

Response to comments from referee #2 (Manuscript Ref. NO: acp-2017-214)

Huanhuan Jiang et al.
mjiang@ufl.edu

Thank you very much for the thoughtful and constructive comments. The paper was greatly improved based on your suggestions.

In this work, Jiang et al. studied the oxidative potential of Secondary Organic Aerosol (SOA) generated in laboratory chambers. The authors investigated the role of various oxygenated compounds within complex SOA systems in contributing to oxidative decay of sulfhydryl groups, which is believed to be the main mechanism by which particulate matter causes adverse cardiopulmonary outcomes. The authors systematically varied experimental conditions and carefully measured various functional groups, and relate the composition to OP measured (using the DTT assay). Based on the experimental results, they conclude that organic hydroperoxides (ROOH) are a major source of oxidative potential, and suggest that electron-deficient alkenes (such as acrolein) may also be important too.

The experiments are done with sufficient controls, and carefully interpreted. The DTT activity of SOA has recently become a topic of great interest within the atmospheric community. The work is within the scope of ACP and should be published after considering the following minor comments.

Major comments:

1. The suggestion about electron-deficient alkenes being important is worth noting. Acrolein seems to be the only example that has significant DTT activity. All others are an order of magnitude lower. Are there example compounds that are more relevant to toluene/benzene system? I would imagine that aromatic compounds would yield conjugated compounds, which according to Figure 7 and Section 3.4, would have negligible DTT activity. Also, acrolein has very high vapor pressure and is unlikely to be in the particle phase.

Response: Acrolein, methyl acrolein, 2,4-hexadienal and mesityl oxide were chosen as model compounds to show how electron-deficient alkenes increase DTT activity. The DTT activity of alkenes increases with an electron withdrawing group such as carbonyls but decreases with an electron donating group such as alkyl groups. The electron-deficiency of acrolein is relatively higher than methyl acrolein, 2,4-hexadienal and mesityl oxide (conjugated carbonyl with 2 methyl groups). As discussed in Section 3.4, the toluene SOA contains a large amount of alkene that are coupled with electron withdrawing groups such as carbonyls, nitrates, and carboxylic acids. These electron-deficient alkenes in toluene SOA might contribute to the DTT response.

2. Related to the previous note, in Section 3.1, the decrease in DTT activity is on the same timescale of acrolein. That is not a fair comparison because acrolein is reacting in the gas phase, where the DTT measurement is for compounds in the particle phase, which likely have longer lifetimes than acrolein in the gas phase.

Response: In order to respond to the reviewer, the lifetime of two semi-volatile conjugated carbonyls (4-oxo-2-butenic acid, and 2-hydroxy-3-penten-1,5-dial) that were reported in the previous study (Jang et al., 2001) was estimated using a structure-reactivity relationship for the reaction with OH radicals (chamber condition). The calculation was included in SI (Section S5). If these two electron-deficient carbonyls are oxidized by OH radicals in the gas phase, the estimated lifetime of 4-oxo-2-butenic acid and 2-hydroxy-3-penten-1,5-dial is estimated to be 134 min and 43 min, respectively. The actual lifetime of these compounds will be shorter than our estimation since they can also be oxidized in particle phase.

Reference: Jang, M. and Kamens, R. M.: Characterization of secondary aerosol from the photooxidation of toluene in the presence of NO_x and 1-propene, *Environ. Sci. Technol.*, 35, 3626-3639, 2001.

3. On that note, why is it called “electron-deficient alkenes”? Why not “unsaturated carbonyls”? Reaction with DTT is likely on the oxygenated group, rather than on the C=C double bond. Also, from a fundamental perspective, does the C=C double bond need to be adjacent to the C=O group? Have the authors investigated, say, 3-penten-2-one?

Response: Not all unsaturated carbonyls are reactive to DTT. The reactivity of an alkene is determined by the contribution of all substituents. If conjugated carbonyls have electron-donating groups such as alkyl, alkenyl, and ester group, they are not anymore electron-deficient alkenes and their reactivity with DTT is low. Thus, we prefer to use electron-deficiency of alkenes (Nair et al.) for their reactivity to DTT.

Reference: Nair, D. P. et al., The thiol-michael addition click reaction: a powerful and widely used tool in materials chemistry, *Chem. Mater.*, 26, 724-744, 2014.

4. Also, if acrolein indeed contributes high DTT activity, I would imagine that SOA from 1,3-butadiene would have very high DTT activity. Have the authors done any experiments to suggest that is the case?

