



**An online, DCFH
assay-based method
measuring PM_{2.5} ROS**

L. E. King and
R. J. Weber

**Development and testing of an online
method to measure ambient fine
particulate Reactive Oxygen Species
(ROS) based on the
2',7'-dichlorofluorescein (DCFH) assay**

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Abstract

An online, semi-continuous instrument to measure fine particle (PM_{2.5}) reactive oxygen species (ROS) was developed based on the fluorescent probe 2'7'-dichlorofluorescin (DCFH). Parameters that influence probe response were first characterized to develop an optimal method for use in a field instrument. The online method used a mist chamber scrubber to collect total (gas plus particle) ROS components (ROS_t) alternating with gas phase ROS (ROS_g) by means of an inline filter. Particle phase ROS (ROS_p) was determined by difference between ROS_t and ROS_g. The instrument was deployed in urban Atlanta, Georgia, and at a rural site during various seasons. Concentrations from the online instrument generally agreed well with those from an intensive filter measurement of ROS_p. Concentrations of the ROS_p measurements made with this instrument were lower than reported in other studies, often below the instrument's average limit of detection (0.15 nmol H₂O₂ equivalents m⁻³). Mean ROS_p concentrations were 0.26 nmol H₂O₂ equivalents m⁻³ at the Atlanta urban sites compared to 0.14 nmol H₂O₂ equivalents m⁻³ at the rural site.

1 Introduction

Reactive oxygen species (ROS) when introduced into a biological system have a strong tendency to disrupt the electrochemical balance. The amount of disruption depends on factors such as the amount of ROS introduced or produced within the system, the location of the introduction or production of the reactive species, the duration of the insult and a host of other factors, many of which have yet to be ascertained in nature as well as in scope (Barrett et al., 1999; Morgan et al., 2001; Oberdörster, 2004; Rothe and Valet, 1990; Squadrito et al., 2001; Sugamura and Keaney, 2011; Xia et al., 2006). Human exposure to ROS can occur by a number of known routes. ROS associated with gaseous or particulate pollutants generated in the atmosphere may be transported into the respiratory system. Their deposition generates adverse effects within cells of

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organic concentrations (Wang et al., 2010) have been observed. Some correlations between ROS and ozone have been reported, particularly in the early to mid afternoon (Venkatachari et al., 2005).

A potential drawback to previously reported ROS_p concentrations is that previous studies may be susceptible to sampling artifacts. Filter based studies, particularly for reactive compounds, are likely limited by long sample collection times that may result in under-prediction of concentrations (Hung and Wang, 2001). Previous ROS_p filter studies have also been challenging due to reported high and variable blank concentrations (Hung and Wang, 2001; Venkatachari et al., 2005, 2007).

Some preliminary results exist for an online method to measure ROS_p (Venkatachari and Hopke, 2008; Wang et al., 2011). This instrument couples the particle-into-liquid-sampler (PILS) with a flow system that mixes DCFH and a catalyst, peroxidase from horseradish (HRP) with the PILS sample stream contained soluble PM_{2.5} components (Venkatachari and Hopke, 2008). After utilizing mixing elements to combine and sufficiently react the sample ROS with the fluorescent reagents, the sample is measured using a spectrometer. Results from a summertime week-long field operation in Rochester, NY (Wang et al., 2011) showed an average of 8.3 ± 2.2 nmol H₂O₂ equivalents m⁻³. This study also indicated a diurnal trend in ROS_p with an increase in daytime concentrations, as well as higher values on weekdays than on weekends. These reported values exceeded ambient values found on filters in previous studies in the USA and Taiwan (Hung and Wang, 2001; Venkatachari et al., 2005, 2007). These results appear to indicate that ROS_p loss from ambient filters can be minimized by using a continuous online system with virtually no delay between collection and analysis.

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2 Materials and methods

2.1 Primary materials

For this study the DCFH probe was chosen to provide a comprehensive ROS detector. Of the ROS probes commercially available, DCFH has a long and well-documented record of sensitivity in both cellular and atmospheric aerosol applications (Hung and Wang, 2001; Liu et al., 2007; Venkatachari and Hopke, 2008; Venkatachari et al., 2005, 2007; Wang et al., 2011; Black and Brandt, 1974; Cathcart et al., 1983; LeBel et al., 1992). 2',7'-dichlorodihydrofluorescein diacetate (DCFHDA) was purchased from both Sigma Aldrich (St. Louis, MO, USA) and Calbiochem (EMD Chemicals, Billerica, MA, USA) depending on availability. Horseradish peroxidase (HRP) (Type II) was purchased exclusively from Sigma Aldrich. Hydrogen peroxide (30%, w/w) was purchased from JT Baker through VWR (Atlanta, GA, USA).

Primary analytical equipment included a spectrofluorometer (Maya2000Pro, Ocean Optics, Dunedin, Florida, USA) with cutoff and long-pass filters for wavelengths greater than 515 nm and a 200 μm slit. The spectrometer was further equipped with a flow-through cell of 450 μL volume with a light path of 10 \times 4 mm (FIA-SMA-FL-ULT). The initial excitation source was a blue (475 nm) LED source manufactured by Mikropack (Ocean Optics, LS-475), replaced with a Jasco-manufactured 470 nm wavelength LED with adjustable voltage (LLS-470, Ocean Optics) to accommodate LED intensity loss with bulb age. Fiberoptic cables (SMA-905, Ocean Optics) completed the primary analytical apparatus.

