An On-line Monitor of the Oxidative Capacity of Aerosols (o-MOCA)

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Abstract. The capacity of airborne particulate matter to generate reactive oxygen species (ROS) has been correlated with the generation of oxidative stress both in-vitro and in-vivo. The cellular damage from oxidative stress, and by implication with ROS, is associated with several common diseases such as asthma, chronic obstructive pulmonary disease (COPD), and some neurological diseases. Yet currently available chemical and in-vitro assays to determine the oxidative capacity of ambient particles require large samples, analysis are typically done offline, and the results are not immediate.

In this manuscript we report the development of an online monitor of the oxidative capacity of aerosols (o-MOCA) to provide online, time-resolved assessment of the capacity of airborne particles to generate ROS. Our approach combines the Liquid Spot Sampler (LSS), which collects particles directly into small volumes of liquid, and a chemical module optimized for online measurement of the oxidative capacity of aerosol using the dithiothreitol (DTT) assay. The LSS uses a three-stage, laminar-flow water condensation approach to enable the collection of particles as small as 5 nm into liquid, without subjecting the sample to temperature extremes. The DTT assay has been improved to allow the online, time-resolved analysis of samples collected with the LSS. The o-MOCA was optimized and its performance evaluated using the 9,10-Phenanthraquinone (PQ) as standard redox-active compound.

Laboratory testing shows minimum interferences or carry-over between consecutive samples, low blanks, and a reproducible, linear response between the DTT consumption rate (nmol/min) and PQ concentration (µM). The calculated limit of detection for o-MOCA was 0.15 nmol/min. The system was validated with a Diesel Exhaust Particle (DEP) extract, previously characterized and used for the development, improvement, and validation of the standard DTT analysis. The DTT consumption rates (nmol/min) obtained with the o-MOCA were within experimental uncertainties of those previously reported for these DEP samples. In ambient air testing, the fully automated o-MOCA was run unattended for 3 days with 3-h time resolution, and showed a diurnal and daily variability in the measured consumption rates (nmol/min/m³).

Keywords: airborne particles, oxidative capacity, online DTT assay
1. INTRODUCTION

Although there is ample evidence linking exposure to particulate air pollution to adverse health effects, the mechanisms that lead to those effects are not understood. A leading hypothesis is that inhaled ambient particulate matter generates reactive oxygen species (ROS), which in turn create cellular damage and induce oxidative stress (Fitzpatrick et al., 2014; Kelly, 2003; Birben et al., 2012; Xia et al., 2006). Oxidative stress is associated with many well-known, widely-spread diseases such as Alzheimer’s, atherosclerosis, diabetes, and myocardial infarction (Andersen et al., 2010; Block and Calderon-Garciduenas, 2009; Calderon-Garciduenas et al., 2008; Maritim et al., 2003; Singh and Jialal, 2006; Uttara et al., 2009). The oxidative potential of airborne particles is attributed not only to their chemical composition (organics, trace metals) (Eiguren-Fernandez et al., 2010; Ercal et al., 2001; Jomova and Valko, 2011; Chien et al., 2009; Hawley et al., 2014) but also to their physical characteristics (particle size, shape, etc.) (Bünger et al., 2000; Chien et al., 2009; de Haar et al., 2006; Wessels et al., 2010). Trace metals, such as copper and iron can generate ROS via Fenton chemistry, while organics, including polycyclic aromatic hydrocarbons (PAHs) and quinones, generate ROS via metabolic transformations (Chung et al., 2006; Kumagai et al., 2012; Ercal et al., 2001; Valko et al., 2005).

