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Artigo

PHYSICO-CHEMICAL PROPERTIES OF *meso*-TETRAKIS(*p*-METHOXYPHENYL)PORPHYRIN (TMPP) INCORPORATED INTO PLURONIC™ P-123 AND F-127 POLYMERIC MICELLES

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The physicochemical properties (solubilization, structural organization and stability) of *meso*-tetrakis(*p*-methoxyphenyl)porphyrin (TMPP), a promising photosensitizer for photodynamic therapy, solubilized in polymeric micelles of tri-block copolymers Pluronic™ P-123 and F-127, were studied. The formulations obtained by the solid dispersion method led to monomerization of TMPP in these copolymers. Solubility studies showed that P-123 solubilizes double the photosensitizer than F-127. The self-aggregation phenomenon was affected by the [TMPP]/[poloxamer] ratio and medium temperature. The decrease in the temperature of these systems promoted the formation of different kinds of TMPP aggregates intrinsically connected with the structural changes occurring in the micelles.

Keywords: *meso-tetrakis(p-methoxyphenyl)porphyrin*; polymeric micelles; photodynamic therapy.

INTRODUCTION

Photodynamic therapy (PDT) is a medical procedure used to treat various diseases caused by rapid and disordered tissue growth, such as cancer.[1] Photofrin™ was the first officially approved PDT drug.[2] Temoporphin, a second generation photosensitizer (PS), known as *m*-tetra(hydroxyphenyl)chorin (*m*-THPC) and commercialized by Biolitec Pharma Ltd. under trade name Foscan™, was found to be up to 200 times as potent as Photofrin™,[3,4] but the relationship was less clear for m-THPC and BCA. In vivo/in vitro experiments were performed after Photofrin or m-THPC PDT in order to assess direct tumour kill (immediate plating Methoxyphenyl porphyrins possess physico--chemical properties similar to those of hydroxy-derivatives and have shown efficient photo-activity and selectivity in the induction of tissue necrosis.[5,6] Its *m*-isomer proved to be 25-30 times more efficient than the hematoporphyrin derivative (HpD) in *in-vivo* bioassays performed with tumor tissues.[7] *Meso-tetrakis(p-methoxyphenyl)porphyrin* (TMPP) (Figure 1A) can be considered a candidate to photosensitizer for the study and application in PDT, since photophysical studies have shown that porphyrin derivatives are effective photosensitizers. This class of PS is effective in the formation of singlet oxygen, which is the main cytotoxic specie in PDT. Porphyrin derivatives have been evaluated successfully in the inactivation of several carcinogenic cell types.[8,9]10,15,20-tetrakis(methoxyphenyl

The main disadvantage of this type of PS is its low solubility in aqueous media. Although hydrophobic properties favor the PS absorption by the cell membrane, they make difficult the formulation and administration process. Its biodistribution is influenced mainly by precipitation in the bloodstream and complexation with low density lipoproteins (LDL). Furthermore, high hydrophobicity leads to PS self-aggregation, affecting its photophysical properties and thereby reducing its effectiveness as a PS. Due to these limitations, it is necessary to use biocompatible carriers for this class of PS.[10]

Polymeric micelles have shown attractive solubilizer and carrier systems for PS to applications in PDT. Among other advantages

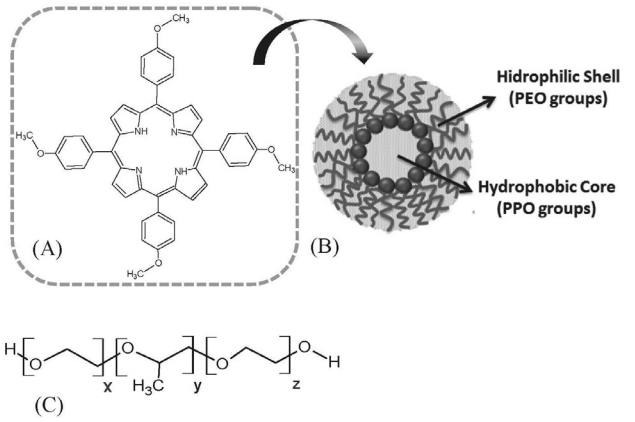

Figure 1. *(A) Molecular structure of TMPP, (B) representation of polymeric micelle and (C) Molecular structures of Pluronic™ P-123 and F-127; x = z = EO groups = 20 and y = PO groups = 70 for P-123 and x = z = EO groups = 106 and y = PO groups = 70 for F-127*

stands out the possibility of modifying the micellar surface with the aim of increasing the selectivity for specific target tissues, as well as use the properties relative to stability of the micelle (pH, temperature and concentration of copolymer) to optimize drug delivery processes through controlled release.[11] For this reason, poloxamer polymeric micelles (Figure 2B) were chosen as carrier systems to evaluate the effectiveness of TMPP as PS in PDT. The use of polymeric micelles is known to be effective as biocarriers of hydrophobic compounds, which can be trapped and/or bound to the micellar core through hydrophobic interactions promoting the stabilization of these molecules in aqueous medium.[12,13]

