Influence of ozone on traffic-related particulate matter on the generation of hydroxyl radicals through a heterogeneous synergistic effect

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A B S T R A C T

Epidemiologic studies suggest that ozone (O_3) and airborne particulate matter (PM) can interact causing acute respiratory inflammation and other respiratory diseases. Recent studies investigated the hypothesis that the effects of air pollution caused by O_3 and PM are larger than the effect of these two pollutants individually. We investigated the hypothesis that ozone and traffic-related PM (PM_{10} and $PM_{2.5}$, diesel and gasoline exhaust particles) interact synergistically to produce increasing amounts of highly reactive hydroxyl radicals ($HO^\bullet$) in a heterogeneous aqueous mixture at physiological pH. Electron paramagnetic resonance (EPR) and spin trapping were used for the measurements. Results showed that $HO^\bullet$ radicals are generated by the catalytic action of PM surface area with ozone and that EPR peak intensities are two to three times higher compared to PM samples without ozone. Incubation of the nucleoside 2′-deoxyguanosine (dG) in aqueous mixtures of ozone and PM at pH 7.4 resulted in the hydroxylation at C(8) position of dG. The formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG) showed a 2–2.5-fold increase over control (PM without O_3). These results suggest that PM and O_3 act synergistically generating a sustained production of reactive $HO^\bullet$ radicals. Partitioning of O_3 into the particle phase depends on the concentration, hygroscopicity and particle size.

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1. Introduction

High levels of ambient traffic-related particulate matter (PM) have been reported to be associated with increased human morbidity and mortality [1]. Ambient PM contains numerous carcinogenic and toxic substances, heavy metals and stable quinoid radicals. Also, traffic-related particles are potent oxidants, oxidizing important biological molecules, producing reactive oxygen species (ROS) and directly attacking cellular compartments [2,3]. Ozone (O_3) is a highly reactive oxidant gas and a major component of air pollution, especially photochemical smog [4].

Many epidemiologic studies have indicated substantial association between ambient ozone concentrations and adverse respiratory lung diseases [5,6]. When inhaled, ozone can induce adverse health effects in human lungs and cause airway inflammation. Ozone induces oxidative stress in two stages, the first by O_3 and its bioactive reaction products, and the second by the activated respiratory tract inflammatory processes [7,8]. A primary target of ozone in the lungs is the alveolar epithelium. Ozone is known

to induce epithelial cytotoxicity, DNA damage and injury through acute chronic oxidative stress, which ultimately produces necrosis, sloughing and increased epithelial permeability [9,10]. Ozone has been shown that is able to induce lung tumors in experimental animals, through free radical mechanisms and especially by the formation of $HO^\bullet$ radicals [11]. Experiments with DNA in aqueous solution showed that ozone itself interacts with DNA in a dose-dependent increase of the 8-oxo-7, 8-dihydro-2′-deoxyguanosine (8-oxo-dG) oxidative damage, which can partially inhibited by hydroxyl radical scavengers. These results suggest that DNA mutagenic damages were caused by ozone via the production of $HO^\bullet$ radicals (enhanced with piperidine treatment) [12].

Ozone and PM have traditionally been considered as separate environmental pollutants, but several recent studies and epidemiological evidence indicate that, there is a positive association between airborne PM and O_3 and hospital admissions for respiratory diseases. The relative risks are mainly for increases of $100 \, \mu g/m^3$ in daily PM_{10} and 50 ppb in daily ozone concentrations [13–15]. Diesel exhaust particles (DEP) generate substantial inflammatory effects in airways of healthy subjects, but when exposed to DEP and ozone together a significant increase in neutrophils and myeloperoxidase was seen in their sputum [16]. Experiments *in vivo* indicate a synergistic or additive effect of ozone and PM. When rats were exposed to mixtures of O_3 and various fine ambient PM there

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was an increase in the toxicity effects [17]. A number of recent studies, showed that ambient concentrations of ozone can increase the biological potency of diesel exhaust particles in rat lungs [18]. Also, inhalation of urban particles at a level that causes few effects in the lung of rats, when inhaled in combination with ozone appeared to potentiate cell injury and interstitial inflammation [19]. Ozone exposure enhances the toxicity of DEP in human airway epithelial cells by augmenting IL-8 gene expression (a potent chemoattractant of neutrophils in the lungs) [20].

