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Microfluidic Electrochemical Sensor for On-line Monitoring of Aerosol Oxidative Activity

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Abstract

Particulate matter (PM) air pollution has a significant impact on human morbidity and mortality; however, the mechanisms of PM-induced toxicity are poorly defined. A leading hypothesis states that airborne PM induces harm by generating reactive oxygen species (ROS) in and around human tissues, leading to oxidative stress. We report here, a system employing a microfluidic electrochemical sensor coupled directly to a Particle-into-Liquid-Sampler (PILS) system to measure aerosol oxidative activity in an on-line format. The oxidative activity measurement is based on the dithiothreitol assay (DTT assay) where after oxidized by PM, the remaining reduced DTT was analyzed by the microfluidic sensor. The sensor consists of an array of working, reference, and auxiliary electrodes fabricated in a poly(dimethylsiloxane) (PDMS)-based microfluidic device. Cobalt (II) phthalocyanine (CoPC)-modified carbon paste was used as the working electrode material allowing selective detection of reduced DTT. The electrochemical sensor was validated off-line against the traditional DTT assay using filter samples taken from urban environments and biomass burning events. After off-line characterization, the sensor was coupled to a PILS to enable on-line sampling/analysis of aerosol oxidative activity. Urban dust and industrial incinerator ash samples were aerosolized in an aerosol chamber and analyzed for their oxidative activity. The on-line sensor reported DTT consumption rates (oxidative activity) in good correlation with aerosol concentration (R^2 from 0.86–.97) with a time-resolution of approximately 3 minutes.

Introduction

Airborne particulate matter (PM) is a prime candidate for the generation of biological oxidative stress.[1,2] Epidemiological and clinical research has demonstrated strong links between atmospheric aerosols and adverse health effects, including premature deaths,[3] impaired pulmonary function,[4] neurodegenerative disorders,[5] and respiratory and

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Supporting Information Experimental details, electrode fabrication diagram, electrode composition optimization results, DTT flow injection profiles and standard calibration, extracted aerosol sample information for method validation test. This material is available free of charge via the Internet at http://pubs.acs.org.

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cardiovascular diseases.[6] Chemical compounds in ambient PM, including aromatic compounds and transition metals such as Fe, V, Cr, Mn, Co, Ni, Cu, Zn, and Ti, may contribute to these effects through the generation of reactive oxygen species (ROS).[4,7,8] The exact mechanism by which PM causes oxidative stress is not completely understood; however, PM-associated ROS can cause damage to lipids, proteins, and DNA and these species have been implicated in pro-inflammatory effects in living tissues.[4,5,7–12] In normal biological systems, generation of ROS as a result of natural aerobic metabolism is balanced by endogenous antioxidants.[13] When ROS levels exceed cellular antioxidant capacity, the redox status of the cell and its surrounding environment changes, thereby triggering a cascade of events associated with inflammation and, at higher concentrations, significant cellular damage.[14,15]

Various approaches for measuring the oxidative activity of PM have been developed to study PM-induced oxidative stress.[16–19] Chemical assays offer the best potential for analysis of effective ROS dose in a format that can support epidemiological research[1,20] and many different types of chemical assays have been developed for assessing PM oxidative activity.[1,16–18,20–22] The oldest of these assays focused on measuring redox-specific chemicals such as transition metals and polyaromatic hydrocarbons (PAH).[22] Other methods have been proposed that make use of chromatography,[18,23] electron paramagnetic resonance (EPR),[24] and fluorescence.[25,26] The dithiothreitol (DTT)-based chemical activity assay is currently the most widely reported technique used to assess the capacity of PM to catalyze ROS generation.[27,28] In this assay, reduced DTT is oxidized to its disulfide in the presence of ROS generated by PM. After the reaction, the remaining reduced DTT is reacted with Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid or DTNB) to produce a chromophore that absorbs light at 412 nm (Scheme 1). Thus, the rate of DTT consumption is proportional to the oxidative activity of the PM sample.[2] Using this assay, redox-active quinones have been shown to catalyze the transfer of electrons from DTT to oxygen, generating superoxide.[27,29] Furthermore, levels of PM oxidative activity measured by this assay have been correlated with increased levels of biological oxidative stress *in vitro*.[28] The traditional assay, however, requires the use of both quenching and developing agents, which results in sample dilution and a higher detection limit. Another major limitation of all current assays for PM oxidative activity is that they rely on classic filter-based collection of PM. These methods require long (up to 110 hrs) aerosol sampling durations to capture sufficient mass for detection.[30] The long sampling times not only reduce the temporal resolution of the measurement but also increase the potential for collected species to react and change composition prior to analysis.[19,21] These methods also require analysis using laboratory-based instrumentation that is not readily integrated into portable, field measurement systems.