To quantify the oxidative potential of airborne particulate matter, both in-vivo and in-vitro studies have been conducted. These studies have found correlations between the oxidative potential of airborne particles and the production of biological markers of ROS formation and oxidative stress (Bardet et al., 2014; Hawley et al., 2014; Li et al., 2004; Swanson et al., 2009; Acworth et al., 1999; Kim et al., 2001; Maier et al., 2008; Oberdorster, 2000). Yet the extent of these measurements is limited. Recently, efforts have been made to reduce and replace the use of animal models through cellular and chemical assays. Chemical assays are more rapid, less costly, and offer a simpler means to screen airborne particulate matter toxicity. Among these methods the dithiothreitol (DTT) assay (Cho et al., 2005a) developed to measure the oxidative capacity of airborne particles, has become widely adopted (Steenhof et al., 2011; Janssen et al., 2014; Charrier et al., 2015; Godri et al., 2011). The DTT assay is based on the ability of redox-active compounds associated with particulate matter to catalyze the reduction by DTT of oxygen to superoxide. Currently the DTT assay is done off-line; particles collected on filters are incubated with DTT over several periods between 10 and 45 minutes, and after each time point, the reaction between the DTT and the redox active components is quenched by the addition of 5,5′-dithiobis-2-nitrobenzoic acid (DTNB), forming a chromophore, which absorbance is measured at 412nm to determine the rate of DTT consumption over time. The total oxidative capacity of the particulate matter is expressed as the rate of DTT consumed per unit particle mass (nmol...
DTT/min/µg) or per sampled air volume (nmol DTT/min/m³). Additionally, this assay has the ability to distinguish between the contribution of metals and of organics to the overall oxidative capacity. The acceptance of this assay is based on several studies that showed a high correlation between the DTT assay and more specific biological markers of oxidative stress such as Heme-oxygenase 1 (HO-1) and inflammatory markers such as interleukin-6 and interleukin-8 (Li et al., 2003; Steenhof et al., 2011; Jiang et al., 2016). Although simpler than the in vivo and in vitro methods, the off-line DTT assay requires many steps, and is still too time-consuming to be widely applied. In addition, the accuracy of the measurement is limited by the integrity of the collected sample, as long collection periods (24-48 h) and the filter extraction processes that follow may alter the chemical and toxicological properties of the particles.

Several on-line systems to monitor the oxidant capacity of airborne particles have been reported previously (Wang et al., 2011; King and Weber, 2013; Sameenoi et al., 2012; Venkatachari and Hopke, 2008). Most of these systems use the 2,7-Dichlorodihydrofluorescein diacetate (DCFH) and horseradish peroxidase assay which is not specific for ROS but instead reports activity related also to the formation of reactive nitrogen species. The most advanced on-line instruments (Sameenoi et al., 2012; Venkatachari and Hopke, 2008) use the Particle-into-Liquid sampler for particle collection prior to DFCM or DTT assay. This sampler uses steam injection to condensationally enlarge the particles, and captures only the water soluble components associated with the collected particles. While it is not known whether the artifacts associated with steam injection are significant, the restriction to soluble components is a distinct limitation. Several studies have shown that the non-water soluble components are important contributors to the oxidative capacity of airborne particles (Shinyashiki et al., 2009; Verma et al., 2012; Wang et al., 2013). Indeed, Shinyashiki et al. (Shinyashiki et al., 2009) showed that 45 % of the ROS activity measured was due to the non-water soluble particles. Thus, it is imperative to collect both insoluble and soluble particle constituents to accurately assess particulate matter oxidative potential.

In this manuscript we present a new approach to provide on-line measurements of the oxidative capacity of airborne particles. Our technology combines the Liquid Spot Sampler with an online new chemical module enabling in-situ, time-resolved characterization of the ability of aerosols to generate ROS. The on-line Monitor of the Oxidative Capacity of Aerosols (o-MOCA) will provide a more complete data set for better understanding the properties of ambient particles that may lead to disease or dysfunction by generating oxidative stress.
2. MATERIALS AND METHODS

