Published in final edited form as:

Environ Sci Technol. 2014 April 15; 48(8): 4266–4272. doi:10.1021/es401770y.

Hydroxyl Radical Generation from Environmentally Persistent Free Radicals (EPFRs) in PM_{2.5}

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Abstract

Hydroxyl radicals were generated from an aqueous suspension of ambient PM_{2.5} and detected utilizing 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO) as a spin trap coupled with electron paramagnetic resonance (EPR) spectroscopy. Results from this study suggested the importance of environmentally persistent free radicals (EPFRs) in PM_{2.5} to generate significant levels of ·OH without the addition of H₂O₂. Particles for which the EPFRs were allowed to decay over time induced less hydroxyl radical. Additionally, higher particle concentrations produced more hydroxyl radical. Some samples did not alter hydroxyl radical generation when the solution was purged by air. This is ascribed to internal, rather than external surface associated EPFRs.

INTRODUCTION

Multiple studies established increases in airborne PM_{2.5} levels promote cardiopulmonary dysfunction and decreased life expectancy.^{1,2} These adverse health effects are induced by oxidative stress triggered when the cell is overwhelmed by reactive oxygen species (ROS).³ However, the exact nature of ROS formation and its sources is still debatable. A wide range of ROS appear in the gas phase of secondary organic aerosols as very unstable intermediate products, such as hydrogen peroxide, organic peroxides, diacyl peroxide, peroxyxynitrite, etc.,

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Supporting Information

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The authors declare no competing financial interest.

and in the particulate phase.^{4–6} Recent evidence of short-lived radicals, such as $\cdot R$, $\cdot RO$, $\cdot RO_2$, $\cdot OH$, and $HO \cdot_2$, in the particle phase was reported using electrospray ionization/tandem mass spectrometry (ESI/MS) in the ozonolysis of α -pinene.⁷ Of all the ROS, including hydroxyl radicals ($\cdot OH$), superoxide anion-radicals ($O \cdot^-_2$), hydrogen peroxide (H_2O_2), etc., $\cdot OH$ is the most damaging.

PM is documented to induce a toxic response whether from wood smoke, other biomass burning, or ambient $PM_{2.5}$.^{8–10} The details of ROS generation from different types of PM were previously reported.^{11,12} Adverse health effects might also be initiated by ROS generated from the red-ox cycling of environmentally persistent free radicals (EPFRs) associated with PM.^{8,13}

EPFRs were identified initially on the surfaces of particles containing redox-active transition metals in the post flame and cool-zone regions of combustion systems.^{8,14,15} In addition to combustion systems, EPFRs were established in ambient $PM_{2.5}$.^{8,11,16,17} Formation of an EPFR occurs when an organic precursor chemisorbs onto a redox metal site (e.g., CuO or Fe_2O_3) subsequently reducing the metal via electron transfer.¹⁴ This mechanism results in the generation of a surface bound radical. EPFRs associated with the surface of the particle imparts additional stabilization to these radicals, allowing them to persist in the environment.^{14,18}

Red-ox cycling of the adsorbed EPFRs was hypothesized as a source for ROS formation.^{12,13,16,18–20} Experimental measurements and calculations implied one EPFR generated $\sim 10 \cdot OH$, suggesting a catalytic cycle.¹⁹ The $\cdot OH$ was most probably attached to the surface, instead of acting free in solution,²⁰ and these results corroborates earlier studies theorizing $\cdot OH$ were surface associated.^{5,12,16,18,21}

Some studies suggest total or soluble redox transition metals present in the PM is the main contributor to ROS generation, because they catalyze the generation of $\cdot OH$ via Fenton-type reaction.^{22,23} However, the majority of previous studies testing PM samples only generated ROS with the addition of H_2O_2 ,^{24–29} which is necessary for the Fenton Reaction. Only a few examples in the literature report ROS generation without the addition of H_2O_2 .^{11,12,30,31} One study proposed ROS generation without the external addition of H_2O_2 results from the synergistic effect of transition metals and persistent quinoid/semiquinone radicals in ambient PM.¹⁶ In this work, the generation of superoxide radicals from PM in DMSO solution was clearly evidenced, while the formation of $\cdot OH$ radicals, observed as the DMPO- $\cdot OH$ adduct, was not straightforward.