Solutions were pumped through the flow-through cell using an 8-channel peristaltic pump (Ismatec, Opfikon, Switzerland). Any tubing, glassware or other vessels for working solution storage or transport were shielded in aluminum foil to prevent photooxidation.

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2.2 Reagent preparation

According to previously published methods (Cohn et al., 2008; LeBel et al., 1992) DCFHDA was dissolved in HPLC grade ethanol in a portable darkroom (Silver Edition HydroHut, Flora Hydroponics, Atlanta, Georgia, USA), and either used immediately or stored in the freezer in an amber bottle, sealed to prevent evaporation of the solvent. To prepare the working solution of a desired concentration of DCFH, 0.01 N NaOH was added to the DCFHDA solution to remove the acetate. After thirty minutes, the solution was buffered with a sodium phosphate buffer (pH 7.4) to halt the reaction, dilute the DCFH concentration and bringing the overall pH to 7.2 (allowable range, 7.2–7.4). pH was verified with a handheld pH monitor (VWR). A quantity of HRP (Type II, Sigma Aldrich) was then added to the solution to bring it to 0.5–1 units HRP mL⁻¹ solution, based on the specific purpogallin units per milligram for each lot of HRP. The working solution (DCFH + HRP) was then stored in an amber vessel or foil-wrapped flask in the laboratory refrigerator at 2 °C and discarded after a period of 2 days. The original standard working solution was 5 μM DCFH with 0.5 units mL⁻¹ HRP.

3 Offline system for calibration and sensitivity analysis of DCFH

3.1 Initial method and analysis

Initial evaluation of DCFH was conducted following the method described by Hung and Wang (Hung and Wang, 2001) in which the probe was used to quantify the ROS concentrations from ambient particle filter extracts. 3 mL of DCFH-HRP solution were pipetted in the darkroom into 15 mL amber centrifuge vials, which were then covered with predrilled caps (1/16" diameter) and sealed with paper laboratory tape. These preloaded vials were stored in the refrigerator until use.

For analysis, the tape was briefly removed from the vials and 0.1 mL of either deionized water (dl) or a hydrogen peroxide standard was pipetted through the cap hole. The

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tape was replaced and the vial briefly inverted to ensure that no peroxide remained uncombined with the DCFH solution. The vials were incubated in a 37 °C water bath for fifteen minutes. The solution was then briefly agitated by hand and placed in line with the analytical system, shown in Fig. 1. A peristaltic pump moved the DCFH-HRP-H₂O₂ solution at 0.4 mL min⁻¹ through the flow-through cell, while a selector valve directed dl through the cell when not measuring DCFH intensity. A small glass debubbler was also employed to reduce the signal interference by small air bubbles that may be introduced into the system, also run from the peristaltic pump. Volume loss from the discarded flow was approximately 10 % of the overall sample flow. Sufficient dl was allowed to move through the system to return the fluorescent signal to a baseline value before analyzing the next sample. The entire system was plumbed with green PEEK tubing (1/16" outer diameter, 0.030" inner diameter, Upchurch Scientific, Oak Harbor, Washington, USA).

As the solution moved through the flow cell, it was excited by the 475 nm light source, causing the DCF* product to fluoresce at 530 nm. This light intensity is transmitted via the fiber optic cable to the spectrophotometer. Fluorescent intensity at 530 nm was measured using SpectraSuite from Ocean Optics and recorded with an integration time of 500 ms and average of 10 scans. Prior to any analysis a dark spectrum signal was measured while blocking all light to the spectrometer and subtracted automatically from the light spectrum. Intensity values reported are average intensities of measurements made once the fluorescent signal was stabilized, after a minimum of ten minutes from powering on the excitation source. Deionized water also provided a measureable signal at 530 nm, which was used as a surrogate for light source intensity and system flow stability over time.

An example of an initial calibration made with H₂O₂ solution concentrations of 100–400 nM is shown in Fig. 2. This sample range was chosen as a representative span for the concentrations reported in ROS_p analysis for typical filter measurement setups (Hung and Wang, 2001; Venkatachari et al., 2005, 2007).

