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Exploring the Differences in the Response of SARS-CoV-2 Delta and Omicron to Ultraviolet Radiation

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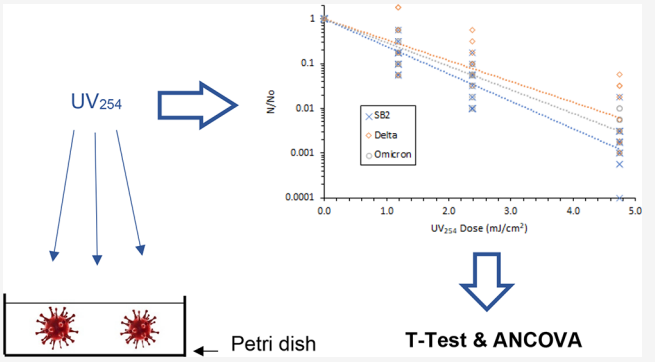
ABSTRACT: One method that can help slow the spread of coronaviruses is disinfection with UV light. The Delta and Omicron variants of the COVID-19 virus (SARS-CoV-2) have come to dominate the later stages of the pandemic due to their higher rates of transmission. In this work, it is shown that a 17% higher UV_{254} dose is required for the disinfection of Delta and Omicron variants when compared to the ancestral strain of SARS-CoV-2. The UV_{254} disinfection rate constants for SARS-CoV-2 and the Delta and Omicron variants were found to be 1.4 ± 0.3, 1.1 ± 0.2, and 1.1 ± 0.2 cm^2/mJ, respectively. The rate constants of Delta and Omicron were statistically different from the ancestral strain of SARS-CoV-2 at the 95% confidence level based on at least three replicate experiments. It is suggested that the reason for this difference is the absence of repeating uracil (U) bases in the genome of the two variants. The UV_{254} sensitivity of repeating pyrimidine bases is well-established. There are 2.6 and 3.7% fewer uracil triplets (UUU) in the Delta and Omicron variants, respectively, when compared to SARS-CoV-2. This difference in UV_{254} sensitivity is relevant to a range of UV disinfection applications including upper-room disinfection, air handling equipment, aircraft sanitization, and others.



KEYWORDS: *SARS-CoV-2, COVID-19, Delta, Omicron, UV*

INTRODUCTION

As of late December 2022, there have been 641 million cases and 6.6 million deaths[1] caused by COVID-19. There have been 3.0 million new cases in the past week, several years into the pandemic. COVID-19 is the latest in a series of global coronavirus outbreaks, including severe acute respiratory syndrome (SARS) in 2003 and the Middle East respiratory syndrome (MERS) in 2018.[2] The current pandemic is not resolved after almost three years, and based on the recurrence of different coronavirus diseases, we can expect additional global coronavirus outbreaks in the future.

Germicidal UV disinfection, often at a wavelength close to 254 nm, has been suggested for the disinfection of the virus that causes COVID-19 (SARS-CoV-2) in a wide range of applications. Since the start of the COVID-19 pandemic, UV disinfection has been suggested for robotic disinfection in hospital rooms,[3] respirator disinfection,[4] airborne infection control in clinical settings,[5] and sanitization of passenger aircraft.[6] There are many applications where UV disinfection can be used to help slow the spread of coronavirus outbreaks.

The sensitivity of different organisms to UV disinfection can be quantified using the first-order disinfection rate constant shown below, where the fraction of surviving organisms (N/N_o) is given by

$$\frac{N}{N_o} = e^{-kD} \tag{1}$$

Dose, D, is the product of the UV radiation fluence rate (mW/cm^2) the organism receives and the duration of exposure (s). The parameter k is the disinfection rate constant (cm^2/mJ). The first-order model has been applied with good results ($R^2 > 0.94$) in tests on MS2, ΦX174, Φ6, and T7 viruses.[7] Disinfection kinetics shapes UV reactor design, and finding a good estimate of the rate constant is often the first step in the design process.

However, there are exceptions to this simple first-order model. For example, the response in the MS2 virus to UV light in aqueous suspension has been shown to follow a phenotypic persistence and external shielding (PPES) model.[8] This model may indicate the presence of more UV-resistant subpopulations that can dominate UV disinfection kinetics at higher

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doses (>50 mJ/cm^2). However, this work and others have shown that lower doses, a simple first-order model, can often be used.

COVID-19 is known to be transmitted mainly via aersols.[9,10] Previous research on other diseases transmitted this way, such as tuberculosis, has shown that the effectiveness of UV disinfection will depend on many factors, including droplet size and humidity.[11] Significant differences can be observed between the disinfection rates in aerosols and aqueous suspension.[8] However, UV effectiveness will also be affected by differences in fundamental sensitivity of the organisms, as indicated by the UV disinfection rate constant, which is the subject of this research.

