J. Braz. Chem. Soc., Vol. 20, No. 1, 31-36, 2009.
Printed in Brazil - ©2009 Sociedade Brasileira de Química
0103 - 5053 $6.00+0.00

Article

Singlet Oxygen Quantum Yields (ϕ_Δ) in Water using Beetroot Extract and an Array of LEDs

Juliano A. Bonacin, Fábio M. Engelmann, Divinomar Severino, Henrique E. Toma and Mauricio S. Baptista*

Instituto de Química, Universidade de São Paulo, Av. Prof. Lineu Prestes, 748, CP 26077, 05513-970 São Paulo-SP, Brazil

É proposta uma estratégia simples e barata para determinar rendimentos quânticos (ϕ_Δ) de oxigênio singlete ($^1O_2$) de fotossensibilizadores (PS) em água utilizando extrato de beterraba contendo betacianina (Bc) e um conjunto de diodos emissores de luz (LEDs) para excitação. Bc, um corante catiônico natural, foi obtida por purificação através de cromatografia a partir do extrato de beterraba vermelha (*Beta vulgaris*), e utilizada como uma sonda para detecção de $^1O_2$. Soluções do Bc e PS foram iluminadas com um arranjo de LEDs adaptado no compartimento de um espectrofotômetro comercial e a diminuição da absorvância de Bc foi seguida em função do tempo. O fotobranqueamento de Bc diminuiu em solução purgada com nitrogênio e aumentou em D_2O, indicando o envolvimento de $^1O_2$. A constante de velocidade de fotobranqueamento observada (k_{obs}) foi proporcional à intensidade do LED, à concentração e ao ϕ_Δ do PS. Mantendo a fonte de luz constante pudemos estimar a integral de sobreposição (R) entre a absorção do PS e a emissão do LED para diferentes concentrações de PS. A inclinação da curva de R em função de k_{obs} é o valor da constante de velocidade de fotobranqueamento (k), que foi mostrada ser proporcional a ϕ_Δ. Valores de ϕ_Δ obtidos por este método foram comparados com aqueles obtidos através da medição da emissão no NIR (infravermelho próximo) para uma série de corantes fenotiazínicos.

It is proposed a simple and inexpensive strategy to determine singlet oxygen ($^1O_2$) quantum yields (ϕ_Δ) of photosensitizers (PS) in water using beetroot extract containing betacyanin (Bc) and a set of light emitting diodes (LEDs) for excitation. Bc, a cationic natural dye, was obtained by flash chromatography purification from the red beet extract *(Beta vulgaris)* and employed as a convenient probe for $^1O_2$ detection. Solutions of Bc and PS were illuminated with an array of LEDs adapted in the cuvette compartment of a commercial spectrophotometer, and the decrease in Bc absorbance was followed as a function of time. Bc photobleaching decreased in de-aerated solution and increased in D_2O, indicating the involvement of $^1O_2$. The observed photobleaching rate constant (k_{obs}) was proportional to the LED intensity, concentration and ϕ_Δ of the PS. By keeping the light source constant we could estimate the overlap integral (R) between the LED emission and PS absorbance for different PS concentrations. The slope of R *versus* k_{obs} is the value of the photobleaching rate constant (k), which was shown to be proportional to ϕ_Δ. Values of ϕ_Δ obtained by this method were compared with those obtained by measuring NIR (near infrared) emission for a series phenothiazine dyes.

Keywords: beetroot, betacyanin, quantum yield, singlet oxygen, phenothiazines, photodynamic therapy

Introduction

Singlet oxygen ($^1O_2$) is the most reactive form of molecular oxygen. Its $^1\Delta_g$ electronic configuration allows efficient reactions with double bounds presented in unsaturated lipids, proteins and nucleic acids.[1,2]

$^1O_2$ has deleterius effects to human beings, for example, it is involved in skin damage caused by sun exposition.[3,4] However, it is also the main species responsible for killing diseased cells in the treatment of tumors or other pathological conditions by photodynamic therapy (PDT).[5-7] In PDT, the electronic excitation of photosensitizers (PS) leads to triplets that undergo energy transfer, yielding $^1O_2$. The efficiency of $^1O_2$ generation by a specific PS, which can be measured as its $^1O_2$ quantum yield (ϕ_Δ), is an important

