Semi-automated system for quantifying oxidative potential of ambient particles in filter extracts using dithiothreitol (DTT) assay

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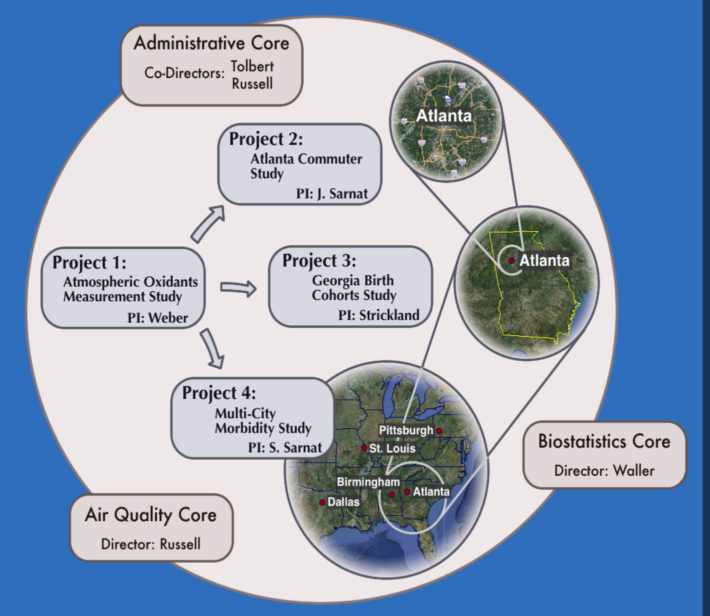
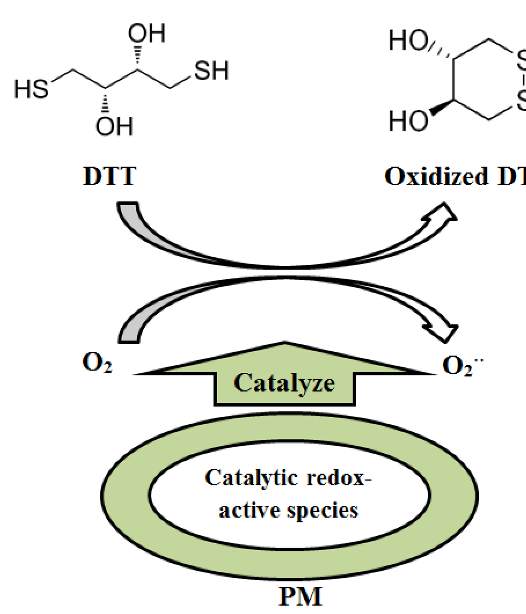


Abstract

Aerosol oxidative potential may be a more relevant indicator of adverse health effects of particulate matter (PM) than the mass concentration. Methods have been developed to measure PM oxidative potential. The DTT assay is one commonly used method and has been shown to correlate with several health markers[1][2]. The conventional manual protocol for the DTT assay is labor-intensive and time-consuming with low detection sensitivity, limiting applications of the method. To address these concerns, we developed a semi-automated system with a simplified protocol, for high throughput measurements at roughly 1 hour per sample. A scaled down system with higher sensitivity to measure samples with much lower mass loadings than Hivol samples was also developed. By automating the analysis, our goal is to generate large data sets to assess the utility of the DTT assay.

