Journal of Physics: Conference Series

OPEN ACCESS

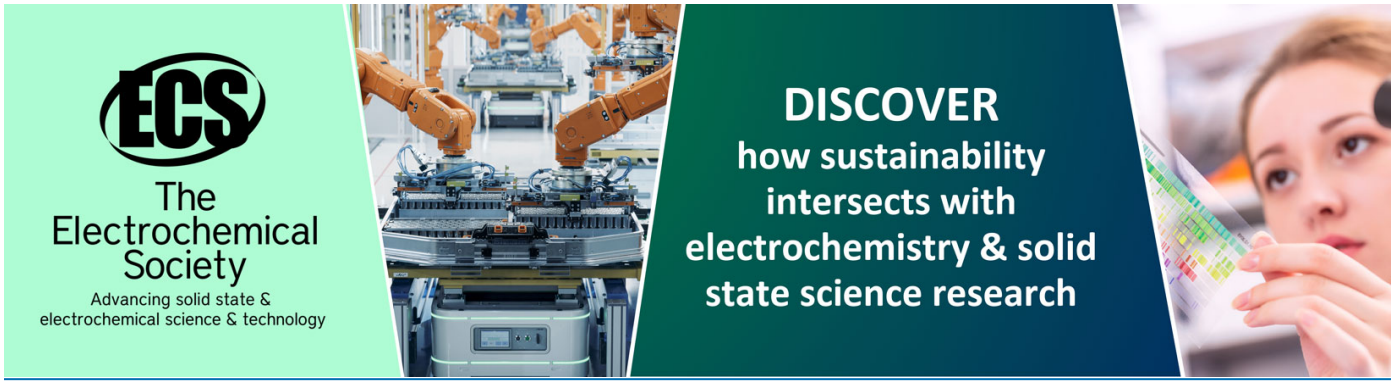
In Vitro Studies of Photosensitizer fluorescence changes on singlet oxygen mediated photobleaching

To cite this article: M Atif 2013 *J. Phys.: Conf. Ser.* **414** 012025

View the article online for updates and enhancements.

You may also like

- Photobleaching of organic fluorophores: quantitative characterization, mechanisms, protection
Alexander P Demchenko
- Extending the photobleaching lifetime in the presence of an optical tweezers by wavefront engineering
Z Zhang and J N Milstein
- Photostability of organic electro-optic polymer under practical high intensity continuous-wave 1550 nm laser irradiation
Yukihiro Tominari, Toshiki Yamada, Takahiro Kaji et al.



This content was downloaded from IP address 3.138.174.195 on 05/05/2024 at 19:34

In Vitro Studies of Photosensitizer fluorescence changes on singlet oxygen mediated photobleaching

M. Atif

[a]Research Chair for Laser Diagnosis of Cancer, Physics and Astronomy Department, College of Science, King Saud University, Riyadh, Saudi Arabia

[b]National Institute of Laser and Optronics, Nilore, Islamabad, 45650, Pakistan

E-Mail: atifhull@gmail.com

Abstract:

The effects of photosensitizer concentration on the singlet oxygen mediated photobleaching have been investigated in keratinocytes. Cells having different photosensitizers concentration were identified on the basis of the fluorescence signal amplitude and their fluorescence decay then studied to see if there was a correlation with concentration. The experimental results shows that the photobleaching was more rapid at the higher concentration as compared to lower concentration which mean that singlet oxygen plays a vital role in the rapid m-tetrahydroxyphenylchlorin (mTHPC) induced fluorescence photobleaching causing the sensitizer to degrade.