2.1. Liquid Spot Sampler

The Liquid Spot Sampler (LSS) is the front end of the system – it deposits both soluble and insoluble components of airborne particulate matter into a small volume of liquid. The LSS uses the three-stage water condensational growth technology (Eiguren Fernandez et al., 2014; Hering et al., 2014; Lewis and Hering, 2013) to enlarge sub-micrometer particles to form micrometer-sized droplets that are subsequently deposited into a water-filled vial. The three-stage approach provides a very gentle water condensation process. The supersaturation required for condensational growth is created in a laminar flow through a wet-walled tube, the first portion of which is cooled to ~5 °C, the second portion is warmed to ~37 °C, and the final portion is again cooled to ~12 °C. As shown by Lewis and Hering (2013), this approach activates the condensational growth of sub-10 nm particles. In contrast to steam injection condensation methods cited above, the temperature of the majority of the flow remains below 30 °C. Further, at the exit of the condensation region, the water vapor content of the droplet laden flow is below saturation at ambient temperature, enabling direct collection into liquid without excessive water condensation from the vapor. The Liquid Spot Sampler directs the condensationally enlarged particles through 3, 1.1-mm jets to impact gently the droplets directly onto the surface of water (200 to 500 µL) contained in a small vial. The sample flow rate can vary from 1.5 to 4.0 L/min. Uniquely, our approach provides particle growth and collection at moderated temperatures, reducing artifacts and maintaining sample integrity.

The collection efficiency of the LSS was evaluated in our laboratory (Berkeley, CA) using laboratory generated aerosols containing sulfate and nitrate. Particle collection efficiency was inferred through measurement of particle penetration through the sampler after delivery into the liquid. For submicrometer particle sizes, the aerosol was generated through atomization, mixing 10:1 with dry air, charge neutralized using the TSI soft X-Ray source and size selected using a high-flow differential mobility analyzer (HF-DMA, (Stolzenburg et al., 1998)). The DMA was operated with single-pass, 15 L/min sheath flow comprised of filtered laboratory air (with relative humidity of 30 % – 40 %). Particle concentrations at the inlet to the sampler and in the exiting flow were monitored using a pair of water-based condensation particle counters (TSI Model 3783 upstream, and 3787 downstream). These tests were done as a function of particle size (10 to 200 nm) and sampling flow rates (1.5 to 3.5 L/min).
2.2. Chemical module

The DTT assay is conducted in the chemical module after sample collection. The main components of the module are: 2 precision syringe pumps (Cavro XLP6000, Tecan), a 6-port analytical injection valve (EV-750, Alltech), a homemade heating/shaking system consisting of an aluminum block, a thermoelectric device, and a shaker (VWR), which contains the reaction vial where the DTT (Sigma Aldrich) and the sample mix and react, a “mixing Tee” (Upchurch) where the DTT-sample containing solution and DTNB (Sigma-Aldrich) join and are mixed, and a Diode the Array Detector (DAD, Agilent 1100). PEEK and Teflon were selected as the materials for all liquid handling tubing in order to reduce unwanted chemical reactions.

The flow diagram of the o-MOCA is shown in Figure 1. As soon as the predetermined sample collection time ends, the whole sample volume is transferred to the reaction vial using syringe pump 2. Once the sample is transferred, the collection vial and lines are rinsed with Chelex treated water, and 500 µL are added to the vial to initiate a new collection. After these steps, pump 2 starts the continuous delivery of the solution containing DTNB in Chelex water (2 µmol) to the detector. Few minutes later, once the system is equilibrated, 500 µL of DTT solution (100 nmol) in phosphate buffer (0.1 M, pH 7.4) are added to the reaction vial via syringe pump 1. The solution containing the sample and DTT is incubated at 37 °C with gentle shaking. At the designated times of 1, 3, 5, 7.5, 10, 12.5 and 15 minutes into the reaction, syringe pump 1 extracts 75 µL of solution from the reaction vial, and through a 150 µL loop delivers it via the injection valve to the mixing tee, where it combines with the DTNB flow prior to reaching the detector, forming 5-mercapto-2-nitrobenzoic acid that is measured by absorbance at 412 nm. The reaction between the DTT in solution and the DTNB is fast and since delivery to the detector is quick there is no need for adding stabilizing agents such as trichloroacetic acid or Tris Buffer (Charrier and Anastasio, 2012;Charrier et al., 2015;Cho et al., 2005a). Removing these extra processing steps, which dilute the sample, results in a more concentrated solution and increased sensitivity. Once the analysis is finished, and prior to delivering the next sample, the reaction vial is rinsed twice with 18 MΩ distilled, de-ionized water (DDW). All solution deliveries and rinsing steps are controlled via computer (Labview, National Instruments). A separate analysis routine (Igor, Wavemetrics) is used to post-process the DAD signal data to identify and measure maximum absorbance corresponding to each aliquot injection.

