Environmental Pollution 238 (2018) 638–646

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Generation of hydroxyl radicals and singlet oxygen by particulate matter and its inorganic components☆

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ARTICLE INFO

Article history:
Received 17 November 2017
Received in revised form
12 February 2018
Accepted 20 March 2018

ABSTRACT

Particulate matter (PM) can strongly affect redox biochemistry and therefore induce the response of the immune system and aggravate the course of autoimmune diseases. Nanoparticles containing transition metal compounds possessing semiconductor properties (TiO_2, ZnO) may act as photocatalysts and accelerate the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS). In this study, the NIST standard reference material, SRM 1648a, has been analyzed in terms of this consideration. Organic compounds present in SRM 1648a were removed by cold oxygen plasma treatment. Samples of SRM 1648a with removed organic content (<2% of organic carbon, <1% of nitrogen) were obtained within 2 h of this treatment. The treatment did not affect the morphology of the powder. The reference material and PM2.5 collected in Kraków are composed of smaller particles and nanoparticles forming aggregates. The efficiency of (photo)generation of hydroxyl radicals and singlet oxygen was compared for original and organics-free samples. The analyzed samples showed the highest activity towards ROS generation when exposed to UV-vis-NIR light, moderate under UV irradiation, and the lowest in dark. Data collected in the present study suggest that the organic fraction is mostly responsible for singlet oxygen generation, as almost twice higher efficiency of $^1O_2$ generation was observed for the original NIST sample compared to the material without the organic fraction. However, particulate matter collected in Kraków was found to have a five times higher activity in singlet oxygen generation (compared for original NIST and Kraków dust samples).

1. Introduction

It is widely recognized that inflammation and dysregulations of immune system play pivotal roles in the pathogenesis of the majority of civilization diseases. Environmental agents, such as volatile organic compounds (VOCs), gases, and particulate matter (PM), have a significant impact on the breakdown of tolerance leading, among other, to autoimmunity *via* numerous mechanisms. Particulate matter is a heterogeneous mixture of solid nanoparticles and liquid droplets suspended in the air (World Health Organization, 2013). The composition of PM varies in space and time, but it mainly consists of transition metal compounds (of copper, iron, manganese, nickel), inorganic ions (sulfate, nitrate, ammonium), adsorbed reactive small molecules (NO_x, ozone, sulfur dioxide, hydrogen peroxide), organic compounds (polycyclic aromatic hydrocarbons (PAHs), nitro-substituted PAHs, chlorinated pesticides, *etc.*), biological materials (endotoxins, viruses, cell fragments), minerals (quartz, soil dust) and carbonaceous species (soot) (Aust et al., 2002). In general, particulate matter is divided into three main categories: PM1, PM2.5 and PM10, involving particles of aerodynamic diameters smaller than 1, 2.5 and 10 μm, respectively. In air, PM10 constitutes 44% of the total PM mass, while 36–65% of PM10 exists as PM2.5 (Brook et al., 1997), whereas the PM1 fraction represents around 60–70% of PM2.5 (Samek et al., 2017). Smaller PM can penetrate the lower respiratory tract more efficiently and therefore more harmful to human health. The specific surface area of particles and their reactivity are directly associated with the

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☆ This paper has been recommended for acceptance by B. Nowack.
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https://doi.org/10.1016/j.envpol.2018.03.068
0269-7491/

diameter of PM (Valavanidis et al., 2008). When the particle size decreases, the surface area increases. Consequently, a larger number of reactive groups exposed at the particle surface favors the efficiency of generation of ROS. Several physicochemical properties of PM (*e.g.,* particle size, solubility, chemical composition) can strongly affect their redox biochemistry and therefore also their toxicity toward human cells (Nel et al., 2006). On the surface of nanoparticles, numerous chemical processes essential for human health, including catalytic ones and ROS generation, can take place. Reactive oxygen species are involved in the oxidation of various biologically important macromolecules, such as proteins, nucleic acids, lipids, and carbohydrates and thus, induce oxidative stress (Ogilby, 2010).

The solubility of PM components is another important factor that influences the reactivity and toxicity of dust. Soluble species like nitrates or sulfates may easier internalize into cells and cause adverse health effects (Kelly and Fussell, 2012; Reiss et al., 2007; Schlesinger and Cassee, 2003). Insoluble particulate compounds can induce reactive oxygen species production in phagocytic cells (Costa and Mollenhauer, 1980). In addition to the solubility, the composition of PM also plays a crucial role in their toxicity. Costa et al. postulated that the amount of transition metal compounds bioavailable for cells is responsible for inflammatory response, rather than the dosage of particulate matter (Costa and Dreher, 1997). Because of the redox properties of transition metal ions (iron, nickel, chromium, and copper), they are able to generate reactive species like ROS *via* redox cycles operating in biological systems. Iron(II) ions, in the presence of hydrogen peroxide and light, can generate $HO^\bullet$ and Fe^{3+} as the result of photo-Fenton reactions (Szaciłowski et al., 2005). Non-redox metals, like aluminum and lead, can intensify or reduce toxic effects of ROS (Milnerowicz et al., 2015; Valko et al., 2016; Verstraeten et al., 2008). Particles of metal compounds possessing semiconducting properties, often work as photocatalysts and enhance the generation of ROS in the presence of light of suitable photon energy. Photoexcited semiconductors offer strongly reductive electrons in the conduction band (e^-) and strongly oxidative holes in the valence band (h^+). The main redox process taking place at the surface of particles are those well recognized for *e.g.* titanium dioxide and zinc oxide, *i.e.* oxygen reduction to superoxide and water oxidation to hydroxyl radicals (Szaciłowski et al., 2005).

