The photodynamic inactivation of *Staphylococcus aureus* in water using visible light with a new expanded porphyrin

G. Rossi, D. Goi and C. Comuzzi

ABSTRACT

In this work, the results of the application of organic expanded porphyrins in the disinfection of water by the photodynamic inactivation (PDI) technique are presented. The photoinactivation properties of a novel, expanded porphyrin, namely 20-(4-carboxyphenyl)-2,13-dimethyl-3,12-diethyl-(22π) pentaphyrin (PCCox), were tested in the disinfection of water using *Staphylococcus aureus* as a Gram-positive bacteria model. The data showed that PCCox was effective against *S. aureus* bacteria at nanomolar concentrations. The variation with irradiation time and concentration was studied using both a multi-LED monochromatic light ($\lambda = 470$ nm) and an incandescent light bulb with a wide emission spectrum. A PCCox dosage of 5 μM was sufficient to achieve a 99.997% abatement of *S. aureus* within 1 h of 40 W/m^2 irradiation with monochromatic light ($\lambda = 470$ nm), whereas under the same conditions using irradiation with white light, the abatement was 99.9997%.

Key words | organic pentaphyrin, photodynamic inactivation (PDI), photosensitisation, *Staphylococcus aureus*, water disinfection

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INTRODUCTION

The rapid development of industrialisation and population growth and the long-term projection of water scarcity have produced an increasing demand for clean water sources. To address this problem, various useful strategies and solutions have been adopted, including new forms of water treatment, recovery and reuse. The availability of clean water can be increased by introducing low cost and highly efficient water treatment technologies, and many efforts have been recently carried out in this field.

Recently, many studies have demonstrated that photo processes can be applied for both the decomposition of organic pollutants and the disinfection of water (Hijnen *et al*. 2006; Chong *et al*. 2010; Sunnotel *et al*. 2010). In most cases, the catalysts are semiconductors such as TiO_2 and the light source is ultraviolet radiation, and photo water treatments using organic dyes and visible light are still in their infancy. In recent years, the application of porphyrin derivatives in photodynamic inactivation (PDI) (Lambrechts *et al*. 2005) is boosting greater interest in water disinfection processes (Kuznetsova *et al*. 2007; Ergaieg *et al*. 2008). Although only a few studies have been conducted in this area, the preliminary evaluations show that these organic compounds and their derivatives have potential application in water treatment and other environmental purposes (Jemli *et al*. 2002; Magaraggia *et al*. 2006; Carvalho *et al*. 2007; Almeida *et al*. 2009).

Natural porphyrins and their synthetic analogues are macrocyclic systems consisting of four pyrrole subunits linked by =CH– bridges located in the α position with respect to the heterocyclic nitrogen. Porphyrins are known as photosensitising agents; in the presence of light radiation and oxygen, they generate cytotoxic reactive oxygen species (ROS) (Miller 1999; Kochevar & Redmond 2000; De Rosa & Crutchley 2002). The reaction proceeds by light excitation of the molecule to higher energy states from which it may decay back to the ground state via fluorescence or the

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so-called Type I and Type II pathways, which occur simultaneously and are in competition (De Rosa & Crutchley 2002). The Type I pathway is characterised by the electron transfer between a photosensitiser in an excited state and a molecular substrate to produce radical ions that can then react and produce ROS as cytotoxic species. In the Type II pathway, the energy is transferred from the photosensitiser in a triplet state to the ground-state molecular oxygen to produce excited-state singlet oxygen $^1O_2$. These intermediates can initiate the photo-oxidation reaction of biomolecules in the surroundings, and they are able to attack a wide variety of organic substrates, including proteins, nucleotides, unsaturated lipids and steroids. The subsequent irradiation of porphyrins at specific wavelengths in the visible spectrum causes the death of a wide variety of pathogens; in particular, the effectiveness of singlet oxygen has been tested *in vitro* against Gram-positive and Gram-negative bacteria (Hamblin & Hasan 2004), including antibiotic-resistant strains (Wainwright 1998), viruses (Costa *et al*. 2011), fungi (Donnelly *et al*. 2008), protozoa (Jori *et al*. 2006) and parasites in the cystic and vegetative stages (Kassab *et al*. 2002; Kassab & Al-Herrawy 2005).

The recent studies on porphyrins and their analogues have led to the engineering of chemical architectures that promote rapid and preferential connections with several types of cells (Banfi *et al*. 2006; Comuzzi *et al*. 2006b; El-Aldy 2008).

Furthermore, it can be highlighted that at photochemically active doses (micromolar concentrations), porphyrins do not show significant toxicity to most higher organisms, as confirmed by their use in the food industry (Lukšiene 2005), fish farming (Almeida *et al*. 2009) and photodynamic therapy for infectious disease (Hamblin & Hasan 2004).

The variety of porphyrins and their derivatives that are able to cause the photoinactivation of Gram-positive bacteria is large; however, their cationic derivatives have to be used to effectively disable Gram-negative bacteria (Merchat *et al*. 1996; Minnock *et al*. 1996; El-Aldy 2008). There is an interesting relationship between the effectiveness of the photosensitisers used in antimicrobial therapy and the structure and distribution of the intramolecular charges. Positive charges on the porphyrins promote electrostatic interactions with the negative sites on the phospholipid membranes of Gram-negative bacteria by altering the texture of the outer membrane and making it susceptible to photosensitisers (Malik *et al*. 1992; Alves *et al*. 2009).

