Construction of Polyelectrolyte Microcapsules envisaging potential Cancer Therapy

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Abstract

This thesis proposes a new porphyrinoid delivery system in order to increase the efficiency of photodynamic therapy with the possibility of light-tracking for Cancer therapy. Two different porphyrins: a negatively charged 5,10,15,20-(tetrakis)-(4-sulfonatophenyl) porphyrin (H2TSPP-4) and a positively charged 5,10,15,20-(tetrakis)-(N-methyl-4-pyridinium-yl) porphyrin (TMPyP) were used as photosensitizers and adsorbed in pH-sensitive core-shell polyelectrolyte microcapsules (PECs) of poly(styrenesulfonate) (PSS) and poly (allylamine hydrochloride) (PAH). The characterization of the PECs system was made by UV-Vis absorption, steady state and transient spectroscopy, Fluorescence Lifetime Imaging Microscopy (FLIM), zeta potential and Transmission Electron Microscopy (TEM). The key characteristics of this delivery system are: pH dependence on encapsulation efficiency; a high load release (87.7%) in acidic pH (~3.0) environments; a high stability with very low release (2.18%) in neutral pH environments and non-degradation of the system by light in non-oxidative environments. It is also reported the organized self-assembly of TSPP on PECs where the CaCO3 core works as a nucleation site for the formation of radially distributed needle-like structures of porphyrin aggregates. These last results were published on 11th July of 2017 in *Langmuir (ACS Publications).*

1. Introduction

Cancer remains one of the most prominent diseases that affects humanity. The predicted increase in number of cases and deaths motivates the continuous research in developing new cancer therapeutics or improving current ones [1].

Current treatments, such as chemotherapy or surgery, are limited by specificity, selectivity, therapeutic index, solubility, stability and induction of chemoresistance or physical trauma [2].

Previous limits might be overcome by using polyelectrolyte microcapsules as a delivery system of a therapeutic drug.

An alternative therapeutic method such as Photodynamic Therapy (PDT) presents potential advantages: Non-invasive; Can be target accurately and selectively in early or localized cancer; It can improve quality of life and lengthen survival (in case of advanced disseminated disease); Repeated doses can be given without total-dose limitation; has less side-effects; it

doesn't scar tissue; can be implemented with low infrastructures investments [3].

One of the key components for PDT is the photosensitizer. It should be characterized by high chemical purity, activation with wavelength appropriate for tissue penetration, selectivity for tumor cells and formation of a long-lived triplet excited state. The most important factors are the period of photosensitivity, availability, concentration and the activation wavelength. The activation of the fluorophore accompanied by a formation of a triplet excited state promoted by intersystem crossing should occur in adequate yields as the energy transfer from this state to the molecular oxygen in its fundamental state (triplet) promotes the formation of singlet oxygen. This cytotoxic specie at certain concentrations can damage and kill the disease cell.

The photosensitizer should also be able to accumulate in the diseased tissue in order to achieve a high local concentration increasing the photodynamic efficiency. The natural characteristics of cancer cells already promote this accumulation by having a higher supply of blood and a reduced lymphatic drain when compared with healthy tissue. Nevertheless, is important to reduce the systemic clearance and increase even further the fluorophore concentration inside cancer cells.

It is reported in this study the development of a drug delivery system that is expected to promote an increase in local concentration of photosensitizer and the possibility of application as a tracking delivery method.

The polyelectrolyte microcapsule system is a microstructure formed by an inorganic core and a polyelectrolyte shell. These systems, have already been introduced in the end of the last century and have been described as promising systems for biotechnology applications [4]. These engineered micro- carriers offer advantages such as high loading/release of several drugs (as photosensitizer used in PDT), surface characteristics feasible for specific targeting, protection of drugs enhancing their stability, minimize their systemic clearance and also structural characteristics that enable their compartmentalization providing an easy way to incorporate different drugs and simultaneous therapeutics.

The porphyrinoids derivatives present interesting photochemical properties that makes them prominent photosensitizers for this system, such as low dark toxicity, adequate quantum yields and kinetic and thermodynamically stability. It was chosen two porphyrins in order to conduct this study. A negatively charged porphyrin, 5, 10, 15, 20 - (tetrakis) - (4-sulfonatophenyl) porphyrin (H_2TSPP^{-4}) and a positively charged, 5,10,15,20-(tetrakis)-(N-methyl-4-pyridinium-yl) porphyrin (TMPyP).