Keywords: Photosensitizer concentration, singlet oxygen mediated photobleaching, keratinocytes, fluorescence signal amplitude and their fluorescence decay

Published under licence by IOP Publishing Ltd

1. Introduction

Singlet oxygen $^1O_2$ was first found in 1924 [1] and widely believed to be a more reactive form of oxygen, which oxidizes biological substrates [2]. It is not a free radical but an electronically excited form of oxygen. The generation of $^1O_2$ is carried out by photosensitization reactions [3]. An important characteristic of $^1O_2$ is that it readily combines chemically with a variety of biomolecules. Kautsky's investigations showed that singlet oxygen might be a reaction intermediate in dye-sensitized photo-oxygenation [4]. Several later observations support this idea and demonstrated that during detrimental oxidation of biomolecules, singlet oxygen is an important intermediate species. Moreover, the role of singlet oxygen during PDT is that it attacks the critical sites e.g., proteins, DNA, mitochondria and lipids. When the oxidative injury to cells exceeds a certain threshold level, the cell death occurs.

Zhao [5] addressed the impact of singlet oxygen as a higher energy state molecular oxygen species. It is considered the most active intermediates involved in chemical and biochemical reactions. In order to fully understand the reactivity of $^1O_2$, it is important to know about its physical and chemical nature. His work reviews the generation, quenching and detection of $^1O_2$. Moreover, the properties, possible chemical reactions and its biological role in photodynamic therapy (PDT) are also discussed. Fuchs et al, [6] described the current information on cutaneous oxygen physiology and oximetry, the role of oxygen and singlet oxygen in PDT.

The approach to modulate skin oxygen tension is also discussed. Their results reveal that effectiveness of PDT can be manipulated by modulating skin oxygen tension.

Georgakoudi et al, studied Photofrin bleaching via a singlet oxygen mediated mechanism [7]. Their results show that photobleaching is confined to regions of singlet oxygen abundance. Moreover, photosensitizer degradation results in decreased photodynamic oxygen consumption and as laser exposure precedes the supply of oxygen to cells at long distances from the source is due to diffusion. Due to this, it will gradually extend the distance from the oxygen supply over which significant singlet oxygen is produced. An important conclusion of this investigation is that sensitizer photobleaching is fundamentally linked to spatial distribution of photodynamic dose.

In the current study of normal keratinocytes, the observed sensitizer fluorescence changes using constant laser power are analyzed. The experimental results show that the availability of free oxygen may be limited even in the case of formalin fixed cells. This complex interaction between fluence rate and oxygen status during the fixed cell system in vivo determine the PDT dose delivered. This paper describes a performance study of the effects of photosensitizer concentration on singlet oxygen mediated photobleaching in keratinocytes.

2 Materials and methods

The cells used for this study are keratinocytes derived from human foreskin. The culture conditions and Foscan administration are the same as described in our previous reported studies [8-9].

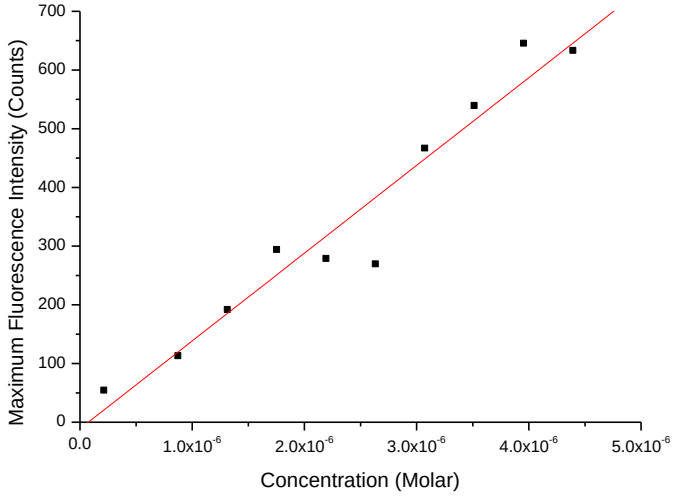
2.1 Absorption spectrum of mTHPC at different concentration

Absorption spectrum of foscan has been recorded at different concentration using either a spectrophotometer or fluorimeter, respectively. On the basis of this absorption spectrum the absorption coefficient of mTHPC in solution is calculated using equation 1.2 of Beer-Lambert Law. Moreover, its maximum fluorescence intensity is also measured. Fig 1 (a) shows the plot of foscan at different concentration versus maximum fluorescence intensity while figure 1 (b) represents the absorption coefficient of mTHPC at different concentration against maximum fluorescence intensity.

