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Contribution of Water-Soluble and Insoluble Components and Their Hydrophobic/Hydrophilic Subfractions to the Reactive Oxygen Species-Generating Potential of Fine Ambient Aerosols

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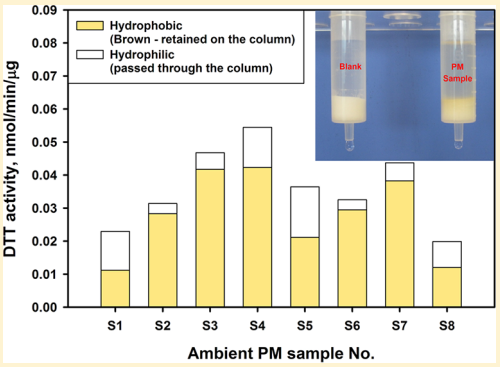
Supporting Information

ABSTRACT: Relative contributions of water- and methanol-soluble compounds and their hydrophobic/hydrophilic subfractions to the ROS (reactive oxygen species)-generating potential of ambient fine aerosols (D_p < 2.5 μm) are assessed. ROS-generating (or oxidative) potential of the particulate matter (PM) was measured by the dithiothreitol (DTT) assay. Particles were collected on quartz filters (N = 8) at an urban site near central Atlanta during January−February 2012 using a $PM_{2.5}$ high-volume sampler. Filter punches were extracted separately in both water and methanol. Hydrophobic and hydrophilic fractions were then subsequently segregated via a C-18 solid phase extraction column. The DTT assay response was significantly higher for the methanol extract, and for both extracts a substantial fraction of PM oxidative potential was associated with the hydrophobic compounds as evident from a substantial attenuation in DTT response after passing PM extracts through the C-18 column (64% for water and 83% for methanol extract; both median values). The DTT activities of water and methanol extracts were correlated with the water-soluble (R = 0.86) and water-insoluble organic carbon (R = 0.94) contents of the PM, respectively. Brown carbon (BrC), which predominantly represents the hydrophobic organic fraction (referred to as humic-like substances, HULIS), was also correlated with DTT activity in both the water (R = 0.78) and methanol extracts (R = 0.83). Oxidative potential was not correlated with any metals measured in the extracts. These findings suggest that the hydrophobic components of both water-soluble and insoluble organic aerosols substantially contribute to the oxidative properties of ambient PM. Further investigation of these hydrophobic organic compounds could help identify sources of a significant fraction of ambient aerosol toxicity.



INTRODUCTION

Several epidemiological studies have established the associations of adverse human health with ambient particulate pollution.[1−4] Toxicological studies indicate that oxidative properties of some aerosol constituents (e.g., organic compounds, transition metals, and particulate ROS (reactive oxygen species)) cause damage to cellular macromolecules (e.g., DNA, lipids, etc.).[5−8] Numerous chemical and biological methods have been developed to measure the ability of ambient particles to generate ROS. In chemical methods, specific reactions relevant to cellular mechanisms, such as oxidation of important antioxidants, are mimicked in a nonbiological environment; examples include dithiothreitol (DTT[9]), ascorbic acid (AA[10]), and respiratory tract lining fluid (RTLF[11−13]) assays. In-vitro assays measure the ROS generation using fluorescent probes by directly exposing live cells (e.g., macrophages) to the particles.[14,15] An increasing number of studies have linked the response of these assays with specific chemical components of PM.[16−18] The most commonly used medium to extract the particles in many of these studies was water. Although there have been studies using other solvents (e.g., RTLF, methanol, and dichloromethane[13,19−21]), these are generally limited compared to those focused on the water-soluble species.

Most of the water-insoluble aerosol species can be characterized for their chemical and toxicological properties by extracting them in organic solvents such as methanol, hexane, and dichloromethane. However, which components of aerosol extract obtained by these different solvents (including water) are toxic remains unresolved. An approach to narrow down the specific groups of compounds responsible for toxic

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properties of PM is the bulk separation of extracts based on specific physical and/or chemical characteristics and evaluating their individual contribution to PM toxicity. Based on their molecular configuration, ambient PM compounds exhibit a wide range of hydrophobicity, which can be used as a means for their segregation. A major class of organic aerosols speciated by their strong hydrophobicity is known as HULIS (humic-like substances). These organic macromolecules may comprise a sizable fraction of $PM_{2.5}$ mass (up to 50%[22−24]) and can be of both primary (e.g., biomass burning[23,25]), and secondary origin.[26−28] HULIS resembles terrestrial/aquatic humic and fulvic acids to some extent and a number of studies have investigated its properties, although detailed chemical characterization is not possible (as summarized by Graber and Rudich[29]). Lin and Yu[30] measured the oxidative potential of HULIS isolated from aerosol samples and recognized it as one of the major redox-active components in ambient PM. An enhanced knowledge of the toxicological characteristics of organic aerosols and relative contributions of their subcomponents (e.g., hydrophobic (HULIS) and hydrophilic fractions) would be useful to assess the overall health risks associated with ambient particles.