Recent experiments have achieved the photoinactivation of bacteria found in drinking and sewage water by exposure to sunlight in the presence of organic photosensitisers (Villen *et al*. 2006; Kuznetsova *et al*. 2007). The effectiveness of photosensitisers has even been observed in the cases of environmental viruses (Costa *et al*. 2008), although the exact mechanism of viral inactivation is not yet well known. The variation in physicochemical properties of the photosensitisers may result in the observed differences between their association with the biological molecules of viruses, including the plasma proteins and viral capsids (Casteel *et al*. 2004).

As an alternative to the traditional disinfection techniques (chlorination, ozonolysis, irradiation with UV light), the photo-oxidation process is a promising technology characterised by low cost, low environmental impact and easy management. We can consider that the use of very small concentrations of a chemical agent, the organic photosensitiser, combined with the application of a natural light source, sunlight, ensures that the environmental impact of the technology is very low.

Over the past 3 decades, a new class of ligands termed 'expanded porphyrins' has been developed; these are macrocyclic compounds that result from the expansion of the π-electron conjugation by increasing the number of heterocyclic rings (Sessler & Weghorn 1997). The resulting chromophores show strong absorption in the visible and near-infrared region of the light spectrum. Since the start of the study and application of these compounds, there has been a great deal of interest in the synthesis of new macrocyclic compounds with different core atoms and cavity sizes because of their interesting spectroscopic, chemical and physical properties, which can be applied in many fields (Comuzzi *et al*. 2006a), including material science (Suslik *et al.* 2000) and catalysis (Benaglia *et al.* 2002).

In this work, we studied the effectiveness of a new expanded porphyrin, 20-(4-carboxyphenyl)-2,13-dimethyl-3,12-diethyl-(22π) pentaphyrin (PCCox) on Gram-positive bacteria, to evaluate its possible applications in the disinfection of waterborne pathogens.

In these tests, the well-known *Staphylococcus aureus* has been used as a model of Gram-positive bacteria and is

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a pathogenic microorganism that causes respiratory and cutaneous infections in humans; furthermore, the water-borne hazard presented by this bacteria is underlined in many recent studies, as shown in its presence in water bodies (Tolba *et al.* 2008; Ralston *et al.* 2011) and its resistance to traditional antibacterial treatment (Barker-Reid *et al.* 2010).

MATERIALS AND METHODS

Microorganisms and media

Two model microorganisms were selected to provide a representation of Gram-positive and Gram-negative bacteria. We used two model bacteria: *Escherichia coli* DSM 11250 and *S. aureus* ATCC 6538 (Leibniz Institut DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH).

The bacterial strains were cultured in 10 mL of nutrient broth No. 2 (Sigma-Aldrich) and allowed to grow to the logarithmic phase at 37 °C. The culture was then centrifuged at approximately 3,200 rpm for 15 min, and the pellet was suspended in 0.01 M sterile phosphate-buffered saline (Sigma-Aldrich) at a bacterial concentration of approximately 10^8 CFU/mL (optical density of 0.1 at 600 nm). The absorbance was measured with a Jasco SSE-343 spectrophotometer. This suspension was added to a calculated volume of photosensitiser solution and was irradiated during the photo-oxidation tests. Control experiments were carried out by illuminating the bacterial suspension in the absence of photosensitiser and by incubating the bacterial suspension with photosensitiser in the dark.

Samples were withdrawn from each reaction well and serially diluted in 1 mL of 0.01 M sterile phosphate buffer saline (Sigma-Aldrich). The concentration of bacteria in the diluted samples was determined according to the agar-germi method 9215 B proposed by the *Standard Methods for the Examination of Water and Wastewater* (APHA 1999), where 0.1 mL was spread on the appropriate agar plates, which were then incubated at 37 °C for 18–24 h. We used *E. coli* direct MUG agar (Fluka) for *E. coli*, and mannitol salt agar (Fluka) for *S. aureus*. The morphologies of the colonies were observed to confirm that they were typical, and then the colonies were counted and the results expressed as CFU/mL. The bacterial survival was expressed as the percentage of the bacterial final concentration of the treated samples with respect to the bacterial concentration of the negative control sample (illuminated bacterial suspension in the absence of photosensitiser).

Photosensitisers

Pentaphyrin macrocycle 20-(4-carboxyphenyl)-2,13-dimethyl-3,12-diethyl-(22π) pentaphyrin (PCCox) was used in the photo-oxidation tests (Goi *et al.* 2011). This molecule is an expanded porphyrin with an aromatic macrocycle belonging to the class of pentaphyrins (1.1.1.1.1) (Figure 1(a)). In methanol (ACS reagent, >99.9% Sigma-Aldrich), the molecule exhibited a UV–vis spectrum with a typical high-intensity

