

Response to reviewers' comments

Thank you and the reviewers for handling the manuscript (manuscript number: amt-2021-407). Responses to reviewers are in *italics*. The changes in the manuscript have been marked in [blue](#). Please refer to the point-by-point response to the reviewers' comments and concerns.

Thank you again and the reviewers for such detailed suggestions for revision.

Comment on amt-2021-407

Anonymous Referee #3

The author's present a methodology where they incorporate a dithiothreitol (DTT) based assay into a previously established online method for particle composition analysis (MARGA). The authors make alterations compared to previous online DTT methods in the literature, including the use of nitrogen carrier gas and shielding from light in order to reduce the DTT background signal. The optimised DTT method is compared to current methods existing in the literature and deployed in ambient measurement campaigns where they correlate DTT activity with a range of inorganic ions, trace gasses and black carbon. However, there is a lack of technical detail in places, and the comparison between online and offline measurements requires additional clarification. I would recommend publication after addressing the following comments:

Line 111-113 - There is a lack of technical details in general in this section – is nitrogen continuously flowed through, if so what flow rate? How is the DTT reaction vial actually protected from light? As these are key modifications, there should be substantially more technical details added.

Response: *Thanks for the suggestion, Nitrogen does not flow continuously, we fill it with nitrogen before the experiment starts, and nitrogen is used to remove air from the instrument. We use aluminum foil to wrap the tubing and instruments to protect from light. We add a new text to line 124 in the revised manuscript:*

“In the DTT reaction module, to avoid the influence of light and air on the experiment, all pipelines, reaction flasks and mixing flasks are sealed and protected from light by aluminum foil. The whole DTT experimental part was filled with N₂ by pump A and pump B before the experiment started.”

Line 172 – Additional details should be given regarding how offline PQN experiments were performed.

Response: *We add a new text to line 184 in the revised manuscript:*

“First, we select PQN with concentrations of 0.01, 0.02, 0.025, 0.05, 0.085 nmol L⁻¹ to compare online and offline DTT activity detection to determine the error of online and offline experiments. The details of PQN analysis can be found in Supplement S1.”

Supplement S1:

“First, we configure PQN solutions with concentrations of 0.01, 0.02, 0.025, 0.05, 0.085 μM. Then, take 1.5 mL PQN solution and 5 mL 0.1 M potassium phosphate solution (adjust the pH to 7.4 after preparation) and mix in a 15 mL reaction flask. Next, add 0.5 mL of 1mM DTT to the reaction mixture, and place it in a constant temperature oscillator (THZ-D, Suzhou Peiying Experimental Equipment Co., Ltd.) at 37 °C and a rotation speed of 250 r/min. At the specified time interval (0, 10, 20, 30, 40 minutes), take out 0.5 mL of the reaction mixture and transfer it to another vial containing 0.5 mL of 10% w/v trichloroacetic acid (TCA) for termination reaction between DTT and sample solution. Then, add 50 μL of 1 mM DTNB (5,5'-dithiobis (2-nitrobenzoic acid)) to react with the remaining DTT in the solution. Finally, add 2 mL of 0.4 M Tris buffer (0.4 M Tris + 20 mM EDTA, adjust the pH to 8.9 after preparation), and use a

spectrophotometer to detect the absorbance at a wavelength of 412 nm, where the spectrophotometer includes an ultraviolet-visible (UV-VIS) light source (Ocean Optics DT-mini-2) and a multi-wavelength light detector (USB4000 micro fiber spectrometer), and the data acquisition software (Spectra Suite) to record the absorbance intensity at 412 and 700 nm (selected as the baseline absorbance of TNB).”

Line 174 – What are traditional samples, please specify.

Response: *Traditional sample trial production of real samples collected by high flow samplers. Here, it refers to the samples collected continuously for 24 hours using a large-flow PM_{2.5} 188sampler (KC-6120) in Xuzhou. I have changed to offline samples.*

Line 184 – What are the PM_{2.5} mass loadings on the filters collected for analysis?

Response: *The PM_{2.5} mass loadings on the filters collected for analysis is between 110µg/m³ and 140µg/m³ per sampling film.*

Line 204 – Again, more detail required here regarding the nitrogen and light-reducing modifications.

Response: *Thanks for the suggestion, we add a new text to line 123 in the revised manuscript:*

“In the DTT reaction module, in order to avoid the influence of light and air on the experiment, all pipelines, reaction flasks and mixing flasks are sealed and protected from light by aluminum foil. The whole DTT experimental part was filled with N₂ by pump A and pump B before the experiment started.”

