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# Photodynamic inactivation of *Escherichia coli* immobilized on agar surfaces by a tricationic porphyrin

Daniel A. Caminos and Edgardo N. Durantini*

*Departamento de Química, Universidad Nacional de Río Cuarto, Río Cuarto, Agencia Postal Nro. 3, X5804BYA Río Cuarto, Córdoba, Argentina*

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**Abstract**—The photodynamic activity of 5,10,15-tris[4-(3-*N*,*N*,*N*-trimethylammoniumpropoxy)phenyl]-20-(4-trifluoromethylphenyl)porphyrin iodide ($A_3B^{3+}$) has been studied in vitro on a typical Gram-negative bacterium *Escherichia coli* immobilized on agar surfaces. The results obtained for the tricationic $A_3B^{3+}$ porphyrin were compared with those of 5,10,15,20-tetra(4-*N*,*N*,*N*-trimethylammoniumphenyl)porphyrin *p*-tosylate ($TTAP^{4+}$), which is a standard active sensitizer established to eradicate *E. coli* in cellular suspension. The photobleaching of these porphyrins in solution was evaluated by decay in absorbance and in fluorescence. In both cases, a higher photostability was found for $A_3B^{3+}$ than for $TTAP^{4+}$. Photodynamic inactivation capacities of these sensitizers were analyzed in *E. coli* cells immobilized on agar surfaces. Small colonies were treated with different amount of sensitizer (0–14 nmol) and irradiated with visible light for 3 h. The light source used was either a projector or midday sun. The $A_3B^{3+}$ porphyrin produced a growth delay of *E. coli* colonies on agar surfaces. Similar result was obtained irradiating only one isolated colony through an optical fiber. Under these conditions, $A_3B^{3+}$ porphyrin shows a high activity to inactivate localized bacterial cells. The higher photodynamic activity of $A_3B^{3+}$ was confirmed by mechanical spreading of the colonies before treatment. This procedure produces complete inactivation of *E. coli* cells on the agar surface. Therefore, tricationic $A_3B^{3+}$ porphyrin is an interesting sensitizer with potential applications in photodynamic inactivation of bacteria growing as localized foci of infection.


## 1. Introduction

New approaches to the treatment of bacterial infections have been developed because of the changing patterns of infectious disease and the emergence of bacterial strains resistant to current antibiotics.[1] In this way, bacterial photodynamic inactivation (PDI) represents an interesting alternative to inactivate microorganisms.[2,3] This methodology is based on the administration of a photosensitizer, which is preferentially accumulated in the microbial cells. The subsequent irradiation with visible light, in the presence of oxygen, specifically produces cell damages that inactivate the microorganisms. Two oxidative mechanisms are considered to be principally implicated in the photodamage of cells. In the type I photochemical reaction, the photosensitizer interacts with a biomolecule to produce free radicals, while in the type II mechanism, singlet molecular oxygen, $O_2(^1\Delta_g)$, is produced as the main species responsible for cell inactivation.[4,5]

It was discovered that in general Gram-positive bacteria are efficiently photoinactivated by a variety of porphyrins, whereas Gram-negative bacteria are usually resistant to the action of anionic or neutral agents.[6–9] The resistance of Gram-negative bacteria to the action of photoactivated sensitizers has been ascribed to the presence of highly organized outer membrane, which hinders the interaction of the photosensitizer with the cytoplasmic membrane and intercepts the photogenerated reactive species.[7,10,11] Alternatively cationic porphyrins have been shown to photoinduce direct inactivation of Gram-negative bacteria without the presence of an additional permeabilization agent.[6–10,12] The positive charges on the photosensitizer molecule appear to promote a tight electrostatic interaction with negatively charged sites at the outer surface of the Gram-negative bacteria, increasing the efficiency of the photoinactivation processes.[3] Besides efficacy, PDI has shown other benefits. First, the sensitizers used are highly selective, that is,

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* Corresponding author. Tel.: +54 358 4676157; fax: +54 358 4676233; e-mail: edurantini@exa.unrc.edu.ar

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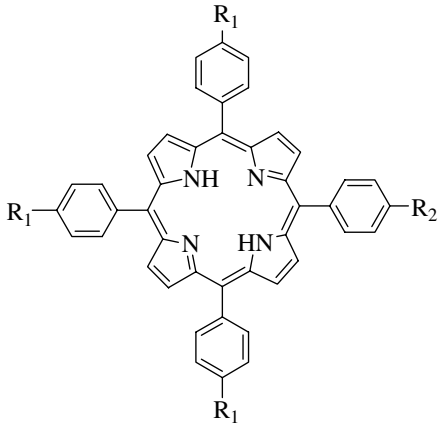
bacteria were killed at combinations of drug and light doses much lower than that needed for a similar effect on mammalian cells. Second, all investigated photosensitizers lack mutagenic activity and the risk of selection of drug-resistant bacterial strains was not still reported.

