

AN EXPLORATORY STUDY FOR THE IMPLEMENTATION OF THE OXIDATIVE POTENTIAL ASSESSMENT OF PARTICULATE MATTER IN PORTUGAL

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ABSTRACT

Particulate matter (PM) is a harmful air pollutant that damages human health by inducing oxidative stress through the excessive generation of reactive oxygen species (ROS). Oxidative Potential (OP) is a proposed metric to measure PM's capacity to generate ROS (Almetwally et al., 2020; Jiang et al., 2019).

This study aims to implement the OP^{DTT} assessment methodology at C²TN (Portugal). A set of PM_{2.5} samples was evaluated using a widely used protocol (Chirizzi et al., 2017), and the results were compared to the values previously obtained at DITEBA, where the protocol is well established.

Moreover, the use of the reference material SRM 1648 (Urban Particulate) as a standard for determining OP^{DTT} was also evaluated. Analyzing a 10 mg.L⁻¹ solution of the reference material, it was possible to conclude that the standard solution presented an average DTT activity (normalized to the mass) value of 27.60 ± 3.79 pmol.min⁻¹.µg⁻¹.

INTRODUCTION

Reactive oxygen species (ROS) can be present in particulate matter (PM), be produced in atmospheric reactions, or result from oxygen metabolism inside the cells. Increased production of ROS in the cell can unbalance the ratio between ROS and available antioxidants, resulting in adverse health effects, such as oxidative stress (Hedayat et al., 2015; Jiang et al., 2019). Oxidative potential has been used to measure PM's capacity to promote the formation of ROS in the human body. This parameter, aligned with information from the composition and sources of particulate matter, can be of extreme importance in creating new directives on air quality and air pollution management (Cho et al., 2005).

Among the developed assays to determine OP, the dithiothreitol (DTT) assay is one of the most widely applied (Jiang et al., 2019). The principle of this method is that redox active species present in PM samples oxidize DTT, a strong reducing agent, to its disulfide form. Simultaneously, oxygen molecules are reduced to form superoxide. The OP determination using this assay presupposes the determination of DTT concentration at different moments of the reaction time. The quantification of DTT is performed using a colorimetric method, where the addition of 5,5'-ditiobis-2-nitrobenzoic acid (DTNB) leads to the formation of 5-mercapto-2-nitrobenzoic acid (TNB), a colored substance with a high molar extinction coefficient ($\epsilon=14\ 150\ \text{M}^{-1}\text{cm}^{-1}$) at 412 nm (Hedayat et al., 2015; Kumagai et al., 2002; Li et al., 2009). The lack of a standard method to perform this assessment makes it difficult to compare results from different studies and to promote results quality management (Frezzini et al., 2022; Jiang et al., 2019). Despite being vastly investigated, in Europe, most of the published studies on OP only present limited sampling periods not exceeding a few months (Paraskevopoulou et al., 2019; Weber et

al., 2018), and for Portugal, there are no studies on OP assessment of aerosols. Thus, the main goal of the present study is to implement the methodology of OP^{DTT} assessment at C²TN (Portugal). For this, a set of PM_{2.5} samples was selected to perform the OP^{DTT} assessment by applying one of the most used protocols (Chirizzi et al., 2017).

Moreover, the evaluation of the use of the reference material SRM 1648 (Urban Particulate) as a standard for determining OP using the DTT assay was also conducted. This could be a valuable tool to evaluate the quality of the results in such type of analysis.

METHODOLOGY

The OP of the water-soluble fraction of a quarter of the PM_{2.5} sampled filters (from an urban-industrial environment), which chemical characterization and source apportionment has already been described elsewhere (Gamelas et al., 2023), was assessed following the DTT assay described by Chirizzi et al. (2017). A solution of the reference material SRM 1648 (Urban Particulate - NIST, USA), with a concentration of 10 mg.L⁻¹, was prepared with deionized water (DI, Milli-Q; > 18 MΩ). This solution was used as a standard and evaluated regarding its OP level following the same procedure as the PM_{2.5} extracts.

