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Synthesis of Cationic Polymers Based on Porphyrins for Photoinactivation of Bacteria †

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Abstract: 5,10,15,20-tetrakis-(pentafluorophenyl)porphyrin ($TPPF_{20}$) was synthesized by acid catalyzed condensation between pentafluorobenzaldehyde and pyrrole catalyzed by $BF_3{\cdot}OEt_2$ in dichloromethane. Subsequent oxidation reaction with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone afforded $TPPF_{20}$ in 38% yield. After was treated with Zn(II) to form the complex $ZnTPPF_{20}$. This porphyrin can be modified by nucleophilic aromatic substitution (S_NAr) with the displacement of the four *para*-fluoro atoms, then $TPPF_{20}$ and $ZnTPPF_{20}$ were reacted with *N*,*N*,*N′*,*N′*-tetrakis(3-aminopropyl)-1,4-butanediamine as the dendrimeric structure by S_NAr to obtain two polymers, $PTPPF_{16}$ and $PZnTPPF_{16}$, respectively. The reactions were carried out in *N*,*N*-dimethylformamide achieving 100% conversion. The polymers were purified by precipitation in water and washing the solid with petroleum ether. Moreover, spectroscopic and photodynamic studies of polymers indicated that the porphyrin unit retains its properties as PS. Thus, these polymers are an interesting material with potential applications to form photoactive aseptic surfaces.

Keywords: polymer; porphyrin; photosensitizer

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1. Introduction

In hospitals, surfaces are one of the main components of possible reservoirs of bacteria, which can cause a notable incidence in nosocomial infections [1]. The growing appearance of resistant microbial strains has promoted the development of viable alternatives for the eradication of infectious diseases. In this sense, photodynamic inactivation (PDI) of microorganisms has been proposed as useful therapy. This therapy uses a photosensitizer, visible light and oxygen to produce highly reactive oxygen species (ROS), which can react with several cell components. These molecular modifications induce a loss of biological functionality that causes cell death [2]. In most PDI studies, photosensitizers are added to a microbial suspension from a homogeneous solution. In this methodology, after PDI treatment the photosensitizer remains in the place of action, producing an undesired photodynamic effect and contaminating the medium. In addition, under these conditions the photodynamic agent is difficult to recover for its reuse in subsequent applications. This drawback can be avoided by using photosensitizers chemically bound to a support [3]. Thus, porphyrins immobilized on a surface have been proposed for the inactivation of microorganisms, considering economic and ecological subjects. In this sense, the coating of surfaces with photosensitizers (PSs) that are immobilized in a film are of great interest to maintain aseptic surfaces in public health [4]. Therefore, the development of new phototherapeutic agents and photoinactivation strategies are of great interest to maintain aseptic conditions in public health.

In this study 5,10,15,20-tetrakis-(pentafluorophenyl)porphyrin ($TPPF_{20}$) was synthesized by acid catalyzed condensation between pentafluorobenzaldehyde and pyrrole catalyzed by $BF_3 \cdot OEt_2$ in dichloromethane. Subsequent oxidation reaction with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone afforded $TPPF_{20}$ in 38% yield. This porphyrin was treated with Zn(II) to form the complex $ZnTPPF_{20}$. After, TPPF20 and ZnTPPF20 were reacted with *N,N,N′,N′*-tetrakis(3-aminopropyl)-1,4-butanediamine as the dendrimeric structure by S_NAr to obtain two polymers, $PTPPF_{16}$ and $PZnTPPF_{16}$, respectively. The polymers were purified by precipitation in water and washing the solid with petroleum ether. Moreover, spectroscopic and photodynamic studies of $PZnTPPF_{16}$ indicated that the porphyrin unit retains its properties as PS. Thus, this polymer is an interesting material with potential applications to form photoactive aseptic surfaces.

2. Materials and Methods

2.1. Materials

Chemicals were obtained from Sigma-Aldrich (Milwaukee, WI, USA). They were used without further purification. Tryptic soy (TS) broth and agar from Britania (Buenos Aires, Argentina) were used in microbial cultures. Microtiter plates (96-well) were acquired to Deltalab (Barcelona, Spain). Organic solvents (GR grade) from Merck (Darmstadt, Germany) were distilled and maintained on molecular sieves. Ultrapure water was obtained from a Labconco (Kansas, MO, USA) equipment model 90901-01.

