**Antibacterial photoactivity and Thermal stability of Tetra-cationic porphyrins immobilized on Cellulosic Fabrics**

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**Abstract**

The thermal stability and photo-bactericidal effect of several tetra-cationic porphyrins and their zinc ion compounds immobilized onto cellulosic fabrics against *S. aureus*, *P. aeruginosa* and *E. coli* were investigated and compared using a 100 W tungsten lamp. Immobilization of various concentrations of these photo-sensitizers onto cellulosic fabrics was carried out and characterized by ATR-FT-IR, DRS, TGA and SEM. Applied cellulosic fabrics with the photo-sensitizers exhibited remarkable photo-stability, thermal stability and antimicrobial activity against these studied strains.

***Keywords:*** *Cellulosic fabrics; Photo-antimicrobial activity*; *Photo-bactericidal; Photo-inactivation; Tetra-cationic porphyrin;* thermal stability*.*

**Introduction**

In order to remove pathogenic bacteria from various surfaces and decrease their survival rates, the fabrication of new bactericidal cellulosic fabrics can play an essential medical role. The most frequent perpetrators, both in hospitals and also in other environments, being *Pseudomonas aeruginosa* (*P. aeruginosa*), *Escherichia coli* (*E. coli*) and *Staphylococcus aureus (S. aureus*) [1], [2]. The increasing use of antimicrobial drugs against these bacteria and their consequent antimicrobial resistance is well documented [3]. Therefore, research aimed at producing effective surfaces with antimicrobial properties is ont only necessary, but also crucial.

In past years, photodynamic inactivation (PDI) as an effective method, has been used to inactivate a broad spectrum of microorganisms that can cause serious clinical problems. Photodynamic antimicrobial chemotherapy (PACT) has recently been studied against a wide range of bacteria, yeasts, fungi and viruses [3]–[10]. In this process, depending on the type of a photo-sensitizer and used light source, free radicals or more probably singlet oxygen can be formed. These species are highly reactive and able to interact with virtually every cell component such as protein, lipid, and nucleic acid. This interaction can lead to generation of a number of reactive by-products such as reactive oxygen species (ROS). These species can induce further damage and lead to cell death [11], [12]. Porphyrin compounds are a variable class of photosensitive compounds with numerous applications in bio-mimetics [13],[14] catalysis and photodynamic therapy. Among the various porphyrin compounds, cationic porphyrins have been shown to be most effective on Gram-negative and Gram-positive bacteria [7], [9], [15]–[19]. Gram-negative bacteria are less sensitive to PACT than Gram-positive bacteria but cationic porphyrins have been shown to inactivate Gram-negative bacteria without using a permeabilization agent [20],[21]. For example, the effect of photodestruction of meso-tetra(N-methyl-4-pyridyl)porphine tetratosylate salt (TMPyP(4)) have been evaluated by Romanova group [22], Pudžiuvytė group [23], Hanakova group [24], Malara group [25] and Tabrizi and et al. [26] by various lamp sources and strains of bacteria. Meso-tetra(3-hydroxyphenyl)chlorin, cationic tetrakis (N-ethylpyridinium-4-yl) porphyrin tetratosylate, zinc phthalocyanine tetrasulfonate, aluminium phthalocyanine have been studied by Peèkaitytë group [8] by LED against *E. coli*, *Salmonella enterica serovar*, *Bacillus subtilis* strain and *Bacillus thuringiensis*. Caminosand Durantini were investigated various cationic porphyrin compounds by Xe lamp, halogen lamp for inactivation of *E. coli* [27], [28], [29]*.* Also,Oliveira and et al. [30] have been studied the effect of various cationic porphyrin compounds by a quartz ⁄halogen lamp against *Bacillus cereus*. According to these literatures, there is no a constant method to study PACT of photo-sensitizers; and various parameters are variable including: strains of bacteria, light source type of photo-sensitizers, illumination time etc. It is difficult to make an accurate comparison between the research work done. But, in all of these researches, tetracationic porphyrin compounds have been identified as effective compounds. In this study, it was decided to investigate and compare the effect of several tetracationic compounds for inactivation of bacteria by choosing three strains of bacteria and a constant visible light source.

Solid supported porphyrins arouse much attention for they not only have increased stability and selectivity but can also be reused for PACT. Various antibacterial surfaces or fabrics have been developed by grafting or incorporating antibacterial compounds into/onto coating polymers[31]. Among the supports that can be used to immobilize porphyrins, cellulose or its derivatives are often employed for their cheapness and ready availability [32]. Cellulosic surfaces or fabrics with antibacterial properties have been prepared using quaternary ammonium salts (such as cetylpyridinum and benzyldimethylhexadecyl ammonium chloride), antibiotics, *N*-halamines, chitosan, phosphonium salts, ureas and related compounds, formaldehyde derivatives and amines, protoporphyrin-based, light-activated singlet oxygen generators, poly (ether ketone) or guanidine polymer [1], [2], [16], [33], [34]. In addition, bactericidal polymers have also been synthesized through cellulose modifications [29–31]. Also, the photo-bactericidal activity of cellulosic surfaces treated with various meso-arylporphyrins have been studied. Tabe 1 shows various cellulosic support porphyrins with photo-inactivation property and various light source that have been used in litrature.

