The responses below address all the points raised by the reviewer. Our responses are shown in blue, deleted text in red, and added text in gold.

General comments

In this manuscript, a method using UV-sensitive dye for UV dose determination is developed. Although the amount of work is much appreciated, there is very limited data analysis and discussion. The experimental design is questionable and many methods used are not well described and need clarification.

The major concern I have is the applicability and feasibility of using the UV-sensitive dye for UV dose measurement as described in the manuscript. The main challenge for measuring the UV dose exposed to pathogens in aerosols in air disinfection studies is that it is very difficult to account for physical shielding provided by the aerosol chemical constituents, which normally from saliva or nasal fluids that contains proteins and absorb UV. The study proposed using DEHS as the dye carrier but it is unclear whether DEHS can simulate the physical shielding and other potential interactions in real pathogen containing aerosols. In addition, the authors failed to demonstrate an actionable procedure using the proposed method for UV dose determination in other experimental settings.

We are truly grateful that you have dedicated the time and effort to providing thoughtful suggestions and critical comments. We have thoroughly considered the reviewer's comments and implemented significant enhancements to this manuscript. These improvements encompass the inclusion of additional data, expanded discussions, and the provision of more detailed experimental information to enhance overall understanding and clarity.

It's worth noting that DEHS, being an oily organic substance, exhibits significant absorbance under UVC (200-300 nm) irradiation. This characteristic is clearly demonstrated in the newly added Figure 6a, which illustrates the UV-Vis spectrum of pure DEHS. The degradation of dye within DEHS droplets when exposed to UVC light may closely resemble the behavior of pathogens in saliva, as proteins in saliva also exhibit UVC light absorption.

Given that the primary goal of this research is to showcase the feasibility of estimating UV irradiation doses delivered to aerosols using dye-laden droplets, we intend to further explore various experimental parameters, including relative humidity and temperature. Additionally, we plan to develop a mathematical model to better

Specific comments:

Line 42: Please consider using other references here. UV inactivation mechanisms were not investigated in these two studies.

We substituted the original citations with alternative references related to research on UV inactivation mechanisms.

"(Budowsky et al., 1981; Kowalski, 2009; Beck et al., 2016)"

Line 93: You mean "no observable sedimentation"?

Thank you for highlighting this. Indeed, we used observable sedimentation as a criterion for exclusion to maintain a stable solution with a consistent dye concentration. We have clarified this sentence in the revision.

Line 105: Need more background on DEHS and why it has been selected for droplet generation. Any previous studies? Are there any other factors that should be considered for the carrier liquid selection, such as density, viscosity, absorbance, and potential chemical reaction with the dye?

We added several sentences to provide more background about the wide application of DEHS in aerosol science.

"In aerosol science, DEHS is widely used to generate liquid particles with extended lifetimes due to its extremely low saturation vapor pressure. This quality is crucial for studying particle size effects (Ren et al., 2021; Li et al., 2020). Besides, DEHS aerosols, recommended for aerosol filtration testing, allow for more accurate particle size measurement of spherical liquid particles compared to non-spherical solid particles like salt and test dusts, which tend to agglomerate (Gustavsson, 2003)."

Additionally, since DEHS is an oily, organic substance, it exhibits notable absorbance under UVC irradiation, a characteristic evident in Figure 6a, which displays the UV-Vis spectrum of pure DEHS. The degradation of dye in DEHS droplets when exposed to UVC light might closely resemble the behavior of pathogens in saliva, as proteins in saliva similarly absorb UVC light.

"While pure DEHS demonstrates significant absorbance under UVC irradiation, as shown in Figure 6a, dyes dissolved in DEHS undergo photodegradation upon UVC exposure, similar to that observed in water-dissolved dyes (Putt, et al. 2012). This behavior of dyes in DEHS under UVC irradiation might closely mimic the response of pathogens in saliva, where proteins also absorb UVC light."

Line 112: "solutions".

This word has been corrected in the revised manuscript.

Line 117: I don't understand why having a similar "UV susceptibility" to nucleic acids is necessary here. You may have different relative sensitivity to DNA/RNA across wavelengths and simply having a sensitivity spectrum documented. Also, how did you determine the "UV susceptibility"? Both absorbance the quantum yield are needed to estimate the susceptibility but it seems you only have the absorbance documented.

We apologize for any confusion caused and have revised the relevant section for improved clarity.