Background

- Atmospheric aerosols pose health risks, possibly through the generation of **reactive oxygen species (ROS).**
- The dithiothreitol (DTT, $HSCH_2(CH(OH))_2CH_2SH$) assay is currently one of the most widely used cell-free measures of particle oxidative potential. It is an indirect method measuring the ability of particles to generate ROS.
- DTT is oxidized to its disulfide form in the presence of ROS generated by particulate matter (PM). Therefore, **the rate of DTT consumption represents the oxidative potential of PM.**

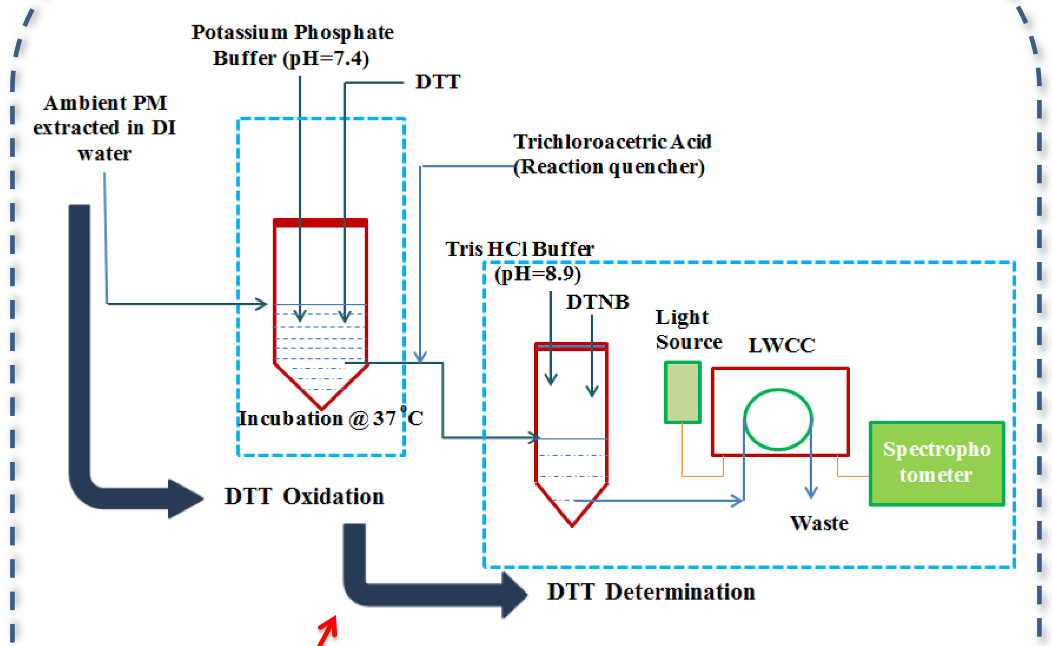


Objectives

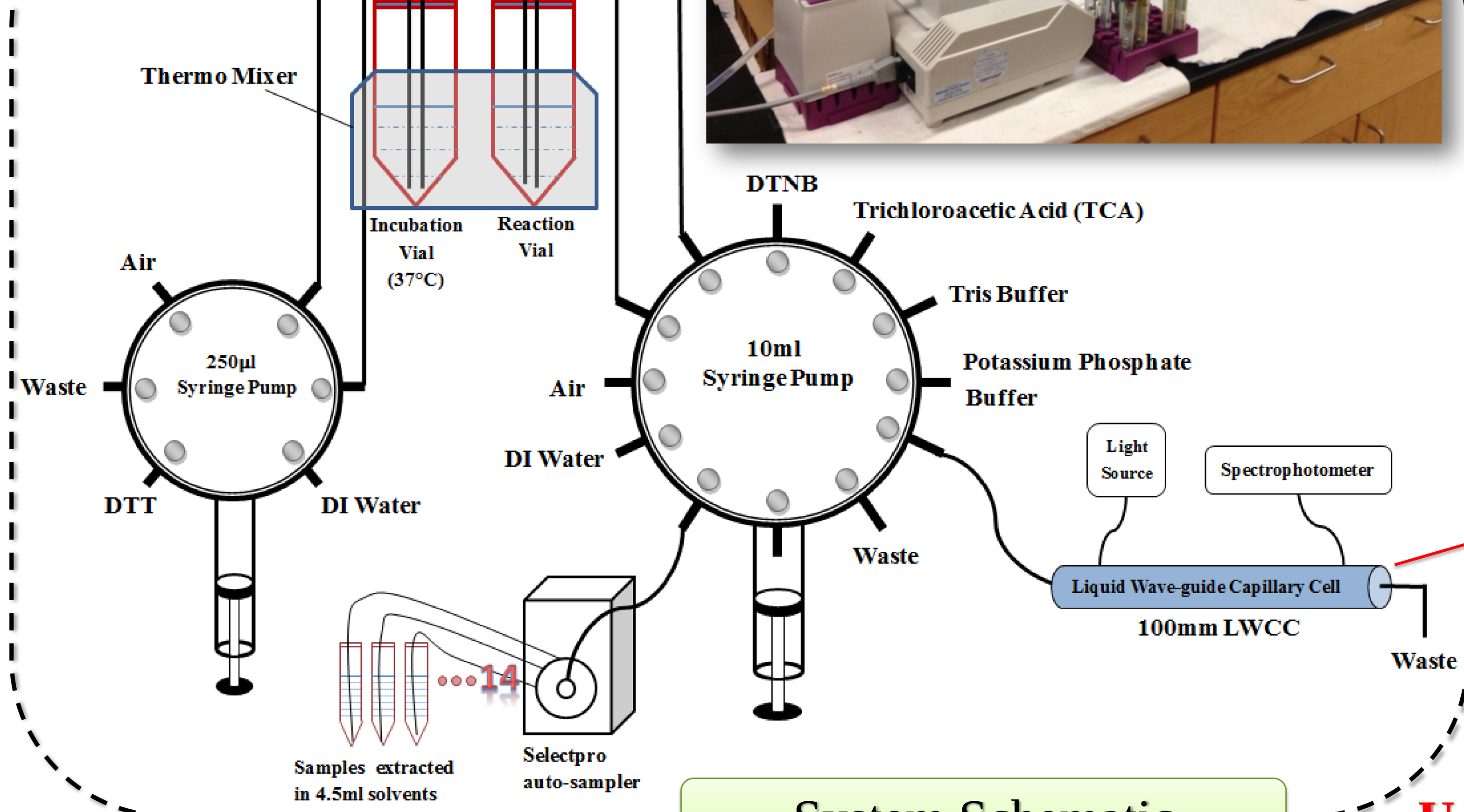