The role of ambient traffic-related particles in the atmosphere has elicited a great deal of recent interest due to the possible influence of heterogeneous reactions with ozone and other gases. The heterogeneous uptake of ozone by mineral dust aerosols, the influence of relative humidity and the process of catalytic transformation at active surface sites have been studied [21]. A recent study showed that ozone-initiated oxidation reactions of volatile organic compounds significantly affected concentrations of ultra-fine particles in confined spaces [22].

Also, relevant studies observed the transformation of ozone into HO• from experiments measuring its high oxidation efficiency in water treatment. Ozone's oxidation potential increases substantially with the presence of activated carbon (with high basicity pyrrol groups) or carbon black, enhancing its transformation into HO•. Activated carbon acts as an initiator or promoter for the ozone decomposition. Textural characteristics, such as large pores and surface area, of activated carbon are governing factors for ozone decomposition into HO• [23,24].

Additionally, it has been shown by various experimental results that ROS produced by airborne particulate matter, including the highly reactive and damaging HO•, can cause severe oxidative stress within cells through the formation of oxidized cellular macromolecules, including nucleic acids, proteins and lipids [25,26]. The formation of the oxidatively damaged DNA nucleosides such as 8-hydroxy-2′-deoxyguanosine (8-OHdG) has been associated with aging and carcinogenesis [27,28].

The intent of the present study was to determine the ability of ozone and traffic-related airborne PM (in aqueous buffered mixtures, at various pH values) to generate synergistically increasing amounts of hydroxyl radicals (HO•), compared to individual action of O_3 or PM. Particles used in this study include: PM_{10}, $PM_{2.5}$, diesel exhaust particles and gasoline exhaust particles (GEP). This work was set-up to test also the mutagenic oxidative damage caused by synergistic or additive effect of O_3 and PM in aqueous mixtures (at physiological pH 7.4) by the hydroxylation of 2′-deoxyguanosine (dG) in the C(8) position and the formation of 8-hydroxy-2′-deoxyguanosine (8-OHdG), as a measure of direct generation of ROS.

2. Experimental

2.1. Chemicals

5,5-Dimethyl-1-pyrroline-*N*-oxide (DMPO) was purchased from Aldrich Chemical Co., Milwaukee, WI. 2′-Deoxyguanosine was from Aldrich. Other fine chemicals of reasonable purity were purchased from Merck and Fluka. Water used in our experiments was filtered with ion-exchange resins and double distilled (also, water was checked for iron ions).

2.2. Particulate matter samples

Three samples from PM_{10} and $PM_{2.5}$ of ambient suspended particles were collected from a sampling site (considered as highly polluted urban area) in the center of Athens (Division of Atmo-

spheric Pollution and Noise Control, Ministry of Environment, Regional Planning and Public Works). Sampling was carried out by high-volume pump on a 24-h basis on pre-weighed glass microfiber filters (Whatman, GF/A 20.3 cm × 25.4 cm). The sampling system was equipped with a six-stage slit cascade impactor (Andersen), which effectively separates the particles into seven fractions. The PM_{10} fraction contains particles in the range of 10.21–4.2 μm of aerodynamic diameter (a.d) and the $PM_{2.5}$ fraction contains particles in the range of 2.5–0.73 μm (a.d). Three different samples of exhaust soot from diesel and gasoline (without catalytic converter) vehicles were collected on annealed glass-fiber filters using a low volume air sampler, at a distance of 0.5 m from the exhaust pipe. Vehicle exhaust emissions sampling represent warm-engine operation at 800 rpm (idle mode) and 2500 rpm (simulating urban driving conditions).

All samples were stored in acid cleaned aluminum foil and kept at −40 °C until used.

2.3. Experimental procedures

Ozone was generated by flowing pure oxygen in a silent arc generator (Ozonisator, Sander Model 200 (Uetze/Eltze, Deutschland)), producing 200 mg of O_3/h. Concentration of ozone in the aqueous solution was determined spectrophotometrically, using ε (260 nm) = 3300 dm^3/mol [29]. The maximum concentration achieved, after 1 h of addition, was 0.05–0.06 mM.