The direct role of stable semiquinone radicals bound to $PM_{2.5}$ as a generator of $\cdot OH$ needs additional clarification. For this reason, experiments were initiated to assess the redox potential of EPFRs in ambient $PM_{2.5}$ without prior addition of H_2O_2 .

EXPERIMENTAL SECTION

$PM_{2.5}$ was collected by us in an industrial environment. Hydroxyl radicals were detected from an aqueous suspension of $PM_{2.5}$ and measured by utilizing 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) as a spin trap coupled with electron paramagnetic resonance (EPR)

spectroscopy. We demonstrated the impact of EPFRs in PM_{2.5} on hydroxyl radical generation without adding H₂O₂. To eliminate any confounding effects, there was no addition of biologically relevant reducing agents or chelating agents.^{19,20} Chelating agents, such as deferoxamine mesylate, can extract the tetrachlorosemiquinone radical anion, suggesting the possibility of eliminating other semiquinone type radicals,³² and they can also affect the redox potential of metals.³³ Instead, a comparative method,^{19,20} where the same sample was utilized under different conditions (aeration vs N₂ purging, fresh vs aged), was chosen to monitor the ·OH generating capacity of ambient PM_{2.5} particles collected from the Louisiana industrial corridor. Our observations and precautions about using Chelex treatment of PM containing suspensions, effects of well documented chelating agents DFO and DETAPAC, and ·OH generation dependence on metal content in PM_{2.5} are presented in the Supporting Information (SI).

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), deferoxamine mesylate (DFO, assay 92.5%, TLS), and 0.01 M phosphate-buffered saline pH 7.4 (PBS, NaCl 0.138 M, KCl 0.0027 M) were all purchased from Sigma-Aldrich. High purity 5,5-dimethyl-1-pyrroline-*N*-oxide (DMPO, 99% +, GLC) was obtained from Enzo Life Sciences and used without additional purification. Hydrogen peroxide (Assay, 30%) and diethylenetriaminepentaacetic acid (DETAPAC, 99%) were purchased from Fluka Analytical. In the few experiments using DFO and DETAPAC, the solutions were made to a final concentration of 0.1 mM DFO or DETAPAC in sample solution. A 0.03% H₂O₂ solution was made by diluting 100 μL of H₂O₂ in 100 mL ultrapure H₂O. The concentration was verified by UV–vis absorption to be 0.0104 M. This was further diluted with sample to give a final concentration of approximately 2 mM H₂O₂.

Metals were extracted from the PM_{2.5} using hot nitric acid and analyzed using an Inductively Coupled Plasma Atomic Emission (ICP-AE) spectrometer.

Compressed air was utilized to prepare aerobic samples and compressed UHP N₂ for anaerobic samples. Unless otherwise stated, aeration or N₂ purging times were 10 min.

Sampling

PM_{2.5} samples were acquired from May to September of 2011 at a Louisiana Department of Environmental Quality (LDEQ) ambient air monitoring station situated 30 ft away from roadside and 10 ft above the ground. This site is located north of the LSU campus in Baton Rouge, LA near heavy interstate traffic along a major industrial corridor of the Mississippi River. Samples were collected using a Thermo Scientific Partisol-Plus model 2025 equipped with a PM_{2.5} fractionator. The flow rate was 16.7 L/min, and samples were collected on a Whatman 2 μm PTFE 46.2 mm diameter filter with a polypropylene supported ring. Samples were accumulated for 48 h to capture enough PM_{2.5} for extraction.

EPR Characterization

All EPR spectra for PM_{2.5} loaded filters were measured with a Bruker EMX 10/2.7 EPR spectrometer (X-band) equipped dual cavity with modulation and microwave frequencies of 100 kHz and 9.516 GHz, respectively. Parameters for the radical signal measurement were:

2.05 mW power; modulation amplitude of 4.0 G; scan range of 100 G; time constant of 40.96 ms corresponding to a conversion of 163.84 ms; sweep time of 167.77 s; receiver gain 3.56×10^4 ; and three scans. Radical concentrations for the filters were calculated by comparing the area of the peak, as calculated from the $(H_{p-p})^2$ multiplied by the relative intensity, to a five point DPPH standard calibration curve (Figure S1 in the SI).³⁴ The g-factors were determined by the WinEPR program. All parameters remained the same for DMPO–OH adduct detection except for the modulation amplitude, 0.80 G, power of 10.25 mW, and two scans.

