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# Active oxygen generation and photo-oxygenation involving temporfin (*m*-THPC)

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**Abstract**

The active oxygen generating ability of *m*-THPC, including the formation of singlet oxygen and superoxide anion radical species, was studied via spin trapping ESR spectroscopy. The mechanisms and the products associated with the self-sensitized photo-oxygenation of *m*-THPC in different solvents were also characterised with the aid of quenching experiments in tandem with UV–visible and HPLC analyses. © 2001 Elsevier Science Ltd. All rights reserved.

*Keywords:* m-THPC; Active oxygen; Self-sensitized photo-oxygenation

## 1. Introduction

*meta*-Tetrahydroxyphenylchlorin (*m*-THPC; **1**) [1] is a second-generation sensitiser used in pre-clinical trials involving photodynamic therapy (PDT). It has several advantages over hemato-porphyrin derivatives (HpD), including high purity, strong absorption in the NIR region, good selectivity for cancerous tumors [2], and low skin sensitizing activity. Concerning the lattermost advantage, *m*-THPC exhibits short-lived skin sensitivity (17 days), while photofrin causes photosensitivity for 3–6 months [3].

The photodegradation of *m*-THPC has been attributed to the formation of singlet oxygen via the triplet-state of the sensitiser, in the so-called type II mechanism [4]. In this regard, the sensitiser is degraded by the attack of self-generated singlet oxygen ($^1O_2$), leading to photo-oxidation. Jones [5] and Kasselouri [6] have reported the products from self-sensitized photo-oxygenation of *m*-THPC; however, they did not investigate the mechanism of the reaction. In this paper, we report the active oxygen generating ability of *m*-THPC and provide an explanation for its photodegradation when used in PDT.

## 2. Material and method

### 2.1. Chemicals

*m*-THPC was synthesized by a published method [1] and its purity was determined to >95% by HPLC. 5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO), 2,2,6,6,-tetramethyl-4-piperidone (TEMP), and 1,4-diazabicyclo[2.2.2]octane (DABCO) were obtained

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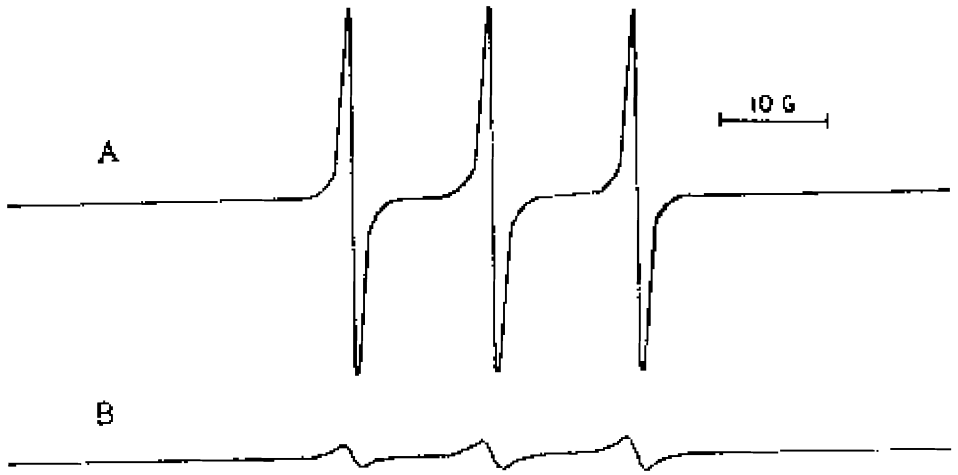
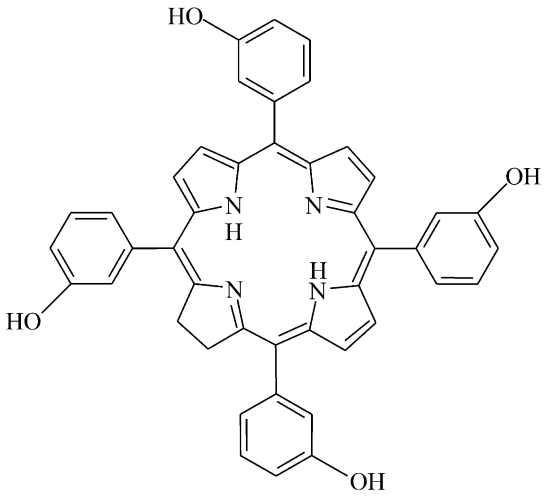