2.2 Acquisition of fluorescence spectrum for Keratinocytes

A microscope with filtered light was used in order to locate and correctly position the individual cells. Moreover, laser was focused close to the nuclear region. Fluorescence spectra were collected from the area close to the nuclear region of the cells and analysed using Grams spectroscopy software (Grams/AI, Thermo Galactic, Salem, USA). The spectral region of investigation was in the range (635 – 675 nm) including the principal mTHPC emission peak (652 nm). A total of twenty spectrums were collected from each cell.This procedure was repeated for a set of 46 cells. Each spectrum was recorded over 2 seconds; sequential spectra were acquired in order to investigate the dynamics of mTHPC photobleaching.

a)



b)

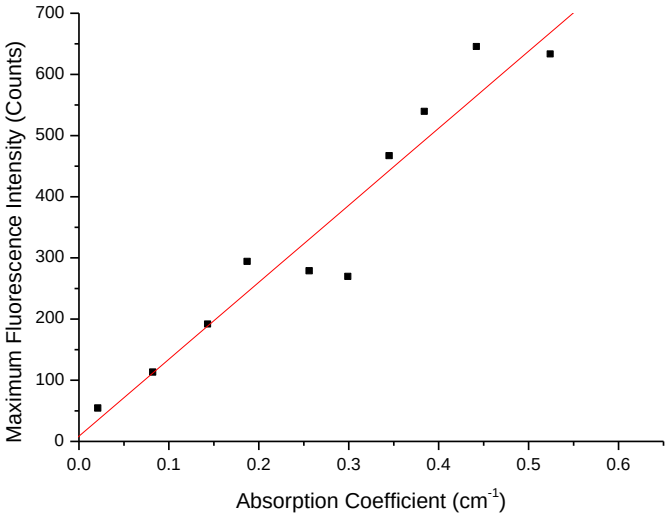


Fig 1 (a) mTHPC at different concentration versus maximum fluorescence intensity (b) represents the absorption coefficient of mTHPC at different concentration against maximum fluorescence intensity.

2.3 Continuous exposure

On irradiation of individual cells with a laser power of 0.0099 mW over a period of 40 seconds, twenty sequential spectra were recorded close to the nucleus of the cell. This procedure was repeated for a set of 46 cells and the mean of the 10 datum points at each exposure time calculated. These 46 cells are divided into six groups e.g., G1, G2, ….G6 etc on the basis of the fluorescence intensity observed. The different combinations of groups are investigated using constant laser power. This allowed comparison of the spectra within six groups of keratinocytes during a 40-second irradiation for photobleaching.

Different individual cells will take up the photosensitizer at different rates and there will be a subsequent distribution of the sensitizer into different parts of the cells and hence the fluorescence photobleaching.

Using the data acquired during the study an analysis was performed upon the shape of the fluorescence emission peak in order to assess possible perturbations due to self-absorption (inner filter effect). The analysis results reveal that the role of self-absorption is significantly small in determining the spectral profile of the fluorescence.

3 Results

3.1 Autofluorescence

Fluorescence spectra were also acquired from keratinocytes in the plateau phase, without mTHPC incubation. A low level (<2% of typical mTHPC signal) of autofluorescence was observed in the spectral regions of interest, and this was detected to bleach rapidly during the initial period of laser irradiation. Hence, autofluorescence has not been subtracted from the mTHPC spectra recorded. No fluorescence was found from regions outside the cells.

3.2 Continuous exposure at constant irradiance

mTHPC fluorescence spectra recorded from fixed keratinocytes show the progressive degradation of the 652 nm peak, following incubation with 0.044 mM mTHPC for 3 hours, during a continuous laser irradiation of 0.0099 mW for 40 seconds. The fluorescence intensity recorded from a set of 20 cells. When it is plotted against time, a significant variation in the magnitude of the fluorescence is found from different

cells (normalized mean = 1 ± SD .23 around the mean for the first spectrum in each sequence).