Line 223 – If the slope is lower compared to Puthussery et al, does this not mean the response as a function of PQN concentrations is less, and thus the method is less sensitive to PQN? Is this slope corrected for baseline, accounting for background DTT consumption? This should be elaborated in more detail in the manuscript.

Response: *Thanks for the question, at pH 7.0, almost 100% of DTT was transformed to DTT-Disulfide by the catalyst 9,10-PQ (Li et al., 2009). This slope is corrected for the baseline to account for background DTT consumption. In addition, we think it is meaningless to compare the magnitude of the slope with Puthussery et al. Therefore, we delete all the comparisons about the slope size in the manuscript. We add a new text to line 240 in the revised manuscript:*

“At pH 7.0, almost 100% of DTT is transformed to DTT-Disulfide by the catalyst 9,10-PQ (Li et al., 2009). The analytical measurement part of the online DTT instrument is calibrated by measuring the DTT activity of PQN at different concentrations. As shown in Figure 4, the linear graph of DTT consumption rate and PQN concentration, which is after subtracting the blank DTT consumption rate.”

Line 231 – How does this LOD compare to the other methods mentioned in the literature? And how does this compare to the LOD of the offline method used?

Response: *We add a new text to line 247 in the revised manuscript:*

“The limit of detection (LOD) of the system is defined as 3 times the standard deviation of the deionized water blank ($N = 23$), i.e., $0.024 \text{ nmol min}^{-1}$, which is significantly lower than the LOD of Puthussery et al. ($0.24 \text{ nmol min}^{-1}$) and Fang et al. ($0.31 \text{ nmol} \cdot \text{min}^{-1}$).”

Line 254 – It is unclear to me what the purpose of the “PQN correction” is? Please elaborate.

Response: *When using PQN to compare online and offline, we found that the DTT consumption rate was deviated between online and offline conditions of the same concentration of PQN. We believe that it is caused by the experimental error of the online instrument. Therefore, when using the online instrument to measure the real sample, the experimental error is compensated by correction.*

Line 257 – Referring to the comparison of online and offline measurements in Figure 5. The authors state in the introduction that online methods are advantageous due to the online method capturing reactive components that offline methods currently do not, which is valid. Therefore, we would expect the online DTT signal to be higher than that of the offline method for an equivalent sample once normalised, due to the rapid capture of particles in the online method compared to offline. This is not the case in Figure 4 where the PQN slopes are the same, but the offline values are higher compared to online, why? Is this due to the optimisation of the online method? It is not clarified clearly. In Figure 5, the ambient samples measured offline have a higher DTT activity compared to online, and after the “PQN correction”, the offline DTT activity is roughly equivalent to the online, if not still slightly higher for some samples. Puthussery et al (2018), frequently cited in this manuscript, for instance show a higher online signal compared to offline. There is really limited description of the data in Figure 4 and Figure 5 in the manuscript, and as the online vs offline comparison is a key feature of implementing the DTT assay into an online methodology, this should be explained more clearly and in more detail.

Response: *We add a new text to line 269 in the revised manuscript:*

“The manual detection results are slightly higher than the automatic detection results, we assume that this is due to the instrument error caused by the complicated piping system of the online instrument.

As shown in Figure 6, the online and offline analysis of the DTT activity of 10 ambient particles, the slope (manual/automatic) obtained by orthogonal fitting is 1.14, the intercept is 0.19, and the correlation coefficient (R^2) is 0.954. We found that the real samples tested also had slightly higher offline results than online results. This is similar to our assumption.”

Line 522 – Figure caption 1 is not sufficient to describe the method, please expand substantially to include more technical detail.