2.3. System Optimization and Validation

System optimization was done using the 9,10-phenanthraquinone (PQ) a very active redox compound commonly used for evaluating the DTT assay. Its redox active behavior has been well characterized, and several studies have shown a linear relation (first-order reaction) between the rate of DTT loss and the PQ concentration in solution (Charrier and Anastasio, 2012;Fang et al., 2014). The carry-over and cross...
contamination between injections was examined by running consecutive samples containing different amounts of the PQ standard. To evaluate if higher activity samples had an effect over subsequent samples of lower activity we measured the DTT loss rate of blanks followed by high PQ concentrations (0.25 µM) followed by low PQ concentrations (0.05 µM).

An important parameter to consider while developing the online system to measure time-resolved oxidative capacity of airborne particles is the sensitivity of the assay. A previous study conducted by Sameenoi et al. (Sameenoi et al., 2012) showed that increasing the initial DTT added to solution resulted in a reduction of the sensitivity of the assay. Although their method of analysis was different to ours, we considered this parameter when optimizing our on-line assay. The effect of the initial amount of DTT in solution on the observed consumption rate was evaluated through systematic increases in the mass of DTT added to the reaction vial (50 nmol, 100 nmol and 140 nmol) at a fixed PQ concentration (0.25 µM).

Our long-term goal is to develop a field deployable o-MOCA that can run unattended for extended periods of time. However, as the DTT is a chemical assay, and both the DTT and the DTNB are known to undergo degradation when exposed to light and high temperatures, optima conditions to maintain the integrity of these solutions for long sampling periods were determined. Photodecomposition was eliminated by keeping the reagent containing reservoirs in the dark (wrapped in aluminum foil). The effect of ambient temperature on the stability of the reagent was evaluated in 2 different ways: i) by measuring the consumption rate of a blank solution for consecutive runs over a 7-hour period, and ii) by measuring and comparing daily the absorbance of the DTT-DTNB mixture at t=0 for solutions kept in the refrigerator (4 °C).

Once the chemical module was optimized the system was run continuously for several hours to measure the DTT consumption rate for different concentrations of PQ and determine if a linear response with PQ concentration was obtained.

Validation of the on-line method was conducted using a diesel exhaust particle extract (DEP) (courtesy of Dr. Arthur Cho and Debra Schmitz, University of California Los Angeles). This DEP was used to develop the original DTT assay and later as control for the improved method (Cho et al., 2005a). It has been used as a positive control when measuring the activity of ambient particulate matter in all the studies conducted by Dr. Cho’s group. It is also used by Dr. Ning Li (Michigan State University) as positive control for in-vitro assays when measuring biomarkers of oxidative stress.

2.4. On-line measurement of the oxidative capacity of ambient aerosols

The fully automated o-MOCA was tested for continuous, on-line measurement of ambient aerosols in our laboratory (ADI, Berkeley, CA). Our prototype o-MOCA ran unattended over a 3-day period and ambient...
particulate matter samples were collected for 3 hours. The oxidative capacity of the sample was measured at the end of the 3-hr collection period. While the DTT assay was conducted a new sample was collected. The particle oxidative capacity of the ambient particles is reported as the rate of DTT consumption per unit m$^3$ of air collected (nmol DTT/min/m$^3$).