3.2 Offline sensitivity analysis

Previous work has reported the need to control the DCFH-HRP working solution storage temperature, storage time (solution age), pH, and operational parameters including reaction temperature, pH and reaction time to achieve maximum reaction of DCFH with hydrogen peroxide. Parameters were optimized to reduce auto-oxidation and thus lengthen the usable lifespan of the DCFH-HRP solution, hereafter referred to as working solution (Cathcart et al., 1983; Black and Brandt, 1974) and to provide the greatest method response. Parameters were assessed primarily by comparing hydrogen peroxide calibration slopes relative to the base case, previously described. DCFH age was examined by comparing the calibration slope of a fresh solution with the calibration slope hours and days later. Reaction temperature was assessed by comparing calibration slopes of solutions incubated at varying temperatures from ambient (23 °C) to 65 °C prior to analysis. A similar method of assessment was used to determine the optimal DCFH concentration and DCFH:sample volumetric ratios, e.g. the ratio of the volume of working solution to the volume of calibration standard. A 30 : 1 ratio, used for the base case, was not considered practical for a future online system. Finally, reaction time was assessed using a different setup in order to measure reaction progress. In those tests, 0.1 mL of a hydrogen peroxide standard or dl was pipetted into the mixing vials as described previously with 3 mL of DCFH solution, briefly inverted to ensure that all liquids were combined and immediately placed in line with the detector. The sample line from vial to detector was shortened as much as possible to reduce delay time from which DCFH and peroxide were combined to initial detection of fluorescence, using the same 0.030" ID PEEK tubing as in the standard flow analysis setup from Fig. 1. The residence time in line prior to detection was 30 s. The findings from these offline assessments are summarized in Table 1 and used in the application of the online instrument.

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4 Online instrument development

4.1 Mist chamber

Originally known as the Cofer scrubber, mist chambers were developed to collect water soluble gases and particles for online analysis (Cofer III et al., 1985; Cofer III and Edahl, 1986; Anderson et al., 2008; Spaulding et al., 2002). Mist chambers are generally a cylindrical glass structure with an air sample inlet at the bottom, a port on the side for introduction and removal of scrubbing liquids, and a nebulizing nozzle, as shown in Fig. 3. Sample air enters the chamber through the bottom nozzle. Inside the chamber, a capillary runs from just above the base and alongside the air nozzle for some vertical distance. Some minimum volume of liquid, usually water, is placed inside the mist chamber via the injection port. Venturi forces created by the airflow accelerate through the nozzle, draw liquid from the reservoir into the airstream and create a fine mist. Affixed to the top of the mist chamber is a filter pack (University Research Glassware (URG), Carrboro, NC) equipped with a 1.0 μm pore size hydrophobic filter (TefSep, Pall Corporation). This filter prevents the liquid from exiting the chamber, which refluxes down from the top of the mist chamber back to the reservoir. This liquid scrubs the soluble gases and particles from the air-stream as it is continually recycled through the chamber. Some liquid is inevitably lost as water vapor in the exhaust flow. When sample air-flow is halted, the liquid and its components in solution are withdrawn from the chamber for analysis. Analysis of the sample can occur while the next mist chamber sample sampling cycle starts.

Benefits of the mist chamber include operation without heating the sample and the ability to vary integration time in order to concentrate samples. The mist chamber has been shown to effectively collect compounds with a Henry's law constant, K_H , of over 10^3 Matm^{-1} (Spaulding et al., 2002) The potential drawbacks of the system include the need for a batch operation process, which tends to consume more water as opposed to a continuous monitoring system. Mist chambers are also effective scrubbers

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of gases, unlike condensation-based systems, indicating the need for an effective gas phase control to effectively measure solely particle concentrations. A mist chamber was chosen as the particle collection for the online ROS_p instrument in part due to control of sample integration times to overcome analytical detection limits.

4.2 Online method setup

The overall setup of the mist chamber-based ROS instrument is shown in Fig. 4. Ambient air is drawn through a cyclone (PM_{2.5}, 16.7 L min⁻¹, URG) and a copper inlet line. The airflow enters an automated valve that either directs it through a 47 mm filter pack (URG) containing a 2.0 μm Teflon filter (Zefluor, Pall Corporation) for measurements of ROS_g, or bypasses the filter for a measurement of ROS_g + ROS_p, or total ROS (ROS_t). The sample air then enters the mist chamber, after which the scrubbed airstream exits through the hydrophobic filter. The airflow finally passes through a water trap followed by a mass flow controller (GFC-47, Aalborg), set at nominally 20 L min⁻¹, and then to a vacuum pump (carbon vane, Gast 1/4 hp). These sampling system components were specifically chosen to maximize retention of PM_{2.5} and thus ROS_p.

A syringe pump equipped with an 8 port valve (V6 pump with 48k resolution, Norgren Kloehn, Las Vegas, NV) and a continuously operating peristaltic pump (4 channel, Ismatec) control the liquid portion of the instrument. One port of the syringe pump is connected to the mist chamber liquid inlet, the other ports led to a reservoir of the DCFH-HRP working solution, dl, waste, and a 15 mL amber centrifuge tube (mixing vial) as described in the offline analysis method. The remaining three ports can be used for up to two hydrogen peroxide standards for automatic calibrations, and for an open port for introduction of air, as required. The V6 pump also electronically controls the power to the vacuum pump through a solid state relay as well as the position of the sample air selector valve and a two position liquid selector valve to change the source of the liquid flow into the flow cell.

The peristaltic pump continuously moves liquid from either the mixing vial or the dl reservoir through the flow cell. This amber mixing vial contains two PEEK tubing lines

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inserted through the hole in the cap. The line from the peristaltic pump extended to the vial bottom while the line from the syringe pump valve extended only a short distance past the cap. This setup allows for all liquid to be withdrawn completely via the longest line from the mixing vial, while the shorter line does not reach the liquid level within the vial at any time. A third channel on the peristaltic pump also controls flow from a glass debubbler in line just prior to the flow-through cell. The fourth channel drains liquid from the water trap to protect the mass flow controller. The syringe and all other clear portions of this system are shielded from light with aluminum foil to inhibit photo-oxidation of the DCFH.