**Table 1.** Photobactericidal cellulosic surfaces with various porphyrin compounds

|  |  |  |  |
| --- | --- | --- | --- |
| **Support** | **Porphyrins** | **Light source** | **Sensitive bacterial strains** |
| Cellulosic fabric | TPP-NH2, TPPS-NH2, trans-MePy+‏-NH2[16] | LED | *S. aureus, E. coli* |
| Cellulosic plastic film | 5-[4-(3-carboxypropyloxy)phenyl]-10,15,20-tri(4-methylphenyl) porphyrin); 5-[4-(10-carboxydecanoxy)phenyl]-10,15,20-tri(4-methylphenyl) porphyrin [38]  | four 150W tungsten bulbs | *S. aureus, E. coli* |
| Cellulosic plastic films | protoporphyrin IX; 5-[4-(3-propargyloxy) phenyl]-10,15,20- tritolylporphyrin; 5-(4-hydroxy phenyl)-10,15,20-tritolylporphyrin; Protoporphyrinato IX (Zn II) dipropargyl ester; Protoporphyrin IX dipropargyl ester [39]  | ten 23 W bulbs | *E. coli, S. aureus* and *P. aeruginosa* |
| Cellulosic plastic films | Monopyridyltritolylporphyrin [40]  | 150W tungsten bulb | *S. aureus, E. coli* |
| Cellulosic fabric | Monohydroxyphenyltritolylporphyrin [41]  | white light | *S. aureus, E. coli* |
| Cellulosic plastic film | protoporphyrin IX [11]  | 150W tungsten bulb | *S. aureus, E. coli* |
| Cellulosic paper | 5-(4-Nitrophenyl)-10,15,20-(4-pyridyl) porphyrin; 5-(4-Aminophenyl)-10,15, 20-tri(4-*N*-methylpyridinium)Porphyrin[42] | LED | *S. aureus, E. coli* |
| nanofibrillated cellulose (NFC) and paper | 5-(4-aminophenyl)-10,15,20- tris-(4-N-methylpyridinium)porphyrin, [5-(4-aminophenyl)-10,15,20-tris-(4-N- methylpyridinium)porphyrinato]zinc(II) [43] | ALumaCare USA model  | *S. aureus, E. faecium, A. baumannii,* *K. pneumoniae (KP;*  |
| Cellulose nanofiber | protoporphyrin IX [44] | Xe lamp | *S. aureus, E. coli* |

In previous work [45], have been investigated the photodynamic activity of tetrakis(4-*N*,*N*,*N*-trimethylanilinium)porphyrin **(TAPP)** and its zinc metal ion complex grafted onto cellulosic surfaces. In this paper, we have evaluated the photodynamic activity of meso-tetrakis(*N*-methyl-3-pyridyl)porphyrin chloride **(TMPyP(3))**, meso-tetrakis(*N*-methyl-4-pyridyl)porphyrin chloride **(TMPyP(4))**, meso-tetrakis(4-*N*,*N*,*N*-trimethylanilinium)porphyrin chloride **(TAPP)** and their zinc compounds as tetracationic porphyrin compounds to inactivate *E. coli,* *P. aeruginosa* and *S. aureus* cells in vitro and immobilized them on cellulosic surface. The photo-inactivation behavior of these tetracationic porphyrins was compared with our previous work to choose the best photo-sentisizer in terms antibacterial activity. Also, the thermal stability of the porphyrin compounds on the cellulosic fabric was investigated by thermogravimetric analysis.

**Experimental**

*General Methods*

*A*ll reagents and solvents, nutrient agar and nutrient broth were purchased from Merck Company. The cellulosic fabric was purchased from the Isfahan Company using 162.5 g/m2, unfinished 100 % cellulosic fabric. As in our previous work [45], all fabrics were of plain (woven) construction, laundered and dried. They were then cut along the fiber direction in (2 × 5 cm) strips and pre-washed in hot deionized water. Laboratory temperature was 25 ± 2 ˚C. All porphyrin compounds in this work were purchased from MidCentury (Chicago, Illinois). The structures of the photo-sensitizers are shown in figure 1. The information of 1H NMR, FT-IR and UV-Vis spectra of the used porphyrin compounds were recorded and given as follow:

***TMPyP(3):*** *1H NMR (D2O)* δ: 4.53 (s, 12H); 8.44 (t, 4H); 8.92 (s, 8H); 9.23 (d, 4H); 9.30 (d, 4H); 9.71 (s, 4H); *FT-IR (cm-1):* 700-900 (porphyrin ring vibration), 975 (N-H bending), 1000-1300 (C-N stretching), 1475-1600 (C=C stretching), 1631 (C=N stretching), 2920 (C-H stretching), 2952 (N-H stretching); *UV-Vis in H2O (λmax, nm):* 417, 516, 550, 582, 642 nm.

***TMPyP(4):*** *1H NMR (D2O)* δ: 4.65 (s, 12H); 8.80 (d, 8H); 9.05 (d, 8H); 9.17 (d, 8H); *FT-IR (cm-1):* 700-900 (porphyrin ring vibration), 970 (N-H bending), 1000-1300 (C-N stretching), 1400-1500 (C=C stretching), 1637 (C=N stretching), 2952 (C-H stretching), 3031 (N-H stretching); *UV-Vis in H2O(λmax, nm):* 422, 515, 554, 556, 641 nm.

