



## REVIEW PAPER

Anna Ciesielka 

# Singlet oxygen discovery

Student's Scientific Club "English Division" at the Medical College of Rzeszów University, Rzeszów, Poland  
supervisor: David Aebisher

### ABSTRACT

**Introduction.** Singlet oxygen is perfectly suited to interact with biological macromolecules and cellular composition.

**Aim.** The goal was to present an information about singlet oxygen discovery.

**Material and methods.** In this article a narrative review regarding singlet oxygen discovery.

**Analysis of the literature.** The desire to summarize information about generation and basic application of singlet oxygen is presented.

**Conclusion.** The history of singlet oxygen is well documented in literature.

**Keywords.** discovery, history, singlet oxygen

### Introduction

Oxygen was independently discovered by Scheele in about 1771 and Priestley in 1774. Carl Wilhelm Scheele's first publication, "Chemische Abhandlung von der Luft und dem Feuer" was delivered to the printing house in 1775, but was not published until 1777. During this time, Joseph Priestley and Lavoisier published their experimental data and conclusions regarding oxygen. Thus, Carl, who made the discovery chronologically earliest, was credited with the discoverer of oxygen along with Joseph Priestley, the English exponent, and Antoine Lavoisier, the father of modern chemistry. Undoubtedly, however, each of the three chemists made a contribution to the discovery of this element; Scheele was the first to isolate the gas, Priestley first published "An Account of further Discoveries in Air" in the journal *Philosophical Transactions* and pointing to the connection between air and blood, and the French physicist and chemist Lavoisier, describing it as "purified air itself in without changing", giving it the name oxygenium and explaining, for the first time, its meaning without using the then erroneous theory of phlogiston, and at the same time putting an end to it.

### Aim

The aim of this work is to present the review of oxygen discovery.

### Material and methods

This article is a review done in regards to discuss the role of singlet oxygen.

### Analysis of literature

Amadeo Avogadro in 1811 described oxygen as a diatomic molecule. In 1848 Michael Faraday announced that the oxygen molecule is a paramagnet and is attracted by a magnet, differing from other gases such as helium gases by a specific electronic structure.<sup>1-3</sup> In his 1867 publication, Fritzsche first described a reaction involving singlet oxygen. This was a precipitation reaction from a solution of 2,3-benzanthracene exposed to sunlight and air, but the true nature of the reaction was not yet suspected. The Frenchman Louis Cailletet was one step ahead of the Swiss Raoul Pictet by producing a few drops of liquid oxygen in 1877. The following year, Sir Dewar conducted an oxygen liquefaction demonstra-

**Corresponding author:** Anna Ciesielka, e-mail: [ciesielka.ania@gmail.com](mailto:ciesielka.ania@gmail.com)

Received: 01.08.2021 / Revised: 30.08.2021 / Accepted: 08.09.2021 / Published: 30.12.2021

Ciesielka A. *Singlet oxygen discovery*. *Eur J Clin Exp Med*. 2021;19(4):318–321. doi: 10.15584/ejcem.2021.4.5



tion in front of members of the Royal Institute during the Friday Discussions. Over the next five years, he improved his method of liquefying oxygen and had a large amount of this element in the liquid state, thanks to which its characteristics could be studied comprehensively. He found that liquid oxygen (ozone) is attracted to magnetic poles. In 1891, at one of the "Friday Discussions", he presented his discovery, using a magnet of a large size and a vacuum bottle, known to this day in laboratories as the Dewar vessel. The liquid oxygen flowing from the bottle hangs in the air between the poles of the magnet, hanging like a large drop until it turns into gas and disappears.<sup>4</sup> It was only in 1925 that this phenomenon was explained by Robert Mulliken, thanks to the newly formulated quantum theory.