4.3 Sampling automation and analysis

The following describes a typical sample collection and analysis cycle using a looping routine in the syringe pump (Kloehn Control) software. The start of the sampling cycle begins with the syringe pump injecting 10 mL of dl into the mist chamber. The vacuum pump is started for a period of 5 min to collect soluble ROS ambient species in the mist chamber collection liquid. The vacuum pump is shut off and the syringe pump withdraws 1.5 mL of the ROS-laden solution from the mist chamber; 0.5 mL of this from the top of the syringe is immediately discarded to remove any air from the liquid system. 9 mL of working solution are then added to the syringe. The combined total 10 mL of sample liquid and working solution is forced into the mixing vial via the higher level tube. This process mixes the sample of dissolved ROS components and working solution. During this time, the peristaltic pump is running continually, pumping dl through the flow cell and the mixed sample-reagent solution from the vial is sent to waste, until after 1 min, at which point the vial liquid has reached the selector valve, which then is actuated to direct vial liquid to the flow cell (dl now to waste). The reacted sample solution moves through the flow cell for 2.5 min, at the end of which the fluorescent signal is recorded. This results in a fluorescent signal quantified after 4.5 min of reaction time. Deionized water in between samples provides a baseline as previously discussed.

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The resulting collection efficiency (mist chamber sulfate to PILS sulfate) shown in Fig. 5 shows that this specific mist chamber should be operated at a minimum flow rate of 15 L min⁻¹. Maximum flow rates for this particular setup were limited to approximately 25 L min⁻¹ due to pressure drop across various elements of the system, mainly from the wetted hydrophobic refluxing filter.

While the refluxing hydrophobic filter that retains liquid in the mist chamber is effective, inevitably some liquid loss occurs. The final volume of liquid retained by the mist chamber is measured periodically to account for this loss to account and to determine the ambient concentration. This potential loss was also a motivating factor in the short sampling periods employed during the majority of the instrument's field deployment.

4.5 Online calibration and dynamic liquid blanks

The ROS instrument is calibrated with the mist chamber offline in an automated process using the analytical system just described. Instead of liquid from the mist chamber being combined with the DCFH working solution in the mixing vial, the same volume of a standard is used. This allows the verification of known concentrations as part of routine operation. “Blank”, or auto-oxidation measurements to adjust for working solution age and subsequent increase in baseline fluorescent intensity, were also made using dl. Figure 6 shows a typical plot from such a calibration, using standard concentrations in the range anticipated for ambient sampling with the online instrument.

As “blank” measurements in this system are truly measurements of the auto-oxidation state of the working solution, they must be measured regularly during the sampling operation to allow for dynamic correction of the sample fluorescent signal over time. The blank signal is measured after every 6 ambient measurements, or approximately every hour. Figure 7 shows a sample of the drift over roughly a 48 h sample period of the blank signal during field-testing of the instrument.

4.6 Calculation of ambient ROS concentration

The ROS concentration in the ambient air in H₂O₂ equivalents is calculated by:

$$C_a = \left(\frac{l - b}{a} \right) \left(\frac{V_s}{1000Q_a t} \right) 1000 \text{Lm}^{-3} \quad (1)$$

where l is the intensity of the fluorescent signal (counts), b the intercept from calibration linear fit, a the slope from calibration linear fit, V_s final solution volume (mL) in mist chamber, Q_a the average air flow through the mist chamber (Lmin⁻¹, ambient T and p) for a sampling period of t (min). Multiplying C_a by 1000 Lm⁻³ results in an ambient ROS concentration in nmol H₂O₂ equivalentsm⁻³.

4.7 Evaluation of gas phase removal and ROS_p calculation by difference

Several compounds were assessed as dry scrubbers or as annular denuder coatings for use in ROS_g removal to improve determination of ROS_p. A glass annular denuder (URG) coated with a slurry of MnO₂ and a diffusion dryer filled with Carulite (a dry MnO₂ compound) (Carus Corporation, Peru, IL) were evaluated for their ability to remove ROS_g. MnO₂ was of primary interest since this has been an ROS removal compound used in previous studies (Hwang and Dasgupta, 1986; Stobbe et al., 1999). Ti(IV) oxalate was also used in an annular denuder coating given its use in scrubbing hydrogen peroxide (Possanzini et al., 1988; Possanzini and Di Palo, 1995).

Denuder efficiency tests were done by consecutive denuder-on versus denuder-bypass ROS measurements with the automated mist chamber system by placing the Teflon filter upstream of the complete system, and the denuder in the position formerly occupied by the filter controlled by the air selection valve. The annular denuder coated with a MnO₂ slurry removed an average of 9% of the ambient ROS_g, with a maximum removal efficiency of 57% (standard deviation of removal efficiency was 21% ($n = 111$)). Dry MnO₂ packed into the diffusion dryer removed an average of 18%, with

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equivalents m^{-3} for the normal operational values of the mist chamber, in which the final liquid volume is 9.7 mL, air flow rate is 20 L min^{-1} and the sample collection time is 5 min. For the field-deployed instrument, ROS measurement LOD was similar for consecutive blanks.