The goal of this study was to examine the relative contribution of water-soluble and insoluble PM species and their hydrophobic/hydrophilic subfractions on the oxidative potential (defined as the ability to generate ROS) of atmospheric aerosols. Ambient fine particles (D_p< 2.5 μm) were collected at an urban site near central Atlanta. Particles collected on high-volume filters were extracted in both water and methanol from separate filter punches to analyze water-soluble and insoluble species. An aliquot of the PM extracts (both water and methanol) was passed through a C-18 solid-phase extraction column to separate the compounds into hydrophobic and hydrophilic fractions. Oxidative potential of the PM for both the water and methanol extracts was measured by the dithiothreitol (DTT) assay. Chemical analyses of the filter samples, including water-soluble organic carbon (WSOC), water- and methanol-soluble brown carbon (BrC) and metals, and filter organic and elemental carbon (OC/EC), were also conducted to investigate the association of these aerosol properties with the measured oxidative responses.

■ EXPERIMENTAL METHODS

Sampling Location. Sampling for this study was conducted at the SEARCH (Southeastern Aerosol Research and Characterization) Jefferson street field site (JST). The site is located approximately 4.2 km northwest of central Atlanta and is sufficiently far (at least 2 km) from nearby freeways (I-75/85) not to be characterized as in an immediate "zone of influence". The pollutant sources impacting the site are representative of a typical mixed land-use urban environment, dominated by vehicular and industrial emissions, added to a larger regional background that includes measurable levels of biomass burning during cooler periods.[31] Further information about the site location and the results of earlier PM investigations conducted there can be obtained from previous publications.[32−34]

Sampling and Filter Extraction Protocol. Ambient fine particles ($PM_{2.5}$) were collected via prebaked 8 × 10 in. quartz filters (Pallflex Tissuquartz, Pall Life Sciences) using a high-volume sampler (Thermo Anderson, undenuded, nominal flow rate 1.13 m^3/min, $PM_{2.5}$ impactor). Eight integrated samples and two field blanks were collected during different 24-h periods over two weeks in January−February 2012. Table S1 in the Supporting Information (SI) provides the sampling schedule and $PM_{2.5}$ mass loadings. After collection, the filters were immediately wrapped in prebaked aluminum foil and stored in a freezer (−18 °C). Given the experimental condition of sampling (high-volume and relatively long duration), semivolatile aerosol species are not expected to be significantly collected on the filters and thus our study is not designed to capture the oxidative potential of these species.

Prior to chemical and DTT analysis, the filters were cut and the particles were extracted separately via sonication (15 min) using two solvents: deionized water (DI, Milli-Q; >18 MΩ) and methanol (HPLC grade). All the extracts were filtered using PTFE 0.45-μm pore syringe filters (Fisher brand) to remove insoluble materials and any filter fibers. The methanol extracts were evaporated to near dryness using a rotary evaporator (Büchi model R-205 with B-490 heating bath (56 °C)) and then reconstituted to a volume of 15 mL with potassium phosphate buffer (0.1 M, pH = 7.4). As a less polar solvent than water, methanol is likely to dissolve a majority of the water-insoluble organic species in addition to some water-soluble compounds. Chen and Bond[35] reported a very high extraction efficiency (∼ 95%) with methanol for the organic compounds present in laboratory generated particles from controlled wood pyrolysis experiments. Although it might be inappropriate to assume similar extraction efficiency for ambient particles from different sources, the assumption that the methanol extract contains more organics and a different subgroup of compounds than the water extract is reasonable. Although evaporating the methanol at 56 °C might cause loss of semivolatile species and associated DTT activity,[36] as noted earlier the filter sampling method was not designed to collect semivolatile PM components.

A fraction of these extracts, with unadjusted pH (varying from 4.9 to 7.1 among different samples), was passed through a C-18 solid phase extraction (SPE) column (octadecyl carbon chain bonded silica; 60A, 40−75 μm, 100 g, 20% carbon load; Sorbent Technologies) to separate the PM organic compounds into hydrophobic and hydrophilic fractions, as done in past aerosol HULIS studies.[22,37] The fraction that passed through the column ("hydrophilic") was collected, and the column was subsequently rinsed with methanol to (partially) recover the compounds retained on the column ("hydrophobic"). The methanol in the hydrophobic fraction was then evaporated and the samples were reconstituted in DI water. Thus, for each filter extracted in either solvent (DI water or methanol), a total of three samples were generated for chemical and toxicity analyses; the initial extract and hydrophobic/hydrophilic subfractions. A figure illustrating the experimental protocol of filter extraction and separation into hydrophobic/hydrophilic fractions is shown in the SI (Figure S1).