These two systems are derived from a tetrapyrrolic parent compound with four pyrrole rings connected by methine bridges and have a delocalized 18 π conjugated electron system. Porphyrins have intense absorption bands in the visible range in particular, the optical spectrum shows a very strong absorption band at the 400-450nm region (Soret Band)) and in the 500-700nm bands (the Q bands) [5].

H_2TSPP^{-4} is a tetra-anionic porphyrin with four sulfonatophenyl groups as *meso*-subsitituents ($C_{44}H_{28}N_4NaO_{12}S_4^-$; Mr = 955.954 g/mol) that upon protonation of the macrocycle nitrogen atoms (pKa≈4.8) forms H_4TSPP^{-2} with a zwitterionic character and high capacity of self-assembly [6].

TMPyP is a tetra-cationic porphyrin with four pyridinium groups as *meso*-subsitituents with high affinity with nucleic acids [7], preferential localization in tumor tissues, anti-HIV [8], antibacterial activity [9], reported use as active compound for singlet oxygen imaging of single cells [10] and for singlet oxygen photosensitization in skin fibroblasts [11]

In literature, it has already been described drug delivery applications of microcapsules and impacts of surface modification. Cheng *et al*. [12] showed that nanoparticles coated with LbL films can be conjugated with branched PEG improving stability and lifetime in a physiological environment. Caruso *et al*. [13] demonstrated the increase in cell uptake in a line of colorectal cancer cells of LbL core-shells functionalized with the monoclonal antibody huA33. Luo *et al*. [14] prepared pH-responsive microcapsules with interaction between polyaldenhyde dextran-graft-adamantane (PAD-g-AD) and carboxymethyl dextran-graft-β-cyclodextran (CMD-g-β-CD). Wu *et al*. [15] described a proof-of-concept application of a multilayer vehicle for drug delivery and NIR light-controlled release induced by the film-loaded AuNPs.

For the characterization of the polyelectrolyte microcapsule system adsorbed with porphyrin it was studied the influence of pH on the system by potential zeta, the adsorption efficiency using UV-Vis absorption and the optical properties were studied by using steady state and time-resolved emission and fluorescence lifetime imaging microscopy (FLIM). The photosensitizer release profile was studied in solutions that mimic the environment of the Human body (in pH and ionic strength) and was followed by steady-state emission.

The release of H_2TSPP^{-4} from $CaCO_3(PAH/PSS)_2$ PAH in acidic pH environments is reported as successful, achieving 87.7% of unloading in less than five hours. Releases under 5% are observed from ninety minutes to seven hours in neutral pH conditions showing the stability of the system during systemic circulation. Light-mediated release actually does not affect the release of porphyrin allowing the possibility of light-tracking without degradation of the drug delivery vehicle.

Two systems with two different porphyrins adsorbed are reported and characterized for the use of PDT in cancer therapy. By combing an inorganic core ($CaCO_3$) with pH-sensitive polyelectrolytes (PAH/PSS) is possible to control and optimize the encapsulation efficiency of the system as well to increase the release load in different pH environments. It is also reported the formation of H_4TSPP^{-2} J-aggregates on polyelectrolyte microcapsules core-shells where is clarified the role of the inorganic core revealing a potential of the system for biomedical and biotechnological techniques.

2. Experimental Section

Materials. Poly (sodium 4-styrenesulfonate), PSS (MW ~ 75000, 18% wt.%), poly (allylamine hydrochloride), PAH (MW ~ 15000), TSPP (ε_{413} = 5.1x10^5 M^{-1}cm^{-1}) and TMPyP (ε_{422} =2.49x10^5 M^{-1} cm^{-1}) were obtained from Sigma-Aldrich. Diammonium ethylenediaminetetraacetate monohydrate, EDTA (MW ~326) was obtained from TCI. Sodium hydroxide (NaOH) and Hydrochloride acid (HCl) were used to control pH and were purchased from Sigma-Aldrich. All reagents were used as obtained. Polyelectrolyte solutions (3 mg/mL, 0.5 M NaCl) were prepared in bi-distilled water and adjusting pH at 6.5 (NaOH) or pH 3 (HCl). TSPP, TMPyP solution (3x10^{-4}M) and EDTA were prepared in bi-distilled water.

Preparation of $CaCO_3$. $CaCO_3$ used for the polyelectrolyte core was obtained by adding equal volumes of $CaCl_2$ (0,1M) and Na_2CO_3 (0,1M) and mixed for one minute under intense stirring and let to rest for 5 minutes. $CaCO_3$ microparticles were recovered after supernatant removal followed by three washing/centrifugation cycles.