*e-mail: Baptista@iq.usp.br

property to be evaluated concerning the application of PS in PDT. Because of the low costs of LEDs and of other non-coherent light sources, PDT has now the possibility to be widely used and relatively inexpensive protocols have been proposed.[8-11] It is also important that inexpensive new drugs can be found and consequently it is quite relevant to develop inexpensive methods to estimate ϕ_Δ.[8-10]

The most specific method to determine $^1O_2$ is to measure its emission at 1270 nm.[12,13] Usually, ϕ_Δ is obtained by measuring the slope of emission *versus* energy plots and comparing them with standard compounds.[14-16] However, expensive lasers and detection schemes are required, specially if the detection is to be performed in aqueous solutions where the lifetime of $^1O_2$ is only *ca.* 4 μs.[10-16]

Perhaps the most commonly used method to quantify the production of $^1O_2$ is based in chemical trapping,[12,13,17-19] *i.e.*, $^1O_2$ reacts with compounds that absorb in the UV-Vis region, causing the decay of its absorption and/or emission bands and/or the rise of the absorption/emission bands associated with the oxidized species. Several compounds have been proposed and used as scavengers including, Rubrene, DPBF (1,3-diphenylisobenzofuran) or other water-soluble compounds.[17-19] Highly selective probes for $^1O_2$ have been recently reported.[20,21]

In this work, we propose a rather simple, convenient and inexpensive chemical trapping method to measure ϕ_Δ, based on the use of beetroot extract containing betacyanin (Bc) as chemical trap and an array of LEDs, which is adapted in the cuvette compartment of a spectrophotometer, as excitation source.

Experimental

Chemicals

The PS utilized in the experiments were Methylene Blue (MB), Azure A, Azure B and thionine. They were recrystallized from ethanol. Water was double distilled and de-ionized.

Extraction and purification of Bc

Betacyanin (B*c*) was extracted from beetroot or beet red (*Beta vulgaris*) following literature procedure.[22-24] Two hydro-soluble compounds: betacyanin (B*c*) and betaxanthins (Bx), which present absorption bands at 540 and 480 nm, respectively are obtained from a aqueous extraction of beet red.[22-24] Extraction was realized by addition of the triturated beetroot (50 g) on 30 mL of milli-Q water under stirring by 90-120 min. Heating should be avoided because Bc is very susceptible to oxidation upon heating. In fact, the color of the extract changes from pink to brown when the solution is heated. While Bc is very sensible to oxidation by $^1O_2$, Bx is not (Figure 1). Therefore, in order to obtain a sensitive probe for $^1O_2$ it was necessary to separate Bc from Bx. The separation of Bc from the other components of the extract was carried out by column chromatography using silica gel as stationary phase and water as eluent. The first fraction was predominantly Bc, as determined by UV-Vis spectroscopy.[22-24]

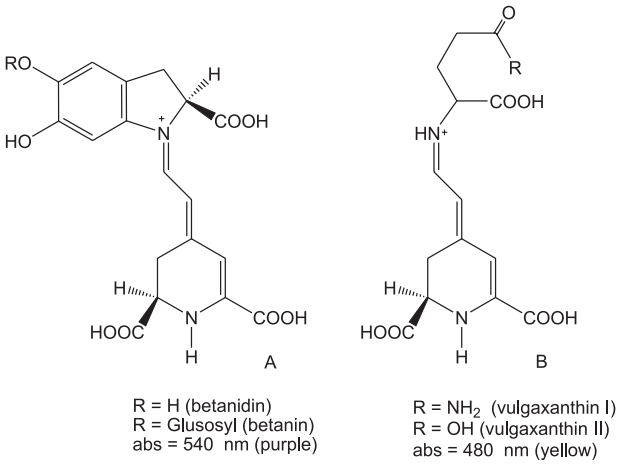

Figure 1. Structure of the main beetroot dyes. (A) betacyanins (Bc) also known as betanin (with sugar moiety) and betanidine without sugar and (B) betaxanthins (Bx).

