Carbon-centered free radicals in particulate matter emissions from wood and coal combustion

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RUNNING HEAD: Carbon-centered free radicals in PM emissions

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ABSTRACT:

Electron Paramagnetic Resonance (EPR) spectroscopy was used to measure the free radicals in the particulate matter (PM) emissions from wood and coal combustion. The intensity of radicals in PM dropped linearly within two months of sample storage and stabilized after that. This factor of storage time was adjusted when comparing radical intensities among different PM samples. Relative to the PM samples collected on glass fiber filters, those duplicate samples collected on Teflon filters demonstrated smaller g-values and higher spin intensities. The g values of the free radicals were close to the free electron g value of 2.0023. This was indicative for carbon-, rather than oxygen- or nitrogen-centered free radicals. Furthermore, the strong correlation between intensities of free radical and elemental carbon suggested the radical species may be predominantly graphitic carbon. The redox and biology activities of these carbon-centered radicals are worthy of evaluation.
Introduction

Although extensive epidemiological evidence associate airborne particulate matter (PM) with increases in human mortality\textsuperscript{1,2}, the mechanisms of PM-related health effects are still incompletely understood. One mechanistic hypothesis for PM injury is the ability of certain PM properties to induce oxidative stress, which refers to the situation of a serious imbalance between the production of reactive oxygen species (ROS) and antioxidant defense. Oxidative stress occurs in an organism when the concentration of ROS generated exceeds the antioxidant capability of that organism. ROS is a collective term that includes both oxygen-centered radicals (O$_2$•$^-$ and OH$^-$) and non-radical derivatives of O$_2$ such as hydrogen peroxide (H$_2$O$_2$). A free radical is a molecular fragment with an unpaired valence electron, conventionally symbolized by a superscript dot "•". The presence of the unpaired electron causes free radicals to be attracted slightly to a magnetic field, thus it is called paramagnetic and can be measured by Electron Paramagnetic Resonance (EPR) spectroscopy. Most previous studies on PM-induced oxidative stress have not made the difficult distinction between endogenous and exogenous sources of free radicals, so the contribution of each source to the PM effect is unknown\textsuperscript{3}. To differentiate the contribution of each source to the PM effects, we need to first measure the free radicals that already exist on the particles before inhaled. This is particularly important for combustion-generated carbonaceous particles, which may contain not only transition metals and polycyclic aromatic hydrocarbons (PAHs) that are responsible for in situ production of free radicals in the lung, but also pre-existing free radicals.
The current paper presents measurements of pre-existing free radicals in PM emissions from wood and coal combustion. The coal samples were taken from Xuan Wei, China, where the extremely high indoor air pollution due to coal combustion was linked to the highest women lung cancer rate of China. Besides examining the chemical speciation and spin intensities of radicals in the particulate matter, we also explored the effects of sample storage time and filter substrate type on the measurement of free radicals.

**Experimental section**

**Combustion experiment and air sampling.** The experimental methods for combustion and air sampling were reported elsewhere and are only summarized here. Fifteen types of coal and one pine wood sample were burned under controlled conditions in the laboratory to measure the gaseous and particulate emissions. An open fire was assembled with firebricks to simulate the household fire pit used in Xuan Wei, China, where the extremely high indoor air pollution was linked to the highest women lung cancer rate of China. The combustion experiment involves heating 1.5 kg of water from ambient temperature to boiling, and kept simmering for 10 minutes. Air is drawn from the exhaust duct through 1/4" stainless steel sampling probes placed 0.5 m downstream of the duct inlet and 1 m upstream of the damper. Duplicate sets of particulate matter (PM) was collected with various kinds of filter media for different analytic techniques: quartz fiber filters for EC/OC analysis, Teflon membrane filters (SKC Inc.) and Teflon-coated glass fiber filters (Pallflex, Gelman Sciences Inc.) for free radical analysis. Filter samples were stored in -10 °C freezers before analysis. Over 90% of the particulate mass was attributed to submicrometer particles.
**EC/OC analysis.** The thermal/optical reflectance (TOR) method was applied to determine elemental and organic carbon (EC/OC). The TOR method is based on the principle that different types of carbon-containing particles are converted to gases under different temperature and oxidation conditions. Quartz fiber filter samples were analyzed for EC/OC by DRI Model 2001 Thermal/Optical Carbon Analyzer. The protocol involves heating a 0.526 cm\(^2\) punch aliquot of a filter stepwise in a non-oxidizing helium (He) atmosphere to measure organic carbon, and in an oxidizing atmosphere of 2% oxygen in a balance of helium to measure elemental carbon\(^7\).