To overcome problems with filter collection and off-line laboratory analysis, an on-line analysis system was developed by the Hopke group.[25,26,31] Their system uses the Particle Into Liquid Sampler (PILS)[32,33] for aerosol sampling and a dichlorofluorescein (DCFH) based assay to determine particle-bound ROS activity. The PILS offers the potential for direct, real-time measurement of aerosol-bound ROS and represents the first step towards on-line measurement of aerosol oxidative activity. However, stability of the DCHF reagent, due to photobleaching and photo-oxidation, proved problematic during analysis, resulting in larger than desired variability.[34,35] In addition, an internal standard was not used to account for sample dilution by the PILS system.[25,31,35] Temporal resolution of this system was also limited (>20 min/sample) by the long sampling periods needed for sufficient mass capture and subsequent sample flushing/rinsing periods to ensure proper detector performance.[35]

Here we present a microfluidic electrochemical sensor for on-line monitoring of aerosol oxidative activity that is smaller, less expensive, and more portable than previously reported systems. Microfluidic devices can handle small sample volumes efficiently and thus, they are attractive for field-based measurements.[36,37] They can also be multiplexed to carry out multiple types of chemistry at the same time.[38–40] Electrochemical sensing is also well-suited for microfluidics because of the ease of integration and low cost.[41,42] By the choice of detection potential and/or electrode modification, electrochemistry also provides high sensitivity and high selectivity even when working with low analyte masses.[43–45] The sensor reported here is based on the existing DTT assay, with several simplifying modifications. A schematic of the procedure for both the traditional and new DTT assays is shown in Scheme 1. Following the reaction of DTT with PM, the remaining DTT is analyzed directly by the sensor, eliminating the need for quenching and developing reagents associated with UV-Vis detection. The electrochemical sensor is highly sensitive and capable of detecting small changes in the DTT electrochemical signal following reaction with a small amount of PM. Reducing the required sample mass also increases temporal resolution of the instrument, as less mass is needed for each individual measurement. Reducing the number and quantity of reagents also simplifies the system, making it more portable.

To create an electrochemical sensing device for DTT detection, cobalt (II) phthalocyanine (CoPC)-modified carbon paste electrode (CPE) was used as an electrode material. CoPC-CPE has shown good selectivity for the catalytic oxidation of thiol compounds like DTT, is stable for long periods of time, and can be fabricated in a microfluidic device.[46,47] The electrode design and fabrication is based on our prior work and utilizes PDMS-containing binder to generate a CPE with high physical stability and good electron transfer properties. Electrode composition and system operating parameters were optimized using cyclic voltammetry. System performance was then characterized off-line using flow injection analysis and amperometric detection to establish the linear range, detection limit, and sensitivity of the electrode towards DTT. Then to characterize DTT assay, the working range and sensitivity of the assay chemistry were then determined using a model oxidant, 1,4-naphthoquinone (1,4-NQ). Reactions of DTT and 1,4-NQ were performed off-line and the remaining DTT was directly measured by the sensor. The sensor performance was found to depend on the starting concentration of DTT, with lower concentrations giving higher sensitivity but a lower working range. As a final off-line validation step, 14 extracted filtered samples of ambient urban PM and biomass burning aerosols were analyzed. Results showed no significant difference in the oxidative activity measured by the sensor versus the traditional method. Finally, to demonstrate that the sensor can be applied for the measurement of aerosol oxidative activity in-situ, we connected the sensor to an on-line aerosol sampling system (Scheme 2). PM collected by the PILS reacted with DTT in sample transfer lines and the remaining reduced DTT was analyzed directly. Lithium fluoride was used as an internal standard to account for aerosol dilution by the PILS. A strong linear correlation between aerosol concentration and the measured oxidative activity (DTT consumption rate) was observed at concentrations similar to those found in polluted air (4 – 120 $\mu g\ m^{-3}$). High temporal resolution was obtained; at least 3 aerosol samples were analyzed every 10 min. To the best of our knowledge, the system gives the fastest time information on the aerosol oxidative activity available, which can greatly contribute to the future understanding of how aerosols affect human health during short-term exposure events.