Photochemical set up

All experiments were carried out in a diode-array spectrophotometer (Agilent model 8453) working in kinetic mode and using a quartz cuvette with magnetic stirring. For the irradiation, we have built a photo device using a set of commercial LEDs positioned at 90° to the analyzer beam of the spectrophotometer (Figure 2). They were assembled in groups of four and placed at the laterals of a perforated cuvette holder. A picture of this device taken under working conditions can be seen in Figure 2B. The LEDs had maximum emission around 640 nm with total nominal power output of 16 mW (2 mW/LED, as measured in the Power meter Fieldmate-Coherent) and were connected in series, powered by an electric source of 3.0 V (DC). The photobleaching experiments were performed using 1 mL of milli-Q water, 10 μL of the Bc extract and a PS with maximum absorption smaller than 1 a.u. Under continuous irradiation, spectra were acquired every three seconds.

Near Infrared (NIR) emission

Phosphorescence decay curves at 1270 nm were recorded with a time-resolved NIR fluorometer (Edinburgh

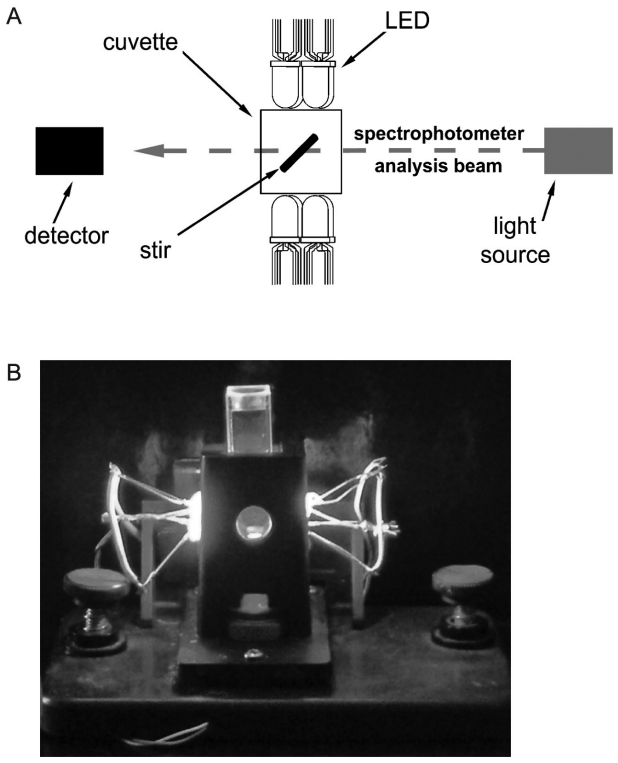

Figure 2. (A) Top view of the schematic arrays of LEDs arranged around the spectrophometric cuvette. The LEDs were connected in series and powered by a 3.0 V (DC) source. (B) Picture of the irradiation device at working conditions; the LEDs exhibited maximum emission at *ca.* 640 nm.

Analytical Instruments) equipped with a Nd:YAG laser (Continuum Surelite III) for sample excitation at 532 nm. The emitted light was passed through a silicon filter and a monochromator before detection by NIR-PMT (Hamamatsu Co. R5509). Data acquisition was performed by a MSA 300 MHz acquisition board (Becker&Hickl GmbH, Berlin).[16,25,26] The experiments were performed at room temperature, in air-saturated aqueous solution. The sample absorbance was adjusted to 0.1 a.u. at 532 nm ([Dye] *ca.*70 μmol L^{-1}). To avoid dimerization at these relatively large dye concentrations sodium dodecyl sulfate (SDS) was added to the solution at final concentrations of 10 mmol L^{-1}.[26,27] In the absence of dimerization the value of singlet oxygen quantum yield (ϕ_Δ) for MB is 0.52 in several media (even in naflon films) and measured with different techniques.[12,26-29] ϕ_Δ values were calculated using equation 1:[12-16]

$$\phi_\Delta{}^b = \frac{\phi_\Delta{}^a}{I^a} I^b \qquad (1)$$

where $\phi_\Delta{}^a$ and I^a are, respectively, the quantum yield and initial phosphorescence intensity of $^1O_2$ at 1270 nm of the MB species, which was used as standard. I^b is the phosphorescence emission intensity of the other cationic phenothiazine derivatives.