***TAPP:****1H NMR (D2O)* δ: 3.66 (s, 36H); 8.09 (d, 16H); 8.68 (s, 8H); *FT-IR (cm-1):* 700-900 (porphyrin ring vibration), 900 (N-H bending), 1000-1300 (C-N stretching), 1469, 1487 (C=C stretching), 1606 (C=N stretching), 3029 (N-H stretching); *UV-Vis in H2O (λmax, nm):* 412, 515, 552, 580, 634 nm.

***ZnTMPyP(3):*** *1HNMR (D2O)* δ: 4.60 (s, 12H); 8.36 (t, 4H); 8.91 (s, 8H); 9.16 (d, 4H); 9.24(d, 4H); 9.64(s, 4H); *FT-IR (cm-1):* 700-900 (porphyrin ring vibration), 1000 (Zn-N stretching), 1000-1300 (C-N stretching), 1475-1600 (C=C stretching), 1633 (C=N stretching), 2966 (C-H stretching);*UV-Vis in H2O (λmax, nm):* 428, 558, 594.

***ZnTMPyP(4):*** *1HNMR (D2O)* δ: 4.35 (s, 12H); 8.50 (d, 8H); 8.75 (d, 8H); 8.87 (d, 8H); *FT-IR
(cm-1):* 700-900 (porphyrin ring vibration), 1000 (Zn-N stretching), 1000-1300 (C-N stretching), 1400-1500 (C=C stretching), 1639 (C=N stretching), 2923 (C-H stretching); *UV-Vis in H2O (λmax, nm):* 436, 563, 606.

***ZnTAPP:****1HNMR (D2O)* δ: 3.36 (s, 36H); 7.68 (s, 8H); 7.38(s, 8H); 8.53 (s, 8H); *FT-IR
(cm-1):* 700-900 (porphyrin ring vibration), 1000-1300 (C-N stretching), 1473,1494 (C=C stretching), 1606 (C=N stretching); *UV-Vis in H2O (λmax, nm):* 421, 556, 596.

**<Figure 1>**

*Spectroscopic Measurements*

Absorption spectra in the range (400-700) nm, ATR-FT-IR at the range (400-4000) cm-1 and reflectance UV-Vis spectroscopy at the range (400-700) nm were recorded on a UV-1700 pharma Spec (Shimadzu), Shimadzu FT-IR-8400S spectrophotometer and Shimadzu (MPC-2200) spectrophotometer, respectively. For thermo-gravimetric analyses of the samples used, a TGA V5.1A Dupont 2000 instrument with a heating rate of 10 ˚C /min in the air was used and all samples were heated from 20-600 ˚C. The surface morphology of cellulosic samples was observed by SEM using a VEGA TESCAN scanning microscope. Electron micrographs of the sample were recorded at 600×magnification.

*Photo-stability* *study of prepared porphyrins*

The photo-stability of porphyrins was determined in distilled water and nutrient broth at pH=7.4 upon illumination with our irradiation system as same as S. Banfi,s article [7]. During irradiation, the solution was magnetically stirred at room temperature and the UV-Vis absorption measured in 10 min intervals; photo-stability was expressed as the percentage residual absorbance compared to absorbance measured before irradiation.

*Preparation of photosensitive cellulosic fabric*

For impregnation of the porphyrins onto cellulosic fabrics, at first, the fabrics were immersed in an alkaline compound containing 1 g/100 ml Na2CO3 at 50 ˚C for 30 min. Afterward, the fabrics were prepared and applied to the selected concentrations of porphyrins for 60 minutes at 50 ˚C as in Rahimi et al. [35]. This was followed by washing cycles with hot water to remove unbound photosensitizers. After drying in an oven at 50 ˚C, the resulting cellulosic fabric samples gained. The percent of the molar grafting ratio of porphyrins to cellulosic fabric was calculated by the method of Ringot et al. [16]:

$Grafting ratio \left(\%\right)=\left[1-\frac{\frac{A\_{soret }}{E\_{soret }}×V ×d}{n\_{initial}}\right]×100$ *(eq 1)*

*ASoret* =Soret band absorbance of free photo-sensitizer in solution of the final reacting solution; *𝓔Sore*t = Molar absorption coefficient of the free photo-sensitizer at the Soret band;
*V* = volume of prepared solution for obtaining an absorbance value between 0 and 1; *d* = dilution factor done for UV-Vis measurement; *ninitial* = the initial amount of photo-sensitizer (mol)

*Washing durability photosensitive cellulosic fabric*

Durability of the treated cellulose was tested in soap solution following ISO 1 Test Method [46]. In the current study, the samples rinsed with a 50:1 liquor ratio in a vessel with 5g/l standard soap solution for 30 min. The washing temperature was 40 °C. The solution was stirred slowly and followed by washing cycles with water to remove unbound photosensitizers and was shown by spectrophotometry.

