General Comments

This manuscript investigates an important source of artefact in fluorescence-based measurements of particle-bound reactive oxygen species (ROS), namely the suppression of BPEAnit fluorescence in the presence of carbonaceous particles. The use of BPEAnit-Me as a reference compound to characterize particle-induced signal loss is potentially useful. The experiments clearly demonstrate a concentration- and time-dependent change in fluorescence following the addition of Printex 90. However, several key aspects require further clarification before the proposed correction approach can be considered quantitatively robust, particularly the assumptions underlying Eq. (1), the mechanistic interpretation of the fluorescence loss, and the applicability of the batch experiments to online PINQ measurements. These issues should be addressed before the proposed correction can be reliably applied to quantitative ROS measurements.

Dear Reviewer,

We would like to thank you for your valuable comments. Based on your suggestions and comments, we have conducted a series of additional measurements, which have led to revised results and modified conclusions. We present first a summary for a quick overview, while below we have addressed all of your comments one by one.

Summary:

- We have performed new series of experiments investigating the probe-particle interactions using the same molar concentration of BPEAnit and BPEAnitMe. The trends and observed mechanisms remained the same, however the differences between BPEAnit-Me and BPEAnit (ROS attributed fluorescence intensity) have different values
- Additional filtration measurements (with and without particles) in BPEAniMe confirmed that absorption of light by particles present in solutions is not the main mechanism for the decrease in fluorescence intensity
- Due to particle-probe suppression, ROS generated by Pintex90 must be assessed after allowing the time for the reaction (1-3h), which is not captured by online methods.
- We suggest in parallel to online measurements, a collection of the particles on filters for subsequent offline ROS/OP assessment with correction for observed artefacts.
- A method (first approach) to correct for the carbonaceous particle-induced fluorescent decrease for offline ROS measurements with BPEAnit, namely by using a stable derivative of BPEAnit (i.e. BPEAnit-Me) is suggested
- Our results indicate that using BPEAnit as a probe for offline ROS and OP determinations is possible and should be explored

Once again thank you for your comments and suggestions. We have responded to all of your comments point by point below.

Authors of the manuscript

Major Comments

1. **The key assumption underlying Eq. (1) requires stronger justification.** Eq. (1) assumes that BPEAnit and BPEAnit-Me experience equivalent particle-induced fluorescence suppression, and further assumes that the fluorescent product formed after reaction of BPEAnit with ROS is affected to the same extent as

BPEAnit-Me. This assumption is central to the correction procedure but has not been directly validated. This is particularly relevant because BPEAnit and BPEAnit-Me were used at substantially different concentrations, 4 µM and 130 nM, respectively. The authors should provide further justification or experimental validation of this assumption. If such validation is not available, the applicability and uncertainty of Eq. (1) should be more explicitly constrained.

We agree. We think that the similarities between the probes (only one bond changed) make it probable that the interactions between probe and particles are equivalent. We have expanded description of the chemical structure of the probes in introduction and added Fig 1 that shows the structural similarity of the probes.

We have also stated more clearly that this is an assumption based on the chemical similarities and literature describing how the probes were developed and tested

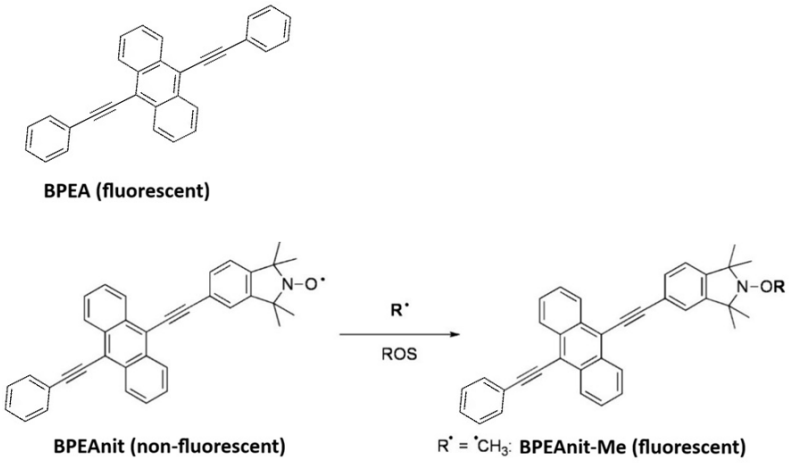


Figure 1. Adapted from Elkaee et al., 2025: Chemical structure and fluorescence description of BPEA, BPEAnit, and BPEAnit-R, including the reaction with ROS that elicits BPEAnit-R (primarily BPEAnit-Me).

We have also performed additional experiments using BPEA, the fluorophore part of BPEAnit. We compared the fluorescence to BPEAnit-Me (Fig.A3) and found that BPEA and BPEAnit-Me are affected to almost equal extent by the addition of Printex 90. This indicates that it is the fluorophore part, not the nitroxide (where the only difference between BPEAnit and BPEAnit-Me is located) that is affected by the particle-probe interactions. Previous studies have shown that a majority of the product from reactions between ROS and BPEAnit is BPEAnit-Me (Miljevic et al. 2010). We have repeated all experiments and used identical concentrations for BPEAnit and BPEAnit-Me. This led to changed results and conclusions. We state that equal probe concentration should be used by comparing the fluorescence and attempting to correct for the artefact.

