Oxidative potential of ambient aerosols: an Indian perspective

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Aerosols are tiny particles suspended in ambient air and therefore follow the air transport pathway. Many species present in ambient aerosols such as transition metals or some organics have the potential to generate reactive oxygen species (ROS) in situ. These ROS affect oxidizing capacity of the atmosphere. On inhalation, aerosols can generate ROS in the respiratory system of humans, which may result in a variety of cardiopulmonary diseases. Further, ROS can also affect plant growth and productivity. India has a wide variety of pollutant sources with perennial emissions. Although there are reasonable number of studies on the physical, chemical and optical properties of aerosols, virtually no information is available on the oxidative potential of ambient aerosols and its consequences. Therefore, it is important to assess the oxidative potential of ambient aerosols on a temporal and spatial scale over sites dominated by different emission sources and meteorological conditions in India.

Keywords: Ambient aerosols, oxidative potential, reactive oxygen species, pollutants sources.

AMBIENT aerosols are tiny particles suspended in the air with size predominantly ranging between 10 nm to 10 μm. Aerosols consist of a wide variety of organic and inorganic chemical species such as major cations and anions, transition metals, heavy metals, elemental carbon (EC) and organic carbon species. They are injected into the atmosphere from a variety of natural (e.g. mineral dust, sea-salt, forest fire, volcanic eruption) and anthropogenic (e.g. biomass burning, fossil-fuel burning, vehicular and industrial emissions) sources, either directly (primary particles) or through the chemical processing of their gaseous precursors (secondary particles), or both. Aerosols are known to affect the earth's climate (radiation budget, hydrological cycle), aquatic ecosystem (biogeochemistry of oceans and lakes), and air quality (visibility and human health)[1]. Aerosols react with several atmospheric trace gases that leads to change in their chemical and physical properties, which can affect their optical and hygroscopic properties as well as bioavailability of nutrients. Although all the effects of aerosols are important, the effect on air quality is gaining specific interest and attention, as it directly relates to public health. There are numerous studies in the literature showing a direct relationship between high concentrations of ambient fine particles, and morbidity and mortality[2–6]. One of the widely proposed mechanisms related to the effect of aerosols on human health is that several types of aerosols produce reactive oxygen species (ROS) *in situ* in the human respiratory system, while breathing. Production of ROS causes imbalance between oxidants and antioxidants in the body and leads to several cardiopulmonary diseases like bronchitis, breathing problem, asthma, lung cancer, heart attack and even death[2–4].

ROS are oxygen-containing chemical species with strong oxidizing capability. Although molecular oxygen is not very reactive due to spin restriction in its outermost orbits, when paramagnetic centres (different aerosol species) react with O_2, ROS are generated. They include families of oxygen-centred or related free radicals, ions and molecules. The free-radical family includes, but is not limited to, hydroxyl, hydroperoxyl and organic peroxy radicals, ions consist of superoxide, hypochlorite and peroxynitrite ions, and molecules are represented by hydrogen peroxide, organic and inorganic peroxides (Figure 1). These ROS can be exogenous (ROS are constituents of the particle) and/or endogenous (formed by *in situ* reactions where particles act as catalysts). Exogenous ROS are also called particle-bound ROS, whereas endogenous ROS are called particle-induced ROS. Here, it is important to note that ambient particles act as a catalyst for the generation of endogenous ROS and therefore, they can be active until they are removed from the place of generation (atmosphere, human respiratory system, plants). Reactivity of ROS is much higher than that of molecular oxygen. As a result, residence time of ROS is very short (a few seconds to minutes). ROS contain unpaired electrons which always look towards pairing and can interact with metabolites such as DNA, pigments,

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proteins, lipids and other essential cellular molecules, and eradicate them. ROS not only affect the health of humans and plants, but also affect atmospheric oxidizing capacity and secondary aerosol formation (Figure 2).