ϕ_Δ by trapping with Bc and LED excitation

There are several chemical methods used for the determination of ϕ_Δ.[17-21] In the simplest one, which use monochromatic light sources, the solution absorbance at the irradiation wavelength of the standard and sample is set at the same value, in order to guarantee that the same number of photons is absorbed. During the irradiation $^1O_2$ is formed and reacts preferentially with the scavenger, which is in large excess compared with PS, causing a decrease in the scavenger absorption band. The absorbance change monitored as a function of time is typically fitted by a monoexponential function.[18] The exponential constant gives the observed photobleaching rate constant (k_{obs}), which should be proportional to ϕ_Δ. Strategies based in varying the concentration of trapping agents are also common.[12]

In this work, another strategy was implemented in order to allow the use of an inexpensive polychromatic light source (LED) to promote irradiation. k_{obs} was obtained using different PS concentrations (Bc being always in large excess) and a relationship between k_{obs} and ϕ_Δ was found and used, considering several assumptions as described bellow.

The actual value of k_{obs} depends on the quantum yield of $^1O_2$ production (ϕ_Δ), on the photon flux absorbed by the PS, on the bimolecular rate constant of the chemical reaction between Bc and $^1O_2$, on the concentration of Bc and on the $^1O_2$ lifetime.[12,13,29] Because Bc is photochemically inert, we only have to consider the photochemical reactions that start with PS excitation.

Calculation of the exact photon flux absorbed by the PS is not trivial because the LED device exhibits a spectral band of emission. It is necessary to calculate the absorption factor of the sensitizer ($1\text{-}10^{-Abs_{PS}}$) integrated on the overall spectral region of the LED emission, which should be expressed in photonic units (PE_{LED}), and this integral should also be corrected by the fraction of incident light transmitted by the Bc ($10^{-Abs_{Bc}}$), (equation 2).

$$R = \int (1 - 10^{Abs_{PS}})\, PE_{LED}\; 10^{-Abs_{BC}}\, d\lambda \qquad (2)$$

Because the main idea of this work is to provide an easy method to calculate ϕ_Δ, it is fundamental to simplify this calculation. Considering that measurements will be made at different PS concentrations and compared with a standard, even if the simplified equation proposed bellow has systematic errors, these errors will be the same for samples and standard.

The first assumption is related with the Bc absorption. Although Bc does absorb the LED emission, this absorption basically does not change during irradiation, *i.e.*, the LED emission maximum is just in an isosbestic point of the Bc absorption change profile during the photochemical reaction (Figure 3). Also the Bc concentration was kept constant in all experiments. Therefore, we will not consider the Bc absorption in the calculations, assuming that the changes that are due to Bc absorption are the same for samples and standard.

In terms of the PS absorption factor and LED emission, because the PS absorption is not large and does not change during irradiation, we propose to estimate the relative fraction of PS excited by the LED at each PS concentration by calculating the overlap integral (R) between PS absorption (ABS_{PS}) and LED emission (E_{LED}) (equation 3), instead of calculating the exact photon flux absorbed by the PS (equation 2).

$$R = \int ABS_{PS}\, E_{LED}\, d\lambda \qquad (3)$$

Depending on the acceptor and on the solvent, $^1O_2$ lifetime may vary during irradiation. However, in water the total quenching of $^1O_2$ by Bc (physical and chemical reactions) is negligible compared to quenching by the solvent (we have always obtained $^1O_2$ lifetime of 4 ± 0.5 μs in all experimental conditions). Consequently, the PS absorption and the $^1O_2$ lifetime did not change during irradiation.