3. RESULTS

3.1. Collection Efficiency of the Liquid Spot Sampler

The collection efficiency with particle size and sampling flows are shown in Figure 2. For all particle sizes and flows, collection efficiencies $>90\%$ were obtained. Higher collection efficiencies ($>97\$%) were observed for sampling flow rates varying between 1.5 and 2.5 L/min; the efficiency decreased slightly as the sampling flow rate increased to 3.0 L/min (95\%) and 3.5 L/min (93\%). A sampling flow rate of 3.0 L/min was selected to run the Liquid Spot Sampler as to maximize the air sample volume collected without compromising the high collection efficiency over a wide range of particle sizes.

During these experiments, the volume of the collecting liquid was adjusted according to the sampling flow. For lower flow rates, smaller volumes could be used (down to 200 µL) without observing changes in the collection efficiency. For the higher flow rates, the minimum collection volume was increased to 500 µL. If a smaller volume was used the incoming jets were unstable disrupting the liquid surface and depositing the particles onto the dry surface of the bottom of the collection vial. In this case, although the collection efficiency was not greatly affected, resuspension of the deposited particles was required. Gentle agitation was enough to remove particles from the surface and into the liquid. For the 3 L/min sampling flow rate used with the o-MOCA, 500 µL was selected as the volume for optimum liquid collection.

3.2. System Optimization and Validation

3.2.1. Equivalence of DAD to Benchtop UV-VIS detector

In our online system we use a continuous-flow DAD instead of a batch fixed wavelength UV-VIS detector. Our first step was to evaluate the equivalence between the readings of the bench top UV-VIS detector (Spectronic 20D, Milton-Roy) and the online method with the DAD detector for the same sample. We ran the standard off-line DTT assay (Cho et al., 2005a) using PQ as our standard compound, and the absorbance of the same solution after 0, 5, 10 and 20 min reaction was measured with both detectors. Good linear relation was observed between the detectors ($r^2=0.99$). As the analogue output signal of the DAD is not recorded as the AU but as volts, the absorbance signal measured directly by the DAD and the output...
voltage recorded by the system were also compared. Although the range of the processed voltage output was lower than the signal displayed (AU), the correlation between both measurements was good indicating that the analog output can be used to accurately determine the absorbance of the solution.

3.2.2. Injection integrity, cross-contamination and carry over

As in any other on-line system, the potential for carry-over from injection to injection, and sample to sample is of concern. Although the internal volume of the lines has been minimized, the dead volume of the syringe pumps could lead to cross-contamination. To eliminate interferences and cross contamination between consecutive injections, we added a 150 µL loop and a 6-port injector valve between the reaction vial and syringe pump. The loop line, filled with water, prevents the reaction solution from reaching the syringe pump eliminating undesired mixing. We also added a step to rinse the delivery lines between injections by injecting 75 µL water following the 75 µL of reaction solution using pump 1. The resulting peaks after implementation of the loop and rinsing step for a blank and PQ solutions are shown in Figure 3. No interferences were observed for the sample peak height suggesting that the rinsing step with water injection reduces cross contamination without interfering with the sample signal.

After this improvement we tested the new configuration for possible carry over using different standard amounts. The DTT consumption rate (nmol/min) obtained for each sample is shown on Table 1.

The difference obtained for the duplicates suggests that, although small (<10 %), there may be some carry over effect when considerable changes in concentration (e.g. a factor of 5 as in this test) occur between consecutive samples. This small effect could be further reduced with design improvements.