Results and Discussion

Electrode Composition and System Optimization

Carbon paste electrodes, a mixture of graphite and binders (mineral oils, non-conducting polymers, etc.), have shown potential as electrochemical sensors in microchip devices[48,49] because of their ease of fabrication and the ability to the modify the electrode with a range of chemically-selective dopants.[50] Various methods have been reported for carbon paste electrode fabrication on microfluidic devices including the insertion of tube sleeves into the device and screen printing.[49,51] Of these methods, screen printing is particularly attractive because it can be performed directly on-chip with electrode dimensions controlled by screens or channels on the device itself. In this work, an electrode fabrication method analogous to screen-printing was used to produce on-chip electrodes using carbon paste with a custom designed binder (detail for electrode fabrication provided in Supporting Information Figure S-1).[44] We found that these electrode systems provided robustness and well electrochemical properties. The electrochemical sensor can be reused over a month by rising with de-ionized water daily. They were also characterized with catecholamines that have limited fouling potential. DTT requires the addition of a catalyst, however, to reduce the oxidation potential and reduce fouling.[55,56] Cobalt phthalocyanine (CoPC) is one of the more common electrocatalytic agents used and acts as a redox mediator that lowers the overpotential for thiols.[52–54] The two-step electrocatalytic mechanism starts with the electrochemical oxidation of Co(II)phthalocyanine to Co(III)phthalocyanine, followed by the chemical oxidation of DTT and regeneration of the Co(II)phthalocyanine.[52] Since both solution pH and CoPC composition impact DTT detection, the signal for 1 mM DTT as a function of %CoPC and solution pH was studied using cyclic voltammetry.[52,55–57] A two-variable experimental design was used for this optimization study.[58] Optimal values giving the highest signal of anodic current (scan range −0.1 to 1 V vs. unmodified CPE) were obtained at a CoPC concentration of 12% (w/w) and a solution pH of 7 (detailed discussion provided in Supporting Information Figure S-2). Therefore, this composition of CoPC was used for CPE modification and a solution pH of 7 was used as the running buffer for all subsequent experiments.

As a first step to test the performance of the sensor, a flow injection analysis system was designed for off-line measurements of aerosol oxidative activity. The voltammetric behavior of the systems was established first (Figure 1A). The voltammogram shape is different from most hydrodynamic voltammograms where the current plateaus at higher potentials because of mass transport. The unusual behavior shown here can be attributed to many factors such as additional oxidation and decomposition of the phthalocyanine ring at higher potentials and irreversible complexation of the Co(III) center.[57,59,60] While the highest signal-to-noise ratio (S/N) was observed at +0.5 V, we selected a potential of +0.2 V for selective DTT detection to avoid potential interference (i.e., oxidation signal) from other redox-active species typically present in ambient aerosols. These species include metals such as Fe, Cr, V, Ni and a broad spectrum of organic compounds such as PAHs (for example, pyrene, fluoranthene, chrysene), redox cycling agents (hydroquinones), olefins, aldehydes, ketones, nitro-compounds.[61,62] Although some chemicals can be oxidized at 0.2 V (according to standard reduction potentials), our electrochemical sensor is chemically modified as discussed above for selective detection of DTT.[63] For each measurement, we also injected an aerosol sample extract in the absence of DTT (i.e., as a negative control) to ensure the sample did not contribute to the electrochemical signal. Since aerosol composition is highly variable, fourteen different filter samples were employed to test for interferences. These samples included biomass burning aerosol, and urban aerosols collected during both summer and winter seasons. All samples showed negligible inferences at the DTT detection potential (data not shown). Example results (Figure 1B) show a high signal for DTT (20 μM) and no signal for the extracted aerosol sample.