Fig. 1. ESR spectra produced by irradiating an oxygen saturated DMSO solution containing *m*-THPC (0.1 mM) and TEMP (25 mM) in the absence (A) and presence (B) of DABCO (5 mM).

from Aldrich Chemical Company. All solvents were purchased from Beijing Chemical Plant, Beijing, China and distilled before use.




## 2.2. Methods

ESR spectra were recorded at room temperature, using a Bruker ESP-300E spectrometer operating at 9.80 GHz and X-band with 100 KHz field modulation. Samples (30 μl) were injected into quartz capillaries that were specially made for ESR analyses and irradiated directly in the cavity of the ESR spectrometer with a Q-switched Nd:YAG nanosecond laser apparatus (full width at half-maximum, 35 mJ pulse$^{-1}$). The excitation wavelength was 532 nm and the sample absorbances at 532 nm were

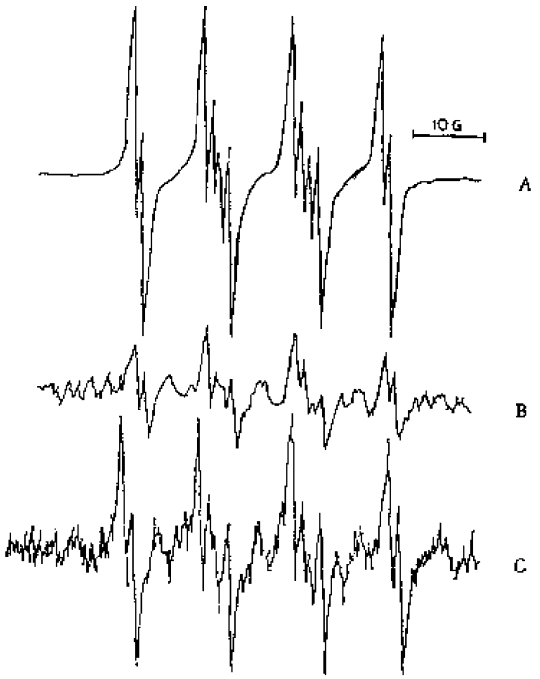



Fig. 2. ESR spectra of the DMPO–$O_2^{\cdot-}$ radical adduct produced from the excitation (532 nm) of an oxygen-saturated DMSO solution of *m*-THPC (0.1 mM) with DMPO (20 mM) (A), *p*-benzoquinone (5 mM) (B), and HA (0.1 mM) (C).

adjusted to be the same. ESR spectra were recorded, stored and manipulated by using an IBM personal computer. Spectrometer settings were microwave power, 5.05 mW; modulation amplitude, 1.05 G; time constant, 20.48 ms; scan range, 200 G; receiver gain, A = $3.5\times10^{4}$; B, C = $2\times10^{-4}$.

UV–visible spectra were recorded on a Shimadzu UV-160A spectrophotometer and HPLC analyses were carried out on a Shandon spherisorb ODS-2 column (25 cm×4.6 mm, 5 μm particle size). The mobile phase was 0.1% trifluoroacetic acid×water in methanol (23:77, v/v), pumped at a flow-rate of 0.5 ml/min using a Shimadzu LC-10AS pump. An SPD-10V UV detector set at 416 nm was used.

### 2.3. Irradiation studies

Solutions of *m*-THPC (10 μmol/l) were prepared in DMSO, acetone and acetonitrile and the absorbances of these solutions at 514.5 nm were adjusted to be the same. Air-saturated *m*-THPC solutions (4 ml) were irradiated in 1-cm quartz cuvettes using 514.5 nm light from a medium-pressure sodium lamp equipped with a narrow-band interference filter.