The method LOD for determining ROS_p is substantially higher, however, since it involves a difference between two large of relatively close magnitude. During the field measurements described below, frequent negative ROS_p values resulted from the difference calculation. An alternate and conservative LOD was use based on the variability in the negative ROS_p values. For example, once ROS_p was determined for a specific study period (e.g. a specific site) and after basic quality control removed erroneous measurements of erratic highs or lows, the LOD for the particle measurements was estimated from one standard deviation of all negative values calculated from the difference method. The calculated LOD by this method varied between 0.07 to 0.19 nmol H₂O₂ equivalents m^{-3} , with an average value of 0.15 nmol H₂O₂ equivalents m^{-3} . The high LOD associated with this difference method emphasizes the importance of reducing or eliminating the gas signal from the particle measurement. Future progress is needed in this area to improve the ROS_p method used in this work.

5 Results and discussion

5.1 Field deployment

The ROS mist chamber was evaluated for ambient sampling by deployment at a number of sites during various seasons. Sites included the Southeastern Aerosol Research and Characterization (SEARCH) network Jefferson Street site (JST) from 7 February to 2 March 2012, and 8 May to 31 May 2012. Located in central urban Atlanta, GA, the site is considered representative of urban Atlanta (Hansen et al., 2006). Measurements were made at Yorkville (YRK), the SEARCH rural pair to JST located approximately 80 km northwest of Atlanta, 8 to 29 June 2012. Finally, measurements were made from

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the LOD of 0.15 nmol m^{-3} , but the urban sites had fewer values below LOD, consistent with generally higher ROS_p values.

The GT location is much closer to a congested 16 lane highway that runs through the middle of the city. Online measurements of elemental carbon (EC), a tracer for primary aerosol, show a greater influence of roadway emissions at GT. For example, GT/JST EC ratio was 1.36 for July 2012. This comparison, though, is somewhat ambiguous since the ROS_p measurements made at the two sites were at different times (JST in May 2012, GT in July 2012), but still no large difference is observed for a site expected to be more impacted by highway emissions. The lower concentrations at the rural site suggest urban emission may be related to ROS_p .

These offline and online measurements of ROS_p are generally lower than what has been reported by other investigators, as summarized previously (Wang et al., 2011) and shown in Table 4. Filter-based studies have reported ROS_p concentrations in the range of 0.54 to $15.1 \text{ nmol H}_2\text{O}_2 \text{ equivalents m}^{-3}$, about an order of magnitude above levels measured in this study. The few online studies conducted over much shorter time periods (1 week) report a mean ROS_p concentration of $8.30 \pm 2.19 \text{ nmol m}^{-3}$ (Wang et al., 2011). The highest concentrations are found in studies conducted next to roadways, possibly suggesting that extremely fresh emissions from these locations can lead to higher measured concentrations. It is not clear why the concentrations in Atlanta and vicinity are significantly lower. This could be due to different emission characteristics, linked to different measurement methods, or due to some of the substantial challenges associated with using this chemical probe.

7 Conclusions

An automated flow system and online instrument was developed for analysis of ROS using a mist chamber collection system coupled to an analytical system employing DCFH as a fluorescent probe. The system was operated using a set of operational

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parameters optimized based on extensive laboratory experiments. The analytical system LOD was 0.28 nM. This detector was coupled to a mist chamber for collecting and concentrating ROS components in water. PM_{2.5} ROS (ROS_p) was determined by difference between total gas plus particle (ROS_t) and filtered air (i.e. gas-phase ROS, ROS_g). This method was chosen since experiments with various denuders showed low and variable effectiveness for removing ROS_g. Higher LODs are associated with this method versus potential direct online methods due to high ROS_g levels relative to ROS_p. For the three months of measurements reported in this study the percentage of ROS_g to total, ROS_g/ROS_t, was 96±124% (one standard deviation). Online measurements were above the detection limit of nominally 0.15 nmol H₂O₂ equivalents m⁻³ approximately 25% of the time. ROS_g artifacts can be reasonably expected to dominate other liquid bases systems. During the field deployment of the instrument, concentrations of ROS_p were higher in urban areas relative to a rural site, averaging 0.25 nmol H₂O₂ equivalents m⁻³ for urban Atlanta in May and July, versus 0.14 nmol H₂O₂ equivalents m⁻³ at the rural Yorkville site during June. These online results were consistent with a series of filter samples using the same ROS analytical system designed for the online method. The ROS_p reported in this study is significantly below what has been reported by other investigators, with ranges between 0.54 to 15.1 nmol H₂O₂ equivalents m⁻³. Application of the DCFH probe to measurement of ambient particle ROS is challenging due to a number of factors, including auto-oxidation of the working solution over a short period of time as well as and photosensitivity and potential for large interferences from ROS_g.

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Research Institute (EPRI) and the Southern Company. This work does not necessarily represent the views of any of the above parties, nor do they endorse any of the commercial products mentioned within this text.

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Table 1. Optimal parameters for use in online instrumentation.