Preparation of Polyelectrolyte Microcapsules. The polyelectrolyte microcapsules were prepared by dispersing the $CaCO_3$ template in an aqueous PAH (3mg/ml, 0.5M NaCl) solution. After stirring for 30 minutes and centrifugation (6000 rpm, 10 minutes), the particles are recovered and the supernatant removed. After three washing/centrifugation cycles to remove the excess PAH, the particles are resuspended in an aqueous PSS (3mg/ml, 0.5 NaCl) solution. This layer-by-layer deposition technique of opposite charged polyelectrolytes was repeated until four or five polyelectrolyte layers were adsorbed.

Porphyrin Adsorption onto Polyelectrolyte Microcapsules. TSPP (H_4TSPP^{-2} and H_2TSPP^{-4}) and TMPyP were adsorbed onto polyelectrolyte microcapsules with PAH and PSS as the last layer, respectively. A volume of 1.5mL of capsule solution was added to a porphyrin solution (3µM, pH 6.5 or pH 3.0) and mixed for one hour. After centrifugation the supernatant is removed and stored and the functionalized microcapsules are washed three times with distilled water (pH 6.5 or pH 3.0).

Polyelectrolyte Microcapsules Porphyrin Release. It was prepared two solutions that simulate gastric and intestinal conditions [16]. For the gastric solution, it was prepared an aqueous solution with 0,2% (w/v) NaCl with an adjusted pH 2.0 (HCl) and for the intestinal solution it was prepared an aqueous solution with 0,68% (w/v) KH_2PO_4 with an adjusted pH = 7,2 (NaOH). The polyelectrolyte microcapsules are added to the release solution in a cuvette and while mixing slowly, the supernatant fluorescence emission is measure in a spectrofluorometer.

Preparation of Hollow Polyelectrolyte Microcapsules. Hollow polyelectrolyte microcapsules were obtained by suspending microcapsules in EDTA (10 mL, 0.1 M) and stirring for 30 min at room temperature. This process was repeated twice. The prepared hollow polyelectrolyte microcapsules were washed three times with distilled water before further use.

Formation of J-aggregates on Polyelectrolyte Microcapsules. The polyelectrolyte microcapsules were left overnight at room temperature in an aqueous solution of H_4TSPP^{-2} at pH =3.0 without stirring. Afterwards, the supernatant is removed and the PECs are washed with distilled water at pH 3.0.

Methods. A Jasco V-560 spectrophotometer and a Hitachi (U-2000) spectrophotometer were used in UV–vis absorption measurements. Corrected fluorescence measurements were recorded with a SPEX Fluorolog spectrophotometer (Horiba Jobin Yvon).

FLIM measurements were performed with a time-resolved confocal microscope (MicroTime 200, PicoQuant GmbH). The excitation at 639nm was carried out by a pulsed diode laser at a repetition rate of 10/20 MHz. The fluorescence lifetime was detected with a single-photon counting avalanche diode (SPAD) (PerkinElmer) whose signal is processed by TimeHarp 200 TC-SPC PCboard (PicoQuant) working in the time-tagged time resolved (TTTR) operation mode. For point-by-point measurements, fluorescence decays of more than thirty-pixel points were collected from random points chosen within microcapsules.

The zeta potential values were measured in a Doppler electrophoretic light scattering analyser, Zetasizer Nano ZS from Malvern Instruments Ltd.

Transmission Electron Microscopy (TEM) images were obtained by a Hitachi 8100 transmission electron microscope operating at 200 kV with an energy dispersive X-ray spectrometer (EDS).

The light-mediated release was done using with a mounted High-Power LED (595L3) from THORLABS with a nominal wavelength of 595nm allowing to achieve an irradiance of 0,5mW/cm^2.

3. Results and Discussion

H_2TSPP^{-4} interaction with core-shell polyelectrolyte microcapsules. After the TSPP adsorption onto both polyelectrolyte microcapsule systems, it has done a pH optimization in order to obtain the different H_2TSPP^{-4} forms separated (Figure 1,2).

