University of Leicester

RAFT: Real-time Air Fingerprinting Technology

SoeMac Project – Summary Report

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Summary Report

This document provides a summary of the work conducted by the University of Leicester and SOE Health Ltd aimed at investigating the SoeMac device: a product developed with the aim of providing 'Activated Oxygen Therapy' based on the generation of excited state $^1O_2$ using a photosensitizer catalyst. This project was split into two main activities; an in-depth literature review followed by an experimental study of the SoeMac system.

Key findings from the literature study and experimental work are outlined below in Sections 1 and 2.

Based on these findings, the following claims can be stated:

- Based on experimental results, with the SoeMac photosensitizer on, oxidation within ambient air is promoted.

- The SoeMac produces an oxidised species (based on experimental results) and based on peer-reviewed literature. It is likely that this is Singlet Oxygen.

- When Singlet Oxygen exits the SoeMac and returns to the stable state (the exact timeframe for this process is yet to be determined - literature gives varying opinions on this, see Section 2), it releases energy which is here referred to as Singlet Oxygen Energy. The nature of this energy is discussed below in Section 3.

1.0 Literature Study and Stage Report Summary - Executive Summary

- The University of Leicester conducted research with the aim of better understanding how devices such as the SoeMac affect ambient air during the use photosensitizers. An in-depth literature study was performed in order to ascertain whether published evidence exists of changes to the composition of normal air resulting from the operation of the SoeMac-like devices.

- A list of 75 documents was obtained covering various aspects of SoeMac devices with much of the work pertaining to recently published literature reflecting the on-going and continued interest in the chemistry and physics of singlet oxygen in the gas phase.

- Much of the literature reviews the successes of photodynamic therapy and other biological effects of singlet oxygen, however the fundamental therapeutic mechanism of devices such as the SoeMac are not well documented.

- Singlet oxygen, along with several other reactive oxygen species, form a group of molecules which have been used in medical applications such as photodynamic therapy, however little has been reported on the toxicity of singlet state oxygen as used in gas phase applications. Furthermore, no peer-reviewed literature was identified that describes the deployment of these devices in clinical applications.

- Whilst much work has been conducted on the investigation of singlet state oxygen, none or very little has investigated 'Activated Air', leading to the possibility that both terms refer to the same product. If this were established experimentally, it would provide the technique with scientific basis for further development.

- Widely disparate results were obtained for reported lifetimes of singlet oxygen due to a strong dependence on the surrounding environment where it had been created.

- **T**he SoeMac device is claimed to generate singlet oxygen (or another form of reactive oxygen species) in an unknown quantity and duration, resulting in an unknown change in the composition of the surrounding air. The University of Leicester performed preliminary experiments to investigate changes in gaseous composition by measuring a group of compounds known to interact with singlet oxygen: volatile organic compounds.

- During preliminary tests under controlled conditions, a measurable change in the composition of air was detected, however further tests are needed to define the exact nature of this change and the impact of the SoeMac device on ambient air.

2.0 Laboratory Investigation

The following points are a management Summary of the findings of the tests performed on the SoeMac device.

2.1 Scope and Experimental Set-up

An important goal of the experimental stage of this research was to establish whether an oxidative process was involved in the operation of the SoeMac device. The results obtained would point to further stages of investigative work.

The SoeMac device was modified so that the illuminating element could be switched on or off while leaving the circulating fan running and was mounted into a sealed container. A dual-oven diluter (Model 491 KinTek, UK) provided a constant flow of nitrogen (400 sccm, N6.0 Grade, BOC gases, UK) into the SoeMac enclosure with a T-junction to vent ensuring excess flow did not lead a rise in pressure above ambient levels. 150 ml per min was drawn off for measurement by PTR-ToF-MS (See summary stage report for more details).

Successive runs took measurements using firstly air only and then air spiked with limonene at a predetermined concentration (initially 35 ppb and later 70 ppb). For comparison, the same procedure was repeated with the device turned off, leaving only the fan running.