The water-soluble fraction was extracted from 1/4 of each filter in 15 mL deionized water (DI, Milli-Q; > 18 MΩ) by sonication in a water bath for 30 min. The extracts were filtered through 0.45 μm pore PTFE (polytetrafluoroethylene) syringe filters to remove insoluble materials and residual fibers. The standard solution was not submitted to the filtration process. A 3.5 mL sample from each extract was then incubated at 37 °C with DTT (100 μM) in 0.1 M potassium phosphate buffer at pH 7.4 (5 mL total volume) for 90 minutes. At specified times (5, 10, 15, 20, 30, 45, 60, and 90 minutes), a 0.5 mL aliquot of the incubation mixture was collected, and 0.5 mL of 10% trichloroacetic acid was added to stop the reaction. At the end of the incubation period, 2 mL of 0.4M Tris-HCl, pH 8.9 containing 20 mM EDTA and 25 μL of 10 mM DTNB was added to the mixture. The concentration of TNB formed was measured by its optical density absorption at 412 nm using a UV-1800 spectrophotometer (Shimadzu Corp.). The rate of DTT consumption (δDTT , pmol/min) was determined from the slope and intercept of linear regression of measured absorbance versus time (Cho et al., 2005).

Three replicates were made for each sample, and the variability (standard deviation) between replicates was taken as the uncertainty of the measured DTT activity. The final DTT activity for a sample was calculated by subtracting the average value of the blanks from the value of each sample. The final DTT activity was normalized concerning 1) the sampled air volume (V), which provides OP_V^{DTT} , and 2) the collected aerosol mass (M), which provides OP_M^{DTT} , that represents an intrinsic property of particles linked to sources (Chirizzi et al., 2017).

Regarding the standard solution, the effect of the order of addition of trichloroacetic acid (TCA) was studied, i.e., in half of the tests, TCA was added to the aliquot, and in the other half, the aliquot was added to the TCA solution. Blanks were performed using only deionized water, with TCA added to the aliquot.

RESULTS AND DISCUSSION

Oxidative potential of SRM 1648 (Urban Particulate - NIST, USA)

The OP of a solution of 10 mg.L⁻¹ of the reference material SRM 1648 was determined. In each test, one blank and two solution samples were analyzed. TCA was added to the aliquots in one of the samples, and in the other sample, the aliquots were added to the TCA solution. The experiment was performed 10 times to provide the statistical significance of the data. The results, normalized to the mass, are presented in [Table 1](#). The total value comprises all the DTT activity results from the solution analysis. The average DTT consumption on the blanks was 547.81 ± 133.40 pmol.min⁻¹.

Table 1. Oxidative potential of solution of SRM 1648 (Urban Particulate - NIST, USA), with a concentration of 10 mg.L⁻¹.

Parameter	Total	TCA Before	TCA After
Average DTT activity (pmol.min ⁻¹ .µg ⁻¹)	27.6	27.5	27.7
Standard deviation (pmol.min ⁻¹ .µg ⁻¹)	3.8	4.8	2.7
Coefficient of variation	13.7 %	17.5 %	9.7 %
Number of replicas	20	10	10

The obtained results were submitted to the Mann-Whitney test. It was possible to verify that the order of addition of TCA does not significantly influence the results once the average DTT activity values are not statistically different (p value=0.666, significance level=5%). However, when the aliquot is added to the TCA, there is a bigger variation between the obtained results (higher coefficient of variation, increasing from 9.7% to 17.5%), and, therefore, it is more suitable to perform the addition of TCA to the aliquot.

From the presented results, it is possible to conclude that a solution of SRM 1648 (concentration of 10 mg.L⁻¹) can be applied as a standard for OP determination (obtaining a mean value of 27.6 ± 3.8 pmol.min⁻¹.µg⁻¹), thus creating a form of quality control for this assay.

Oxidative Potential of Real Samples

In a previous study, the OP of thirty PM_{2.5} samples (out of a total set of 128 filters) was assessed by the DTT method at DISTEBA, Italy (for training purposes). Samples presented mean levels of DTT activity (normalized to the volume, OP_V^{DTT}) of 209 ± 174 pmol.min⁻¹.m⁻³, ranging from 23 to 845 pmol.min⁻¹.m⁻³. The OP_V^{DTT} showed a significant positive association with PM_{2.5} levels (R²=0.71). The results are presented in [Figure 1a](#). The average DTT consumption on the blanks was 947 ± 119 pmol.min⁻¹ (4 different filter blanks, with 2 replicates each).

In the present study, the OP of 18 PM_{2.5} samples of the same set of filters were analyzed, and the results were compared with the previously studied samples. The average DTT consumption on the blanks was 889 ± 113 pmol.min⁻¹ (2 different filter blanks, with 2 and 3 replicates).