Fig 2 shows the rate of photobleaching vs dose at constant irradiance for six groups of cells. From these results, we came to the conclusion that cells demonstrating high initial fluorescence (concentration) undergo bleaching at a faster rate then those with lower fluorescence.

A histogram of the fluorescence intensity for six groups of cells from keratinocytes is also shown in figure 3. Each group represents the mean 652nm emission intensity at constant laser power as indicated by the first spectrum of a bleaching sequence. These trends indicate that G1 has lower initial fluorescence implying lower concentration and this initial fluorescence goes on increasing till the last group with the increase in the concentration. The standard deviations in this histogram result from the large variation in fluorescence intensity for all groups and the ability of the cells to take up mTHPC at the incubation time selected.

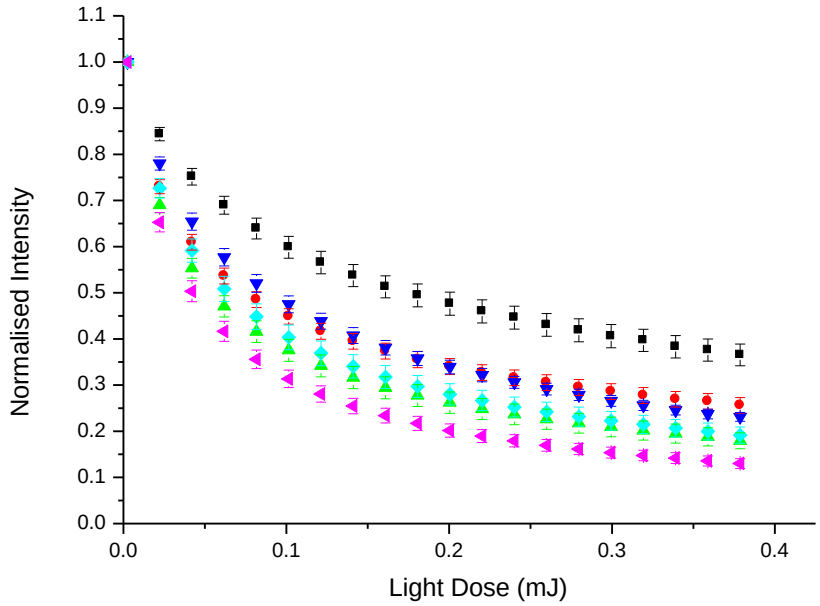


Figure 2: Photobleaching plot of mTHPC showing the normalised averaged data points from six groups (G_1 ■ G_2 ●G_3 ▲ G_4 ▼G_5 ◆ G_6◀) of Keratinocytes (0.044 mM mTHPC for 3 hours) exposed to constant laser power: 0.0099 mW.

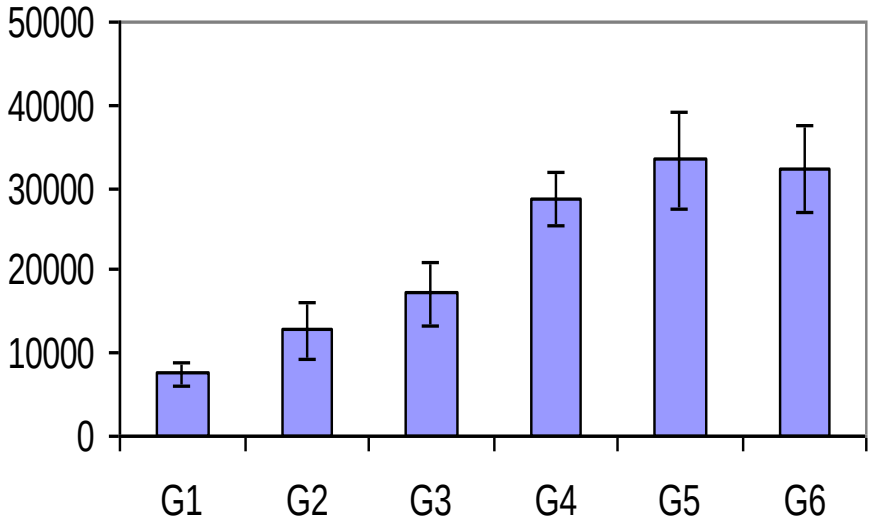


Figure 3: Initial mTHPC (652 nm) fluorescence intensity for keratinocytes among various groups