Robert S. Mulliken, Nobel laureate in chemistry, in 1928 showed that the paramagnetic property of oxygen is due to the parallel spins of two outer electrons in an oxygen molecule. This paramagnetic, unpaired pair of electrons is triplet oxygen. They determine its chemical properties, making it difficult to select electrons, which is related to the low capacity of oxygen to bind with other compounds. The electron spin reversal produces one pair of electrons and one free electron in the orbit, allowing oxygen to react by removing the constraining spin function. The first evidence of the existence of a metastable and highly reactive oxygen species was given in 1931 by Kautsky. Gerhard Herzberg, also a Nobel laureate in chemistry, thanks to infrared spectroscopy in 1934 confirmed the existence and accurately described the possible states of molecular oxygen, including singlet oxygen as a higher energy state of oxygen. In 1943, Schenck discovered the one reaction with singlet oxygen. This reaction is one of the most studied processes in organic chemistry today.<sup>5,6</sup> The first purposeful use of singlet oxygen took place in 1954. Schenck and Ziegler then carried out the oxidation of  $\alpha$ -terpinene in the presence of chlorophyll, as a result of which ascaridol was produced.<sup>7,8</sup>

The next discoveries about singlet oxygen came from photo-oxidation experiments by Christopher Spencer Foote and Wexler and Elias Corey and Taylor in 1964. Foote, a faculty member at UCLA, made a groundbreaking discovery of the role of singlet oxygen as an excited form of oxygen in air in the reactions of organic molecules caused by light and ultraviolet radiation. Foote's discovery of developing an independent chemical pathway for the formation of this form of oxygen was made in 1964, while he was still a lead at the University of California, Los Angeles. This has led to a large amount of research into the interaction of singlet oxygen with a wide variety of chemicals, DNA and nanomaterials.<sup>9</sup> His research has led to important discoveries about why molecular oxygen is both essential to life processes and a major factor in biological damage.

Many aspects of singlet oxygen chemistry are derived from the work of Christopher S. Foote and his colleagues. Singlet oxygen is an interesting molecule with an extraordinary history behind its discovery. Foote and Wexler conducted experiments in the 1960s, where they obtained evidence of singlet oxygen generation through two independent pathways: 1) photochemical reaction (photooxidation with dye) and 2) chemical reaction ( $\text{NaOCl}$  with  $\text{H}_2\text{O}_2$ ). An important factor in the discovery of singlet oxygen as an intermediate in the photooxidation reaction with the dye was Foote's reassessment of the 1930s chemical literature, where it has already been suggested. Experiments using silica gel beads provided evidence for the presence of a volatile diffusive oxidant such as singlet oxygen.<sup>10</sup> Soon after Foote's first research was published in 1964, the idea of singlet oxygen as an intermediate in photooxidation chemistry gained more and more recognition and validation in organic, gaseous and biological processes. Foote's 43-year research career has led him to become a world leader in organic chemistry. His earliest work focused on the effect of bond angle deformation on the property of organic molecules. He established a quantitative correlation between spectroscopic properties and reactivity.<sup>11</sup>

Foote's main interest was the generation and reaction of reactive oxygen species. Foote has released over 250 research papers, many of which focus on singlet oxygen. Scientific advances in the chemistry of singlet oxygen and its reaction with organic compounds have occurred rapidly over the past 25 years. The great importance of the reaction with singlet oxygen has been noticed in medicine, biochemistry, organic chemistry, food chemistry and environmental chemistry.<sup>12</sup>

Molecular oxygen comes in two forms: singlet and triplet. In its ground state, an oxygen molecule has two electrons with opposite spin, occupying the  $\pi$ -bonding orbitals perpendicular to each other. Molecular oxygen exhibits paramagnetic properties due to a spin quantum number of 1. As a result of the supply of a certain amount of energy, a triplet oxygen molecule is excited and two electrons with opposite spin pairing. The spin quantum number is then zero. The oxygen molecule excited in this way is singlet oxygen and has a higher energy. The amount of delivered and absorbed energy determines the form of singlet oxygen, two of which are distinguished: delta and sigma with different distribution of electrons in molecular orbits:  $1\Delta\text{gO}_2$  - in which there are two paired electrons in one orbital  $\pi^* 2p$  and  $1\Sigma\text{g} + \text{O}_2$  - in which it occurs after one electron in each of the  $\pi^* 2p$  orbitals, and the electrons have opposite spins. The difference between the ground state and the excited states is respectively: 22.5 kcal mol<sup>-1</sup> and 31.5 kcal mol<sup>-1</sup>. Singlet oxygen is an excited form of molecular oxygen, but it is not a free radical.<sup>13</sup>