Extraction and Spin Trapping

The extraction procedure closely followed extraction procedures in the literature.²⁸ After collection, the filter's support ring was removed, analyzed for an initial radical concentration, and transferred into 0.01 M PBS solution. This solution was prepared in ultrapure double distilled H₂O (UP H₂O), and PBS was used to maintain the pH at 7.4. The solution with filter was shaken for 15 min on a Daigger Vortex Genie 2, sonicated 5 min (Fisher Scientific FS20) at 40 W, then shaken again for 15 min. The filter was removed from suspension, dried, and the difference in weight determined how much PM_{2.5} was removed.^{11,28} The stock solution for PM_{2.5} suspension was divided into two solutions; the first portion was purged by pure N₂ to remove dissolved oxygen and referred as a control solution, and the second portion was aerated and referred as a sample solution (vide infra). PM_{2.5} suspensions were made for each sample then subsequently diluted with additional PBS solution to a volume of 190 μ L. The final particle concentration for this suspension was approximately 400 μ g/mL. DMPO (10 μ L from a freshly prepared solution of 3 M) was added to the dilutions and vigorously shaken for 30 s at a final volume of 200 μ L. This concentration of DMPO (150 mM) was found to prevent secondary reactions, such as dimerization³⁵ in addition to decomposition reactions with molecular oxygen. Twenty μ L of the suspension was transferred to an EPR capillary tube (i.d. $\sim$ 1 mm, o.d. 1.55 mm) as well as sealed at one end with sealant (Fisher brand). The capillary was inserted in a 4 mm EPR tube and fastened in the EPR resonator. The time from sample removal to initial EPR measurement was approximately 4 h. The EPR spectra of DMPO–OH adducts were recorded at specified times from the initial DMPO addition. The resulting 4-line peak areas for DMPO–OH adducts, as calculated by the H_{p-p}^2 multiplied by the relative intensity for each peak and reported in arbitrary units, were summed together.

RESULTS AND DISCUSSION

Our proposed red-ox cycle for EPFRs associated with metals in PM_{2.5}^{8,12,13,16,19,20,36} is displayed in Figure 1. In this cycle, the surface bound EPFR is deprotonated in water and reduces oxygen to the superoxide anion. The superoxide anion undergoes a dismutation reaction to form H₂O₂ followed by the Fenton reaction using the surface bound metal to generate $\cdot$ OH and an oxidized metal.

Overall, this represents an in situ red-ox cycle where a semiquinone anion radical bound on Cu(I) is the reducing agent, while the quinone (Q) bound on Cu(II) is the oxidant. In the presence of H⁺, the quinone can convert to the initial chemisorbed oxy-phenol bound on Cu(II),

The reducing agents in this stage may result from several sources. For example, some organic compounds from the redox cycle of EPFRs;³⁸ phenolic as well as oxy-PAHs compounds adsorbed on PM_{2.5};^{39–41} or the transition metals existing on the accessible surface of PM_{2.5} may serve as the participating species. During the aging process, the concentration of these reducing agents naturally decreases in solution due to either oxidation by dissolved oxygen or by reaction of the aforementioned organic compounds with ·OH. The aging negatively affects the generation of ·OH by an observed decrease of the DMPO–OH adduct concentration. If biologically equivalent reducing agents were utilized, then ·OH generation would be enhanced as observed in our previous work in a similar model system.²⁰

