Relationships between composition and pulmonary toxicity of prototype particles from coal combustion and pyrolysis

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Abstract

The hypothesis that health effects associated with coal combustion fly-ash particles are exacerbated by the simultaneous presence of iron and soot was tested through two sets of experiments. The first set created prototype particles from complete and partial combustion, or oxygen free pyrolysis of a high iron Illinois bituminous coal in an externally heated drop-tube furnace. The second experiment created prototype particles consisting of iron and soot in various concentrations from doped ethylene Burke–Schumann flames. Size-classified samples from the coal tests were separated into coarse (>2.5 μm), fine (0.5–2.5 μm) and ultrafine (<0.5 μm) fractions, and analyzed for total carbon, elemental composition, and detailed iron and sulfur speciation. In a similar manner, ultrafine particles from the ethylene flame tests were also analyzed for total carbon and elemental composition. Pulmonary inflammatory responses were determined after intratracheal aspiration of 100 μg samples in female CD1 mice. IL-6 and neutrophil responses were monitored as markers of inflammation. With carbon present, the coal data suggested that the ultrafine particles containing soot were more toxic than fine or coarse particles containing char, even though the iron and sulfur speciation varied only slightly with particle size. Iron and sulfur chemistry were, however, dependent on the extent of carbon burnout achieved. In the absence of carbon, ultrafine particles (high in bisulfates and semi-volatile alkali metals) were less toxic than the fine fraction (high in oxidized iron and sulfates). Iron-soot particles created from ethylene flames were more toxic than an equivalent physical mixture of...
irons and soot, and the toxicity depended primarily on the soot concentration. However, taken as whole, these data do not support the notion that iron and soot interact to enhance pulmonary inflammatory responses.

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1. Introduction

Epidemiological data have suggested a link between adverse health effects and particles containing transition metals, including iron [1]. Toxicological studies have also suggested a link between inhalation of particles containing transition metals and oxidative stress in lung cells [2–4] and it has been postulated that inhalation of coal combustion fly-ash particles causes oxidative stress [5] because of the presence of iron [6]. Furthermore, iron is present in atmospheric particles from cities including St. Louis [7], where increased mortality has been attributed to levels of PM$_{2.5}$ [8]. Subsequently, it was suggested from in vitro studies that ferric iron in an aluminosilicate glass phase served as the source of bio-available iron from coal fly-ash [9], and that the process to make iron bio-available depended on ash particle size [10]. Kennedy and co-workers have further investigated particle toxicity of ultrafine iron and soot aerosol from ethylene gas diffusion flames and concluded that oxidative stress resulted from a synergistic interaction between soot and iron [11,12]. They also found that soot burnout mechanisms often resulted in the formation of mixed iron-soot particles, and iron oxide particles largely separate from soot.

In a previous paper, we found a link between coal ash toxicity and carbon in the ash for a range of coals burned in a variety of combustion equipment [13]. In these experiments, only two ultratine samples contained significant amounts of carbon thought to be soot. This is consistent with reports by Veranth et al. [14] indicating over a third of the carbon in fly-ash is present as soot, and that this is affected by the application of combustion modifications for NO$_x$ control. Coal ash particulate matter is emitted with a size distribution containing three modes: a vaporization mode (containing particles denoted as ultrafine, <0.5 μm), a fine fragmentation mode (fine, 0.5–2.5 μm) and a coarse fragmentation mode (coarse, >2.5 μm) [15]. The vaporization mode contains, and is often enriched in, volatile and semi-volatile components, including sulfur and metals, which have vaporized in the flame and subsequently nucleated. Any carbon found in this fraction would most likely be in soot form [14]. Previous X-ray absorption fine structure (XAFS) spectroscopy analysis showed the sulfur on particles containing soot is present enriched in thiophenic forms [13]. The two fragmentation modes have similar bulk mineral compositions, including silicates, although they may be coated with condensed or reacted semi-volatile metals, such as arsenic and selenium [16], while most of the carbon mass likely consists of unburned char. Although one might suspect that the vaporization mode might be the most toxic, previous work suggests that the fine fragmentation mode may also have toxic effects. The objective of this study was to test the hypothesis that particles from coal combustion cause lung injury due to the simultaneous presence of iron and carbon. Experiments were conducted on a single high iron content coal that allowed systematic variations of carbon content in the particles emitted. These tests involved various degrees of carbon burnout, including a pyrolysis condition with little or no carbon burnout. These experiments produced ultratine samples with ~8% iron and low (nearly zero) carbon, ~2% iron and 80% carbon, and <0.5% iron and 95% carbon. The coal tests were complemented by studies on soot particles containing iron from ethylene diffusion flames. These latter experiments, similar to those conducted by Kennedy and co-workers [11,12], were designed to complement the coal ash toxicity tests by using similar toxicological markers for both fuels. They also allow for independent control over a wider range of the amounts of iron and carbon in the samples.