In previous works, we have investigated the photodynamic activity of cationic porphyrin derivatives in different biomimetic media and in vitro as sensitizers to eradicate Gram-negative bacteria.[9,13] Porphyrins bearing three cationic charges and a highly lipophilic trifluoromethyl group showed to be active photosensitizers to inactivate *Escherichia coli* cells in liquid suspension. In particular, 5,10,15-tris[4-(3-*N*,*N*,*N*-trimethylammoniumpropoxy)phenyl]-20-(4-trifluoromethylphenyl)porphyrin iodide ($A_3B^{3+}$, Scheme 1) is tightly bound to *E. coli* cells still after three washing steps.[13] This porphyrin contains the cationic centers isolated from the macrocycle by a propoxy bridge, which provides a higher flexibility of the chain containing the charged N atom, facilitating the interaction with the outer membrane of the Gram-negative bacteria.[14] The tight binding of $A_3B^{3+}$ to cells is accompanied by a high PDI of *E. coli* cultures in solutions.[13]

In this paper, we have evaluated the photodynamic activity of $A_3B^{3+}$ porphyrin in vitro to inactivate *E. coli* cells immobilized on agar surfaces. The behavior of this tricationic porphyrin was compared with those obtained for 5,10,15,20-tetra(4-*N*,*N*,*N*-trimethylammoniumphenyl)porphyrin *p*-tosylate ($TTAP^{4+}$, Scheme 1), which is a standard active sensitizer established to eradicate *E. coli* in cellular suspension (Scheme 1).[6,7] Also, the photostability of both porphyrins was analyzed in solution. The photobleaching produced a reduction in the photosensitizer activity and it could be conveniently used to eliminate photosensibility in the place of treatment.[15] In this study, the photoinactivation efficiencies of $A_3B^{3+}$ and $TTAP^{4+}$ porphyrins were evaluated in growth delay experiment of *E. coli* colonies immobilized on agar surfaces. These procedures can be used to establish conditions for the treatment of pathogenic microorganisms growing as localized foci of infection, on skin or on accessible area to be irradiated with either artificial visible light or natural sunlight.[1] The basics promise is that the sensitizer should be capable of local, topical or intracavitary administration into the infected area and, after a suitable time, irradiated with light through a optic fiber or by direct illumination of a surgically exposed area.[3,16,17]




$A_3B^{3+}$ $R_1$: $-OCH_2CH_2CH_2N^+(CH_3)_3\ I^-$ $R_2$: $-CF_3$

$TTAP^{+4}$ $R_1$=$R_2$: $-N^+(CH_3)_3$ *p*-$Tos^-$

**Scheme 1.** Molecular structures of porphyrins.

## 2. Materials and methods

### 2.1. General

Absorption and fluorescence spectra were recorded on a Shimadzu UV-2401PC spectrometer and on a Spex FluoroMax fluorometer, respectively. Spectra were recorded using 1 cm path length quartz cuvettes at 25.0 ± 0.5 °C. All the chemicals from Aldrich (Milwaukee, WI, USA) were used without further purification. Solvents (GR grade) from Merck were distilled. Ultrapure water was obtained from Labconco (Kansas, MO, USA) equipment model 90901-01.

### 2.2. Sensitizers

5,10,15-Tris[4-(3-*N*,*N*,*N*-trimethylammoniumpropoxy)-phenyl]-20-(4-trifluoromethylphenyl)porphyrin iodide ($A_3B^{3+}$) was synthesized as previously described.[14] 5,10,15,20-Tetra(4-*N*,*N*,*N*-trimethylammoniumphenyl)-porphyrin *p*-tosylate ($TTAP^{4+}$) was purchased from Aldrich.

### 2.3. Sensitizer photobleaching

Spectra were recorded using 1 cm path length quartz cuvettes. Solutions of photosensitizer ($\lambda = 420$ nm, absorbance ~0.3) in 2 mL DMF were irradiated with visible white light in the same conditions described below for the treatment of *E. coli* cultures. The kinetics of photobleaching were studied by following the decrease of the absorbance ($A$) at Soret band ($\lambda \sim 420$ nm). Also, photobleaching of the porphyrins was analyzed by measuring fluorescence intensity ($F$) at $\lambda = 650$ nm exciting the sample with 515 nm light. Control experiments showed that under these experimental conditions the fluorescence intensity is linearly correlated with the porphyrin concentration. The observed rate constants ($k_{obs}$) of photobleaching were obtained by a linear least-squares fit of the semilogarithmic plot of $\mathrm{Ln}\, A_0/A$ or $\mathrm{Ln}\, F_0/F$ versus irradiation time. All the experiment was performed at 25.0 ± 0.5 °C. The pooled standard deviation of the kinetic data, using different prepared samples, was less than 5%.

