Environmental Pollution 219 (2016) 72–79

Contents lists available at ScienceDirect

Environmental Pollution

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

Urban $PM_{2.5}$ oxidative potential: Importance of chemical species and comparison of two spectrophotometric cell-free assays☆

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ARTICLE INFO

Article history:
Received 1 July 2016
Received in revised form
15 September 2016
Accepted 15 September 2016

Keywords:
Oxidative stress
Reactive oxygen species
Dithiothreitol
Ascorbic acid
Particulate matter

ABSTRACT

Oxidative potential (OP) of particulate matter (PM) – defined as the capacity of PM to oxidize target molecules generating reactive oxygen species (ROS) – has been proposed as a more health relevant metric than PM mass. In this study two cell-free methods were used to assess the OP of PM filters collected at an urban site and to evaluate correlation with PM mass and PM composition.

Among the different assays existing, two inexpensive and user-friendly methods were used both based on spectrophotometric measurements of depletion rate of target reagents oxidized by redox-active species present in PM. One assay measures the consumption of dithiothreitol (OP_{DTT}) and the other the ascorbate (OP_{AA}).

Although both assays respond to the same redox-active species, i.e., quinones and transition metals, no correlations were found between OP_{DTT} and OP_{AA} responses to compounds standard solutions as well as to ambient samples. When expressed in relation to air volume, OP_{DTT} m^{-3} strongly correlates with $PM_{2.5}$ mass whereas no correlation was found for OP_{AA} m^{-3} with $PM_{2.5}$. When expressed on mass basis, both OP_{DTT} μg^{-1} and OP_{AA} μg^{-1} show a strong dependence on the sample composition, with higher OP for summer samples. OP_{DTT} m^{-3} were highly correlated with the determined metals (Cu, Zn, Cr, Fe, Ni, Mn) whereas OP_{AA} m^{-3} showed only moderate correlation with Cu and Mn.

Thus, the two assays could potentially provide complementary information on oxidative potential characteristic of PM. Consequently, the combination of the two approaches can strengthen each other in giving insight into the contribution of chemical composition to oxidative properties of PM, which can subsequently be used to study health effects.

1. Introduction

Numerous studies have linked exposure to airborne particulate matter to a wide range of adverse health end points, including, but not limited to, cardiovascular diseases, respiratory problems, and adverse neurodevelopmental effects [Delfino et al., 2005, Ghio and Madden, 2012, Lodovici and Bigagli 2011, MohanKumar et al., 2008].

In most cases, effects were linked to PM mass concentration whereas evidences indicate that PM sources and constituents are more closely linked to the induction of toxic responses [Borm et al., 2007]. In fact, much of the ambient particle mass consists of low toxicity components such as chlorides, sulphates and nitrates, while relatively tiny masses of transition metals and organic species may make a major contribution in worsening human health [Mudway et al., 2011].

The mechanisms of PM related to health effects are still incompletely understood but an emerging hypothesis is that such toxic effects are mediated by inflammatory responses originated from PM-induced oxidative activity, leading to the generation of reactive oxygen species upon the interaction of PM with epithelial cells and macrophages [Squadrito et al., 2001]. Oxidative stress results when the generation of ROS, or free radicals, exceeds the available antioxidant defences [Janssen et al., 2014]. Inflammation is initially a protective mechanism which removes the injurious stimuli and produces ROS able to induce cell killing. In the early phase of inflammation, oxidant stress does not directly cause cell damage and can induce the transcription of stress defence genes including antioxidant genes. This preconditioning effect of ROS enhances the resistance against future inflammatory oxidant stress and promotes the initiation of tissue repair processes. The

☆ This paper has been recommended for acceptance by David Carpenter.

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http://dx.doi.org/10.1016/j.envpol.2016.09.047
0269-7491/

additional release of cell contents amplifies the inflammatory process and consequently can induce tissue injury [Kelly 2003].

ROS are families of compounds containing oxygen radicals and/or strong non-radical oxidative agents, including hydroxyl ($^{\cdot}OH$), hydroperoxyl ($^{\cdot}HO_2$), superoxide ($^{-}O_2$), organic peroxy radicals and hydrogen peroxide (H_2O_2). Free radicals are potentially very dangerous since they can react indiscriminately with neighbouring molecules. Within the affected cells, ROS are formed through the reduction of oxygen by biological reducing agents such as nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH). These agents react with the redox-active chemical species with the catalytic assistance from electron transfer reactions [Dellinger et al., 2001, Squadrito et al., 2001]. This process has the potential to both deplete antioxidant species in the cell and generate reactive oxygen species, contributing to induction of oxidative stress.

Furthermore, it has been found that ROS are present in the atmosphere on respirable particles to which we are exposed [Hasson and Paulson 2003, Venkatachari et al., 2007].

The overall oxidative capacity of PM has been proposed as a metric that is more closely related to biological responses to particles exposure and thus could be more informative than PM mass alone [Borm et al., 2007]. Oxidative potential is an attractive measure because it integrates various biologically relevant properties, including size, surface and chemical composition.

A few chemical components of ambient PM have been identified to catalyze redox reactions in biological systems, as active redox cycling catalysts, such as quinones or quinone-type compounds and transition metals (e.g., Fe, Mn, Cu, V, and Ni) [Charrier and Anastasio 2012, Kelly 2003, Lin and Yu 2011].