2. Materials and methods

2.1. Generation and collection of size-classified coal combustion and pyrolysis particles

Nine size-classified pulverized coal residue particle samples were generated using a three-zone, programmable bench-scale (1 g/h) drop-tube furnace. For this study, a single high iron, high sulfur Illinois bituminous coal was used. The coal was burned in such a manner as to produce particles with no (or very low) carbon, medium carbon, and very high carbon (under pyrolysis conditions). In each case, particles were size-classified into coarse, fine, and ultratine fractions. Table 1 provides the proximate, ultimate, and elemental analyses of this coal. Details regarding the furnace are provided elsewhere [13]. All three furnace zones were maintained at 1350 °C. Flow rates of the annular and transport gases (air or nitrogen) were maintained at 12.0 and 0.5 L/min,
respectively, resulting in a calculated Reynolds number and residence time within the alumina reactor of approximately 340 and 2 s. From the furnace exit, the combustion gases and residue particles, at a flow of 12.5 L/min and at 80 °C to eliminate water condensation, were directed to a five-stage cascade cyclone (Thermo Electron Corp.) to collect particles greater than approximately 0.5 μm diameter [17]. Ultrafine particles (here defined as <0.5 μm diameter) were collected on 47 mm polycarbonate membrane filters located immediately after the cyclone. Once collected, the cyclone catches were combined to yield fine (0.5–2.5 μm) and coarse (>2.5 μm) particle samples.

The oxidizing environment within the drop-tube furnace was varied to control the amount of carbon in the particle samples. This was accomplished by sequentially substituting nitrogen for first the annular air and then both the annular and transport air. Thus, the first set of experiments were performed using air for both the annular and transport flows and this produced an overall extremely lean stoichiometric ratio (SR) of ~115. The second set of experiments used air for the transport flow and nitrogen for the annular flow and produced an overall SR ~ 4.6. However, oxygen diffusion from the transport flow likely produced an effective local SR much less than 4.6 in the vicinity of the coal particles and this resulted in only partial oxidation and significant unburned carbon in the ash. Finally, the third set of experiments used nitrogen for both flows producing pyrolysis in the complete absence of oxygen (SR ~ 0).

### 2.2. Generation and collection of iron-soot particles from ethylene Burke–Schumann flames

Three additional samples containing varying ratios of iron and carbon (soot) were generated using an ethylene-fueled Burke–Schumann co-annular laminar diffusion burner following designs of Santoro et al. [18]. Iron was added to the ethylene fuel using vaporized ferrocene with the relationship between the vapor pressure of ferrocene in ethylene and temperature, between 4 and 87 °C, provided by Jacobs et al. [19] The ferrocene vaporization system followed a design by Zhang and Megaridis [20]. The ethylene and over ventilated air flow rates for these experiments were 0.188 and 55.8 L/min, respectively. The ethylene was further adjusted to control the iron concentration in the resulting soot.

