

Photosensitizing Properties of Ionic Porphyrins Immobilized on Functionalized Solid Polystyrene Support

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ABSTRACT: The photogeneration of singlet oxygen ($^1\text{O}_2$) by tetrasodium salt of *meso*-tetrakis(4-sulfonatophenyl)porphyrins (H_2TPPS) and some of its metal complexes (MTPPS; $\text{M} = \text{Cd}^{2+}, \text{Zn}^{2+}$), immobilized on cationically functionalized polystyrene (PS) beads, was studied. The generation of $^1\text{O}_2$ was monitored by a *N,N*-dimethyl-4-nitrosoaniline (RNO) bleaching assay. The rate of RNO bleaching was seen to be retarded in the presence of DABCO, a specific $^1\text{O}_2$ scavenger, providing evidence for the involvement of $^1\text{O}_2$ as

an active intermediate. The relative efficiencies of the generation of $^1\text{O}_2$, with respect to immobilized rose bengal (PS-RB), by different polymer-supported porphyrins, were determined to be 0.29, 0.27, and 0.16 for PS- H_2TPPS , PS-ZnTPPS, and PS-CdTPPS, respectively. Binding of porphyrins to a polymer has been found to decrease the efficiency of the $^1\text{O}_2$ generation, which could be attributed to the structural deformation of the appended porphyrins. © 2003 Wiley Periodicals, Inc. *J Appl Polym Sci* 89: 3925–3930, 2003

INTRODUCTION

Studies on the inactivation of a variety of bacteria and viruses upon exposure to light, in the presence of oxygen and certain sensitizing dyes, are reported and their potential uses indicated.^{1,2} However, development of any water or wastewater disinfection procedures based on this principle, employing dissolved or homogenized sensitizers, has the drawback of undesirable contamination by the sensitizer itself, the removal of which from the treated water often is difficult. The approach using sensitizers bound on solid polymers or other insoluble supports has been, hence, suggested as a solution for avoiding this inherent drawback involved in employing sensitizers in solution conditions.³ Recently, there has been growing scientific and commercial interest in the development of techniques for selectively immobilizing molecules in a polymer matrix.^{4,5}

Porphyrins, which are tetrapyrrole derivatives with extensive conjugation, have strong absorption characteristics in the visible region and exhibit interesting photophysical, photochemical, and photoredox properties.⁶ The potential use of some of their metallo-derivatives as sensitizers in photobiological applications such as photodynamic therapy and inactivation of microorganisms has attracted active interest.^{7–10} The photoactivity of porphyrins could be changed

upon chemically attaching them with an insoluble polymer.¹¹ It was reported that grafting of porphyrins on a solid polymer backbone is found to impart interesting modifications on the photochemical and photophysical properties of these sensitizers.^{11–13} Spectroscopic and photochemical studies of phthalocyanines bound to insoluble polystyrene, especially with regard to their ability for $^1\text{O}_2$ generation and charge-transfer reactions, were reported.¹⁴

Since singlet oxygen is known to be an interesting phototoxic species for *E. coli* and similar microorganisms, porphyrins with significant $^1\text{O}_2$ yield efficiency are attractive candidates for varied purposes. The ability of water-soluble tetrasodium salt of *meso*-tetrakis(4-sulfonatophenyl)porphyrins (H_2TPPS) and some of its metallo derivatives (MTPPS) to photogenerate $^1\text{O}_2$ has been demonstrated recently.¹⁵ We have been interested in developing polymer-immobilized metalloporphyrins to model efficient enzyme functions by employing MTPPS and also in demonstrating the polymer effect on the porphyrin moieties.^{16–21} In the present study, we report the photosensitizing properties of some of such metalloporphyrins anchored on a functionalized solid polystyrene (PS) surface.