Response: Acrolein was tested to describe the reactivity of functional group in organic compound. Actually, acrolein may not be present in aerosol due to their high volatility. The SOA yield of 1,3-butadiene is as low as 0.4-0.5% for 1.0-2.2 ppm 1,3-butadiene (Angove et al.). In addition, the major aerosol products (75-80%) of 1,3-butadiene are not electron-deficient alkenes, based on the study of Angove et al.

Reference: Angove, D.E. et al., The characterization of secondary organic aerosol formed during the photodecomposition of 1,3-butadiene in air containing nitric oxide, *Atmospheric Environment*, 24, 2006

5. Another suggestion for additional experiments (optional but would really strengthen the argument): if the authors can pass ozone over the SOA (e.g. collected on the filter), the ozone should selectively remove the double bonds. This way the authors can isolate the contribution to DTT from these electron deficient alkenes. While this may create additional organic hydroperoxides, the authors can easily correct for that increase by measuring total OHP with the NPBA assay.

Response: The electron-deficiency of alkenes increases the reactivity to DTT while electron-deficiency can significantly reduce reactivity to ozone. In addition, the products can be off-gassing from the filter by passing ozone through the filter and increase artifacts in chemical assays. Some products that have weak reactivity to DTT may become reactive to DTT due to the ozone reaction.

6. Does the UNIPAR model describe the abundance of electron deficient alkenes under low-NO_x? I would imagine under high NO_x there would be more electron deficient alkenes where C-C bond scission and ring-opening reactions are dominant, but the “unaccounted” DTT activity seems to be higher under low NO_x for toluene (from Fig. 6).

Response: In order to response to reviewer, the quantity of conjugated compounds was estimated using the UNIPAR model. The quantity of conjugated compounds in low-NO_x toluene SOA is similar to or slightly more than that in high-NO_x toluene SOA. Within the error bar, the “unaccounted” DTT activity of low-NO_x toluene SOA was close to that of high-NO_x toluene SOA (Fig. 6 in the old version, Fig. 5 in revised manuscript).

7. I understand that the proposal about measuring DTT_m as a proxy for maximum capacity (described in Section 3.1) is useful for a chemist. But I wonder what the biological relevance is. What is the lifetime of particles deposited in the lungs? I am not a lung expert, but I wonder if mucociliary clearance would make the lifetime shorter than 2 hours, and therefore DTT_m at 2 h may not be that relevant.

Response: The DTT assay enables the evaluation of aerosol reactivity to a sulfhydryl group using a small amount of aerosol mass and simple chemical procedures, and illustrates of the potential toxic mechanisms of aerosols. However, chemical assay is limited to a screening method and the results of chemical assay need to be compared with *in-vitro* and *in-vivo* studies. According to the study of Oberdorster et al. (1988), the lung clearance of particles ranges from several minutes to one day, depending on the solubility, particle size, etc. Binding with and to tissue and cellular components can increase the retention halftimes of particles to days and months. Also, the lifetime of particles in biological systems can also be affected by the lung diseases. The clearance of particles can be retarded if the lung is not healthy.

Reference: Oberdörster G., Lung Clearance of Inhaled Insoluble and Soluble Particles, Journal of Aerosol Medicine, 1, 1988.

8. For DTTm to be meaningful, DTT must be in large excess compared to amount of SOA. What is the estimated ratio of DTT to SOA (100 uL of DTT, 700 uL of SOA solution)? Is DTT in large excess? This is not an issue when looking at the catalytic activity, because both DTT and quinones can be regenerated through catalytic cycles. But this issue arised when it is a one-step reaction with these particulate oxidizers.

Response: The excess amount of DTT was used to determine oxidative potential of SOA. A sentence was added and reads now,

“To ensure the pseudo-1st-order reaction between DTT and redox-active species in SOA, the SOA mass applied to DTT assay was constrained to ensure that the DTT consumption remained less than 50 % of DTT₀.”

9. If hydroperoxides (and PANs) are indeed unstable at room temperature (as suggested in Section 2.3.2), did the decrease in ROOH amount correspond to a decrease in DTTt or DTTm?

Response: The QA/QC of chemical assays used in this study was reorganized and included in the supporting information (Section S4). The stability of H₂O₂ and peracetic acid with storage time was measured by NPBA assay (Fig. S8) and by DTT activity (Fig. S4). When the solution was kept at 4 °C over a 6-day period, the decrease of DTT response of peracetic acid was 22% and the decrease of NPBA assay was 11%. However, SOA samples of this study were analyzed within 24 h after collection. The decrease of DTT response and NPBA response of peracetic acid stored at 4 °C were insignificant compared to those of the fresh solution. Thus, we think that instability of organic peroxides will not influence of our chemical analysis. The QA/QC information of the stability of compound was shown below:

The stability of peroxides. To investigate the impact of the stability of organic compounds on the DTT activities, the DTT consmptions (Δ DTT at reaction time=100 min) by 400 μ L H₂O₂ (100 μ M) and 300 μ L peracetic acid (CH₃C(O)OOH; 100 μ M) were measured as a function of storage time. As shown in Fig. S4, the DTT response of H₂O₂ and CH₃C(O)OOH stored at room temperature (RM) decreased upto 80 % and 36 % after 8 days, respectivley. The DTT response of CH₃C(O)OOH stored at 4 °C also decreased 22% after 6 days.