4. Discussion

The aim of this study is to analyze the effects of photosensitiser concentration in mTHPC induced photobleaching. Fuchs et. al. [6] demonstrated that within single cells there are large oxygen concentration gradients, especially close to the mitochondria. Similarly large variations are also observed at the tissue level in oxygen concentration over small distances particularly in skin, which possesses a complex morphological structure and angioarchitecture. Hence all measurements of oxygen concentration include averaging heterogenous oxygen concentrations.

Due to the observed sensitizer fluorescence changes, it has been classified into six groups according to the fluorescence intensity / counts as explained previously in Table 1. The rate of photobleaching can be explained in term of a bi-exponential decay, as a function of dose delivered. As the values of χ^2 are lower in terms of a bi-exponential decay rate, so the data from keratinocytes (3 hours mTHPC incubation) follow this bleaching function. This is similar with Moan et al. [10] works where a bi-exponential model was used to examine measurements of the loss of fluorescence intensity from PpIX during irradiation of WiDr cells. The main reason for using a bi-exponential decay rate is that first order decay kinetics reveals that photoproducts from one chromophore cannot cause the decay of a neighbouring chromophore, which is not true in the present case, since the decay rates increase with the increasing PpIX

concentration which is in contrast to what one might expect for a pure second order process. An analysis of the described results indicate that in ALA-PCT PpIX is photodegraded mainly according to two or more first order processes i.e. a PpIX molecule is degraded by the photroproducts produced by itself, but that higher order processes definitely play significant roles.

In Fig 2 the bleaching is shown plotted against dose, and is seen to depend on the initial mTHPC fluorescence intensity. Constant laser power is used and the results show that group of cells demonstrating high initial fluorescence (concentration) undergo bleaching at a faster rate then those with lower fluorescence apart from one group of cells (G3). The results observed are in good agreement with the investigations of Bagdonas et al., (2000) [11] where for PpIX a similar dependency upon initial concentration was found and linked to the generation of singlet oxygen, and the subsequent reaction with the PpIX molecule.

In summary, singlet oxygen appears to play a major role in the rapid mTHPC induced fluorescence photobleaching causing the sensitizer degradation rate to vary as a function of the rate of photodynamic $^3O_2$ consumption i.e., photodynamic $^3O_2$ consumption increases with the available drug concentration. Moreover, it results in a fluence rate dependence of photobleaching. Therefore, groups of cells showing high initial fluorescence (concentration) bleach faster than those with lower fluorescence. This is in agreement with a number of in vivo and in vitro studies [12-21].

5. Conclusion:

The results of this study show that sensitivity of mTHPC to photo-oxidation exists alongside the increased PDT efficiency of this compound, compared to porphyrin sensitisers. For example, the localization of mTHPC to cellular lysosomes (rather than mitochondria) has been shown to reduce the light dose necessary for in vitro cell necrosis found by Leung et al, 2002 [22]. Also, inhibiting the intracellular accumulation affected a reduction in PDT efficacy. In our study it was not possible to monitor the oxygen partial pressure, photosensitiser localisation or photodynamic damage. However, it is evident that mTHPC is highly photolabile within a cellular environment, and that oxygen plays a crucial role in the degradation of the fluorescent species. The assumption that the fluorescence bleaching signal can be used as a PDT dosimetry metric relies upon a constant partition between the effect of $^1O_2$ upon sensitiser and cell substrate. New methods of direct detection of the weak near

infrared luminescence may ultimately allow parallel monitoring of f1uorescence bleaching and $^1O_2$ concentration as described by Niedre et al, 2002 [23].