Response: *We add a new text to line 558 in the revised manuscript:*

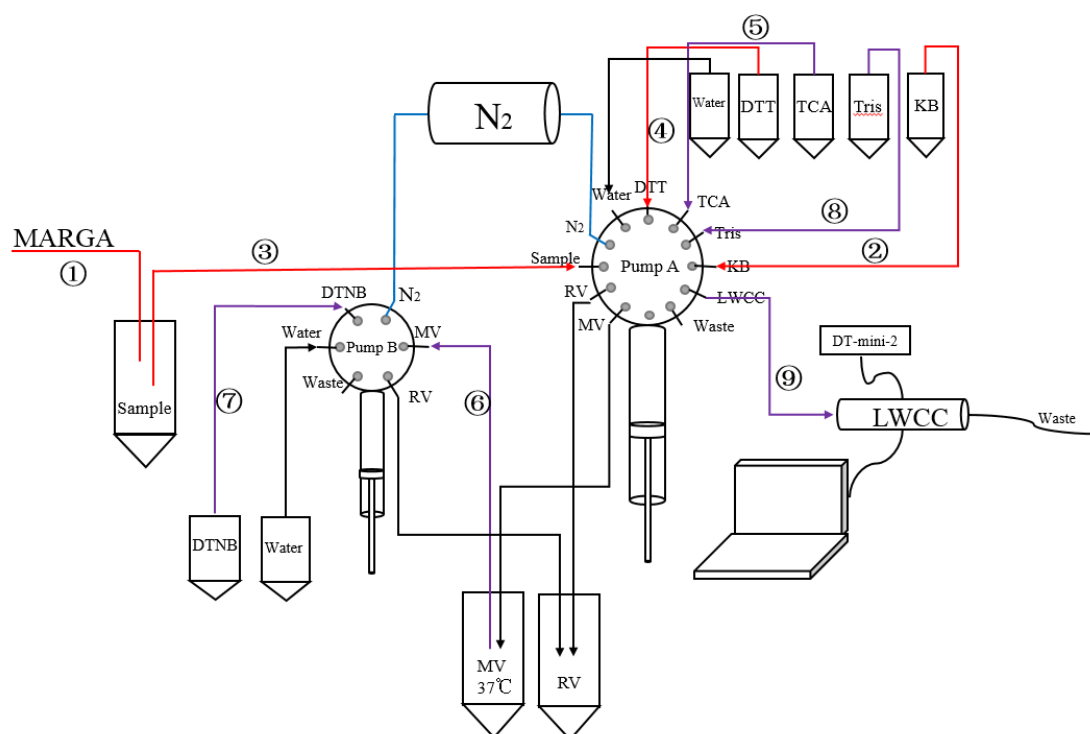


Figure.2 Schematic diagram of DTT reaction part. (①-④) represents the DTT oxidation step, ⑤-⑨ represents the DTT determination step. Blue indicates the ventilation line, all pipelines are wrapped in aluminum foil to protect from light.)

Technical corrections:

The manuscript could benefit from an additional proof reading for English as there are confusing and, in some cases, incomplete sentences throughout the manuscript.

Line 26 – reactive oxygen (species?) typo?

Response: *The full name of ROS is reactive oxygen species.*

Line 150 – deionized water (deionised water) typo.

Response: *Thanks for pointing it out, I have corrected it.*

Line 180 – the amount of what?

Response: *Thanks for pointing it out, we add a new text to line 193 in the revised manuscript:*

“The MARGA is calibrated using internal and external standards. The internal standard is a 10 mg L⁻¹LiBr solution. The external standard calibration is performed after replacing the anion and cation columns, and the replacement cycle is generally 4 to 5 months. At the same time, the MARGA system is cleaned with 1% hydrogen peroxide and 10% acetone solution, and the airflow is calibrated every two months. In the DTT experimental module, DTT and DTNB solutions are prepared every 4 days. Before each test, perform a comprehensive light and nitrogen bag inspection. To ensure the accuracy of the experimental data, a standard curve was measured before each experiment. The instrument pipeline is cleaned once a week, as shown in Figure 1. The programmable pump A and pump B are connected to the ultrapure water channel.

During the cleaning process, all pipelines, reaction tubes and mixing tubes are cleaned.”

Line 294-296 – ng m-3, typo? ug m-3?

Response: *Thanks for pointing it out, we have revised the manuscript:*

“The average concentration of PM_{2.5} during the sampling period is 9.97±6.53 ug m⁻³, the average concentration of PM_{2.5} before rain is 11.13±7.21 ug m⁻³, the average concentration of PM_{2.5} after rain is 7.80±4.18 ug m⁻³. The concentration of PM_{2.5} is a significant drop.”

Line 526 – no x-axis title on Figure 2 (left), label both graphs (e.g. A and B)

Response: *Thanks for pointing it out, I have corrected it*

Line 533 – error given in Figure 3 but not in Figure 4 for the same data?

Response: *Figure 3 shows the rate of DTT consumption from 3 parallel experiments with different concentrations of PQN under offline conditions, so there is a standard deviation. Figure 4 shows the Comparison of the automated system with manual operation using PQN (9,10-phenanthraquinone), with no standard deviation.*