*Bacteria Culture*

*S. aureus* (Gram-positive bacterium)*, E. coli* and *P. aeruginosa* (Gram-negative bacteria) were obtained from the microbiology laboratory of University of Guilan. All strains were inoculated in nutrient broth and incubated at 37 ˚C for 18 hours under aerobic conditions in an incubator. The stock suspensions of liquid culture medium were diluted to obtain 108 colony forming units/ml (CFU/ml).

*Antibacterial activity*

30 μl of broth culture (108 CFU/ml) was aseptically transferred onto nutrient agar plates and spread on the surface. Wells (diameter 5 mm) were made in nutrient agar seeded with the target strain, using sterile Pasteur pipette ends. A stock solution of porphyrins was prepared in distilled water at various concentrations and added to these wells. The plates were first incubated at 37 °C for 20 minutes in the dark, followed by illumination for 30 minutes. They were then incubated at 37 °C overnight in the dark.

Two control cultures were used for incubated of bacteria: with water in the same conditions as negative control and using porphyrins not activated were done. The experiments were carried out in triplicate. Bacterial growth was examined visually by measuring inhibition zones around the wells and also by colony counts of serially diluted samples of overnight incubated broth cultures inoculated onto nutrient agar plates. A diameter larger than 10 mm was formally considered as a positive response.

Minimum inhibitory concentration (MIC) for photo-sensitizers was determined by preparing nutrient broth inoculated with the culture of bacterial strains (108 CFU/ml) in test tubes and adding varying concentrations of the porphyrins. The culture was incubated at 37 °C overnight and examined for bacterial growth under the microscope. The test tubes were first incubated at 37 °C for 20 minutes in the dark, followed by illumination for 30 minutes. They were then incubated at 37 °C overnight in the dark. Two control cultures were used for incubated of bacteria: porphyrins with activated and porphyrins not activated. Cultures exhibiting MIC were further analyzed to determine whether minimum bactericidal concentration (MBC) had been attained.

MBC was determined for porphyrin concentrations giving a negative culture reaction in the MIC assay. Briefly, 100 μl of culture exhibiting MIC was spread onto the surface of a nutrient agar plate and incubated at 37 °C overnight. Bacterial growth was examined visually and the absence of growth indicated MBC.

The antibacterial activity of the porphyrin compounds were shown as the percentage of photo-inactivation. The percentage of the photo-inactivation shows the percentage of killing of the bacteria. This parameter were calculated by *(eq 2)*:

% photo-inactivation= $\frac{\left[c\_{0}\right]-[C]}{[c\_{0}]}×100$ *(eq 2)*

[c]: the number of CFU/ml of the bacteria at the end of the experiment

[c]0: the number of CFU/ml of the bacteria at the beginning of the experiment.

*Antibacterial activity of photo-sensitive cellulosic fabric*

Antibacterial activity of the photo-sensitive cellulosic fabric was determined according to Rahimi et al. [45]. Briefly, the bacterial culture was diluted in nutrient broth to obtain a cell density of 108 CFU/mL, transferred onto solid culture medium plates and spread on the surface. Sterile photosensitive cellulosic fabric (1 × 1 cm) was put on the inoculated Petri dish. After incubation in the dark for 20 min, the plates were illuminated at various times and finally incubated at 37 ˚C overnight in a humidified incubator.

Control cultures, with untreated cellulosic fabrics, were exposed to the light for the same period. Also, a second control was employed with cellulosic fabric in the dark. A diameter larger than 10 mm was considered as a positive response.

100 μL of undiluted and serially diluted treated and control cultures were placed on nutrient agar medium and the percentage of photo-inactivation was calculated [45].

*Irradiation system*

A 100 Watt tungsten lamp (1250 lumen) with an average intensity of ~0.36 mW.cm-2 was used as light source and placed at a distance of 20 cm from the sample in a shaker incubator. To absorb heat and cool the system, inlet and outlet water fluid was used. All the experiments were carried out in a dark room to avoid light reflection (figure 2).

**<Figure 2>**

**Results and discussion**

The effect of various concentrations of the photo-sensitizers compare with several antibiotics against the bacterial strains on agar surface is shown in Table 2. Inhibition zones larger than 10 mm were considered as a positive response. As shown in Table 1, ZnTMPyP(3) (10 µg/well), TMPyP(3) and ZnTAPP at 30 µg/well against *S. aureus*; TMPyP(3) and ZnTMPyP(3) at 60 µg/well against*P. aeruginosa*; and TMPyP(3),ZnTMPyP(3), TMPyP(4) and ZnTAPP at 60 µg/well were effective against *E. coli.* Chloramphenicol was shown to be more effectiveagainst *E. coli* and *S. aureus* than the other antibiotics; Ampicillin did not show any appreciable effect against *P. aeruginosa* and *E. coli.* This data was provided as control and for comparison against our studied photo-sensitizers.