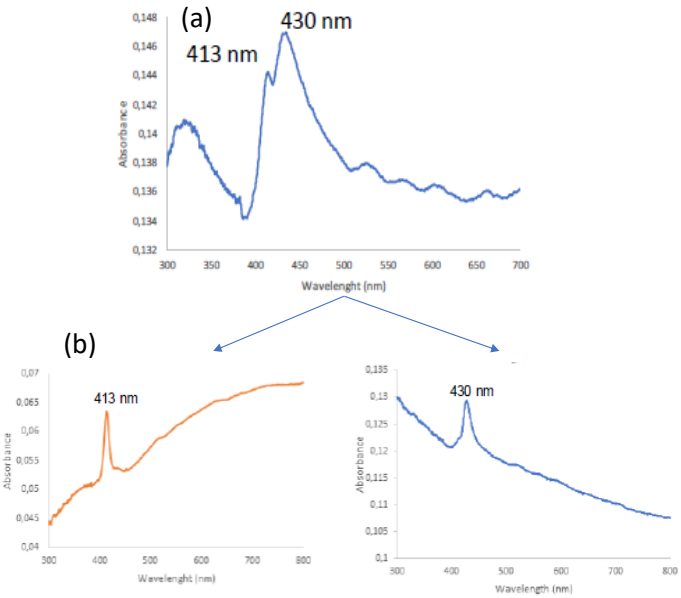

Figure 1 - UV-Vis spectra of $CaCO_3(PAH/PSS)_2PAH$ H_2TSPP^{-4} (before (a) and after pH optimization(b)) and $CaCO_3(PAH/PSS)_2$ H_2TSPP^{-4} systems in water.

It is interesting to note that the nature of the polyelectrolyte last layer as well as the surface charge has a direct outcome on H_2TSPP^{-4} interactions within polyelectrolyte microcapsules. For $CaCO_3(PAH/PSS)_2PAH$ microstructures with a positive last layer, only one band in with a maximum wavelength at 413 nm was observed. In contrast, when H_2TSPP^{-4} was

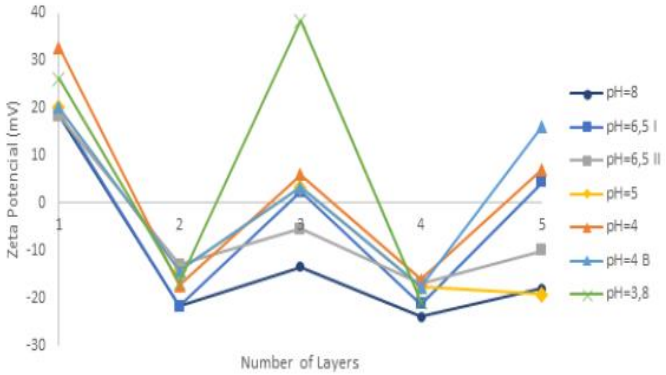

Figure 2 - Effect of the pH of the washing solution on the zeta potential of the polyelectrolyte microcapsule. It was done two different measurements for pH =6.0,5.0 and for pH=4.0 B, only the last layer was washed with pH=4.0 between the rest of them washed with pH=5.0.

adsorbed onto polyelectrolyte microcapsules $CaCO_3(PAH/PSS)_2$ with a negative last layer, the only absorption band observed in the absorption spectra has a maximum at 430 nm.

In $CaCO_3(PAH/PSS)_2PAH$, the low pH should increase the protonation of PAH, leading to a higher adsorption of H_2TSPP^{-4} in interstitial water rich environments, as reflected by the presence of the absorption band at 413 nm. In the second system, the lower protonation of PAH and the last layer PSS increased the electrostatic repulsion of the system. In this case the adsorption of H_2TSPP^{-4} near PSS, probably mediated by counterions, or its location in more hydrophobic environments should be preferred.

H_2TSPP^{-4} interaction with polyelectrolyte solutions. In polyelectrolyte core-shells, the interaction of H_2TSPP^{-4} with each one of the polyelectrolytes was studied by UV-Vis absorption, fluorescence emission and excitation (Figure 3).

The interaction of H_2TSPP^{-4} with PAH is electrostatically favored. This interaction is verified by a broadening of the Soret band in the H_2TSPP^{-4} absorption spectrum and a hypsochromic shift of the maximum wavelength from 413nm to 400 nm, characteristic of H-aggregates formation.

