Assessment of Oxidation Potential of Ambient Particulate Matter

Optimization and Application of Analytical Assays for the Measurement of
Oxidative Potential of Fine Particulate Matter in Chiang Mai, Thailand

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Abstract

Production of particulate matter (PM) has been a growing problem in Chiang Mai and has well-known adverse side effects on human health. Recent studies indicate that the mass concentration of PM alone is not the best parameter to assess the health effects and that the chemical composition of the particles plays an important role. Oxidative potential (OP) measurements of chemical substances bound on PM, which have been shown to lead to oxidative stress after exposure, are being proposed as an alternative way to assess and index toxicity. Dithiothreitol (DTT) assay is an often-used acellular technique for measuring the oxidative potential of PM. This study is on the calculation of the Oxidation potential of fine particulate matter in the air for the reactive oxidative species (ROS) production in our body and the assessment of the PM-bound metals in the Chiang Mai Province Thailand during the Biomass burning Season. This study involved evaluating three incubation methods for measuring the oxidative potential in particulate matter: sonicating, shaking, and submerging in a water bath. Following the optimization, water bath incubation was found to be the only viable method that gave high correlational data. Volume-normalized OP (OPv) were evaluated for 3 $PM_{2.5}$ concentrations: 38.25, 38.33, and 44.63 $\mu g\ m^{-3}$. Results show OPv values of 0.078, 0.041, and 0.056 $nmol\ min^{-1}\ m^{-3}$ respectively. Correlations between PM concentration and OP were also evaluated, showing a weak correlation between PM and OPv with an r^2 of 0.000008.

1 Introduction

Air pollution is a critical environmental issue impacting human health globally, and particulate matter (PM) is one of the most harmful components. Fine particulate matter (PM 2.5 and PM 1.0) can penetrate deep into the respiratory tract, reaching the lungs and even entering the bloodstream. This can lead to a range of adverse health effects, including respiratory and cardiovascular diseases, and has been linked to increased mortality rates.

In Chiang Mai, Thailand, air quality is significantly affected by two primary sources: biomass burning and vehicular emissions. Biomass burning, particularly from agricultural activities and forest fires, is a common practice in the region, especially during the dry season. This results in the release of large quantities of PM and other pollutants into the atmosphere. Additionally, the increasing number of vehicles contributes to high levels of road emissions, further deteriorating air quality.

The oxidative potential (OP) of PM is an emerging metric for assessing the health risks associated with particulate pollution. OP measures the ability of PM to generate reactive oxygen species (ROS) when inhaled, leading to oxidative stress and subsequent cellular damage. Understanding the OP of PM from different sources can provide insights into their relative toxicity and guide targeted mitigation strategies.

This study aims to assess the oxidative potential of fine particulate matter in Chiang Mai, focusing on biomass burning and road emissions. By employing advanced sampling and analytical techniques, we aim to provide a comprehensive understanding of the temporal and chemical variations in PM's oxidative potential, contributing to more effective air quality management and public health protection.

Objective: To assess the oxidative potential of fine particulate matter (PM) in Chiang Mai to aid in the classification of risks caused by air pollution

2 Materials & Methodology

2.1 Water Extraction

A high-volume air sampler was used to collect $PM_{2.5}$ samples from sites around Chiang Mai to quantify PAH concentrations. The PM samples were brought back to Chiang Mai University for chemical analysis. The samples were contained within UV-sensitive tubes to limit UV exposure which could possibly deteriorate sample quality. The particulate samples were punched out using a 12 mm puncher to regularize sample size.

To prepare the samples for the DTT assay, the samples were placed in UV-sensitive tubes with 30 mL of deionized (DI) water. DI water was chosen as the solvent to avoid contaminating the results and introducing any additional ions or contaminants that could interfere with the results. DTT assays are highly sensitive to contamination as well as ions, necessitating the need for DI water. To ensure thorough mixing of the particulate matter with the water, sonication was used to ensure a homogeneous mixture. The UV-sensitive tubes containing the particulate matter and water were placed in the sonicator, which was then run at a temperature of 37°C for 30 minutes. The controlled temperature and duration were selected to optimize the mixing process without causing any degradation or alteration of the particulate matter.