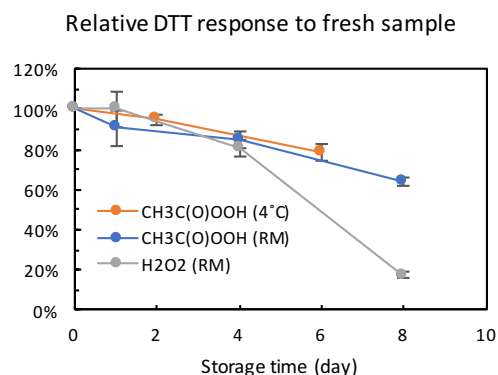


Figure S4. The DTT responses of H₂O₂ and peracetic acid stored at room temperature (RM) or 4 °C. The errors was estimated using the standard deviation of three replicates.

The stability of peroxides. To investigate the impact of the stability of peroxides on NPBA assay, the NPBA responses for 400 μL H_2O_2 (100 μM) and 300 μL peracetic acid (100 μM) were measured as a function of storage time. Fig. S8 illustrates that the NPBA response of H_2O_2 and $\text{CH}_3\text{C}(\text{O})\text{OOH}$ stored at room temperature decreased 70 % and 27 % after 8 days, respectively. The decrease of the NPBA response in $\text{CH}_3\text{C}(\text{O})\text{OOH}$ stored at 4 $^\circ\text{C}$ was similar to that stored at room temperature.

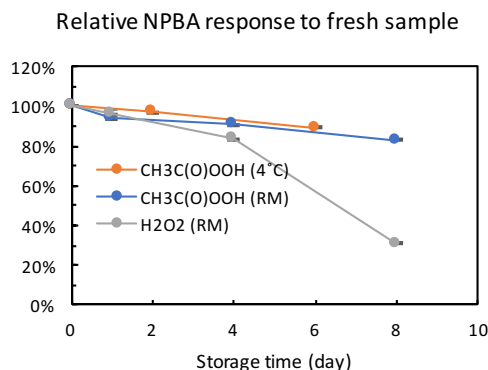


Figure S8. The NPBA responses of H_2O_2 and peracetic acid stored at room temperature or 4 $^\circ\text{C}$. The error bar was estimated using the standard deviation of three replicates.

10. There are large fluctuations in experiment temperatures (more than 20K). Are there systematic differences in chemical composition?

Response: Thanks much for your comment. The UF-APHOR chamber uses the ambient sunlight and meteorological conditions. Thus, the temperature profile for each experiment has a diurnal pattern. Although temperature can influence the chemical composition of SOA due to the impact of temperature on the gas-particle partitioning of organic products, temperature is less influential on DTTt than aging time or NO_x levels. For example, DTTt values of toluene were modulated mainly by aging time (Fig. 3 in old version, Fig. 2 in revised manuscript). For isoprene SOA, DTTt was affected mainly by NO_x conditions. We included the discussion of temperature on DTT activity in the revised manuscript (Section 3.1).

“The DTT_t values of isoprene SOA was, however, higher than those observed by Tuet et al. (Tuet et al., 2017b) and Kramer et al. (Kramer et al., 2016). This difference might be caused by the degree of aerosol aging under different NO_x conditions, initial OH radical sources, humidity and temperature.”

Reference: Tuet, W. Y., Chen, Y., Xu, L., Fok, S., Gao, D., Weber, R. J., and Ng, N. L.: Chemical oxidative potential of secondary organic aerosol (SOA) generated from the photooxidation of biogenic and anthropogenic volatile organic compounds, *Atmos. Chem. Phys.*, 17, 839-853, 2017b. Kramer, A. J., Rattanavaraha, W., Zhang, Z., Gold, A., Surratt, J. D., and Lin, Y.-H.: Assessing the oxidative potential of isoprene-derived epoxides and secondary organic aerosol, *Atmos. Environ.*, 130, 211-218, 2016

Minor comments:

1. Section 2.2: Please provide define PILS and provide details of the PILS method. Was steam used to grow particles for impaction and collection? If so, is there concern about the high

temperatures leading to decomposition of thermally labile compounds important for oxidative potential?