Particle emissions were sampled and collected on 47 mm polycarbonate membrane and 47 mm quartz fiber filters for subsequent chemical and toxicological analysis. Particle size analysis using a Scanning Mobility Particle Sizer (TSI Inc., model 3080/3022A) confirmed that these particles were all ultrafine (<0.5 μm). The three experimental conditions produced soot particles without iron (0% Fe), low iron (2.6% Fe), and high iron (13.3% Fe). Without iron addition, the ethylene soot produced a unimodal particle size distribution with a mean diameter of ~95 nm. As iron (a known soot oxidation catalyst) is added the mass emissions are notably reduced. Iron reduced the 95 nm mode, but produced bimodal behavior with the addition of a 25 nm mode.

### 2.2. Chemical characterization of size-classified particles

Samples between 500 and 1000 mg were needed for the multiple analyses which, depending on the combustion condition, required up to several weeks to collect. As expected, the medium and high carbon coal samples and soot-only ethylene samples were easier to collect (higher mass emission rates) than the zero carbon and iron-soot samples. All samples were analyzed by wavelength dispersive X-ray fluorescence (WD-XRF) spectroscopy [21]. Knowing the sample mass, the WD-XRF software can quantify elements with atomic numbers ≥9 (fluorine). For fly-ash samples the most stable metal oxide was assumed. Other (unidentified) sample mass was initially reported as unknown. For these samples the unknown sample component was subsequently positively identified to be carbon using a Sunset Laboratory Inc. analyzer (model 107 A). The carbon analysis, based on thermal optical transmittance, provided information on total carbon and the partitioning between organic carbon (OC) and elemental carbon (EC).

The coal residue samples were also analyzed by the element-specific techniques, 57Fe Mössbauer and XAFS spectroscopies. Mössbauer spectroscopy was conducted for the coal particle samples using a Halder Mössbauer (model 351) driving system operating in the symmetric
edge (XANE) spectra [23,24] was then performed for the sulfur X-ray absorption near edge (XANES) spectra [22]. Initial analysis of sulfur XAFS spectra followed conventional data reduction practice; least-square fitting for the sulfur X-ray absorption near edge (XANES) spectra [23,24] was then performed to quantify the distribution of sulfur among the different forms.

2.4. Toxicological characterization of the coal and iron-soot particles

Pulmonary instillation studies were carried out in female CD1 mice to compare the relative toxicity of all samples gathered from both the coal fed drop-tube furnace and the ethylene flame. Briefly, the mice were anesthetized with isoflurane and instilled via involuntary aspiration with 100 μg of particles suspended in 50 μL of sterile saline. The size-classified particle suspensions in saline were sonicated and then vigorously vortex-mixed immediately before instillation in an attempt to minimize particle agglomeration. Four or eighteen hours later, mice were euthanized and the lungs were cannulated via the trachea and lavaged with three volumes of sterile saline. The lung washes were analyzed for interleukin-6 (IL-6) and neutrophils (polymorphonuclear leukocytes, PMNs) as markers of pulmonary inflammation. Additional animals were instilled with saline to produce baseline control data or lipopolysaccharide (LPS) as a pro-inflammatory positive control.

3. Results and discussion

3.1. Samples examined

Nine particle samples from the complete combustion, partial combustion, and oxygen free pyrolysis of the Illinois coal were obtained and divided into three groups based on carbon burnout. The first group represented particles obtained under high excess air conditions (SR ~ 115) to eliminate all but trace quantities of carbon in the sampled particles (denoted as “zero carbon”). Unlike the medium and high carbon samples, it was possible to close the elemental mass balance for the zero carbon samples using only the XRF results. Carbon analysis of these samples indicated EC amounts always at blank levels and OC amounts that sometimes varied slightly above blank levels. The second group of tests produced prototype particles from coal combustion at SR ~ 4.6, that contained moderate amounts of carbon (denoted as “medium carbon”). The third group of tests produced prototype particles from coal pyrolysis with no oxygen (SR ~ 0), and produced particles containing all the residual (fixed) carbon with zero burnout (denoted as “high carbon”). Each group contained each of the three size classifications (coarse, fine, and ultrafine particles). Using the iron-doped ethylene diffusion flame three ultrafine particulate samples were produced with iron concentrations of 0% (100% soot), 2.6% (96.3% soot), and 13.3% (81.0% soot). These were augmented by two additional samples consisting of (1) 100% γ-Fe₂O₃ nanoparticles (Nanostructured and Amorphous Materials Inc.); and (2) a physical mixture of the γ-Fe₂O₃ nanoparticles and flame-formed soot (15.8% Fe). The form of γ-Fe₂O₃ was chosen because previous work [13] had shown this form of iron to be identified with coal ultrafine particles.