The UV disinfection rate constants for SARS-CoV-2 and its variants are uncertain. Since working with SARS-CoV-2 requires access to a Biosafety Level 3 laboratory, tests using the actual virus are rare. Moreover, estimates using closely related viruses often suffer from a lack of standardization, where UV sensitivity estimates can vary by a factor of 10.[12,13] For example, irradiating multiwell plates containing the virus, though convenient, makes it difficult to estimate the true UV dose received by the virus. As such, this work uses a standardized collimated beam bioassay that has been used in the wastewater industry for many years.[14−16] This test is done with the pathogen in an aqueous suspension and is highly standardized. In addition, a recent meta-analysis reports a critical need for additional studies that specifically evaluate disinfection kinetics of coronaviruses in an aqueous environment.[17] Finally, given safety constraints around aerosolizing SARS-CoV-2, conducting UV inactivation tests in liquid medium is one of the few viable alternatives.

The UV sensitivities of the Delta and Omicron variants are even more uncertain since there has been little time to test them. The Delta variant, first identified in India, shows evidence of increased household transmission rates and displaced α variant to become the dominant variant in England in January 2022.[18] The Omicron variant emerged in South Africa and has shown a +105% increase in transmission compared to the Delta variant.[19] It has been shown that the transmission of aerosols containing the Delta variant can be attenuated using UV_{254}.[20] However, the rate constant needed to effectively design this equipment remains uncertain.

UV disinfection rate constants are fundamental to the application of UV disinfection technology. Currently, there are few systematic estimates of the UV disinfection rate constant for SARS-CoV-2 and even less for the recently emerged Delta and Omicron variants. As such, the objective of this research is to make systematic estimates of these rate constants using a standardized approach.

MATERIALS AND METHODS

UV Exposure. The collimated beam bioassay used here requires irradiating a mixed viral suspension under a collimated UV source. Since mixing can aerosolize small droplets, this presents a safety hazard when working with SARS-CoV-2 and its variants in Biosafety Level 3 (BSL3) environments. To solve this problem, an airtight containment vessel with a circular UV-transparent quartz window was constructed (Figure 1). The containment vessel was constructed from a resealable food container, with a quartz window sealed with silicone. The fluence rate applied to the suspension was measured using a calibrated IL1400A research radiometer (International UV Light Technologies) equipped with a SEL240 vacuum photodiode detector placed inside the containment vessel. Collimated UV radiation was provided by a custom-built apparatus supplied by Trojan Technologies of London, ON, Canada. The apparatus contains a low-pressure mercury vapor lamp and a collimating tube with a length of 23 cm and a diameter of 6 cm. The lamp height was adjusted until a radiation fluence rate of 100 ± 2.0 μW/cm^2 inside the containment vessel at the Petri dish location was observed. A 50 mm Petri dish containing 5 mL of the virus suspension in Dulbecco's modified Eagle medium (DMEM) was used. A magnetic stirrer was used to mix the Petri dish contents. Estimating the UV dose received by viruses in the suspension requires adjusting the observed fluence rate for the UV absorbance of DMEM (0.76 cm^{-1}), reflection factor (0.975), a Petri factor (0.987), and a divergence factor (1.0). The UV transmission of DMEN was determined using a Thermo Fisher NanoDrop Microvolume UV−Vis spectrophotometer and the other factors according to the cited reference.[16]

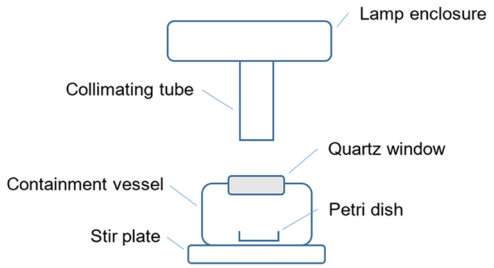

Figure 1. Experimental setup for UV exposure tests. Radiation is applied at 254 nm to a 5 mL sample in a Petri dish inside a containment vessel with a quartz window.

Virus Quantification. The concentration of SARS-CoV-2 can be estimated by counting the copies of a genome sequence present using quantitative polymerase chain reaction (qPCR) or by measuring their ability to infect cells. Since a virus may still have a detectable genome when it is inactivated or noninfective, viral quantification based on infectivity is used here.

All manipulations of SARS-CoV-2 and the Delta and Omicron variants were conducted in the Combined Containment Level 3 Unit at the Temerty Faculty of Medicine, University of Toronto. The ancestral SARS-CoV-2/SB2 virus was isolated in Toronto, Canada in early 2020, as previously described.[21] The SARS-CoV-2/Delta and SARS-CoV-2/Omicron variants were obtained from BEI Resources (respectively, NR-55672, Source: Johns Hopkins University; NR-56461, Source: Johns Hopkins University). The sequences of all SARS-CoV-2 viral stocks were confirmed before proceeding with experiments. The HCoV-OC43 virus was sourced from the American Type Culture Collection (VR-1558). All manipulations of biological agents and UV radiation sources were conducted according to local regulations as permitted and approved by the Environmental Health & Safety Office at the University of Toronto.

Virus quantification was determined using an end-point titration assay to estimate the median (50%) tissue culture infectious dose ($TCID_{50}$). For the ancestral SARS-CoV-2 and its variant experiments, a 96-well culture plate was seeded with 3 × 10^5 Vero E6 cells (CRL-1586, American Type Culture Collection) in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum and supplemented with 100 IU/mL penicillin and streptomycin (Pen/Strep).