Considering that the Bc concentration was kept constant and in excess compared with PS, the Bc bleaching (k_{obs}) should follow pseudo first-order kinetics and can be calculated by mono-exponential fitting. Also the plot of k_{obs} *versus* R can be approximated by a linear fit. The slope of this curve is the rate constant of the chemical reaction of Bc with singlet oxygen (k), which is theoretically proportional to the quantum yield of $^1O_2$ production (ϕ_Δ). Note that the other factors that could affect the value of k, *i.e.*, the bimolecular rate constant of the chemical reaction between Bc and $^1O_2$ and the $^1O_2$ lifetime are unchanged. In these experiments small dye concentrations were used and therefore there was no need to add SDS to avoid dimerization.

Results and Discussion

Before starting the chemical trapping experiments, it was important to test for the stability of the Bc solutions under illumination. The Bc solution was illuminated for several minutes using either a laser source that specifically excites Bc (Nd:YAG laser, 20 mW, 532 nm) or LEDs that do not excite Bc (640 nm, set-up mount, see Experimental section). There was no absorbance change in both control experiments. Therefore, although Bc is susceptible to oxidation upon heating, it is photochemically inert.

When an aqueous solution containing Bc (1.0 a.u. in 540 nm) and MB (*ca.*10 μmol L^{-1}) was irradiated with the LED set up (Figure 2) the Bc absorbance decreased during the 8 min irradiation (Figure 3) and the color of the solution changed from purple to blue. Note that the MB absorption did not change during the experiment, indicating that electron transfer reactions that could lead to MB photobleaching, are not taking place under such experimental conditions.[25,30,31] In none of the phenothiazines tested, there was any change in the dye absorption during irradiation.

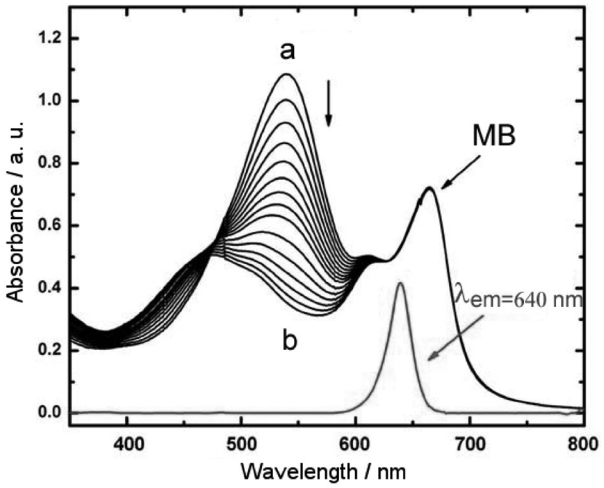

Figure 3. Absorption spectra of the Bc and MB (11.2 μmol L^{-1}) solution under. LED light illumination. The spectra were taken every 30 seconds from a (time=0) to b (time=8 min). The LED emission is shown in red.

Decrease in Bc absorbance at 540 nm during the irradiation followed an exponential profile and fitted well to a single exponential function, allowing the calculation of k_{obs} (Figure 4A). The temporal decay of the Bc photobleaching in the presence of MB was also compared in solutions with different oxygen concentrations. The bleaching efficiency increased with the increase in the oxygen concentration suggesting again the presence of type II oxidation processes (Figure 4A). Photobleaching induced by electron transfer reactions are usually accelerated in nitrogen-purged samples, because oxygen can suppress triplet and radical intermediates.[30,31]

Lifetime of $^1O_2$ is longer in D_2O (35-40 μs) than in water.[6,12,13] Assuming that no other interference occurs, the steady-state concentration of $^1O_2$ would be *ca.*10 times larger giving consequently *ca.*10 times faster reaction rate in *ca.*100% D_2O. This effect has been used to study the role of $^1O_2$ in specific photochemical events by carrying