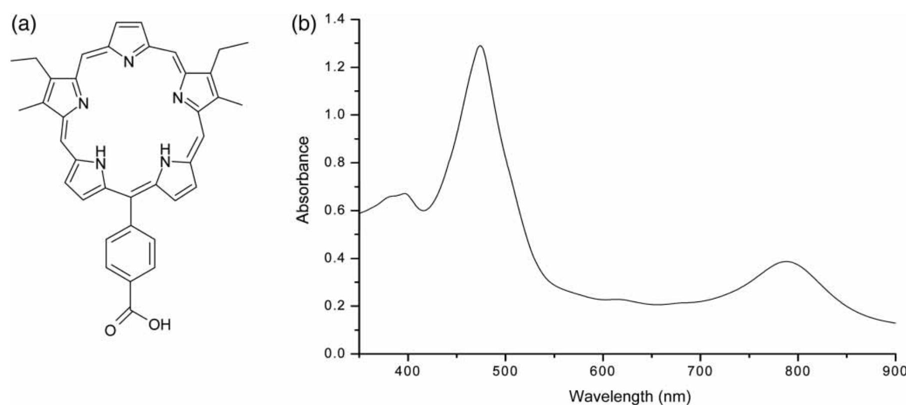

Figure 1 | (a) The molecular structure of PCCox and (b) the PCCox absorption spectra in MetOH: 1 mg of the molecule was dissolved in 200 μL of MetOH; the solution was measured using cuvettes with a 0.1 cm path length.

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band at 470 nm (log $\varepsilon = 3.2$) and a broad low-intensity band at 800 nm (log $\varepsilon = 2.6$) (Figure 1(b)).

The photosensitiser stock solution was obtained by solubilising a weighed amount of photosensitiser in dimethyl sulphoxide (DMSO, ACS reagent, >99.9% Sigma-Aldrich) to reach a final concentration of 330 μM.

The stability of PCCox when exposed to illumination was verified. It was demonstrated that a PCCox solution does not undergo degradation within 60 min of irradiation with either a blue light (470 nm) or an incandescent light bulb.

Arrangement of the light source for the irradiation system

During the tests, two different light sources were used. The first one was a homemade multi-LED lamp specifically designed for the photo-oxidation tests. The spectral emission of the LED (TLWB7600 Vishay) had a maximum peak at 470 nm, and the light intensity of the lamp was controlled electronically by a power supply (Agilent) to obtain a variable fluence rate. The distance between the lamp and the irradiated multi-well plate was kept constant at 10 cm. A second light source was a 100 W incandescent light bulb set at a fluence rate of 40 W/m^2 with a maximum peak at 620 nm (emission spectrum from 340 to 1,100 nm). A polystyrene vessel containing a 1 cm thick layer of purified water was used to avoid overheating the culture and to cut off the UV wavelengths under approximately 300 nm from the incandescent light bulb.

Irradiation procedure

The final concentrations of the photosensitiser in the cell suspensions (optical density 0.1 at 600 nm) were 0.05, 0.5 and 5 μM. The mixtures of the bacterial suspension and photosensitiser solution were introduced into a plastic 48-well flat-bottomed microlitre plate containing 1 mL in each well and maintained under moderate stirring to obtain a homogeneous reaction medium. The irradiation procedure was carried out by filling the multi-well plate and turning on the lamp at the same time as the stirring device. After the reaction time, 0.1 mL of each sample was drawn for analysis. The dark controls were used to determine the toxicity of the photosensitiser in the absence of light during the photoinactivation experiments. The influence of DMSO on bacterial survival was determined even if the solvent concentration in the bacterial solution was under the toxicity threshold (Wadhwani *et al.* 2009). All photo-oxidation tests were performed in triplicate at least.

The fluence rates were determined before sample irradiation using a radiometer (DeltaOhm HD 2302.0 connected to an LP 471 RAD probe).

RESULTS AND DISCUSSION

The photo-oxidative effect of PCCox on Gram-positive and Gram-negative bacteria

The use of PCCox has never been tested in disinfection, so the first set of experiments was to investigate its efficiency against Gram-positive and Gram-negative bacteria and to determine its toxicity towards microorganisms. Many studies have demonstrated that neutral or anionic photosensitising agents were not able to effectively interact with Gram-negative bacteria, which can be treated with positively charged photosensitisers instead (Minnock *et al.* 1996; Alves *et al.* 2009). For this reason, a preliminary assay of the photoinactivation effectiveness of PCCox against different types of bacteria was carried out to confirm that the molecule was efficient only against Gram-positive bacteria.

The photoinactivation experiments were conducted on *E. coli*, a Gram-negative species, and *S. aureus*, a Gram-positive species, at the same bacterial density (10^8 CFU/mL). The same PCCox concentration (5 μM) was used on *E. coli* and *S. aureus* under a blue multi-LED light (470 nm) at 24 W/m^2. As shown in Table 1, PCCox had no activity against *E. coli* but was efficient in the photoinactivation of *S. aureus*, obtaining a bacterial inactivation of 99.981% after 1 h of treatment. The rate of inhibition for *E. coli* was negative, indicating that the bacterial population, which was in the exponential phase of growth during the test, kept its growth capability without being influenced by the photosensitiser.

Influence of the light source on the PDI treatment

To trigger the photodynamic reactions, the radiation must have the proper wavelength (related to the energy carried

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Table 1 | A comparison between the inactivation rates (%) of *S. aureus* and *E. coli* treated with 5 μM PCCox using the multi-LED blue light (470 nm) at 24 W/m^2 at different treatment times

Treatment time (min)	*S. aureus* (% inactivation)	*E. coli* (% inactivation)
0	0	0
15	96.281 ± 3.322	−3 ± 2.37
30	99.865 ± 0.077	−15 ± 3.56
60	99.981 ± 0.007	−56 ± 5.71

by photons) and intensity (related to the number of photons). When penetrating a water body, the spectrum and intensity of white light changes, as the water acts as a filter. Blue light (450–495 nm) is the most penetrating wavelength of the electromagnetic spectrum. To apply the photodynamic processes to the disinfection of water, it is then crucial to test the photosensitiser under different irradiation conditions that should somehow simulate the drop in intensity and the cutoff of wavelength as white light passes through water.