Ozone was added to the mixture at two different modes: mode 1, ozone was bubbled deep inside the solution through a capillary tube; mode 2, ozone was streaming over the aqueous surface of the mixture causing a slight vortex. The experimental vessel was covered by aluminum foil to avoid light. Four different pHs were used in our experiments: 4.5, 6.0, 7.4 and 7.8. Higher values of pH could not be used due to inability of DMPO to trap hydroxyl radicals. The pH at 7.4 represents the physiological pH of aerobic organisms. Four different amounts of PM were used in our experiments: 20, 40, 50 and 80 mg in a 10-mL aqueous solution. The heterogeneous aqueous mixture was stirred continuously with a small PTFE coated magnetic stirring bar (particles floating throughout the solution). One microliter of DMPO aqueous solution (50 mM) was added to the mixture.

A sample of 0.5 mL of the mixture was withdrawn quickly (at intervals (10, 15, 20 and 40 min) and the filtrate was inserted into a quartz flat electron paramagnetic resonance (EPR) cell. The EPR spectra were collected using a Varian E-4 EPR spectrometer. Typical parameters: 100 kHz X-band; microwave frequency 9.4 GHz; attenuation power 20 mW; modulation amplitude 1.0 G; scan range 100 G, scan time 4–8 min; time constant 1 s and receiver gain in the range 2.5×10^2 to 8×10^3.

Control experiments for the generation of hydroxyl radicals (HO•) were performed using only aqueous PM mixtures without ozone, and similarly, ozone solution without the presence of PM. Also, we conducted limited controlled experiments to test the generation of HO• in the presence of 0.01 M H_2O_2 in the mixture of PM and O_3. It is known that traffic-related PM contain high concentrations of transition metals with redox potential, which with H_2O_2 produce HO• by the Fenton reaction $(M^{n+} + H_2O_2 \rightarrow M^{(n+1)+} + HO^- + HO^\bullet)$ [26].

2.4. Hydroxylation of 2′-dG into 8-OHdG at pH 7.4

The nucleoside 2′-deoxyguanosine (dG) was incubated at an ambient temperature for 20 min in the dark with three different amounts of PM (0.1, 0.5 and 1.0 mg/mL of aqueous buffered mixtures at pH 7.4. The dG concentration was 0.1 mM. The aqueous mixture containing PM was stirred gently and continuously with a

small PTFE coated magnetic stirring bar (particles floating throughout the solution). Ozone was streaming over the aqueous surface of the mixture causing a slight vortex (mode 2).

Following incubation, the reaction mixture was filtered through a spin-X 0.45 mm nylon centrifuge tube filter and the resulting filtrate was analyzed for dG and 8-OHdG by HPLC. We used two methods for the HPLC analysis of 8-OHdG with small changes as described previously [30,31]. In the first HPLC analysis the 8-OHdG was monitored at 254 nm and by an electrochemical detector (Coulochem II EC, ESA, Inc., Chelmsford, MA) set at 400 mV and 20 nA full scale. The eluting solution was 7% methanol–93% buffer solution (KH_2PO_4, 50 mM, pH 7.4) and flow rate 1.0 mL/min. In the second HPLC with UV–vis detection at 293 nm the eluting solution was 3% acetonitrile and 0.1% aqueous acetic acid at flow rate 1.0 mL/min. The quantitative measurements of 8-OHdG are presented in microgram 8-OHdG/10^6 dG.

3. Results and discussion

3.1. Generation of HO• in O_3 or PM aqueous mixtures

The science of electron paramagnetic resonance spectroscopy is very similar in concept to the more familiar nuclear magnetic resonance (NMR) technique. EPR is used to record the spectra of free radicals, i.e. an atom, molecule or ion containing one unpaired electron. Free radicals (like the hydroxyl radical, HO•) are extremely unstable, thus spin traps (nitroxides, e.g. DMPO) are used to stabilize them temporarily in order to obtain their EPR spectra by forming the DMPO–OH adduct.

Experimental results showed that without the presence of PM samples there are detectable EPR signals. Ozone, without the presence of PM, was bubbled at an ambient temperature (in the dark) through the aqueous buffered solution at four different pH values (in the presence of 50 mM of the spin trap DMPO). No signals were detected after 10, 20, 40 and 120 min (Fig. 1, 1b), only very weak EPR signals, covered by noise, appeared at pH 7.8 (Fig. 1, 1a). Ozone is known to dissociate at alkaline solution [23]. Also, no EPR signals were obtained from solutions containing only 50 mM DMPO.

In Fig. 1, we included a typical EPR spectrum of DMPO–OOH (1c) adduct which is formed temporarily (when PM samples are included in the mixture) and a representative EPR spectrum of the [DMPO–COO]•−, when HCOONa is added into the mixture to check the formation of hydroxyl radicals (HO•) (1d). The scale on the y-axis of all spectra is indicated by the 10-G line. The height of the peak intensities is in arbitrary units.