2.2 DTT Assay

Once the samples had been prepared, they were subjected to a dithiothreitol (DTT) assay to quantify their oxidative potential. The oxidative potential of the PM was determined by measuring the rate at which a reducing agent, specifically DTT is consumed in the presence of the particulate matter. The DTT assay began by placing the PM samples in UV-sensitive tubes to prevent any light-induced reactions that could alter the results. Six UV-sensitive tubes were placed in a rack and labeled with the times at which they would be analyzed: 2, 6, 10, 14, 18, and 22 minutes. 1 mL of the sample was added into each tube along with 50 µl of 1 mM DTT solution and 1 mL of 0.5 M phosphate buffer solution (K-buffer). The phosphate buffer solution was added to provide a stable pH for the reaction. Once the reagents were added, the tubes were incubated in a water bath mixer to ensure thorough mixing of the contents. A stopwatch was started to track the duration of the reaction for each sample. At each time interval (2, 6, 10, 14, 18, and 22 mins) the corresponding tube was removed from the mixer and 500 µl of 1 mM DTNB (5,5'-dithiobis(2-nitrobenzoic acid)) was added to the tube. DTNB acts as a chromogenic reagent that reacts with the remaining DTT to produce a yellow-colored chromophore product with absorbance at 412 nm. The intensity of the yellow color, which correlates with the amount of unreacted DTT, was measured using a spectrometer set to a wavelength of 412 nm.

The spectrometer measured the absorbance of each sample, providing a quantitative measure of the DTT consumption. By comparing the absorbance readings at different time points, the rate at which DTT was consumed could be determined. This rate is indicative of the oxidative potential of the particulate matter with a higher rate of DTT consumption suggesting a higher oxidative potential, which can be correlated with potential health impacts.

The dithiothreitol (DTT, 1 mM) solution was prepared by dissolving 7.7 mg of DTT in 50 mL of DI water. The 5,5'-Dithiobis-2-nitrobenzoic acid (DTNB, 1 mM) solution was prepared by adding 19.82 mg of DTNB into 50 mL of methanol. A 0.5 M buffer solution (pH 7.4) was prepared by mixing 13.47 g of monopotassium phosphate (KH2PO4) and 69.85 g of dipotassium phosphate (K2HPO4) in 1000 mL DI water. The buffer was also treated with Chelex 100 resin to remove trace metals.

3 Results and Discussion

The oxidative potential of the sample can be expressed in terms of the rate of DTT consumption (nmol/min) due to collected particles per unit sampling air volume ($V_{a}ir$, m3) to yield OPv (extrinsic OP) as shown in Eq. (1):

$$OP_v = \frac{\Delta DTT_{Sample} - \Delta DTT_{Blank}}{V_{air}} \qquad (1)$$
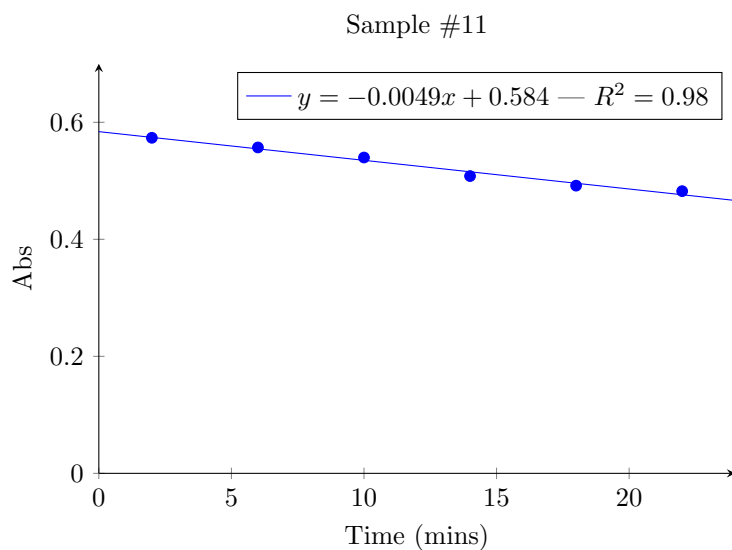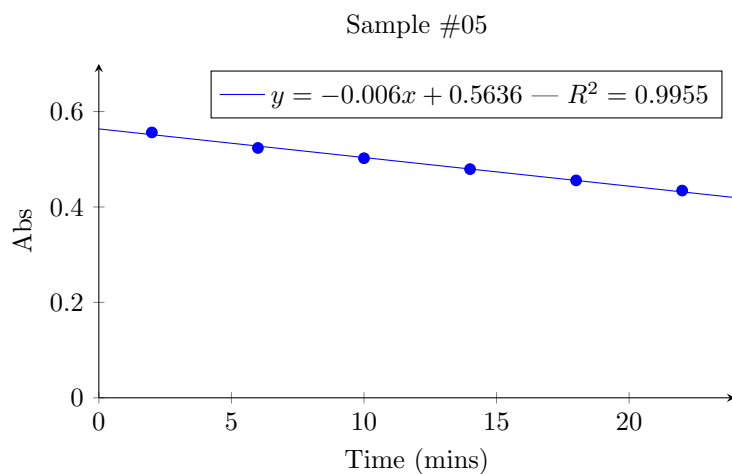
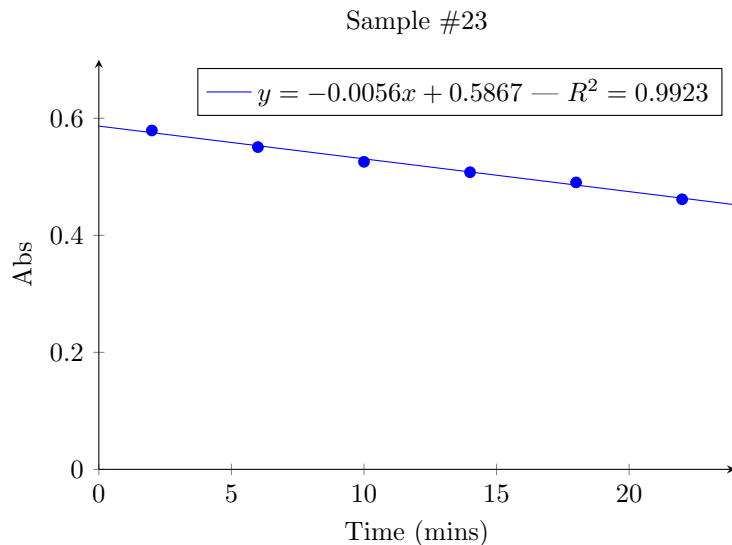Where the consumption rate of DTT (ΔDTT) is calculated by Eq. (2):