Therefore, the influence of the emission spectrum of the light source on the photoinactivation efficiency of PCCox was determined (Figure 2). The incandescent light bulb, which emitted white light with the UV radiation cut off, was used to simulate the radiation in the surface layers of a water body whereas the monochromatic lamp emitting a wavelength of 470 ± 1 nm (the multi-LED lamp) was used to simulate the radiation reaching the deeper layers of water. Figure 2 also reports the microbial abatement efficiency of PCCox when illuminated with the two lamps at

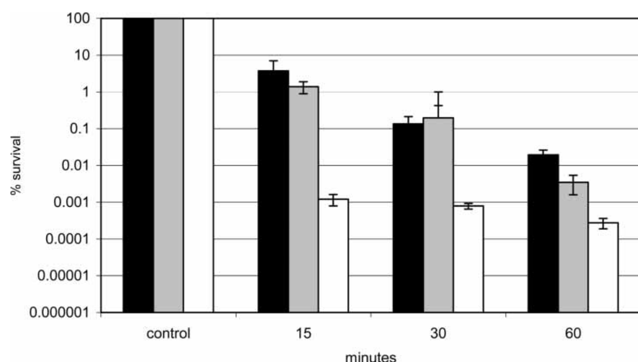

Figure 2 | The inactivation effect of PCCox on 10^8 CFU/mL cultures of *S. aureus*. The photosensitiser concentration was 5 μM, while the treatment time and fluence rate of the light source used varied: multi-LED light (470 nm), 24 W/m^2 (black bar); multi-LED light (470 nm), 40 W/m^2 (grey bar); and incandescent light bulb, 40 W/m^2 (white bar).

different fluence rates. The incandescent light bulb was maintained at 40 W/m^2 to simulate the average value of natural irradiation on a cloudy winter day at 12:00 AM (southern part of Europe), and the multi-LED lamp (470 nm) was used at 40 W/m^2 for comparison and at 24 W/m^2 to simulate the drop in radiation intensity that occurs when penetrating the deeper layers of water. In these experiments, the concentration of the photosensitiser was kept constant at 5 μM.

The bacterial colonies were counted after 15, 30 and 60 min of treatment. Illuminating the bacterial solution containing PCCox with an incandescent light bulb at 40 W/m^2 resulted in a bacterial reduction of 99.9988% (5 logarithmic units) within 15 min of treatment and reached almost 6 logarithmic units after 60 min. When only a very small part of the absorption spectrum of PCCox was used to produce ROS (Ballico *et al.* 2011) by irradiation, that is, when the multi-LED light (470 nm) was used to excite PCCox, the bacterial reduction was only 2 logarithmic units after 15 min of treatment and reached almost 5 logarithmic units after 60 min treatment. These data suggest that the best performance is obtained when a light source with a complete spectrum is used to excite the photosensitisers, confirming that sunlight could be an efficient radiance source. Because UV radiation penetrates only a few millimetres into the water body, when sunlight is used to excite the photosensitisers in the disinfection of water, a high efficiency is commonly noted at the water surface, where both visible and UV light contribute to microbial inactivation. Therefore, the complete sunlight spectrum is needed to excite the complete absorption spectrum of PCCox.

These data also highlight that using an organic photosensitiser with a strong absorption in the blue region of the light spectrum (such as PCCox) also guarantees an efficient disinfection in the deeper layers of the water body, where TiO_2 is not useful.

Moreover, the data obtained regarding *S. aureus* disinfection (Table 2) showed that, when halving the fluence rate of the lamp with a reduced emission spectrum (multi-LED lamp, 470 nm) from 40 to 24 W/m^2, a sharp drop in the bactericidal activity of PCCox was not observed and the reduction of the *S. aureus* concentration by approximately 4 logarithmic units after 1 h of treatment was maintained.

This result suggests that the disinfection of the solution with a higher fluence rate did not significantly improve the

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Table 2 | The inactivation rates (%) of *S. aureus* (10^8 CFU/mL) treated with 5 μM PCCox, at different treatment times and fluence rates for the light source used

Treatment time (min)	Inactivation (%) Multi-LED blue light (470 nm, 24 W/m²)	Multi-LED blue light (470 nm, 40 W/m²)	Incandescent light bulb (40 W/m²)
0	0	0	0
15	96.281 ± 3.322	98.613 ± 0.497	99.9988 ± 0.0004
30	99.865 ± 0.077	99.804 ± 0.229	99.9992 ± 0.0001
60	99.981 ± 0.007	99.997 ± 0.002	99.9997 ± 0.0001