## 3. Results and discussions

### 3.1. Singlet oxygen quantum yield

ESR spin trapping involving TEMP was used to study the generation of $^1O_2$ from *m*-THPC. In this regard, the irradiation of oxygen-saturated solutions of *m*-THPC and TEMP led to a typical three-line

Table 1
The relative rates of *m*-THPC self-sensitized oxygenation in different solvents

| Solvents | Relative rate | Singlet oxygen lifetimes (μs) [16][a] |
|---|---|---|
| DMSO | 1 | 19 |
| Acetone | 0.7 | 26, 51, 50, 43, 65, 46, 39 |
| Acetonitrile | 0.3 | 30,77, 65, 54, 58, 65 |

[a] Multiple $^1O_2$ lifetimes in acetone and acetonitrile reflect results from different studies.

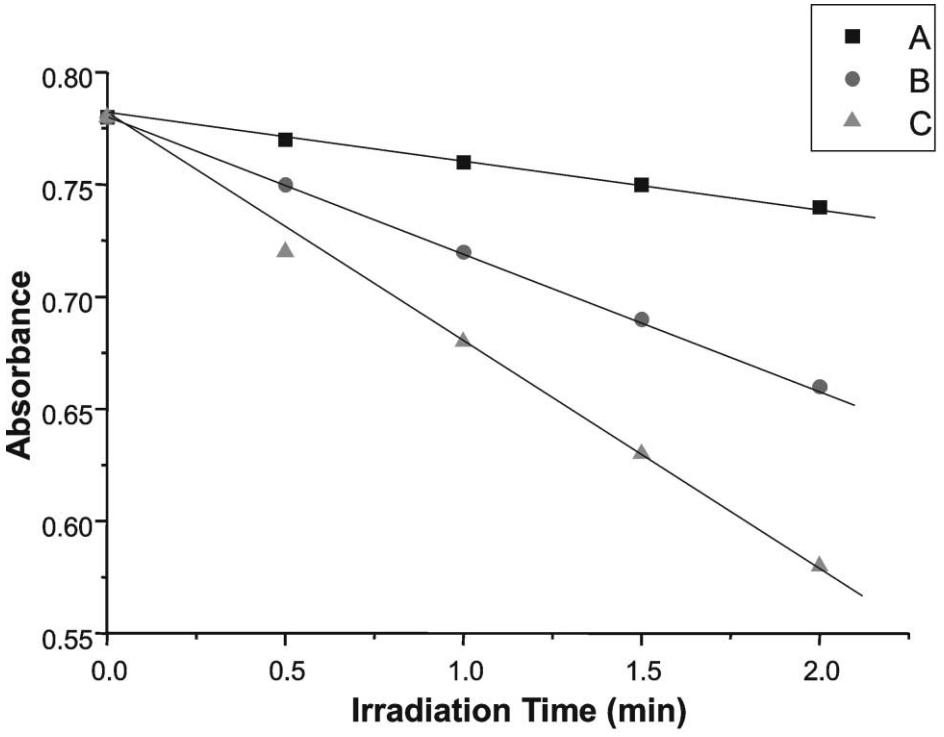



Fig. 3. Changes in absorbance at 650 nm versus irradiation time for a solution of *m*-THPC ($10^{-5}$ mol/l) in DMSO (A), acetone (B), and acetonitrile (C).

ESR spectrum with $a^N = 13.6$ G and $g = 2.0056$ in DMSO (Fig. 1), which was in agreement with the results of prior work [7]. The addition of DABCO ($^1O_2$ inhibitor) effectively inhibited this ESR signal. The quantum yield for m-THPC induced $^1O_2$ generation was 0.40 in oxygen-saturated DMSO solution by using hypocrellin B ($\Phi = 0.76$) as the reference [8].