Parameter	Value
Maximum viable DCFH useful age	2 days
Reaction temperature	ambient
DCFH concentration	10 μ M
Volumetric ratio	Arbitrary (9 : 1–30 : 1)
Minimum reaction time	3 min

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Table 3. Comparison of online and offline ROS_p measurements, 12–27 July 2012.

Method	ROS _p Mean (nmol H ₂ O ₂ equivalents m ⁻³)	Range	Standard Deviation
Online (mist chamber)	0.16 ± 0.010	0.01–0.70	0.17
Offline (filters) (<i>N</i> = 19)	0.15 ± 0.019	0.05–0.34	0.079

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Table 4. Summary of ROS_p studies.

Location	Dates	Concentration (nmol m ⁻³)	Reference
Flushing, NY	Jan–Feb 2004	0.87 ± 0.18	Venkatachari et al. (2007)
Singapore (roadway)	Dec 2005	15.10 ± 0.10	See et al. (2007)
Singapore (ambient)	Dec 2005	5.71 ± 2.30	See et al. (2007)
Taipei (Taiwan)	Jul–Dec 2000	0.54 ± 0.40	Hung and Wang (2001)
Rubidoux, CA	Jul 2003	5.90 ± 1.70	Venkatachari et al. (2005)
Rochester, NY	Aug 2009	8.30 ± 2.19	Wang et al. (2011)
Atlanta, GA (online)	May–Jul 2012	0.25 ± 0.01	This study
Atlanta, GA (filters)	Jul 2012	0.15 ± 0.019	This study

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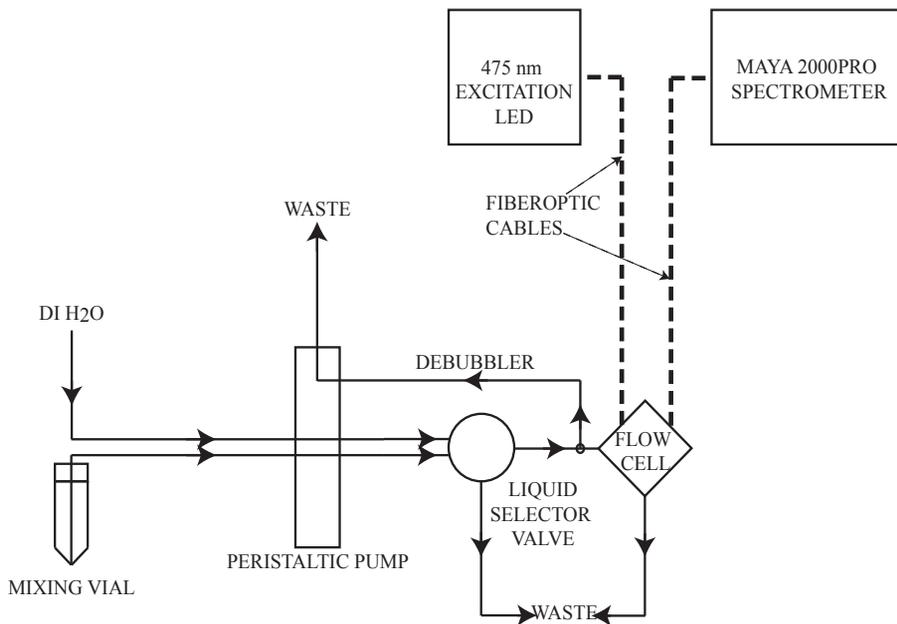


Fig. 1. Schematic for offline ROS analysis of standards and reagents combined in a mixing vial. DCFH + ROS (H₂O₂ for calibrations) is pumped via the peristaltic pump through the flow cell, in which the concentration of DCF is measured at 530 nm by the spectrometer. A selector valve switches between sample and deionized water to provide a baseline and to clear out the flow cell between samples.

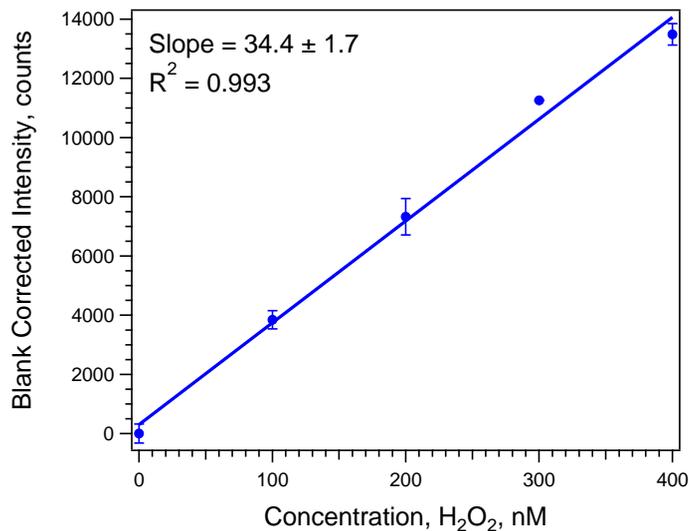
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Fig. 2. Calibration of offline ROS assay using DCFH with known concentrations of H₂O₂. Error bars represent the standard deviation of multiple measurements ($n = 5$).