"Given the varying susceptibility of different UV-sensitive dyes to UVC exposure, dyes with an absorption peak near 260 nm (below 300 nm in this study) were selected for further study. This is because the chemical structure, crucial for the replication of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), is more sensitive to UV irradiation near 260 nm, where it also exhibits an absorption peak (Abkar et al., 2022). ~~those with UVC susceptibility similar to deoxyribonucleic acid (DNA) or ribonucleic acid (RNA), were identified for further investigation.~~"

In this study, we focused only on measuring the UV-visible spectra of various dyes to determine their suitability. We specifically selected dyes that show significant absorbance below 300 nm, which we assume to be near the 260 nm peak absorbance of DNA/RNA, for further investigation

Line 119: I don't understand how the solubility is calculated here. From absorbance and concentration, you may calculate the molar absorptivity according to Beer's law, no sure they are related to the solubility. Also, it is not clear why solubility should be considered for the dye selection here.

Substances can be classified as "insoluble" if their solubility falls below 100 µg/mL in a given solvent (2007 et al. Stegemann). In our study, dye solutions prepared at this concentration (100 µg/mL) level showed varying degrees of sedimentation after 48 hours of observation. Therefore, we used UV-Vis spectroscopy to further estimate the solubility of "insoluble" substances. We have amended the relative section for better clarity and understanding.

"As noted by Stegemann et al. (Stegemann et al., 2007), substances are typically classified as 'insoluble' when their solubility is below 100 µg/mL in a solvent. Technique like UV-Vis spectroscopy can be used to estimate the solubility of insoluble substances, particularly with those with very low solubility (such as below 100 µg/mL)…. According to Beer's law, we expect that the maximal absorbance obtained correlates with the solute concentration, ideally forming a linear standard curve. If the measured data do not exhibit a linear regression, the corresponding substance is excluded."

Line 141: Droplet evaporation likely occurs in the real-world. Does it affect the accuracy of UV fluence measurements using the dye?

In practical scenarios, when a droplet (such as water-based droplet) undergoes evaporation, it leads to a reduction in its volume, yet the quantity of dye remains unchanged. This process causes the concentration within each droplet to increase. Consequently. the dye concentration measured with the collected liquid using an impactor might be biased towards the concentration within individual droplet particles in the real. However, in our study, we utilized DEHS droplets, known for their stability in maintaining volume over several hours, even at nanoscale dimensions. Therefore, we hypothesize that the concentration we measured after droplets collection can represent the true concentration within the individual droplet particles.

Line 160: Is 275 nm the peak emission wavelength? Need to show the UV LED emission spectrum, especially considering LED tends to have a wide emission peak, covering up to 20 nm.

Yes, 275 nm is peak and dominant wavelength, as specified by the manufacturer. This information has been updated in the revised manuscript. We acknowledge that the peak emission of the LED, typically around λ_{max}±10 nm, is broader than that of a low-pressure mercury lamp. In this study, we did not independently measure the emission spectrum of the LEDs to verify their spectrum width. However, this question also provides an interesting aspect to consider for future research.

Line 164: What is the dimension of the cuvette. I'm assuming there is no mixing for the dye solution in the cuvette so the UV irradiance decreases across the solution depth, which could be problematic.

We have included the dimensions of the cuvette in our revised manuscript (see line 190 on page 6).

"The collected liquids were transferred to a quartz submicron cuvette (Part number: 6610024100, Agilent Technologies, Santa Clara, USA), having a minimum capacity of 80 µL (external dimensions: 45×12.5×12.5 mm, opening aperture: 10 mm, path length: 10 mm)."

During UV irradiation, we refrained from using any mixing techniques, like a stirrer magnet, as they might affect the irradiation process. However, the dye solution was thoroughly mixed prior to the UV-Vis measurement. Our observations indicate that the volume and depth of the liquid lead to uneven UV irradiation, resulting in different levels of dye degradation across various parts of the liquid. Therefore, the UV light dosage determined in this study is specifically used for quantitative analysis, aimed at assessing the degradation of the selected dyes to LED UV light.

Line 165: Need more details on the UV light meter. Is it calibrated specifically for this UV LED? UV light meter normally has different sensitivity across wavelengths and often needs to be calibrated for specific UV device, especially for UV LED considering the wide emission peak. Also, what is the size the meter sensor aperture and is this the same as the size of the cuvette?

