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Photo-Bactericidal Property and Characterization of Cellulosic Fabric Treated with Two Tetra-Cationic Porphyrin Compounds

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Abstract

The cellulosic fabrics bearing the porphyrin as a photo-sensitizer were done in order to prepare efficient polymeric materials for antimicrobial applications. The obtained porphyrin-grafted cellulosic fabrics were characterized by ATR-FTIR, DRUV spectroscopy, TG and SEM image. The antimicrobial activity of the products was tested under visible light irradiation against *Staphylococcus aureus*, *Pseudomunas aeroginosa* and *Escherichia coli.*

Keywords: Cellulosic fabrics; Photo-bactericidal activity; Photodynamic Antimicrobial Chemotherapy; Tetra-cationic porphyrins; Thermo-gravimetric analysis; Zinc porphyrin compound.

Introduction

The photosensitizers (PS) such as porphyrin compounds have been intensively studied for their use as photo-bactericidal agents against both Gram negative and Gram positive bacteria in photodynamic antimicrobial chemotherapy (PACT) [1-5].

PACT relies on the intracellular accumulation of a (PS) upon illumination with visible light and produces singlet oxygen or generates free radicals. Singlet oxygen ($^1O_2$) is able to react with almost every cellular ingredient, bringing about irreversible damage that ultimately leads to cell death [2]. This method has recently been studied against a wide range of clinically important bacteria, yeasts, fungi and viruses [6-9].

A number of water-soluble cationic porphyrins and phthalocyanine complexes of biocompatible metals (such as: Zinc, gallium and silicon), porphyrin compounds such as meso-tetrakis-(4-aminophenyl)-porphyrin, meso-tetrakis-(N-methylpyridyl)porphyrin and other photoactive dyes were used as biocompatible porphyrins for using in PACT. Photo-bactericidal cellulosic surfaces have been synthesized from cellulose and natural or synthetic porphyrins [1,2,10-13].

Due to the cellulose is a compatible molecule with no cytotoxic properties and perfect support for immobilization of bioactive molecules for application in medical and biological fields [14,15] we applied various concentrations of tetrakis(4-N,N,N-trimethylanilinium)porphyrin and its zinc metal ion complex to the cellulosic surface for the first time and characterized porphyrin-grafted cellulosic fabrics using various analysis methods (ATR-FITR, DRUV, SEM and TG). In addition, photo-bactericidal activity of these treated cellulosic fabrics was tested against *E. coli* (urinary tract infections [16]), *P. aeruginosa* (infection in patients with endotracheal and urinary cathetersis common [17]) and *S. aureus* (infection in patients with prosthetic devices, venous catheters, and peritoneal dialysis catheters etc. [18]) under illumination with visible light.

Materials and Methods

All chemicals were purchased from Merck Company and used without further purification. The porphyrin, tetrakis(4-N,N,N-trimethylanilinium)porphyrin (TAPP) and its zinc ion complex (ZnTAPP) were synthesized as reported previously [19]. All fabrics were of plain (woven) construction, weighing 162.5 g/m^2, unfinished 100% cellulosic fabric, laundered, dried and the preparation of photoactive cellulosic fabrics and porphyrin treatment with them were done according to our previous article [20].

Bacterial strain and preparation of cultures

The bacterial strains *S. aureus, E. coli* and *P. aeruginosa* were obtained from microbiology laboratory of University of Guilan and were inoculated in liquid culture medium [nutrient broth (Source: Merck Company)] and incubated at 37°C overnight under aerobic conditions in an incubator. The stock suspensions of liquid culture medium were prepared approximately ≈10^8 colony forming units/mL (CFU/mL). Antibacterial activity of photosensitive cellulosic fabric was done according to our previous article [20].

Irradiation system

All the experiments were carried out in a water-jacked reactor irradiated with a 100 W tungsten lamp (1250 lumen), as a visible light source with an average intensity of ~0.36 mWcm^{-2} at a distance of 20 cm from the sample. To avoid light reflection, the reactor was placed in a dark room.