2.2. Synthesis

5,10,15,20-tetrakis-(pentafluorophenyl)porphyrin (TPPF-20) was synthesized following the previously described methodology**Error! Bookmark not defined.**. This porphyrin was metaled with Zn(II) acetate, for this 58 mg, 0.01 mmol was dissolved in 10 mL of DCM. Then, 5 mL of a saturated solution of Zn(II) acetate in methanol was added. The mixture was stirred for 2 h under argon at room temperature. The organic phase was washed three times with 15 mL of water. The organic solvent was evaporated under reduced pressure to obtain 32 mg (98%) of ZnTCP.

After that, were obtained two polymers, PTPPF-16 and PZnTPPF-16, by aromatic nucleophilic substitution using N,N,N′,N′-Tetrakis(3-aminopropyl)-1,4-butanediamine as the dendrimeric structure. The reaction was carried out in N, N-dimethylformamide at room temperature for 44 h, followed by heating at 80 ° C for 4 h, with 100% conversion. The polymers were purified by precipitation in water and washing the solid with petroleum ether.

2.3. Spectroscopic Studies

UV-visible absorption and fluorescence emission measurements in *N,N*-dimethylformamide (DMF) were achieved as previously described [**Error! Bookmark not defined.**]. Fluorescence emission spectra were recorded by exciting the samples at λ_{exc} = 426 nm. The fluorescence quantum yield (Φ_F) of each porphyrin was calculated using Zn(II) 5,10,15,20-tetrakis(4-methoxyphenyl)porphyrin (ZnTMP) as a reference ($\Phi_F = 0.049$) in DMF [5].

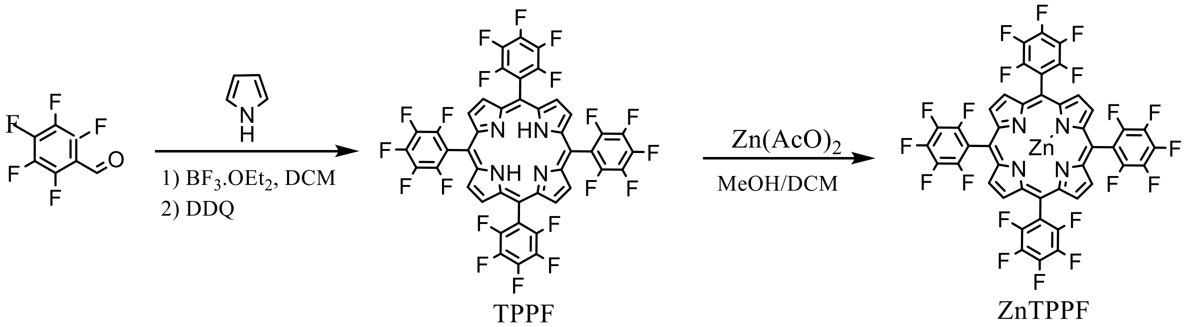
2.4. Determination of $O_2(^1\Delta_g)$ Production

9,10-Dimethylanthracene (DMA, 35 μM) and the photosensitizer in DMF (2 mL) were irradiated with visible light. The kinetics of DMA photooxidation were analyzed by the decrease in the absorption band at $\lambda_{max} = 379$ nm. The observed rate constants k_{obs}^{DMA} of DMA oxidation and quantum yields of $O_2(^1\Delta_g)$ production (Φ_Δ) of the porphyrins were obtained as previously reported [**Error! Bookmark not defined.**]. ZnTMP was used as a reference ($\Phi_\Delta = 0.73$) [**Error! Bookmark not defined.**].