The UV light meter has a spectral measurement range of 250-410 nm. We do not have additional calibration for this UV LED (with a peak wavelength of 275 nm). The detector of this UV light meter has a circular area with a 10 mm diameter, matching the opening aperture of the cuvette used in this work. The required details on the UV light meter have been added.

"The detector of this UV light meter has a circular area with a 10 mm diameter, matching the opening aperture of the cuvette used in this work."

Line 181: The measurement taken here is likely not the average irradiance across the tube. Light irradiance is proportional to the inverse square of the traveling distance (inverse square law), not a linear decrease.

We thank the reviewer for highlighting this issue. The sentence has been revised to underscore that the intensity measured along the center line might not accurately represent the intensity experienced by all particles.

"It should be noted that the intensity measured along the center line might not accurately represent the intensity experienced by all particles. Predicting the equivalent radiation intensity for aerosol droplets in the UV chamber requires developing simulation models, which was not addressed in this study. These mathematical models would need to consider numerous factors, including the optical field of UV irradiation (accounting for reflections and refractions at interfaces between air, quartz, and nitrogen) and the flow field, which affects UV scattering and shadow effects related to particle sizes and trajectories (Kowalski, 2009). Therefore, in our research, we utilized the irradiation intensity measured at the chamber's largest cross-section to inform feedback for our reactor design."

Line 210: Please explain why 100 ug/mL is tested here. A dye could still be considered as a good selection as long as it can provide sensitivity and measurable responses to UV irradiation at a lower concentration, even the solubility less than 100 ug/mL.

We selected dye concentration of 100 ug/mL to align with studies by Putt, who investigated the degradation of chromophores in water solutions for determining UV light radiation doses.

We revised the following sentence.

" To rapidly screen UV-sensitive dyes soluble in DEHS, solutions were prepared at a dye concentration of 100 µg·mL-1, aligning with the concentration used by Putt et al. (Putt et al., 2012), who studied chromophore degradation in water solutions for evaluating UV radiation doses."

Line 218: It is not true. The 260 nm peak absorbance only applies to > 240 nm. Higher absorbance can be observed in the far UVC range (<230 nm).

Thanks for pointing this out. We have revised this sentence to enhance its clarity and comprehension.

Line 224: Please explain why board peaks indicate chemical reactions or polarization?

We revised this sentence for better clarity and added citations to support the explanation that potential alterations in the chromophore structure might occur.

"…, as indicated by ~~broad absorption peaks~~ their UV-Vis spectra. These spectra show absorption across the whole visible wavelength spectrum and the appearance of the absorption peaks becomes broaden and obscure. For a given organic chromophore, the absorption peaks or shoulders are common for the presence of the conjugated systems and might become broad when extensive conjugation occur with the solvent dependent (Hemdan, 2023; Zheng et al., 2018)."

Figure 6: Recommend just showing the germicidal UV range (200 – 320 nm). The data in the visible range is not useful in this study.

As previously clarified, we utilized DEHS's UV-Vis spectra from 270 to 800 nm as the baseline for measuring the spectra of pure dyes, since its absolute absorbance exceeds 1 at wavelength below 270 nm (see figure 6 in revised manuscript). Due to significant baseline fluctuations from 200-270nm, the UV-Vis spectra below 270 nm was excluded. We have included a statement and discussion about this baseline. Furthermore, the data in the visible range exhibits a distinctive peak that represents the specific fine structure of a particular dye and exclude the error caused by the DEHS baseline. We utilize the variations in the absorbance peak to calculate the changes in dye concentration caused by degradation after UV exposure.

Figure 7. I don't understand why the relationship between concentration and absorbance is needed here. It is well known that the absorbance is proportional to concentration (Beer's law).

Beer Lambert law is valid only when the sample being analyzed is a homogeneous solution, which we cannot know by sedimentation observation. We used UV-Vis spectroscopy to further estimate the solubility of the selected dyes. According to Beer's law, we expect that the measured maximal absorbance correlates with the solute concentration, ideally forming a linear standard curve. If the measured data do not exhibit a linear regression, the corresponding substance exhibits inhomogeneous distribution in DEHS liquid and is excluded for further study.

Line 245: Please include references.

Accordingly, the corresponding references have been included in the revised manuscript.

Line 295: Move to introduction.