All four PM samples (without ozone) exhibited similar EPR signals in aqueous buffered mixtures at four different pH values in the presence of the spin trap DMPO. The methodology used and the corresponding results were presented in detail in our previously published study [32]. Initially, the spin-trapped superoxide anion (O_2•−) was detected very briefly, as the DMPO–OOH radical adducts, but it was short-lived and was converted into the DMPO–OH radical adduct. Weak to medium EPR signals were detected for all samples. The pH values did not influence significantly the EPR signal intensities, but the strongest EPR signals were observed at pH 7.4 and 7.8, which developed in the first 20 min of mixing (Fig. 2, 1a–d). The 1:2:2:1 quartet EPR signal observed is the typical DMPO–OH spin adduct [33]. This EPR spectrum was observed when 50 mg/10 mL solution of individual particles (PM$_{2.5}$, PM10, DEP and GEP) was stirred for 20 min in aqueous mixtures at pH 7.4. Confirmatory test for the HO• radical formation (and not an artefactual case) was obtained by the addition of 0.05 M HCOONa. The distinctive six-line spec-
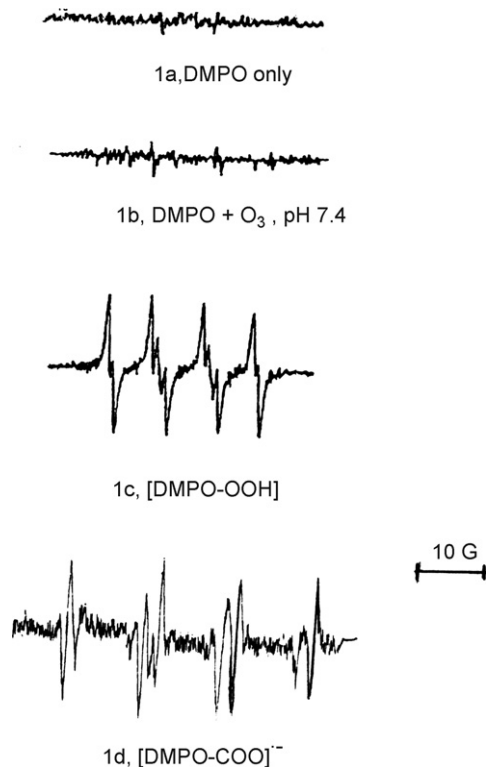

Fig. 1. Typical electron paramagnetic resonance (EPR) spectra (spin trapping) for control experiment: (1a) DMPO in aqueous solution for 20 min; (1b) DMPO plus ozone bubbled through the solution; (1c) typical EPR spectrum of short-lived spin-trapped adduct of superoxide anion, the DMPO–OOH generated in aerated solutions of PM samples in the presence of 100 mM DMPO, and (1d) typical six-line EPR spectrum of [DMPO–COO]•−, in the presence of HCOONa, confirming HO• radical generation.

trum is the [DMPO–COO]•− spin adduct (Fig. 1, 1d). These results provide additional evidence for HO• radical formation by PM mixtures and were explained in detail in our previous papers [32,34].

Finally, ozone was added through a solution of 0.05 M of H_2O_2 in the presence of DMPO. After 30 min no detectable EPR signals appeared. This is another typical confirmatory experiment that can produce artefactual signals in the EPR spectra. The significance of this experiment is the low physiological concentrations of H_2O_2, which have been detected in cell compartments or as a result of an inflammatory response. Hydrogen peroxide can be another oxidative candidate in the aqueous environment of the lung tissues, especially in the alveoli [35].

3.2. EPR study of spin-trapped HO• generated by samples of PM with O_3

PM samples with ozone bubbled through the solution exhibited much stronger EPR signals in the presence of the spin trap DMPO, when compared to only individual PM samples. The samples of PM$_{2.5}$ and DEP generated EPR signals with the highest line intensities, followed by weaker EPR signals from GEP and PM$_{10}$. The optimal concentration of PM was the 50 mg/10 mL mixture. The ozone concentration in the mixture range of 0.05–0.06 mM and the best results were for sampling after 20 min. Longer sampling did not produce higher intensity EPR signals. The best EPR signals were obtained at pH 7.4 and 7.8.