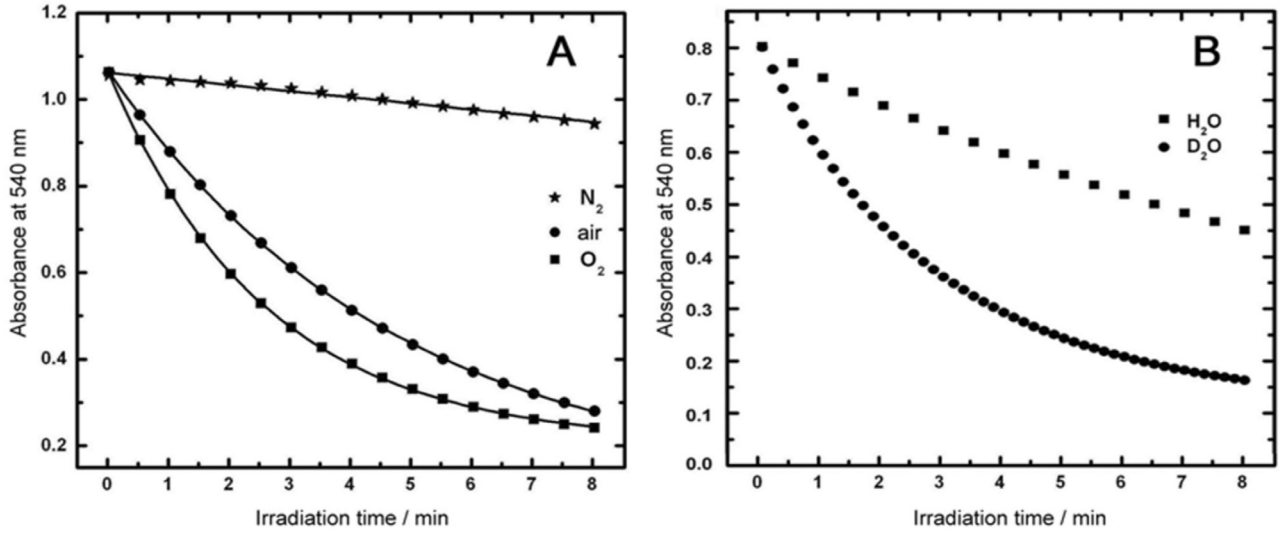

Figure 4. Absorbance at 540 nm as function of irradiation time: (A) in water samples purged with nitrogen (★), air-saturated (●), oxygen (■); (B) air saturated solutions in water (■) and in deuterium oxide (●). The data were obtained in solutions similar to those used to obtain data of Figure 3.

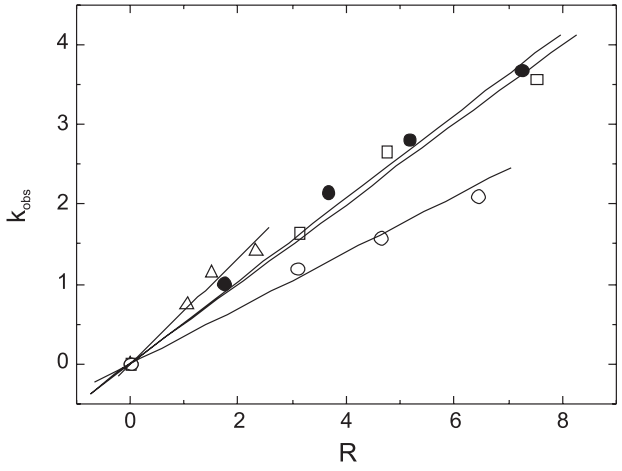

Figure 5. Observable kinetic decays (k_{obs}) of Bc absorbance at 540 nm as a function of overlap integral (R, equation 2) obtained for different concentrations of MB (●,[MB] in μmol L^{-1}= 2.6; 5.3; 7.4; 11.2), Azure A (□, [Azure A] in μmol L^{-1} = 5.1; 8.1; 11.4), Azure B (○,[Azure B] in μmol L^{-1} = 3.4; 7.1; 9.4) and thionine (△, [thionine] in μmol L^{-1} = 4.9; 8.0; 10.1).

out experiments in water and in D_2O under the same experimental conditions (Figure 4B). However, it is important to emphasize that we were not using *ca.*100% D_2O in our experiments because the reaction mixture is prepared *in situ* by using Bc solution in water (giving around 90% D_2O:H_2O mixture). Even so, we have observed *ca.* 4 times faster rate indicating that the bleaching of Bc by MB excitation is more efficient in D_2O than in water, which proves that $^1O_2$ is the main species responsible for the bleaching. These observations also indicate that Bc is a specific $^1O_2$ scavenger and therefore, a good probe for estimating ϕ_Δ.