Nevertheless, we agree that the correction method needs further validation, and we acknowledge that this is the first approach to such a correction. We have now more clearly described the limitations of the assumption regarding the correction

method, both at the introduction of the equation in the Methods, as well as in the discussion.

" ...and assuming that particle-probe interactions suppress the fluorescence of BPEAnit and BPEAnit-Me to the same extent"

" We assume that the fluorescent product formed after reaction between BPEAnit and ROS is suppressed to the same extent as BPEAnit-Me"

"Note that this is a first attempt at correcting for the observed artefact, and further investigations are required to validate the correction method and the equations."

2. **The proposed adsorption/inactivation mechanism remains inferential.** The manuscript attributes the observed fluorescence reduction primarily to adsorption of BPEAnit/BPEAnit-Me onto Printex 90 surfaces, leading to fluorophore inactivation. However, the present experiments demonstrate particle-induced fluorescence loss rather than adsorption itself. Other mechanisms, including static or dynamic fluorescence quenching or other surface-mediated interactions, cannot be excluded. The authors should either provide more direct evidence for adsorption or consistently describe it as a proposed mechanism rather than a demonstrated one, particularly in the Conclusions.

We agree, thank you for commenting on this. It is true that we attribute the fluorescence reduction to adsorption onto particle surfaces without validating it. We relied mainly on previous experience and knowledge of the most likely soot surface interaction, however we cannot prove it. We have therefore changed the phrasing throughout the manuscript to a more descriptive manner (e.g. suppression, interaction). We explain that adsorption to particle surfaces is one of the possible mechanisms.

3. **The attribution of the BPEAnit–BPEAnit-Me difference to ROS formation requires further support.** The consistently higher fluorescence response of BPEAnit relative to BPEAnit-Me is interpreted as ROS-dependent probe activation. This interpretation is plausible, but it relies on the assumption that non-ROS particle interactions are equivalent for the two compounds. Differential adsorption or quenching of BPEAnit and BPEAnit-Me could also contribute to the observed difference. The authors should provide stronger justification for attributing this difference primarily to ROS formation and discuss this alternative interpretation. Independent validation, if available, would further strengthen the conclusion.

With the new experiments, where we use equal concentrations for the comparison of BPEAnit and BPEAnit-Me, and where we investigate suppression of fluorescence of BPEA, we have provided further support for ROS formation. The updated results show a much lower reactivity, and we state that the reaction between BPEAnit and Printex 90 takes time. The fluorescent increase that we are now attributing to ROS is mainly an increase over time, where we see that the fluorescence from the BPEAnit-particle suspension becomes higher than in the particle-free solution and BPEAnit-Me, indicating that the probe has reacted with the particles. Together with results from previous measurements done with BPEAnit (e.g. Miljevic et al. 2010),

we do think that ROS-attributed fluorescence is a major part of the observed difference.

4. **The quantitative relationship between Eq. (1), Fig. 4, and Fig. 5 is unclear.** Eq. (1) is defined using normalized relative fluorescence and therefore yields a dimensionless quantity, whereas Fig. 5 presents "Calculated ROS" in fluorescence units (a.u.). The complete calculation procedure from raw fluorescence counts to normalized fluorescence and subsequently to the values plotted in Fig. 5 should be explicitly described. If the corrected response was converted back to fluorescence counts or another quantity, the corresponding conversion and units should be clearly defined.

   Thank you for pointing it out. We have now clarified this relationship by explaining the calculations and introducing new equations in the revised manuscript. A new set of equations shows how the normalization was done (Eq. 2 and 3), and shows the connection between counts and relative fluorescence (Eq. 6). All equations have been moved to the Method section. Figure 6 has been updated to visualize the equations.

5. **The representativeness of the experimental time scale for online PINQ measurements should be clarified.** The earliest measurements were conducted within 5 min after particle addition, whereas the manuscript states that PINQ analysis occurs within approximately 1 min after particle collection and mixing. Because the particle–probe interaction evolves rapidly at early times, measurements obtained within 5 min cannot necessarily be assumed representative of the online PINQ time scale. The authors should either demonstrate the relevance of this time scale more clearly or qualify the applicability of the results to real-time PINQ operation.

   To improve comparability with PINQ, all new suspensions that were made for the new experiments were measured within 2 minutes after particle addition. The practical work of preparing the suspensions and placing them in the measurement cell, together with flow paths in the tubing, unfortunately makes the residence time longer than online PINQ conditions. We are not aware how these measurements (which require manual sample preparation) could be made within the same time scale as online/real-time measurements with PINQ. This is the inherent difference between real-time and off-line analysis, we physically are not able to do it in shorter time. During our experiments, we measured each suspension over a period of two minutes, to ensure a stable response, already this is longer than the time between mixing of the aerosol and probe liquid in PINQ, where the measurements are done on a continuous liquid flow.

