Micro-Encapsulated Porphyrins and Phthalocyanines – New Formulations in Photodynamic Therapy

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Abstract. Photodynamic therapy (PDT), as an innovative method for cancer treatment, is based on a concerted action of some drugs, called sensitizers, which generate reactive oxygen species via a photochemical mechanism, leading to cellular necrosis or apoptosis. The present work aims at loading some sensitizers, as porphyrins (P) and phthalocyanines (Pc) into alginate particles. Particles were prepared by dropping alginate into an aqueous solution containing P or Pc and CaCl$_2$, which allows the formation of particles through ionic crosslinking. It was obtained P or Pc loaded alginate beads with an average diameter of about 100 $\mu$m. For these systems, this paper analyses the spectroscopic properties, encapsulation into microcapsules, controlled releasing action and their photosensitizer capacity (singlet oxygen generation).

1. Introduction

In photodynamic therapy (PDT), as one of the most efficient methods for cancer treatment, the photosensitizer (PS) used as drug, should be administered and subsequently activated by light of a specific wavelength, leading to selective damage of the tumoral tissue [1].

Due to their essential role in PDT, and to their reduced stability, the photosensitizers should be incorporated in different delivery systems, such as hydrogels and liposomes, as some of the potential strategies to improve their efficiency in various cancer type [2,3].

Porphyrins are the most important sensitizers for PDT, due to its strongest absorption band in the region 600-800 nm, which is recognized as “therapeutic window”, which enhances their selectivity [4]. In addition, the phthalocyanines as the second - generation PSs similar to porphyrins, have superior photophysical and photochemical properties [5], could be easily synthesized, have good photo- and chemical-stability, long-wavelength absorption in the 650–750 nm region and high singlet oxygen quantum yields [6].

Polymers offer several advantages in PDT due to their ability to deliver a large amount of PS to the target area, flexibility toward surface modification for better efficiency, and the possibility of being loaded in specific position [7,8]. Polymers are playing an important role in the preparation of nanoparticles and being used widely, and natural polymers composed of polysaccharides such as chitosan and alginate represent an important issue [9,10]. Normally a particle with 10-500 nm size is considered nanoparticle and the classification of nanoparticles in nanocapsules and nanospheres has been evolved [11].
Alginate is a naturally occurring linear unbranched polysaccharide extracted from brown seaweed, such as *Laminaria hyperborea*, *Ascophyllum nodosum* and *Macrocystis pyrifera*. Alginates are composed of (1-4)-linked β-R-mannuronic acid (M units) and α-L-guluronic acid (G units) monomers [12]. Sodium alginate is a sodium salt of alginic acid, a naturally occurring polysaccharide obtained from marine brown algae, figure 1.

![Figure 1. The structure of alginate.](image)

Alginate can easily be gelled with different cations by the stacking of guluronic acid (G) blocks with the formation of “egg-box” calcium linked junctions (figure 2). The alginate particles are formed through external gelation and typically have a diameter of 100 –5000 μm [13].

![Figure 2. The structure of the complex “egg-box” calcium linked junctions.](image)

The use of alginate based drug delivery systems presents many advantages mainly related to the encapsulation process which takes place at room temperature and without the use of organic solvents [14].

In this perspective, the present work is aimed at the preparation and characterization of alginate based polymeric particles loaded with different porphyrins, a free base (TSPP) and its metallic complex (ZnTSPP); and a free base phthalocyanine (TSPc) and its metallic complex (ZnTSPc).
2. Materials and methods

2.1. Materials
Sodium Alginate (250 cps at 25°C, 2% solution), were purchased from Sigma Aldrich. Calcium Chloride was purchased from Carlo Erba. Porphyrins: TSPP (5,10,15,20-p-tetra-sulphonated-porphyrin) and its metallic complex (ZnTSPP) and phthalocyanine TSPc (2,9,16,23-tetra-sulphonated-phthalocyanine) and its metallic complex ZnTSPc (zinc-3,4′,4′′,4′′′-tetrasulfonated-phthalocyanine) have been synthesized in our laboratory, after literature receipts [15-17].