**Table 2.** The effect of various concentrations of the photo-sensitizers against selected strains on agar surface.

|  |  |  |  |
| --- | --- | --- | --- |
| **Sample** | **Diameter of inhibition****zone (mm)*****S. aureus*** | **Diameter of inhibition****zone (mm)*****P. aeruginosa*** | **Diameter of inhibition** **zone (mm)*****E. coli*** |
| **Concentration µ)g/well)** | 10 | 30 | 60 | 10 | 30 | 60 | 10 | 30 | 60 |
| **Untreated****TMPyP(3)** | -9 | -11 | -12 | -6 | -6 | -11 | -7 | -8 | -1011 |
| **ZnTMPyP(3)** | 10 | 11 | 11 | 6 | 6 | 11 | 8 | 9 |
| **TMPyP(4)** | 6 | 7 | 11 | 6 | 6 | 7 | 6 | 8 | 10 |
| **ZnTMPyP(4)** | 7 | 7 | 8 | 6 | 6 | 7 | 6 | 6 | 7 |
| **TAPP** | 8 | 9 | 9 | 6 | 6 | 6 | 7 | 9 | 9 |
| **ZnTAPP** | 9 | 10 | 11 | 6 | 6 | 6 | 6 | 7 | 10 |
| **Ampicillin**  | 22 | - | - | - | - | - | 8 | - | - |
| **Cefotaxime** | - | 26 | - | - | 20 | - | - | 11 | - |
| **Chloramphenicol** | - | 28 | - | - | 20 | - | - | 36 | - |
| **Tetracycline** | - | 26 | - | - | 24 | - | - | 28 | - |

Photodynamic activity of all porphyrin compounds was evaluated *in vitro* against three strains of bacteria: Gram-positive bacterium *(S. aureus*) and Gram-negative bacteria (*E. coli* and *P. aeruginosa*). Minimum inhibitory concentrations and minimum bactericidal concentrations of the photo-sensitizers were determined as the percent of photo-inactivation. Figure 3 shows the percentage of photo-inactivation in the presence of these porphyrin compounds at various concentrations.

For negative, the samples of bacteria without photo-sensitizers were illuminated. *S. aureus* was only sensitive strain to illumination in the absence of the compounds. It was reduced about 1 log. The effect of the photo-sensitizers under dark conditions, without light activation, was also determined, although the results are not shown in this figure. According to the results, only TMPyP(4) and ZnTAPP at a concentration of 60 µg/ mL and TAPP at a concentration of 15 µg/ mL exhibited MBC against *S. aureus* and are 100 % photo-bactericidal. Other photo-sensitizers had no MBC effect against studied strains at any concentrations. But these photo-sensitizers exhibited photo-inactivation effect against the Gram-negative bacteria. The highest levels of photo-inactivation effect was observed with ZnTMPyP(3) and ZnTAPP against *P. aeruginosa* and *E. coli*, with about 45 % inactivation, respectively. Gábor et al. [47] have shown that the symmetrically substituted structure is preferable for association to both type of *E. coli* and *Enterococcus hirae.* According to our results, it seems that *S. aureus* is most effeciently photo-inactivated effect by more symmetric molecules, such as TAPP, ZnTAPP, TMPyP(4) and ZnTMPyP(4). .

**<Figure 3>**

Table 3 shows the effect of the studied tetra-cationic porphyrins against the bacteria under dark conditions. Our results showed that the maximum inactivation was achieved with TAPP and ZnTAPP against *S. aureus.* These compounds did not show significant activity against Gram-negative strains. For cationic compounds, toxicity and activity under dark conditions is probably due to the presence of the quaternary ammonium charge, known to disorganize bacterial cell walls without light irradiation [48]. Nitzan *et al.* had shown that cationic porphyrins were active against Gram-positive bacteria and Gram-negative [18], [49]–[52]. Cationic porphyrin such as TMPyP are excellent photosensitizers for the photo-inactivation of gram-positive and gram-negative bacteria[53].

**Table 3.** The effect of the photo-sensitizers at a concentration of 60 μg/ml against selected strains under dark condition

|  |  |  |  |
| --- | --- | --- | --- |
| Samples at Concentration 60 μg/ml | % inactivation *S. aureus* | % inactivation *P. aeruginosa* | % inactivation *E. coli* |
| TMPyP(3) | 5 | 5.16 | 2.3 |
| ZnTMPyP(3) | 6 | 8.5 | 1 |
| TMPyP(4) | 24.2 | 1 | 1.3 |
| ZnTMPyP(4) | 5 | 0.8 | 2.6 |
| TAPP | 35 | 7.7 | 5.3 |
| ZnTAPP | 30 | 6.28 | 4.5 |

Photo-stability of the porphyrins was determined in distilled water and nutrient broth at pH=7.4 upon illumination after 10, 20 and 30 min with the irradiation system; the results are shown in Figure 4. Photo-stability is expressed as the percentage of residual absorbance relative to the absorbance measured before irradiation.

In distilled water after 30 min irradiation, the residual absorbance for ZnTMPyP(3),TMPyP(3), ZnTMPyP(4), TMPyP(4), TAPP and ZnTAPP was determined to be 92.2 %, 82.5 %, 87.5 %, 87 %, 74.6% and 81%, respectively; whereas in nutrient broth under the same conditions, the residual activity was 90 %, 88.8 %, 91.8%, 92, 91% and 92%, respectively. Therefore, ZnTMPyP(3) is generally more stable than the other photo-sensitizers in distilled water. All of the photo-sensitizers showed approximately the same photo-stability in nutrient broth. The amounts of photo-stability of these compounds in nutrient broth media confirm that stability/instability is not a determinative factor in their performance.