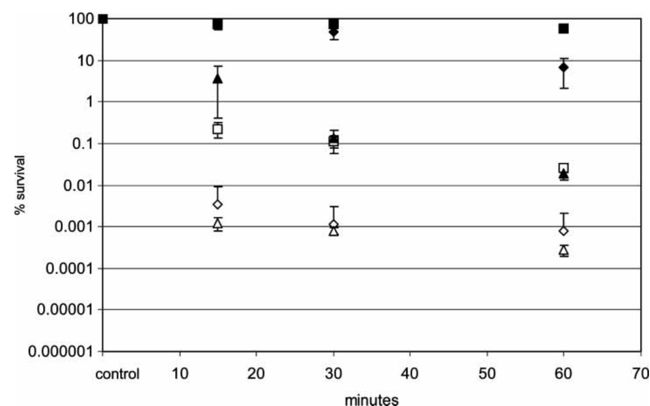

Figure 3 | The inactivation effect of PCCox on *S. aureus* (10^8 CFU/mL) when varying the photosensitiser concentration, treatment time, light emission spectrum and fluence rate of the light source (multi-LED light (470 nm) and incandescent light bulb). ≤: PCCox 0.05 μM, incandescent light bulb, 40 W/m²; ◊: PCCox 0.5 μM, incandescent light bulb, 40 W/m²; △: PCCox 5 μM, incandescent light bulb, 40 W/m²; ■: PCCox 0.05 μM, multi-LED light (470 nm), 24 W/m²; ♦: PCCox 0.5 μM, multi-LED light (470 nm), 24 W/m²; ▲: PCCox 5 μM, multi-LED light (470 nm), 24 W/m².

efficiency of the treatment, especially when a shorter treatment time is applied. Very good efficiencies were obtained at the longest treatment time even when using a lower fluence rate. When considering a full-scale application, this could be an interesting feature that requires less power and therefore lower energy costs to perform the treatment.

Trends in the inactivation rate

The PDI potential of PCCox was tested by varying the photosensitiser concentration. Suitable volumes of PCCox were added in the bacterial suspension to obtain different photosensitiser doses (0.05, 0.5, and 5 μM) to evaluate the efficiency of *S. aureus* inactivation.

Two opposite experimental conditions were chosen to span between strong and weak photoinactivation conditions. The multi-LED lamp (470 nm) was set at a fluence rate of 24 W/m² and the incandescent light bulb was set at a fluence rate of 40 W/m². The treatment times were 15, 30 and 60 min.

Figure 3 shows how the treatment efficiency depends on the photosensitiser concentration. In general, the greatest effects on bacteria were obtained using a PCCox concentration of 5 μM. When the incandescent light bulb was used, a high inactivation effect was observed even at nanomolar concentrations of PCCox. In fact, the bacterial inactivation trend was very similar at 0.5 μM of PCCox compared to 5 μM, reaching 5 and 6 logarithmic units of abatement, respectively, after 60 min. Further dropping the photosensitiser concentration to 0.05 μM caused a decrease in efficiency, but the efficiency was nevertheless maintained at high levels, reaching 4 logarithmic units within 60 min of irradiation. When the multi-LED blue light (470 nm) was used at a fluence rate of 24 W/m², the treatment was much more sensitive to the photosensitiser concentration. Under these irradiation conditions, the bacterial solution containing PCCox at 0.5 and 0.05 μM showed a low *S. aureus* abatement within 30 min of treatment, reaching 1 logarithmic unit after 60 min of irradiation only at 0.5 μM. When PCCox was used at a concentration of 5 μM, the abatement was 4 logarithmic units after 60 min of irradiation.

Table 2 reports the data of the PDI treatment of *S. aureus* (10^8 CFU/mL) with 5 μM PCCox. The test confirmed that irradiation with a blue light (470 nm) at 24 W/m² for 60 min yielded the same inactivation result as irradiation with a white light at 40 W/m² using a PCCox concentration of 0.05 μM.

These results suggest that, by varying the fluence rate, light source and photosensitiser concentration, the PDI process can be optimised in terms of its efficiency and energy costs.

Figure 4 depicts the inactivation effect of 5 μM PCCox on suspensions with different concentrations of *S. aureus* that were irradiated for 60 min with an incandescent light bulb at a fluence rate of 40 W/m².

It can be noted that the photodynamic irradiation on the bacterial suspension with a concentration of 10^{13} CFU/mL resulted in an inactivation degree of more than 10 logarithmic units. This abatement was approximately five times higher than when the concentration of the bacterial suspension is 10^8 CFU/mL.

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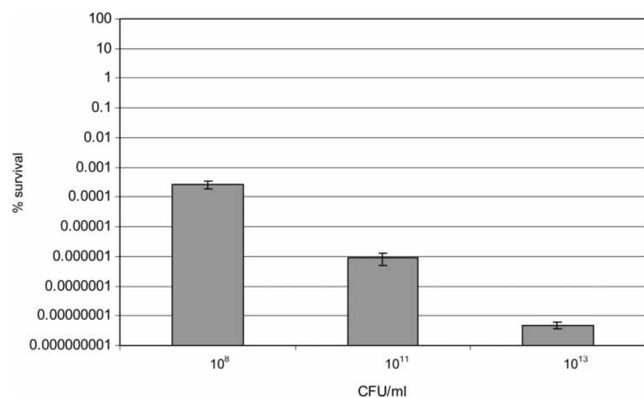

Figure 4 | The inactivation effect of PCCox on *S. aureus* after 60 min of treatment with 5 μM PCCox using an incandescent light bulb (40 W/m^2). In abscissa the initial bacterial concentrations used in the tests are reported.

