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***Abstract*—Carbonaceous aerosol pollutants, particularly black carbon (BC) nanoparticles derived from diesel emissions, can produce reactive oxygen species (ROS), such as singlet oxygen ($^1O_2$), through photodynamic activity. This study mapped $^1O_2$ emission from individual diesel-derived BC nanoparticles using confocal microscopy and time-resolved fluorescence. High-resolution spectral imaging enabled the quantification of localized photochemical reactions, revealing a singlet oxygen quantum yield of approximately 7% for single nanoparticles. The findings indicate that individual BC particles exhibit significant oxidative potential, with localized impacts that may not be detected by conventional steady-state analytical methods.**

***Index Terms*—singlet oxygen, confocal microscopy, two-photon excitation, soot, nanoparticles**

I. Introduction

Carbonaceous aerosol pollutants are primarily generated by the incomplete combustion of fossil fuels, biomass, and uncontrolled fires [1]. These pollutants have been linked to various toxicological effects, including respiratory diseases, infant mortality rate [2], suppression of enzymatic regulators [3], and skin aging, along with related epithelial disorders such as inflammatory processes induced by oxidative stress [4].

Among urban pollutants, stable black carbon (BC) particles stand out; they are mainly composed of an elementary carbon (EC) core, aggregating polycyclic aromatic hydrocarbons (PAHs) and other organic gas pollutants [5], [6]. Its dissolved part can generate reactive oxygen species (ROS), such as singlet oxygen, upon sunlight excitation. [7] The photodynamics action (PDA) involves a triplet-state process in photosensitive chromophore that leads to ROS production [8]. In aggregated forms, PDA occurs similarly, with triplet states playing a crucial role [9]. The mechanisms of ROS generation are classified as Type I and Type II, both requiring a high density of triplet states to facilitate electron or energy transfer to surrounding molecules. [10]

In this study, we map singlet oxygen generation from individual carbonaceous nanoparticles, specifically black carbon derived from diesel engines, using high-resolution spatial and a time-resolved technique. Confocal microscopy, previously utilized to identify BC in biological tissues, [11], [12] is further developed here to investigate photophysical phenomena through spectral imaging and co-localized signals of fluorescent probes and standard carbon nanoparticles.

II. Methodologies

A. Diesel Exhaust Particles (DEP)

Diesel exhaust particles were collected from a diesel engine using a particle retainer, a bimetallic filter designed to capture particulate matter emitted from the exhaust. The sampling was performed on a 2004 Volkswagen model bus equipped with a 6-cylinder MWM x-10 engine. The vehicle, operated by a private company in Campo Belo, São Paulo, Brazil, runs approximately 100 to 150 km daily. The particle retainer was attached to the exhaust pipe for one week during September and October 2015.

The collected DEP sample was received as a powder in an unsealed plastic container, provided by the Experimental Air Pollution Laboratory (LIM-05), Department of Pathology, Faculty of Medicine, University of São Paulo. Samples of DEP were suspended at a concentration of

1 mg/mL in Milli-Q water and subjected to ultrasonic treatment at 30 °C for 30 minutes. [3].

B. Photo-bleaching and Nanoparticle Singlet Oxygen ($^1O_2$) Quantum Yield

Photo-bleaching experiments were conducted using a Zeiss LSM ®780 confocal microscope equipped with a 63x water-immersion objective (NA = 1.4, working distance = 0.2 mm). A droplet containing 20 mg/L of DEP and 200 μM of 9,10-Anthracenediyl-bis(methylene)dimalonic acid (ABDA) was excited with a 405 nm Argon laser at a fluency of 0.4 J/cm^2(93 μW). The pixel size was set to 0.133 µm. 512x512 resolution. The exposure time per pixel was 1.27 μs. ABDA molecules undergo a decrease in fluorescence upon reaction with $^1O_2$, leading to photo-bleaching centered on their aromatic groups.

To identify individual nanoparticles by fluorescence, a two-photon laser at 800 nm was used to selectively excite single DEPs nanoparticles detected via GaAsP detector featuring ($\Delta\lambda = 8nm$) generating spectral images.

Following localized excitation of the NPs, subsequent laser scanning captured the kinetics of the photochemical reaction within the region, recorded via fluorescence detection in the 410–430 nm wavelength channel (ABDA fluorescence). Analysis of the region's fluorescence pixels profile determined the dependency of singlet oxygen generation on laser excitation.

The resolution limit and 405 nm absorption light value by one photon excitation of the BCs nanoparticles were made by transmitted light images (Non-descanned detector).

The singlet oxygen quantum yield $\Phi_{\Delta NP}$ was calculated as the ratio of molecules of $^1O_2$ formed to photons absorbed by the nanoparticle:

$$\Phi_{\Delta NP} = \frac{\text{molecules of } {}^1O_2 \text{ formed}}{\text{photons absorbed by NP}} \tag{1}$$

The formation of singlet oxygen molecules was indirectly estimated based on the concentration of ABDA within the calculated volume of the measured profile. [13]

The high spatial resolution of confocal microscopy enabled the quantification of photochemical kinetics around single nanoparticles, excluding contributions from dissolved molecules presented in the middle.