### 2.4. Bacterial strain and preparation of cultures

The *E. coli* strain (EC7), recovered from clinical urogenital material, was used as previously described.[9,13,18] The *E. coli* strain was grown aerobically at 37 °C in 30% w/v tryptic soy (TS) broth overnight. Aliquots (~40 μL) of

this culture were aseptically transferred to 4 mL of fresh medium (30% w/v TS broth) and incubated at 37 °C to middle of the logarithmic phase (absorbance ~0.6 at 660 nm). Cells in the logarithmic phase of growth were harvested by centrifugation of broth cultures (3000*g* for 15 min) and re-suspended in 4 mL of 10 mM phosphate-buffered saline (PBS, pH 7.0). Then the cells were diluted 1/1000 in PBS, corresponding to $\sim 10^6$ colony forming units (CFU)/mL, and subsequent dilutions were performed according to the experiment.

### 2.5. Photosensitization of *E. coli* cells growing in colonies on TS agar

Suspensions of *E. coli* ($\sim 10^2$ CFU/mL) in PBS were spread on a 10 cm diameter TS agar dish and grew at 37 °C by 13 h. This procedure gives between 5 and 10 small colonies per plate. The sensitizers were added from a $4 \times 10^{-4}$ M stock solution in water. The colonies were treated with different amount of sensitizers. The colonies were spread with porphyrin using 5 μL (~2 nmol) of the stock solution. Before adding a new dose of sensitizer, drop of the solvent was dried to avoid an increase in the area of treatment. At this time, the cultures were incubated for 10 min at 37 °C and irradiated with visible light from different sources. (A) The dish was illuminated with a slide projector (Novamat 130 AF) equipped with a 150 W halogen lamp. The light was filtered through a 2.5 cm glass cuvette filled with water to absorb heat. A wavelength range between 350 and 800 nm was selected by optical filters. The light intensity at the treatment site was 90 mW/cm$^2$ (Radiometer Laser Mate-Q, coherent).[18] (B) The Petri dish was placed under direct natural sunlight at midday (80 mW/cm$^2$). During the irradiation, the temperature of the cultures was about 35 °C. (C) One selected colony was irradiated with the light from the projector described above by an optic fiber localized on the plate cover with a spot of 0.5 cm diameter on the colony (9 mW/cm$^2$) on the TS agar surface. During the experiment the culture was kept at 37 °C. After irradiation, the plates were incubated for additional overnight at 37 °C. Controls with and without photosensitizers kept in the dark as well as illuminated controls without porphyrin were carried out. The variation in the area of *E. coli* colonies was estimated considering 100% to the increase in the area of the control. Each experiment was repeated separately three times.

## 3. Results and discussion

### 3.1. Porphyrin properties and photobleaching

The absorption spectra of $A_3B^{3+}$ and $TTAP^{4+}$ porphyrins in DMF show the typical Soret (~420 nm) and four Q-bands (~515, 550, 592, and 650 nm), characteristic of free-base porphyrins (Fig. 1A).[19] In PBS, the spectra of these porphyrins show a broadening of Soret band, indicating that aggregation occurs, as is typical of many porphyrin derivatives.[13,20–22] The steady-state fluorescence emission spectra of these cationic porphyrins in DMF show two bands (~655 and 720 nm) in the red spectral region, which are characteristic for similar free-base porphyrins (Fig. 1B). The fluorescence quantum yield ($\phi_F$) of these cationic porphyrins in DMF (Table 1) presents appropriated values for quantification in biological media.[23]