Analytical Figures of Merit

After determining the optimal electrochemical conditions, the figures of merit for DTT were determined to ensure that the experimental conditions provide effective analysis for the remaining reduced DTT using flow injection analysis for DTT concentrations of 10–100 μM. A plot of average peak current (nA) (n=3) as a function of DTT concentration (μM) gave a linear calibration curve from 10 to 100 μM ($y = 0.037\times - 0.43$, $R^2 = 0.997$) (see

Supporting Information Figure S-3). The relative standard deviation from ten consecutive injections of 100 μM DTT was 7.0 % and electrode fouling was not observed (data not shown). The limit of detection for DTT defined as the concentration that gives a signal 3× larger than the baseline noise was 2.49 ± 0.20 μM (n=5) (equivalent to 24.9 pmol for 10 μL injection), which is comparable to similar microfluidic electrochemical sensors.[57,64]

Sensor Performance Study

Following calibration, the sensor was used to measure PM oxidative activity. The effect of DTT starting concentration on the assay dose response curve using 1,4-NQ as a model oxidant was studied for sensitivity and working range. Results shown in Figure 3 demonstrate that at low starting DTT (25 nmol), the signal dropped quickly with increasing 1,4-NQ concentration, providing the highest sensitivity of all three conditions tested (sensitivity of −1.50, −0.75, −0.50 %DTT remaining/ng 1,4-NQ, for 25, 50 and 75 nmol, respectively). The decrease in signal at higher starting DTT levels (75 nmol) is more gradual but provides a larger assay working range. These results indicated that the assay sensitivity and working range can be tuned according to the levels of DTT present in solution. In all remaining off-line assays, 25 nmol (50 μL 0.5 mM) DTT was used to provide high sensitivity at the low oxidative activity of our PM samples.

Finally, the new sensor was compared to the traditional DTT assay for aerosol oxidative activity using 14 representative aerosol filter samples (Supporting Information Table S-1). The 1,4-NQ equivalent values obtained by the two methods were compared using a paired t-test and plotted for correlation (Figure 4). There was no significant difference ($t_{observed}$ = 1.621, $t_{critical}$ = 2.179, p = 0.05) in the 1,4-NQ equivalent values obtained using the electrochemical DTT sensor and the traditional DTT assay. A good correlation for the 1,4-NQ equivalent values determined by the two methods was observed (R^2= 0.96). The equivalence between the two methods demonstrates that the new electrochemical DTT assay is suitable for the measurement of oxidative activity from PM samples collected on filters. Moreover the electrochemical assay for filter samples requires 100 times less sample for detection when compared to the traditional assay (10 μL vs. 1000 μL).[27–29,65] This reduction allows for a commensurate reduction in field sampling duration, representing a significant advantage over the traditional DTT assay. The inclusion of several aerosol types (biomass burning smokes, urban winter aerosols, urban summer aerosols) indicates that this finding is not restricted to a small class of aerosol types.

On-line Aerosol Oxidative Activity Measurement

After the electrochemical microfluidic sensor was validated for measurement of DTT consumption by PM from filters, the sensor was connected to a PILS to create an on-line aerosol oxidative activity analysis system (Scheme 2).

As a first step, the system was tested to show the ability to detect reduced DTT in the presence of non-oxidizing aerosols. Sodium chloride (NaCl) aerosol, which has no oxidative activity, was created in the chamber at various concentrations and the DTT signal measured. Results shown in Figure 4A demonstrated a decreasing DTT signal for increasing NaCl aerosol concentration (and thus increased water) delivered to the PILS impaction plate, which is indicative of sample dilution. The internal standard was used to account for this dilution effect and to correct the measurement of aerosol oxidative activity. A decrease in measured fluoride concentration, proportional to the salt aerosol concentration, is also shown in Figure 4A and demonstrates our ability to account for this phenomenon.

In the on-line system, various experimental conditions were different from our validated off-line assay, including temperature (~37 °C to ~28 °C) and the chemical mixing environment.

The online DTT assay was therefore tested using 1,4-NQ as a model oxidant. For this test the PILS was allowed to sample only filtered, particle-free air. To simulate exposure to an oxidant under the conditions of the PILS, 1,4-NQ was injected through a T-valve at the entrance to the PILS which is above the impaction plate. Decreasing DTT signal for higher concentrations of 1,4-NQ demonstrated DTT consumption by a standard oxidant under on-line operation (Figure 4B). The amount of 1,4-NQ used was equivalent to what was used in the off-line system, and the DTT consumption of the standard oxidant was of the same magnitude observed in the off-line system indicating that the viability of the on-line DTT assay.