Chemical Analysis. An aliquot (approximately 2 mL) of the water extracts was used to measure the water-soluble organic carbon (WSOC) concentrations in PM samples using a Sievers Total Organic Carbon (TOC) Analyzer (model 900, GE Analytical Instruments; Boulder, CO). Due to interference from the residual organic solvent, neither of the reconstituted methanol extracts, hydrophilic and hydrophobic fraction of PM extracts, could be analyzed for WSOC content. However, the organic aerosol color (here referred to as brown carbon, BrC, which is the light absorption between wavelengths of 360 and 370 nm), which provides insight on organic aerosol sources/composition, was measured on both water and methanol

extracts and their hydrophobic/hydrophilic subfractions using a UV/vis spectrophotometer (USB4000 spectrometer, Ocean Optics, Dunedin, FL), coupled with a Liquid Waveguide Capillary Cell (LWCC-2100, World Precision Instruments, Sarasota, FL). The exact details of both WSOC and BrC measurement are provided in Hecobian et al.[38] Elements including metals were analyzed on both the water and methanol extracts of PM filters ($N = 8$) using magnetic sector inductively coupled plasma mass spectrometry (ICP-MS). To investigate the partitioning behavior of metals in SPE, the hydrophilic fractions (both water and methanol extracts) of three PM samples (S3, S6, and S8) were also analyzed for elemental concentrations.

A section (1.45 cm^2) of the quartz filters was used to measure the elemental and organic carbon content of the deposited PM using a thermal/optical transmittance (TOT) analyzer (Sunset Laboratory[39]) by NIOSH (The National Institute for Occupational Safety and Health) method. Independent of the filter samples, ambient concentrations of inorganic ions (sulfate (SO_4^{-2}), nitrate (NO_3^-), and ammonium (NH_4^+)) were measured using an Aerosol Chemical Speciation Monitor (ACSM; Aerodyne Inc.). Since ACSM measures PM_1 (instead of $PM_{2.5}$ collected on our filters), inorganic ions data were scaled by comparison to a $PM_{2.5}$ PILS-IC system (Particle-Into-Liquid-Sampler coupled to an ion chromatograph[40]) that ran concurrently at the site, but only for a fraction of the filter sampling period. Comparisons (ACSM and PILS-IC) showed good correlation ($R^2 > 0.85$ for all species, regression parameters shown in SI Table S2). $PM_{2.5}$ mass concentrations were monitored by a tapered element oscillating microbalance (Thermo Scientific TEOM 1400a), operating at 30 °C with an upstream nafion dryer.

Oxidative Potential. The oxidative potential of the ambient aerosols was measured by the DTT assay, which was conducted on both the water and water-reconstituted methanol extracts of PM, and also on the C-18 hydrophobic/hydrophilic subfractions. This assay monitors the ability of redox-active PM components to catalyze the reduction of oxygen (O_2) to superoxide radical by DTT.[41] At conditions of excess DTT, the reaction exhibits a linear rate proportional to the concentration of redox-active species in the test PM. The DTT assay analysis followed the methods described by Cho et al.[9] In brief, DTT oxidation was carried out by incubating the mixture containing PM extract (0.7 mL), DTT (1 mM; 0.1 mL), and potassium phosphate buffer (0.2 mL; 0.5 M, pH = 7.4, no Chelex or EDTA treatment) in seven separate 2-mL vials (conical microcentrifuge polypropylene tubes) submerged to near vial top in a water bath at 37 °C. At designated time intervals (0, 3, 6, 9, 12, 15, and 20 min), TCA (10% v/v, 1 mL) was added to individual incubation vials for quenching the reaction. An aliquot (0.5 mL) of this reaction mixture was withdrawn and combined with 5,5′-dithiobis-2-nitrobenzoic acid [(DTNB), 10 mM in methanol, 25 μL) in 1 mL of Tris-buffer (0.4 M Tris HCl, pH 8.9 in 20 mM EDTA). Light absorption of the product (2-nitro-5-mercaptobenzoic acid; TNB) formed by the reaction of DTNB with residual DTT was measured at 412 nm using a spectrometer (USB4000, Ocean Optics, Dunedin, FL). Overall DTT consumption (i.e., DTT activity) was determined from the linear regression slope of the 7 data points. DTT activity of multiple blanks ($N = 18$; sections of field blanks collected during sampling) processed along with samples was stable with a mean $\pm 1\ \sigma$ of 0.75 ± 0.07 nmol/min for water extracts and a higher value (1.12 ± 0.09 nmol/min) for methanol extracts. The reproducibility of the entire analysis was determined by repeating the test on three different equal sections of the same filter, which were extracted and analyzed for DTT separately. The results of the reproducibility test were highly consistent with a minimal standard deviation of calculated slope (<2% of mean; shown in Figure S2) for triplicate analysis.