Singlet oxygen is produced by a photosensitization

reaction in which an endogenous photosensitizer, such as a porphyrin, is excited by light. When a quantum of ultraviolet radiation (or higher energy radiation) is absorbed, the excitation energy is transferred to oxygen and transformed into singlet oxygen, and the photosensitizer returns to its ground state. In order to obtain singlet oxygen, therefore, one needs an oxygen source (it may be air), light of the appropriate wavelength (usually sunlight) and photoactive particles. The efficiency of the process is determined by a parameter called quantum efficiency. Singlet oxygen can also be generated in chemical reactions without the use of light, e.g. decomposition of calcium peroxide, molybdenum, tungsten and lanthanum peroxides, thermal decomposition of ozonophosphine adducts (Myrray method), thermal decomposition of aromatic endoperoxides or decomposition of hydrogen peroxide. Singlet oxygen is also produced by an oxygen explosion in phagocytes during an inflammatory reaction, while hypochlorous acid is formed, which reacts with hydrogen peroxide and as a result of lipid peroxidation, i.e. the reaction of two peroxy radicals.<sup>14</sup>

Singlet oxygen interacts with other molecules by transferring excitation energy - this is the so-called quenching singlet oxygen, it then goes into a triplet state or by entering into a chemical reaction. It can easily react with other singlet molecules. Chemical reactions involving singlet oxygen are often accompanied by visible light emission - chemiluminescence (e.g. blue during the oxidation of luminol). The high reactivity of singlet oxygen is used in a number of chemical reactions in organic chemistry, especially with unsaturated compounds. The first step of the reaction with unsaturated compounds is usually the formation of  $R_2C=C(R)-O-O-H$  allyl hydroperoxide or the  $R-O-O-R$  peroxide bridge. These compounds often undergo secondary reactions - simultaneous rupture of the  $O-O$  and  $C-C$  bond with formation of two carbonyl groups. The reaction products of singlet oxygen with cholesterol and tryptophan are characteristic. The possibilities of singlet oxygen are limited by inactivation with water. Heavy water is less likely to quench the excited state of oxygen, therefore it is used in experiments to determine the role of singlet oxygen in the studied reactions. If the reaction presumably caused by singlet oxygen works better in heavy water than in ordinary water, we have a right to consider our suspicions to be valid.<sup>15-17</sup>

Singlet oxygen is more electrophilic and has better oxidizing properties than basic oxygen, therefore it is considered a universal oxidant. It reacts with: lipids leading to peroxidation; proteins leading to oxidation of side chains, inactivation, misfolding, or enhanced degradation in proteasomes; nucleic acids, resulting in base modification and strand breaks. The most susceptible to damage by singlet oxygen are histidine, methionine, tryptophan, tyrosine, cysteine and guanine residues.

The very strong oxidizing properties of singlet oxygen can find application in environmental protection, e.g. treatment of industrial wastewater containing phenols derived from clothing from the paper industry. The singlet oxygen method to remove 2-chlorophenol from industrial wastewater uses oxygen from the air and sunlight to generate singlet oxygen, and the immobilization of photoactive Bengal red particles in silica gel enables their recovery and multiple use. We also find many uses of singlet oxygen in medicine, e.g. for the sterilization of blood donations by scientists working for the Swiss Red Cross. In medical applications, photoactive molecules must meet stringent requirements, such as high antimicrobial activity and non-toxicity to humans, e.g. methylene blue. The mechanism of action is based on the interaction of singlet oxygen with cells of foreign organisms. First, the photoactive molecule is excited, and then, as a result of energy transfer, molecular oxygen is formed, oxidizing bacterial cell walls, nucleic acid fragments and enzymes. Due to the high degree of adsorption of phenothiazines, such as methylene blue, they can be successfully used in local antibacterial therapy in the fight against microorganisms such as, for example, *Helicobacter pylori*, *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus faecium*. Phenothiazine derivatives are called the "antibiotics of the future" because they may soon become a modern alternative to currently used antibacterial agents.<sup>13-14</sup>

Singlet oxygen is believed to be the primary reactive form of oxygen responsible for damage to leaf cell components and the light-induced loss of photosystem II activity.

