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Applications of lead phthalocyanines embedded in electrospun fibers for the photoinactivation of *Escherichia coli* in water

Olawale L. Osifeko, Tebello Nyokong*

Department of Chemistry, Rhodes University, Grahamstown 6140, South Africa

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ABSTRACT

Lead (II) pyridyloxyphthalocyanine (PbTpyPc) and its quaternized form (PbTepyPc) were synthesized and the photophysical behavior examined. Low fluorescence quantum yields (Φ_F) of 0.01 and 0.02 were observed for PbTepyPc and PbTpyPc, respectively. The singlet oxygen quantum yields (Φ_Δ) were 0.60 and 0.68, for PbTepyPc and PbTpyPc, respectively using DPBF as a quencher in DMF. Singlet oxygen production of the embedded sensitizers in electrospun fiber were quantified using ADMA and were found to be $\Phi_\Delta = 0.41$ and $\Phi_\Delta = 0.21$ for PbTepyPc and PbTpyPc, respectively. Photodynamic inactivation of *Escherichia coli* (*E. coli*) with the quaternized photosensitizer at 5 μM, totally inactivated the *E. coli* (with log CFU = 10 decrease). Only 0.4 log CFU decrease was obtained with PbTpyPc. The embedded non-quaternized photosensitizer (PbTpyPc) was less active on the gram negative bacteria but the quaternized photosensitizer (PbTepyPc) was effective towards inactivation of *E. coli*.

1. Introduction

Phthalocyanine (Pc) derivatives have gained significance in a number of fields including their use as photosensitizers in photodynamic therapy (PDT) of cancer [1]. The PDT activity of Pcs may also be applied to the inactivation of bacteria [2]. Similar to PDT, photodynamic antimicrobial chemotherapy (PACT) is based on the concept that a non-toxic photosensitizer, localized in microorganisms, can be activated by visible light of appropriate wavelength to generate reactive oxygen species (ROS) that are cytotoxic to these microorganisms [3–5]. Photo-inactivation of *Streptococcus sanguis* by aluminum phthalocyanine [6], has been reported. Metal-free tetra (tert-butyl) phthalocyanine has been incorporated into polymer films and used for photoinactivation of *Staphylococcus aureus* (*S. aureus*) [5]. When the antibacterial efficiency of anionic, cationic and neutral zinc phthalocyanines was tested against Gram-positive and Gram-negative bacteria, the results showed that only the positively charged species, a cationic water soluble pyridinium zinc phthalocyanine was active [7]. It has also been shown that the cationic silicon phthalocyanine (Pc-4) can incite the photoinactivation of blood borne pathogens such as *Plasmodium falciparum* and *Trypanosoma cruzi* [8–10]. Hence in this work we use a cationic Pc complex lead (II) 2,9,16,23-tetrakis[4-(N-ethylpyridyloxy)]-phthalocyanines (PbTepyPc, Scheme 1). *Escherichia coli* was chosen since the photo-inactivation of Gram-negative bacteria is known to be favored in the presence of the cationic photosensitizers [11]. The use of catalyst supports is important for removal after use, thus electrospun fibers were used for support in this work. The main interest in using electrospun nanofibers, compared to other supports, is their ability to host a variety of molecules which in turn can improve their properties for a specific application.

Unsubstituted zinc phthalocyanine (ZnPc) embedded in electrospun polyurethane fibers has been used for the inactivation of *E. coli* [12]. In this work, we also embed metallophthalocyanines (MPcs) in electrospun fibers as support. Incorporation of phthalocyanines into electro-spun polymer fibers has been reported with the functionality of the phthalocyanines maintained in the solid fiber core [13–17]. Low symmetry Pc (mono substituted) showed improved antimicrobial activity [17]. In this work we use PbPc complexes (due to their low symmetry) embedded in electrospun polystyrene fibers for PACT studies. PbPcs are of low symmetry due to the structural deformation caused by the metal being out of the plane of the ring in a "shuttle-cock" arrangement [18,19]. An additional reason for employing PbPc is that heavy metals (such as Pb) increase singlet oxygen quantum yields through the heavy atom effect, which enhances intersystem crossing to the triplet excited state. However heavy metals are known for their toxicity. We have reported before that once electrospun into fibers, Pcs do not leach out [15]. This will also be confirmed in this work.

* Corresponding author. Tel.: +27 46 6038260; fax: +27 46 6225109.
E-mail address: t.nyokong@ru.ac.za (T. Nyokong).

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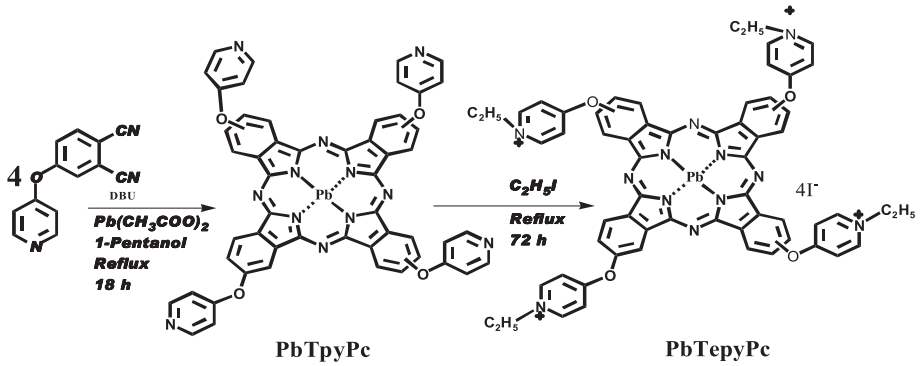

PbTpyPc PbTepyPc

Scheme 1. Synthesis of PbTpyPc and PbTepyPc.