RESULTS AND DISCUSSION

Ambient Aerosol Composition. Figure 1 shows the chemical composition of ambient particles for the various days' filter samples were collected at the site. Reconstituted mass from measured chemical species accounted for more than 85% (average 93 ± 8% (1σ)) of $PM_{2.5}$ mass measured by the TEOM. Note, a conversion factor of 1.6 was applied to calculate the organic matter (OM) concentration from OC.[42,43] Missing mass includes insoluble metals and mineral dust,[44,45] and possibly other organic material (i.e., actual factor possibly larger than 1.6) and semivolatile material lost from the filter. Comparison between OC measured on the filters and a colocated online OC/EC monitor (Sunset Laboratory) shows good agreement ($R^2 = 0.98$; slope = 1.19; SI Figure S3). The chemical composition varied widely during the sampling period, with 40−80% OM, 4−11% EC, 4−15% NH_4^+, 6−14% NO_3^-, 3−20% SO_4^{-2}, and 2−8% water-soluble metals in the $PM_{2.5}$ mass. Fractions of various chemical components in $PM_{2.5}$ are generally consistent with previous observations that show organic matter dominates the fine particulate mass in this region.[31,33,46,47] The figure shows that approximately half of the total OM (30−60%) is water-soluble (WSOM). Earlier studies conducted in a similar season (January−February) have indicated that the two major sources of WSOC at the site are biomass burning and secondary photochemical reactions,[31] however, a non-negligible fraction can also be attributed to primary vehicular emissions.[47] The significant variation in PM chemical composition among different samples facilitates the

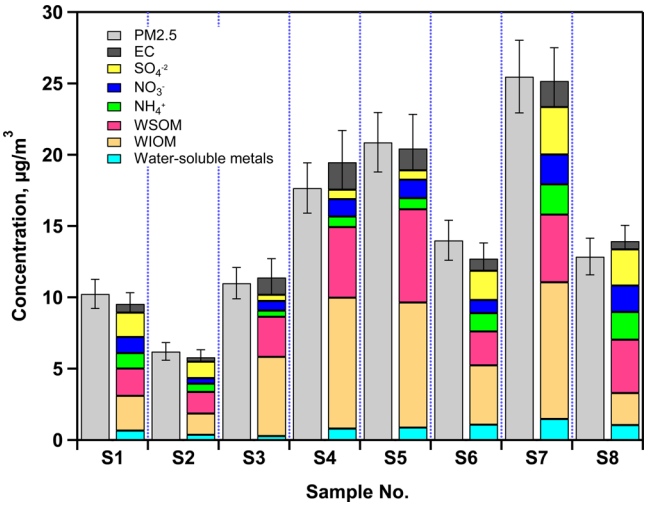

Figure 1. Chemical composition of the 8 $PM_{2.5}$ samples. WSOM: water-soluble organic matter; WIOM: water-insoluble organic matter (OM-WSOM); EC: elemental carbon. Samples S1−S8 represent different filters collected from JST site on different days in January−February 2012. Error bars denote the propagated total uncertainties from that in individual analysis.

use of statistical approaches for investigating the associations of oxidative potential with aerosol constituents.

Oxidative Potential: Water Extracts versus Methanol Extracts. Figure 2 shows the results of DTT assay conducted on water and methanol extracts from the eight filter samples. The DTT activity was normalized by particle mass (i.e., mass on the extracted filter section) and thus expressed in the units of nmol (of DTT consumed)/min./μg (of PM mass). The results expressed in these units represent oxidative potential as an intrinsic property of the particles and thus allow us to make a direct comparison among different PM samples and between their water and methanol extracts, independent of ambient concentrations.

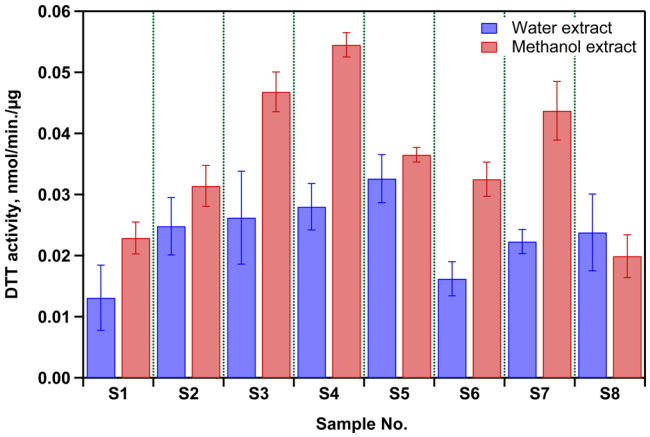

Figure 2. Results of the DTT assay conducted on the water and methanol extracts of various $PM_{2.5}$ samples. Error bars denote the standard error in slope (DTT reaction rate) of single analysis.