- To develop a simplified protocol compared to the conventional labor intensive manual DTT assay method.
- To build a high throughput system that generates large data sets to assess the utility of the DTT assay.
- To achieve measurements of the DTT activity of filter samples with low mass loadings.

Semi-automated System Setup

Protocol Development



A small aliquot (100µl) withdrawn at five different time intervals.

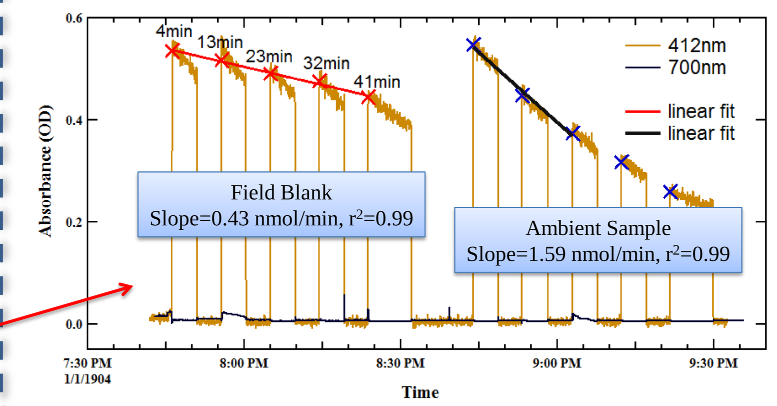


System Schematic

DTT Activity Analysis

$$\text{DTT activity} = \frac{Slope_{(Sample)} - Slope_{(Blank)}}{\mu g \text{ of PM mass or } m^3 \text{ of air}}$$

The consumption rate of DTT is proportional to the concentration of catalytic redox-active species in PM.