To demonstrate on-line performance more fully, standard reference samples of urban dust and fly ash (industrial incinerator ash) were aerosolized, sampled, and analyzed for their oxidative activity directly. These aerosols were selected because of their varying chemical composition and because they represent typical toxicants found in outdoor air. For each sample, aerosol concentrations generated in the chamber were in the range of those found in urban atmosphere (4–120 μg m^{-3}). Results shown in the top panel of Figure 5A show DTT consumption corresponding to urban dust aerosol concentrations. As aerosol concentration increased, DTT consumption rate increased. Furthermore, the system provided high temporal resolution, reporting an independent measurement approximately once every three minutes. To the best of our knowledge, this is the highest temporal resolution for an aerosol oxidative activity measurement system that has been reported.[25,26,31] Higher temporal resolution could ultimately be obtained by reducing the volume of the injection loop and increasing buffer flow rate through the system. In terms of aerosol mass, the on-line system required between 7 and 214 ng of particle mass per injection to observe quantifiable DTT consumption. This range was calculated using the PILS air sampling rate (12.5 L min^{-1}), aerosol concentrations measured in the chamber (4–120 μgm^{-3}) and a 10 μL injection loop. This mass range is approximately three orders of magnitude lower than amount required for the traditional DTT assay (5–40 μg).[27–29,65] A correlation plot between DTT consumption rate and aerosol concentration was constructed and a strong linear correlation coefficient was obtained ($R^2 = 0.97$) (Figure 5A, bottom panel). Performance of the system was further confirmed with a fly ash test aerosol. In this example, the fly ash aerosol concentration was varied faster and DTT consumption rate was analyzed (Figure 5B, top panel) continuously. The on-line system was able to measure DTT consumption rates that were strongly correlated with aerosol concentration ($R^2 = 0.86$), even during periods of rapid concentration change. The oxidative activity of the urban dust and fly ash samples were comparable. The oxidative activity of the fly ash aerosol is believed to result from the transition metal content of the sample.[66,67] ROS generated from urban dust aerosol might be attributed to PAHs and nitro-PAHs which are major components of such sample.[68]

Conclusions

We present here for the first time a high temporal resolution on-line sampling/analysis system for aerosol oxidative activity using a microfluidic electrochemical sensor coupled with an on-line aerosol collection system. The determination of aerosol oxidative activity was based on the widely-reported DTT assay but used electrochemical detection instead of photometric detection. The sensor was validated off-line for its performance in aerosol oxidative activity measurement. No significant differences for the aerosol oxidative activity expressed as the 1,4-NQ equivalent was observed between the traditional assay and the sensor for 14 extracted ambient aerosol and biomass burning smoke filter samples. Using on-line monitoring of aerosol oxidative activity, high correlations between aerosol concentration and DTT consumption rate were observed for two representative test aerosols. The on-line system developed here shows promise as an eventual tool for field studies of aerosol oxidative activity. Such studies may lead to a better understanding of how PM can affect human and environmental health.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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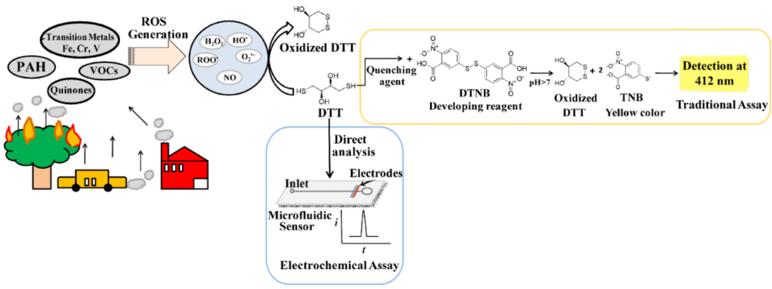
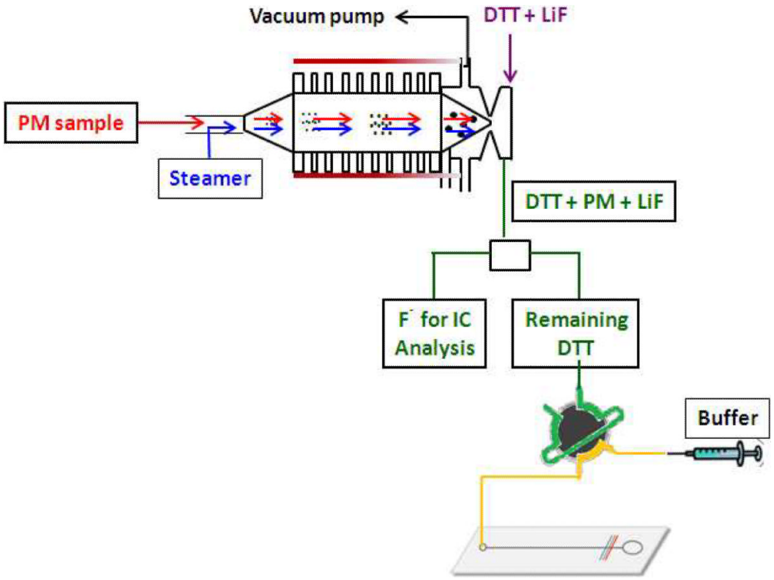