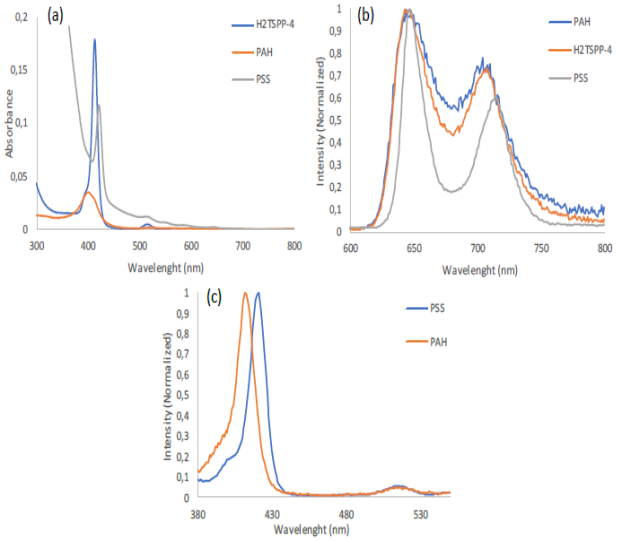

Figure 3 - UV-Vis absorption (a), fluorescence ($\lambda_{exc}H_2TSPP^{-4}$, PAH = 413 nm; λ_{exc}PSS= 420 nm) (b) and excitation spectra(λ = 650 nm) (c) of H_2TSPP^{-4} in free and polyelectrolyte solution.

It was chosen an excitation wavelength of 400 nm to obtain the emission spectrum of H_2TSPP^{-4} in a PAH solution. Nevertheless, the emission spectrum has the same form and both two maximum wavelengths for 650 nm and 708 nm as H_2TSPP^{-4} in aqueous solution at pH=7 (figure 3 b). Also, the excitation spectra obtained at 650 nm (Figure 3 c) overlaps in each point its absorption spectra of H_2TSPP^{-4} in water. Therefore, the fluorescence observed should be a result of the remaining monomeric H_2TSPP^{-4}.

The H_2TSPP^{-4} interaction with PSS is not electrostatically. Nevertheless, the interaction of H_2TSPP^{-4} with PSS promotes a bathochromic shift of the Soret band from 413 nm to 420 nm. When using 420 nm as

an excitation wavelength, the maximum emission wavelength for the solution of H_2TSPP^{-4} and PSS is 650nm and 718nm. The second maximum is the only with a visible change (~10nm).

By comparison of the spectra of H_2TSPP^{-4} in PAH solution with the ones observed previously for microcapsules resuspended in water at pH 6.5, is possible to infer that the interaction of PAH as the microcapsules external layer, does not induce the formation of H-aggregates as it occurs in solution. Two main reasons may be in the origin of this observation: a lower concentration of PAH adsorbed or a preferential location of H_2TSPP^{-4} in more hydrophilic environments such as interstitial water as revealed by the presence of the absorption band at 413 nm and the typical emission bands of H_2TSPP^{-4} in water. Another interesting point is the changes observed in the absorption spectra of H_2TSPP^{-4} due to the presence of PSS. Although the wavelength maxima of the unknown band at 430 nm is 10 nm red shifted when compared to the Soret band of H_2TSPP^{-4} in PSS solution the possibility that the two are due to the same type of interaction should not be disregarded, since the differences accounted for may be related with adsorption.

TMPyP interaction with polyelectrolytes solutions. Similarly to H_2TSPP^{-4}, the adsorption of TMPyP onto polyelectrolyte microcapsules $CaCO_3(PAH/PSS)_2$ was also followed by UV/Vis absorption and Fluorescence Spectroscopy. (Figure 4,5).

It is expected that these differences also arise from an interaction with the polyelectrolyte film.

The interaction of TMPyP with PSS changes the absorbance spectra of TMPyP

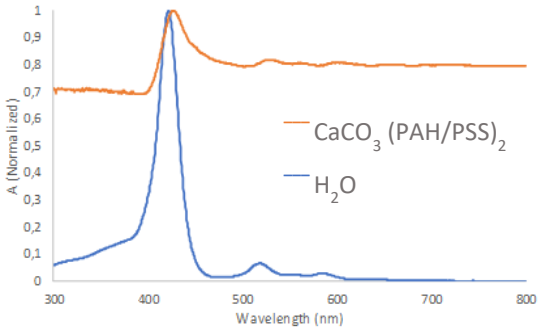

Figure 4 – UV-Vis absorbance spectra of TMPyP absorbed onto polyelectrolyte microcapsules (orange) and in aqueous solution (blue) (pH=7).

in solution. The maximum of the Soret band changes from 422 nm to 433 nm. The interaction with PAH is not favored and the absorbance spectrum does not change from one of the free base porphyrin (figure 5a).