EXPERIMENTAL

Preparation of polymer-supported metalloporphyrins

Tetraphenylporphyrin (H_2TPP) was prepared by Adler's method²² and was sulfonated under controlled conditions²³ to get tetrasulfonated derivatives (H_2TPPS). Their metallo derivatives MTPPS [M

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= Cd(II) and Zn(II)] were prepared in aqueous conditions with suitable metal carriers by the procedure reported earlier.¹⁸

The polymer support used was 2% divinylbenzene (DVB)-crosslinked PS after chloromethylation. The solid polymer bearing chloromethyl groups on the surface was then reacted with pyridine to get quaternized moieties on the support.¹⁸ The anionic porphyrins H₂TPPS and MTPPS were grafted onto the surface of the cationic polymer beads by careful stirring with water or a methanol solution of the porphyrins. In a typical reaction, 2 g of PS was suspended in a solution containing 0.2 mM of H₂TPPS/MTPPS and stirred carefully for about 2 h in a water bath to get the immobilized systems.

Preparation of polymer beads bonded to rose bengal

An aqueous solution of rose bengal (RB) was prepared by dissolving 97.4 mg (1×10^{-4} M) in a minimum amount of water. About 1 g *N*-alkylpyridinium-functionalized chloromethylated 2% DVB-crosslinked PS beads was added and heated in a water bath for 24 h. During this time, almost the entire RB was found to be immobilized on the polymer surface as evident by the color change observed for the solution. The modified beads were collected by filtration and were thoroughly washed with water and methanol. No immobilized RB was eluted out during the washing process, indicating strong chemical bonds between RB and the functionalized polymer beads.

Spectral measurements

The characterization of the porphyrins was carried out by electronic and IR spectral measurements using Shimadzu UV-160 A and IR-470 spectrophotometers, respectively. The electronic spectra (in solution) of the ionic porphyrins were measured either in water or methanol. IR spectra were recorded in the solid state in the form of KBr pellets. The electronic spectra of the polymer-supported porphyrins were measured in the solid state by grinding the solid sample with nujol and spreading the fine paste obtained uniformly on a strip of Whatman 41 filter paper. The reference strip contained the paste made from the porphyrin-free polymer beads.

Generation of ¹O₂: RNO bleaching assay

The detection of singlet oxygen was performed by an RNO bleaching assay. The sensitizers (30 mg) were exposed to light in the presence of imidazole (0.01M) and RNO (0.05M) in 0.05M phosphate buffer (pH 7.4). The bleaching rate of RNO was followed at 440 nm. This method is based upon secondary bleaching of RNO as induced by the reaction of ¹O₂ with imidazole

(transannular peroxide, Im-¹O₂). The experiment was performed as described by Kraljic and El Mohsni.²⁴

A 150-W xenon lamp (Photon Technology International, USA) was used as the light source. A filter combination of 10 cm of a potassium iodide solution (1 g in 100 mL water) and 1 cm pyridine was used to cut off below 300 nm. The solution, taken in a quartz cuvette placed at a distance of 12 cm from the light source, was continuously stirred during irradiation. The irradiation was carried out in an open cuvette in equilibrium in the atmosphere.²⁵ The irradiation experimental setup and spectroscopy instruments were set nearby and absorbance measurements followed by irradiation were carried out periodically (every 2 min) using a UV-vis double-beam spectrometer.

Generation of O₂⁻: SOD inhibitable cytochrome c reduction

The formation of superoxide was assayed using the SOD inhibitable cytochrome c reduction method. PS-porphyrins (30 mg) were photolyzed in the presence of ferricytochrome c (40 μM) in 50 mM phosphate buffer (pH 7.4). The reduction was monitored spectrophotometrically at 550 nm using $\Delta OD_{550} = 20,000 M^{-1}cm^{-1}$ for reduced-oxidized cytochrome c.²⁶

