

# Thermal Release of Nitric Oxide from Ambient Air and Diesel Particles

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In recent years there have been numerous reports of an association between daily increases in particle air pollution ( $PM_{10}$ ) and daily increases in mortality and morbidity. Despite these reports, there continue to be unresolved issues with these studies, including the issue of a plausible biological mechanism by which small increases in daily particle levels could cause adverse human health effects. Chemical characterization of particles is a critical step in testing any biological mechanism. We have characterized gas-phase material associated with particles from various sources and found that measurable amounts of nitric oxide (NO) can be thermally released from these particles. NO is well-known to be an effective vasodilating agent at very low concentrations. In this work, release of NO from four NIST reference materials, two ambient air particulate samples, and two diesel particulate samples was quantified after heating the samples for 1 h. The amount of NO originating from particles depends on the temperature used to evolve this gas. NO levels ranged from 0.005 ng of NO/mg of sample at 37 °C to 1900 ng of NO/mg of sample at 140 °C. These experiments demonstrate that nitric oxide can be released from some particles at physiological temperatures (37 °C).

## Introduction

Over 50 peer-reviewed recent scientific papers report that daily increases in particle air pollution ( $PM_{10}$ , aerodynamic diameter less than  $10\ \mu m$ ) are associated with daily increases in adverse human health effects, primarily among the elderly (1–5). There have also been reports suggesting that daily increases in particle pollution are associated with daily increases in hospitalization of children with respiratory diseases (6, 7). However, some studies show that adverse human health effects are not associated with daily increases in  $PM_{10}$  or that these health effects are more strongly associated with other pollutants (8–12). None of the above statistical associations, however, establish a causal link between daily changes in particle concentrations and health effects because there is no known biological mechanism for such a link. Hypotheses advanced by several investigators are based on specific chemical compounds or classes of compounds, such as acidic aerosols (13–15) or transition metals (16–18), that could cause adverse human health

effects. Thus, the chemical characterization of particles is a necessary and critical step in testing such hypotheses. Such characterizations are experimentally important because particles from different sources may have different compositions, and future regulatory decisions should take into account a mechanistic understanding of the health effects of particles.

We have characterized gas-phase material associated with particles from various sources and found that measurable amounts of nitric oxide (NO) can be thermally released from these particles. It is known that nitric oxide in many ways can act like a hormone in that very small concentrations can result in a biological cascade yielding a significant biological effect such as vasodilation, neurotransmission, platelet regulation, and bronchodilation (19, 20). It is not known if ambient air concentrations of NO (several hundred ppb in urban areas) are high enough to cause vasodilation of lung tissue (21). However, clinical studies using high concentrations (80 ppm) of NO in the treatment of infants with persistent pulmonary hypertension of the newborn or adults with respiratory distress syndrome suggest that nitric oxide receptor sites are found in pulmonary tissue and that NO can cause pulmonary vasodilation (22, 23). Thus, NO ought to be considered in an investigation of the mechanism of particle toxicity.

Nitric oxide is a ubiquitous byproduct of high-temperature combustion, and there is substantial literature regarding the adsorption of NO onto a variety of materials including activated carbon, silica, molecular sieves, and so forth (24–27). Therefore, we would not be surprised to find that NO can be associated with combustion particles. It is plausible that combustion particles inhaled into the deep lung could deposit onto alveolar cells and then release NO into these cells, resulting in localized concentrations sufficient to trigger a response. For example, an elderly individual with partial left-ventricular failure would have pulmonary hypertension (28). It is well-known that small ambient air particles deposit on the alveolar epithelium or penetrate into the interstitial space and deposit on the pulmonary capillaries. If these particles released sufficient quantities of NO to the cell membranes, they could cause vasodilation of pulmonary vessels, which are already under pressure by virtue of the pulmonary hypertension. In turn, vasodilation of blood vessels would cause the cell membranes to expand and become “leaky”, resulting in plasma and proteins flowing from the pulmonary vessels into the alveolar sacs causing or exacerbating pulmonary edema. This paper demonstrates that NO is bound to ambient and combustion-derived particles and that NO can be thermally released from these particles in vitro.

