomaterials are present in some sunscreens, toothpastes, sanitary ware coatings and even food products.

A new and revolutionary method for stabilizing porphyrins (as organic part), by their encapsulation into supports with controlled porosity as macronets (as anorganic parts), obtaining some hybrids materials, are presented.

Included are also their properties and potential applications into photochemotherapy (PCT).

To avoid the above limitations of TSPP, we have developed mesoporous nanoparticles that encapsulate the porphyrin (TSPP) in biocompatible nanoshells that act as a carrier, delivery, and targeting system.

They were nearly 300 nm in diameter with narrow size distribution, spherical shape, porous surfaces. The nanoparticle-encapsulated macronet (MN) remained stable in aqueous media. We tested the in vitro intracellular uptake of TSPP on EL-4 cancer cells, studying the effect of concentration and kinetics of uptake for nanoparticles and free TSPP.

Our results demonstrate that MN nanoparticles provide ideal vehicles for tumor delivery of TSPP. The nanoparticles stabilize TSPP in aqueous media, enhance intracellular uptake of TSPP by tumor cells, increase the accumulation and retention of TSPP in various organs, and show promise of photodynamic anticancer activity.

2. MATERIALS AND METHODS

2.1. Chemicals

Classified into three categories (after their dimensions) in this paper has been used only macronets from Group II, with area surface as 900-1200 m²/g and a pore volume of inside of a range 0,6-0,8 ml/g; (MN 150, MN250). The well defined structures of MN prevent the aggregation of the phthalocyanine molecules because of their location in the intracrystalline voids and galleries of the supports. The polymeric sorbents as **Macronet MN 150** have a unique structure which induces for them special advantages: big internal surfaces 1000-1500 m²/g, a transport region that allows rapid access of the adsorbant molecules, functionalization.

5,10,15,20-tetra-p-sulphonato-phenyl-porphyrin (TSPP), Figure 1, has been prepared and purified in the lab after a literature receipt [5].

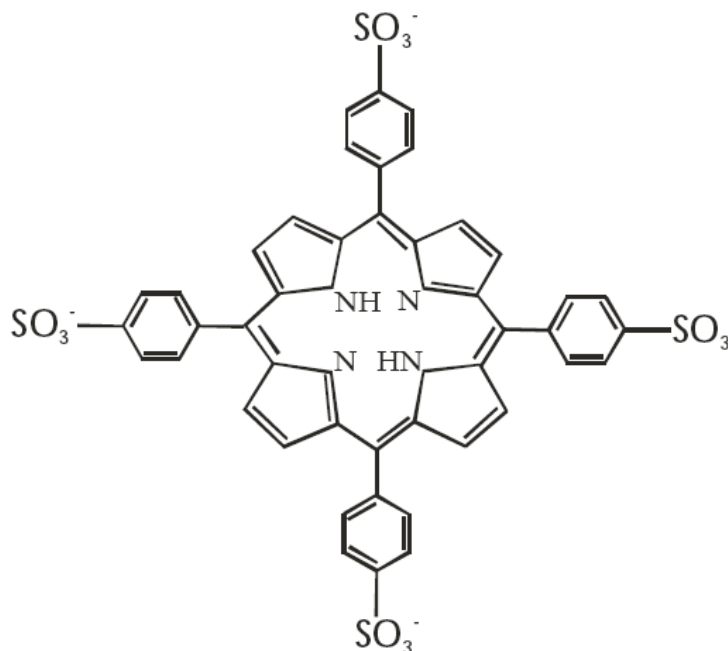


Figure 1. Chemical structure of meso-5,10,15,20-sulfonatophenyl porphine ($\text{H}_2\text{TPPS}_4^{4-}$)

2.2. Measurements

Ultraviolet-visible (UV-Vis) absorption spectra were recorded on a SPECORD M400 spectrometer.

Using a 30-mW diode laser operating at 632,8 nm, we established the photodynamic properties of the new systems.

For TSPP encapsulation into macronets pores, the flexible ligand method has been used. The method use the direct contact between TSPP solution 10^{-4} M and 1 gram of macronet for 24 hours in argon atmosphere. The characteristics of the macronets are shown in Table 1.

Macronet	Medium diameter of pore (Å)
MN 150	392
MN 250	372

Table 1. The characteristics of macronetics suport with controled porosity

3. RESULTS AND DISCUSSION

Porphyrins are the most exciting sensitizers for PCT, due to its strongest absorption band is around 800 nm and its most intense emission is around 820 nm (*in vivo*), which enhances signal selectivity.

Aggregation of the photosensitizers used in the so called photodynamic (photochemical) therapy of tumors (PDT) has *empirically* been found to play a decisive role for their photodynamic activity [6]. The principle of the photodynamic therapy is a very promising modality for the non-invasive treatment of tumors and other diseases.