RESULTS AND DISCUSSION

Most of the reported procedures for immobilizing porphyrins on a polymer support involved interaction between the functionalized solid polymer and suitably functionalized porphyrins, leading to mainly covalently bonded porphyrins. Such systems generally lead to porphyrins surrounded by a hydrophobic environment. For generating an effective polymer-supported photosensitizing system suitable in an aqueous condition, it is highly desirable that the porphyrins should be in a hydrophilic environment. In the present study, a surface-functionalized polymer support (bearing a CH₂Cl group) was made ionic by interaction with pyridine to get cationic *N*-alkylated pyridinium functions (PS) as shown in Figure 1. The porphyrins (H₂TPP and MTPP) are made anionic by sulfonation at the *para* position of the mesophenyl groups by a known procedure.²³ The sodium salt of the resulting tetrasulfonated porphyrins H₂TPPS and MTPPS [M = Zn(II) and Cd(II)] are then interacted with the cationic PS to get immobilized porphyrin systems with the desired ionic environment. Due to the presence of sufficient ionic sites, the resulting polymer-porphyrin system becomes compatible with aqueous medium and, hence, would be effective for photoinactivation of microorganisms.

The immobilization of H₂TPPS or MTPPS on PS was done by a simple ion-exchange method in an aqueous

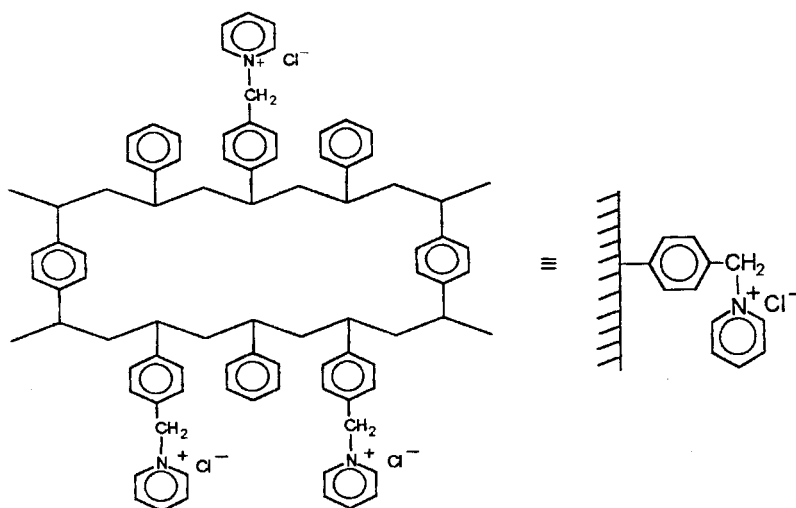


Figure 1 Structure of *N*-alkylpyridinium-functionalized, chloromethylated DVB-crosslinked PS.

condition. The appending of porphyrins on the polymer support was confirmed by the color change of the polymer beads and also by electronic and IR spectral measurements. The nature of the supported porphyrin is shown in Figure 2. Once ionically bonded to the support, these porphyrins are found to be not exchangeable easily and the support system seems to be very stable, both in polar and nonpolar solvents.

The extent of appended porphyrin could be estimated spectrophotometrically by measuring the decrease in the absorbance of the Soret peak of the H₂TPPS/MTPPS solution after treating it with known amount of solid PS. However, in the present study, we restricted the porphyrin uptake to 0.1 meq/g of the PS in all the cases so that the photosensitization of the various porphyrins could be compared. This was achieved by taking the calculated amount of the porphyrins in solution and reacting it with a required amount of PS. The uptake by solid PS was seen to be

complete within 2 h in all cases, as evident from the complete decolorization of the solution. Since the Cl capacity of the original PS was about 3.8 meq/g of the polymer and the extent of porphyrin grafting was only to the extent of 0.1 meq/g of resin, it could be tacitly assumed that the porphyrins are widely separated on the polymer surface. We also ruled out any possibility of porphyrin aggregation at this low level of uptake. In the case of RB also, the grafting was restricted to be the same extent as in the case of porphyrins.