3.2. Elemental compositions

Figure 1 shows the elemental composition of each of the size-classified zero carbon, medium carbon and high carbon (pyrolysis residue) particle samples, as well as for the five ethylene flame related samples (all with diameters <0.5 μm). For the zero carbon particles, the coarse and fine fragmentation size classes had similar compositions, except for iron which was enriched in the fine particles. The source of this iron enrichment in the fine fragmentation mode is consistent with a hypothesized size range of extraneous pyrites, which here contained half of the total coal sulfur (Table 1). As expected, the ultrafine particles were rich in semi-volatile alkali metals but also contained appreciable calcium and iron. Consistent with surface condensation/reaction mechanisms, sulfur was present in increasing amounts as the particle size decreased.

The medium carbon particle samples contained appreciable amounts of carbon (50–80%). It is reasonable to assume that the form of carbon in the ultrafine fraction is soot, while the large amounts of carbon in the larger particles suggest the presence of char in both the fine and the coarse (fragmentation mode) fractions. The inorganic compositions of the fine and coarse fractions were similar, while the ultrafine fraction was enriched in semi-volatile alkali metals. Sulfur was present in all samples, but was enriched in the ultrafine fraction. The high carbon particle compositions followed similar inorganic component trends as the medium carbon samples, with the obvious exception that these samples contained significantly higher amounts of carbon. For this group, the coarse and fine fractions each contained 80% carbon (presumably primarily as char) and the ultrafine fraction contained over 95% car-
bon (as soot). The carbon partitioned similarly in both the medium and high carbon ultrafine samples (~5% OC and ~95% EC). These relatively low OC values suggest soot-like chemistry with relatively minor amounts of organic species. The ethylene derived samples contained only carbon (soot) and iron (oxide assumed), as shown by Yang et al. [11]. The physical mixture of γ-Fe₂O₃ nanoparticles and flame-formed soot contained 15.8% iron which is similar to the flame-formed (ferrocene doped) mixture containing 13.3% iron.

### 3.3. Iron and sulfur speciation

Examples of the iron Mössbauer and the sulfur XAFS spectra for selected samples are shown in Fig. 2 and a summary of results are presented for the nine particle samples in Fig. 3 for iron (upper panel) and sulfur (lower panel). The sample mass for the ultrafine fraction of the zero carbon particles was insufficient to allow Mössbauer analysis. However, previous analysis [13] of a carbon free ultrafine samples from a Utah coal showed the iron formed under those conditions to be γ-Fe₂O₃. For the zero carbon particles, the coarse and fine fractions contained all the iron as either hematite (α-Fe₂O₃) or amorphous Fe³⁺ in glass. For the medium carbon particles, the iron appeared as magnetite (Fe₃O₄ and γ-Fe₂O₃) and several reduced forms of iron. For the high carbon particles, the iron was present in even more reduced forms (including metallic iron) in the coarse and fine fractions suggesting that carbon was present in the char. This iron speciation trend from the high carbon to zero carbon samples is consistent with the study by Huffman et al. [25], and undoubtedly reflects the oxidation conditions of the combustion and the breakdown and oxidation of pyrite in the coal, first to iron sulfides (FeS + Fe₁₋ₓS) and metallic phases and then to iron oxides (magnetite, Fe₃O₄). Interestingly, only the fine medium carbon and high carbon samples exhibited carbide phases (Fe₃C) or alloys (austenite, Fe–C). Such phases did not appear to be present in either the corresponding coarse or ultrafine samples, and while their formation is rather unusual, it is consistent with the hypothesis that the fine fragmentation mode is enriched in extraneous pyrite particles. Interestingly, iron appeared almost exclusively as amorphous Fe³⁺ in the ultrafine fraction, consistent with the oxidation state of iron nanoparticles in sooting ethylene flames reported by Yang et al. [11], suggesting that the sooting ethylene flame can generate similar prototype particles at least in regards to iron and soot. Iron speciation data for the ferrocenedoped ethylene flame samples were not available but might be assumed to also consist of Fe₃O₄ (see Yang et al. [11]).