Results of EPR spectra for the HO• radical (as DMPO–OH spin adduct) generation by four PM samples (50 mg) with ozone, when
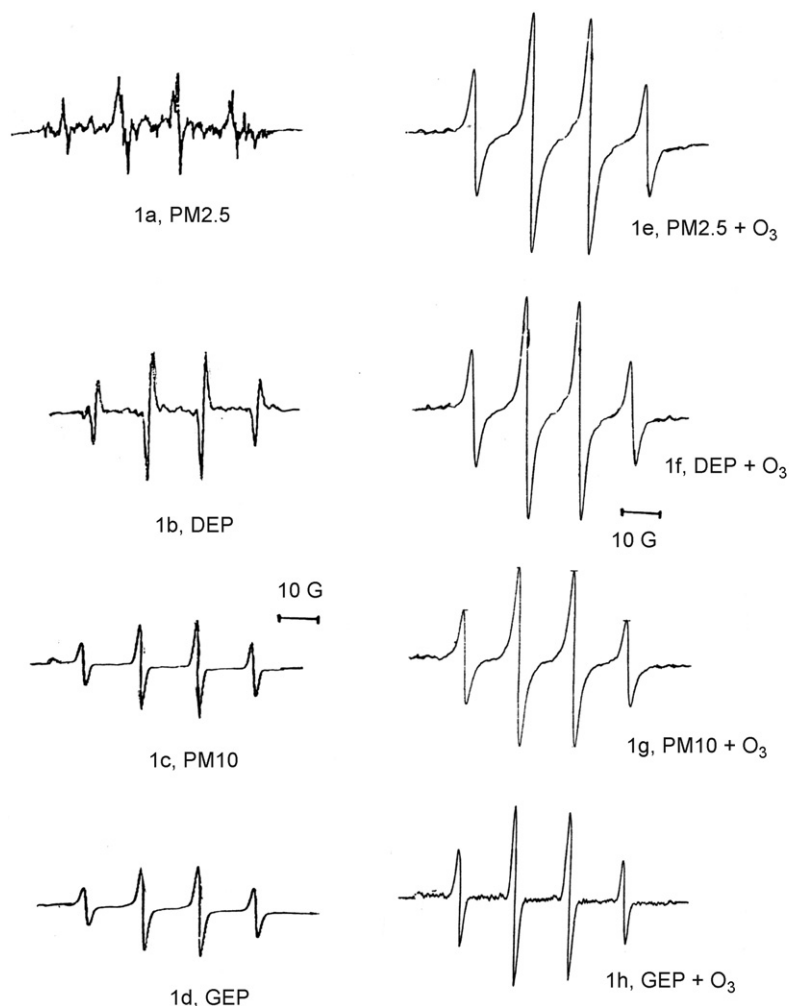
Fig. 2. Typical EPR spectra of radicals, first, generated by PM samples without ozone, as the 1:2:2:1 quartet of DMPO–OH spin adduct: (1a) $PM_{2.5}$; (1b) PM_{10}; (1c) DEP; (1d) GEP. Secondly, typical EPR spectra of PM samples with ozone bubbled over the top of mixture (mode 2). (1e) $PM_{2.5} + O_3$; (1f) $PM_{10} + O_3$; (1g) DEP + O_3; (1h) GEP + O_3.

bubbling was over the aqueous mixture (mode 2) (Fig. 2, 1e–h). The EPR signal intensities are higher, approximately, by 10–20%, compared with the intensities obtained when ozone was bubbled deep inside the aqueous mixture (mode 1).

Quantitative measurements of EPR peak intensities of all four PM samples with O_3 show that these EPR signals are 2–2.5 times higher than when individual PM were used only, without ozone. The measurements are in arbitrary units, i.e. EPR peak intensities are the sum of the height, in centimeter, of the four lines of the 1:2:2:1 quartet). The summary of the results is presented in Fig. 3. Together, these results indicate that PM can catalyze the transformation of O_3 resulting in the increase of HO$^\bullet$ radical concentrations, compared to controls (individual PM only). Increasing the amount of PM over 50 mg/10 mL (while ozone concentration remains constant) did not increase the EPR signal intensities.

A slightly different experimental procedure was used for the addition of ozone. Blowing ozone over the reaction vessel of the stirred mixture and not deep inside the mixture (method 2), caused a 10–20% increase in the EPR signal intensities, compared to method 1. Changing the pH also influenced the EPR signal intensities. Higher signals, by, approximately, 20–30%, were recorded at pH 7.4 (sampling at 20 min) compared to lower pH. The results are presented in Fig. 3.

