Coating of glass with tetra-cationic porphyrin and its zinc compound used as photo-inactivation of bacteria

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Abstract

The meso-tetrakis (4-N, N, N-trimethylanilinium) porphyrin and its zinc compound deposited on glass slide at high temperature and at a pressure of 15 PSI were used as photo-inactivation surface. Also, the effect of two porphyrin compounds irradiated with tungsten lamp, on a Gram negative bacterium, *Pseudomonas aeruginosa*, and a Gram positive bacterium, *Bacillus subtilis* was investigated.

Keywords: *Pseudomonas aeruginosa*; *Bacillus subtilis*; Photo-inactivation; Tetra-cationic porphyrin.

Introduction

Severe health hazards and diseases can be concluded by the adhesion and propagation of bacteria on the surfaces of various compounds [1]. In recent years, preparation of materials with antibacterial properties, for use in a wide range of fields such as sanitary materials, food packaging, household, medical, and military items has grown. Antimicrobial surfaces can be created by incorporating antimicrobial types via interaction covalent bonding or non-covalent to the surface [2].

Photo-sensitizers (PS) such as porphyrin compounds have been intensively studied for their use as photo-bactericidal agents in photodynamic antimicrobial chemotherapy (PACT) against Gram negative and Gram positive bacteria [3-8]. PACT relies on the accumulation of a photosensitizing agent intra-cellular and illumination with visible light. The photodynamic process involves energy absorption by the photo-sensitizer which, brought to its excited triplet state, either activates ground state molecular oxygen into singlet oxygen or generates free radicals. Photo-toxicity primarily relies on the formation of singlet oxygen ($^1O_2$) after

illumination. This highly reactive species is able to react with almost every cellular component, bringing about irreversible damage that ultimately leads to cell death [1]. This technique has recently been studied against a wide range of bacteria, fungi, yeasts and viruses that can cause serious clinical problems [9-13].

The effect of meso-substituted cationic porphyrins has been investigated on Gram negative and Gram positive bacteria [3, 13-16]. For example, photodynamic treatment of N-alkyl-pyridyl porphyrins has been used on various bacteria using different light sources [5-7, 17].

Our aim of present work is to investigate the effect of tetrakis (4-N, N, N-trimethylanilinium) porphyrin and its zinc metal ion on the photo-inactivation of *P. aeruginosa* and *B. subtilis* by means of an irradiation source of visible light. Also, coating of these compounds on the glass slides can be used as a surface with photo-inactivation properties.

Materials and methods

All of the used chemicals in this work were purchased from Merck and used without further purification. Gram positive bacterium *B. subtlis,* and Gram negative bacterium *P. aeruginosa* were obtained from the microbiology laboratory of Guilan University. Electronic spectra were measured on a UV-1700 pharma Spec (Shimadzu) with a quartz cuvette. A 100 Watt tungsten lamp was used as light source. To absorb heat, plate filled water was used. The wavelength range for the lamp was approximately 400-800 nm.

Preparation porphyrin and coating on glass

The porphyrin, tetrakis (4-N, N, N-trimethylanilinium) porphyrin (TAPP) and its zinc ion complex (ZnTAPP) were synthesized as reported previously [18-21]. A stock solution of porphyrins was prepared in water at concentration 2×10^{-3} M and glass slides (1 × 1 cm) was immersed in it. These samples were placed in an autoclave at temperature 121˚C and at a pressure of 15 PSI for a period of time. Then glass slides removed and washed with water until no porphyrin could be detected in the supernatant by UV–visible spectrophotometer.