3.2.3. Optimization of the initial DTT mass in solution

Figure 4 shows the average consumption rates (+ STDEV) for each initial DTT concentration. Results showed that at low initial amounts of DTT in the reaction solution the consumption rate was slightly lower than expected, with higher variability in the calculated rate. For initial concentrations of 100 and 140 nmol, the consumption rate was very similar, with lower variability for consecutive samples. Based on these results, initial amounts of at least 100 nmol should be used. A similar study was conducted to evaluate the effect of DTNB concentration on the intensity of the solution after reaction. Two different DTNB concentrations, 1 µmol and 2 µmol, were used and the results (not reported here) indicate no difference between concentrations.
3.2.4. Stability and integrity of the reagent for long-term field deployment and sampling

When reagents were kept at ambient conditions, a slow decrease in the absorbance peak of the DTT-DTNB mixture, estimated for \( t=0 \), was observed overtime (\( n=7 \)); however, the consumption rate for all samples (blanks and PQ) remained constant and similar to those obtained in previous and subsequent runs. For solutions kept in iced water, the estimated absorbance at \( t=0 \) was very similar over the entire period of analysis (\( n=7 \)), with again no changes in the consumption rates. In the longer study, DTT and DTNB solutions were kept in the refrigerator (4°C) and aliquots were taken out at the beginning of each day for 7 consecutive days. The measured absorbance intensity showed a small, but not significant decrease, over the 7-day period, which did not affect the measured consumption rate. Based on these results we improved the o-MOCA by adding a small cooling bath with a recirculating chiller (VWR) to maintain DTT and DTNB solutions at ~6°C for further studies.

3.2.5. System Performance for continuous runs using PQ standard

Figure 5 illustrates how the oxidative capacity of the samples is obtained with the o-MOCA system. Shown are data obtained for duplicate blanks and PQ samples. Figure 5a shows the raw absorbance data for each of four different quantities of PQ after addition of 500 µL of standard solution for final concentration in the reaction vial, after adding 500 µL of DTT, of 0.025 µM, 0.05 µM, 0.1 µM and 0.25 µM, plus a blank. Each group of peaks shows the decay in DTT concentration over time due to consumption by the PQ. Figure 5b shows the transformed data, calculated as the fraction of DTT remaining in solution (average ± STDEV) versus the reaction times. The amount remaining is obtained by normalizing the absorbance values by the initial absorbance that is inferred by the zero-intercept in a linear fit (corresponding to 100 nmol) to the absorbance values obtained at each reaction time (excluding the first two peaks corresponding to 1 and 3 min). For all samples a good linear correlation between the fraction of DTT remaining and reaction time was observed, with a small standard deviation for duplicates. The rate of DTT loss was determined for each PQ concentration from the slope and zero-intercept of the linear regression the measured absorbance, as shown in Figure 5b. Multiplying the blank subtracted values by the DTT added to initiate the reaction gives the consumption rate for each PQ concentration in solution. The average rate of DTT consumption (±STDEV) obtained from all runs is 9.8 ± 1.2 nmol DTT/min/µM PQ.

Figure 5c presents the regression equation for DTT consumption for this range of PQ concentrations. As expected, a linear correlation is obtained with near-0 intercept, indicating a good system response to this standard compound. The small standard deviation and consistency of these data which span an order of magnitude variation in the PQ concentrations indicates that the on-line DTT method works properly.
The limit of detection of the system, defined as five times the standard deviation of the slope obtained for our blanks (n=12 over different days) is 0.15 (nmol/min). This value is slightly lower than previous limits of detection reported in the literature for the DTT assay (Charrier and Anastasio, 2012; Fang et al., 2014). If we express the limit of detection as equivalents of PQ mass in solution using the regression obtained in Figure 2c, our LOD is close to the 0.025 µM PQ (25 pmol) in the reaction solution, we have been using as our lowest test concentration. These results validate our on-line DTT method, which eliminates the need for quenching agents through rapid assay, proving a method that is both simpler and more sensitive, yet retains the robustness of the original method.