**Free radical analysis.** Electron Paramagnetic Resonance spectroscopy (EPR) analysis was carried out using the standard continuous wave method in which the microwave frequency, \(v\), is held constant and the magnetic field strength, \(H\), is swept. The EPR spectra were obtained with a Bruker Instruments model EMX EPR spectrometer using filter samples placed in quartz tubes. One quarter of a Pallflex glass fiber filter or Teflon membrane filter was cut out, folded to cover the particle collection side, rolled into a small cylinder, and inserted into a quartz EPR tube until reaching 1.5 cm from the bottom of the tube. The EPR parameters were: 100 kHz, X-band; microwave frequency, about 9.25 GHz; attenuation 7dB; modulation amplitude, 4 G; time constant, 1 s; receiver gain, 2500; scan time, 2 min; scan range, 200 G. All measurements were done at room temperature. The radical intensities were quantified using a standard curve of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The \(g\)-values that describe the EPR peak positions were estimated using DPPH (\(g=2.0036\)) as a standard. In order to determine how the
radical intensity changes over time, we measured two duplicate quarters from one same glass fiber filter loaded with PM for 5 times within 6 months. Filter samples placed in quartz tubes were stored in -10 °C freezers before each measurement. In order to examine the effect of filter type on the radical measurement, we compared six pairs of duplicate samples: one set on glass fiber filters and the other on Teflon filters.

Results and discussion

**Decay of free radicals over storage time.** The intensity of radicals dropped by 15% in the first two months since first measurement, and stabilized after that (Figure 1). The g-value and peak-to-peak line width $\Delta H_{p-p}$ had no significant changes over storage time. The first EPR measurement was not performed until 8 days after the particulate sample was taken. It was likely that an even sharper decrease already occurred before the first EPR measurement at Day 9. Decay of thermally generated free radicals in nuts was studied by Yordanov. Free radical intensity in thermally treated flakes of almond decreased over time: a sharp decrease by 50% was observed in the first two days and remains practically unchanged over a period of 15 days. It took only two days for the radical intensity to stabilize in the study of almond nuts, while in the present study of wood and coal emissions it took two months. The contrast here may reflect the effects of different source materials, thermal treatment procedures and sample storage conditions. One possible reason for the decrease of radical intensities within the first two months of the storage time is the ‘oxygen effect’ \(^9\)\(^10\), the rapid decrease of radical intensities when the oxygen diffuse through the carbonized specimen. The decrease in radical intensity may be caused by the physical interaction between the radicals in the specimen and
oxygen, a paramagnetic gas. There are probably two types of initially formed radicals: one short-lived and the other environmentally persistent. The environmentally persistent radicals are thought to account for the stabilized radical intensities 2-6 months after the samples were taken (Figure 1). These radicals are inactive and chemically stable due to the delocalization of the unpaired electron over many conjugated or aromatic chemical bonds \(^{11}\). The lifetime of some unpaired electrons, strongly delocalized over the condensed aromatic rings in charcoal or coal, can be extremely long \(^{12}\). Autopsy lung tissue from coal miners exhibited similar free radical activity as that of coal dust \(^{13}\). The intensity of unpaired electrons in charcoal produced today is comparable to that found in the pyramids in Egypt \(^{9}\). Some radicals found in cigarette tar have an essentially infinite lifetime \(^{14}\). Radicals on PM2.5 collected from urban air are remarkably persistent and can be observed in samples that had been stored for several months \(^{15}\).