## Materials and Methods

**Analysis of NO.** Nitric oxide was analyzed using two techniques, chemiluminescence and Fourier transform infrared (FTIR) spectroscopy. The chemiluminescent analyzer used in this study is the instrument used in the Airtrak 2000 ambient air analyzer (Mineral Control Instrumentation). The flow analyzer was operated at 50 Torr and 10 °C. Gas samples in a gastight syringe were injected through a septum port into the analyzer and the signal was integrated over 10 s. The NO analyzer was calibrated by injecting representative volumes of a known NO concentration in the range 5–50 ppm. The analyzer response was linear from 5 to 50 ppm and gave the following typical results:

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2 cm<sup>3</sup> sample:  $I(\text{nA}) = \text{dark current} + 0.4 [\text{NO}] (\text{ppm})$

4 cm<sup>3</sup> sample:  $I(\text{nA}) = \text{dark current} + 1.1 [\text{NO}] (\text{ppm})$

The dark current is typically 2 nA. The FTIR infrared spectrometer used in this study is commercially available as the Real time Emissions Analyzer (REA) system (Pierburg Instruments). A variable path length, multipass gas cell (Wilks, 20 m, model 9020) with potassium bromide windows, was used exclusively at the 14th order setting (21.75 m path length) to improve the sensitivity of the system. The accuracy of this system was routinely verified using NIST standards in the same concentrations range as the samples analyzed. Analysis of the NIST standards were typically within  $\pm 10\%$  of the label values. Sample spectra were analyzed using specialized software developed for the quantification of carbon monoxide, carbon dioxide, nitric oxide, and nitrogen dioxide (29). A 10 cm<sup>3</sup> sample in a gastight syringe was injected through a septum port into the evacuated Wilks multipass gas cell and pressurized to 700 Torr using ultrapure nitrogen gas.

**Particulate Samples.** Four NIST standard reference materials were used in this study. SRM 1648 Urban Particulate Matter is a sample of particulate matter from ambient air and was collected in St. Louis, Missouri in a baghouse over a 12 month period. SRM 1649 Urban Dust/Organics is a sample of urban dust collected from ambient air in Washington, DC using a baghouse over a period of slightly greater than 1 year. SRM 1650 Diesel Particulate Matter is composed of particles collected from the heat exchangers of a dilution tube facility after 200 h of diesel engine operations. Several direct-injection four-cycle diesel engines operating under different conditions were used to generate this sample. SRM 2975 Diesel Particulate Matter (Industrial Forklift) is a new NIST standard reference material; however, no details are available on how this material was collected (30).

**Experimental Procedure.** A particle sample was weighed and placed in a 4.8 cm<sup>3</sup> vial which was sealed with a septum and cap. For each vial the septum was pierced with two needles and purged with nitrogen for 10 min. After the sample was purged, the needles were removed and the sealed vials were placed in the well of a heating block for 1 h at the desired temperature. The temperature was stable to  $\sim 1\%$  over the heating period. At the end of an hour, a gastight syringe was used to withdraw a sample of gas which was injected into the chemiluminescent NO analyzer within 30 s. Another sample was taken within 2 min of the first and injected into the NO analyzer. Before and after the sample measurement, standard NO concentrations were injected into the NO analyzer to calibrate the current output. Measurements were also made of the analyzer dark current. These calibrations were used to determine the NO concentration within the syringe. Withdrawing a gas sample from the vial reduces the pressure of the initial sample. Furthermore, the gas sample cools when removed from the vial. Corrections for these temperature and pressure effects were made to convert the syringe NO concentration to the NO concentration in the vial prior to sampling.

This procedure is illustrated as follows. A 2.06 mg sample of SRM 1649 was placed in a vial, purged, and heated in a block at 120 °C for 1 h. A gastight syringe was used to withdraw a 2.0 cm<sup>3</sup> sample, which when injected into the NO analyzer, gave a signal of 12.3 nA. A second, 4.0 cm<sup>3</sup> sample was withdrawn and injected into the NO analyzer, giving a signal of 20.3 nA. Using the calibration curves and a dark current of 2.3 nA, the NO concentration in the gastight syringe was calculated to be 28.6 ppm for the 2.0 cm<sup>3</sup> sample and 14.6 ppm for the 4.0 cm<sup>3</sup> sample. After correcting for temperature and pressure, the mass of thermally released NO in the vial was calculated. The thermally released NO from SRM 1649 at 120 °C for 1 h was calculated to be 116 ng of NO/mg of

sample and 109 ng of NO/mg of sample for the 2 and 4 cc injections, respectively.