3.3. Validation of the online DTT assay by Comparison with UCLA Standard Method

As no standards have been established for DTT consumption rate equivalence, we ran our new on-line DTT assay using diesel exhaust particles (DEP) extract (in DMSO) obtained from UCLA (courtesy of Dr. Arthur Cho and Debra Schmitz). In our validation test we measured the DTT consumption rate for 3 different initial amounts of DEP (n=2) in the incubation solution as done at UCLA (data obtained from Emma Di Stefano). Table 2 shows the DTT consumption rate obtained using our o-MOCA and the ones obtained by the UCLA group. Similar linear regression curves were obtained for the DTT consumption rate (nmol/min) as a function of DEP mass for both methods, with y=0.031+0.929 for the o-MOCA and y=0.036+0.591 for UCLA.

These results indicate that our method is comparable to the original DTT method and that the reported consumption rates will be directly comparable to laboratory based assays with equal accuracy.

3.4. On-line measurement of the oxidative capacity of ambient aerosols

The interface of the chemical module with the Liquid Spot Sampler was done connecting a port from one of the syringe pumps to the sample collection vial. The line is connected to the bottom of the collection vial to remove the entire particle containing suspension. The liquid suspension, containing both water soluble and insoluble material, is then delivered to the reaction vial where the reaction with DTT takes place. After sample delivery, the sample collection vial is rinsed twice with DDW (Chelex) and filled again for the next sample collection. All these steps are conducted automatically. After some preliminary testing, the original collection vial was modified slightly to provide more complete sample transfer into the chemical module. We also found that suspension transfer was aided by turning the sampling flow off during the transfer and rinsing steps. A solenoid valve under software control was added to enable automatic switching off/on of the sample flow. The improved and optimized o-MOCA interface was initially evaluated using different standard
solutions of PQ (as shown in Figure 5). Finally, to accommodate the analysis of an air sample containing insoluble as well as soluble particulate matter, we added a stainless steel frit (Upchurch) in the line through which sample is extracted from the reaction vial and delivered to the injection valve. This way, particles are physically present in the suspension while conducting the reaction, but do not reach the injection valve, the mixing Tee, or detector, avoiding interferences and clogging of the lines. No differences were observed in DTT consumption rates for PQ solution with and without the frit.

The fully automated o-MOCA was tested for continuous on-line measurement of ambient aerosols in our laboratory. Our prototype o-MOCA was operated unattended over a 3-day period. Ambient PM samples were collected for 3-hr and the oxidative capacity of the sample measured at the end of the collection. While the DTT assay was conducted a new sample was collected. It is important to note that DTT and DTNB stability, measured as the obtained intensity of the mixture at t=0, was good, with a small, but not considerable change between injections over the 3-day period.

Figure 6 shows the oxidative capacity of the consecutive samples measured as the consumption rate of DTT nmol/min/m^3. At the beginning of the study the consumption rate was below our limit of detection. During these first days the weather was not favorable with considerable rain. The rain lasted from the beginning of the sampling to midnight on Friday (Weather Underground historical data), which is consistent with the <LOD consumption rate measured during this period. After the rain, changes in the oxidative capacity were observed over time, with considerable diurnal and daily variability. We compared the consumption rate measured for our 3-hr samples with hourly concentrations of ambient PM_{2.5} and Black Carbon measured at the Air Quality Management District monitoring station closest to our location. The oxidative capacity measured for ambient particles using the o-MOCA followed a similar trend to ambient PM_{2.5} and Black Carbon concentrations (Figure 6). The measured activity for airborne particles collected in Berkeley, reported as the DTT consumption rate (nmol/min/m^3), is within the range of activities reported by other studies for ambient particulate matter (Cho et al., 2005b; Figueroa-Fernandez et al., 2010; Verma et al., 2009). Our results illustrate that the oxidative capacity of ambient particulate matter changes rapidly, and thus good time resolution is important to characterize the oxidative potential of ambient aerosols. Average consumption rates obtained from long sampling periods may fail to measure and report peak oxidative activities.
4. CONCLUSIONS