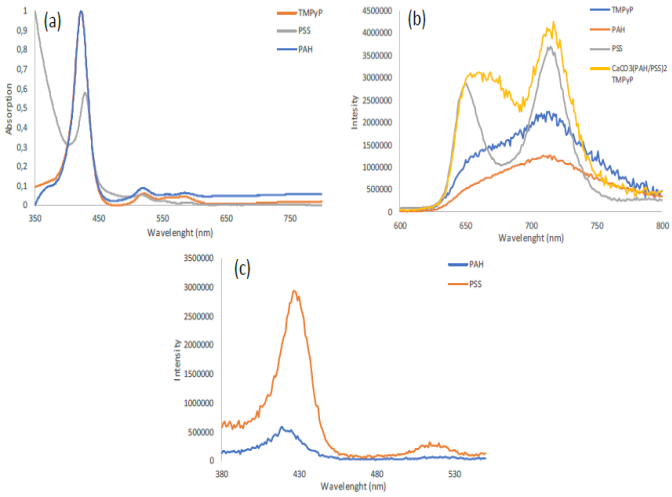

Figure 5 - UV-Vis absorption (a), fluorescence emission (b) and excitation spectra (λ = 650 nm) (c) of TMPyP in polyelectrolyte aqueous solution and adsorbed onto PECs

The broadening observable in the spectrum of TMPyP adsorbed microcapsules are a result of the interaction with the shell. This interaction also promotes the resolution of the emission spectrum and allows a visible separation of the Q (0,0) and Q (0,1) band. This resolution occurs due to the modification of the degree of coplanarity of

the pyridinium group and the electron transfer to the sulfonate group of PSS (figure 5b).

The excitation study of TMPyP in polyelectrolyte solution was done, revealing a maximum at 422 nm in PAH and a maximum at 428 nm in PSS. As the PAH interaction is not favored, the excitation spectrum is overlapping with the absorbance spectrum of TMPyP in aqueous solution. The interaction of TMPyP with PSS is favored and, by the absorbance and emission spectrum is notable this influence. In the excitation spectrum this is confirmed, being obtained a spectrum overlapping the absorbance spectrum of the same solution (figure 5c).

Photosensitizer adsorption by polyelectrolyte microcapsules

The efficiency of a drug delivery system depends on the amount of pharmaceutical that can transport. To characterize and compare our systems the encapsulation efficiency was calculated.

For this, we measured the absorption of the supernatant of a porphyrin solution (H_2TSPP^{-4}/TMPyP, 0.3mg/ml) before and after one hour in contact polyelectrolyte microcapsules. The difference found in absorption is proportional to the concentration of porphyrin adsorbed by polyelectrolyte microcapsules, according to Beer-Lambert law. After separation of the supernatant from the polyelectrolyte microcapsules by centrifugation, the microcapsules were dried in vacuum and weighed (Table 1).

Table 1 – Encapsulation Efficiency calculated for the systems being studied. The values presented were calculated using the mean values of three samples.

System	Encapsulation Efficiency (µg Porphyrin/µg PECs)
$CaCO_3(PAH/PSS)_2PAH$ H_2TSPP^{-4}	2.32 ± 0.06
$CaCO_3(PAH/PSS)_2$ H_2TSPP^{-4}	0.42 ± 0.02
$CaCO_3(PAH/PSS)_2$ TMPyP	1.43 ± 0.08

The system more efficient for encapsulation of porphyrin was the $CaCO_3(PAH/PSS)_2PAH$ H_2TSPP^{-4}. The encapsulation efficiency of this system was almost 1% higher than $CaCO_3(PAH/PSS)_2$ TMPyP and approximately six times higher than $CaCO_3(PAH/PSS)_2$ H_2TSPP^{-4}. These results are as expected as the electrostatic interactions between opposite charged polyelectrolytes and porphyrins are favored.

Characterization by Fluorescence Lifetime Image Microcopy (FLIM) H_2TSPP^{-4} and TMPyP polyelectrolyte core-shell.

FLIM measurements were done to compare both H_2TSPP^{-4} and TMPyP adsorbed in polyelectrolyte microcapsules. The polyelectrolyte microcapsules non-functionalized are non-emissive upon irradiation at λ_{exc}=635nm therefore is possible to conclude that the emission observed only arises from the adsorbed porphyrin (Figure 6). The point-by-point measurements (30 points by system) were

done in the periphery and the core of the polyelectrolyte microcapsules.