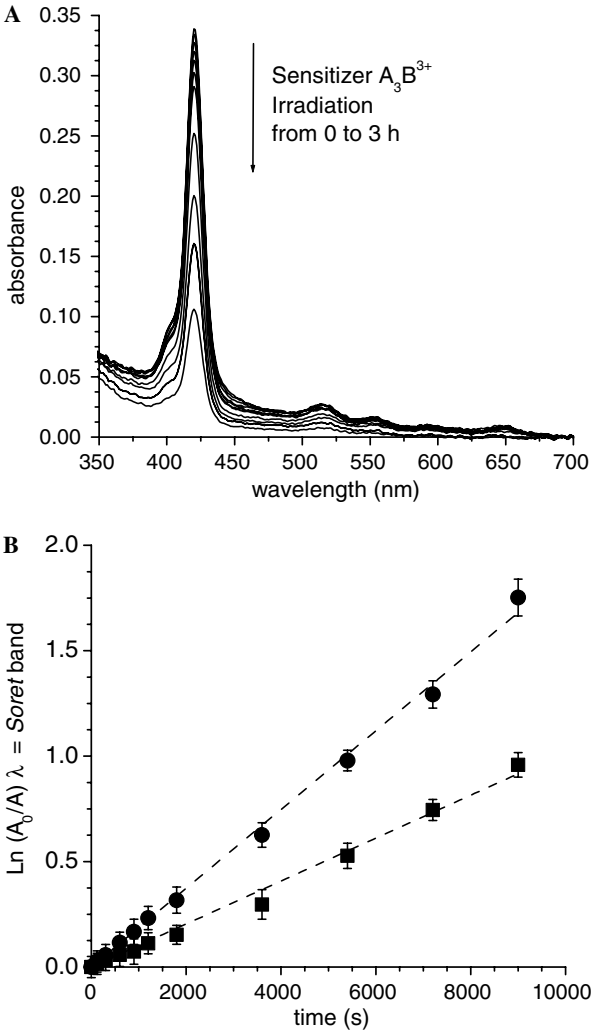



**Figure 1.** (A) Absorption spectra changes of $A_3B^{3+}$ in DMF irradiated with visible light (90 mW/cm$^2$) and (B) first-order plots for the photobleaching of $AB_3^+$ (■) and $TTAP^{4+}$ (●). Values represent means ± standard deviation of three separate experiments.

Both sensitizers present a high efficiency in the quantum yield of $O_2(^1\Delta_g)$ production ($\Phi_\Delta$) in DMF (Table 1). However, the values of $\Phi_\Delta$ can significantly change in a different medium, diminishing when the sensitizer is partially aggregated.[4,5] Also, the biological microenvironment of the sensitizer can induce important modifications in the photophysics of the porphyrin established in solution.[24] In consequence, there are limitations to predict photodynamic efficiencies of sensitizers in biological systems on the basis of photophysical investigations in homogeneous solution.

Photobleaching of cationic porphyrins was carried out under the same irradiation conditions (A) used for photoinactivation of *E. coli* cultures (see Section 2). The decompositions were analyzed following the decrease

**Table 1.** Kinetic parameters of the sensitizer photobleaching, fluorescence quantum yield ($\phi_F$), and singlet oxygen quantum yield ($\Phi_\Delta$) in DMF

| Porphyrin | $k_{obs}$ ($s^{-1}$)[a] | $\tau_{1/2}$ (h)[a] | $k_{obs}$ ($s^{-1}$)[b] | $\tau_{1/2}$ (h)[b] | $\phi_F$[c] | $\Phi_\Delta$[c] |
|---|---|---|---|---|---|---|
| $A_3B^{3+}$ | $(1.1 \pm 0.1) \times 10^{-4}$ | $1.8 \pm 0.1$ | $(1.4 \pm 0.1) \times 10^{-4}$ | $1.3 \pm 0.1$ | 0.13 | 0.53 |
| $TTAP^{4+}$ | $(1.8 \pm 0.1) \times 10^{-4}$ | $1.1 \pm 0.1$ | $(2.1 \pm 0.1) \times 10^{-4}$ | $0.9 \pm 0.1$ | 0.12 | 0.65 |

[a] From absorption spectra at Soret band.
[b] From emission spectra at 650 nm.
[c] From Ref. 13.

in absorption of the porphyrin Soret band. Figure 1A shows the absorption spectra changes observed on photolysis of $A_3B^{3+}$ in DMF. As can be observed, the diminishments in the Soret bands are not accompanied by the formation of a new band in this region. The photobleaching reactions follow a first-order kinetic as shown in Figure 1B. Similar behavior was observed for $TTAP^{4+}$. The values of observed rate constant ($k_{obs}$) are shown in Table 1. Under these conditions, the photodegradation lifetime ($\tau$) of $A_3B^{3+}$ porphyrin was found to be higher for about 45 min than that of $TTAP^{4+}$. Also, porphyrin photobleaching was studied by fluorescence. The decay in emission intensity during irradiation was monitored at $\lambda_{em}$ = 650 nm under excitation at 515 nm. Figure 2A shows the result for $A_3B^{3+}$ porphyrin. From the semilogarithmic plots in Figure 2B, describing the progress of the reaction, the values of $k_{obs}$ were obtained (Table 1). The photobleaching rates calculated from the fluorescence data are about 1.2 times greater than that monitored by decay in absorbance. In previous studies, this difference was attributed to preferential photobleaching of photolabile monomeric forms as compared to aggregates.[25,26] In our cases, no large difference was found probably due to the fact that both porphyrins mainly occur as monomer in DMF.[13] However, the mechanism of photodegradation can be complex and differ in solution and in biological media.[15,25,27] For PDI treatment, if the sensitizer bleaches too rapidly during irradiation, microbial cell inactivation may be incomplete. However, an appropriate photosensitizer dosages and photostability rate allow to use PDI efficiently. Thus, photobleaching can be an advantage to eliminate the photosensibility of the medium.[27,28]