In the atmosphere, ROS can be generated through a variety of processes such as photochemical reactions in polluted air containing different precursor pollutants such as volatile organic compounds (VOCs) and NO_x (ref. 7). For example, VOCs react with ozone to form ROS (eq. (1)); alkyl radical reacts with oxygen to form a peroxy radical (eq. (2)). ROS generation also occurs through Fenton chemistry-type reactions (eqs (3) and (4)).

$$R_1CH = CHR_2 + O_3 \rightarrow OH^{\bullet} + \text{products}, \quad (1)$$

(terpenes, unsaturated compounds)

$$R^{\bullet} + O_2 \rightarrow RO_2^{\bullet}, \quad (2)$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + HO^{\bullet} + OH^{-}, \quad (3)$$

$$Fe^{3+} + H_2O_2 \rightarrow Fe^{2+} + HOO^{\bullet} + H^{+}. \quad (4)$$

Production of ROS in the atmosphere not only affects its oxidizing capacity, but also leads to secondary aerosol formation. The formation of secondary particles results in chemically reactive species. Oxidation of SO_2 and NO_2 results in the formation of strong acids. Oxidation of organic compounds may again produce oxidative radicals and peroxide compounds[8].

In the human body, ROS are naturally produced and are essential for several biological activities, e.g. immune system control, defence against pathogens and vascular smooth-muscle function. They also defend the body against environmental challenges such as unknown organisms. However, their overproduction, lower depletion, or inhalation can become damaging for epithelial cells. They could also damage DNA and proteins, cause lipid peroxidation and oxidation of enzyme. Our body produces antioxidants in lung fluids to protect the surface tissue from exposure to ROS. It is important to have a balance between oxidants and antioxidants in such a way that tissues that are damaged as a result of oxidant-based defence are repaired. An increase in imbalance towards oxidants leads to oxidative stress, inflammation and cell death[2–6].

In plants, ROS generation is triggered by different kinds of environmental stresses, such as light intensity, temperature, salinity, drought, nutrient deficiency and pathogen attack[9–11]. Plants also generate a host of antioxidants and antioxidative enzymes (e.g. superoxide dismutase, peroxidase, catalase, ascorbate peroxidase and glutathione reductase), and other small molecules to dissipate ROS. Here also, imbalance between ROS production and their detoxification by enzymatic and non-enzymatic reactions causes oxidative stress. As a result of higher net ROS formation, there could be photo-oxidative damage to DNA, proteins and lipids and ultimately cell death. ROS also act as a signalling device for molecules involved in growth and developmental processes, pathogen defence responses such as hypersensitive reaction and systemic acquired resistance, stress hormone production, acclimation and programmed cell death[9–11].

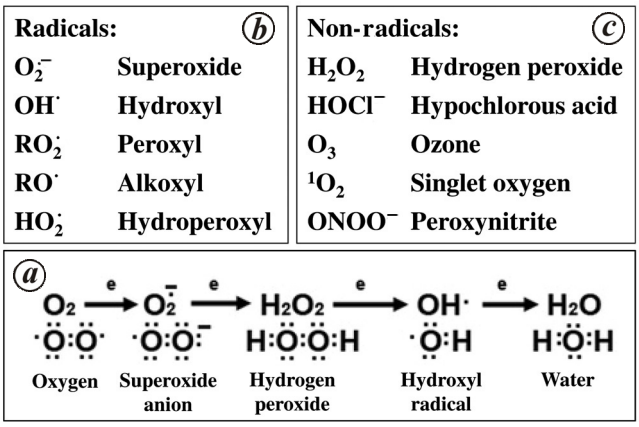

Figure 1. ***a***, In a sequential univalent process by which O_2 undergoes reduction, several reactive intermediates are formed such as superoxide, hydrogen peroxide and the extremely reactive hydroxyl radical, collectively termed as reactive oxygen species (ROS). These ROS can be radicals (***b***) or non-radicals (***c***).

ROS measurement

Among the available methods for the measurement of ROS generated through particles, those widely used are dithithreitol (DTT), *p*-hydroxyphenylacetic acid (POPHAA), and dichlorodihydrofluorescein (DCFH) methods[12–14]. However, none of these methods covers the entire range of ROS which can be produced by particles. DTT assay is documented to be mainly used for ROS produced by quinones and some trace metals, whereas POPHAA and DCFH assays measure H_2O_2 concentration[8,15].