Detecting the red-ox reactions of EPFRs associated in a matrix with other metals and organics is challenging. Many types of spin traps are utilized for spin trapping experiments, for example, DMPO, DEPMPO (5-diethoxyphosphoryl-5-methyl-1-pyrroline-N-oxide), and fluorescent reagents, for example, dichlorofluorescein, dithiothreitol. All these assays are sensitive to different types of organics and metals in PM³. Furthermore, there are many types of PM used in these experiments, for example, wood smoke, diesel exhaust, coarse (PM₁₀), fine (PM_{2.5}), and ultrafine (PM_{0.1}) particles.^{12,16,21,24,42–45} With so many changing parameters found in particulate matter, the comparative method for such complex systems was promising to solely demonstrate the generation of ROS due to the presence of EPFRs.^{19,20} A control and sample suspension (vide infra) with the same composition and the same experimental conditions were compared assuming all secondary processes, if they occur, may have the same contribution in both control and sample suspensions. The same approach was utilized for assessing ·OH generation of ambient PM_{2.5} collected from the southern Louisiana industrial corridor.

The EPR examination of all PM_{2.5} samples, Table 1, displayed as a single, unstructured peak, Figure 2. The average H_{p-p} was 5–8 G, and this implied multiple organic species of the same radical family present or broadening by organic–metal interaction.^{11,13–15} All sample g-factors were in the range of 2.0030–2.0047, characteristic for a group of semiquinone-type or oxygen centered radicals^{13–18,29,46,47} (cf. Figure 2). The radical concentrations were $\sim 10^{16}$ – 10^{17} radicals/g of PM_{2.5}. This concentration is comparable to the same concentration range from cigarette smoke¹³ corresponding to 1.8–18 ppm as a semiquinone radical.

Transition metal concentrations in representative PM_{2.5} samples are presented in Table 2; however, the displayed data is for total metals, while we are only interested in surface-associated metals. Because the metal concentrations varied significantly from sample to sample, the variation in hydroxyl radical generation could not be tested between samples. Parallel to the comparative method described elsewhere,^{19,20} another alternative approach was also employed. The suspended PM_{2.5} was allowed to decay over time, and the

difference in the hydroxyl radical generation between the original and decayed radical samples were compared.

Aerated vs Nitrogen-Purged PM_{2.5} Suspensions

Typical 1:2:2:1 spectra, indicative of the DMPO–OH spin adduct,^{24,25} were observed in PBS solutions containing PM_{2.5} and DMPO with an example spectrum displayed in the SI. Preparing control suspensions without EPFRs were a problem due to the complexity of the collected PM_{2.5}. For this reason, we considered a control suspension as part of the PM_{2.5} stock suspension purged by pure N₂ to remove dissolved O₂ from the system. Without O₂, the control suspension (purged by N₂), while containing EPFRs, cannot generate hydroxyl radicals due to blocking the superoxide and H₂O₂ formation channel (cf. Figure 1). Under such circumstances, the differences of DMPO–OH adduct accumulation between the aerated (sample) and N₂ purged (control) suspensions is due solely to the ability of EPFRs to generate ·OH during that time, Figure 3A. The aerated samples generated a 13% greater quantity of ·OH than the purged sample, Figure 3A. This is similar to the literature, where formation of ·OH was eliminated by N₂ purging, unless H₂O₂ was added.¹² This was also consistent with our previous work where the largest differences between the control (CuO on amorphous Si) and the EPFR model system were observed when the suspensions were aerated.²⁰ This resulted in the EPFR model system exhibiting the larger DMPO–OH adduct formation.²⁰

Following a 19 day decay in solution, the aerated sample decreased by 18% from when it was fresh, while only a 10% diminution was observed between the fresh and aged N₂ purged samples (Figure 3B). A 5% decrease in DMPO–OH intensity was observed between the aged aerated and N₂ purged sample. The lesser effect in the aged sample is thought due to a reduced presence of the organic radical. Overall, there was a 21% decrease from the fresh aerated sample to the decayed N₂ purged sample. This decrease demonstrates the EPFRs impact on ROS formation in this sample, because the oxidation state of Fe, the main contributor to the Fenton reaction in PM_{2.5}, was documented to change very little over the course of 40 days.⁴⁴