This result is evidence that the PCCox effect is amplified when the bacterial concentration in the mixture increases. This might indicate that PDI is more appreciable when there is an enhanced interaction between the photosensitiser and the cells and indicates that the photo-oxidant activity of PCCox is not caused by its crossing the cellular membrane of the bacteria but is most likely mediated by ROS-oxidation reactions which occur near the cell membrane and lead to the disruption of the cell membrane. Further studies are needed to support this hypothesis.

When comparing the data obtained from the inactivation experiments with PCCox with those reported in the literature, it can be highlighted that PCCox ranks among the most effective photosensitisers, it is more active than many neutral porphyrins and it demonstrated a potential bactericidal effect comparable to or higher than that of many cationic porphyrins tested in other studies (Minnock *et al.* 1996; Banfi *et al.* 2006; El-Aldy 2008).

CONCLUSIONS

A photosensitising agent, 20-(4-carboxyphenyl)-2.13-dimethyl-3,12-diethyl-(22π) pentaphyrin, called PCCox, never before used in antibacterial therapy, has been tested and its effectiveness has been validated on the Gram-positive bacteria *S. aureus*. This bacterial species was chosen because it is related to human healthcare and it is potentially pathogenic in water, for example, in swimming pools and baths. *S. aureus* was studied as a Gram-positive bacteria model to represent the photo-oxidation process on other Gram-positive bacteria which are targeted in the disinfection of water. The disinfection activity of PCCox was not evident in Gram-negative bacteria; because the molecule is neutral, it is not attracted to the membranes of these bacteria.

The effectiveness of the inactivation of *S. aureus* (and potentially other Gram-positive bacteria) by PCCox was high and comparable to that of many other positively charged porphyrins.

A bacterial abatement of approximately 5 logarithmic units was achieved after only 15 min of treatment using the photosensitiser at micromolar concentration and applying a 100 W incandescent light bulb at a very low fluence rate (40 W/m^2). Good efficiencies were also obtained when nanomolar concentrations of PCCox (0.05 and 0.5 μM) were used, achieving an abatement that was between 4 and 5 logarithmic units after 60 min of treatment under the same irradiation conditions.

The data obtained on *S. aureus* open new perspectives on the application of PDI by PCCox on antibiotic-resistant *S. aureus*. The inactivation efficiency of PCCox on these strains could be an interesting application to study; indeed, developing new strategies to improve the disinfection of bacteria that are resistant to traditional treatment is a hot topic in the hygiene–sanitary field of applied research, and PDI has been already evaluated as a good option (Wainwright 1998). A significant advantage of applying PDI to the disinfection of waterborne pathogens is that this technique has been repeatedly shown to be independent of the antibiotic resistance spectrum (Tavares *et al.* 2010); for this reason, it could be used to treat resistant bacteria such as the several epidemic healthcare-associated (HA) and community-associated (CA) methicillin-resistant *S. aureus* (MRSA) present in river, sea and swimming pool waters (Tolba *et al.* 2008).

The new molecule used (PCCox) is insoluble in water, which is an important feature for future applications due to the possibility of easily recovering it at the end of the photo-induced process. New chemical architectures of PCCox derivatives could be synthesised to improve the disinfection effectiveness of other types of microorganisms in water (e.g. Gram-negative bacteria, protozoa, fungi and viruses).

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The above reasons present an interesting argument for the potential use of photo-oxidation via organic molecules for the treatment of waterborne microbes. The employment of this photo-oxidative process in water treatment has significant potential if the photosensitisers are immobilised on polymeric materials; this would make the photoinactivation technology more relevant in water disinfection.

The use of photosensitisers that can be excited by shorter wavelengths of light associated with blue colours has a very interesting application. This technique was developed from the initial application of photodynamic therapy in cancer, where organic photosensitisers were traditionally selected and arranged to absorb red light. Here, the technique overcomes the absorption of light by water and ensures a better disinfection activity not only on the water surface, as seen in traditional UV technology and even some recent solar disinfection technologies (Graf *et al.* 2010), but even at greater depths.

In addition, PDI does not induce bacterial resistance to the treatment (Tavares *et al.* 2010). PDI is not mutagenic or genotoxic (Costa *et al.* 2011), so it has a lower environmental impact than other traditional disinfection techniques.

The application of PDI in water treatment also has many advantages related to the possibility of reducing costs, large-scale operational management, personnel qualifications and logistic deficits.

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REFERENCES

Almeida, A., Cunha, Â., Gomes, N. C. M., Alves, E., Costa, L. & Faustino, M. A. F. 2009 Phage therapy and photodynamic therapy: low environmental impact approaches to inactivate microorganisms in fish farming plants. *Mar. Drugs* **7**, 268–313.

Alves, E., Costa, L., Carvalho, C. M. B., Tomé, J. P. C., Faustino, M. A., Neves, M. G. P. M. S., Tomé, A. C., Cavaleiro, J. A. S., Cunha, Â. & Almeida, A. 2009 Charge effect on the photoinactivation of Gram-negative and Gram-positive bacteria by cationic meso-substituted porphyrins. *BMC Microbiol.* **9**, 70–83.

APHA 1999 Standard Methods for the Examination of Water and Wastewater, 20th edition. American Public Health Association/American Water Works Association/Water Environment Federation, Washington, DC.

Ballico, M., Rapozzi, V., Xodo, L. & Comuzzi, C. 2011 Metallation of pentaphyrin with Lu(III) dramatically increases reactive-oxygen species production and cell phototoxicity. *Eur. J. Med. Chem.* **46**, 712–720.