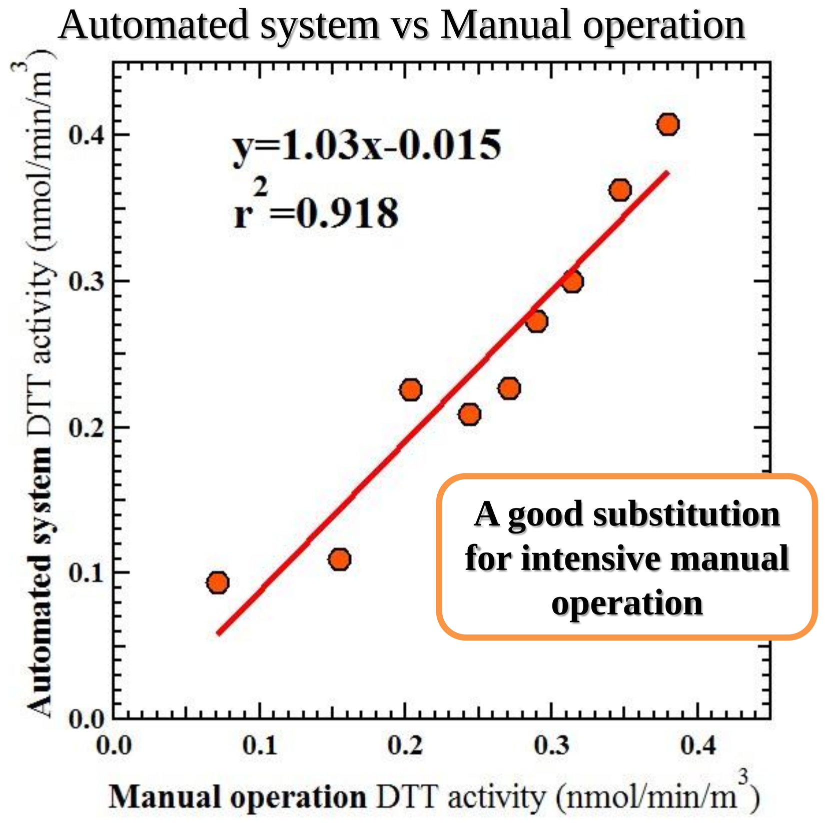


Graph 1. Example absorption spectral time series of a field blank and a sample analyzed via semi-automated system.

Unattended analysis of 1 hour/sample

System Performance

Automated system vs Manual operation



Graph 2. DTT automated system and manual operation comparison on 9 Hivol PM samples extracted in DI water.

Table 1. System reproducibility on blanks , standards and samples

Sample	Sample Size	Standard Deviation, nmol/min (▲%CV)
DI Blank	37	0.12 (27)
Field Blank* extracted in DI water	34	0.10 (28)
Field Blank* extracted in MeOH	27	0.098 (28)
Standard (9,10-Phenanthrenequinone)	30	**0.16 (12)**
Equal sections of the same filter* extracted in DI water	7	**0.081 (4)**