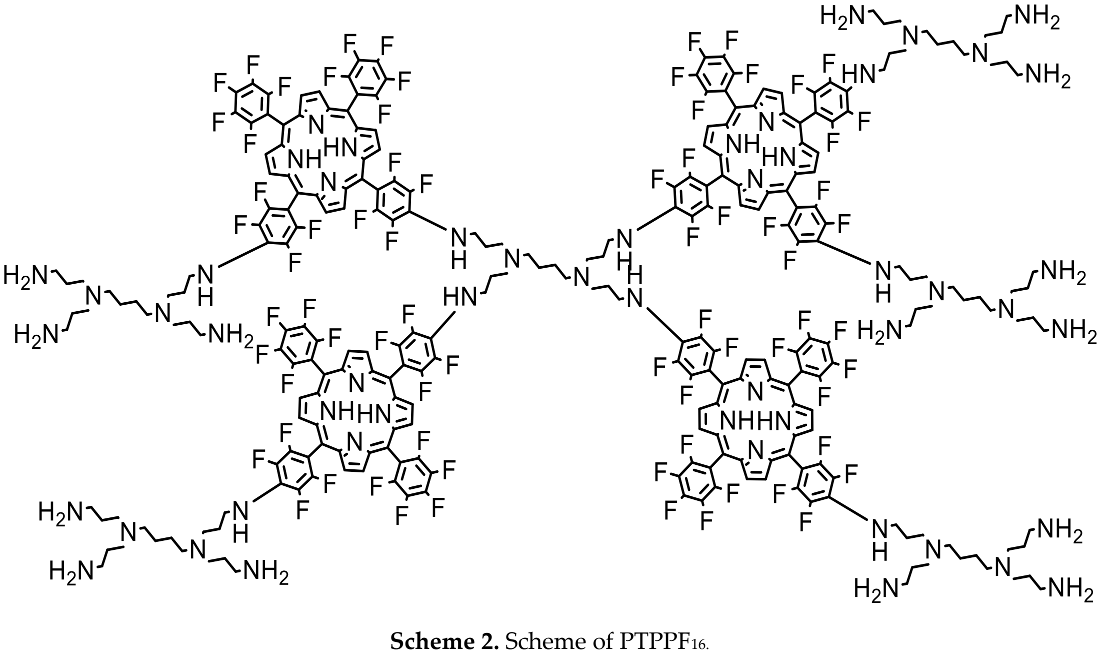
3. Results and Discussion

3.1. Synthesis of Porphyrins and Polymer

The synthetic procedure to obtain porphyrins are shown in **Error! Reference source not found.**. This porphyrin was metaled with Zn(II) acetate in DCM/methanol to produce the complex $ZnTPPF_{20}$ in 98% yield. After that, $TPPF_{20}$ and $ZnTPPF_{20}$ were reacted with *N,N,N′,N′*-tetrakis(3-aminopropyl)-1,4-butanediamine as the dendrimeric structure by SNAr to obtain two polymers, $PTPPF_{16}$ and $PZnTPPF_{16}$, respectively. The reactions were carried out in *N,N*-dimethylformamide at room temperature for 44 h, followed by heating at 80 °C for 4 h. This approach produces the polymers in 100% conversion. The polymers were purified by precipitation in water and washing the solid with petroleum ether.



Scheme 1. Synthesis of $TPPF_{20}$ and $ZnTPPF_{20}$.



Scheme 2. Scheme of $PTPPF_{16}$.

3.2. Photophysical Characterization

The UV-visible absorption spectra of $TPPF_{20}$, $ZnTPPF_{20}$, $PTPPF_{16}$ and $PZnTPPF_{16}$ were performed in DMF. These spectra were also compared with that for ZnTMP, which was used as a reference. The main optical characteristics of these compounds are summarized in **Error! Reference source not found.**. The spectra of $TPPF_{20}$, $ZnTPPF_{20}$, $PTPPF_{16}$ and $PZnTPPF_{16}$, show the typical Soret band around 420 nm and Q-bands in the 500–600 nm range, characteristic of the corresponding Zn(II) substituted porphyrins [6]. The sharp absorption of Soret bands indicated that porphyrins are dissolved as monomer in this medium. Furthermore, both polymers essentially retained the spectroscopic properties of the porphyrin-based chromophore despite to be an extensively aggregated system. The UV-visible absorption observations also confirm the polymerization of $TPPF_{20}$ and $ZnTPPF_{20}$. The Soret and Q bands of both polymers exhibit a red-shifted maximum of around 15 nm in comparison with those of monomers in DMF, together with a small broadening of both bands. These results indicate only slight interaction between the porphyrin units in the polymer structure [**Error! Bookmark not defined.**].