In clinical/pharmaceutical applications it is interesting to use neutral surfactants such as poloxamers, known commercially as Pluronic™, due to their low toxicity when compared to conventional surfactants.[14,15] particularly, anthracycline antibiotics. Furthermore, Pluronic affects several distinct drug resistance mechanisms including inhibition of drug efflux transporters, abolishing drug sequestration

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in acidic vesicles as well as inhibiting the glutathione/glutathione S-transferase detoxification system. All these mechanisms of drug resistance are energy-dependent and therefore ATP depletion induced by Pluronic block copolymers in MDR cells is considered as one potential reason for chemosensitization of these cells. Following validation using in vitro and in vivo models, a formulation containing doxorubicin and Pluronic mixture (L61 and F127 Poloxamers are triblock copolymers formed by a central block composed of repetitive units of propylene-oxide (PO) bonded to the lateral blocks of ethylene-oxide (EO) (Figure 1C). The central block (PPO group) form the core of the polymeric micelle which is capable of accommodating molecules that have little affinity with the aqueous medium outside the micelle. The lateral blocks (PEO groups) form external shell of the micelle which has relative affinity for water and serves as an intermediary region between the aqueous medium and the hydrophobic core (Figure 1B).[16-18]

Moreover, polymeric surfactants are more stable, having a relatively low CMC, about 7.0 μmol L^{-1} at 25 °C for P-123 (Figure 1C). Therefore, they are more resistant to the dilution effects that occur during drug administration.[19] Their biocompatibility and relatively small size help to prevent the recognition of the micelles by proteins and macrophages, allowing a greater circulation time.[15] Furthermore, a greater selectivity can be achieved by modifying the peripheral chains with the addition of selective ligands.[20,21]

In the particular case of porphyrin derivatives was observed that the environment exerts a strong influence on the excited state of the PS, since the tetrapyrrole ring establishing hydrogen bonds between the nitrogen atoms and the water molecules.[11] This favors the dissipation of energy through non-radiative processes, impairing photophysical processes fundamental for PDT such as lifetime in the excited triplet state. Porphyrin derivatives generally show similar behavior in organic solvents and micellar solutions, with high sensitivity to variations in polarity and low tendency of a molecule to join another via self-aggregation process. Is important to emphasize that the PS in it self-aggregated form loses the efficiency of generation of singlet oxygen which makes it unfeasible to photodynamic action.

Thus, in this work polymeric surfactants Pluronic™ P-123 and F-127 (Figure 1C) were used to formulate/solubilize TMPP in aqueous media and evaluate relevant properties suitable for PDT application, such as molecular organization, stability and the influence of temperature on their physicochemical properties.

EXPERIMENTAL

Materials

Meso-tetrakis(*p*-methoxyphenyl)porphyrin (TMPP 95%, MM = 786.34 g mol^{-1}) and polymeric surfactants P-123 (MM = 5800 g/mol) and F-127 (MM = 12600 g mol^{-1}) (Figure 1C) were purchased from Sigma-Aldrich. The ethanol used for solubilization into copolymers was of high purity. The compounds were used as purchased.

Solubilization of TMPP in F-127 and P-123 poloxamers

Formulations containing TMPP solubilized in aqueous system of poloxamer (varying from 1.0 to 8.0%, w/V) were prepared using the solid dispersion method:[22] this method consists on the solubilization of TMPP (weights varying from 0.50 to 4.00 mg, according with the solubilization capacity of the surfactant system) and block copolymer surfactant in dichloromethane. The solvent was removed by rotatory evaporation at 50 °C for 20 min, resulting in a thin solid TMPP/poloxamer matrix film. The matrix was kept in a desiccator under reduced pressure for 24 h. The TMPP/poloxamer matrix was rehydrated by heating the samples to 60 °C under stirring (Dubnoff metabolic shaking) for approximately 7 h. The final volume of the TMPP/poloxamer solutions to obtain the desired concentrations was 25 mL. Background samples (without TMPP) were prepared for spectroscopic by the same technique.

Subsequently, the solutions were transferred to 25 mL test tubes with 3.0 cm diameter and rested for 24 h for precipitation of not-bound water-insoluble TMPP. Next, three aliquots of supernatant were carefully taken from three different parts of the tubes (top, middle and just above the bottom) and transferred to other test tubes to ensure that the encapsulated TMPP was homogeneously distributed within the micellar solutions. UV-Vis spectra of the samples were recorded for quantification of the solubilized photosensitizer within the micellar microenvironment.