Fresh vs Aged PM_{2.5} Samples

The PM samples were aged at room temperature in PBS solution. The comparisons of hydroxyl radical generation in two samples are depicted in Figure 4. When the PM_{2.5} was suspended in solution for 1 day, an 11% reduction in the DMPO–OH intensity was observed in one sample, Figure 4A, and when suspended for 2 days, a 35% reduction in a second sample, Figure 4B. A reduction in ·OH generation after aging was also observed by others in the presence of added H₂O₂.²⁹ Likewise, a reduction in integrated fluorescence activity or oxidative capacity after aging was observed.^{4,43,48,49}

Unfortunately, due to small extraction weights from the filter (on the order of 200–300 µg) and subsequent measurements using the same small sample volume, an exact radical concentration could not be established after the aging occurred. Previous experiments demonstrated polar solvents can extract EPFRs from model particles but are eliminated after

extraction either through hydrogen abstraction, radical dimerization, or radical–radical recombination.³⁸

The blank solution (PBS + DMPO) did not display any significant contribution to the formation of $\cdot\text{OH}$; however, the extracted blank PTFE filter consistently generated DMPO–OH, Figure 4A. This was expected as a result of sonication readily removing any loosely bound metals in the filter. Additionally, DMPO is documented to easily hydrolyze, in the presence of metals, into DMPO–OH as an experimental artifact.^{19,20} The ability of the PTFE filter to generate low levels of the DMPO–OH adduct was also reported elsewhere.⁵⁰ All sample signals in this study were at least 2 $\times$ greater than the blank filter at a low incubation time.

Effect of Particle Concentration

The effectiveness of red-ox cycling (Figure 1) may be seen in dependence of the DMPO–OH adduct concentration generated vs incubation time at two different particle concentrations, Figure 5. The larger particle concentration exhibited a larger DMPO–OH adduct intensity. Similar behavior was reported for PM samples with the addition of H_2O_2 and other ROS detection methods.^{12,51} A lack of linear dependence between the two particle concentrations may result from the inhomogeneity of the sample aliquots despite careful efforts. Considering $\text{PM}_{2.5}$ can vary dramatically when collected within short time ranges, especially over the course of 2 days, a large range of particle dimensions and characteristics are present.

Addition of H_2O_2

Addition of H_2O_2 into the $\text{PM}_{2.5}$ suspension resulted in an average doubling of the DMPO–OH formation (data not shown). With the blank filter, there was a 60% increase in DMPO–OH production, further demonstrating the leeching of metals from the PTFE filter during extraction. Data generated from H_2O_2 addition is consistent with literature data demonstrating metals in $\text{PM}_{2.5}$, or other analogous systems, can catalyze $\cdot\text{OH}$ formation in the presence of H_2O_2 .^{12,19,20,24,26,27,31,52} Addition of H_2O_2 to the system facilitates ROS formation via the exogenous Fenton reactions (Figure 1). However, in our experiments, external addition of H_2O_2 was not needed to generate $\cdot\text{OH}$. In this study, a combination of EPFRs and surface metals worked *in tandem* to form ROS.

“Passive” vs “Active” $\text{PM}_{2.5}$

There were a few samples which did not alter hydroxyl radical formation under the different experimental conditions, referred to as passive. These passive samples exhibited no dependence on aging time or aeration, Figure 6A, and they were also found slightly inversely proportional to the radical concentration, Figure 6B. This is in contrast to the samples developing differences in hydroxyl radical generation, referred to as active. The active set of samples exhibited an average increase of the DMPO–OH signal when the samples increased in radical concentration, Figure 6B.

The active samples probably contain external (or sterically available) radicals enabling reaction on the exposed surface. The passive samples, due to their “non-reactivity” in the

generation of $\cdot\text{OH}$ radicals, are believed to contain internal (or otherwise inaccessible) radicals where the interaction with reagents is limited. For instance, DEP samples generating little $\cdot\text{OH}$ may also result from internal radicals.¹² Other researchers reference the presence of internal radicals, and no change in the EPR signal of combusted plastics after 6 months.¹⁸

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

ACKNOWLEDGMENTS

We gratefully acknowledge support of this research from NSF Grant CHE-115-0761. We also thank the Louisiana Department of Environmental Quality for the use of their ambient air monitoring site to collect samples.