Banfi, S., Caruso, E., Buccafurni, L., Battini, V., Zazzaron, S., Barbieri, P. & Orlandi, V. 2006 Antibacterial activity of tetraaryl-porphyrin photosensitizers: an *in vitro* study on Gram negative and Gram positive bacteria. *J. Photochem. Photobiol. B* **85**, 28–38.

Barker-Reid, F., Fox Ellen, M. & Faggian, R. 2010 Occurrence of antibiotic resistance genes in reclaimed water and river water in the Werribee Basin, Australia. *J. Water Health* **8**, 521–531.

Benaglia, M., Danelli, T., Fabris, F., Sperandio, D. & Pozzi, G. 2002 Poly(ethylene glycol)-supported tetrahydroxyphenyl porphyrin: a convenient, recyclable catalyst for photooxidation reactions. *Org. Lett.* **4**, 4229–4232.

Carvalho, C. M. B., Gomes, A. T. P. C., Fernandes, S. C. D., Prata, A. C. B., Almeida, M. A., Cunha, M. A., Tomé, J. P. C., Faustino, M. A. F., Neves, M. G. P. M. S., Tomé, A. C., Cavaleiro, J. A. S., Lin, Z., Rainho, J. P. & Rocha, J. 2007 Photoinactivation of bacteria in wastewater by porphyrins: bacterial b-galactosidase activity and leucine-uptake as methods to monitor the process. *J. Photochem. Photobiol. B* **88**, 112–118.

Casteel, M. J., Jayaraj, K., Gold, A., Ball, L. M. & Sobsey, M. D. 2004 Photoinactivation of hepatitis A virus by synthetic porphyrins. *Photochem. Photobiol.* **80**, 294–300.

Chong, M. N., Jin, B., Chow, C. W. K. & Saint, C. 2010 Recent developments in photocatalytic water treatment technology: a review. *Water Res.* **44**, 2997–3027.

Comuzzi, C., Cogoi, S., Overhand, M., Van der Marel, G. A., Overkleeft, H. S. & Xodo, L. E. 2006a Synthesis and biological evaluation of new pentaphyrin macrocycles for photodynamic therapy. *J. Med. Chem.* **49**, 196–204.

Comuzzi, C., Cogoi, S. & Xodo, L. E. 2006b Spectroscopic characterization of the oxidation control of the iso-pentaphyrin/pentaphyrin system. *Tetrahedron* **62**, 8147–8151.

Costa, L., Alves, E., Carvalho, C. M., Tomé, J. P., Faustino, M. A., Neves, M. G., Tomé, A. C., Cavaleiro, J. A., Cunha, A. & Almeida, A. 2008 Sewage bacteriophage photoinactivation by cationic porphyrins: a study of charge effect. *Photochem. Photobiol. Sci.* **7**, 415–422.

Costa, L., Tomé, J. P., Neves, M. G., Tomé, A. C., Cavaleiro, J. A., Faustino, M. A., Cunha, A., Gomez, N. C. M. & Almeida, A. 2011 Evaluation of resistance development and viability

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recovery by a non-enveloped virus after repeated cycles of aPDT. *Antivir. Res.* **91**, 278–282.

De Rosa, M. C. & Crutchley, R. J. 2002 Photosensitized singlet oxygen and its applications. *Coordin. Chem. Rev.* **233–234**, 351–371.

Donnelly, R. F., McCarron, P. A. & Tunney, M. M. 2008 Antifungal photodynamic therapy. *Microbiol. Res.* **163**, 1–12.

El-Aldy, A. A. 2008 Photoactive anionic porphyrin derivative against Gram-positive and Gram-negative bacteria. *J. Appl. Sci. Res.* **4**, 1817–1821.

Ergaieg, K., Chevanne, M., Cillard, J. & Seux, R. 2008 Involvement of both Type I and Type II mechanisms in Gram-positive and Gram-negative bacteria photosensitization by a meso-substituted cationic porphyrin. *Sol. Energy* **82**, 1107–1117.

Goi, D., Comuzzi, C., Rossi, G. & Fedele, R. Use of derivatives of pentaphyrine as antimicrobial and disinfectant agents. 2011 Patent PCT/IB2011/053992, yet to be granted.

Graf, J., Togouet, S. Z., Kemka, N., Niyitegeka, D., Meierhofer, R. & Pieboji, J. G. 2010 Health gains from solar water disinfection (SODIS): evaluation of a water quality intervention in Yaounde, Cameroon. *J. Water Health* **8**, 779–796.

Hamblin, M. R. & Hasan, T. 2004 Photodynamic therapy: a new antimicrobial approach to infectious disease? *Photochem. Photobiol. Sci.* **3**, 436–450.

Hijnen, W. A. M., Beerendonk, E. F. & Medema, G. J. 2006 Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. *Water Res.* **40**, 3–22.

Jemli, M., Alouini, Z., Sabbahia, S. & Gueddari, M. 2002 Destruction of fecal bacteria in wastewater by three photosensitizers. *J. Environ. Monitor.* **4**, 511–516.

Jori, G., Fabris, C., Soncin, M., Ferro, S., Coppellotti, O., Dei, D., Fantetti, L., Chiti, G. & Roncucci, G. 2006 Photodynamic therapy in the treatment of microbial infections: basic principles and perspective applications. *Laser Surg. Med.* **38**, 468–481.