Evaluation of TMPP solubility in Pluronic™ P-123 and F-127 polymeric micelles

The molar absorption coefficient values (ε) of TMPP in the micellar microenvironment were evaluated for the quantification of the incorporated photosensitizer. TMPP (4.76 μmol L^{-1}) incorporated into P-123 micelles 2.0% (w/V) was prepared and used to determine the molar absorption coefficient. This low TMPP concentration ensured the total solubilization of the photosensitizer. The molar absorption coefficient for the main bands was estimated using the Beer-Lambert treatment from electronic absorption spectra obtained at 30 ºC.[20] The molar absorption coefficient of TMPP (6.0 μmol L^{-1}) incorporated into F-127 2.0 (% w/V) was determined by the same procedure.

The solubility of TMPP in surfactant aqueous solutions was determined at P-123 concentrations between 8.7×10^{-4} mol L^{-1} (0.50% w/V) and 1.3×10^{-2} mol L^{-1} (8.0% w/V), and F-127 concentrations between 4.0×10^{-4} mol L^{-1} (0.50% w/V) and 6.4×10^{-3} mol L^{-1} (8.0% w/V), at 30 °C. The TMPP concentrations were determined by spectrophotometry using analytical wavelength (λ) at 421 nm.

The solubilization capacity, χ (mol of TMPP/ mol of poloxamer units) was calculated using general Equation 1 for micellar solubilization:[23]

$$S_{total} = S_w + \chi C_{s,mic} \quad (1)$$

where S_{total} is the total solubility of TMPP, S_w is the solubility of TMPP in water, C_s is the total molar concentration of surfactant (monomer and micellar form) and $C_{s,mic}$ is the molar concentration of surfactant only in the micellar form, which can be estimated as (C_s-CMC). Since the CMC values of poloxamers are very small (in the order of 10^{-6}-10^{-5}), and as in Equation 1 $C_{s,mic} = C_s$ - CMC, it was considered approximately equal to C_s, and for pure TMPP S_w was considered negligible in aqueous medium, leading to the final equation:

$$S_{total} = \chi C_s \quad (2)$$

Stability of TMPP encapsulated in P-123 and F-127 polymeric micelles submitted to freeze-drying/rehydration process

Formulations containing TMPP (4.0×10^{-5} mol L^{-1}) in either P-123 or F-127 8% (w/V) were frozen in liquid nitrogen and freeze-dried in drying equipment (MicroModulyo – Freeze-Dryer) for 48 h. The solid obtained was stored at -5 °C and thereafter rehydrated with water at 60 °C. The UV-Vis spectra of the solutions were recorded before the lyophilization process, immediately after the lyophilization/rehydration procedure, and also monitored every day by one month.

Effect of temperature on the self-aggregation of TMPP encapsulated into P-123 or F-127

Critical micellar temperature (CMT) is the lowest temperature that can be found micelles formed by N > 1 unimers of the copolymer, where N is called aggregation number (N_{agg}). The CMT is a function of copolymer concentration and at temperatures below the CMT value the unimers are individually matted , forming what the literature describes as "monomeric micelles".[19] Solutions (10 μmol L^{-1}) of TMPP incorporated into P-123 and F-127 1 at 8% (w/V) were used in this study. The changes in the UV-Vis spectra of TMPP as a function of time and at several temperatures above and below the poloxamer critical micellar temperature (CMT) (30 °C, 25 °C, 20 °C, 15 °C, 13 °C and 10 °C) were monitored. CMT values were obtained by Equation 3.[19]

$$CMT = CMT° - A\,(w) \quad (3)$$

where CMT° is the value obtained at infinite dilution, *w* is the surfactant concentration (% w/V) and *A* (0.38 and 0.59 for P-123 and F-127, respectively) is a constant equivalent to the slope of the graph TMC versus poloxamer concentration.[19] The CMT° values for P-123 8% and 1% (w/V), are 14.8 and 17.4 °C, respectively, and for F-127 8% and 1% (w/V) are 15.7 and 19.8 °C respectively.[24] This equation is valid for these poloxamers, as long as their concentrations are lower than 30% (w/V). Additionally were estimated aggregate constants of TMPP in temperature of 15°C (below CMT), fitting the experimental data with a kinetic model of order 1.

Depth-allocation evaluation of PS into the micelle of the Pluronic™ P-123 and F-127

Studies of fluorescence quenching are used for determining the distribution coefficients and depth allocation of the hydrophobic molecules in biological membranes models such as micelles and liposomes. Accessibility of a water-soluble fluorescence suppressor to the encapsulated drug is the parameter obtained from the decrease in fluorescence emission intensity and calculation of Stern-Volmer constants (K_{SV}).[25] Fluorescence quenching studies with iodide as a hydrophilic quencher were performed by adding aliquots of a KI solution (1.0 mol L^{-1}) to 3.0 mL of TMPP (2.6×10^{-7} mol L^{-1}) in either P-123 or F-127 2% (w/V) (both at 30 °C). The emission spectra were recorded 5 min after each addition.