Acknowledgements:

This project was supported by King Saud University, Deanship of Scientific Research, College of Science Research Center.

References

1. H. H. Wasserman, Introductory remarks. In: Wasserman H. H. ed. Singlet Oxygen. Vol. **40** Academic Press, (1979).
2. H. Barry, M. C. John, Free Radical in Biological and Medicine. Second Edition (1982) Clarwndon Press. Oxford.
3. N. I. Krinsky, Biological roles of singlet oxygen. In: Wasserman H. H; ed. Singlet Oxygen. Vol. **40** Academic Press. pp. 597-641(1979).
4. D. R. Kearns, Chem. Rev. **71**, 395-427 (1971).
5. L. Zhao, Singlet Oxygen. Department of Radiology, The University of Iowa, Iowa City, USA (2001).
6. J. Fuchs and J. Thiele, Free Rad. Bio. and Med. **24,** 835-847 (1998).
7. I. Georgakoudi, M. G. Nichols and T. H. Foster, Photochem. and Photobio. **65,** 135-144 (1997).
8. M. Atif, M. R. Stringer, J.E. Cruse-Sawyer, P.E. Dyer, and S.B. Brown, Photodiagn. Photodyn. Ther. **2**, 235–238 (2005).
9. M. Atif, M. R. Stringer, J. E. Cruse-Sawyer, and S. B. Brown, Photodiagn. Photodyn. Ther. **1**, 173–180 (2004).
10. J. Moan, G. Streckte, S. Bagdonas, O. Bech and K. Berg, International J. of Can. **70,** 90-97 (1997).
11. S. Bagdonas, L. W. Ma, A. Iani, R. Rotomskis, P. Juzenas and J. Moan, Photochem. and Photobio. **72,** 186-192 (2000).
12. D. J. Robinson, H. S. deBruijn, W. J. deWolf, H. J. Sterenborg, and W. M. Star, Photochem. and Photobio. **72,** 794-802 (2000).
13. N. de Veen, K. M. Hebeda, H. S. deBruijn and W. M. Star, Photochem. and Photobio. **70,** 921-929 (1999).
14. I. Van Geel, H. Oppelaar, J. P. Marijnissen and F. A. Stewart, Rad. Res. **145,** 602-9 (1996).
15. J. Schlothauer, S. Hackbarth and B. Röder, Laser Phys. Lett. **6**, 216-221 (2009).
16. D. Chorvat Jr. and A. Chorvatova, Laser Phys. Lett. **6**, 175-193 (2009).

17. R. S. Cavalcante, H. Imasato, V. S. Bagnato and J. R. Perussi, Laser Phys. Lett. **6**, 64-70 (2009).
18. L. G. Sabino, L.M.V. de Negreiros, J.D. Vollet-Filho, J. Ferreira, D.P.C. Tirapelli, P.C. Novais, L.F. Tirapelli, C. Kurachi and V.S. Bagnato, Laser Phys. Lett. **8**, 239-246 (2011).
19. Y. Shen, H. Y. Lin, Z. F. Huang, D. F. Chen, B. H. Li and S. S. Xie, Laser Phy. Lett. **8**, 232-238 (2011).
20. E. S. Tuchina and V. V. Tuchin, Laser Phys. Lett. **7**, 607-612 (2010).
21. L. Buriankova, Z. Nadova, D. Jancura, M. Refregiers, I. Yousef, J. Mikes and P. Miskovsky, Laser Phys. Lett.**7**, 613-620 (2010).
22. W. N. Leung, X. Sun, N. K. Mak, C. M. Yow, Photochem. Photobiol. **75,** 406-11 (2002).
23. M. Niedre, M. S. Patterson, and B. C. Wilson, Photochem. Photobiol. **75,** 382-391 (2002).