

General Comments

This study by Enarsson et al. investigates the interference and suppression of fluorescence from carbonaceous particles in reactive oxygen species (ROS) when analyzed using the BPEAnit probe in an online instrument.

The topic of this manuscript is of interest and relevance to AMT and is useful for improving the performance of online ROS measurements by reducing the effects of experimental artefacts. The intention and objective of the study are unique.

However, I have several concerns regarding the methodological approach the authors have employed to make their inferences. Some of the authors' interpretations are not directly derived from the results and need more clarity before they can be verified. I think the manuscript needs to be revised to address these concerns before I can recommend it for publication.

Specific Comments

- 1.) Page 2: Lines 84 – 86: Please cite a couple of papers that mention this interference or have quantified it while measuring OP or ROS. I don't think the four papers cited by the authors in the earlier sentence give interference of insoluble particles as a reason for choosing water-soluble ROS/OP measurement approaches. To my knowledge, at least two of these papers are using an online instrument with microfluidic connections (Wragg et al. 2016 and Puthussery et al. 2018) or narrow flow cells (Utinger et al. 2023). Insoluble particles in their methods can, in turn, affect the results, likely by being deposited onto the microfluidic tubing, leading to clogging, or being difficult to clean when using a narrow flow cell, which may be possible reasons for those studies to not use insoluble PM. The authors have cited studies that have shown interference for non-ROS or non-OP measurements, but this still does not provide a justification as to whether insoluble particles interfere with OP measurements.
- 2.) Page 3: Lines 93 – 95; Authors are requested to substantiate the claim that BC concentrations are elevated in indoor environments due to candle burning. I don't fully agree with their assessments as several studies have reported high BC emissions from candles only when the candles are sooting and not burning cleanly. (Fine et al., 1999; Pagels et al., 2009; Stabile et al., 2012; Subramanian et al., 2025) The authors are expected to make this distinction as well, as clean combustion of a candle releases only a very small fraction of BC (Fine et al., 1999; Pagels et al., 2009; Stabile et al., 2012; Subramanian et al., 2025).

- 3.) Line 139: Have the authors investigated if the insoluble particles are deposited in the tube, as it has a pretty small diameter? This may also lead to a reduction in fluorescence. The authors are requested to provide more details validating this.
- 4.) Lines 186 - 191: This is not a strong enough analogy for selecting these concentrations without providing more details on the manuscript in preparation. $150 \mu\text{g}/\text{m}^3$ of BC is very high and is likely a concentration obtained from chamber experiments, I believe. These concentrations are environmentally relevant except on rare occasions such as maybe during wildfires, or during highly polluted wintertime PM in China and India. Moreover, I am not sure how the authors converted the mass concentrations in the air to liquid concentrations. It seems to be that 1 mL of liquid corresponds to 16.67 L of Air according to this statement. How did the authors come about this number?
BC only constitutes a small fraction of ambient PM (5 - 20%). Moreover, the study the authors cite is not a study using PM in real-world DCFH-DA, albeit one using nanoparticles.
The authors are requested to cite relevant studies of ambient PM using DCFH-DA or other assays and requested to verify if the concentrations of BC they used are environmentally relevant. I think $25 \mu\text{g}/\text{mL}$ of Printex 90 is a very high concentration that may not be environmentally relevant.
- 5.) Lines 229 – 235: Please be more specific about the chamber experiments and add more details about the methodology in the SI. What were the concentrations of SOA and soot in the final liquid that was analyzed using fluorescence? In Figure 1, the intensity in the first peak with SOA is only 20% of the background. Were these experiments performed in triplicate, and the LoD of the measurement ascertained? What was the sampling time point for this measurement: 5 mins, 30 mins, 1h, 2 h, 3h, or 24 h?
- 6.) Lines 243 – 244: DMSO is not fluorescent. Printex 90 particles are likely not fluorescent in the presence of only DMSO, as they need a reducing agent to oxidize and release ROS. Therefore, I am not sure about the inference the authors are trying to make for this statement.
- 7.) Lines 245 – 250: Why were these measurements only performed at $5 \mu\text{g}/\text{mL}$ Printex concentration and not at all three concentrations? In table A2, the relative decreases (1 – observation) would be 45 and 42%, respectively, instead of 55 and 58%. And are the authors referring to Printex 90 while mentioning particles or different particles?
- 8.) Line 251: The authors are requested to be specific that they are referring to Printex 90 particles and not SOA or candle emissions here.

- 9.) Lines 270 – 275 and figure 3: Have the authors statistically substantiated their claim that the fluorescence of BPEAnit increases with sampling time? To me, the error bars are overlapping, and the replicate size ($n=3$) is too small to make this inference statistically significant. Please validate if this statement is statistically significant.
- 10.) Why are these changes in lines 275-280 for 5 $\mu\text{g/mL}$ of Printex 90 very different from those in Appendix 2?
- 11.) Lines 280 – 287: Have the authors validated whether the normalization at no particles holds true in the presence of particles? The authors mention that BPEAnit-Me is non-reactive in line 25, so it is likely that their quantum yields may not be the same at no particle vs. particle conditions.
- 12.) Lines 313 – 318: I am not sure if the authors can make the statement that the ab/adsorption are similar between the two probes without validation.
- 13.) Line 335: I am not sure if a 5% decrease makes the fluorescence decrease evident. Are these differences statistically significant?
- 14.) Lines 358 – 362: I think the correction factor should not be 0.63, but rather $[0.63/0.83 = 0.76]$ as only 84% of the BPEAnit fluorescence remains at 5 mins.
- 15.) Conclusions: Adsorption/Absorption were not measured to conclude those as reasons for the reduction in fluorescence.