In this regard, our study focused on the redox activity of particulate matter standard SRM 1648a. SRM 1648a from the National Institute of Standards and Technology (NIST) is atmospheric particulate matter collected in St. Louis and used as a reference material. The particle size varies from 0.2 to over 100 μm with a predominance of particles with a size of 10–20 μm. Elemental composition was investigated by many analytical techniques (INAA, WDXRF, PAA, SS-GFAAS, PIXE, PGAA, ICP-MS) and is reported in the certificate provided with the material (Wise, 2008). This material was used as a quality reference dust of air pollution.

The main goal of this work was to assess the ROS generation photoactivity of the SRM 1648a material and its inorganic components. In particular, this study focused on the photocatalytic and thermal generation of reactive oxygen species. The work is a part of the APARIC project (Air Pollution versus Autoimmunity: Role of multiphase aqueous Inorganic Chemistry) aimed to study the influence of transition metal compounds as components of air pollutants on the induction of autoimmune disorders (Samek et al., 2017).

2. Materials and methods

2.1. Materials

SRM 1648a was supplied by the National Institute of Standards and Technology (NIST). Terephthalic acid was purchased from Aldrich (98%), methanol 99,9% and sodium hydroxide from POCh, deuterated methanol from Merck.

2.2. Particulate matter sampling and extraction

Particulate matter was collected in the city center of Kraków, Poland, between March 2 and May 15 of 2015 for 24 h exposure (low volume LVS-3 samplers, flow rate: 2.3 m^3/h) on Teflon PTFE filters (46.2 mm diameter). Filters were extracted with methanol and sonicated for 1 min in a water-bath sonicator (SONIC-5, Polsonic, 620 W). Subsequently, PM2.5 material was dried overnight at 60 °C.

2.3. Removal of organic components

The Plasma Zepto system (Diener Electronic GmbH) was used for the removal of organic compounds present in the reference material. Plasma treatment is a well-known method for the elimination of organic contaminants, which are converted into volatile products and sucked off by the pump. In the case of an oxygen plasma, hydrocarbons are oxidized, mainly to CO_2 and H_2O. The process takes place at low temperatures (cold plasma) and is environmentally safe (Petasch et al., 1997). Samples (17–20 mg) were treated with a low-temperature plasma for 5, 10, 15, 20, 30, 40, 50, 60 and 120 min at the maximum power of the device (100 W). Plasma treated dust will be further referred to as SRM/5′, SRM/10’, *etc*. The contents of carbon, hydrogen, nitrogen, and sulfur were determined by elemental analysis (Elementar, Vario Micro Cube; one measurement for times from 0 to 60 min and 3 independent measurements for samples after 120 min of plasma treatment) and total organic carbon analyzer (Shimadzu, TOC-V series with Total Nitrogen accessory) was used to determine the organic and inorganic carbon content.

2.4. Physicochemical characterization of particulate matter

Particles shape, morphology, and chemical composition of grains were analyzed by scanning electron microscopy (SEM) by Tescan Vega3 LMU microscope equipped with the LaB_6 cathode and EDS detector (Oxford Instruments, X-act, SDD 10 mm^2). EDS spectra were collected in several areas of dust samples in order to assess the distribution of the elements and evaluate its homogeneity in the tested samples.

2.5. Detection of hydroxyl radicals

Terephthalic acid (TA) was used as a fluorescence probe to detect $HO^\bullet$ radicals (Ishibashi et al., 2000). Hydroxylation of TA leads to a highly fluorescent product, hydroxyterephthalic acid (HTA) (Fig. 1). The concentration of the formed product is proportional to the hydroxyl radical concentration produced during irradiation. 11 mg of PM2.5 AGH, SRM 1648a, plasma-treated SRM 1648a (30 and 120 min of treatment) were suspended in 16 mL of terephthalate solution (3 mM TA, dissolved in 10 mM NaOH) and placed in a quartz cylindrical cuvette (5 cm dia., 1 cm optical path, 16 mL volume). The suspensions were irradiated with a 150 W xenon lamp equipped with a water-cooled housing (Instytut Fotonowy), 320 nm cut-off filter and a 10 cm thick copper sulfate solution filter (0.1 M in water) or 10 cm thick water filter for UV-vis or UV-vis-NIR irradiation conditions, respectively. Similar suspensions were magnetically stirred in the dark to evaluate their thermal reactivity. The average irradiance was kept at the level of 0.85 $mW\,cm^{-2}$. Samples of 2 mL were collected every 20, 30 or 60 min during irradiation and filtered through PTFE filters (pore