Table 1. Quantum yield of singlet oxygen in water using Methylene Blue as reference (ϕ_Δ = 0.52), and red light emitting LED sources (λ_{em} = 640 nm)

photosensitizers	k[a]	ϕ_Δ	ϕ_Δ (literature)
Methylene Blue	0.47 ± 0.05	0.52 (reference)	0.52[b,c]
Azure A	0.41 ± 0.06	0.45	0.47[d]
Azure B	0.28 ± 0.05	0.31	0.29[d]
Thionine	0.54 ± 0.10	0.60	0.58[c]

[a]Slope k_{obs} × R. Slope and standard deviation of three independent measurements. [b]References 32 and 33; [c]reference 34. [d]Values obtained by NIR emission as described in the experimental section in 10 mmol L^{-1} SDS.

Monoexponential decays of Bc absorbance as a function of time were observed for all dyes and concentrations tested, facilitating the calculation of k_{obs}. k_{obs} was plotted as a function of R, which was calculated for each dye concentration (Figure 5). The slope of these curves, found by least square fitting of the experimental data, allows the calculation of k (Table 1, first column). Assuming the proportionality between k and ϕ_Δ and using MB as standard it was possible to calculate ϕ_Δ values of azure A, azure B and thionine (Table 1, second column). It is clear that the calculated values are very similar to those found in the literature or obtained by measuring NIR emission (Table 1, third column).

Conclusions

The chemical trapping protocol based in Bc and LED provides an efficient, inexpensive and simple method to

quantitatively determine $^1O_2$ formation by a PS. Although killing organisms and treating diseases with dyes and light is a very old process in humanity, now a days, people have to pay thousands of dollars to be treated by PDT. However, inexpensive PDT protocols can be easily established. Considering that finding new drugs represents an increasingly difficult and expensive task, the discovery of new PS for PDT can be a practical solution, especially for underserved populations. The method here presented may be of relevance in pursuing such solution.

Acknowledgments

The authors gratefully acknowledge the financial support and fellowships from Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Rede de Nanotecnologia Molecular e Interfaces (RENAMI) and Instituto do Milênio de Materiais Complexos (IMMC).