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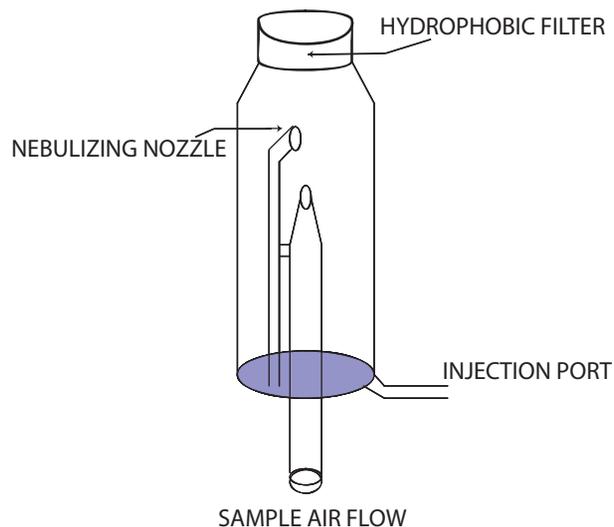


Fig. 3. Schematic of a mist chamber. Liquid is injected and extracted via the port at the bottom of the chamber. Sample air is drawn through the opening in the base of the chamber, nebulizing the liquid inside and creating a mist of droplets. Liquids are retained by means of a hydrophobic filter attached to the top of the mist chamber, which also refluxes liquid back down the sides of the glass.

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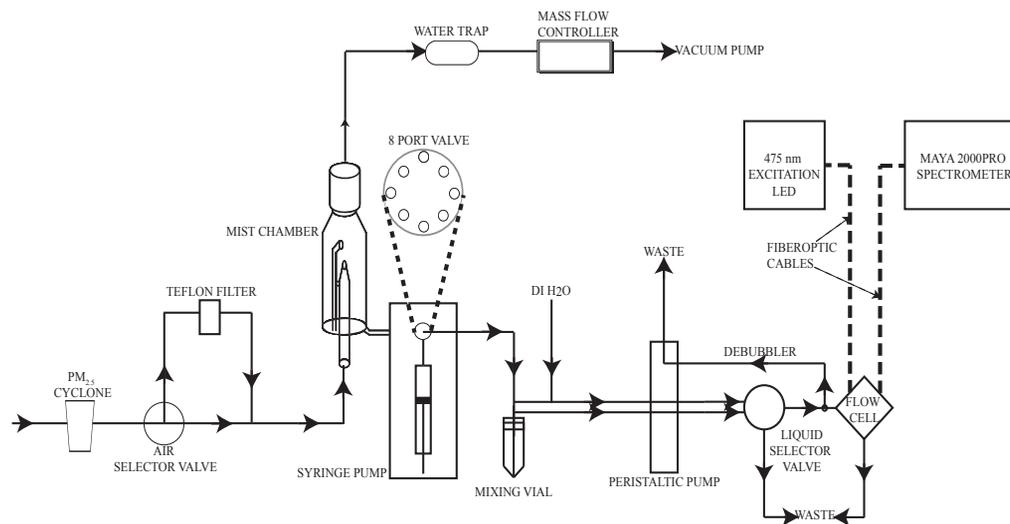


Fig. 4. Schematic of online PM_{2.5} ROS measurement approach using a mist chamber particle collection system and fluorometric probe.

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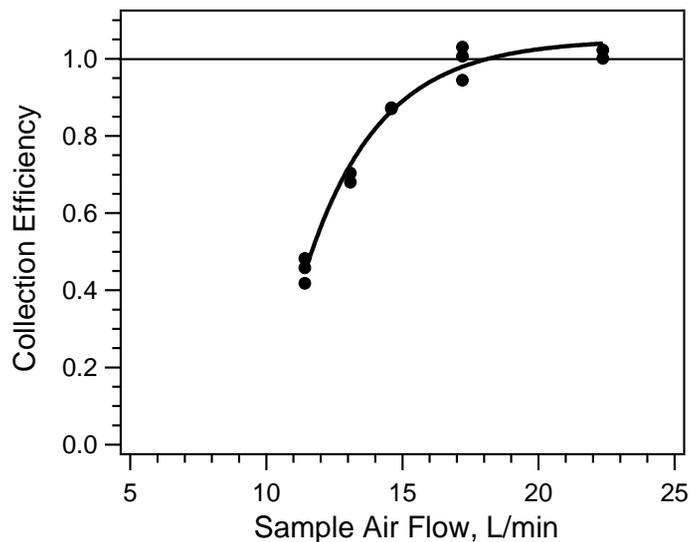


Fig. 5. Mist chamber collection efficiency. Collection efficiency determined by ratio of ambient sulfate measured with the mist chamber-IC to that from a PILS-IC system.