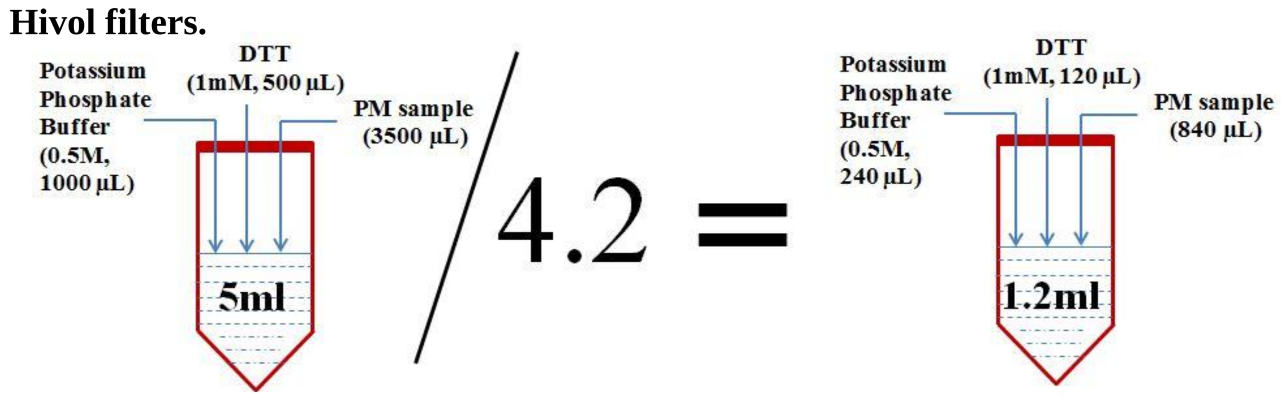
*Whatman Hivol quartz filters
▲Coefficient of Variation (%CV) = StdDev/Avg*100

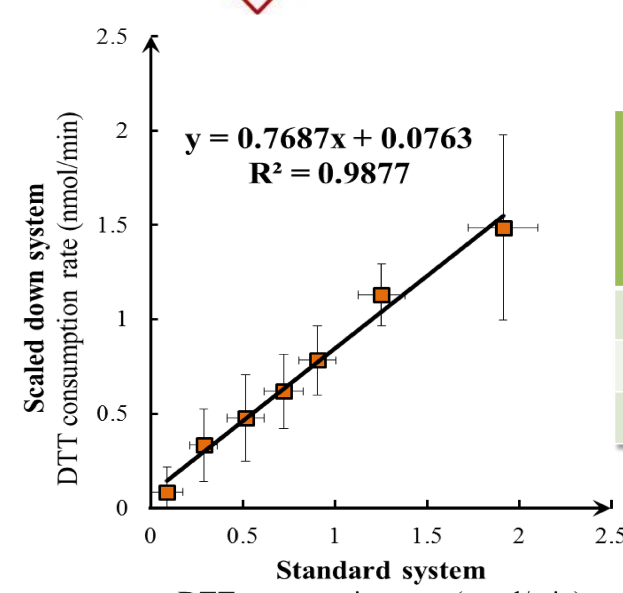
LOD=0.37 nmol/min (typical ambient level 0.8~2.6 nmol/min from Hivol filters);
Low standard deviations for standards & samples;

- **Minimum mass concentration of sample solutions ≈ 20 µg/mL; Minimum sample volume=5 mL;**
- **Theoretical PM mass loading ≈ 100 µg (depends on extraction efficiency, filter type and size)**

Scaled Down Version

In order to increase the sensitivity of the semi-automated system, a scaled down version was developed to measure samples with much lower mass loadings than Hivol filters.





Graph 3. Two automated systems comparison on standards with different concentrations

Table 2. System reproducibility on blanks and standards

Sample	Sample Size	Standard Deviation, nmol/min (▲%CV)
DI Blank	6	0.19 (51)
Field Blank	10	0.21 (36)
Standard	13	0.41 (35)

▲Coefficient of Variation (%CV) = StdDev/Avg*100
Standard - 9,10-Phenanthrenequinone

Minimum mass concentration of sample solutions ≈ 20 µg/mL
Minimum sample volume=1 mL
Theoretical PM mass loading ≈ 20 µg (depends on extraction efficiency, filter type and size)

Acknowledgement

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References

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2) Delfino, R. J., N. Staimer, T. Tjoa, D. L. Gillen, J. J. Schauer & M. M. Shafer (2013) Airway inflammation and oxidative potential of air pollutant particles in a pediatric asthma panel. *J Expos Sci Environ Epidemiol*, 23, 466-473.