References

1. Foote, C. S.; *Science* **1968**, *162,* 963.
2. Foote, C. S.; *Photochem. Photobiol.* **1991**, *54,* 659.
3. Ichihashi, M.; Ueda, M.; Budiyanto, A.; Bito, T.; Oka, M.; Fukunaga, M.; Tsuru, K.; T. Horikawa; *Toxicology* **2003**, *189*, 21.
4. Baier, J.; Maisch, T., Maier, M.; Engel, E.; Landthaler, M.; Bäumler, W.; *Biophys. J.* **2006**, *91*, 1452.
5. Brown, S. B.; Brown, E. A.; Walker, I.; *Lancet Oncol.* **2004**, *5*, 497.
6. Jarvi, M.T.; Niedre, M.J.; Patterson, M.S.; Wilson, B.C.; *Photochem. Photobiol.* **2002**, *75*, 382.
7. Silva, A. R. ; Pelegrino, A. C. ; Tedesco, A. C. ; Jorge, R. A.; *J. Braz. Chem. Soc.* **2008**, *19*, 491.
8. Tardivo, J. P.; Del Giglio, A.; Paschoal, L. H.; Baptista, M. S.; *Photomed. Laser Surg.* **2006**, *24*, 528.
9. Tardivo, J. P.; Del Giglio, A.; Oliveira, C. S.; Gabrielli, D. S.; Junqueira, H. C.; Tada, D. B.; Severino, D.; Turchiello, R.; Baptista, M. S.; *Photodiagn. Photodyn. Ther.* **2005**, *2/3,* 175.
10. Allison, R. R.; Downie, G. H.; Cuenca, R.; Hu, X.-H.; Childs, C. J. H.; Sibata, C. H. *Photodiagn. Photodyn. Ther.* **2004**, *1*, 27.
11. Menezes, P. F. C.; Bagnato, V. S.; Johnke, R. M.; Bonnerup, C.; Sibata, C.; Allison, R.R.; Perussi, J. R.; *Laser Phys. Lett.* **2007**, *4*, 546.
12. Wilkinson, F.; Helman, W. P.; Ross, A. B.; *J. Phys. Chem. Ref. Data* **1993**, *22*, 113.
13. Kochevar, I. E.; Redmond, R.W.; *Methods Enzymol.* **2000**, *319*, 20.
14. Engelmann, F. M.; Mayer, I.; Araki, K.; Toma, H. E.; Baptista, M. S.; Maeda, H.; Osuka, A.; Furuta, H.; *J. Photochem. Photobiol., A* **2004**, *163*, 403.
15. Engelmann, F. M.; Mayer, I.; Gabrielli, D.; Araki, K.; Toma, H. E.; Kowaltowski, A.; Baptista, M. S.; *J. Bioenerg. Biomembr.* **2007**, *39*, 175.
16. Uchoa, A.F.; Knox, P.P.; Turchielle, R.; Seifullina, N.K.h.; Baptista, M.S.; *Eur. Biophys. J.* **2008**, *37*, 843.
17. Spiller, W.; Kliesch, H.; Wohrle, D.; Hackbarth, S.; Roder, B.; Schnurpfeil, G.; *J. Porphyrins Phthalocyanines* **1998**, *2*, 145.
18. Aubry, J. M.; Rigaudy, J.; Nguyen, K.C.; *Photochem. Photobiol.* **1981**, *33*, 149.
19. Nardello, V.; Aubry, J. M.; *Tetrahedron Lett.* **1997**, *38*, 7361.
20. Tanaka, K.; Miura, T.; Umezawa, N.; Urano, Y.; Kikuchi, K.; Higuchi, T.; Nagano, T.; *J. Am. Chem. Soc.* **2001**, *123*, 2530.
21. Li, X.; Zhang, G.; Ma, H.; Zhang, D.; Li, J.; Zhu, D.; *J. Am. Chem. Soc.* **2004**, *126*, 11543.
22. von Elbe, J. H.; Schwartz, S.J.; Hildenbrand, B.E.; *J. Food Sci.* **1981**, *46*, 1713.
23. Escribano, J.; Pedreño, M. A.; García-Carmona, F.; Muñoz, R.; *Phytochem. Anal.* **1998**, *9*, 124.
24. Gasztonyi, M.N.; Daood, H.; Hájos M.T.; Biacs, P.; *J. Sci. Food Agric.* **2001**, *81*, 932.
25. Gabrielli, D.; Belisle, E.; Severino, D.; Kowaltowski, A. J.; Baptista, M.S.; *Photochem. Photobiol.* **2004**, *79*, 227.
26. Severino, D.; Junqueira, H. C.; Gabrielli, D.; Gugliotti, M.; Baptista, M. S.; *Photochem. Photobiol.* **2003**, *77*, 459.
27. Junqueira, H. C.; Severino, D.; Dias, L. G.; Gugliotti, M.; Baptista, M. S.; *Phys. Chem. Chem. Phys.* **2002**, *4*, 2320.
28. Wetzler D. E.; Garcia-Fresnadillo, D.; Orellana, G.; *Phys. Chem. Chem. Phys.* **2006**, *8*, 2249.
29. DeRosa, M C.; Crutchley, R. J.; *Coord. Chem. Rev.* **2002**, *233/234*, 351.
30. Baptista, M. S.; Indig, G.L.; *Chem. Commun.* **1997**, *18*, 1791.
31. Baptista, M. S.; Indig, G. L.; *J. Phys. Chem. B* **1998**, *102*, 4678.
32. Usui, Y.; Tsukada, M.; Nakamura, M.; *Bull. Chem. Soc. Jpn.* **1978**, *51*, 379.
33. Tanielian, C.; Golder, L.; Wolff, C.; *J. Photochem.* **1984**, *25*, 2.
34. Usui, Y.; *Chem. Lett.* **1973**, *2*, 743.

Received: July 3, 2008
Web Release Date: October 16, 2008

FAPESP helped in meeting the publication costs of this article.