   The experiments with suspensions that are presented in the manuscript are different to PINQ analysis in several ways. We were not claiming that the performed experiments are exactly the same as for online PINQ measurements. Nevertheless, the observed and described effect of Printex 90 particles on

BPEAnit is relevant to understand when using PINQ, since that effect is visible during PINQ operation at certain circumstances.

The transferability of the batch experiments to actual PINQ operation requires a more explicit discussion. The present experiments were conducted using Printex 90 directly suspended in DMSO, whereas PINQ continuously collects aerosol particles into a BPEAnit-containing liquid. Differences in solvent composition, particle dispersion state, collection process, and contact time may influence the magnitude of the particle–probe interaction. The limitations associated with transferring the present batch-suspension results to actual PINQ operation should therefore be discussed more explicitly, and the proposed correction should be clearly framed as requiring further evaluation under online operating conditions. In addition, the manuscript should clarify the choice of 4 µM BPEAnit and whether this represents an updated PINQ operating protocol.

We agree. The choice of 4 µM, which is higher than previous experiments with PINQ, is an adapted PINQ protocol to the version used in our laboratory. After testing with this version, it was concluded that 4 µM gave the best performance. This was the probe concentration used in the mentioned chamber experiments (Figure 2 in the revised manuscript), therefore it was used in this study. This has been clarified in the Appendix.

"Note that this probe concentration is higher than originally used in PINQ (1 µM). This updated protocol was chosen to optimize the performance of our setup."

The possible differences in particle dispersion state and collection process are listed as possible limitations affecting the magnitude of the particle–probe interaction in comparison to real-time PINQ measurements. However, one of the main conclusions of our study is that if there is suspicion of artefacts in online measurements, particles should be collected on filter for offline analysis and the corrections done during offline ROS determination after 1-3h.

6. **Basic physicochemical information on Printex 90 should be provided.** Because the proposed mechanism is explicitly related to particle surface interactions, particle mass concentration alone provides limited mechanistic information. The manuscript currently reports the preparation and sonication procedure but provides little information on primary particle size, specific surface area, or other relevant properties of Printex 90. At minimum, basic physicochemical properties from the manufacturer or literature should be reported. The atmospheric relevance of the tested concentrations should also be discussed more carefully. In particular, the description of 1 µg mL^{-1} as a "low concentration" should be qualified, since this corresponds to an atmospheric particle concentration of approximately 63 µg m^{-3} under the stated assumptions.

We added the basic physicochemical properties of Printex 90 obtained from the manufacturer. To call 1 µg/mL "low concentration" is not a claim that the corresponding atmospheric particle concentration is low, but rather compared to the other two concentrations measured in this study. We have changed the wording from "low" to "lower" in several places. The atmospheric relevance is discussed in more depth in the revised manuscript. We want to emphasize that

the particle concentrations are highly relevant for chamber studies, which is where the effect was first observed, as well as laboratory emission studies, laboratory and field assessment of combustion sources, extreme pollution events, occupational exposure assessment in workplaces, or measurements in indoor environments

7. **The uncertainty and validity range of Eq. (1) should be addressed.** At the highest Printex 90 concentration, the BPEAnit-Me response approaches zero, making the correction in Eq. (1) mathematically unstable and strongly amplifying the calculated ROS response. The authors acknowledge this limitation, but the corresponding values are still presented and discussed quantitatively in Fig. 5. The uncertainty associated with Eq. (1) should be evaluated, and the range over which the correction can be meaningfully applied should be clearly defined or discussed.

   After increasing the concentration of BPEAnit-Me to 4 µM, the issue with probe depletion and the denominator in the equation has been mitigated. We also performed additional experiments investigating the reduction in fluorescence at different probe concentrations, which showed the probe concentration dependence for given particle concentration. When discussing the correction method, we now suggest that using the equation when we have clear fluorescence suppression could elicit non-linear responses in ROS formation. When performing offline analysis, we suggest using concentrations that do not cause fluorescence suppression of BPEAnit-Me higher than 80%.

Minor Comments

8. Units, numerical formatting, and figure labels should be standardized throughout the manuscript. For example, use µg mL^{-1} rather than µg/ml and 0.5 h rather than 0,5 h. Typographical errors in figure labels should also be corrected.

   It has been corrected in the revised manuscript. Thank you for making us aware on this matter.

9. Printex 90 is a commercial carbon black used here as a model for soot-like particles. The observed interference should therefore not be generalized to atmospheric black carbon or other insoluble particles without appropriate qualification.

This is correct. This caveat should be highlighted. This is now listed as one of the limitations of the study. Printex 90 was in part chosen to create a reproducible “worst case scenario” where the large surface area would allow us to observe the effect. Atmospheric soot particles can have different coating, changed structure, etc hence behave differently.