In DTT assay, particulate matter (PM) species such as quinones or transition metals take an electron from DTT and transfer it to molecular oxygen, forming various types of ROS through the pathways described in Figure 1 *a*. Remaining DTT is then allowed to react with 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) that leads to the conversion of DTNB to 2-nitro-5-thiobenzoic acid (TNB) (stable for 2 h at room temperature), which is quantified at 412 nm by UV–Vis spectroscopy. The linear rate of DTT consumption is then calculated to quantify the oxidative capacity of PM[12]. DCFH is also a fluorescence technique based on the oxidation of deacetylated (via NaOH) 2′–7′-dichlorodihydrofluorescein diacetate (DCFH-DA) to its fluorescent product, 2′–7′-dichlorodihydrofluorescein (DCF) in the presence of horse radish peroxidase (HRP)/H_2O_2, with excitation and emission

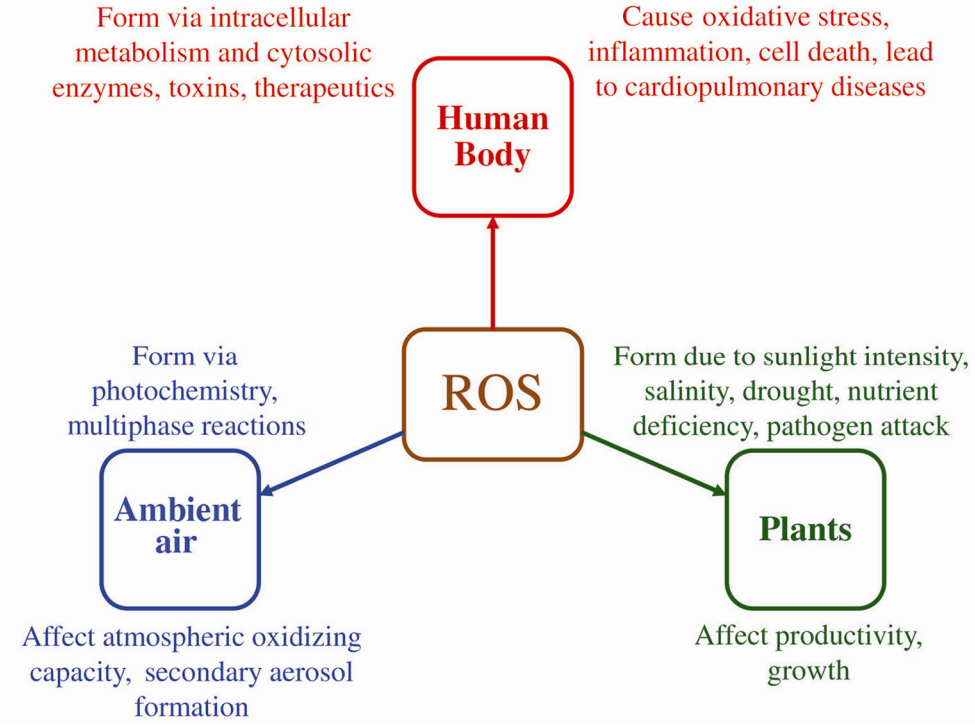

Figure 2. Schematic diagram depicting how ROS affect atmospheric chemistry as well as health of humans and plants.

wavelengths of 485 and 530 nm respectively[13]. In the POHPAA method, a dimer is produced by the peroxidase enzyme-catalysed reaction of hydrogen peroxides with POHPAA that fluoresces strongly under alkaline conditions at excitation and emission wavelengths of 320 and 400 nm respectively[14].

Measured ROS concentrations are usually reported in the form of DTT consumption (in molar concentration) per minute in the presence of PM, which is described as redox-based DTT activity[12]. Cho *et al.*[12] documented the redox-based DTT activity of ultrafine particles at the Los Angeles Basin, USA, which varied from 0.05 to 0.25 activity/μg of PM. ROS concentrations are also reported as equivalent H_2O_2 concentration (in μmol/m^3) in PM[16]. Venkatachari *et al.*[16] reported typical ROS concentration (equivalent H_2O_2 concentration) in the range 0.3–$0.9 \times (10^{-6})$ M/m^3 at Rubidoux. Verma *et al.*[17] showed that DTT was well correlated with water-soluble organic carbon, whereas equivalent H_2O_2 was found to be associated with soluble metals at Los Angeles.