Our measurements of DTT activity are in the range of typical levels observed for ambient particles in other studies (0.005–0.170 nmol/min./μg).[48] In general, the response of the DTT assay was significantly (p = 0.02 for a paired t test) higher for the methanol versus water extracts (mean $\pm 1\sigma$ methanol to water extract ratio of 1.6 $\pm$ 0.4). Evidence for significant oxidative potential (measured by DTT assay) associated with insoluble PM species was also suggested by Akhtar et al.[49] In a recent study comparing the oxidative properties of ambient aerosols collected by different samplers (filter, impaction, and biosampler), Daher et al.[50] reported the highest oxidative potential (on a per PM mass basis) for the method that was thought to be most efficient in capturing both the insoluble and soluble species (biosampler). Our results are in agreement with these studies and demonstrate an important finding of the present work; that water-insoluble components of the ambient PM possess significant oxidative properties that may be relevant in toxicological studies. The increase in DTT activity of the methanol extracts was much higher for some of the samples (e.g., S3, S4, S6, and S7) than others. This differential increase in mass normalized DTT activity suggests that aerosol chemical composition has a major role in PM oxidative potential and is discussed later in the paper.

Hydrophobic and Hydrophilic Fractions. The fractional split in DTT activity from hydrophobic and hydrophilic PM fractions is shown in Figure 3. The contribution from the hydrophobic fraction was calculated by subtracting the remaining activity after passing the PM extract through the C-18 column (hydrophilic DTT), from the total activity prior to SPE separation (i.e., hydrophobic = total − hydrophilic).

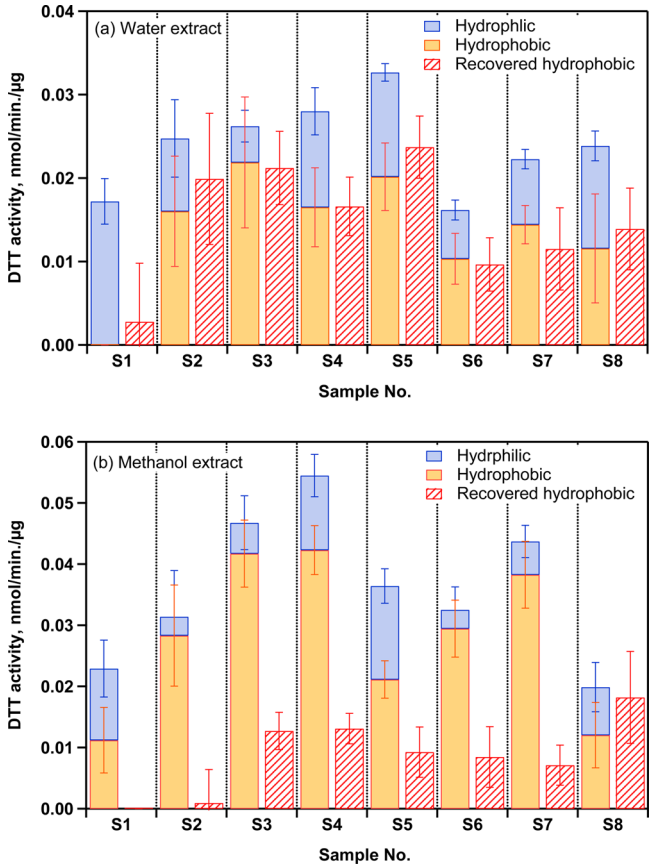

Figure 3. Contribution from the hydrophobic (both calculated by difference method, i.e., total hydrophilic, and that recovered from the C-18 column) and hydrophilic (penetrated C-18 column) fractions of ambient $PM_{2.5}$ in the DTT activity of their water (a) and methanol extracts (b). Error bars denote the standard error in slope (DTT reaction rate) and are propagated in calculating the DTT activity of hydrophobic fraction by difference method.

DTT activity measured directly on the eluted hydrophobic fraction was also compared with that obtained from the difference method and is shown in the same figure (hatched bars). These tests show that passing the PM extracts through the C-18 column removed a large fraction of the aerosol oxidative potential (with the sole exception of S1-water extract, whose activity is all contributed by its hydrophilic components). Contribution from hydrophobic compounds to DTT activity was higher in the methanol extracts (median = 83%) compared to water extracts (median = 64%). This is likely due to the additional water-insoluble hydrophobic compounds in methanol extracts that apparently make a significant contribution to DTT activity.