RESULTS AND DISCUSSION

Spectral characteristics of TMPP incorporated into Pluronic™ P-123 and F-127

The spectrum of TMPP incorporated into P-123 8% (w/V) by the solid dispersion method is shown in Figure 2.[22] TMPP in P-123 presented a Soret band characterized by $S_0 \rightarrow S_2$ (λ = 421 nm) transitions and four Q bands, Q_{IV}, Q_{III}, Q_{II} and Q_I, at 516, 553, 593 and 650 nm, respectively, relative to $S_0 \rightarrow S_1$ transitions.[26] The profile and absorption maximum values in this spectrum are similar to those obtained for monomeric TMPP in dichloromethane indicating that PS was incorporated in monomeric form in this system. Additionally the profile of absorption spectra of TMPP in F-127 is similar to P-123. The characteristic spectrum of the monomeric form is an indication that PS undergoes a chemical environment similar to that found in nonpolar organic solvent, in other words, it is situated in the hydrophobic core of the micelle, in a deeper region than the hydrophilic corona. This is an important result in studies of TMPP for Photodynamic Therapy in aqueous systems because PS in aggregate form has the quantum yield of singlet oxygen generation strongly hindered. Biological fluids are composed mostly of water, an environment that leads hydrophobic molecules as TMPP to self-aggregation process. However, the Pluronic™ micelles of P-123 and F-127 have been good solubilizer-carrier systems for TMPP, keeping it free of self-aggregation to achieve the target tissues.

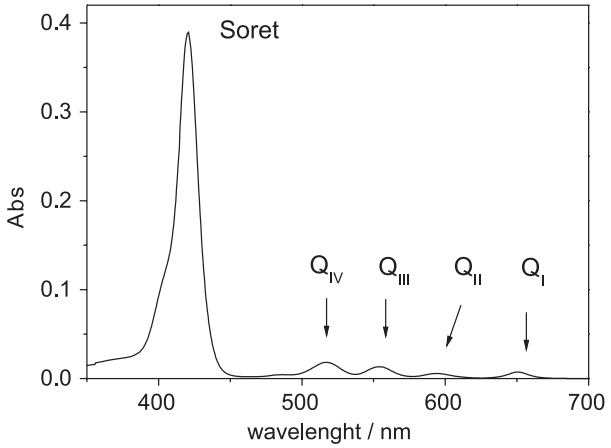

Figure 2. *Electronic absorption spectrum of TMPP (1 × 10^{-3} mol L^{-1}) incorporated into P-123 8% (w/V; 1.3 × 10^{-2} mol L^{-1}) at 25 °C*

The ε values of TMPP in P-123 and F-127 micellar media were obtained using the Beer-Lamber law and are presented in Table 1.

Table 1. Molar absorption coefficient of TMPP in Dichloromethane, P-123 2% (w/V; 3.5 mmol L^{-1}) and F-127 2% (w/V; 1.6 mmol L^{-1}) aqueous solutions at 25 °C

		Dichloromethane	P-123	F-127
Band	λ_{max} (nm)	ε (10^3 L mol^{-1} cm^{-1})	ε (10^3 L mol^{-1} cm^{-1})	ε (10^3 L mol^{-1} cm^{-1})
Soret	421	476	391	374
Q_{IV}	516	13.9	13.4	12.8
Q_{III}	553	9.30	9.90	9.40
Q_{II}	593	4.30	3.40	3.70
Q_I	650	5.10	4.90	4.70

The emission fluorescence spectra of TMPP in P-123 (Figure 3) also show that it is in monomeric state in homogeneous media.

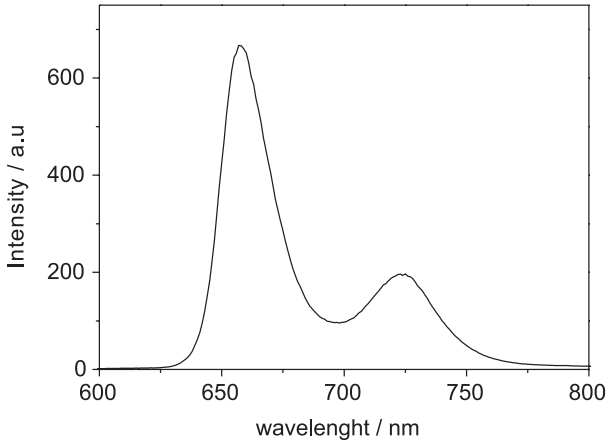

Figure 3. *Emission spectra of TMPP (1.8 × 10^{-6} mol L^{-1}) incorporated into P-123 8% (w/V; 1.3 × 10^{-2} mol L^{-1}) at 25 °C (λ_{exc}: 420 nm)*