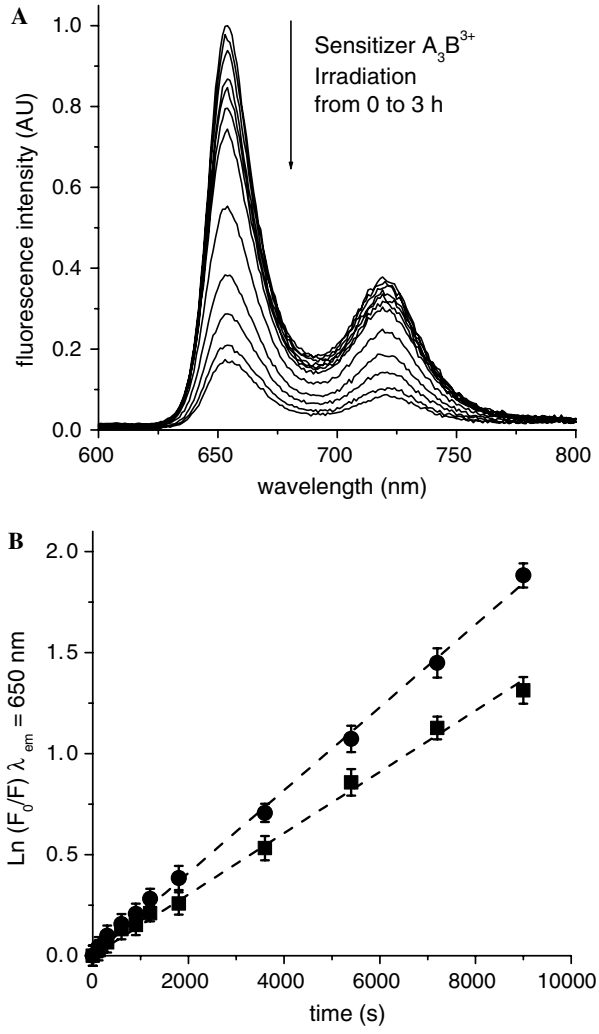



**Figure 2.** (A) Fluorescence emission spectra changes of $A_3B^{3+}$ in DMF irradiated with visible light (90 mW/cm$^2$) and (B) first-order plots for the photobleaching of $AB_3^+$ (■) and $TTAP^{4+}$ (●) ($\lambda_{exc}$ = 515 nm, $\lambda_{em}$ = 650 nm). Values represent means ± standard deviation of three separate experiments.

### 3.2. Photodynamic inactivation of *E. coli* cells immobilized on agar surfaces

The capacity of these porphyrins to bind to bacterial cells of *E. coli* was previously studied in PBS suspension.[13] When *E. coli* cultures are treated with 1 μM of sensitizer, the $A_3B^{3+}$ porphyrin is rapidly (<5 min) bound to bacterial cells reaching a binding value of ~0.8 nmol/$10^6$ cells. This behavior suggests that compound $A_3B^{3+}$ has particularly high binding affinity for bacterial cells. Under similar conditions, the binding of $TTAP^{4+}$ porphyrin to *E. coli* cells reaches a value of ~0.4 nmol/$10^6$ cells. Photosensitized inactivation of *E. coli* cells treated with 1 μM of sensitizer for 30 min at 37 °C in PBS suspension indicates that $TTAP^{4+}$ produced a ~4.0 log decrease (~99.984%) of cell inactivation after a light fluence of 108 J/cm$^2$. Under these conditions, the tricationic $A_3B^{3+}$ porphyrin exhibits a photosensitizing activity causing a ~4.5 log decrease of cell survival, which represents a value greater than 99.997% of cellular inactivation.[13]