Figure 1. The change of radical intensities over storage time. The first EPR measurement was not performed until 8 days after the particulate sample was taken. The intensity of radicals dropped by 15% in
Influence of filter type on free radical measurement. The radical intensities are higher and the g-values of the radicals are smaller on Teflon filters than on the duplicate glass fiber filters (Figure 2). On average, radical intensities are 15% higher on Teflon filters. The mean g-values are 2.0027 and 2.0035 for the PM samples on the Teflon filters and glass fiber filters, respectively. Both of the intensity and g-value differences by filter type may be caused by the interaction of the radicals with inorganic impurities in the glass fiber filters. The g-values give information on localization of unpaired electrons in free radical molecules. Free radicals in the PM may couple magnetically with the inorganic matter in the filter. Silbernagel 16 suggested that interactions with the inorganic matter may render some radicals invisible in the EPR, and change the properties of others; demineralized coals were thus suggested for EPR experimental work when studying radicals in the coal. Transition metal impurities, especially Mn(II) and Cu(II) ions, can suppress the detection of organic radicals in humic substances.17

Figure 2. The effect of filter type on free radical measurement. Six pairs of duplicate samples collected on
glass fiber filters and Teflon filters were compared. The glass fiber filters (Pallflex) were from Gelman Sciences Inc. and Teflon filters from SKC Inc.

Accurate determination of g-values is crucial in understanding the nature of the free radicals. The g-values of EPR spectra can be used to assess whether a radical is carbon-centered or oxygen-centered: whether the unpaired electron is located on a carbon or oxygen atom. Carbon-centered radicals have g-values that are close to the free electron g-value 2.0023\textsuperscript{10, 18}. Carbon-centered radicals with an adjacent oxygen atom have higher g-values in the range of 2.003-2.004, while for oxygen-centered radicals have g-values that are >2.004\textsuperscript{18}. The impact of filter type on g-value measurement warrants attention when we interpret different results in the literature.

**Chemical speciation of radicals.** EPR spectra from samples collected on Teflon filters were examined in more detail. Figure 3 shows the EPR spectra of the DPPH standard and two typical PM samples from wood and coal combustion. The g values of the PM samples are close to the free electron g value of 2.0023. This is indicative for carbon-, rather than oxygen- or nitrogen-based free radicals\textsuperscript{10}. The g-values do not fall in the range of 2.0033-2.0038 that is characteristic of semiquinones\textsuperscript{19}. The line width $\Delta H_{p-p}$ for PM from wood combustion is 5.8 G and that from coal combustion 6.4 G. The EPR signals are very similar to those from diesel particulate matter which had a g value of 2.0029 and line width of 5.3-6.0 G\textsuperscript{10}, and different from the principal radical species, semiquinone, in cigarette smoke with g-value=2.0035 and $\Delta H_{p-p}$=6.6 G
Figure 3. Typical EPR spectra of the radicals on PM emissions from wood and coal, and that of the radical standard DPPH (g=2.0036). The g-values of the radicals on PM emissions are close to the free electron g-value (2.0023) which is indicative for carbon-, rather than oxygen- or nitrogen-based free radicals.

Figure 4 shows the relation between the intensity of radicals and the percentage of elemental carbon in the PM from wood and coal combustion. The strong correlation between intensities of free radical and elemental carbon (R=0.87, p<0.0001) suggests that the predominant radical species may be graphitic carbon. It has been claimed that free radicals are generated during the thermal breaking down of the carbon-hydrogen bonds in the formation of the condensed carbon rings\(^9\). The unpaired electron becomes highly stabilized in the \(\pi\)-bond system over the ring structure. They are similar to the type II radicals from the oxidative pyrolysis of tobacco, which are surface-associated or bulk polymeric, carbon-centered radicals containing vicinal oxygen\(^{21,22}\). While oxygen-centered radicals are the most studied and are involved in the pathogenesis of many
pulmonary diseases\textsuperscript{23}, carbon-centered free radicals may be also reactive to living cells and thus important in biology. The redox and biological activities of these carbon-centered radicals are worthy of evaluation.

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