### 3.2. Superoxide anion radical generation

Irradiation of an oxygen-saturated DMSO solution of *m*-THPC and DMPO at 532 nm afforded a typical 3×2×2 ESR spectrum (Fig. 2), which is characteristic of the DMPO–$O_2$(H) radical adduct [9]. The DMPO–$O_2$(H) signal was not observed in the absence of either oxygen or *m*-THPC. Similarly, when *p*-benzoquinone ($O_2^{-\cdot}$ quencher) [10], was added to the system, the DMPO–$O_2$(H) signal was not observed. These results are consistent with the generation of $O_2^{-\cdot}$ upon the irradiation of *m*-THPC in solution. The results in Fig. 2 also show that *m*-THPC is at least 18 times as effective as hypocrellins in $O_2^{-\cdot}$ generation, though the latter photosensitisers have been the substrates used in PDT clinical trials [11,12].

Generally the main cause of the photodegradation of Photofrin II, a widely used PDT agent, has been attributed to the formation of $^1O_2$ [13]. Other workers reported that Photofrin II lacked the ability to generate $O_2^{-\cdot}$ and concluded that its utility in PDT arises from type II reactions [14]. However, the above results indicate that *m*-THPC has a greater ability to generate $O_2^{-\cdot}$ than Photofrin II and hypocrellins, with the preservation of $^1O_2$ generation. This suggests that a type I mechanism also plays an important role in *m*-THPC therapy and could explain why *m*-THPC is more effective than Photofrin II in PDT.

### 3.3. Self-sensitized photo-oxygenation of m-THPC

The ideal sensitiser for PDT does not stay in the human body for too long [15]. Consequently, in this

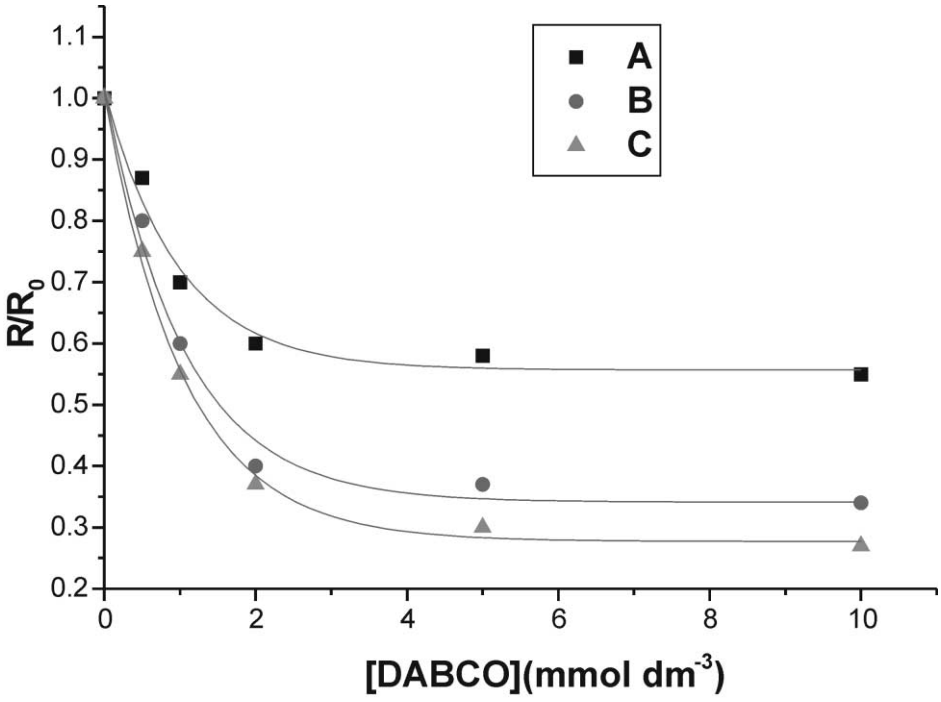



Fig. 4. Results from DABCO quenching experiments involving the photo-oxygenation of *m*-THPC ($10^{-4}$ mol/l) in DMSO (A), acetone (B), and acetonitrile (C). $R_0$ = the relative rate of *m*-THPC self-sensitized photo-oxygenation in the absence of DABCO. $R$ = the relative rate of *m*-THPC self-sensitized photo-oxygenation at different DABCO concentrations.