Sample characterization

UV-spectra were recorded on a UV-1700 pharma Spec (Shimadzu) with a quartz cuvette. ATR-FTIR spectra of untreated and treated cellulosic fabrics were recorded on Shimadzu FT-IR-8400S

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spectrophotometer. DRUV spectra were prepared with a Shimadzu (MPC-2200) spectrophotometer. Thermo-gravimetric analyses of samples were carried out using a TGA V5.1A Dupont 2000 instrument with a heating rate of 10 °C/min in air and all samples were heated from 20-600°C. Surface morphology of untreated and treated cellulosic samples was observed by SEM using a VEGA TESCAN scanning microscope. Electron micrographs of the sample were recorded at 600×magnification.

Results

The effect of photosensitizers on cellulose surface was confirmed by attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, diffuse reflectance UV-Vis (DRUV) spectroscopy, SEM and thermo-gravimetric analysis (TG).

ATR-FTIR spectra of treated and untreated cellulosic fabrics are presented in Figure 1a. Classic spectral data have been found: 3340 cm^{-1} (OH stretching), 2850 and 2900 cm^{-1} (symmetric and asymmetric C-H stretching), 1325, and 1045 cm^{-1} (C-O stretching), (600-800) cm^{-1} (alcoholic –OH out-of-plan bending, out-of-plan ring stretching in cellulose β-linkage) [21-24]. The samples of treated cellulose display a weak signal at 3340 cm^{-1} and (600-800) cm^{-1}, corresponding to OH groups and the changes in the bands of (C-O stretching) at the range of (800-1325) cm^{-1}. These observations confirmed the linkage between porphyrins and the cellulosic fabric [1,25].

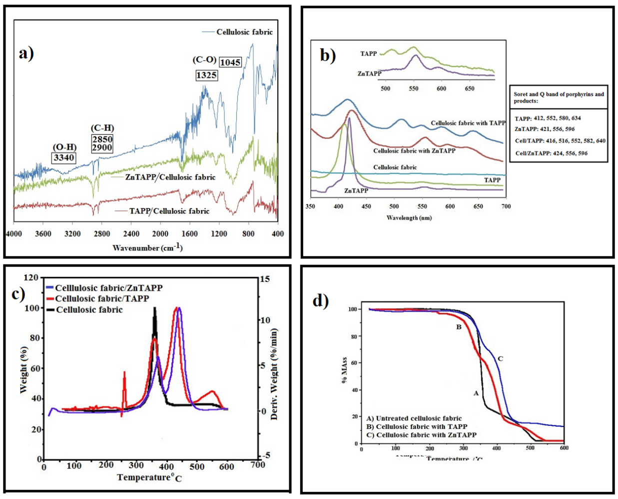

Figure 1: a) ATR-FTIR spectra (600-4000 cm^{-1}) of untreated cellulose, treated cellulose with $TAPP^{4+}$ and treated cellulose with $ZnTAPP^{4+}$; b) DRUV spectra (350-700 nm) of treated and untreated cellulose. Porphyrins spectra recorded at 2×10^{-6} M in water; c) TG and DTG thermograms of untreated and treated cotton fabric.

DRUV analysis of porphyrin solutions were shown Soret band near 412-424 nm and Q bands between 500-700 nm clearly show up (Figure 1b). Upon grafting the porphyrins onto cellulosic fabrics, Soret bands broadened that can be attributed to π-electron interaction with surface hydroxyl groups [9]. Therefore, DRUV analyses confirmed that porphyrins are either attached onto the surface via interaction between porphyrin and cellulose.

Thermo-gravimetric analyses (TG and DTG) were used to investigate the thermal properties of porphyrins attached to cellulose (Figure 1c and 1d). The pyrolysis of untreated cellulosic fabric comprises different major stages. The main pyrolysis stage of cellulosic fabric occurs in the temperature zone between 300-370°C [23,24].

At higher temperatures, treated cellulosic samples showed multistep mass loss due to decomposition of photo-sensitizers, or degradation of polymeric material or the backbone itself [25]. Also, residual mass percent of samples at 600°C were 15.08% and 1.74% respectively for $ZnTAPP^{4+}$ and $TAPP^{4+}$, whereas that for untreated cotton fabric was 1.63%.

The surface of untreated cellulose and porphyrin-grafted cellulose was examined by scanning electron microscopy (SEM) and did not show any destruction of cellulose fibers (similar diameter and structure) (Figure 2).