3.3. Hydroxyl radical generation by PM and O_3 in the presence of H_2O_2

Experiments were conducted to test the effect of H_2O_2 (0.01 M) in the aqueous mixture of PM and O_3 at pH 7.4. The physiological presence of low concentrations of hydrogen peroxide in cell compartments makes this observation interesting. The EPR measurements of DMPO–OH adduct showed even higher EPR signal intensities, when compared to the mixture of only PM with O_3. These results are presented in Fig. 3. Increases in the formation of HO$^\bullet$ can be explained by the presence of transition metals with redox potential, in the traffic-related particles and the Fenton reaction.

3.4. Hydroxylation of nucleoside 2′-deoxyguanosine

The results of the comparative study dealing with the C(8) hydroxylation of the purine moiety of dG by PM and O_3 are shown in Table 1. Control experiments with only 2′-deoxyguanosine (0.1 mM) in aqueous solutions at pH 7.4 showed very small concentrations of 8-OHdG, in the range of 8–10 8-OHdG/10^6 dG. Solutions of dG exposed to O_3 alone (streaming on the surface, pH 7.4, at an ambient temperature in the dark) showed small increases, in the range of 19–23 8-OHdG/10^6 dG. Mixtures of
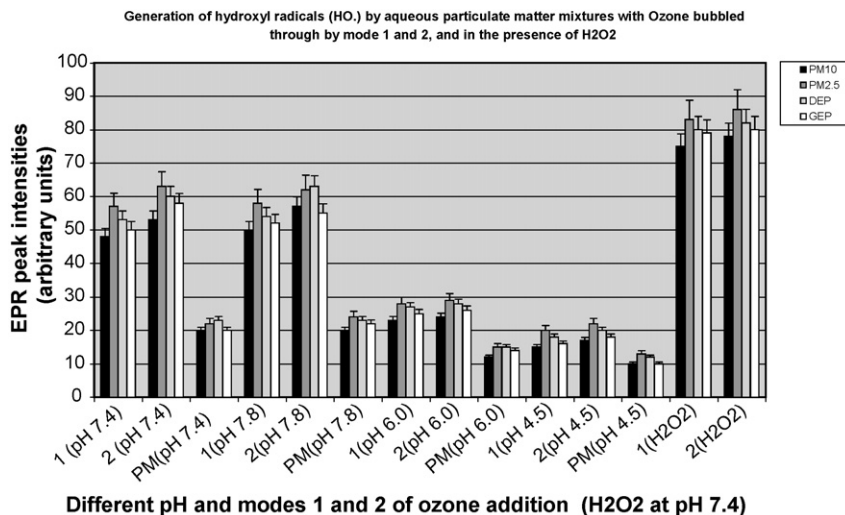
Fig. 3. Generation of HO• radicals by four PM samples (50 mg/10 mL) and ozone in the presence of DMPO. Ozone was bubbled through the mixture mode 1 (into the aqueous mixture) and mode 2 (over the top of the aqueous mixture). EPR peak intensities are expressed in arbitrary units (the sum of the height (cm) of four lines of the 1:2:2:1 quartet). The results are expressed as the mean ± S.D. (n = 3). Error bars represent the S.D.

dG with PM alone showed increases in concentrations, ranging from 72 to 110 8-OHdG/10^6 dG. Finally, incubation of dG with mixtures of PM and O_3 (ozone was added over the surface of the stirred mixtures) showed substantial increases of 8-OHdG formation. Results showed 2.0–2.5-fold increases of C(8) hydroxylation. Concentrations were in the range of 240–190 8-OHdG/10^6 dG. The highest concentrations were the result of the incubation of dG with $PM_{2.5}$ and O_3. Results are presented in Table 1.