The emission spectrum of TMPP in P-123 (Figure 3) presents two bands at maximum wavelengths (λ_{max}) of 658 and 722 nm. These bands refer to $S_1 \rightarrow S_0$ transitions; however, involving vibrational energy levels between these two electronic states.[27]

Solubility studies of TMPP in P-123 and F-127

After the determination of the ε values of TMPP in P-123 and F-127 aqueous solutions, the relationships between the photosensitizer incorporation capacity and the concentration and type of poloxamer were evaluated. The actual amounts of PS solubilized in the formulations were determined from ε values (Table 1) and maximum absorbance of the Q bands for each formulation concentration. It is noteworthy that the average solubility value was estimated considering the preparation of at least six formulations for each poloxamer concentration (% w/V).

Figure 4 shows the TMPP solubility plots varying concentrations of P-123 and F-127. TMPP solubility increased linearly with the increase in the surfactant concentration, as shown in Equation 1. Although the amounts of TMPP incorporated for each concentration (% w/V) of P-123 and F-127 were close, the molar concentrations of surfactants used were about two times higher for P-123 than for F-127.

The solubility constant, obtained from the plot slope in Figure 4 was 0.00975 $n_{TMPP}/n_{P\text{-}123}$ for P-123, which provides approximately one TMPP molecule for about 103 polymer molecules. It is noteworthy that at 30 °C the aggregation number of P-123 is 228;[19] therefore, leading to two TMPP molecules per P-123 micelle on average at this temperature. In the studies with F-127, the solubility constant obtained from the plot slope in Figure 4 was 0.00205 $n_{TMPP}/n_{F\text{-}127}$, which provides approximately one TMPP molecule for about 49 polymer molecules. At 30 °C, the aggregation number of F-127 is 67;[19] therefore, leading to approximately one TMPP molecule per F-127 micelle at this temperature.

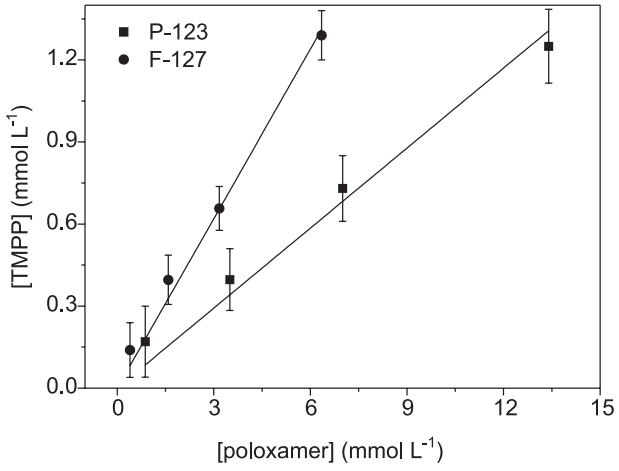

Figure 4. *Solubility curve of TMPP in P-123 and F-127 (concentration range for P-123: 0.14 × 10^{-3} to 1.30 × 10^{-3} mol L^{-1} and for F-127: 0.15 × 10^{-3} to 1.25 × 10^{-3} mol L^{-1}) at 30 °C to determinate the solubility coefficients*

The higher solubility of P-123 in comparison to F-127 can be related to structural difference between both copolymers, where the P-123 has a lower number of EO (ethylene oxide - about five times lower - Figure 1C). This structural difference makes P-123 a more hydrophobic copolymer than F-127. It is worth mentioning that it was not possible to obtain a more efficient and simpler method of direct solubilization of TMPP into the micellar aqueous solutions, i.e., the direct addition of TMPP to P-123 and F-127 micellar solutions under constant agitation at room temperature. Hydrophobicity of the TMPP makes its molecules remain mostly self-aggregated in aqueous medium and even precipitate in solid form (large aggregates). Therefore, only the solid dispersion method produced homogeneity and a significant incorporation rate of TMPP into the micellar systems, which reflected on macroscopic characteristics and photochemical properties of the aqueous media.[22,28]

Additionally the formulations TMPP/copolymers were evaluated as their stability after process of lyophilization-rehydration process using UV-Vis technique (Figure 5).