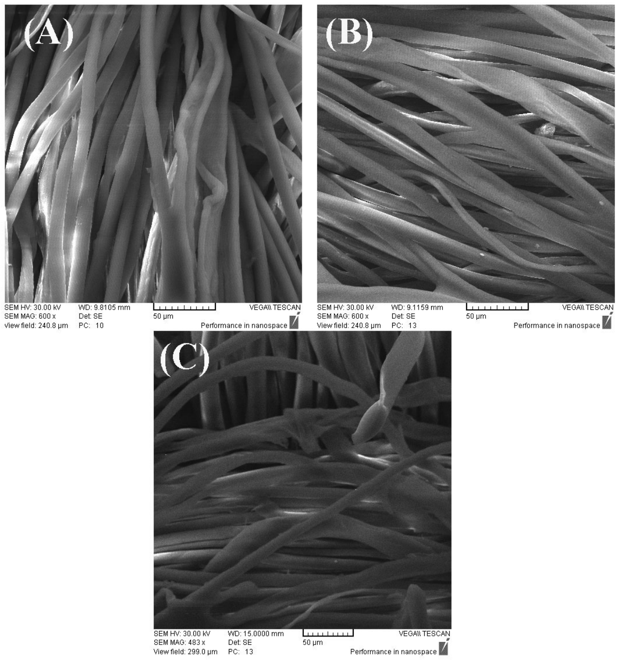

Figure 2: SEM photomicrographs (50 µm scale) of cellulose fibers. Untreated cellulosic fabric (a) and treated cellulosic fabric with TAPP4+ (b) ZnTAPP4+.

Photodynamic activity of treated cellulose was evaluated *in vitro* against bacteria. Extensive research on the effect of singlet oxygen on photo-inactivation of bacteria [11,26-29], indicates that singlet oxygen diffusion is an effective factor. Figure 3 shows the percentage of photo-inactivation against three bacterial strains. Untreated samples in the dark allow bacterial growth; but *S. aureus* growth under light irradiation for 90 min on untreated samples was reduced by about ~12.7%. Samples treated with $ZnTAPP^{4+}$ and $TAPP^{4+}$ (in the dark) allow bacterial growth and have little effect on reducing the number of bacteria; about 1-6%. Both types of treated cellulose have photo-bactericidal activities against *S. aureus*. At low concentrations, these compounds have photo-inactivation property against this strain. *E. coli* and *P. aeruginosa* are Gram-negative bacteria and are less susceptible to PACT than Gram-positive bacteria. $ZnTAPP^{4+}$ ([PS]=10^{-4} M, 90 min) exhibits photo-bactericidal effect against *P. aeruginosa* whereas TAPP4+ exhibits 52.4% photo-inactivation. It seems that the Zinc atom in the $TAPP^{4+}$ is clearly playing a synergistic inhibitory role in bacterial growth.

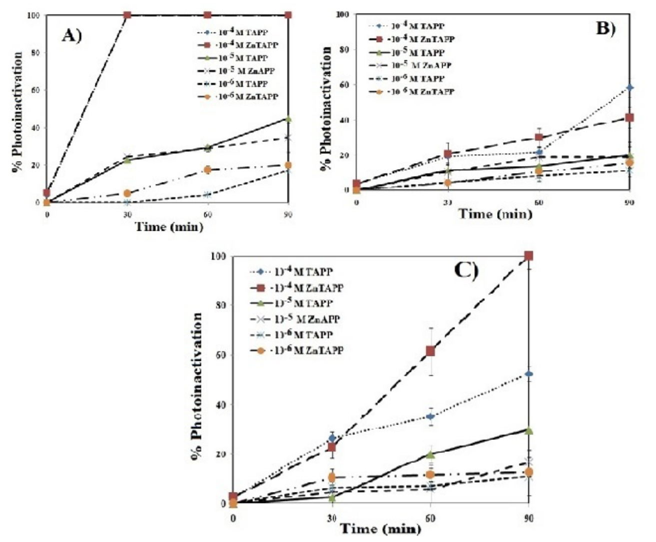

Figure 3: The percent of photo-inactivation of (A) *S. aureus* (B) *E. coli* and (C) *P. aeruginosa* by treated cellulosic fabric with $TAPP^{4+}$ and $ZnTAPP^{4+}$.

Furthermore, both products have photo-inactivation effect on *E. coli*. The percentages of photo-inactivation for TAPP4+ were: 19%, 21.6% and 58.5% and for $ZnTAPP^{4+}$ they were: 20.7%, 30% and 41.3% at a concentration of 10-4 M of [PS] after 30, 60 and 90 min illumination.

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