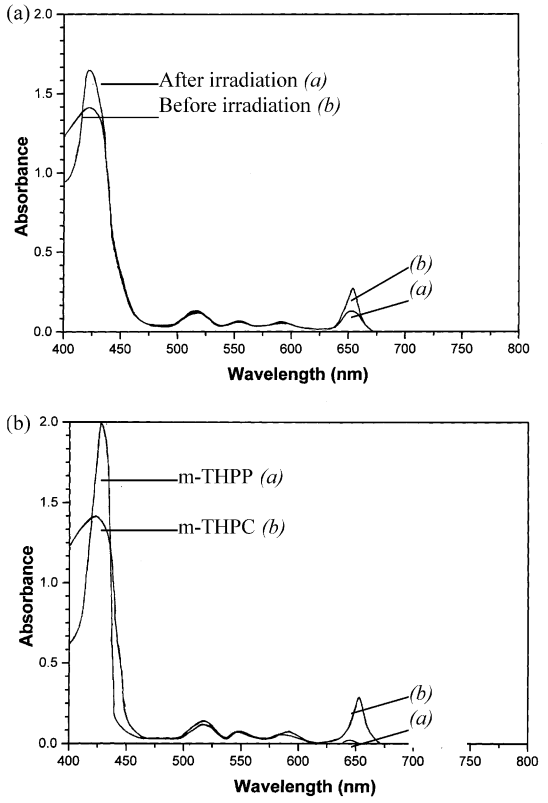



Fig. 5. A: Absorption spectral changes from the photolysis of *m*-THPC ($10^{-4}$ M) at 514 nm. B: Absorption spectra for *m*-THPP and *m*-THPC prior to photolysis.

aspect of our work the mechanism and reaction products from the self-sensitized photo-oxygenation of *m*-THPC were characterised. By observing the decrease in the absorption peak at 650 nm as a function of irradiation time (Fig. 3), the relative rates of *m*-THPC degradation in different solvents were determined (Table 1). Results of control experiments indicate that the sensitiser, oxygen and light are all essential for the self-sensitized photo-oxygenation of *m*-THPC, demonstrating that this is a photodynamic process.

Ordinarily the $^1O_2$ mechanism is regarded as the predominant route to the self-induced photo-oxygenation process. However, the results in Table 1 [16] indicate that the relative rates of *m*-THPC photo-oxygenation in different solvents do not show a direct correlation with $^1O_2$ lifetimes in the solvents employed. This suggests that a free radical ($O_2^{-\cdot}$) mechanism is also involved in the self-sensitized photo-oxygenation of *m*-THPC. To confirm this idea, experiments involving DABCO ($^1O_2$ quencher) and *p*-benzoquinone ($O_2^{-\cdot}$ quencher), respectively were conducted. These results show that the addition of either agent effectively inhibits the rate of *m*-THPC photo-oxygenation, indicating that both reactive species play an important role in the photo-oxygenation process. To determine the relative contribution of the two mechanisms, the rates of *m*-THPC photo-oxygenation at different DABCO concentrations were assessed (Fig. 4). The results show that in DMSO the $^1O_2$ mechanism accounts for about 45% of the photo-oxygenation process while in acetone and acetonitrile the $^1O_2$ contribution rises to 67 and 73%, respectively. The quenching experiments involving *p*-benzoquinone gave essentially the same results. Therefore, it is evident that the $O_2^{-\cdot}$ mechanism predominates during the self-sensitized photo-oxygenation of *m*-THPC in DMSO, while in acetone or acetonitrile the $^1O_2$ mechanism is dominant.