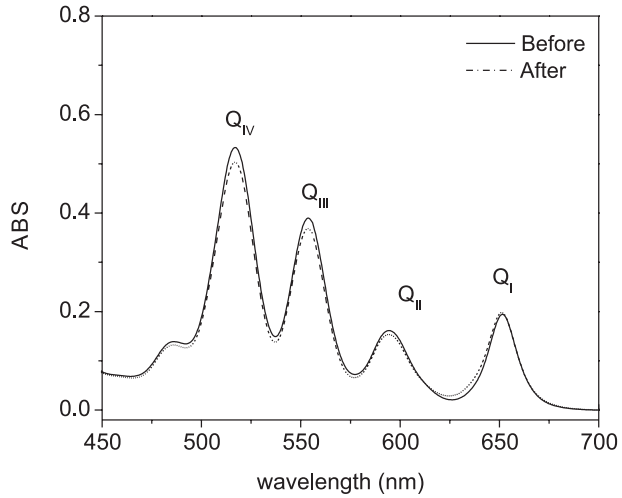

Figure 5. *Variations in the UV-Vis spectra (Q bands) of TMPP (4.0 × 10^{-5} mol L^{-1}) in P-123 aqueous solution 8% (w/V) before and after of freeze-drying/rehydration process. The effect in F-127 was similar*

The absorption spectra of TMPP solubilized in P-123 showed that PS remained stable even after lyophilization-rehydration. The results for formulation TMPP/F-127 were similar (spectra not shown). Therefore, lyophilization of the TMPP-poloxamer formulations followed by re-suspension did not affect the quantity and types of species incorporated (Figure 5). Furthermore none change were observed in these formulations by one month of monitoration. In addition, the lyophilized and rehydrated formulations solubilized faster than the non-treated TMPP/poloxamer matrix. This can be explained by the fact that the structure of the lyophilized material had small pores formed by water sublimation, which must have facilitated rehydration.

Stability of TMPP in copolymers as a function of temperature

The micellization process for polymeric surfactant is known as dependent of temperature. Aggregation number, hydration of the shell and size of the micelle are factors strongly influenced by temperature. Thus, identify and characterize possible changes in the structural organization of the photosensitizer as a consequence of the micellar dynamics is relevant. This study was conducted for the poloxamers in various formulations: P-123 (1 and 8% w/v) at 15 and 10 °C, and F-127 (1 and 8% w/v) at 15 and 13 °C. These temperatures were selected for the respective concentrations of polymeric surfactants in order to monitor the self-aggregation effect on TMPP in conditions above and below the CMT of the copolymer.[19]

When the P-123 (1 and 8%, w/V) samples were cooled from 30 to 20 °C, no significant changes were observed in the TMPP UV-Vis spectra for these systems. Therefore, with the subsequent temperature change from 20 to 15 °C, no effect was observed for the P-123 8% (w/V) system, while for P-123 1% (w/V), the TMPP spectrum changed with the pattern observed in Figure 6A, with CMT lower

than 17.4 °C.[29] By lowering the temperature of the samples to 10 °C, the spectral changes also became noticeable for the P-123 8% (w/V) system (Figure 6B), with CMT of 14.8 °C.[30] This corroborates the hypothesis that the spectral changes observed for TMPP resulted from changes in the structural organization of the polymeric micellar system under the influence of temperature. When the system is submitted to temperatures below the CMT of the copolymer, the micelles begin to destabilize exposing the TMPP to the contact with water molecules, which leads this PS to self-aggregate.

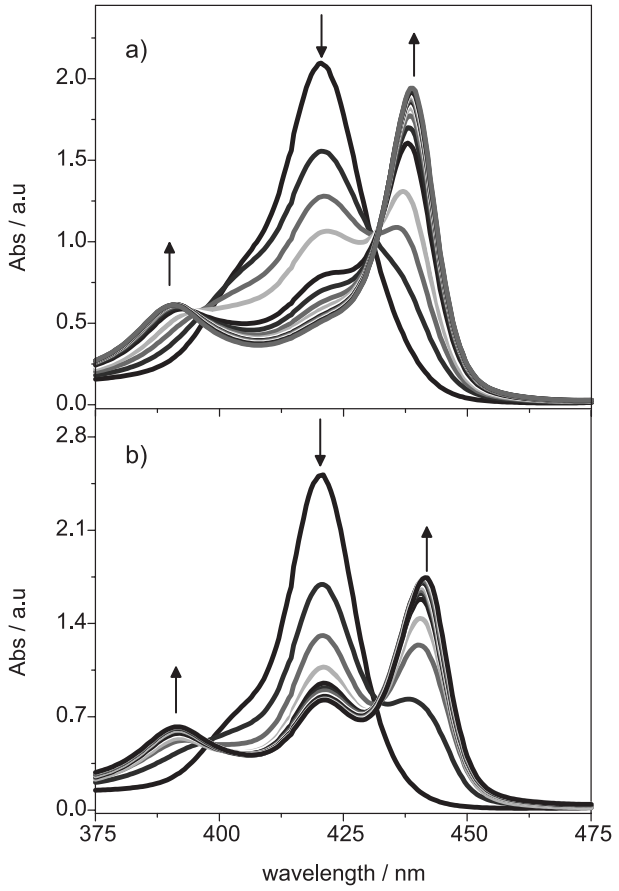

Figure 6. *Variations in the UV-Vis spectra of TMPP (10 μmol L^{-1}) in aqueous solution for (a) P-123 1% (w/V; 1.7 mmol L^{-1}) at 15.2 °C and (b) P-123 8% (w/V; 13.4 mmol L^{-1}) at 10.1 °C with time (min) starting at t = 0. The first 12 spectra were collected every 10 min and the others every 15 min*