Singlet oxygen, unlike its spin-limited sister form, quickly reacts with molecules of organic compounds, but if by chance oxygen existed only in singlet form, it would never accumulate in the atmosphere, and as a result, life would never come from the oceans to land. Singlet oxygen is not ROS produced in the cells of our body in physiologically significant amounts. However, there are situations in which the production and reactions of singlet oxygen become important. This is the case with porphyria, a disease caused by a defect in the metabolism of porphyrins, which causes them to accumulate in the skin. Some drugs are photosensitive and singlet oxygen acts as a mediator of their harmful side effects. St. John's Wort *Hypericum* contains hypericin, which produces singlet oxygen when exposed to light, so that large amounts of St. John's wort by cows or sheep in sunny pastures can lead to photosensitivity reactions.

## Conclusion

Oxygen is hailed as the elixir of life, while arousing fear as a fuel to keep smoking and the poison that causes our death. Undoubtedly, however, it is very important in our lives - we cannot live without it for more than a few minutes.

## Declarations

### Funding

This research received no external funding.

### Author contributions

Conceptualization, A.C.; Writing – Original Draft Preparation, A.C.; Writing – Review & Editing, A.C.

### Conflicts of interest

The authors declare no conflict of interest.

## References

1. Stájer A, Kajári S, Gajdács M, Musah-Eroje A, Baráth Z. Utility of Photodynamic Therapy in Dentistry: Current Concepts. *Dent J (Basel)*. 2020;8(2):43.
2. Panzarasa G. Just Add Luminol to Turn the Spotlight on Radziszewski Amidation. *ACS Omega*. 2018;3(10):13179-13182.
3. Okamura MY, Lubitz W, Allen JP. Remembering George Feher (1924-2017). *Photosynth Res*. 2018;137(3):361-375.
4. Bothe H, Happe T, Trebst S, Rögner M. In memory of Achim Trebst (1929-2017): a pioneer of photosynthesis research. *Photosynth Res*. 2018;137(3):341-359.
5. Rochaix JD, Kim C, Apel W. Klaus Apel (1942-2017): a pioneer of photosynthesis research. *Photosynth Res*. 2018;137(2):153-159.
6. Railkar R, Krane LS, Li QQ, et al. Epidermal Growth Factor Receptor (EGFR)-targeted Photoimmunotherapy (PIT) for the Treatment of EGFR-expressing Bladder Cancer. *Mol Cancer Ther*. 2017;16(10):2201-2214.
7. Chilakamarthi U, Giribabu L. Photodynamic Therapy: Past, Present and Future. *Chem Rec*. 2017;17(8):775-802.
8. Vil' VA, Yaremenko IA, Ilovaisky AI, Terent'ev AO. Synthetic Strategies for Peroxide Ring Construction in Artemisinin. *Molecules*. 2017;22(1):117.
9. Cengel KA, Simone CB 2nd, Glatstein E. PDT: What's Past Is Prologue. *Cancer Res*. 2016;76(9):2497-2499.
10. Kładna A, Marchlewicz M, Piechowska T, Kruk I, Aboul-Enein HY. Reactivity of pyruvic acid and its derivatives towards reactive oxygen species. *Luminescence*. 2015;30(7):1153-1158.
11. Demchenko AP, Heldt J, Waluk J, Chou PT, Sengupta PK, Brizhik L, del Valle JC. Michael Kasha: from photochemistry and flowers to spectroscopy and music. *Angew Chem Int Ed Engl*. 2014;53(52):14316-14324.
12. Jones DP, Radi R. Redox pioneer: professor Helmut Sies. *Antioxid Redox Signal*. 2014;21(18):2459-2468.
13. Kato Y. The formation of lipid hydroperoxide-derived amide-type lysine adducts on proteins: a review of current knowledge. *Subcell Biochem*. 2014;77:21-39.
14. Kładna A, Michalska T, Berczyński P, Kruk I, Aboul-Enein HY. Evaluation of the antioxidant activity of tetracycline antibiotics in vitro. *Luminescence*. 2012;27(4):249-255.
15. Kupferschmidt K. Infectious diseases. Can new chemistry make a malaria drug plentiful and cheap? *Science*. 2012;336(6083):798-799.
16. Voukides AC, Konrad KM, Johnson RP. Competing mechanistic channels in the oxidation of aldehydes by ozone. *J Org Chem*. 2009;74(5):2108-2113.
17. Olsson M, Wilson M, Uller T, Mott B, Isaksson C. Variation in levels of reactive oxygen species is explained by maternal identity, sex and body-size- corrected clutch size in a lizard. *Naturwissenschaften*. 2009;96(1):25-29.