Details on laser power calibration and absorption corrections followed the procedures described by Vicente et al. [14]

III. Results and Discussion

A. Singlet Oxygen Quantum Yield from One Single Nanoparticle

A single carbonaceous particle may be responsible for a high generation of singlet oxygen due to its molecular aggregation structure. Confocal microscopy, a high-resolution spatial technique, is capable of quantifying the kinetic reaction around a single nanoparticle, excluding the contribution of dissolved molecules.

In Figure 1, the intensity curve of the probe reveals an "inverted" Gaussian profile (purple curve). The fluorescence decay around the nanoparticle indicates the presence of ABDA molecules photodegraded by the reaction with $^1O_2$. The orange curve shows the lateral size of the nanoparticle (also shown in image B of Figure 1), limited by the lateral resolution of two-photon excitation microscopy (approximately 200 nm). The emission curve in the 410–430 nm channel reveals a nanoparticle diameter of about 270 nm. Due to the high absorption properties of black carbon nanoparticles, the transmission image (Figure 1C) and its transmission profile in the 410–430 nm range determined the one-photon resolution to be approximately 600 nm. The halo between 600 nm and 2.1 μm contains ABDA molecules outside the nanoparticle, and this volume was considered in the estimation of the number of singlet oxygen molecules generated by the excitation of a single nanoparticle.

Based on the average lifetime of the singlet oxygen of 4 μs [15] in aqueous solution, the reactive species generated by the excitation of a BC particle would not be expected to diffuse over a distance comparable to the Gaussian beam radius (1μm) in the interval of excitations, 640 μs. Therefore, the diffusion time of ABDA molecules following the first laser excitation was estimated in the order of 10^{-5} cm^2/s. [16] Although the diffusion coefficient of ABDA in water is not explicitly reported in the literature, typical values for similar organic molecules range between 10^{-5} and 10^{-6} cm^2/s; the calculated value is thus consistent with expectations for organic probes like ABDA. This supports that our measures represent the kinetics occurring after the BC nanoparticles' excitation.

To determine the singlet oxygen quantum yield, the number of photons absorbed by a single nanoparticle depends directly on the laser illumination time and the absorption power of the particle. The laser power at 405 nm was previously measured at 93 μW. The light absorption by a single nanoparticle was measured by the transmission profile images, resulting in 20% of optical depth at 405 nm and 2% of total absorption [14].

The calculated singlet oxygen quantum yield for a single black carbon nanoparticle derived from a diesel engine was approximately 7%. This value is higher than those typically reported in the literature [17], [18]. One possible explanation is that, in the absence of interfering organic molecules from the surrounding medium, both the absorption efficiency of the nanoparticle and the survival of singlet oxygen are enhanced. In contrast, laboratory studies under controlled ambient conditions often report values around 6.5%, which likely reflect ensemble-averaged measurements, incorporating contributions from all molecules in the system. [19]

The relatively high quantum yield observed here suggests that a single nanoparticle can produce localized photochemical effects that are not captured by steady-state, bulk analytical methods. Understanding such localized effects is particularly important given the ongoing investigations into the toxicity and biological impact of nanoparticulate carbonaceous materials.

This study successfully determined the singlet oxygen quantum yield of individual carbonaceous nanoparticles

using a time-resolved, spatially localized, and novel optical methodology. To fully understand the photophysical processes and dynamics, techniques capable of selectively probing specific sites are essential. Our methodology was able to quantify these point-specific impacts, demonstrating that a black carbon nanoparticle can generate singlet oxygen under visible light illumination, thereby ruling out the hypothesis that only isolated molecules produce reactive oxygen species.

Further studies should be done to focus on optimizing the parameters of this technique for environmental applications, particularly for mapping the photophysical effects of nanoparticles in complex, realistic systems.

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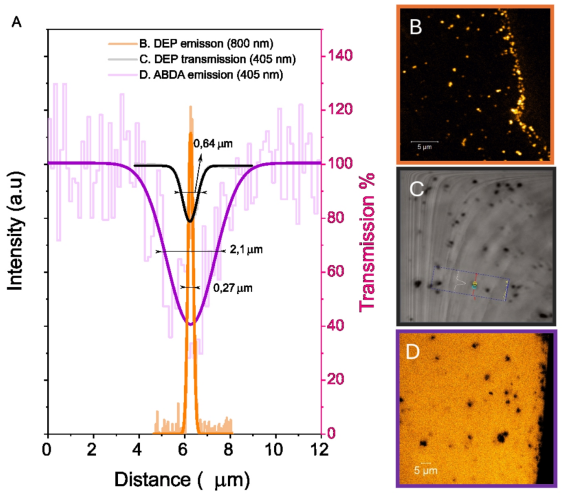

Fig. 1. A. Singlet Oxygen generation by DEP in aqueous solution, intensity profile curves of the corresponding channel images around the circles; B. Channel image from 800 nm two-photon excitation without ABDA; C. Transmission image of DEP with ABDA at 405 nm of excitation; D. Channel image by 405 nm excitation DEP + ABDA solution.

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