The spectral changes observed in both cases were characterized by a marked decrease in the intensity of the Soret band at 421 nm and the rise of two additional bands, one with a blue shift to 389 nm and another with a red shift to 438 nm, relative to the Soret band mainly in the first 50 min of total time of 3.5 h (Figure 6). For the poloxamer formulations with TMPP concentrations lower than 10 μmol L^{-1} (35-10 °C), the spectrum remained the same, probably due to the low [TMPP]/[P-123] ratio, which reduced TMPP self-aggregation in these conditions.

When the F-127 samples (1 and 8%, w/V) were cooled from 30 to 25 °C, no spectral changes were observed. By decreasing the temperature of the samples to 15 °C, the intensity and number of bands in the TMPP electronic spectra changed noticeably for the TMPP solution (10 μmol L^{-1}) for F-127 1% (w/V), CMT of 19.8 °C.[31] However, similar changes were observed for the F-127 8% (w/V) solution only at 13 °C (not shown), CMT of 15.7 °C.[31] At TMPP concentrations lower than 10 μmol L^{-1} (35-10 °C) in these systems no spectral changes were detected.

Additionally, in experiments with TMPP in monomeric state in homogeneous media (dichloromethane and ethanol), a subsequent decrease in temperature to 10 °C produced no spectral changes in this system. This result reinforces the hypothesis that TMPP self-aggregation is due to structural changes in the polymer micelle (micelle to unimer) with decreasing temperature.

The studies of TMPP self-aggregation *versus* temperature showed the rise of two new absorption bands, which indicate the formation of different types of aggregates in these systems. In most cases, the TMPP self-aggregation equilibrium inside the poloxamer monomers was reached just after the decrease of the medium temperature below the CMT. The TMPP self-aggregation process showed to be irreversible once the increase in the temperature system up to 60 °C (much above the poloxamer CMT), did not favor monomer formation. Furthermore, as shown in Figure 6, for P-123 formulations, no isosbestic points were observed. This suggests that two different types of self-aggregates are in equilibrium with the monomer.[32] The same was noticed for the F-127 formulations (not shown).

In general, self-aggregation causes a reduction in the apparent absorptivity of the monomer band of porphyrin.[10] Simultaneously, a decrease or increase in intensity and/or a displacement of the monomer band or even the formation of additional bands, related to the formation of porphyrin aggregates can be observed.[33] The presence of an additional aggregate band is explained by the Exciton Theory proposed by Kasha.[34] The development of these bands depends on the relative orientation of the transition dipole moments of the monomers that form the aggregates.[35] The transitions allowed for J-type dimers have a lower energy, which shifts the aggregate peak to longer wavelengths (red shift). On the other hand, the transitions allowed for H-type aggregates have more energy, which causes a shift of the aggregate peak to shorter wavelengths (blue shift).[34]

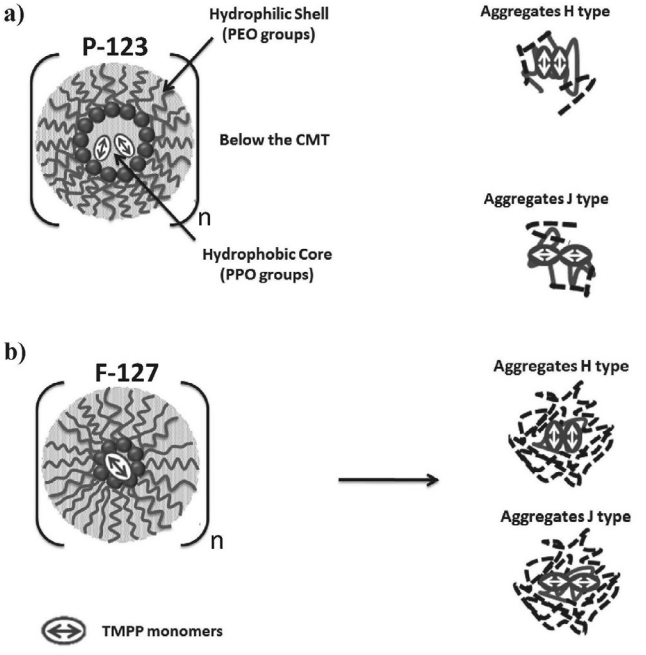

Figure 7. *Simplified representation of the TMPP self-aggregation process below the CMT in P-123 (a) and F-127 (b) polymeric solutions*