Several methods to provide the rapid read out of the oxidative potential have been developed. The only analytical method that provides direct quantification of radical species in the samples is electron spin resonance (ESR). However, ESR is an expensive and complicated instrument which has low sensitivity due to low steady state concentration and short radical's lifetimes [Hedayat et al., 2014]. Other assays are based on the capacity of PM suspensions to oxidize target antioxidants selected to simulate the respiratory tract lining fluids (RTLFs), which represent the first physical interface encountered by inhaled materials. The original method used an antioxidant mixture containing ascorbate (vitamin C), urate and reduced glutathione that were found present in RTLFs at high concentrations [Ayres et al., 2008, Zielinski et al., 1999]. A simplified version based on an only ascorbate solution has also been developed [Mudway et al., 2011, Janssen et al., 2014, Hedayat et al., 2014]. Another common assay is based on dithiothreitol (DTT), a strong reducing agent that simulates cellular reducing species in the biological systems [Charrier and Anastasio 2012, Cho et al., 2005, Janssen et al., 2014, Yang et al., 2014].

Some studies make use of fluorescent (2′,7′-dichlorofluorescin diacetate, profluorescent nitroxide probes, dihydrorhodamine) or chemiluminescent (acridinium ester) reagents which emit after chemical reactions with ROS [Hedayat et al., 2014, Yang et al., 2014].

This study is focused on two cell-free assays commonly used to assess the oxidative capacity of $PM_{2.5}$ samples: dithiothreitol and ascorbic acid assays. In both cases, the redox-active species present in PM oxidize the reagents and the oxidative potential is determined as the rate of reagent depletion measured with spectrophotometric techniques as user-friendly, direct and inexpensive tools. Most of the current literature indicates that the two assays respond differently to the various redox-active species. In particular, the DTT assay is known to be strongly sensitive to organic species, such as polycyclic aromatic hydrocarbons (PAHs) and quinones [Charrier and Anastasio 2012, Cho et al., 2005, Chung et al., 2006, Li et al., 2009], and only recently, it was found by some authors sensitive also to laboratory solutions of transition metal ions, such as Cu (II) and Zn (II), [Charrier and Anastasio 2012, Lin and Yu 2011]. It is well known that the presence of transition metals promotes oxidation reaction of ascorbic acid [Ayres et al., 2008, Buettner and Jurkiewicz 1996, Xu and Jordan 1990], but also quinones have been discovered able to oxidize ascorbic acid [Mudway et al., 2011, Roginski et al., 1999].

Given the limited comparative information on the different methods to measure OP, the primary objective of this study was to compare the two assays by measuring their responses to standard solutions of some quinones, PAHs, oxo-PAHs and metals commonly present in environmental particulate matter. The aim was to highlight different sensitivity to the ROS generating compounds and better understand the effects of redox-active chemical species in ambient PM.

The second step of the current study was to assess the oxidative potential of real-world urban $PM_{2.5}$ samples collected in Northern Italy that is recognized as one of the most worrying air pollution situations in Europe, where high anthropogenic emissions and meteorological factors may cause air pollution episodes and serious risks for human health [Pietrogrande et al., 2016]. The relationship of OP with the PM mass and chemical composition was investigated.

Although some field studies have investigated OP from different locations, i.e., Germany, the Netherlands, Los Angeles [Cho et al., 2005, Mudway et al., 2011, Saffari et al., 2015, Verma et al., 2015], at our knowledge only one study has been recently published concerning Northern Italy [Perrone et al., 2016]. In addition, most of the papers have generally focused on a specific procedure to evaluate OP, while only few compared different measurement methods.

2. Materials and methods

2.1. Standards and reagents

15 compounds representative of different chemical classes of catalytically redox-active species were considered for this study: 4 quinones—namely 9,10-phenantrenequinone (9,10-PNQ), 1,2-naphthoquinone (1,2-NPQ), 1,4-naphthoquinone (1,4-NPQ) and anthraquinone—3 PAHs (naphthalene, phenanthrene and anthracene), 2 oxo-PAH (1,8-naphthalic anhydride and xanthone) and 6 transition metals, i.e., copper (II), manganese (II), Nickel (II), chromium (III), zinc (II) and iron (III).

Individual standard stock solutions were prepared for each analyte by weighting pure standards (Acros Organics, Sigma Aldrich, Dr. Ehrenstorfer, Carlo Erba Reagenti) at a concentration of 10^{-2} M using acetonitrile (for quinones, PAHs and oxo-PAHs) or MilliQ water for metal ions as solvent. The solutions were stored in amber glass vials in the dark at −20 °C.

DTT solution was prepared at a concentration of 10 mM in a 0.1 M phosphate buffer (Na_2HPO_4 and NaH_2PO_4) at pH 7.4. AA solution was made at the same concentration as DTT in MilliQ water. Aqueous solutions of the reagents are unstable at room temperature and DTT solution is also sensible to light, thus they were preserved in amber glass vials in the dark and at −20 °C.

To reduce the background oxidation of DTT and AA in the blank, the phosphate buffer was treated with a cation exchange resin (Chelex 100 resin, sodium form, Sigma Aldrich) to remove trace metals, mainly transition metals, which are commonly, recognize as redox-active species. The resin was poured into an acid-rinsed glass chromatography column that had a permanent glass frit to contain the Chelex. The phosphate solution was allowed to drip through the resin at 4 °C and the resulting treated phosphate buffer was collected into a clean, acid washed, Teflon (PTFE) bottle [Charrier and Anastasio 2012]. Chelex-resin treated buffer has been