Antibacterial activity of photosensitive glass

Gram-positive bacterium, *B.subtilis* and Gram-negative bacterium, *P. aeruginosa* were inoculated into nutrient broth and incubated at 37 °C overnight under aerobic conditions. The stock suspensions were diluted to give a working suspension of approximately 10^7-10^8 colony forming units/mL (CFU/mL). Amount of broth culture was aseptically transferred onto nutrient agar plates and spread on the surface with a sterile spreader. A stock solution of porphyrins was prepared in water at various concentrations. Wells (diameter 0.5 cm) were made in nutrient agar seeded with the target strain. Aliquots of different concentrations of porphyrins were added to these wells. The plates were incubated at 37°C for a few minutes in the dark and illuminated with the tungsten

lamp for 30 minutes. They were then incubated at 37°C overnight in the dark. One well was filled with water in the same conditions, as negative control. Bacterial growth was examined visually by measuring inhibition zones around the wells. A diameter larger than 10 mm was considered as a positive response formally.

For photo-bactericidal test of porphyrins coated on glass slides, the samples were cut (1 × 1 cm) and put on the inoculated Petri dish aseptically. For negative control, one plate was untreated glass slide. The former method was used for Inhibition zones around the samples. We illuminated the samples for 90 min. Each sample was removed and transferred into sterile water. After gentle stirring at 37 °C, serial dilutions of these suspensions were prepared. Aliquots (100 µl) of diluted samples were then spread on nutrient agar plates. After incubation at 37 °C for 24-48 h, plates were examined and the number of CFU was counted manually. Each test was done in triplicate.

Results and discussion

The absorption spectra of two porphyrin compounds were recorded in H_2O (Figure 1). The absorption spectrum of TAPP and ZnTAPP, exhibit a Soret and Q bands at 412, 515, 552, 580, 634 nm and 421, 556, 596 nm respectively.

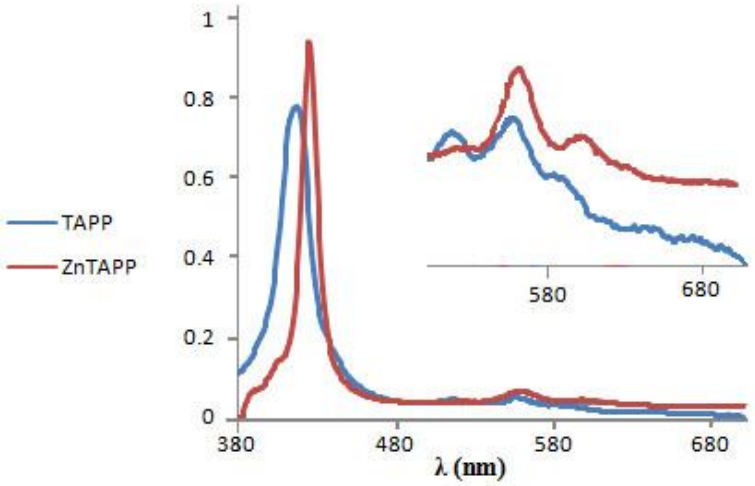

Figure1. The UV-Vis spectra of TAPP and ZnTAPP in water

The effect of various concentrations of TAPP, ZnTAPP and coating of glass with them against two strains of bacteria on agar surface, are shown in Table 1 and 2. Inhibition zones larger than 10 mm were considered as a positive response formally. Photographs of the coating of glass with TAPP, ZnTAPP and inhibition zones for these samples were shown in figures 2 and 3. We illuminated coating of glass for 90 min, because exposure time for 30 min was not effective enough. Probably, it can be attributed to trace porphyrins attached to glass. The optimum concentration of TAPP and ZnTAPP for *P. aeruginosa*, was 60 µg per milliliter. At this concentration

colony number decreased by ~ 3.7 logs and ~ 3.42 logs respectively. For *B. subtilis*, at higher concentrations of 15 µg per milliliter, no colonies were observed.