Spectral properties

The electronic spectra of the porphyrins showed a noticeable change on the grafting of the polymer (Table I). A significant observation of the appended porphyrins was the shift of the absorption peaks to the lower-energy region. While the Soret band of H₂TPPS showed only a marginal red shift (to the extent of 85 cm⁻¹), both ZnTPPS and CdTPPS exhibited pronounced shifts to lower energy (1165 cm⁻¹ for ZnTPPS and 1170 cm⁻¹ for CdTPPS) on polymer immobilization. The Q bands of porphyrins also showed a similar red shift on polymer grafting. This could be explained in terms of the molecular distortion brought about by steric interaction of polymer groups on porphyrin moieties and also the electronic charge variations around the π -framework.²⁰ The LUMO of the porphyrins are a set of two degenerate π^* orbitals which are antibonding with the metal *dxz* and *dyz* orbitals.²⁷ In a planar geometry, these two metal orbitals would interact strongly with porphyrin LUMOs and, correspondingly, the antibonding combinations of the LUMOs of MTPPS would be increased in energy to a considerable extent.²⁸ Any deviation from planarity will reduce the π overlap with the *d* orbitals and, hence, lower the energy of the LUMOs of MTPPS. The strong polymer-porphyrin interaction is also expected to push

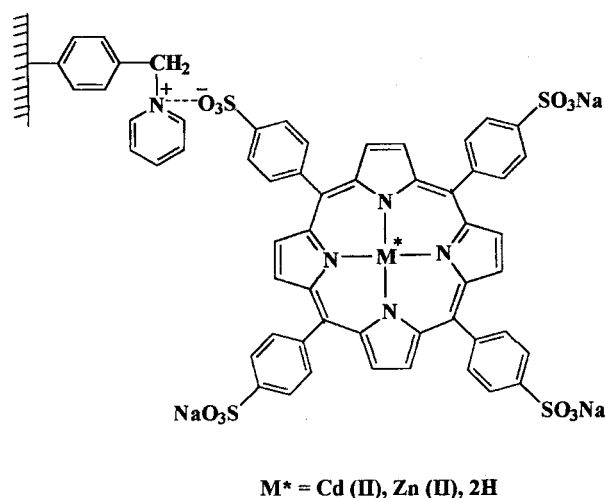


Figure 2 Structure of the PS-MTPPS systems.

TABLE I
Spectral Characteristics of Porphyrin Chromophores

Porphyrin system	Absorption maxima (nm) ^a				
	Soret band	Q bands			
H ₂ TPPS	417	520	556	593	652
PS-H ₂ TPPS	419 (85)	522 (73)	559 (96)	595 (57)	654 (47)
ZnTPPS	414		561	602	
PS-ZnTPPS	446 (1163)		590 (876)	634 (838)	
CdTPPS	422		556	609	
PS-CdTPPS	445 (1170)		583 (833)	638 (797)	

^a The data in parentheses are the red-shift observed for Soret and Q bands (in cm⁻¹) of the porphyrins on the polymer matrices as compared to the corresponding polymer-free porphyrins.

the porphyrin HOMOs energetically up due to enhanced electron density on the π -framework caused by both the sterically tilted anionic aryl sulfonato groups and the freely available Cl⁻ moieties of the PS around the porphyrin framework. As a result, the electronic transition from the low-lying filled π orbital to the π^* orbital would occur at lower energy. Hence, the observed red shift of the immobilized porphyrins can be attributed to the distortion of the porphyrins from the planarity.²⁰

Photogeneration of ¹O₂

Polymer-bound porphyrins are found to sensitize the photochemical production of singlet oxygen as observed by the bleaching of RNO. Photosensitized bleaching of RNO by PS-H₂TPPS, followed spectrophotometrically at 440 nm, is given in Figure 3. The rate of bleaching of RNO by PS-H₂TPPS, PS-CdTPPS, and PS-ZnTPPS as a function of irradiation time is shown in Figure 4. The rate of RNO bleaching by PS-RB is also included in this figure. By taking the ratios of the slope from Figure 4 and a known value