Response:

We added the principal of PILS in Section 2.2 and read now,
“The aerosol particle that enters the PILS grows quickly into a droplet under the supersaturated environment and this droplet is collected on the plate by impaction.”

It is possible that high temperature leads to the decomposition of thermally unstable compounds. However, the residence time of a particle inside PILS is approximately 1 second. In addition, the rapid cooling of water steam by the ambient air sample gives the high supersaturation. The resulting temperature downstream PILS is nearly ambient temperature.

2. Section 2.3.1 Line 1: it is unclear what “two steps” mean in parentheses. Perhaps re-word to “The two-step DTT assay: : :”

Response: This paragraph was modified and reads now

“DTT assay was employed to quantify the oxidative potential of SOA (Cho et al., 2005; Jiang et al., 2016). In the first step (DTT oxidation), a mixture of 700 μ L SOA-PILS sample, 200 μ L potassium phosphate buffer (2 mM, pH=7.4) and 100 μ L DTT (1 mM) was incubated at 37 °C in a sonicator (FS30H Ultrasonic Cleaner, Fisher Scientific). For the second step (determination of the remaining DTT), the reaction between DTT and SOA was quenched by adding 1 mL 1% w/v trichloroacetic acid (a commonly used quencher of thiol oxidation).”

3. Section 2.3.1 Line 2: What does SOA sample mean? Is this the aqueous solution?

Response: “SOA sample” was changed to “SOA-PILS sample” that was the aqueous solution collection by PILS.

4. Section 2.3.1 Page 6 Line 1: What is the pH of the PBS?

Response: The information of pH=7.4 was added to the sentence.

5. Section 2.3.1 Page 6 Line 1: “shaken in a sonicator” is confusing. Particularly since in the later section “shaking” was compared to “sonicating”.

Response: This sentence was modified and reads now,

“In the first step (DTT oxidation), a mixture of 700 μ L SOA-PILS sample, 200 μ L potassium phosphate buffer (2 mM, pH=7.4) and 100 μ L DTT (1 mM) was incubated at 37 °C in a sonicator (FS30H Ultrasonic Cleaner, Fisher Scientific).”

6. Section 2.3.2: Can 4-nitrophenol be formed in high NO_x SOA from toluene? If so, are there any negative controls?

Response: 4-Nitrophenol may be found in toluene SOA. The influence of 4-nitrophenol in SOA on NPBA assay was discussed in Section S4.2 (revised supporting information) and reads now,

“4-Nitrophenol, NPBA product, can also be found in toluene SOA and potentially influences on NPBA data. However, the light absorption of the SOA sample collected using PILS was negligible at 406 nm, suggesting that NPBA data is not influenced by the light absorbing materials in SOA products.”

7. Section 3.1 heading: remove period after title

Response: The period mark after title was removed.

8. Section 3.3 Line 5: Grek et al. does not provide any evidence that PANs can oxidize sulfhydryl groups. They only include peroxyxynitrites as an RNS, but PANs are peroxy nitrates.

Response: For the reaction of PAN with a sulfhydryl group, we cited other references. The new citations are shown below:

Products of the Reaction of Peroxyacetyl Nitrate with Sulfhydryl Compounds, Mudd et al., Archives of Biochemistry and Biophysics, 132, 1969.

Reaction of Peroxyacetyl Nitrate with Glutathione, J. B. Mudd, The journal of Biological Chemistry, 241, 1966.f

9. Section 4 Line 25: remove (DTT modulator: quinones)

Response: “(DTT modulator: quinones)” was removed from this sentence.

10. Section 4 Page 14 Line 8: An assay that is not water-based may not be relevant, since cells are water-based

Response: This sentence was deleted.

11. Table 1 and throughout manuscript: Does HC/NO_x ratio refer to the initial or average HC/NO_x? If it is the average, over what time range is the HC/NO_x calculated?

Response: The HC/NO_x ratio mentioned throughout the manuscript refers to the initial HC/NO_x. The title “HC/NO_x (ppbC/ppb)” in Table 1 was changed to “Initial HC/NO_x (ppbC/ppb)”. The detection of the HC/NO_x ratio over the course of experiment is complex due to the complexity in chemical species and the wall process of organics, nitric acid, and organonitrates.

12. Table 1 Mid-collection time: is this local time?

Response: “Mid-collection time” refers to the local time in Gainesville, Florida. This was clarified in the revised manuscript.

13. Figure 4: why is one data series labeled with experiment date, but others are not?

Response: The date information was removed. The initial HC/NO_x ratio was added.