## Results and Discussion

Samples from the four NIST standard reference materials were analyzed for NO after being held for 1 h at 60, 80, 100, 120, and 140 °C. In addition, NO was detected from NIST SRM 1649 and NIST SRM 1650 at the physiologically important temperature of 37 °C. The release of NO from NIST SRM 1648 and NIST SRM 2975 was not measured at 37 °C because the estimated amount of material required to detect a signal (detection limit = 0.002 ng of NO/mg of sample) at this temperature exceeded our supply of these samples. The chemiluminescent NO analyzer was used to quantify the NO concentration in each vial. The thermal release of NO from the four NIST standard reference materials is shown in Table 1. The release of NO is highly temperature-dependent; the released NO increased by a factor of 90000 and 6000 between 37 and 140 °C for SRM 1649 and SRM 1650, respectively. Experiments were conducted to assess if the NO concentrations reported here are equilibrium values. It was found that 4 h is required at 120 °C for SRM 1649 to reach equilibrium. There was not sufficient material available to assess this in detail for all materials at all temperatures. Hence, the values here are not equilibrium values, which makes interpretation of these temperature-dependent NO release data difficult.

The levels of NO thermally released from these four samples varied substantially. There are a number of factors that could influence the level of NO evolved from these particles including the mix of particles collected during sampling which includes soil particles, combustion particles, and biological particles (e.g., pollen or bacterial spores). In addition, different diesel engine technologies (heavy-duty diesel engines, SRM 1650 versus forklift diesel engines, SRM 2975) could also account for some of the variability in the release of NO from these particles.

FTIR spectroscopy was used to confirm the identity and quantification of NO measured by the chemiluminescent technique. Figure 1 shows the FTIR spectrum of nitric oxide from the headspace of two SRM 1649 samples at 100 and 120 °C and a 30 ppm NO reference. The spectra of the two NIST samples closely match the standard reference spectrum. A comparison of these two techniques (Table 2, Figure 2) demonstrates that these two techniques yield comparable results. The disadvantage of the FTIR technique is the dilution involved in introducing the sample into the IR cell. The large sample size required for a measurable signal makes routine use of the FTIR technique impractical.

The additional features present in the spectra from the NIST samples are due to water vapor and noise. In addition, nitrogen dioxide, carbon monoxide, and carbon dioxide were also quantified using the FTIR. The concentrations of NO, NO<sub>2</sub>, CO, and CO<sub>2</sub> shown in Table 2 are similar to those reported from the thermal release of an activated carbon sample that had been exposed to water, nitric oxide, and oxygen (27). The chemical origin of NO from these particles is not known. It is well-known that the initial binding of NO to carbonaceous particles is a very complex process that involves a variety of chemical reactions that depend on the temperature and concentrations of water and oxygen (27, 31). Likewise, the thermal release of NO is equally complex and may involve breaking weak and strong bonds due to surface binding phenomenon and the rupture of chemical bonds, respectively.

The NO<sub>2</sub> concentrations were markedly different between two samples of SRM 1649 (Table 2). We do not know why there is such large variation in the release of this gas from this material, but it could be due to the sample size required for the analysis. A sample of 500 mg of SRM 1649 fills about

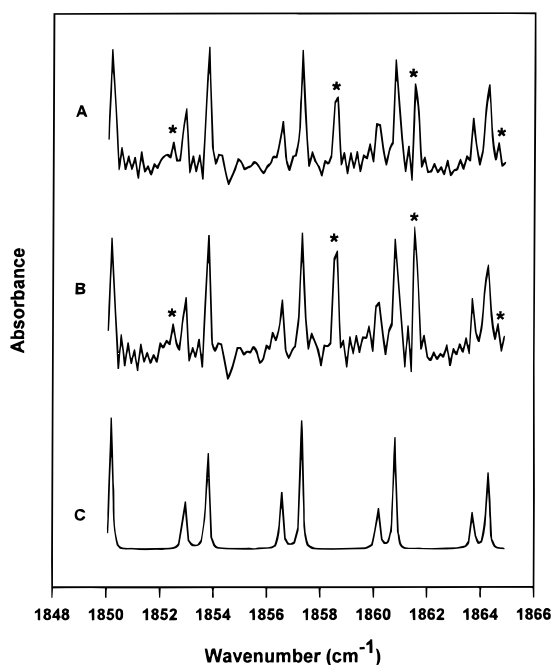
TABLE 1. NO Concentration from NIST Standard Reference Materials after 1 h at the Designated Temperature