Many of these hydrophobic compounds that contributed to DTT activity in the methanol extracts could not be recovered from the C-18 column. Although DTT activity measured directly on the eluted hydrophobic fraction of water extracts was in reasonable agreement with that obtained from the difference method (p = 0.765 for a paired t test to check the statistically significant difference), this was not true for the methanol extracts (p = 0.001). For methanol, a significant fraction of the DTT activity was not recoverable from the column, indicating that highly hydrophobic compounds (i.e., compounds with strong interaction with the C-18 stationary

phase) accounted for most of the methanol-hydrophobic DTT activity.

Overall, for both water and methanol extracts these results indicate the substantial contribution of hydrophobic (e.g., HULIS) compounds to the oxidative properties of ambient particles measured by the DTT assay.

Association between Oxidative Potential and PM Chemical Composition. The wide range of oxidative responses measured by the DTT assay suggests a sensitivity of the analytical method to the chemical composition of various PM samples. A linear regression analysis was performed between measured chemical constituents and the assay response to roughly identify the compounds linked to PM oxidative potential. The results are summarized in Table 1. It is noted that the regression was conducted on the mass normalized levels of PM parameters. These units differ from the traditional expression of air pollutants, which are normalized by the volume of air. Although both approaches have their advantages and disadvantages, in the context of this analysis, the mass-based approach benefits by diminishing the effect of covariability among PM components and any confounding relationships with the assay response.

Table 1. Results of a Univariate Regression Analysis between PM Constituents and DTT Activity of Ambient PM Samples (N = 8)[a]

	correlation coefficient (R) and significance level (p)			
	water extract		methanol extract	
PM constituents	R	p	R	p
OC	**0.78**	0.02	**0.84**	0.01
EC	0.30	0.48	**0.74**	0.03
WSOC	**0.86**	0.01	0.14	0.74
WIOC	0.56	0.15	**0.94**	0.00
BrC	**0.78**	0.02	**0.83**	0.01
SO_4^{-2}	−0.65	0.08	−0.79	0.02
NO_3^-	−0.37	0.37	−0.68	0.06
NH_4^+	−0.57	0.14	−0.83	0.01
S	−0.69	0.06	−0.74	0.04
V	−0.43	0.29	−0.57	0.15
Cr	0.37	0.37	−0.25	0.56
Mn	0.27	0.52	0.26	0.53
Fe	0.27	0.52	−0.43	0.29
Co	0.24	0.57	0.33	0.42
Ni	0.14	0.75	−0.27	0.52
Cu	0.09	0.83	−0.53	0.18
Zn	0.02	0.97	−0.38	0.35

[a]Regression was done on the mass normalized levels of both DTT activity and PM constituents. Bold values represent R > 0.70.

As shown in Table 1, DTT activity of the water extracts is most correlated with the WSOC fraction of the PM (R = 0.86; p = 0.01), as has been observed in a number of other studies.[16,17,51] Although DTT activity for the water extracts is also correlated with OC (R = 0.78; p = 0.02), this association is likely driven by the contribution of water-soluble compounds present in the OC fraction (SI Table S3, R (WSOC vs OC) = 0.58). Note that water insoluble organic carbon (WIOC = OC − WSOC) and DTT (R = 0.56) are less correlated than WSOC and DTT.

In contrast to the regression results on water extracts, DTT activity of the methanol extracts is strongly correlated with WIOC (R = 0.94; p = 0.00), but not with WSOC (R = 0.14) fractions. The results showing the relationship of water-insoluble organic compounds with DTT assay are novel and emphasize the importance of water-insoluble organic aerosol components in toxicological studies. The high oxidative potential of these water-insoluble compounds probably contributes to the correlation of OC with DTT in the methanol extracts (R = 0.84; p = 0.01).

Participation of water-insoluble organic compounds also explains the differential increase in DTT activity of the methanol extracts over water extracts. This is illustrated in Figure 4 which plots the percentage increase in DTT activity against the water insoluble fraction of OC (WIOC/OC). The high coefficient of determination (R^2 = 0.77; p = 0.003) supports the additional contribution from these water-insoluble organic compounds in the DTT activity of the methanol extract. The major sources of these water-insoluble species in an urban environment are likely primary vehicular emissions.[52,53] Thus, the correlation of DTT activity with EC fraction (Table 1; R = 0.74), a marker of primary emissions,[54] may be interpreted as a confounding relationship driven by the colinearity of EC and WIOC (R = 0.82; SI Table S3).