2.2. Instrumentation
UV-Vis absorption spectra measurements and the degree of porphyrins/phthalocyanines sorption has been monitored in the solution with a SPECORD M400 spectrophotometer with monochromator and double beam. Optical microscopy has been achieved with a NOVEX 100 microscope by using proper magnitudes.

2.3. P/Pc Loaded Alginate Particles
P/Pc loaded alginate particles (beads and microparticles) were obtained by means of ionotropic gelation. Alginate was dissolved in physiological solution and mixed with P/Pc in distilled water. The resulting solution was added dropwise, using a syringe equipped with a 22G needle, to a water solution containing calcium chloride, under magnetic stirring.

2.4. Evaluation of P/Pc Encapsulation
The encapsulation efficiency of porphyrin or phthalocyanine was calculated by using formula:

\[
EE \, (\%) = \frac{(P_{\text{loading}} - P_{\text{filtration}})}{P_{\text{loading}}} \times 100
\]

where: EE = encapsulation efficiency, %; \(P_{\text{loading}}\) = total amount of P or Pc loading; \(P_{\text{filtration}}\) = amount of P or Pc in ultrafiltrate (calculated after filtration with CHROMAFIL O-45/15 MS filters (Machinery-Nagel GmbH, Germany).

2.5. In Vitro Release of Porphyrin or Phthalocyanine
The nanoparticles in the filter were diluted with water solution of pH=5.5 to 2 mL and incubated at 37°C. The amount of extract released, was estimated from the ratio between the amount of porphyrin or phthalocyanine determined in the release medium and the initial amount of porphyrin or phthalocyanine in the nanoparticles.

2.6. Measurements of singlet oxygen (\(1^\text{O}_2\)) generation
Porphyrin or phthalocyanine (10 μM) were mixed with 9, 10-anthracenediy-bis(methylene) dimalonic acid (ABDA) (20 μM) in water bi-distilled and placed in a quartz cuvette. The sample solutions were irradiated with polychromatic light from a Hg medium-pressure lamp (400 nm-800 nm) for 2 min and then the fluorescent emission of ABDA was measured at 431 nm (excitation wavelength = 380 nm). The destruction of ABDA is an indicator for the generation of singlet oxygen [18].

3. Results and discussion
The absorption spectra of porphyrins are the most useful technique for characterizing porphyrins. The presence of the conjugated double aromatic aromatic system in the porphyrin structure translates into a characteristic absorption spectrum in the visible field, porphyrins being highly colored substances [19]. In neutral solvents, the free base porphyrins, \(H_2P\), exhibit a very high band (Soret band) around 400 nm in the absorption spectrum, and yet another series of four bands, marked IV, III, II, I in the 500-700 nm [20].

Metalloporphyrins have an absorption spectrum characterized by a very intense Soret band between
380 and 420 nm (known as B (0,0) and by a two-band system in the 500-600 nm area, known to be composed of the bands β [Q (0,0)] and the α band [Q (1, 0)], which are 10-20 times less intense than the Soret band, depending of the central metal ion.

Tetrakis-4-sulfonato-phenyl-porphyrin (TSPP) is an anionic porphyrin, which possesses four negative charges the sulfonate groups from the four corners. In aqueous solutions, at neutral pH, the electronic absorption spectrum of TSPP is characterized by an intense Soret band at around 420 nm and four Q bands in the 500-700 nm range. In acidic medium, new absorption bands (from 490, 707 nm) appear: J-aggregate (edge-to-edge interaction) at 490 nm and H-aggregate (face-to-face interaction) at 422 nm [21].

Figure 3. The absorption spectra of TSPP (full line) and ZnTSPP (dotted line).

The phthalocyanines show electronic spectra substantially different from those of the porphyrins. Phthalocyanines as expected from the extensively conjugated aromatic chromophore, exhibit UV-VIS absorption spectra with intense π–π* transitions, usually referred to as Q bands in the range 660-700 nm (ε > 10^5 M^{-1} cm^{-1}) [22].