PDT is based on a selective retention of a light-sensitive substance, photosensitizer, in tumor cells and its subsequent activation with light at appropriate wavelength. The photodynamic effect arises due to

the energy- and/or charge transfer from the PS to an acceptor molecule and the formation of cytotoxic species such as highly reactive singlet oxygen or free radicals. In the past decades, a great number of PS for PDT have been developed but only a few of them show a more or less satisfactory tumor selectivity and phototoxicity.

Sensitizer molecules, which possess a hydrophobic region in their structure, tend to isolate it from the contact with water molecules and self-associate in order to minimize the free energy of the system.

Formation of J-aggregates of TSPP

Meso-5,10,15,20-sulfonatophenyl porphine ($\text{H}_2\text{TPPS}_4^{4-}$) is a water-soluble tetrapyrrolic dye (Fig.2). It has been shown that $\text{H}_2\text{TPPS}_4^{4-}$ can self-associate to form H- and J- aggregates depending on the dye concentration, pH and ionic strength [7]. In an acidic medium ($\text{pH} < 5$), the nitrogen atoms of $\text{H}_2\text{TPPS}_4^{4-}$ can be protonated resulting in a diprotonated form $\text{H}_4\text{TPPS}_4^{2-}$. The diprotonated tetraphenyl porphyrin shows, in contrast to its neutral form, a near to coplanar conformation of the phenyl rings to the tetrapyrrolic ring. This allows the approach of the sulfonato groups to the central part of the tetrapyrrolic ring thereby forming J-aggregates [8]. The formation of J-aggregates is indicated by the appearance of a sharp intense absorption band that is shifted to the red with respect to the monomeric Soret band [9]. Fig. 2 shows the ground state absorption spectra of TPPS_4 in aqueous solution buffered at pH 7.5, 3.4 and 1.2. The absorption spectrum of $\text{H}_2\text{TPPS}_4^{4-}$ at pH 7.5 is characteristic for the free-base porphyrins. It exhibits four Q bands located at 515, 553, 580 and 633 nm and an intense near-UV band (Soret band) with a maximum at around 414 nm.

The absorption spectrum changes in acidic environment ($\text{pH}=3.4$) exhibiting a red-shifted and slightly broadened Soret band at 433 nm, a narrow intense band at 490 nm, and two broad weaker bands at 642 and 707 nm. The spectral bands at 433 and 642 nm can be attributed to the diprotonated ionic species $\text{H}_4\text{TPPS}_4^{2-}$, whereas the band at 490 nm (B-band) and at 707 nm (Q-band) are characteristic for J-aggregates [10]. A further decrease of the solution pH to 1.2 results in an increase in the intensity of the absorption bands at 490 and 706 nm, and in the disappearance of the absorption bands related to the neutral and diprotonated species of TPPS_4 .

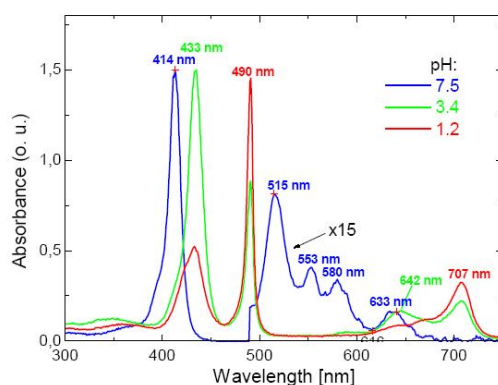


Figure 2. The ground state absorption spectra of TPPS_4 in aqueous solution buffered at pH 7.5, 3.4 and 1.2.

These changes in the absorption spectra have been assigned to the formation of the J-aggregates, derived from the dianionic $\text{H}_4\text{TPPS}_4^{2-}$, as indicated by the diminution of the absorption at 433 nm [1]. A decrease in concentration of $\text{H}_4\text{TPPS}_4^{2-}$ at low pH ($\sim 1-2$) results in a decrease of the amount of J-aggregates, Figure 3.

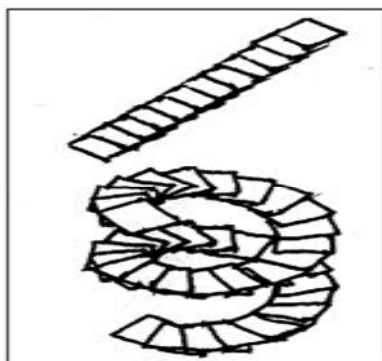


Fig.3. The TSPP aggregates structure H (up) and J(down)

Assuming that the distance between molecules is $\sim 5 \text{ \AA}$, the aggregate length can be calculated to be $\sim 10^{-6} \text{ cm}$, showing that the exciton diffusion length is shorter than the aggregate length. This result suggests that the number of molecules per aggregate determined represents the diffusion-limited size of the aggregate. The number of coherently conjugated molecules obtained in this work is in fairly good agreement with previous findings on the aggregation number of TPPS₄ obtained from UV/VIS absorption spectroscopy ($N=11$) [11], fluorescence anisotropy lifetime measurements ($N=22$) [12] and dynamic light scattering experiments ($N=6-32$) [13]. However, it has been proposed that J-aggregates of TPPS₄ tend to form large fractal-like structures or macroaggregates in organic solvents.