Taking into account these results in PBS solution, the photodynamic activity of these photosensitizers ($A_3B^{3+}$ and $TTAP^{4+}$ porphyrins) was evaluated in *E. coli* cells immobilized on TS agar. This approach can be used to inactivate bacteria growing in vivo as localized foci of infection, on skin or on accessible mucous membrane.[29,30] Also, photodynamic treatment has been proposed as a new possibility for protecting foods from microbial spoilage.[31]

Appropriated dilution of *E. coli* cellular suspensions in PBS was spread on TS agar plates to obtain about ten separated colonies. The cultures were incubated for 13 h at 37 °C to form colonies of ~2 mm diameter. The colonies were treated with different amount of porphyrin (0–14 nmol), which was homogeneously distributed on the colony from a stock solution in water. The cultures were kept in dark for 10 min at 37 °C. During this period, the binding of sensitizer to *E. coli* cells can take place. The plates were then illuminated with visible light from a projector equipped with a 150 W halogen lamp (90 mW/cm$^2$) as described above (see Section 2) for 3 h (~972 J/cm$^2$). Afterward, the plates were incubated overnight at 37 °C in dark. Characteristic results for $A_3B^{3+}$ porphyrin are shown in Figure 3. Control of colony B is larger than the control of colony A and all the treated colonies because the colonies B have been incubated one overnight at 37 °C. As can be observed, growth delay of *E. coli* colonies on TS agar was clearly evidenced for colonies treated with $A_3B^{3+}$ porphyrin with respect to control without sensitizer. Comparable increase in the area size was also obtained for a control containing porphyrin but without irradiation. Thus, the growth delay obtained after irradiation of the cultures treated with the porphyrin is due to the photosensitization effect of the agent produced by visible light. Similar behavior was observed for tetracationic $TTAP^{4+}$ porphyrin. Samples of these colonies were aseptically transferred to fresh TS broth medium and transferred to a new TS agar plate. After additional overnight incubation at 37 °C, the viability of the *E. coli* cells was clearly evidenced by the formation of colonies, indicating no complete inactivation of bacterial cells. The variation in the area of *E. coli* colonies treated with $A_3B^{3+}$ and $TTAP^{4+}$ are summarized in Figure 4A. As can be observed, the enhancement in the colony size was dependent on the amount of sensitizer used. For both porphyrins, the increase was practically not detected (<10%) for colonies treated with 10–14 nmol of sensitizers.

On the other hand, comparable experiments were performed with colonies but exposing the culture dishes directly to midday sunlight (80 mW/cm$^2$) for 3 h, which represented a dose of ~864 J/cm$^2$. During the irradiation, the temperature of the cultures was about 35 °C. This indicates that thermal inactivation of the bacteria does not occur under these conditions, as also shown by control experiments. The results are summarized in Figure 4B. Under these conditions, a lower photodynamic inactivation effect was observed according to a

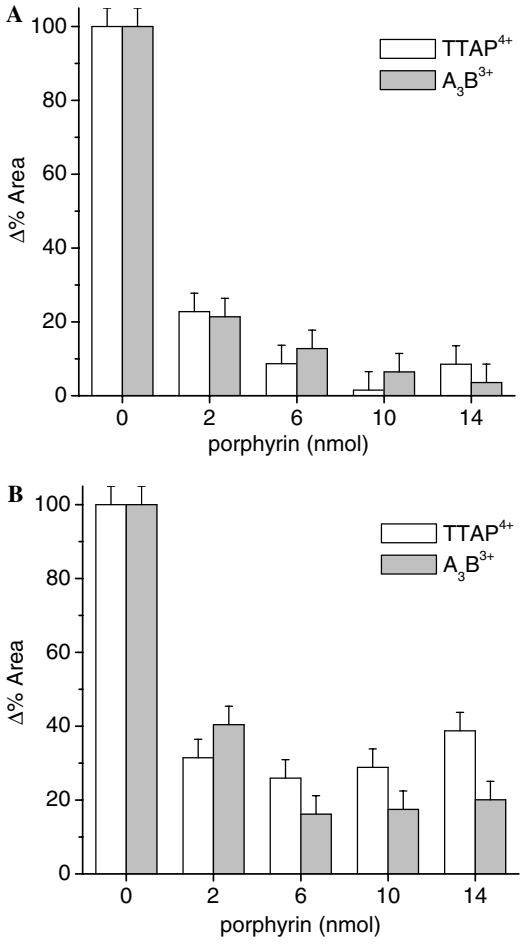



**Figure 4.** Variation in the area of *Escherichia coli* colonies treated with $A_3B^{3+}$ (gray bars) and $TTAP^{4+}$ (white bars) as described in Figure 3. Cultures irradiated for 3 h with visible light using (A) a projector (90 mW/cm$^2$) and (B) midday sun (80 mW/cm$^2$) as light source. Values represent means ± standard deviation of three separate experiments.