The TMPP aggregation constants were determined in temperature below of CMT (10 °C). The electronic absorption intensity was monitored at 421 nm and the aggregation kinetic constant was obtained by fitting of the experimental points using a kinetic model of first order. The values found are shown in Table 2. Kinetics decay of electronic

absorption of the TMPP was monitored for a total time of 3.5 h. The first 12 spectra were recorded at 10 min intervals (others every 15 min) (Figure 6). Also is interesting to note that the TMPP self-aggregation below the copolymer CMT (in the monomer microenvironment of copolymer) is faster in P-123 than in F-127 (Table 2). This is due to faster kinetics of demicellization for P-123 comparing to F-127.

A possible schematic representation of the TMPP/poloxamer system and a simplified mechanism for the TMPP self-aggregation process (H and J self-aggregates) below the CMT for each poloxamer type in the micellar systems studied are presented in Figure 7. Upon analysis of the two schemes (Figure 7) and based on the solubility studies, it becomes clear that in addition to the smaller number of TMPP molecules solubilized in each F-127 micelle (about 1 TMPP molecule per micelle) in relation to P-123 (about 2 TMPP molecules per micelle), the greater size of PEO chains in the F-127 monomers contributes to further stabilize the TMPP molecules in the polymeric microenvironment, because the micelle of F127 takes more time to disassemble at temperatures below the CMT (Table 2). This hinders the diffusion of the TMPP monomers during and after the demicellization process, which in turn is relatively fast (in the *ms* range).[24] As a consequence, the photosensitizer aggregation is slowed down. In this case, the structural transition of both copolymers to monomers did not influence the types of aggregates formed.

Table 2. First order (*k*) aggregation rate constant of TMPP in copolymers below CMT

TMPP/Pluronic (m/V)	k (10^{-3} min^{-1})	R^2
F-127 1%	14	0,9909
F-127 8%	6,4	0,9988
P-123 1%	72	0,9980
P-123 8%	12,8	0,9974

Additionally, it is interesting to distinguish this effect from that observed for the behavior of TMPP molecules incorporated into P-123 and F-127 during the fast temperature change that preceded the lyophilization process, in which monomeric TMPP and the structural features of the polymeric micelles remained nearly intact. In the re-dissolution of the TMPP/poloxamer formulations after the lyophilization process this systems presented similar absorption spectral profile of the original solutions before lyophilization/rehydration (Figure 5). This was attributed to the initial freezing step being extremely faster than the timescale required for significant structural re-organization of the poloxamer micelles and subsequent self-aggregation of the TMPP molecules, detectable by spectroscopic measurements, thus preserving the TMPP monomeric state inside the polymeric micelles.

Finally the results of studies related to depth allocation of PS into polymeric micelles presented a Stern-Volmer constant equal zero ($K_{SV} = 0$), showing that the water-soluble suppressive agent used (iodide ion) does not have access to TMPP molecules. This indicates that it is encapsulated in a deep region of the core micelle.

CONCLUSIONS

Solubilization studies of TMPP into P-123 and F-127 polymeric micelles were conducted by solid dispersion method. TMPP was incorporated into these copolymers in the monomeric form. P-123 solubilizes two times as much this photosensitizer in comparison to F-127 (1 TMPP molecule per F-127 micelle *versus* 2 TMPP molecules per P-123 micelle). The decrease in temperature in these systems below of the CMT promotes the formation of different kinds of TMPP aggregates. Self-aggregation process are intrinsically connected to structural changes occurring in micelles and affect the TMPP photophysical properties, thus compromising its efficiency as a photosensitizer for photodynamic therapy applications. Furthermore, the formulations studied are very stable under sequential lyophilization/re-solubilization without loss of their original features.

Both P-123 and F-127 systems can be used according to specific requirements of each situation. The P-123 micelle encapsulates TMPP molecule in greater quantity and more stable form (slow release), that is ideal for applications in which the longer availability of the PS be necessary. On the other hand, F-127 micelle solubilized less molecules of TMPP but promotes a more rapid release which is suitable for cases where all available concentration of the drug should be delivered in a short time. Additionally, the variation of the concentration of copolymer (% w/V) can also be used to modify the capacity (higher or lower amount of PS) or to optimize drug-delivery system characteristics making it slower or faster.

Therefore, polymeric micelles of poloxamer™ copolymers P-123 and F-127 were effective to formulate TMPP molecules in aqueous environments similar to biological fluids. These results are promising for the formulation of TMPP as a drug for use in photodynamic therapy.

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