After the irradiation of *m*-THPC in DMSO for $\sim$1 h the intensity of the absorption peak at 420 nm increased and the peak at 650 nm decreased (Fig. 5). As the absorption peak at 420 nm is generally attributed to porphyrin and the peak at 650 nm to chlorin, these results indicate that *m*-THPC is converted in part to porphyrin during its self-sensitized photo-oxygenation. The conclusion was further established by HPLC analysis (Fig. 6a), which shows that a peak at retention time of 12 min which is characteristic of porphyrin (Fig. 6b) appears, while the peak for *m*-THPC is less intense after irradiation. When *p*-benzoquinone was added, the peak at 650 nm and retention time of 6 min reduced significantly. These results suggest that porphyrin is obtained during the self-sensitized photo-oxygenation of *m*-THPC in DMSO and that its formation occurs mainly via an $O_2^{-\cdot}$ mechanism.

When the photo-oxygenation process was carried out in acetonitrile or acetone, the absorption peaks at 420 and 650 nm decreased and only a weak signal for porphyrin was evident via HPLC, along with some unidentified components. When DABCO was added, the intensity of these unidentified components reduced sharply, indiating that their formation was quenched by DABCO. It has been reported [17,18] that the irradiation of

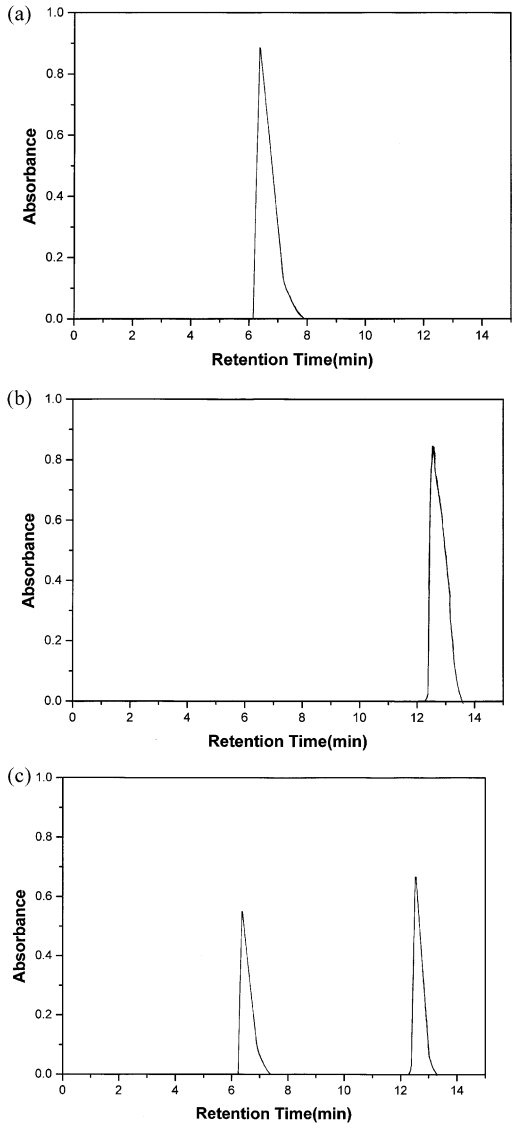



Fig. 6. HPLC chromatograms for *m*-THPC (A) and *meta*-tetrahydroxyphenyl porphyrin (B) prior to irradiation and *m*-THPC after irradiation for 20 min (C).

*m*-THPC leads to a destruction of the molecule, and the present results indicate that some of the degradation products formed in acetonitrile or acetone were produced mainly via a $^1O_2$ mechanism.

## 4. Conclusions

*m*-THPC more efficiently produces superoxide anion radicals than Photofrin II and hypocrellins, which makes it a promising agent for photodynamic therapy. While singlet oxygen and superoxide anion radicals contribute to the self-sensitized photo-oxygenation of *m*-THPC, the main product (porphyrin) obtained arises from superoxide anion radical attack. Only minor products result from singlet oxygen attack.

## Acknowledgements

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