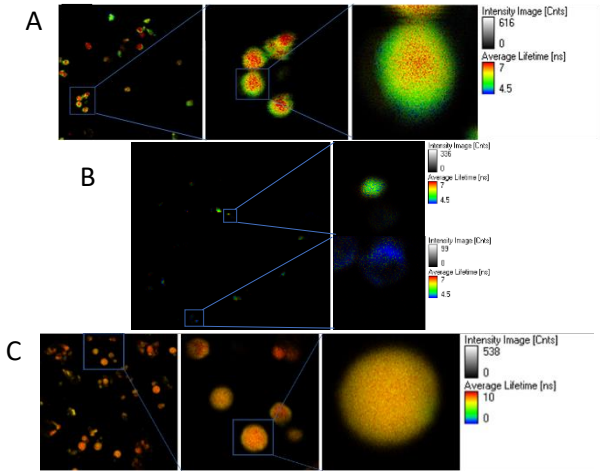

Figure 6 – FLIM images of (a) $CaCO_3(PAH/PSS)_2$ PAH TSPP, (b) $CaCO_3(PAH/PSS)_2$ TSPP and (c) $CaCO_3(PAH/PSS)_2$ TMPyP adsorbed onto PECs at different magnifications.

The FLIM images obtained from the system $CaCO_3(PAH/PSS)_2PAH$ H_2TSPP^{-4} shows micrometric matrix-type fluorescent rounded shapes well dispersed. The fluorescence lifetime results of H_2TSPP^{-4} adsorbed onto PECs can be decomposed in three pre-exponential factors. The longest fluorescence lifetime (6-10 ns) can be attributed to the interaction between H_2TSPP^{-4} and the interstitial waters of the system or with PAH. The medium fluorescence lifetime (2-4 ns) can be attributed to the interaction between H_2TSPP^{-4} and PSS polyelectrolyte. The shorter lifetime (<1 ns) is of complex nature and cannot be easily attributed to a single interaction. $CaCO_3(PAH/PSS)_2$ H_2TSPP^{-4} system shows a heterogeneous system and high variability on fluorescence lifetime decays.

The fluorescence lifetime measures of the TMPyP system revealed complex decays in which two exponential components were always needed to achieve a proper fit is observable a shorter distribution of fluorescence lifetimes (2-4 ns) with a lower contribution that can be attributed to the interaction between TMPyP and the interstitial waters of the system. The longer distribution of fluorescence lifetimes (8-10 ns) with a higher contribution can be attributed to the interactions between TMPyP and PSS.

The decomposition of the fluorescence lifetimes allows to understand the different interactions that occur in the polyelectrolyte microcapsules specially between the porphyrin and the polyelectrolyte multilayer film. Instead of a simple adsorption on the surface of the polyelectrolyte microcapsules, a diffusion of porphyrin towards the core of the system is verified. The porphyrin presence is possible interacting with the polyelectrolyte or in interstitial waters.

Release Study of PECs: Intestinal, Gastric and Light-Mediated conditions. The photodynamic therapy efficiency shows a great dependence on the local concentration of photosensitizer and one of the ultimate goals of this study is to understand if the polyelectrolyte microcapsules may work as an efficient porphyrin delivery method. As the adsorption of porphyrin has already been studied, is important to understand how the system behaves in controlled environments that mimic different parts of the human body. $CaCO_3(PAH/PSS)_2$ PAH H_2TSPP^{-4}, $CaCO_3(PAH/PSS)_2$ H_2TSPP^{-4} and $CaCO_3(PAH/PSS)_2$ TMPyP systems were studied. Its observed that the system $CaCO_3(PAH/PSS)_2$ PAH H_2TSPP^{-4} has

higher amount of H_2TSPP^{-4} released in the intestinal solution (pH = 7.2, 0,68% (w/v) KH_2PO_4). (Table 2).

Table 2 –Release of H_2TSPP^{-4} or TMPyP (%) based on the amount of porphyrin adsorbed onto the polyelectrolyte microcapsules

System	Porphyrin Release (%)
$CaCO_3(PAH/PSS)_2$ PAH H_2TSPP^{-4}	2.18
$CaCO_3(PAH/PSS)_2$ H_2TSPP^{-4}	0.92
$CaCO_3(PAH/PSS)_2$ TMPyP	1.98

The porphyrin release is very low comparing with the overall amount of porphyrin that has been adsorbed in the polyelectrolyte microcapsules. $CaCO_3$ $(PAH/PSS)_2$ TMPyP release values were near the release percentage of $CaCO_3(PAH/PSS)_2PAH$ H_2TSPP^{-4}. In the system $CaCO_3(PAH/PSS)_2$ H_2TSPP^{-4} the release values were even lower (~1%).

The sustained release of porphyrin in a neutral pH environment is not very effective which means that the system is stable is systemic circulation (Data not shown).