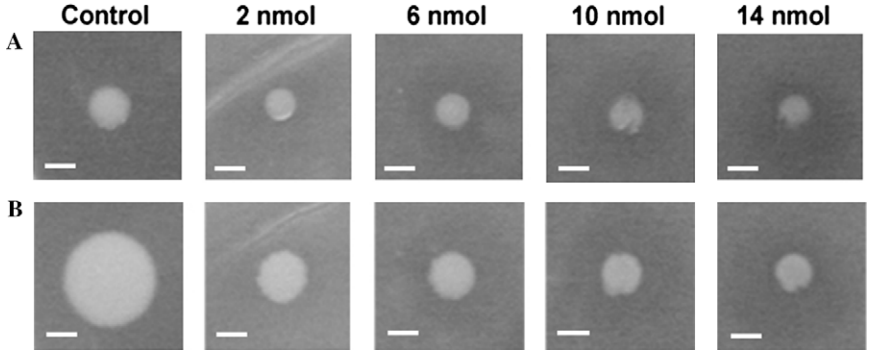



**Figure 3.** Growth delay of *Escherichia coli* colonies on TS agar treated with different amount of $A_3B^{3+}$ porphyrin and irradiated with visible light (90 mW/cm$^2$) for 3 h. (A) Colonies immediately after PDI treatment; (B) colonies incubated overnight at 37 °C. Sensitizer was spread on the colony from a stock solution (~0.5 mM) and the culture was incubated for 10 min at 37 °C before irradiation. Scale bars 2 mm.

lower dose of light with respect to light from projector. Also, Figure 4B indicates a higher efficiency in the growth delay of colonies for cells treated with $A_3B^{3+}$ than those containing $TPPA^{4+}$ porphyrin.

Also, experiment of growth delay of *E. coli* colonies on TS agar was performed irradiating only a separated colony with an optical fiber of 9 mW/cm$^2$ visible light. The isolated colony was treated with 10 nmol of sensitizer, incubated for 10 min in dark and irradiated with a spot of 5 cm diameter for 8 h (260 J/cm$^2$). In this condition, both sensitizers produce similar photosensitization effect and the increase in the colony size was arrested in similar amount to that obtained in Figure 4A for 10 nmol of sensitizer.

The morphological changes of colonies were observed during the photodynamic experiments (Figs. 5A and B). Colonies under PDI treatment and after 18 h of incubation in dark at 37 °C show the appearance of Figure 5A, which is characterized by a cellular growth from the base. In contrast, controls are characterized by convex appearance and glossy colonies (Fig. 5B). The variation in the colony morphology is schematically represented in Figure 5C. As can be observed, the colony is homogeneously covered with sensitizer and after irradiation some levels of cells of the cover are inactivated. Viable cells remain after this PDI procedure in the colony core. Subsequent incubation overnight produces a side development of viable cells. A second growth overnight expands the new viable cells from the base of the colony, producing the colony crater form observed in Figure 5A.

Therefore, the results indicated that under our experimental conditions *E. coli* cells growing in small colonies are difficult to obtain a complete eradication mainly because viable cells are remaining in the core of the colony. However, this experimental problem can be avoided by breaking the colony structure. Thus, when colonies are diffused in a small area of about 0.5 cm diameter on the TS agar and this area is treated with 6 nmol of sensitizer, the irradiation with visible light conduces to a complete eradication of *E. coli* cells. As can be seen in Figure 6A for cells treated with $A_3B^{3+}$ porphyrin, no colony formation was observed even after two overnights incubation at 37 °C, while the irradiated control without sensitizer grew over the spread area containing cells (Fig. 6B).

In conclusion, $A_3B^{3+}$ and $TTAP^{4+}$ cationic porphyrins were evaluated as photosensitizers to inactivate colonies of *E. coli* cells on agar surfaces. The photobleaching of these porphyrins indicates a photodegradation lifetime of about 1.5 h for $A_3B^{3+}$ and 1 h for $TTAP^{4+}$. Although these results are not directly extrapolative to the biological media, the photosensitizing efficiency decreases with the dose of light. This disadvantage could be beneficial to avoid long sensibility in the treatment side. Photodynamic inactivation capacities of these sensitizers were