Kassab, K. & Al-Herrawy, A. Z. 2005 Photosensitized inactivation of cyst forms of the freshwater amoeba *Vahlkampfia hartmanni* by aluminium (III) phthalocyanine tetrasulfonate. *Environmentalist* **25**, 13–18.

Kassab, K., Ben Amor, T., Jori, G. & Coppellotti, O. 2002 Photosensitization of Colpoda inflata cysts by meso-substituted cationic porphyrins. *Photochem. Photobiol. Sci.* **1**, 560–564.

Kochevar, I. E. & Redmond, R. W. 2000 Photosensitized production of singlet oxygen. *Method. Enzymol.* **319**, 20–28.

Kuznetsova, N. A., Makarov, D. A., Kaliya, O. L. & Vorozhtsov, G. N. 2007 Photosensitized oxidation by dioxygen as the base for drinking water disinfection. *J. Hazard. Mater.* **146**, 487–491.

Lambrechts, S. A. G., Aalders, M. C. G., Verbraak, F. D., Lagerberg, J. W. M., Dankert, J. B. & Schuitmaker, J. J. 2005 Effect of albumin on the photodynamic inactivation of microorganisms by a cationic porphyrin. *J. Photochem. Photobiol. B* **79**, 51–57.

Lukšiene, Z. 2005 New approach to inactivation of harmful and pathogenic microorganisms by photosensitization. *Food Technol. Biotech.* **43**, 411–418.

Magaraggia, M., Faccenda, F., Gandolfi, A. & Jori, G. 2006 Treatment of microbiologically polluted aquaculture waters by a novel photochemical technique of potentially low environmental impact. *J. Environ. Monitor.* **8**, 923–931.

Malik, Z., Ladan, H. & Nitzan, Y. 1992 Photodynamic inactivation of Gram-negative bacteria: problems and possible solutions. *J. Photochem. Photobiol. B* **14**, 262–266.

Merchat, M., Spikes, J. D., Bertoloni, G. & Jofi, G. 1996 Studies on the mechanism of bacteria photosensitization by meso-substituted cationic porphyrins. *J. Photochem. Photobiol.* **B 35**, 149–157.

Miller, J. 1999 Second generation drugs and additional application of PDT. *J. Chem. Educ.* **76**, 594.

Minnock, A., Vernon, D. I., Schofield, J., Griffiths, J., Parish, J. H. & Brown, S. T. 1996 Photoinactivation of bacteria. Use of a cationic water-soluble zinc phthalocyanine to photoinactivate both Gram-negative and Gram-positive bacteria. *J. Photochem. Photobiol. B* **32**, 159–164.

Ralston, E. P., Kite-Powell, H. & Beet, A. 2011 An estimate of the cost of acute health effects from food- and water-borne marine pathogens and toxins in the USA. *J. Water Health* **9**, 680–694.

Sessler, J. L. & Weghorn, S. J. 1997 *Expanded, Contracted and Isomeric Porphyrins*. Elsevier, Oxford.

Sunnotel, O., Verdoold, R., Dunlop, P. S. M., Snelling, W. J., Lowery, C. J., Dooley, J. S. G., Moore, J. E. & Byrne, J. A. 2010 Photocatalytic inactivation of *Cryptosporidium parvum* on nanostructured titanium dioxide films. *J. Water Health* **8**, 83–91.

Suslik, K. S., Rakow, N. A., Kosal, M. E. & Chou, J. 2000 The materials chemistry of porphyrins and metalloporphyrins. *J. Porphyr. Phthalocyanines* **4**, 407–413.

Tavares, A., Carvalho, C. M. B., Faustino, M. A., Neves, M. G. P. M. S., Tomé, J. P. C., Tomé, A. C., Cavaleiro, J. A. S., Cunha, Â., Gomes, N. C. M., Alves, E. & Almeida, A. 2010 Antimicrobial photodynamic therapy: study of bacterial recovery viability and potential development of resistance after treatment *Mar. Drugs* **8**, 91–105.

Tolba, O., Loughrey, A., Goldsmith, C., Millar, B. C., Rooney, P. J. & Moore, J. E. 2008 Survival of epidemic strains of healthcare (HA-MRSA) and community-associated (CA-MRSA) meticillin-resistant *Staphylococcus aureus* (MRSA) in river-, sea- and swimming pool water. *Int. J. Hyg. Environ. Health* **211**, 398–402.

Villen, L., Manjon, F., García-Fresnadillo, D. & Orellana, G. 2006 Solar water disinfection by photocatalytic singlet oxygen production in heterogeneous medium. *Appl. Catal. B: Environ.* **69**, 1–9.

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Wadhwani, T., Desai, K., Patel, D., Lawani, D., Bahaley, P., Joshi, P. & Kothari, V. 2009 Effect of various solvents on bacterial growth in context of determining MIC of various antimicrobials. *Int. J. Microbiol.* **7**, 1. Available from: http://www.ispub.com/journal/the-internet-journal-of-microbiology/volume-7-number-1/effect-of-various-solvents-on-bacterial-growth-in-context-of-determining-mic-of-various-antimicrobials.html.

Wainwright, M. 1998 Photodynamic antimicrobial chemotherapy (PACT). *J. Antimicrobiol. Chemother.* **42**, 13–28.

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