**< Figure 4** **>**

To determine bactericidal and bacteriostatic activity of fabric surfaces, the photo-sensitizers were impregnated onto cellulosic fabric. To this end, the fabrics were first washed in distilled water several times to remove all impurities. Then, the fabrics were cut along the fiber direction in (2 × 5 cm) strips and immersed in Na2CO3 solution according to (experimental section). Na2CO3 was used to increase the reactivity of cellulose via reaction with its hydroxyl groups. Using this route, a negative charge on the cellulosic fabric was prepared and it could react faster and more easily with various chemicals [54]. Afterthat, the fabrics was washed in 100 ⸰C distilled water several times untile no trace of porphyrins was shown by spectrophotometry. The degree of grafting porphyrins to cellulosic fabric is shown in Table 4 [16], [45].

**Table 4.** Grafting yield determination of photosensitizers by UV-Vis titration

|  |  |  |  |
| --- | --- | --- | --- |
| **Grafted porphyrin** |  **Concentration** **(µMolar)** |  **Grafting yield (%)** | **Amount of grafted photo-sensitizer****(μmol/mg of cellulosic sample)** |
|  | 100 | 97.8(±0.80) | 3 |
|  **TMPyP(3)** | 10 | 98.5(±0.61) | 0.3 |
|  | 1 | 96/0(±0.48) | 0.029 |
|  | 100 | 99.2(±0.70) | 3 |
| **ZnTMPyP(3)** | 10 | 99.5(±0.42) | 0.3 |
|  | 1 | 94/0(±2.82) | 0.028 |
|  | 100 | 97.4(±0.45) | 2.99 |
|  **TMPyP(4)** | 10 | 98.6(±0.84) | 0.3 |
|  | 1 | 92.6(±5.09) | 0.028 |
|  | 100 | 93.2(±0.40) | 2.86 |
| **ZnTMPyP(4)** | 10 | 98.7(±0.15) | 0.3 |
|  | 1 | 81.4(±0.60) | 0.025 |
|  | 100 | 90.75(±1.06) | 2.79 |
|  **\* TAPP** | 10 | 98.75(±0.35) | 0.3 |
|  | 1 | 82.77(±5.09) | 0.025 |
|  | 100 | 97.75(±0.08) | 3 |
|  **\* ZnTAPP** | 10 | 98.63(±0.15) | 0.3 |
|  | 1 | 90.57(±5.65) | 0.027 |
|  |  |  |  |

\* The data of these compounds were done at our previous work [45].

The amount of grafted photo-sensitizers of cellulosic fabrics is shown as micromol of photo-sensitizer (PS) per milligram of cellulosic samples. The increasing concentration of photo-sensitizers can lead to an increase in the amount of grafting efficiency. The amount of porphyrins used only reddened the color of fabric and did not change in the softness and texture of the fabric in appearance or by touching.

The washing durability of the antimicrobial functions on finished cellulosic fabrics was evaluated in soap solution at maximum concentration according to ISO 1 Test Method [46] after 3 times washing and the results are shown in figure 5. At first, the samples were rinsed with a 50:1 liquor ratio in a vessel with 5g/l standard soap solution at 40 °C for 30 min. The solution was stirred slowly and followed by washing cycles with water to remove unbound photosensitizers and was shown by spectrophotometry. The washing solutions were collected and evaluated by spectrophotometery to determine the existence of any porphyrin in the washing solutions. No trace of porphyrins was shown by UV-Vis spectrophotometery for treated cellulose with TMPyP(4), ZnTMPyP(4), TAPP and ZnTAPP; and did not show any reduction of grafting yield as shown in Table 3. This experiment illustrated that these porphyrins remained linked to cellulosic fabric and showed interaction with the cellulose functional groups. But, for treated cellulose with TMPyP(3) and ZnTMPyP(3) was observed trace of porphyrin after washing with soap solution and reduced grafting yield (see figure 5). It seems that the lower symmetry of TMPyP(3) and ZnTMPyP(3) than the other photosensitizers can be caused to reduce their insertion to cellulosic fabric. The washing durability test can be confirm the linkage of these photo-sensitizers to the fabric.

**<Figure 5>**

The interaction between the photo-sensitizers and cellulosic surface was examined by ATR-FT-IR, UV-Vis, Diffuse reflectance UV-Vis spectroscopy and SEM. TG analysis was used in a comparative study of the resistance of treated and untreated cellulosic fabrics against heat.

ATR-FT-IR spectra of treated and untreated cellulosic fabrics are shown in Fig. 6a. The samples of treated cellulose display weak signals at 3340 cm-1 zone (OH stretching) [16], [41], [45] in comparison to strong signals in the untreated cellulosic fabric; which indicates the interaction between the positive charges of the photo-sensitizers with hydroxyl groups of cellulose. Also, it is observed that the band of C-O stretching at 1045 cm-1[16], [41], [45] in treated cellulose in comparison to untreated cellulose at 1325 cm-1 is reduced. This can be related to the interaction between NH groups or zinc of tetra-cationic porphyrins with OH or C-O bonds of cellulose. These observations confirm the linkabbge of these tetra-cationic photosensitizers to the cellulosic fabric.