Figure 4. The absorption spectra of TSPc (full line) and ZnTSPc (dotted line).
By analyzing the obtained experimental results, some remarks could be formulated:

- The encapsulation efficiencies are completely different at free-base compounds (TSPP and TSPc): 1.11% for TSPP and 10.16% for TSPc;
- for metallo-complexes, the encapsulation efficiencies are higher than for free-base compounds: 17.32% for ZnTSPP, and 36.15% for ZnTSPc.

All the tested compounds have different releasing capacity, figure 5. Microparticles were obtained by ionic gelation lowering alginate concentration to 2% (w/v) and Calcium chloride to 2.5% (w/v) in agreement with literature data [23].

![Figure 5. The controlled release of P and Pc from alginate micro-capsules.](image)

These results could be very well correlated with their sizes, as follows:

- An initial burst release of the encapsulated P or Pc was observed at 2.5 h;
- The values of controlled release capacity are: 97.85% for TSPP, 99.23% for ZnTSPP, 97.75% for TSPc and 93.62% for ZnTSPc;
- Alginate alone is generating spherical micro-capsules with the size of 170.78 μm;
- With TSPP is generating larger sphere, because this porphyrins is able to aggregate, increasing its size from 108.07 μm at initial micro-capsules to 141.68 μm at the micro-capsules after 5 hours (assigned to a dimerisation process) and to 177.12 μm after 24 hours (assigned to aggregation process), and 55.45 μm after 96 hours (assigned to monomerisation process).
- After drying process, the micro-capsules became with unclear and damaged edges (106.44 μm), and release the monomers, which clear fitted visible (30 μm). TSPP is able to disaggregate and to slowly release from the capsules, generating monomers.
- The forms of TSPP could be identified by specific absorption maxima, too, figure 6. monomer form: Soret band at 415-416 nm; dimeric - aggregated form: 417.5 nm - 423.75 nm, in good agreement with literature reports [24];
- Similar behaviour has been observed at TSPc, but due to smaller size of this compound, it is able to encapsulate it more easily into capsules.
Figure 6. The changes of spectra for TSPP as micro-capsule: initial (full line), after 24 h (dotted line), and after 96 h (dried micro-capsule) (brushed line).

The aspects of TSPP encapsulated into alginate micro-capsules in different experimental conditions are visible in figure 7.
TSPP in alginate micro-capsule after 24 h (177.12 μm)

TSPP in alginate micro-capsule after 96 h (55.45 μm)

TSPP in alginate micro-capsule dried after 24 h (106.44 μm)

TSPP in alginate micro-capsule dried after 96 h (30.44 μm)

**Figure 7.** Optical microscopy for microcapsules with encapsulated P or Pc compounds.

To check whether P or Pc maintained its own functional properties after the loading into alginate based beads, singlet oxygen generation were performed on the studied compounds, table 1.

**Table 1.** Singlet oxygen generation in solution and in microcapsules.

| Compound | $\phi [1^\text{O}_2]$ Water solution | $\phi [1^\text{O}_2]$ microcapsules |
|----------|-----------------------------------|-----------------------------------|
| TSPP     | 0.67                              | 0.45                              |
| ZnTSPP   | 0.48                              | 0.25                              |
| TSPc     | 0.11                              | NA                                |
| ZnTSPc   | 0.68                              | 0.25                              |

By light irradiation, has been observed the ability of the porphyrins or phthalocyanines to induce the formation of singlet oxygen and the subsequent controlled release, followed by the disruption of the microcapsules. The obtained results are partially similar with other literature reports for porphyrins as TSPP [25], but not for Pcs. In the present study, Pcs showed not very promising results, they are too light sensitive and unstable. The same has been observed for ZnTSPP, which is very unstable.

### 4. Conclusions

The use of alginate for the production of P or Pc loaded particles showed interesting results. The studied formulation clearly demonstrated that the proposed system posses good properties of alginate particles for therapeutic applications. The porphyrins or phthalocyanines are able to be encapsulated into microcapsules and to induce the formation of singlet oxygen, and the subsequent controlled release, followed by the subsequent disruption of the microcapsules.
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Acknowledgments
This paper received the financial support from MEN-UEFISCDI, through the projects: PNII 185/2014, PNIII 120 BG/2016 and PN 16.31.02.04.03.