3.5. Significance of particle sizes and O_3 for additive or synergistic effect

This investigation, concerning traffic-related airborne particles and ozone, is considered important because of health implication in urban environments. Studies in experimental animals and epidemiologic evidence have indicated that combined exposure to ozone and PM exacerbate asthma symptoms, airway inflammation, hospital admissions for pneumonia and chronic obstructive diseases [13,36].
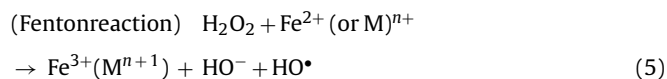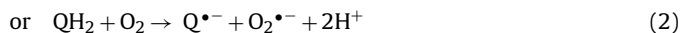
Our results demonstrated that traffic-related PM can generate ROS in aqueous mixtures, especially HO• radicals, but in combination with ozone there is a substantial increase of HO• radicals formation (as DMPO–OH spin adduct), suggesting an additive or synergistic effect. $PM_{2.5}$ and DEP samples, which contain particles with smaller aerodynamic diameter, have the highest EPR signal intensities, followed by GEP and PM_{10}, compared to only PM samples. The increases are in the range of 2.0–2.5-fold, when compared to individual PM alone. This is an indication that surface areas of

particles play an important role in the transformation of O_3 into hydroxyl radicals.

Our experimental results showed that, addition of O_3 over the top of the PM aqueous mixture, than deep inside the mixture, produced higher amounts of HO• radicals. Although the difference is relatively small, this observation is an interesting indication suggesting that the transformation of O_3 occurs in the interface of particle surface and the gaseous pollutant. Also, the results suggest that the smaller the PM size fraction (e.g. PM_{10}) the larger the surface area and their porous cavities, and subsequently the higher the radical-generating capacity. A recent review of studies for the evaluation of toxicological effects of traffic-related PM showed that, in general, the smaller PM size fractions (<PM_{10}) have the highest toxicity, due to its higher concentrations of extractable organic matter and the higher radical-generating capacity [37].

3.6. Reactions of hydroxyl radical generation by PM and O_3

In our previous published experimental work we demonstrated that various samples of airborne traffic-related PM could generate $O_2^{•-}$ (short-lived even in the spin-trapped form of its DMPO–OOH adduct), which subsequently forms the HO• radicals in aerated aqueous mixtures [32]. The various reactions, proposed for the production of ROS by PM alone, involve the redox dynamic mixture of quinoids (QH_2) and their semiquinone radicals (QH•) found in relatively high concentrations in traffic-related PM [2,32].

$$QH_2 + O_2 \rightarrow QH^• + O_2^{•-} + H^+ \tag{1}$$

$$\text{or} \quad QH_2 + O_2 \rightarrow Q^{•-} + O_2^{•-} + 2H^+ \tag{2}$$

$$QH^• + O_2 \rightarrow Q + O_2^{•-} + H^+ \tag{3}$$

$$O_2^{•-} + 2H^+ \rightarrow H_2O_2 + O_2 \tag{4}$$

$$\text{(Fenton reaction)} \quad H_2O_2 + Fe^{2+} (or\ M)^{n+}$$
$$\rightarrow Fe^{3+}(M^{n+1}) + HO^- + HO^• \tag{5}$$

The addition of O_3 in the aqueous mixture introduces some subsequent reactions. $O_2^{•-}$ produced by PM samples can transfers an electron to O_3 and the radical anion $O_3^{•-}$ is transformed, according

Table 1
Quantitative HPLC measurements of 8-OHdG formation from incubation of nucleoside 2′-deoxyguanosine with mixtures of various PM and bubbling with O_3 at physiological pH 7.4

Reaction mixture	PM_{10}	$PM_{2.5}$	DEP	GEP
PM + O_3	190 ± 12	240 ± 30	235 ± 24	210 ± 18
PM (without ozone)	72 ± 8	110 ± 18	100.3 ± 10.8	90 ± 8
O_3 (without PM)	23 ± 3	20 ± 3	21 ± 2	19 ± 1
Control (only dG)	8 ± 1	8 ± 2	10 ± 2	9 ± 2

Values estimated in μg 8-OHdG/10^6 dG, the results are expressed as means ± S.D. (n = 3). Results are for 1 mg/mL of PM mixture and ozone was bubbled on the surface of the mixture for 30 min.

to the reactions:

$$O_2^{\bullet-} + O_3 \rightarrow O_3^{\bullet-} + O_2 \tag{6}$$

$$O_3^{\bullet-} + H_2O \rightarrow HO^\bullet + HO^- + O_2 \tag{7}$$
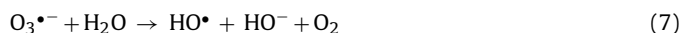
In our limited experiments, with the addition of H_2O_2 in the mixture ($PM + O_3$), we observed a substantial increase in the intensities of the EPR signal. This is the result of the additional $HO^\bullet$ generation by transition metal ions, especially with redox potential, which are constituents of most traffic-related PM [29]. Similar experiments were performed by Dalal et al. [38] with coal mine dusts (generation of $HO^\bullet$, spin-trapped by DMPO at pH 7.4) but in the presence of H_2O_2. Their results showed that higher concentrations of surface iron in coal mine dusts were responsible for the generation of $HO^\bullet$ radicals.