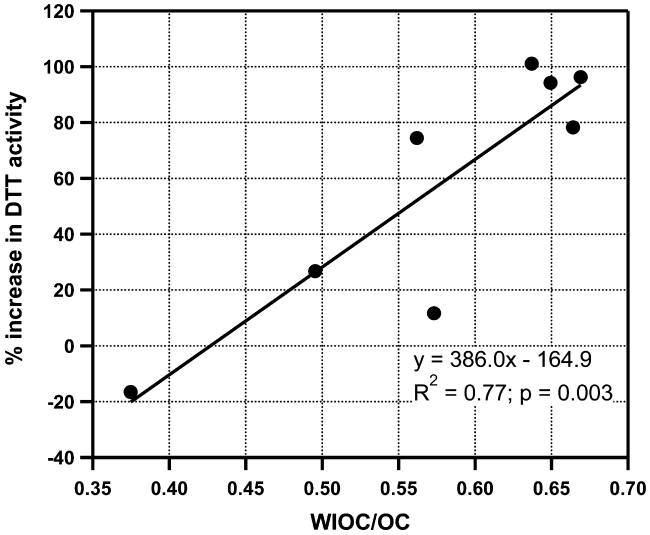

Figure 4. Correlation between percentage increase in DTT activity of methanol extract over water extract and the water insoluble fraction of $PM_{2.5}$ organic carbon (N = 8).

Another parameter of the atmospheric organic compounds, BrC, is also correlated with DTT, in both the water (Table 1; R = 0.78; p = 0.02) and methanol (R = 0.83; p = 0.01) extracts. BrC denotes the light absorbing organic compounds (chromophores) that appear brown.[55] Studies conducted in Atlanta and other southeast locations suggest that biomass burning and fossil fuel combustion are the direct sources of these compounds, along with a regional background BrC component apparently from aged SOA (secondary organic aerosol[38,56]). The brown organic compounds from these filter extracts (both water and methanol) were mostly hydrophobic (SI Figure S4), as seen in other studies on HULIS.[29] Roughly 70−90% of the brown organic matter was retained on the C-18 column, and, similar to DTT activity, a large fraction of the retained BrC from methanol extracts could not be recovered (Figure S4b). This explains the strong correlation of BrC with DTT activity and supports the indirect evidence for higher

oxidative activity associated with the hydrophobic organic PM species compared to hydrophilic. We note that the BrC measurement provides a relatively easy method to estimate the bulk hydrophobic mass of organic aerosols, which may be relevant in assessing the oxidative potential of ambient PM.

We did not observe a strong positive correlation of DTT activity with any metal analyzed in either water or methanol extracts of PM (Table 1; only important elements are shown). Table 2 shows the ambient concentrations of these elements obtained from both water and methanol extracts and their percentage in the respective hydrophilic fractions (average of three of the eight filter samples where sufficient filter remained for analysis). The results of one-sample t test revealed that the mean concentration of some transition metals, such as V, Mn, Zn, and Fe, were significantly ($p < 0.06$) lower in the hydrophilic fractions compared to their initial extracts (containing both hydrophilic and hydrophobic components). In contrast, S (as a reference), Cr, Co, and Cu were less affected by the column ($p > 0.20$) (mostly hydrophilic). We hypothesize that some fraction of these metals (V, Mn, Zn, and Fe) might be complexed with hydrophobic compounds and thus removed on the column along with those organic species.[57−59] Although removal of metals on the C-18 column complicates clearly distinguishing the contribution of organic compounds in PM oxidative potential, an overall poor correlation of metals with DTT in both water and methanol extracts indicates that these elements do not play a major role in the DTT activity of ambient PM relative to organic species.

Table 2. Ambient Concentrations (Average ±1σ of Three Samples: S3, S6, and S8) of Elements (Including Metals) Obtained from the PM Extracts (Both Water and Methanol) and Their Percentage (Average ± 1σ) in the Respective Hydrophilic Fractions[a]

	water extract		methanol extract	
metal	initial extract, ng/m^3	hydrophilic/ initial extract, %	initial extract, ng/m^3	hydrophilic/ initial extract, %
V	0.11 ± 0.08	6 ± 6	0.07 ± 0.05	4 ± 6
Mn	1.48 ± 0.19	41 ± 17	0.70 ± 0.23	55 ± 27
Zn	9.92 ± 1.91	48 ± 9	5.40 ± 0.60	73 ± 30
Fe	5.39 ± 1.83	55 ± 37	3.76 ± 1.26	44 ± 11
Ni	0.13 ± 0.07	186 ± 217	0.12 ± 0.03	54 ± 28
Cr	0.06 ± 0.05	153 ± 32	0.03 ± 0.03	63 ± 9
Co	0.01 ± 0.00	59 ± 14	0.01 ± 0.00	109 ± 30
Cu	4.95 ± 1.82	113 ± 46	6.19 ± 1.10	71 ± 49
S	560 ± 346	95 ± 1	3275 ± 218	100 ± 2

[a]Metals in the upper part of the table tend to have both hydrophilic and hydrophobic components, whereas metals in the lower part tend to be mainly hydrophilic. S serves as a reference for the method.