Preliminary tests showed an increase in most mass channels once the photosensitizer was switched on relative to the analysis before the photosensitizer was used. The mass spectrum taken whilst the photosensitizer was off shows significant elevations above baseline levels for many mass channels indicting that VOCs were present in the chamber before the photosensitizer was switched on (these were presumably present as a result of compounds desorbing from the SoeMac device and chamber walls or from ambient air leaking into the chamber).

Initially, these emissions from the SoeMac device convoluted observation of oxidation. Over several weeks of continuous running and through temperature control, these emissions had decreased and stabilized sufficiently to enable experimental work to proceed.

2.2 Results

Positive results supportive of oxidation processes taking place were found in the observed yield increases of Limonene Oxide and Carvone, both oxidative products of Limonene. The yield of a third product, Carveol, could not be confirmed as its molecular mass is the same as that of Limonene Oxide, and fragments characteristic of Carveol were not observed in the measured spectra.

While consistent results were obtained with the device switched off, the results obtained when switched on varied widely, with yield increases of up to 5 ppbV of the targeted compounds. The results obtained are summarised in Tables 1 and 2.

If the goal for future work is to identify the specific oxidative agent at work in the SoeMac device, then techniques such as detecting the characteristic 1275 nm light emission of Singlet Oxygen should be considered. Consideration should also be given to establishing the presence of other longer lived Reactive Oxygen Species generated by the reaction of Singlet Oxygen. An example of this is the work of Buettner and Kramarenko [1, 2] who showed that Hydrogen Peroxide was generated by the decay of Singlet Oxygen in the presence of Ascorbic acid (Vitamin C).

Table 1 Results for SOEMAC experiments.

Ratio ofMasses (153:137) -Limonene Oxide & Carveol

Expt	Light on	Light OFF	Change	ppb *
SOEMAC 4	0.18±0.02	0.17±0.02	11%	< 1
SOEMAC 5	0.30±0.02	0.24±0.02	25%	4
SOEMAC 6	0.24±0.02	0.35±0.02	-30%	-

Table 2 Ratio ofMasses (151:137) Carvone

Expt	Light on	Light OFF	Change	ppb *
SOEMAC 4	0.23±0.02	0.14±0.02	67.7%	3
SOEMAC 5	0.22±0.02	0.15±0.02	50.1%	5
SOEMAC 6	0.17±0.02	0.16±0.02	3.3%	< 1

Note
Statistical errors only.
* Concentration calculation assumes 30 ncps per ppb

1. Buettner, G. and R. Hall, *Superoxide, hydrogen peroxide and singlet oxygen in hematoporphyrin derivative-cysteine, -NADH and -light systems.* Biochimica et Biophysica Acta 1987. **923** p. 501 - 507.
2. Kramarenko, G., et al., *Ascorbate Reacts with Singlet Oxygen to Produce Hydrogen Peroxide.* Photochem Photobiol., 2006. **82**(6)): p. 1634 - 1637.

3.0 Formation and Decay of Singlet State Oxygen (SSO).

Oxygen in a singlet excited state is usually produced by firstly exciting a photosensitizing agent with light in the 580 nm region. As shown in the typical case in the accompanying illustration, the excited photosensitiser decays to its ground state by a variety of stages, and where oxygen molecules are present, oxygen molecules are excited to a singlet state via an intramolecular transfer. Once in the singlet state, a subsequent direct decay to its triplet ground state is hindered by quantum mechanical selection rules. Where such transitions occur they are usually mediated by reactions with other molecules. In a vacuum the lifetime of an SSO molecule can reach 45 hours, but this is reduces rapidly to nanoseconds when the SSO is in solution.

Photosensitive materials include Rose Bengal and the family of Porphyrins. In Photodynamic Therapy these are applied to the area of treatment, and irradiated with light of the requisite wavelength to produce the SSO in situ. Certain substances in the makeup of live cells exhibit some photosensitive behaviour as well and in this case it is sufficient to apply the activating light directly to the point of treatment without the need of an external photosensitizer. Figure 1 shows SSO decaying to the triplet ground state, but that is only one of several decay modes available.