Despite the lack of similar experiments in the scientific literature, our results can be compared with a study of ozone degradation into $HO^\bullet$ radicals in aqueous buffered solution at pH 4.5, 7.2 and 7.8 (spin trapped by DMPO). The authors observed weak EPR signal of DMPO–OH adduct if ozone was bubbled into the solution, but subsequently the EPR signal intensities were much higher with the addition of 10^{-4} M caffeic acid, phenol, ferrulic or cinnamic acid (reducing agents). They observed that the EPR signal were much stronger when ozone was bubbled over the solution for 60 s (mode 2). The higher EPR signals were detected at pH 7.8 [39].

Other recent studies showed that traffic-related PM (such as PM_{10} and $PM_{2.5}$ from both diesel and gasoline engine exhaust) have the capacity of generating ROS ($HO^\bullet$ radicals). This capacity was found to be significantly correlated with the concentrations of polycyclic aromatic hydrocarbons (PAHs) and transition metals (Cu^{2+}, Fe^{2+}). Reducing agents, like ascorbic acid, in aqueous mixtures of PM stimulated the production of ROS in vitro [40,41]. Obviously, PAHs and metal ions can contribute through redox cycling in the production of ROS. Stable quinoid radicals in airborne particles must play a major role in redox cycling and in the mechanism for sustained free radical generation, especially $HO^\bullet$ [2,42].

3.7. Oxidative stress and toxicity of PM and O_3 shows consistent interactions

Toxicity experiments *in vivo* showed that carbonaceous particles and O_3 increased oxidative stress. Rats exposed alone and in combination to ultrafine carbonaceous particles (median diameter 25 nm) and ozone (1 ppm) showed consistent interactions between particles and ozone and increased oxidative stress. The results indicated that inhaled ultrafine carbonaceous particles for short period (6 h) can induce significant pulmonary inflammation and oxidative stress, modified by age, co-pollutants (ozone) and a compromised (bacterial toxin) respiratory tract [43]. Similar acute pulmonary toxicity effects results were recorded with rats exposed to PM and ozone [44,45].

Partitioning of O_3, $HO^\bullet$, and H_2O_2 (formed as a decomposition product) into the gas and particle phases probably depends on their concentration, but also on the hygroscopicity and porous cavities of the particles as well as their size. The co-occurrence of these pollutants in the ambient atmospheric environment may provide a transport route of reactive oxygen species and free radicals into the respiratory tract with damaging effects into the alveolar region. It must be emphasized that molecular oxygen metabolites, such as superoxide anion ($O_2^{\bullet-}$) and H_2O_2 have been detected in the respiratory tract lining fluid as components of innate immunity. H_2O_2 is extremely water-soluble and can penetrate deeper into the respiratory tract [46].

Increasing interest has developed recently with regard to the role of metals in the particulate air pollution in inducing nuclei

acid damage, especially in the base moieties of DNA [47]. In our study, incubation of urban particulates with dG and exposed at the same time to O_3 resulted in 2.0–2.5-fold increase in 8-OHdG formation compared to controls at ambient temperature at pH 7.4. There are no similar results in the scientific literature, but similar results were reported by Prahalad et al. [26] with coal fly ash, oil fly ash and ambient air particulates (without exposure to O_3) compared to controls. They used free dG, calf thymus and cellular DNA in their experiments. According to their results the enhancement of DNA damage under aerobic conditions by particulates was consistent with the concentrations of water-soluble metals of PM [26].

In summary, our findings provide further evidence for the additive or synergistic effect of ozone and traffic-related PM and in the generation of highly damaging hydroxyl radicals, in aqueous solutions at physiological pH, compared to airborne particles or ozone individually. Although additional work is needed to establish the mechanisms of $HO^\bullet$ generation by the combined effect of PM and ozone, this study indicates that particle size and surface area play an important part. Also, stable quinoid radicals and transition metals of PM may have an important role in the formation of $HO^\bullet$, which is capable to cause cellular injury in lung tissues.

Acknowledgements

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