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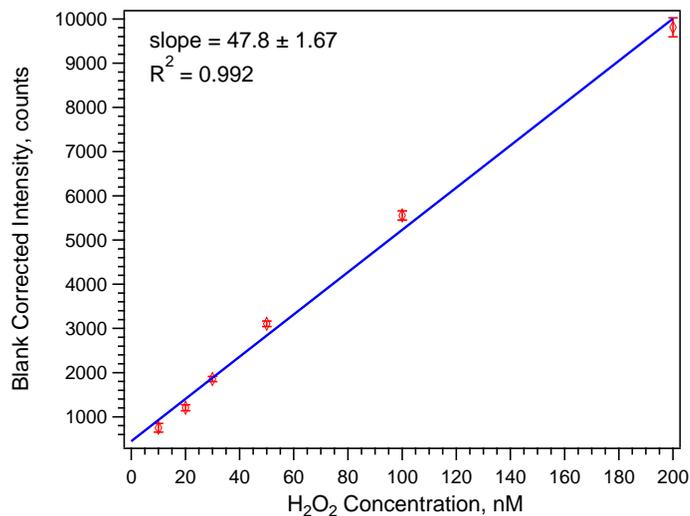


Fig. 6. Calibration of online ROS assay using DCFH with known concentrations of H₂O₂. Error bars represent the standard deviation of multiple measurements ($n = 5$).

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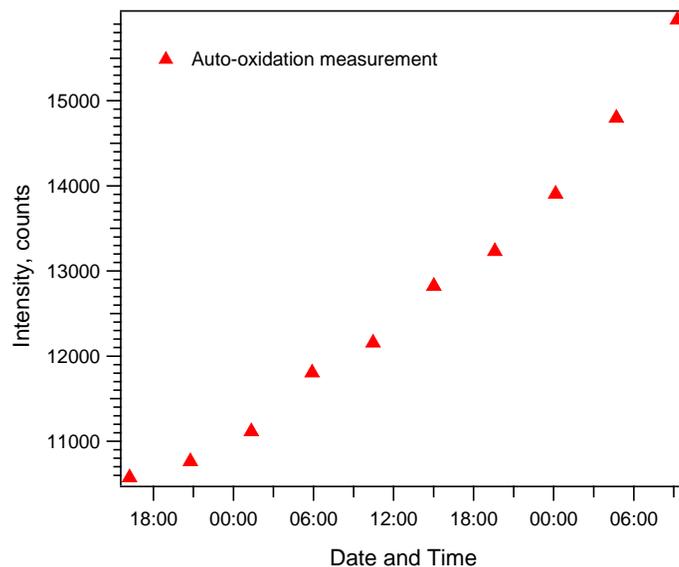


Fig. 7. Auto-oxidation (pure water blank) measurements of DCFH over time, demonstrating the drift of the DCFH working solution during regular operation of the online instrument.

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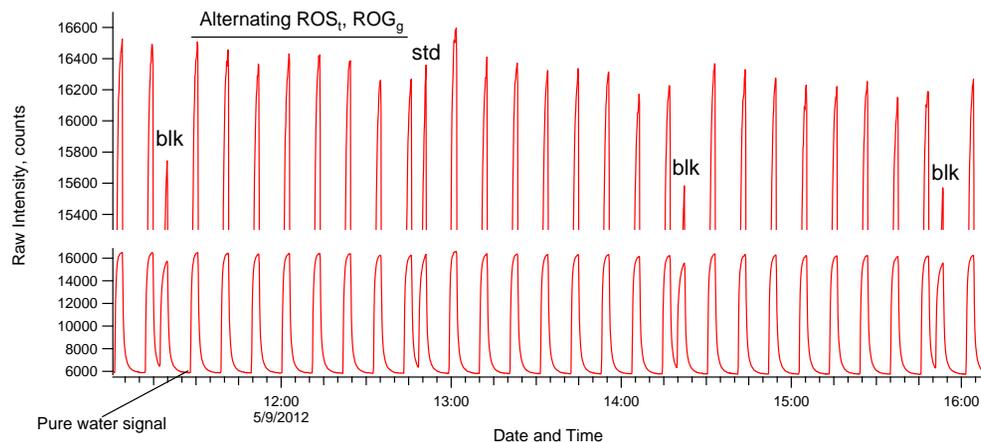


Fig. 8. Time series of raw spectrometer data for ambient ROS measurements showing responses for; pure water, deionized water + DCFH/HRP (blk), online measurements of total and gas ROS, and H₂O₂ standards (std).

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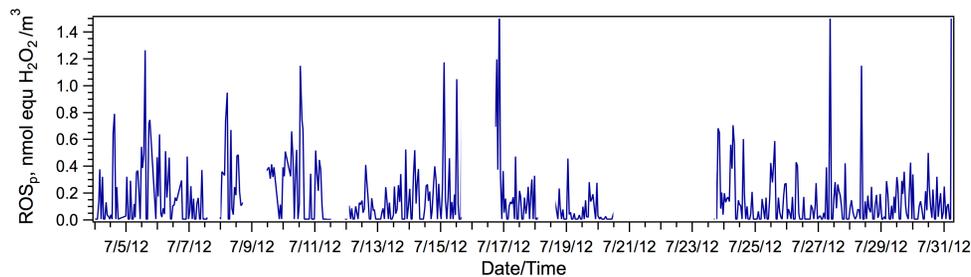


Fig. 9. Time series plot of ROS_p at the Georgia Tech sites. ROS_p below estimated limit of detection is plotted as 0.5 (LOD).

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