The polymers exhibited the two red emission bands, characteristic of porphyrins. The spectra of $ZnTPPF_{20}$ show two bands around 610 and 660 nm, which are typical for similar *meso*-substituted Zn(II) porphyrin derivatives [**Error! Bookmark not defined.,Error! Bookmark not defined.**], while the corresponding polymer $PZnTPPF_{16}$, shows its bands whit a redshift of 5 nm. These emission bands have been assigned to Q_x(0-0) and Q_x(0-1) transitions. This is characteristic of porphyrins with D_{2h} symmetry, indicating that the vibronic structure of the tetrapyrrolic macrocycle remains practically unchanged upon excitation [7]. In addition, both polymers presented good emission proprieties indicating that the spectroscopic characteristics of the porphyrin based-chromophore were retained in the polymeric matrix. These results also indicate that the porphyrin can be embedded in the polymer without substantial aggregation. These minor spectral changes in absorbance spectrum and the fluoresce properties of the polymer suggest that the π-π stacking between the porphyrin cores is impede and only takes place a weak interaction.

3.3. Photodynamic Properties

Photooxidation of DMA induced by ZnTMP, $TPPF_{20}$, $ZnTPPF_{20}$, $PTPPF_{16}$ and $PZnTPPF_{16}$ was determined in DMF. The reaction was followed by the decay of the DMA band at 379 nm due to the formation of the 9,10-endoperoxide product [**Error! Bookmark not defined.,Error! Bookmark not defined.**]. Since DMA quenches $O_2(^1\Delta_g)$ by chemical reaction, it was used as an approach to determine the ability of these PSs to produce $O_2(^1\Delta_g)$ [8]. The values of k_{obs}^{DMA} were calculated from first-order kinetic plots of the DMA absorption vs. time. Also, the results were compared with those using ZnTMP as a reference. **Error! Reference source not found.** summarized the values of k_{obs}^{DMA} calculated from the first order kinetic plots. The value of k_{obs}^{DMA} for the reaction sensitized by $PTPPF_{16}$ is lower than that obtained for ZnTMP, while the value obtained for $PZnTPPF_{16}$ is higher (Table 1. Spectroscopic and photodynamic properties of ZnTMP, $TPPF_{20}$, $ZnTPPF_{20}$, $PTPPF_{16}$ and $PZnTPPF_{16}$ in DMF).

Table 1. Spectroscopic and photodynamic properties of ZnTMP, $TPPF_{20}$, $ZnTPPF_{20}$, $PTPPF_{16}$ and $PZnTPPF_{16}$ in DMF.

PS	λ^{Soret} (nm)	λ_{em}(nm)	Φ_F [a]	Φ_Δ [b]
ZnTMP	426	607	0.049 ± 0.004	0.73 ± 0.03 [c]
$TPPF_{20}$	410	634	0.029 ± 0.003	0.80 ± 0.03
$ZnTPPF_{20}$	419	644	0.037 ± 0.002	0.81 ± 0.004
$PTPPF_{16}$	423	658	0.018 ± 0.002	0.22 ± 0.004
$PZnTPPF_{16}$	433	608	0.025 ± 0.001	0.86 ± 0.03

[a] fluorescence quantum yield, [b] quantum yield of $O_2(^1\Delta_g)$ production, [c] from Ref. [**Error! Bookmark not defined.**].

4. Conclusions

The synthesis of the corresponding porphyrins, and their subsequent polymerization were carried out in a fast and simple way by S_NAr. The polypropylene imine crosslinked porphyrin monomers formed a polymeric structure and retained spectroscopic absorption and fluorescence spectroscopic properties. Through photodynamic studies it was demonstrated that the polymers possess the ability to produce singlet molecular oxygen. According to these results, the polymers have potential applications for the PDI of bacteria, achieving a complete eradication of S. aureus after 15 min of irradiation, and after 30 min in the case of *E. coli.*

Institutional Review Board Statement:

Informed Consent Statement:

Data Availability Statement:

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