Scheme 1.

The analysis of aerosol oxidative activity using the DTT assay with traditional (yellow box) and microfluidic electrochemical detection (blue box) methods.



Scheme 2.
An automated sampling-analysis system for aerosol oxidative activity. Aerosol was collected by the PILS and mixed with DTT reagent and Lithium Fluoride (internal standard LiF). Following reaction within the sample line, the remaining DTT was analyzed directly by a microfluidic electrochemical sensor.

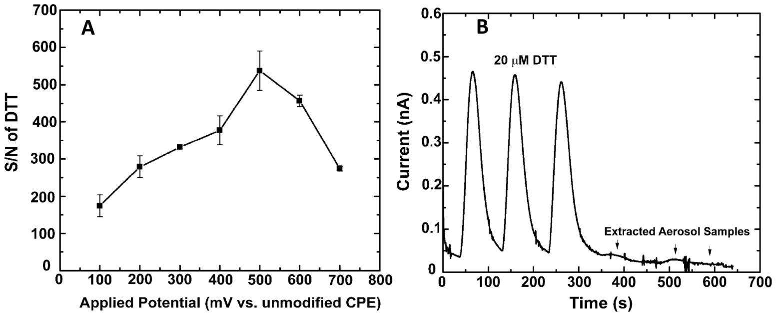


Figure 1.

Selectivity of the microfluidic electrochemical sensor for DTT. (A) Hydrodynamic voltammogram plotted as signal-to-noise ratio as a function of applied potential from 100 μM DTT injection (n=3) (B) Flow profiles from injections of DTT and extracted aerosol samples.

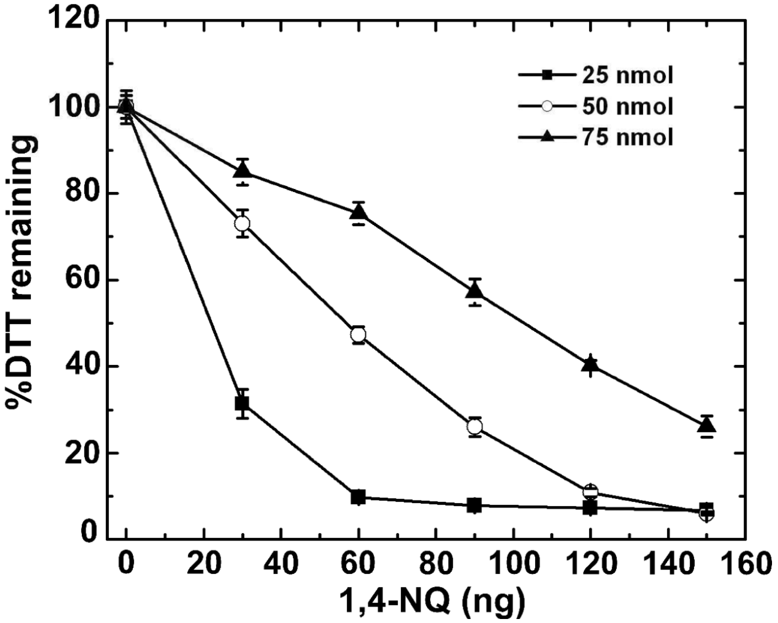


Figure 2.

The impact of initial DTT amount on assay dose-response (n=3).