**<Figure 6>**

Diffuse reflectance UV-Vis spectroscopy was also used to observe and examine the existence of porphyrins on the surface of cellulosic fabric (Fig 6B). Soret band near 420-448 nm and Q bands between 518-648 nm clearly show up, and can be compared with reference molecules as cationic compounds.

**Table 5.** Electronic Diffuse Reflectance UV-Vis spectral data.

|  |  |  |
| --- | --- | --- |
| Products | Soret band maximum (nm) | Q bands (nm) |
| \*TMPyP(3) | 417 | 516, 550, 582, 642 |
| \*ZnTMPyP(3) | 428 | 558, 594 |
| \*TMPyP(4) | 422 | 515, 554, 550, 641 |
| \*ZnTMPyP(4) | 430 | 563, 606 |
| \*TAPP | 412 | 515, 552, 580, 634 |
| \*ZnTAPP | 421 | 556, 596 |
| Cell/ TMPyP(3) | 426 | 518, 554, 588, 644, 688 |
| Cell/ ZnTMPyP(3) | 438 | 562, 600 |
| Cell/ TMPyP(4) | 430 | 524, 560, 594, 648 |
| Cell/ ZnTMPyP(4) | 448 | 570, 610 |
| Cell/ TAPP | 416 | 516, 552, 582, 640 |
| Cell/ ZnTAPP | 424 | 556, 596, 634 |

\*UV-Vis spectra recorded in distilled water

As can be seen in Fig. 6B and Table 5, free porphyrin compounds exhibited Soret bands at 417-430 nm; but when the porphyrins were grafted onto cellulosic fabrics, they exhibited a red shift. The Q bands of treated cellulosic fabrics indicate that the surface of cellulose was not distorted at the surface of porphyrins [16], [45]. The interaction between π-electron of the photosensitizers with hydroxyl groups of the cellulosic fabric can broaden the Soret bands. The latter was attributed to π-electron interaction with surface hydroxyl groups [16]. Diffuse reflectance UV-Vis spectroscopic analyses and the washing durability test confirmed that the porphyrins are attached onto the surface of cellulose and ATR-FTIR showed that there are ionic interactions and probably covalent bonding between NH groups or zinc of porphyrins with OH groups of cellulose. On the other hand, at least for four the photo-sensitizers on the fabric, no trace of porphyrins was shown using UV-Vis spectrophotometery of washings of the fabrics with soap solution, as previously described. All ot these observations confirmed the attachment of the porphyrins to cellulosic fabrics.

To study the resistance of the porphyrins attached to cellulosic fabrics, thermal stability of these products were recorded by TG and DTG thermograms and are shown in figure 7 and Table 5.

The pyrolysis of untreated cellulose includes distinct stages [55]. In the first stage, consisting of two steps, up to 150 ˚C, no mass loss could be observed; and up to 300 ˚C, most remarkable physical changes begin to occur and little mass loss is evident [56]. The main mass loss of cellulosic fabrics occurs between 300–370 ˚C; this step is fast and there is pronounced weight loss. Above 370 ˚C, charring reactions and dehydration tend to be completed. These results are consistent with the literature in this area [57]–[60]. The study of treated cellulosic fabrics showed remarkable changes. At higher temperatures, treated cellulosic samples showed a multistep mass loss due to decomposition of porphyrins, degradation of polymeric material or the backbone itself (at 369 ˚C, 413 ˚C and 452 ˚C for (Cell/ TMPyP(3)); 345 ˚C, 420 ˚C and 510 ˚C for (Cell/ ZnTMPyP(3)); at 347.7 ˚C, 421.7 ˚C; 532.5 ˚C for (Cell/ TMPyP(4)); 343.4 ˚C, 421 ˚C and 532.5 ˚C for (Cell/ ZnTMPyP(4)); at 352 ˚C, 421.7 ˚C; 534.7 ˚C for (Cell/ TAPP) and at 358.6 ˚C, 426 ˚C for (Cell/ ZnTAPP)).

According to the DTG curve and Table 5, the most mass-loss temperature of the untreated fabric occurs at range 300-370 ˚C and its mass-remain percentage is 27 % at 370 °C. Whereas the treated cellulosic fabrics showed a higher mass-loss temperature and also a higher mass-remain percentage than untreated fabric at all temperatures. Also, TG of untreated cellulose has a higher slope than treated samples (i.e. more mass-loss percentage with increasing temperature); and has less residual mass percent than treated samples at 600 ˚C. (See Table 6).

The action of certain materials is to promote the pyrolysis products when the polymer is subjected to thermal degradation [61]. That is, the carbon present in cellulose could be confined to the solid phase during the thermal decomposition, and so degradation could be done through the catalytic dehydration shown below:

**(C6H10O5) x→6*x*C + 5*x*H2O**

These results suggest that treatment of cellulosic fabric with these porphyrin compounds can increase thermal stability of the fabric. Among the studied samples, Cell/ TMPyP(3) and Cell/ ZnTAPP have shown most mass-loss temperature range and mass-remain percentage at 600 °C. So, they have the highest thermal stability.