$$\Delta DTT = -\sigma Abs \cdot \frac{N_{DTT}(nmol)}{Abs_0} \qquad (2)$$

Where σAbs is the slope of absorbance versus time, and Abs_0 is the initial absorbance estimated from the intercept of the linear regression of absorbance versus reaction time. and N_{DTT} is the initial mole concentration of DTT (nmol). OPv, a quantitative measure of OP, can be used to estimate the exposure dosage based on the total inhalation air volume.

Time (mins)	Sample #05	Sample #11	Sample #23
2	0.5562	0.5735	0.5792
6	0.5237	0.5570	0.5508
10	0.5022	0.5398	0.5256
14	0.4793	0.5081	0.5078
18	0.4557	0.4917	0.4904
22	0.4344	0.4823	0.4616

Table 1: DTT consumption over time

Sample #05

$$y = -0.006x + 0.5636 \quad R^2 = 0.9955$$

Sample #11

$$y = -0.0049x + 0.584 \quad R^2 = 0.98$$

Sample #23

$$y = -0.0056x + 0.5867 \quad\text{—}\quad R^2 = 0.9923$$

3.1 Optimization of DTT Assay

OP measurements in this study were carried out using the procedure outlined by Hsiao et al., 2021. One modification was made to this method, which improved the correlation and linearity of the results. The use of water bath incubation was found to greatly improve data and give more reproducible results. 3 different methods of incubating and shaking were tested: sonication, shaking, and water bath incubation. Both sonication and shaking were found to give unstable results, with sonication most likely unevenly heating and mixing due to the varying intensity of the ultrasonic waves, and the shaker being unable to maintain the necessary 37°C which led to unstable results and poor reproducibility. Water bath incubation was the only method that gave high correlational data and was able to maintain the 37°C. The stability and uniformity of temperature in the water bath likely contributed to the improved linearity and reliability of the measurements, making it the preferred choice for this study.

3.2 Mass concentration of $PM_{2.5}$ compared to OP

Sample	Slope (-Abs)	N_{DTT} (nmol)	Intercept (Abs)	ΔDTT	OP(v)	PM 2.5
Sample #05	-0.006	50	0.5636	0.5323	0.0780	38.25
Sample #11	-0.0049	50	0.584	0.4195	0.0410	38.33
Sample #23	-0.0056	50	0.5867	0.4772	0.0600	44.63

Table 2: Oxidative Potential

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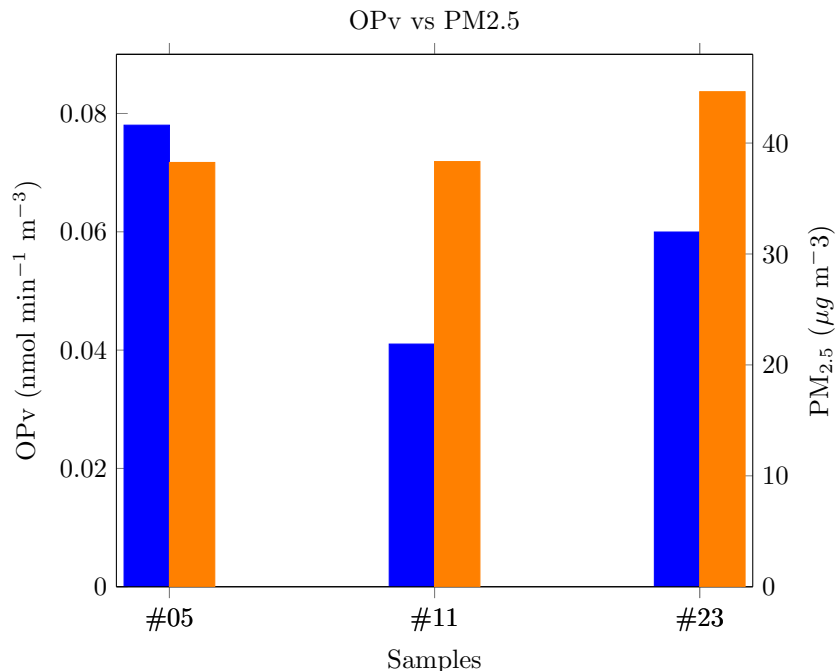
The oxidative potential (OP) values in this study were calculated and normalized for the sampled volume (OPv) to represent the amount of air inhaled, approximating actual human exposure to oxidative stress. This approach directly correlates the oxidative potential of particulate matter (PM) to potential health impacts, giving a more accurate assessment of human exposure to oxidative stress compared to merely considering PM mass concentration.