For the release study at gastric conditions one problem occurring was the low pH degrades the $CaCO_3$ core of the polyelectrolyte microcapsules. This promotes a neutralization of the environment pH causing oscillating inflating-deflating cycles due to CO_2 formation and release, promoting the capsule collapse. It is believed that this effect promotes the higher porphyrin release but also the release of polyelectrolytes to the supernatant as the destruction of the microcapsule occurs. As polyelectrolyte microcapsules do not have enough weight to stay in the bottom part of the cuvette, they rise due to the stirring motion affecting the release kinetics measures. Due to this, only the supernatant measured after centrifugation and removal of the microcapsules, is used for the calculation of the release of porphyrin.

In a sustained release (five hours), where the release solution was replaced in order to maintain the environment pH, it was achieved 87.72% H_2TSPP^{-4} release (Table 3). It was noticeable a reduction of the reddish tone of the polyelectrolyte microcapsule during the release kinetics. Is possible to conclude with this result that if the medium in which the polyelectrolyte microcapsules are present is not neutralized the release of total H_2TSPP^{-4} adsorbed in the system is inevitable after more than five hours.

Table 3 – Sustained Release of H_2TSPP^{-4} or TMPyP (%) based on the amount of porphyrin adsorbed onto the polyelectrolyte microcapsules in gastric conditions.

System	Time (min)	Porphyrin Release (%)
$CaCO_3$ $(PAH/PSS)_2$ PAH H_2TSPP^{-4}	300	87.72
$CaCO_3$ $(PAH/PSS)_2$ TMPyP	450	47.00

Light-mediated burst release was found interesting to study in this system due to the presence of a fluorophore and to study the impact of light irradiation on the polyelectrolyte microcapsule system. The light-mediated release efficiency was equal to release conditions without light irradiation. Previous works already showed the degradation of polyelectrolyte microcapsules functionalized with H_2TSPP^{-4} but only with H_2O_2 present in solution [17].

The difference in results may result due to a lower oxidative environment and weaker LED intensity. Although the results were different from what was expected a light-mediated release is not efficient as a remote physical release method. Nevertheless, it shows that tracking of polyelectrolyte microcapsule delivery can be possible without compromising the drug delivery process.

Core-assisted formation of porphyrin J-aggregates in pH- sensitive polyelectrolyte microcapsules. It is also reported the formation of H_4TSPP^{-2} J-aggregates on the polyelectrolyte microcapsule shell. Using different spectroscopic techniques, it is showed that J-aggregation is favored in core−shell PECs in contrast to hollow polyelectrolyte microcapsules and $CaCO_3$ inorganic microparticles. It is assumed the existence of a core and polyelectrolyte synergistic effect to sustain this observation. Furthermore, three fundamental steps support the directional growth of J-aggregates: porphyrin adsorption in the polyelectrolyte shell and in the core, stabilization of the porphyrin release from the $CaCO_3$ core by its capture in the polyelectrolyte shell network and aggregate growth initiated by the increase in the local porphyrin concentration due to core release. At lower pH values (pH<3.0), the polyelectrolyte shell becomes unstable and core−shell collapse is substantial. Also shifting the last polyelectrolyte layer charge has a negative outcome in porphyrin J-aggregation formation and growth.

Conclusions. A system with two porphyrins is reported and characterized for the use of PDT in cancer therapy. By combing an inorganic core with pH-sensitive polyelectrolytes (PAH/PSS) is possible to control and optimize the encapsulation efficiency of the system as well to increase the release load in different pH environments. Using information collected by various techniques, such as steady-state and time-resolved emission, zeta potential, UV-vis absorption and fluorescence lifetime image microscopy (FLIM) it was possible to further understand the polyelectrolyte interaction with H_2TSPP^{-4} and TMPyP and its importance on optical properties of the system. It is also reported that in mild pH conditions (pH 3.0), the formation of H_4TSPP^{-2} J-aggregates on polyelectrolyte microcapsules core-shells occurs highlighting the core influence and the effects of lowering pH and changing the last polyelectrolyte layer has on the system and J-aggregates formation. It has been reported and analyzed the potential of this system for both biomedical and biotechnological applications.

Future remarks. Is important as future work to increase the potential therapeutic effect of the system. One way to achieve this is to take advantage of pathological characteristics of the disease such as enhanced permeability and retention of macromolecules and porphyrinoids (EPR). Polyelectrolyte microcapsules surface modification must be considered: Adsorption of PEG or the use of thiol groups or thiomers, can be done in order to increase accumulation of the system in the target area. To actively target a specific tumor,

surface or polyelectrolyte modification should be done with the use of aptamers or immunoglobulins that target over-expressed molecules or membrane receptors. Porphyrin chemical modification, such as introduction of halogens should increase intersystem crossing by spin-orbiting coupling interaction increasing singlet oxygen quantum yield.

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