On-line methods for ROS determination have also been developed to understand their characteristics on diurnal basis[18–20]. Sameenoi *et al.*[18] have produced a microfluidic method for DTT analysis, which may be widely utilized in future studies. Venkatachari and Hopke[19] compared the responses of DCFH-DA, POPHAA and DTT to measure ROS compounds in ambient air. King and Weber[20] developed a DCFH-based on-line method for the measurement of particulate, gaseous as well as total ROS. However, none of these on-line instruments is commercially available, which limits their wide use.

ROS in the Indian perspective

Poor air quality is a growing societal concern in India. Mineral dust and emissions from a variety of anthropogenic combustion sources such as crop residue, wood, cow-dung cakes, vehicles, thermal power plants and industries are reported to be major contributors to ambient aerosols over the country[21–23]. As a result, high concentration of fine particles has been documented over different regions of India[21,24]. Inhalation of particles containing transition metals (such as Fe, Cu, Zn, Cr, Co, Ni), and/or organic compounds (such as quinones, PAHs), and/or other unknown species can initiate the formation of ROS *in vivo*, which may cause oxidative stress, inflammation or cell death that leads to various diseases[2–6]. Many researchers have documented optical, physical and chemical properties of aerosols over different regions of India[25–30]. However, studies on oxidative potential of ambient aerosols, i.e. capacity of aerosols to produce ROS, are virtually non-existent in India and limited globally. This is because available methods for ROS measurement are hard to use and yet to be well-established. Efforts to build automated systems have had limited success[8]. There is a need for better methods/tools to assess both exogenous and endogenous ROS concentration. Only then will it be possible

to collect sufficient data for the assessment of relationships of particle-bound and particle-induced ROS with observed adverse health effects. To the best of our knowledge, there are no publications on aerosol oxidative potential from any group working in India. A research group at the Physical Research Laboratory, Ahmedabad has initiated actual measurements of oxidative potential of ambient aerosols, according to presentations in the recent National Space Science Symposium organized at Space Physics Laboratory, Vikram Sarabhai Space Centre, Thiruvananthapuram, Kerala in February 2016 (ref. 31).

It is highly recommended that research groups in India take up such studies so that aerosol oxidative potential can be assessed in time and space over the Indian subcontinent. These measurements are not only important for the assessment of regional air quality, but also for understanding the role of aerosol oxidative potential on atmospheric chemistry and transformation processes during the long-range transport of climatically important gaseous and particulate species. As certain types of aerosols act as catalysts in the formation of ROS, it is necessary to identify these species for better understanding of regional atmospheric chemistry. Identification of these chemical species is also inevitable for understanding their major sources in the atmosphere, which in turn is important in designing appropriate mitigation strategies to reduce these sources. There is also a need for multidisciplinary collaborative research between academic institutions (aerosol chemists) and hospitals (medical doctors/scientists) to relate the oxidative potential of ambient aerosols with not only cardiopulmonary disease patients, but also with registered morbidity and mortality over different regions of India, and understand the mechanism. Outcome of such studies has important implications in various fields of research such as atmospheric chemistry, medicine, epidemiology, toxicology, and agriculture.

Summary

Due to unprecedented increase in growing anthropogenic activities, particulate pollutants (aerosols) are also increasing. These aerosols have high potential to generate ROS in the atmosphere as well as in humans and plants. These ROS not only affect the health of humans and plants, but also the atmospheric oxidizing capacity and secondary aerosol formation processes occurring in the atmosphere. This article suggests that there is immediate need for taking up studies related to oxidative potential of ambient aerosols over regions dominated by different sources and meteorological conditions as well as different micro environments in India. It is also important to relate the oxidative potential with morbidity and mortality through collaboration between research institutions and hospitals. This would help in designing effective mitigation strategies to curb the effects of pollutants on human health. Such studies are also important for regional and global chemical transport models.

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