The sulfur spectra of the three zero carbon samples appeared quite different from those of the medium carbon and high carbon samples and exhibited peaks only at the high end of the XAFS energy range that arise from oxidized sulfur forms;
principally sulfate for the coarse and fine fractions and bisulfate for the ultrafine fraction. In contrast, the carbon-containing samples exhibited sulfur XAFS peaks due to reduced sulfur forms, principally organosulfur (thiophene), elemental sulfur, and iron sulfide, as well as peaks for oxidized forms, organosulfone, and sulfate. The sulfur speciation in the high carbon samples was independent of particle size and consisted only of reduced forms of sulfur, present in relatively small fractions compared to the high carbon contents.

3.4. Pulmonary inflammation

Pulmonary inflammation was quantified by the pro-inflammatory cytokine IL-6 and the recruitment of inflammatory cell neutrophils in the bronchoalveolar lavage fluid (BALF) at 4 and/or 18 h post-instillation. IL-6 (which is a heat shock protein) was chosen as a marker of inflammatory signaling, because it showed a more sensitive response for lung injury than other cytokines such as MIP-2 and TNF-α. For coal samples, the IL-6 response was greatest at 4 h and the neutrophil response was highest at 18 h and these higher-response data were used for further investigation. For flame-generated iron-soot particles, data from a pilot study indicated that IL-6 and neutrophils were greater at 4 h than 18 h, so the full scale bioassay with five types of particles was conducted only at the 4 h time point. Microalbumin in the BALF was also monitored but showed no significant effects for any of the samples (results not shown). The cytokine and neutrophil results are presented in Fig. 4.
3.4.1 Zero carbon particles

Surprisingly, in the absence of carbon, the ultrafine particles were not the most toxic. Compared to the ultrafine and coarse fractions, the influx of neutrophils was significantly higher after exposure to the fine fraction (0.5–2.5 μm) and the corresponding IL-6 response was also elevated, although not as significantly (see Fig. 4). This would suggest that the semi-volatile metals and the condensed bisulfate present in the vaporization-nucleation formed ultrafine fraction were less toxic than the fine fraction that contained partially silicated metals with condensed sulfate. The fine sample also contained the largest amounts of silicated Fe<sup>3+</sup>, consistent with the conclusion of Veranth et al. [9] that glassy Fe<sup>3+</sup> forms of iron are most bio-available to lung tissue in part due to leaching by the alveolar fluid.

3.4.2. Medium and high carbon particles

In the presence of medium and high amounts of carbon, the ultrafine fractions demonstrated the greatest potential for lung injury, especially with neutrophil recruitment, while the IL-6 response was less profound. The neutrophil response to the ultrafine fractions (whose carbon was soot) was significantly greater than for the fine and coarse fractions whose carbon was most likely char. The OC contents of the ultrafine fraction samples were fairly low (~5%), and it is unclear if OC was responsible for the increased toxicity of the ultrafine particles as the OC/EC ratios of the size classified samples (both medium and high carbon) were similar. The ultrafine samples also contained significantly less iron than the fine and the coarse samples, although the iron speciation was similar. The high carbon ultrafine sample, which contained much less iron, caused little noted difference in toxicity compared to the medium carbon ultrafine sample. Therefore, these data do not support the hypothesis that iron and soot in combination enhance the toxicity of the ultrafine particles. Pulmonary inflammation may correlate with soot carbon (as in Linak et al. [13]), but in these tests the soot concentration in the ultrafine samples did not vary by a sufficient amount (it was always over 80%) to allow extraction of a dependence of inflammation on soot alone. When compared with the zero carbon fine fraction, these ultrafine particles induced neutrophils and IL-6 responses to the same level.