$T (^{\circ}\text{C})$	SRM 1648 average $\pm$ std. dev. (M) (ng/mg of sample)	SRM 1649 average $\pm$ std. dev. (M) (ng/mg of sample)	SRM 1650 average $\pm$ std. dev. (M) (ng/mg of sample)	SRM 2975 average $\pm$ std. dev. (M) (ng/mg of sample)
37	N.M. <sup>a</sup>	$0.005 \pm 0.0009$ (2)	$0.33 \pm 0.17$ (2)	N.M.
60	$0.012 \pm 0.003$ (2)	$0.32 \pm 0.01$ (2)	$17 \pm 7$ (2)	$0.12 \pm 0.04$ (4)
70	N.M.	N.M.	N.M.	$1.9 \pm 0.04$ (2)
80	$0.73 \pm 0.10$ (4)	$3.6 \pm 0.9$ (2)	$140 \pm 30$ (2)	$31 \pm 5$ (2)
100	$8.3 \pm 0.3$ (2)	$38 \pm 7$ (2)	$430 \pm 76$ (2)	46 (1)
120	$50 \pm 2$ (2)	$110 \pm 18$ (4)	$940 \pm 120$ (2)	$95 \pm 22$ (2)
140	$160 \pm 4$ (2)	$430 \pm 23$ (2)	$1900 \pm 250$ (2)	$240 \pm 40$ (2)

<sup>a</sup> N.M. = not measured.

TABLE 2. Comparison of Results for SRM 1649 Using FTIR and Chemiluminescent NO Analysis

compound	SRM 1649 120 $^{\circ}\text{C}$ , 1 h ng/mg of sample		SRM 1649 120 $^{\circ}\text{C}$ , 1 h ng/mg of sample		SRM 1649 100 $^{\circ}\text{C}$ , 1 h ng/mg of sample	
	FTIR	NO analyzer	FTIR	NO analyzer	FTIR	NO analyzer
	533 mg	4.9 mg	sample mass 150 mg	2.1 mg	556 mg	10.1 mg
NO	90	120	80	110	19	38
NO <sub>2</sub>	9		56		12	
CO	8		13		4	
CO <sub>2</sub>	450		660		180	

FIGURE 1. FTIR spectra of headspace of (A) 150 mg of SRM 1649 at 120  $^{\circ}\text{C}$  for 1 h, (B) 556 mg of SRM 1649 at 100  $^{\circ}\text{C}$  for 1 h, and (C) a 30 ppm NO reference. Peaks with a asterisk are due to water vapor.

one-half of the reaction vial which reduces the effective surface area of the particle sample that is available for the thermal release of this gas. Additional research will be needed to fully understand the physical chemistry of the release of all the gases from these particles.

These experiments demonstrate that nitric oxide can be thermally released from some particles at physiological temperatures (37  $^{\circ}\text{C}$ ). Two NIST samples (SRM 1650 Diesel Particulate Matter and SRM 1649 Urban Dust/Organics) thermally released NO at 37  $^{\circ}\text{C}$  with SRM 1650 evolving about 70 times the amount of NO. The gas-phase concentrations

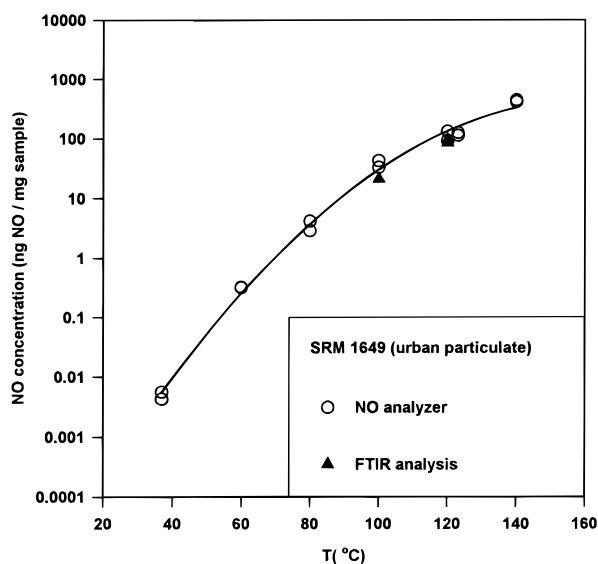


FIGURE 2. NO released from SRM 1649 after 1 h at the given temperature comparing chemiluminescent NO analysis and FTIR analysis.

of NO evolved at 37  $^{\circ}\text{C}$  are low for these two samples. However, once a particle binds to a membrane, the local concentration of released NO may be high enough to induce a physiological response such as vasodilation. This is the first step in characterizing a possible route by which NO bound to particles could be released into human lung tissue. Further research is required to investigate the release of NO from particles in biological media representative of lung fluids and to ultimately test the effect of NO bound to particles in experimental animal models.

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