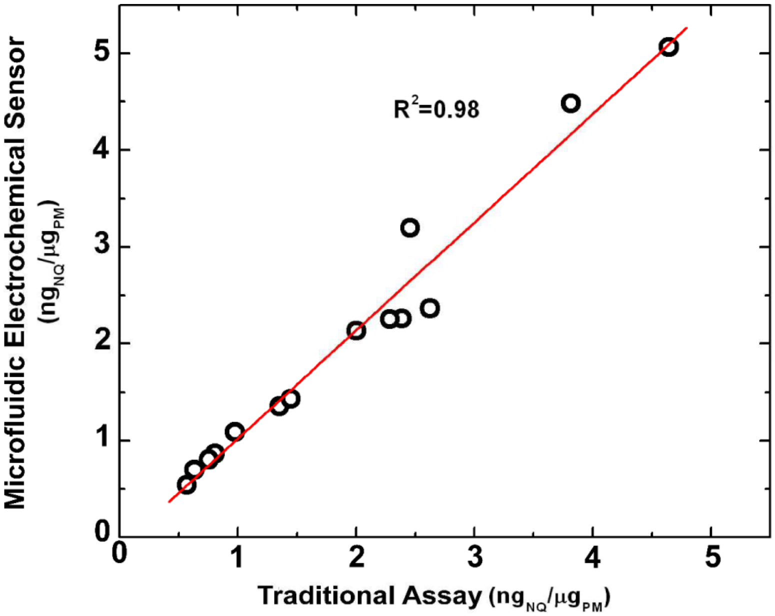


Figure 3.

Comparison of PM oxidative activity (1,4-NQ equivalent unit, $ng_{NQ}/\mu g_{PM}$) between the traditional DTT assay and the microfluidic electrochemical sensor (off-line). Data represent aqueous extracts of 14 different aerosol samples.

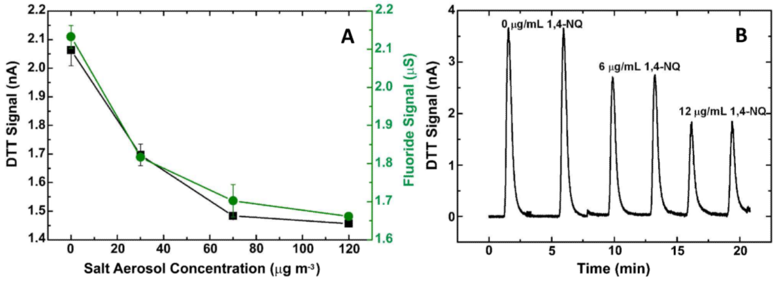


Figure 4.

Initial study of the on-line aerosol oxidative activity system. (A) DTT (black) and fluoride (green) response curve as a function of salt aerosol concentration without oxidative activity. DTT levels were measured using the electrochemical sensor. F^- levels were measured using ion-chromatography. (B) DTT signal response as standard oxidant (1,4-NQ) added to react on-line without aerosol delivered.

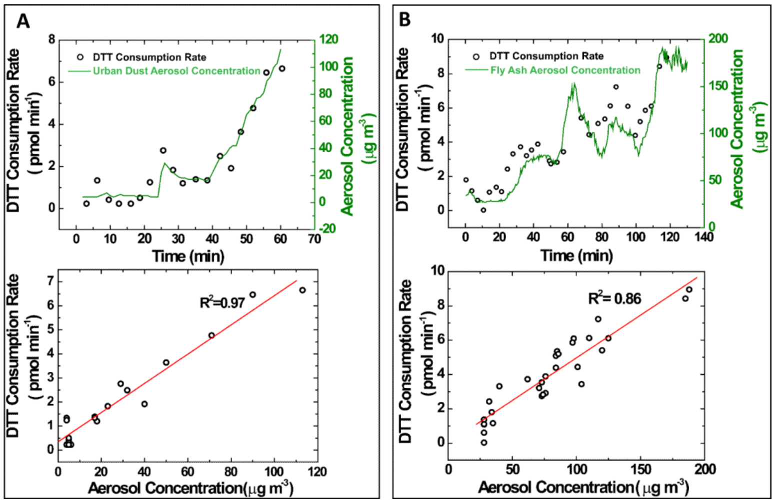


Figure 5.

Correlation of DTT consumption rate with aerosol concentration for (A) standard reference material urban dust and (B) standard reference fly ash. The top panels show DTT consumption rate (black y axis) and aerosol concentration (green y axis) as a function of experiment time. The bottom panels plot DTT consumption rate as a function of aerosol concentration and show the resultant correlation coefficient.