Presented is the o-MOCA system for on-line, time-resolved, un-interrupted measurement of the oxidative capacity of ambient particulate matter. Through laboratory test we have demonstrated a reproducible method with consistency in our blank signal and responsive (linear correlation) to PQ mass. The carry over and interferences are minimal. The limit of detection reported as minimum DTT consumption rate detectable with the o-MOCA is 0.15 nmol DTT/min. In addition, we have validated our o-MOCA by comparing rates obtained for a well characterized diesel exhaust particle extract to those obtained previously using the standard DTT assay (Dr. Cho’s group at UCLA). Finally, we have run the system unattended for 3 days in the laboratory obtaining diurnal and temporal variation of the oxidative activity of ambient particulate matter in Berkeley.

The authors declare that they have no conflict of interest.

5. ACKNOWLEDGEMENTS

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REFERENCES


Steenhof, M., Gosens, I., Strak, M., Godri, K., Hoek, G., Cassee, F., Mudway, I., Kelly, F., Harrison, R., Lebret, E., Brunekreef, B., Jansen, N., and Pieters, R.: In vitro toxicity of particulate matter (PM) collected at different sites in the Netherlands is associated with PM composition, size fraction and oxidative potential - the RAPTES project, Particle and Fibre Toxicology, 8, 26 %U http://www.particleandfibretoxicology.com/content/28/21/26, 2011.


Table 1. DTT consumption rate (nmol/min) for consecutive samples of different activities

<table>
<thead>
<tr>
<th></th>
<th>BLK</th>
<th>PQ (0.25 µM)</th>
<th>PQ (0.05 µM)</th>
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<tr>
<td>Injection #1</td>
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<td>2.93</td>
<td>1.46</td>
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<td>Injection #2</td>
<td>1.09</td>
<td>3.22</td>
<td>1.31</td>
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Table 2. Comparison of consumption rates (nmol/min/µg DEP) for different DEP concentrations obtained at UCLA with the standard DTT assay and o-MOCA

<table>
<thead>
<tr>
<th>DTT consumption rate (nmol/min/µg DEP)</th>
<th>DEP (µg)</th>
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<tr>
<td></td>
<td>2.5</td>
</tr>
<tr>
<td>UCLA</td>
<td>0.07</td>
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<tr>
<td>o-MOCA</td>
<td>0.068±0.002</td>
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FIGURES

Figure 1. Flow diagram for the on-line monitor of the oxidative capacity of aerosols (o-MOCA).

Figure 2. Collection efficiency for the Liquid Spot Sampler with particle size and sampling flow rate.
Figure 3. Peak absorbance for water blanks and PQ (0.25 µM) after rinsing injection with water

Figure 4. DTT consumption rate (nmol/min/µM PQ) for different initial DTT concentrations in solution (PQ concentrations of 0.25 µM).
Figure 5. Laboratory evaluation and performance of the automated system for different PQ solutions and blanks: (a) raw intensity signal (AU); b) DTT remaining in solution at each reaction time (nmol); (c) correlation between DTT consumption rate (nmol/min) and PQ concentration (µM).

(a) Blank PQ (0.025 µM) PQ (0.050 µM) PQ (0.100 µM) PQ (0.250 µM)

(b) DTT remaining in solution (nmol)

(c) DTT consumption (nmol/min)
PQ (µM)

\[ y = 4.59x + 0.81 \]
\[ R^2 = 0.99 \]
Figure 6. DTT consumption rate (nmol/min/m$^3$) for 3-hr samples collected and analyzed using o-MOCA for 3 consecutive days at Berkeley, CA; PM$_{2.5}$ and Black Carbon (µg/m$^3$) data obtained from nearby monitoring station (Air Quality Management District); *: <LOD