Oxidative Properties of Hydrophobic Organic Compounds. Our results show a major contribution of hydrophobic organic compounds in the oxidative properties of ambient particles measured by the DTT assay. Lin and Yu[30] also showed that hydrophobic compounds (HULIS) isolated from ambient PM samples collected in the Pearl River Delta region (China) accounted for about 80% of the DTT activity of water-soluble organic matter. However, a quantitative comparison of the results of these two studies might not be appropriate since, unlike their experiment, we did not chelate our PM samples. The C-18 column used in our tests is one of the several solid-phase extraction techniques which have been traditionally applied to isolate humic substances from soil and aquatic systems.[29] The other resins which have also been extensively employed include Oasis HLB and XADs (e.g., XAD-2, XAD-4, and XAD-8).[22,37,60] The primary force for separation of hydrophobic compounds on these columns is physical adsorption, which is also a function of the solution pH. However, in the atmospheric samples, pH does not markedly affect the retention efficiency for organic compounds. For example, a study conducted by Krivacsy et al.[22] using a C-18 column showed only a marginal increase (∼10%) in the retained fraction of ambient WSOC by decreasing the pH from 7 to 2. Since pH of the aerosol extracts used in our experiments varied significantly among different samples, it could have an effect on the removal of species which could enhance oxidative potential. To test it, we chose two different filters, each extracted separately in DI water (pH of the extracts ∼5.5) and phosphate buffer (0.1 M, pH = 7.4) and measured their DTT activity before and after passing through the C-18 column. No significant difference was observed in removal of DTT activity between the two extracts (water and buffer) for both samples (data not shown). The typical fractions of hydrophobic and hydrophilic substances in ambient WSOC, attained by using different columns (C-18, Oasis HLB, XAD-8) are also comparable (∼50:50%).[22,24,37] This apparent similarity in the recovery of ambient organic fractions suggests that the results of atmospheric studies using different resins to characterize hydrophobic fraction (HULIS) are largely independent of the column type. The characteristics of HULIS reported in other studies may thus be useful in assessing the linkages of specific organic species with DTT activity determined from our study.

There have been attempts to identify the major functional groups present in both hydrophobic and hydrophilic fractions of atmospheric organic compounds. Sullivan and Weber[60] isolated the different fractions of ambient WSOC from Atlanta PM samples by employing size exclusive chromatography coupled with an XAD-8 column. While the hydrophilic fraction was found to contain only aliphatic compounds (short chain aliphatic acids and carbonyls (<4−5 C atoms), saccharides and polyols)), higher C number acids and aromatic compounds (e.g., aromatic acids, phenols) dominated the hydrophobic fraction. Very similar results were obtained from the solid-state ^{13}C nuclear magnetic resonance (NMR) spectra of these fractions measured by Sannigrahi et al.[61] The hydrophilic fraction was enriched with *O*-alkyl and *N*-alkyl groups, while aromatic groups were exclusively associated with the hydrophobic fraction. It is possible that hydrophobic fraction in methanol extracts of aerosols might be chemically different with even a higher aromatic content than in the water extracts. Although detailed chemical characterization of these two fractions have not been possible to date, it appears from the above discussion that the compounds retained on the C-18 column would most likely include some of the redox-active species such as nitro-PAHs and quinones. These compounds have been shown to catalyze the oxidation of cellular antioxidants (including thiols) and generate ROS[9,41,62,63] and thus are strong candidates that may contribute to the oxidative properties of hydrophobic PM fractions. When combined with redox-active metals, these organic compounds can enhance Fenton-type reactions and the production of ROS, making the presence of both organic and metal redox-active species in the hydrophobic fraction of interest.[64,65]

We note that the regression results presented in this work are based on a relatively small sample set ($N = 8$) and thus the

association between PM oxidative potential and chemical constituents deduced from these results should be interpreted with caution. Although hydrophobic organic components appear to make a substantial contribution in the PM oxidative potential, a significant fraction of DTT activity was also associated with the hydrophilic components, especially in water extracts (e.g., nonaromatic organic species and possibly certain metals, such as those not retained on the column, Table 2). Furthermore, the DTT assay used in our experiment represents just one of the many potential modes of aerosol toxicity simulating the interaction of thiols and PM components. Other plausible oxidative mechanisms such as those involving metal-catalyzed hydroxyl radical generation (Fenton reaction)[10,11] are not covered by this assay. Therefore, for a better understanding of the overall ability of ambient particles to generate ROS and the subsequent toxicity mechanisms, further studies should be conducted involving multiple assays and/or biomarkers, while effectively segregating the contributions from different PM components, on a larger data set.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Tables S1−S3 and Figures S1−S6. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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