3.4.3. Iron-soot particles

Neutrophil and IL-6 responses to the ethylene flame particles also failed to support the hypothesis that iron plus soot are more toxicologically active than soot alone. Instead, soot alone was at least (if not more) toxic than the flame-formed iron-soot samples. Neutrophils and IL-6 responses induced by soot alone were comparable or higher than those caused by iron-containing soot particles. Among samples containing iron, the toxicity was greater in the order: soot containing 13.3% iron > soot containing 2.6% iron > physical mix of γ-Fe<sub>2</sub>O<sub>3</sub> nanoparticles with soot > γ-Fe<sub>2</sub>O<sub>3</sub> nanoparticles alone. Especially, IL-6 responses to the flame-generated iron-containing soot particles were significantly greater.

Fig. 4. Indicators of inflammatory response after instillation of 100 μg of different particles in female CD1 mice. Neutrophil and IL-6 concentrations measured in BAL fluid. Neutrophil was measured 18 h post-exposure for the coal test and 4 h for the ethylene flame test. IL-6 was measured 4 h post-exposure for both tests. Statistical difference compared to saline control was indicated as: *p < 0.05; **p < 0.01.
as compared to non-flame-generated particles. The toxicity did not vary monotonically with soot content, as we suggested previously [13]. The results for soot alone are also at variance with those of Zhou et al. [26]. The reasons for this are not clear but may be due to different exposure techniques (inhalation vs. instillation) and/or differences in the biological markers used. Instillation was chosen because the small scale of the experimental equipment needed to control the coal fly-ash carbon composition and size classification produced insufficient particle concentrations for whole animal inhalation exposures. Although instillation does not necessarily model the mechanism by which particles reach the respiratory system, it is an accepted method to administer study particles of different toxicity and allows investigating multiple cell types and functions, which is more realistic than cell based assays.

4. Conclusions

A series of specially engineered tests, in which particles were systematically created to have various amounts of carbon and iron from coal combustion, showed that the presence of soot especially in the ultrafine fraction (<0.5 μm) affected pulmonary inflammation. The inflammatory responses were not, however, associated with total carbon in the coarse and fine samples, since this consisted of both char and soot. Also the tests did not support the hypothesis that the simultaneous presence of iron and carbon in particles might lead to increased pulmonary inflammation. These conclusions were confirmed by additional experiments involving iron-doped sooting ethylene diffusion flames, where the greatest increase in IL-6 production and neutrophil recruitment, two indicators of lung inflammation, occurred for soot particles alone, rather than from flame-formed iron-soot mixtures. Interestingly, a physical mixture of purchased γ-Fe₂O₃ nanoparticles with flame-formed soot gave a lower response than the same concentration of iron in flame-formed iron-soot particles.

Mössbauer and XAFS spectroscopies were used to determine the forms of iron and sulfur present in a suite of nine coal samples that represented three combustion conditions (including pyrolysis) and three particle size ranges. Both iron and sulfur exhibited surprisingly complex assemblages of different forms in the carbon-containing samples and simpler assemblages in the ash-only samples. For both elements, a wide range of oxidation states was present in these samples, especially those that contain significant carbon. The zero carbon samples exhibited iron and sulfur in their most oxidized forms, as Fe³⁺ in oxides and other phases, and as sulfate. In the carbon-containing samples, sulfur existed as iron sulfide, elemental sulfur, and thiophenic sulfur, in addition to lesser amounts of sulfate; whereas iron was found in metallic, sulfide and carbide phases, as well as in oxide phases. More specific observations are as follows: (1) bisulfate forms were present in the ultrafine samples, but were lacking in the fine and coarse samples; (2) thiophene sulfur and iron sulfides were most significant in the carbon-containing samples and absent from the zero carbon samples; (3) iron was present as carbide phases only in the fine size fractions of samples containing carbon; and (4) magnetite (+ minor maghemite) was only significant in the medium carbon samples. The ability to produce combustion particles with varying components and chemical reactivity is important to help understand the relative toxicity of complex combustion products and how the health effects may be related to particular sources of air pollution.

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