The encapsulation rate of TSPP into the macronets are calculated from the absorption spectra and is $0,002 \text{ M}\cdot\text{s}^{-1}$ in MN150 and $1,31 \text{ M}\cdot\text{s}^{-1}$ in MN250.

In MN150 (cationic character), the cavitar space is $392 \text{ \AA}$, is favourable to TSPP association as H-aggregates, while MN250 (anionic character), with cavitar space of $372 \text{ \AA}$, is favourable to J-aggregates formation, but insufficient to encapsulation, and only to over-crowding of MN. The MN cavities are used as host for different molecular guests. H and J-aggregates of some porphyrins are based on the intermolecular interactions of 3-5 Kcal/mol per porphyrin face. Such aggregates have the size of 5-6 nm in solution. The columnar structure formed by porphyrins has a length of 5 to 27 porphyrin unities [14].

TSPP/MN150 do not influence the cellular viability in the area 0-250 mg/ml.

Concentration (mg /ml)	5	15	25	50	100
TSPP/MN150	0,56	0,89	0,25	0,29	0,47

Tabel 2 The proliferative capacity of TSPP/MN150 on EL-4 cell line

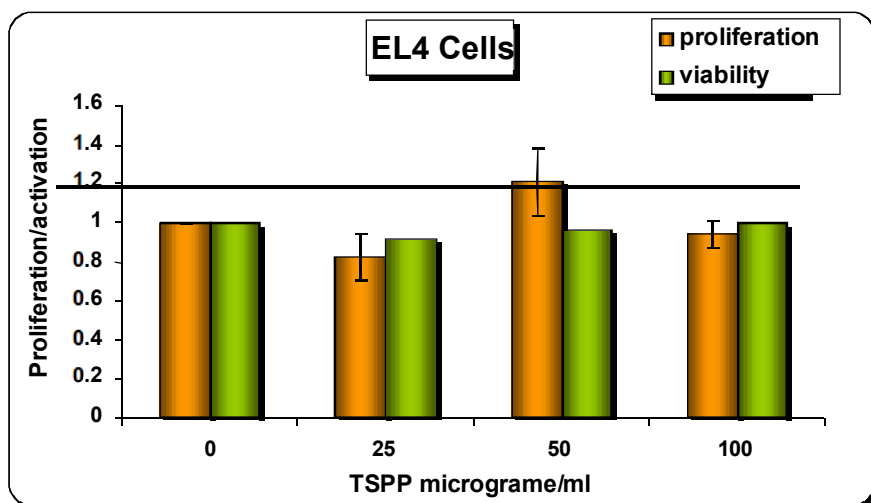


Figure 4- Corelation between viability and proliferation for EL4 cells line, in the presence of TSPP/MN150.

The photodynamic activity of TSPP/MN150 on EL-4 cells, has been evaluated and shown in figure 5

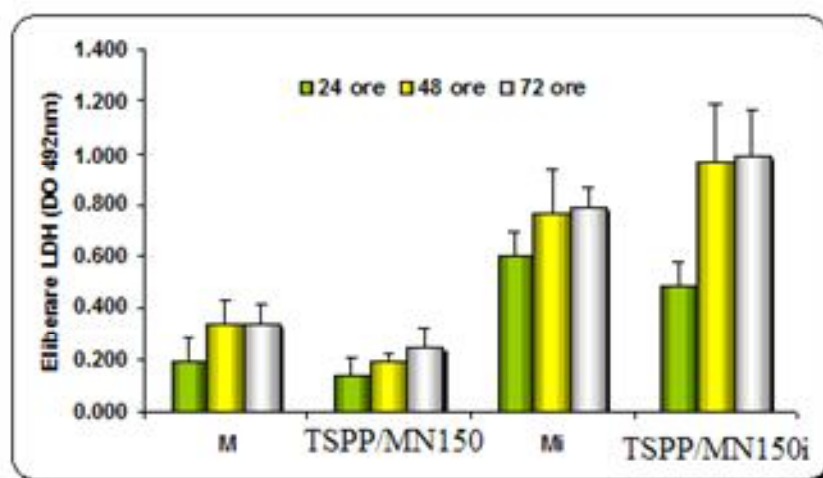


Figure 5 – Time evolution of cellular viability of EL-4 after laser activation of TSPP/MN150

Could be observed that after irradiation, TSPP/MN150 intracellular loaded and activated presents an homogeneous effect on the cells viability.

4. CONCLUSIONS

The porphyrins encapsulated in macronets as MN150, has a intracavitar space of 392 Å, favourable to TSPP association as H aggregates and MN250, with an intracavitar space of 372 Å, is favourable for J-aggregates insufficinet to incapsulation, but is favorable to over-crowding of MN.

After irradiation, TSPP/MN150 intracellular loaded and activated presents an homogeneous effect on the cells viability.

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