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Figure 1.

Proposed mechanism for ROS generation by a semiquinone EPFR-CuO particle system. The reactions in red denote proposed reactions for the reduction of O₂ to O₂^{•-} by a surface bound semiquinone.³⁷ Figure from “Free radicals in tobacco smoke” *Mini-Rev. Org. Chem.* **2011**, 8, 427–433. Reprinted with permission from Bentham Science Publishers.

Figure 2.

EPR spectrum ($H_{p-p} = 5.28$ G, $g = 2.0035$) of EPFR in $PM_{2.5}$. The radical concentration was 5.57×10^{17} radicals/g. The drift in the spectrum is from a transition metal signal.

Figure 3.

DMPO–OH adduct accumulation vs time at different conditions for sample collected from 5/25- 5/27/2011: **A.** Demonstration of an “active” sample with impact from aeration. This results from 10 min aeration (labeled as Aerated Sample) and 10 min N₂ purging (labeled as Purged Sample) of freshly extracted PM. **B.** Fresh and aged (19 days) samples aerated or purged by N₂.

Figure 4.

A. Demonstration of another sample (collected from 5/25–5/27/2011 with a radical concentration of 6.94×10^{16} radicals/g) to depress DMPO–OH adduct formation after aged in the dark for 1 day. **B.** Generation of DMPO–OH adduct from blank solution of PBS + DMPO (green), a blank PTFE filter (red), freshly extracted PM_{2.5} (black), and PM_{2.5} extract suspension aged in the dark for 2 days. This was collected from 5/6–5/8/2011 where the initial radical concentration was 3.37×10^{17} radicals/g.

Figure 5. Impact of particle concentration on DMPO–OH adduct generation. This was collected from 5/25 – 5/27/2011.

Figure 6.

A. Demonstration of a “passive” sample collected from 6/23 – 6/25/2011 with no impact from aeration. The initial radical concentration was 1.84×10^{17} radical/g. (a) Aeration for 10 min, (b) Aeration for 1 h, (c) Aeration for 2 h, and (d) Purged with N₂ for 2 h. **B.** DMPO–OH adducts concentration vs radical concentration for active (solid line) and passive (dashed line) samples.

Table 1

Sample date collection and radical information

date collected	radical concentration ^a	g-factor ^a
5/25–7/27/2011	6.94×10^{16}	2.0036
7/7–7/9/2011	1.34×10^{17}	2.0041
6/23–6/25/2011	1.84×10^{17}	2.0047
7/19–7/21/2011	2.82×10^{17}	2.0032
5/8–5/10/2011	3.05×10^{17}	2.0035
5/6–5/8/2011	3.37×10^{17}	2.0034
7/21–7/23/2011	4.04×10^{17}	2.0030
5/10–5/12/2011	2.92×10^{17}	2.0032
8/3–8/4/2011	5.54×10^{17}	2.0034

^a All measurements were performed immediately prior to extraction and DMPO analysis.

Table 2Transition Metals Found in Representative PM_{2.5} Samples (ppm)

sample name	Al	As	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Si	Zn
6/23-6/25/2011	32.94	0.21	0.06	0.03	0.21	5.04	41.08	1.09	0.43	1.37	77.62	9.57
7/7-7/9/2011	21.76	0.06	0.03	0.02	0.09	3.19	27.30	0.71	0.20	0.56	52.63	8.92
5/8-5/10/2011	19.60	0.10	0.04	0.02	0.08	2.91	25.09	0.66	0.22	0.75	52.47	9.00
5/6-5/8/2011	42.38	0.21	0.03	0.03	0.11	2.62	36.08	1.28	0.20	0.68	90.80	9.85
5/10-5/12/2011	36.84	0.05	0.03	0.03	0.17	3.00	34.46	1.26	0.34	0.62	86.24	5.62
6/23-6/25/2011	331.71	1.65	0.30	0.37	0.93	59.48	253.97	11.23	4.36	4.23	486.89	46.46
5/25-5/27/2011	101.01	0.00	0.09	0.08	0.38	7.32	84.09	3.99	1.27	1.73	181.59	17.39