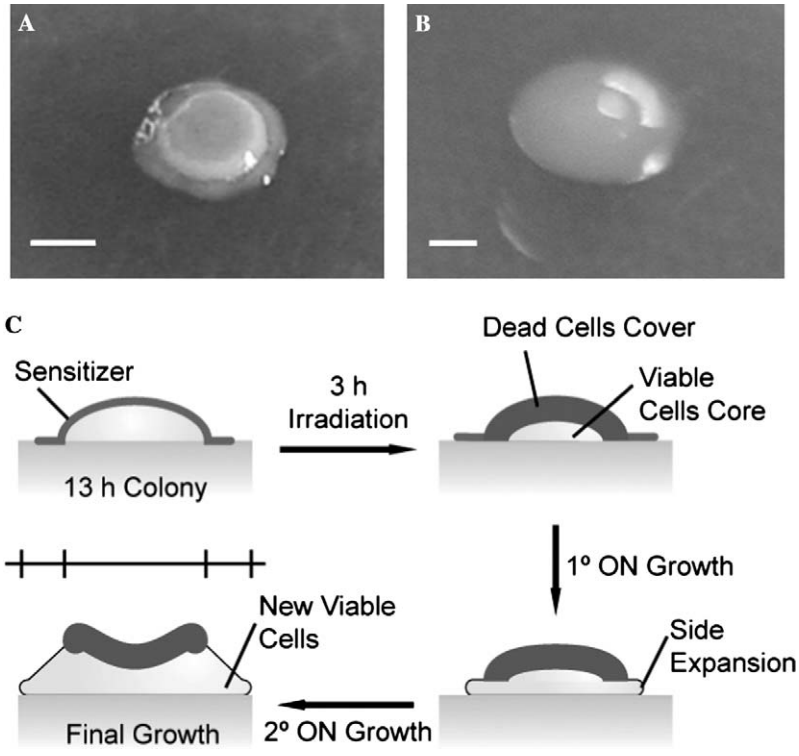



**Figure 5.** Colony morphology variation of *Escherichia coli* cells. (A) Colony treated with sensitizer, irradiated with visible light for 3 h, and grown for two overnights (ON) at 37 °C; (B) colony control without sensitizer; (C) representative scheme of morphological evolution of the colony. First, the colony is spread with the sensitizer and irradiated. After PDI treatment, dead exterior cells inhibit the normal growth of the colony forming a cover. Overnight growth produces a little side development of the viable cells localized in the colony core. A second overnight growth at 37 °C expands the new viable cells taking the colony the final crater form. Scale bars 2 mm.

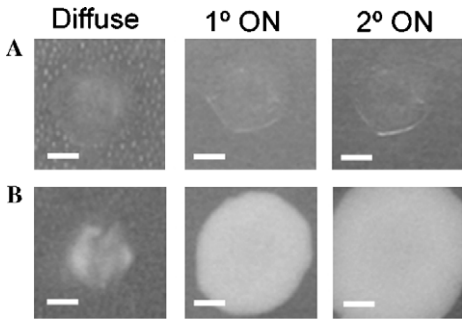



**Figure 6.** Photosensitization of *Escherichia coli* cells spread from a broken colony. (A) Small colony cells were spread on an area of 0.5 cm diameter, treated with 6 nmol of $A_3B^{3+}$ porphyrin, and incubated for 10 min at 37 °C. After that, the area was irradiated with visible light (90 mW/cm$^2$) for 3 h and incubated at 37 °C for one and two overnights. (B) Control colony without sensitizer. Scale bars 2 mm.

analyzed in *E. coli* colonies immobilized on TS agar surfaces. The experiment with small colonies shows that even though the growth delay of the colonies can be arrested, complete eradication of *E. coli* cells is not possible under these conditions. Growth of *E. coli* cells as a colony leads to a large increase in resistance to antimicrobial PDI treatment compared with cultures grown in suspension in conventional liquid media.[13,32] This was confirmed because $A_3B^{3+}$ porphyrin induces absolute growth delay in suspension of *E. coli* cells.[13] Previous studies also show that when the cells were treated with sensitizer in solution and immobilized on TS agar, the $A_3B^{3+}$ porphyrin exerts a higher photodynamic activity than tetracationic $TTAP^{4+}$ porphyrins. Even so, the photodynamic inactivation capacity of $A_3B^{3+}$ porphyrin remains high for immobilized cells on agar surfaces containing an area spread with the sensitizer.[13] In the present study, apparently the main problem of complete bacterial eradication consists in the lack of sensitizer penetration into the colony core. This can be evidenced when the colonies are diffused on the surface before PDI treatment. This procedure produces complete inactivation of *E. coli* cells on the TS agar surface. Therefore, these results indicate that the tricationic $A_3B^{3+}$ porphyrin is an interesting sensitizer with potential applications in photodynamic inactivation of bacteria growing as localized foci of infection.

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