Table1. Inhibition zones of various concentrations of TAPP and ZnTAPP on selected strains

Sample	Concentration (µg/well)	Diameter of inhibition zone (mm) *P. aeruginosa*	Diameter of inhibition zone (mm) *B. subtilis*
TAPP	60	10	11
	30	6	9
	10	6	7
ZnTAPP	60	10	12
	30	6	10
	10	6	8

Table2. Inhibition zones of Coating glass with TAPP and ZnTAPP on selected strains

Sample	Diameter of inhibition zone (mm) *P. aeruginosa*	Diameter of inhibition zone (mm) *B. subtilis*
Coating glass with TAPP	48	49
Coating glass with ZnTAPP	42	43

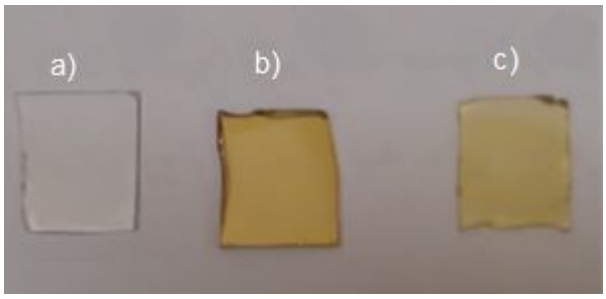

Figure2. Photographs of (a) untreated glass slide; (b) treated glass slide with $TAPP^{4+}$ and (c) treated glass slide with $ZnTAPP^{4+}$

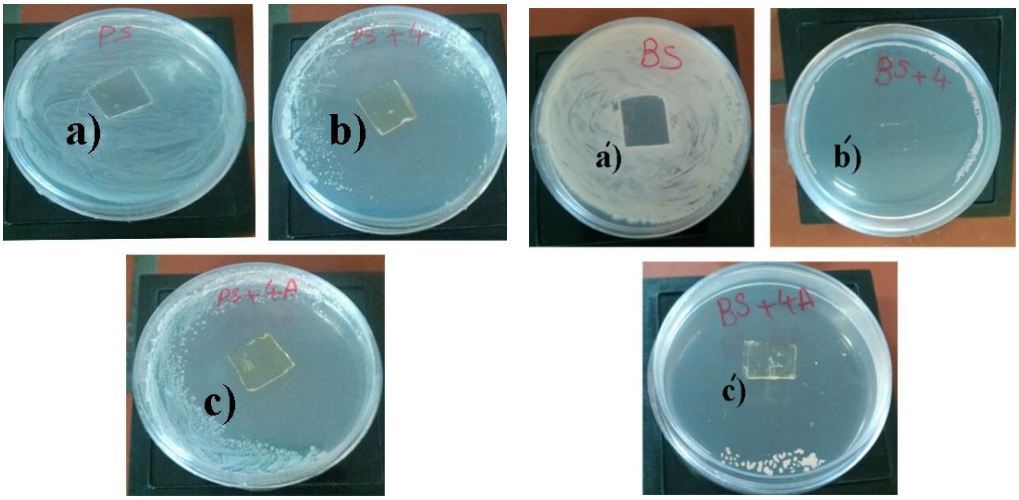

Figure3. Inhibition zones of (a) untreated glass slide; (b) treated glass slide with $TAPP^{4+}$ and (c) treated glass slide with $ZnTAPP^{4+}$ against *P. aeruginosa*. (a′) untreated glass slide; (b′) treated glass slide with $TAPP^{4+}$ and (c′) treated glass slide with $ZnTAPP^{4+}$ against *P. aeruginosa*.

Percentage of photo-inactivation for both porphyrin compounds on the glass slides was100% for two strains of bacteria at illumination time for 90 min. Untreated control samples in the dark and under light irradiation allow bacterial growth. Porphyrin compounds induce singlet oxygen production; it is likely that this property imparts photo-bactericidal activity on $TAPP^{4+}$ and $ZnTAPP^{4+}$.

In the present study, photosensitive glass slides were successfully prepared by treating two tetra-cationic porphyrins onto glass slides. This suggests very interesting potentialities for further industrial applications. These surfaces could be efficiently used in biomedical fields to prevent microbial infections.

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