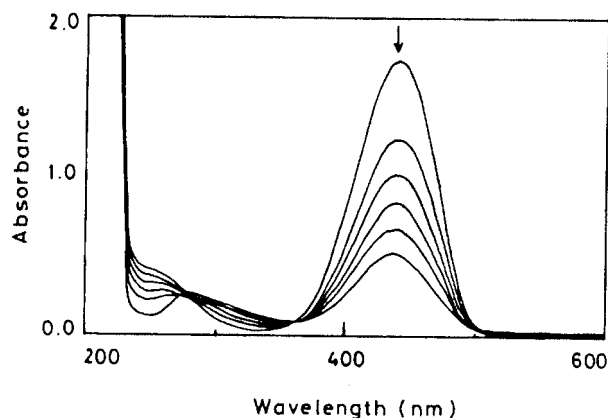


Figure 3 Absorption spectra of RNO in the presence of imidazole (10 mM) in 50 mM phosphate buffer (pH 7.4) during photosensitized reaction of PS-H₂TPPS (30 mg) at each 3-min interval of irradiation time.

for the quantum yield of singlet oxygen generation by illuminated RB (0.76), the quantum efficiencies of ¹O₂ by different polymer-bound sensitizers were calculated. The relative efficiencies of the generation of ¹O₂ by different polymer-bound sensitizers were calculated to be 0.29, 0.27, and 0.16 for PS-H₂TPPS, PS-ZnTPPS, and PS-CdTPPS, respectively.

In the homogeneous phase, the unbound porphyrins are reported to generate ¹O₂ upon photoillumination with the quantum yields of 0.62 and 0.81 for H₂TPPS and ZnTPPS, respectively.¹⁵ This shows that the binding of porphyrin to the polymer has decreased its efficiency of ¹O₂ generation. This is in accordance with the earlier observation for metallotetracarboxy-phthalocyanines bound to the amino groups of Amberlite IRA-93.¹⁴ The observed lowering reported in that study was attributed to aggregation and self-quenching. Even though, for more highly functionalized polymers, self-quenching becomes significant between sensitizer molecules on the polymer chain, we

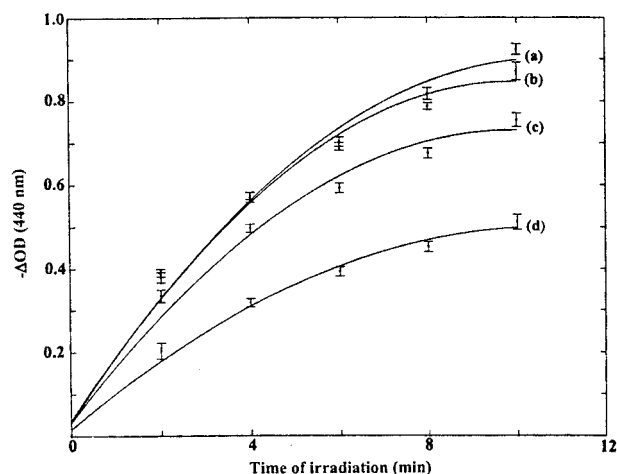


Figure 4 Photosensitized RNO bleaching measured at 440 nm in the presence of imidazole (10 mM) in 50 mM phosphate buffer (pH 7.4) with (a) PS-H₂TPPS, (b) PS-RB, (c) PS-CdTPPS, and (d) PS-ZnTPPS as a function of illumination time in minutes.

believe that in our polymer-supported porphyrins such an aggregation is not possible as we have restricted the porphyrin uptake to the extent of only 0.1 meq/g of resin (as compared to 3.8 meq of the available functionalization sites). To prove this point further, we prepared ESR-active PS-CuTPPS and PS-AgTPPS having the same porphyrin content (0.1 meq/g of PS). The EPR spectra obtained were well resolved and showed all the characteristic hyperfine features indicating wide separation between the porphyrin moieties.²⁰ The observed low efficiency of singlet oxygen generation could therefore be attributed, to a great extent, to the structural modification (as discussed earlier) brought about by the polymer support, rather than due to any aggregation.