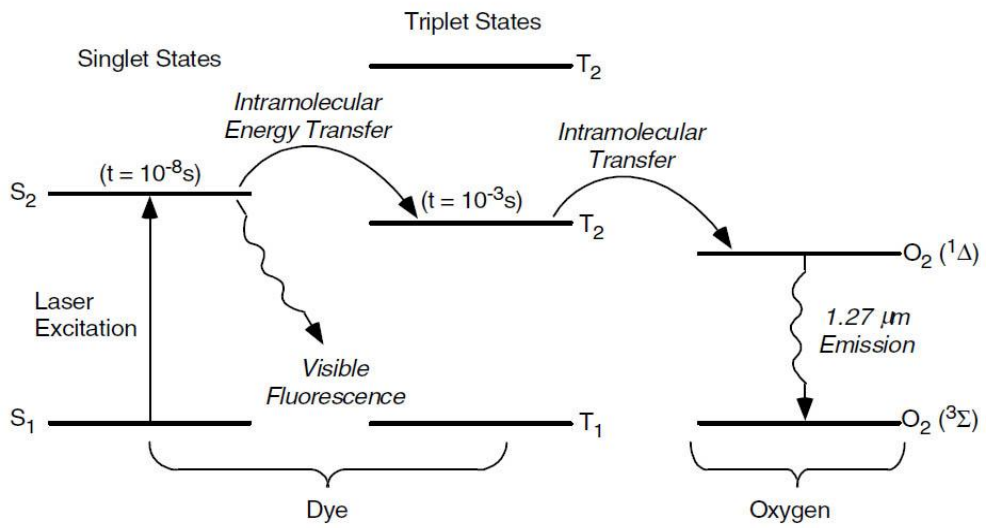

Figure 1 Formation and decay of SSO

The most prevalent modes of decay of SSO are summarized in reactions (1) to (4) below. In the first example, the decay to the ground state involves the emission of a light quantum (*hv*), which although 'forbidden' does have a finite probability of occurring. In the second example, the molecule is de-excited by inelastic collisions with neighbouring oxygen molecules, so for example the characteristic orange glow at 635 nm is due to collision of two singlet state oxygen molecules.

$$^1O_2^* \rightarrow {}^3O_2 + h\nu_P \qquad (1)$$

$$^1O_2^* \rightarrow {}^3O_2 \qquad (2)$$

$$^1O_2^* + M \rightarrow {}^1M + {}^3O_2 \qquad (3)$$

$$^1O_2^* + M \rightarrow \text{products} \qquad (4)$$

In the third example, the singlet oxygen molecule transfers its excitation energy to another species which is left in a singlet state and which can follow its own decay paths. In the final case the SSO molecule reacts with another species and alters its composition. This last route is the one which is of interest in medical applications. It is remains to be proven that radiation emitted by SSO at 1275 nm or even the dual molecule radiation at 635 nm can have any medicinal value.

In simple terms, the energy associated with singlet oxygen follows the first law of thermodynamics: *"energy cannot be created or destroyed; it can only be converted from one form to another".* This energy may well be released as light energy as outlined in example reaction (1) or the aforementioned emissions at 635 and 1275 nm. The examples given above may also give rise to molecules in exited rotational, vibrational or translational states (put colloquially, the molecules 'move' faster). In these cases perhaps it is best to describe this energy as 'heat'. Clearly there are many different routes by which the exited singlet oxygen molecules may decay back down to the stable ground state and therefore there are many different forms that this 'singlet oxygen energy' can take (and no single example alone would best describe this energy).

Example reaction (4) may give rise to a wide range of products in ambient air. These products would best be described as oxygenated VOCs following the oxidation of hydrocarbons. The results from Section 2 indicate that oxidation is indeed occurring and that the SoeMac device is altering the composition of the surrounding air. Reaction (4) also represents the largest toxicological uncertainty surrounding the use of this device, as it is this reaction that is utilised in PDT to kill cells.

In summary:

- The SoeMac generates reactive oxygen species which result in heat and light energy transfer and the creation of oxygenated VOCs into the surrounding air.

- Toxicological studies are needed to quantify the toxic nature of reactive oxygen species generated by the SoeMac device and changes to the composition of the air.