2. Experimental

2.1. Materials

The following chemicals were purchased from SAARCHEM; 1-pentanol, n-hexane, dimethylsulfoxide (DMSO), tetrahydrofuran (THF) and N,N-dimethylformamide (DMF). Agar bacteriological BBL Muller Hinton broth and nutrient agar were purchased from Merck. *E. coli* was purchased from Microbiologics, polystyrene (PS, M_w = 192,000 g/mol), 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU), zinc phthalocyanine (ZnPc), anthracene-9,10-bis-methylmalonate (ADMA), potassium carbonate, 1,3-diphenylisobenzofuran (DPBF), 4-hydroxypyridine, and lead (II) acetate were purchased from Sigma Aldrich. 4-(4-Pyridyloxy) phthalonitrile was synthesized according to literature methods [20]. Phosphate buffer saline (PBS) pH 7.4 was prepared using appropriate amounts of Na_2HPO_4, KH_2PO_4 and chloride salts using ultra-pure water, from a Milli-Q Water System (Millipore Corp, Bedford, MA, USA).

2.2. Equipment

Infrared (IR) spectra were recorded on a Perkin–Elmer Fourier transform-IR (100 FT-IR) spectrophotometer. UV–visible absorption spectra of solutions were recorded on a Shimadzu UV-2550 spectrophotometer. The absorption spectra of the modified fiber were recorded by placing it directly on a glass plate. Fluorescence emission spectra were recorded on a Varian Eclipse spectrofluorimeter.

Scanning electron microscope (SEM) images were obtained using a JOEL JSM 840 scanning electron microscope. Irradiations for singlet oxygen and photodegradation quantum yields of Pcs, and photo-irradiation for the antimicrobial studies were performed using a General Electric Quartz lamp (300 W); 600 nm glass (Schott) and water filters were used to filter off ultra-violet and far infrared radiations, respectively. An interference filter, 700 nm with a band of 40 nm, was placed in the light path just before the cell containing the sample. Light intensities were measured with a POWER MAX 5100 (Molelectron detector incorporated) power meter and were found to be 1.0×10^{19} photons cm^{-2} s^{-1} for photodegradation and singlet oxygen quantum yields, and for PACT studies.

X-ray powder diffraction (XRD) patterns were recorded on a Bruker D8 Discover equipped with a proportional counter, using Cu-K_α radiation (λ = 1.5405 Å, nickel filter). Data were collected in the range from $2\theta = 5^\circ$ to 100°, scanning at 1° min^{-1} with a filter time constant of 2.5 s per step and a slit width of 6.0 mm. Details have been provided before [21].

Mass spectral data were collected with a Bruker AutoFLEX III Smartbeam TOF/TOF Mass spectrometer. The spectra were acquired using dithranol as the MALDI matrix. ^1H nuclear magnetic resonance spectra were recorded on a Bruker AMX 600 MHz NMR spectrometer. Laser flash photolysis system was used for the determination of triplet decay kinetics. Details of the equipment have been provided before [22].

The optical density of the bacteria culture was determined using the LEDETECT 96 from LABXIM PRODUCTS. Vortex mixer and HERMLE Z233M-2 centrifuge from LASIEC were used to mix the bacteria suspension and for the harvesting of the bacteria cells, respectively.

Thermo-gravimetric analysis (TGA) was performed using a Perkin Elmer TGA 7 analyser. The analyses were carried out under nitrogen/air mixture at a flow rate of 120 cm^3 min^{-1}. The weighed samples were heated from 100 to 500 °C at a heating rate of 10 °C min^{-1}.

Fluorescence decay times were measured using a time correlated single photon counting (TCSPC) setup (FluoTime 200, PicoQuant GmbH). The excitation source was a diode laser (LDH-P-670 driven by PDL 800-B, 670 nm, 20 MHz repetition rate, 44 ps pulse width, PicoQuant GmbH). The details of the set-up have been previously described [23].

2.3. Preparation of electrospun nanofibers

Polystyrene solutions were prepared at a concentration of 20% w/v in mixture of DMF/THF (ratio 4:1) as reported elsewhere [24–26]. The solution for the modified fiber was prepared by adding 0.1 g phthalocyanine to solution of the polymer (20% w/v). The mixture was homogenized using a magnetic stirrer at room temperature for 24 h, and then electrospun. The process parameters for electrospinning were: tip to collector distance (TCD) = 12 cm; applied voltage = 15 kV. The flow rate for polystyrene alone was 0.1 ml h^{-1}. The flow rate was increased to 0.5 ml h^{-1} of the polystyrene-metallophthalocyanine composite to avoid clogging at the needle tip and jet instability. The Pc modified fibers are represented as PbTpyPc-PS or PbTepyPc-PS, where PbTpyPc = lead(II) 2,9,16,23-tetrakis[4-(N-pyridyloxy)]-phthalocyanine and PbTepyPc = lead (II) 2,9,16,23-tetrakis[4-(N-ethylpyridyloxy)]-phthalocyanine.

2.4. Antimicrobial studies

E. coli was grown on a nutrient agar plate prepared according to the manufacturer's specifications to obtain individual colony. The colony was then inoculated into Luria nutrient broth and then