Another reason causing a decrease in $^1\text{O}_2$ generation is the relative disposition of porphyrin moieties toward the incoming light source. In homogeneous conditions, the photosensitizers are well distributed within the reaction medium and all the molecules would get photoexcited by the incoming light from the specified direction at a given time. But when the same number of such sensitizer molecules are immobilized on a polymer bead surface having almost a spherical shape, only half of them are likely to be photoactivated at a given time because only half of the polymer surface would be exposed to the direction of the incoming light. Therefore, the amount of light quanta absorbed by the sensitizer molecule at a given time would be low (nearly half) in such heterogeneous conditions when compared to homogeneously dispersed conditions. The combined effect of these two factors might cause the observed lowering of $^1\text{O}_2$ generation (calculated value) in the case of PS-MTPPS/PS- H_2 TPPS. The generation of $^1\text{O}_2$ was confirmed by carrying out the RNO bleaching assay in the presence of a specific singlet oxygen quencher such as DABCO. Figure 5 shows the rate of bleaching of RNO by PS- H_2 TPPS in the presence of an equimolar amount of imidazole and DABCO (0.01M). DABCO and imidazole have comparable quenching rates.²⁹ Hence, the ratio of the slopes of RNO bleaching in the presence and absence of DABCO is found to be nearly half (Fig. 5). This result confirms the generation of $^1\text{O}_2$ during the photosensitization process of PS- H_2 TPPS. Similar results were also observed for other PS-porphyrins.

To check the possible generation of superoxide anion during the photoirradiation of these PS-porphyrins, the SOD inhibitable cytochrome c reduction assay was performed. These polymer-bound porphyrins did not reduce cytochrome c, indicating that $\text{O}_2^{\cdot -}$ was not generated during the photosensitization by PS- H_2 TPPS, PS-ZnTPPS, and PS-CdTPPS. We may conclude that only singlet oxygen was formed.

A mechanism involving the participation of photo-generated singlet oxygen in the inactivation of *E. coli* was proposed.³ Preliminary studies were carried out

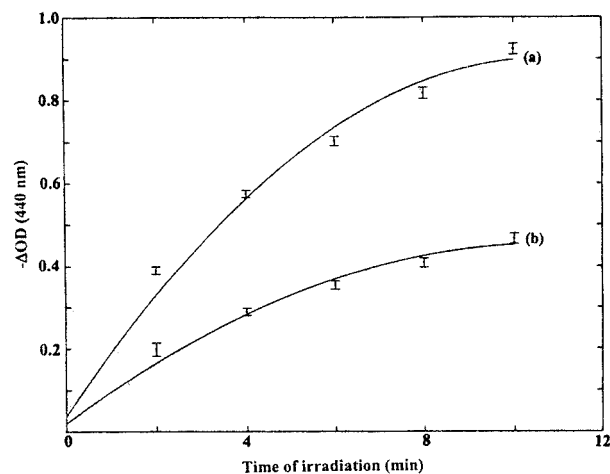


Figure 5 Inhibition of photosensitized RNO bleaching measured at 440 nm by PS- H_2 TPPS (a) in the absence and (b) in the presence of 10 mM DABCO as a function of irradiation time.

on these porphyrin systems immobilized on polymer beads with *E. coli* in the presence of light and oxygen which indicate that these porphyrins were efficient in the photokilling of *E. coli* cells (data not shown). Detailed investigation of the photokilling of *E. coli* and other bacterial organisms using these polymer-immobilized porphyrins and also their possible applications as wastewater disinfectants is in progress.

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