We have revised this section for improved clarity and to prevent any potential confusion.

"~~Currently, most UV disinfection systems for water, air, and surfaces continue to utilize conventional low- or medium-pressure mercury lamps. The primary concern with these lamps is their fragility and their containment of toxic mercury, which poses environmental hazards and requires proper disposal. UV LEDs, on the other hand, are emerging as a popular, environmentally friendly alternative. Their compact size simplifies their integration into sterilization systems, and they offer a diverse range of wavelengths (Kim and Kang, 2018; Song et al., 2016). Considering the above reasons, this study employed UVC LEDs to investigate dye degradation upon UV radiation.~~ In this study, UVC LEDs were utilized to explore the degradation of dyes under UV radiation. Traditional low- or medium-pressure mercury lamps, although effective, raise concerns due to their fragility and the presence of toxic mercury, posing environmental hazards and necessitating proper disposal. Conversely, UV LEDs are emerging as a favored and eco-friendly alternative (Chiappa et al., 2021). Their compact size facilitates easy integration into sterilization systems, and they provide a wide range of wavelengths (Kim and Kang, 2018; Song et al., 2016)."

Figure 11. Why is 2000 to 6000 mJ/cm2 selected here? This range is way too high for disinfection application, and the sensitivity at reasonable UV fluence range is unclear from this study. Also, a mathematical relationship between the UV fluence and absorbance needs to be developed here.

In this study, we irradiated the dye solution with doses ranging from 0 to 8000 mJ/cm^2. Only a few examples are presented in the illustration, as the spectral changes between 2000 and 6000 mJ/cm^2 can be visually identified and compared. The higher irradiation dose required could potentially depend on the species and is also influenced by factors such as the irradiated sample volume and UV absorption of the carrier liquid DEHS. The sensitivity comparison is evident through the peak changes at the same UV dose.

We did not develop a mathematical model for the relationship between UV irradiation dose and dye solution degradation in this study. This decision is because the UV irradiation dose depends on various factors, such as the experimental equipment, solution volume, and the medium used. Initially, we used UV LEDs to irradiate the solution to verify if the selected dyes exhibited degradation upon irradiation. This step provided valuable feedback for designing the aerosol UV irradiation chamber. The corresponding section was revised.

"We initially used UV LEDs to irradiate the dye solution and confirm whether the selected dyes exhibited degradation upon exposure. This information guided us in designing the aerosol UV irradiation chamber. Figure ~~11~~12 presents examples of the measured UV-Vis spectra of dye solutions before and after irradiation with various UV radiation doses, using experimental setup displayed in figure 2. The higher irradiation dose required could potentially depend on the species and is also influenced by factors such as the irradiated sample volume and UV absorbance of the carrier liquid DEHS. As the UV radiation dose increased, the maximal absorbance values in the visible region decreased noticeably. Moreover, despite a similar degradation trend observed for the two tested dye solutions, at the same UV irradiation dose of 6000 mW·s·cm^{-2}, the degradation fraction for dye 4 was 90% while dye 7 was 50%. It can be concluded that dye solution #4 demonstrated a higher sensitivity to UVC irradiation at 275 nm."

Figure 12: How was the "dye survival" determined, absorbance? Again, a mathematical relationship between the UV fluence and "dye survival" needs to be developed here. This relationship needs to be compared with the results in Figure 11.

We have revised several sentences to explain how the calculation of dye degradation was performed, including the procedure details.

"Figure 13b shows *an example of* the UV-Vis spectra *for* the collected droplets, which we used to calculate the fraction of dye degradation. This calculation involved converting the maximum absorbance values at 497 nm (for dye #4) after UV irradiation (using apparatus in figure 3) into a percentage of the original absorbance value before irradiation…."

Although our ability to develop a mathematical model or relationship between dye degradation under UVC irradiation was constrained by limited experimental data, we have made enhancements to Section 3.3 to provide a more thorough explanation. The development of a mathematical relationship between UV radiation dose and dye degradation will be a focus of our future research.

"Two potential mathematical models that might offer insights into dye degradation: the classical exponential decay model (log-linear decay) and the shoulder model, which begins with a horizontal slope before transitioning into full exponential decay (Kowalski, 2009). However, the determination of which model is better suited for the selected dye solution remains inconclusive due to limited experimental data. A dataset that includes 90% dye degradation induced by UV radiation is, at a minimum, required."