**Table 6.** Comparative data concerning TG/DTG curves of untreated and treated cellulosic fabrics.

|  |  |  |  |
| --- | --- | --- | --- |
| Sample | Most mass-loss temperature ranges/℃ | Mass-remain percentage at 370 °C | Mass-remain percentage at 600 °C |
| Cell/ TMPyP(3) | 395-510 | 67 | 13.47 |
| Cell/ ZnTMPyP(3) | 385-480 | 64 | 1.9 |
| Cell/ TMPyP(4) | 375-485 | 75 | 4.4 |
| Cell/ ZnTMPyP(4) | 365-465 | 68 | 3.76 |
| Cell/ TAPP | 380-485 | 57 | 1.9 |
| Cell/ ZnTAPP | 398-510 | 70 | 15.08 |
| Untreated cellulosic fabric | 300-370 | 27 | 1.63 |

**<Figure 7>**

Scanning electron microscopy (SEM) was used to examine the surface of untreated cellulose and cell/porphyrins by typical scanning electron photomicrographs (Fig. 8). No hetrological diameter and disorder structure can be seen for cellulose fibers in any of the samples. So, no change in fiber morphology or physical properties of the fibers was observed.

**<Figure 8>**

Three strains of bacteria were used for evaluating of photodynamic activity of treated cellulose *in* *vitro*: *S. aureus*, *E. coli* and *P. aeruginosa*. The result of the percentage of photo-inactivation of the immobilized porphyrins onto the textile are shown in figure 9. In each diagram a sample of the contact test is inserted.

**<Figure 9>**

Three controls were used this study: untreated fabric in dark and in light conditions; treated fabrics in the dark condition. The untreated control samples allow bacterial growth in the dark; but untreated control samples, reduced *S. aureus* growth about ~1 log under light irradiation. In the dark condition, treated fabrics allow bacterial growth and show about 1-6 % reduction in the number of bacteria.

In this study, the effect of increasing the concentration and irradiation time were two important parameters in the photo-inactivation percentage of these strains of bacteria. For example, at a concentration of 10-4 M, cellulosic fabrics treated with TMPyP(3), TAPP and ZnTAPP exhibited photo-bactericidal activity against *S. aureus* with 30 min illumination. In addition, cellulosic fabrics treated with TMPyP(3), ZnTMPyP(3), TMPyP(4) and Zn TMPyP(4) with 60 min illumination and TAPP with 90 min illumination showed activity against *P. aeruginosa*; and cellulosic fabrics treated with TMPyP(3), ZnTMPyP(3), TMPyP(4) and Zn TMPyP(4) demonstrated photo-bactericidal activity against *E. coli* with 60 min illumination. Other results can be observed in figure 9.

All of the porphyrins in this study were tetra-cationic porphyrins. According to the literature[10- 25], cationic porphyrins show photo-inactivation of Gram-negative bacteria without the presence of additional permeabilization agents, by producing singlet oxygen. In this study, all of the compounds have been shown to have a remarkable effect on the reduction of Gram-positive and Gram-negative bacteria

This work has significant advantages attributed to the other research work, which are, the immobilization of tetracationic porphyrins to cellulosic fabric without the use of organic solvents, their washing durability and their photobactericidal effect against Gram negative and Gram positive bacteria by choosing an inexpensive light source.

**Conclusion**

In conclusion, this study provide information on the photodynamic activity of cationic porphyrin derivatives with equal positive charges on the periphery of the tetrapyrrolic macrocycle. MIC and MBC of TMPyP(3), ZnTMPyP(3), TMPyP(4), ZnTMPyP(4), TAPP and ZnTAPP were investigated against *S. aureus*, *E. coli* and *P. aeruginosa* with a constant light source. According to the results, only TMPyP(4) and ZnTAPP at a concentration of 60 µg/ mL and TAPP at a concentration of 15 µg/ mL exhibited MBC against *S. aureus*. It seems that *S. aureus* photo-inactivation occurs more effectively by more symmetric molecules such as TAPP, ZnTAPP, TMPyP(4) and ZnTMPyP(4). The most photo-inactivation effect was observed by ZnTMPyP(3) and ZnTAPP against *P. aeruginosa* and *E. coli*, with about 45 %, respectively.

Furthermore, photo-bactericidal and thermal stability effects of cellulosic fabrics with the tetra-cationic porphyrinic moieties have been compared with our previous work. Cellulose was impregnated with the selected concentrations of four tetra-cationic porphyrins. The products were characterized and compared by ATR-FT-IR, diffuse reflectance UV-Vis spectroscopy, TGA and SEM. The modified fabrics displayed photo-antibacterial activity against *S. aureus*, *E. coli* and *P. aeruginosa*. According to the washing stability of the products in hot water and their thermal stability, these fabrics may be efficiently used in biomedical and textile fields and as coating surfaces to prevent microbial infections. Among the studied porphyrins, the best photo-bactericidal activities, with 30 min illumination, have been shown by cell/TMPyP(3), cell/TAPP and cell/ZnTAPP against *S. aureus;* with 60 min illumination by cell/TMPyP(3), cell/ZnTMPyP(3), cell/TMPyP(4) and cell/ZnTMPyP(4) against *P. aeruginosa* and with 60 min illumination by TMPyP(3), ZnTMPyP(3), TMPyP(4) and Zn TMPyP(4) against *E. coli*.

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