Samples presented a mean OP_V^{DTT} value of 182 ± 142 $\text{pmol}\cdot\text{min}^{-1}\cdot\text{m}^{-3}$, ranging from 44 to $656\cdot\text{pmol}\cdot\text{min}^{-1}\cdot\text{m}^{-3}$.

Overall, the total of the 48 samples presented a mean OP_V^{DTT} value of 199 ± 161 $\text{pmol}\cdot\text{min}^{-1}\cdot\text{m}^{-3}$, ranging from 23 to $845\text{ pmol}\cdot\text{min}^{-1}\cdot\text{m}^{-3}$. The results are presented in **Figure 1b**, with samples analysed at C²TN described as orange triangles. The DTT activity (normalized to the volume, OP_V^{DTT}) showed a significant positive association with PM_{2.5} levels ($R^2=0.71$).

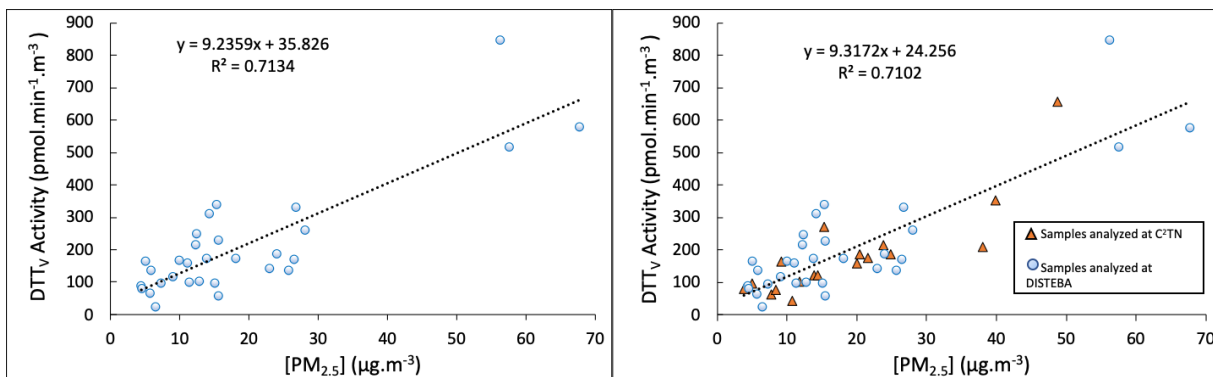


Figure 1 Relation between DTTv Activity and PM concentration. a) Samples analyzed at DISTEBA; b) Total of samples (with samples analyzed at DISTEBA represented as blue circles and the samples analyzed at C²TN represented by orange triangles).

Comparing the results obtained at DISTEBA with the values achieved at C²TN, it is possible to conclude that the assay was successfully implemented in Portugal, once the results are at the same range. Additionally, the new data does not significantly affect the correlation between OP_V^{DTT} and PM_{2.5} concentrations.

CONCLUSIONS

This study aimed to study the application of SRM 1648 (Urban Particulate - NIST, USA) as a potential standard solution for the DTT assay. Analyzing a $10\text{ mg}\cdot\text{L}^{-1}$ solution of this standard material, it was possible to conclude that the standard solution presented an average DTT activity (normalized to the mass) value of 27.6 ± 3.8 $\text{pmol}\cdot\text{min}^{-1}\cdot\mu\text{g}^{-1}$, with a coefficient of variation of 14%

To determine the effectiveness of the DTT assay in assessing the oxidative potential of particulate matter in Portugal, a comparison was made between the results obtained from analyzing a set of samples at DISTEBA, where the method is already well-established and the data obtained at C²TN in Lisbon, Portugal. Based on this comparison, it can be concluded that the implementation of the DTT assay has been successful.

The DTT activity (normalized to the volume, OP_V^{DTT}) of the forty-eight analyzed samples showed a significant positive association with PM_{2.5} levels ($R^2=0.71$).

ACKNOWLEDGMENTS

This work was supported by the Portuguese Foundation for Science and Technology (FCT, Portugal) through the project HypnosAir (reference PTDC/CTA-AMB/3263/2021,

<https://doi.org/10.54499/PTDC/CTA-AMB/3263/2021>), the PhD fellowship PRT/BD/154533/2023 (C. Vicente), the contract 2021.00088.CEECIND (N. Canha) and the financial support to C²TN/IST (UIDB/04349/2020+UIDP/04349/2020).

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