Blank G (ΔDTT_{Blank})	0.2945
Air Vol (m^3)	0.001873
Filter area (m^2)	1627.2

One of the key findings of this study was the lack of significant correlations between the mass concentration of PM and the volume-normalized oxidative potential (OPv). These results underscore the complexity of PM's chemical composition, which varies depending on pollutant sources, their intensity, and the atmospheric conditions. The heterogeneity of PM composition means that mass concentration alone cannot reliably predict or measure the toxicity of PM. This observation aligns with previous studies that have highlighted the inadequacy of using PM mass concentration as the sole indicator of health risks associated with PM exposure.

The negative effects of PM on human health are well-documented, including respiratory and cardiovascular diseases, as well as other chronic conditions. However, the complex and varied chemical makeup of PM presents a significant challenge in establishing clear relationships and correlations between its toxicity and its individual components. PM can originate from multiple sources, including biomass burning, vehicle emissions, industrial activities, as well as natural sources such as dust and sea spray. Each of these sources contributes different chemical constituents to the overall PM mixture, leading to a complex and variable chemical profile. Components such as metals, organic compounds, and reactive oxygen species precursors present in PM can significantly influence its oxidative potential. The interaction between these components and their collective contribution to oxidative stress are not captured by mass concentration measurements alone.

The findings of this study have important implications for health risk assessment and regulatory policies. Current air quality standards primarily focus on PM mass concentration ($PM_{2.5}$ and PM_{10}) to assess air pollution levels and associated health risks. However, results suggest that such standards might not fully capture the health impacts of PM exposure. Incorporating measures of oxidative potential, which provide

insight into the chemical reactivity and potential toxicity of PM, could lead to more comprehensive and accurate assessments of health risks. Future research should focus on further understanding the specific chemical components of PM that contribute most significantly to its oxidative potential and toxicity. This study reinforces the notion that PM mass concentration is an insufficient metric for assessing the health risks of air pollution. The oxidative potential of PM, influenced by its complex chemical composition, provides a more relevant measure of its potential to cause oxidative stress and subsequent health effects. Integrating oxidative potential measurements into air quality monitoring frameworks could improve the assessment and management of air pollution's health impacts.

4   Conclusion

In this study, the oxidative potential (OP) of fine particulate matter (PM) in Chiang Mai was assessed as a method to evaluate PM toxicity, replacing mass concentration evaluations. Through an evaluation and optimization of three incubation methods - sonicating, shaking, and submerging in a water bath — water bath incubation emerged as the most reliable, providing high correlational data for measuring OP. Volume-normalized oxidative potential (OPv) was determined for three $PM_{2.5}$ concentrations: 38.25, 38.33, and 44.63 $\mu g\ m^{-3}$, resulting in OPv values of 0.078, 0.041, and 0.056 $nmol\ min^{-1}\ m^{-3}$, respectively. These values reflect the reactive oxidative species (ROS) production potential of the particulate matter.

The chemical composition of PM plays a significant role in its oxidative potential. PM-bound metals, which are often associated with sources like biomass burning and traffic emissions, contribute significantly to the OP values. The analysis revealed that individual metals, despite constituting a small fraction of the $PM_{2.5}$ mass, greatly influence OP due to their catalytic activity in generating ROS. Furthermore, atmospheric aging processes, such as the formation of secondary organic aerosols (OAs) and secondary inorganic aerosols alter the surface area and catalytic activity of transition metals, thereby affecting ROS generation.

Overall, the findings highlight the complexity of PM toxicity, influenced by both its chemical composition and atmospheric processes. This study suggests that current air quality regulations, which rely solely on mass concentration of $PM_{2.5}$, may underestimate the adverse health effects of particulate matter. In contrast, measuring oxidative potential provides a more direct quantification of PM toxicity, offering a better metric for evaluating its impact on human health.