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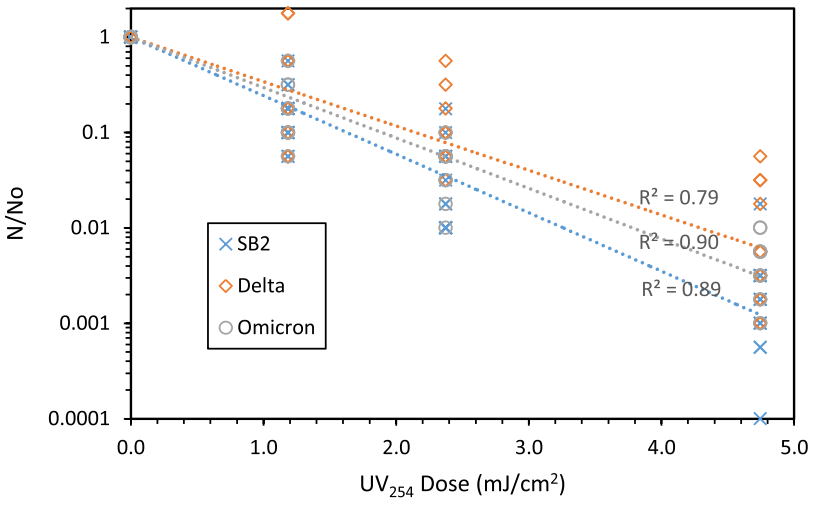

Figure 2. Remaining infectivity of the native strain SARS-CoV-2/SB2 and the Delta and Omicron variants after exposure to UV radiation at 254 nm. The remaining infectivity is a ratio of the mean tissue culture infectious dose (TCID50) at the shown dose (N) divided by the TCID50 with no UV exposure (N_o). See statistical analysis below for more details on the differences between strains.

Table 1. Statistical Analysis of UV Sensitivity Rate Constant Estimates for Four Viruses[a]

virus	mean rate constant ± standard deviation (cm^2/mJ)	T-test results[b] ($P(T > t)$)	significant difference vs. SARS-CoV-2/SB2
SARS-CoV-2/SB2	−1.35 ± 0.28		
HCoV-OC43	−1.40 ± 0.06	0.56	no
SARS-CoV-2/Delta	−1.09 ± 0.24	0.01	yes
SARS-CoV-2/Omicron	−1.14 ± 0.16	0.03	yes

[a]Each has a minimum of three biological repeats of triplicate measurements. [b]Two-tailed T-test. Significant difference if $P(T > t) < 0.05$.

After UV exposure, the virus suspension was serially diluted in DMEM and added to the Vero E6 cells. The cells were then incubated at 37 °C and 5% CO_2. Plates were evaluated microscopically for cytopathic effect after 5 days and the $TCID_{50}$ was calculated according to the Kärber−Spearman method.[22,23]

For the HCoV-OC43 experiments, a 96-well culture plate was seeded with 4×10^5 HCT-8 cells (CCL-244, American Type Culture Collection) in Dulbecco's modified Eagle's medium (DMEM) containing 10% (v/v) fetal bovine serum and supplemented with 100 IU/mL penicillin and streptomycin (Pen/Strep). After UV exposure, the OC43 virus suspension was serially diluted in DMEM and added onto the HCT-8 cells and incubated and counted as above.

RNA Sequencing. To confirm that the viral stock of SARS-CoV-2/SB2, the Delta variant, and the Omicron variant had not mutated, each was sequenced prior to UV testing. The results of the RNA sequencing were used in genome comparisons discussed below. The details of the RNA sequencing are described in the following reference.[21]

RESULTS AND DISCUSSION

Each experiment involved triplicate measurements of infectivity or mean tissue culture infectious dose (TCID50) of the virus at several different UV doses. Each variant experiment was repeated a minimum of three times and the SARS-CoV-2/SB2 experiment was repeated six times. The results of all experimental replicates are shown in Figure 2. Data for all trials can be found in the Supporting Information.

From the data presented in Figure 2, the Delta and Omicron strains are slightly more resistant to UV irradiation at 254 nm than the ancestral SARS-CoV-2/SB2. To further explore differences in UV sensitivity between the variants and the ancestral strain, a Students T-test was performed using the rate constant estimates. These results are summarized in Table 1. The raw data is available in the Supporting Information. A subsequent analysis of covariance (ANCOVA) found that the Omicron and Delta variants had a significantly different dose responses compared to the native SARS-CoV-2/SB2 ($P < 0.01$).

The average rate constant for SARS-CoV-2/SB2 shown in Table 1 (1.35 cm^2/mJ) is identical to the recent estimate, provided by others,[24] of 1.35 cm^2/mJ for SARS-CoV-2, based on a one-log reduction. The rate constant for the seasonal coronavirus HCoV-OC43 in Table 1 (1.40 cm^2/mJ) is quite close to the estimate by the same authors of 1.28 cm^2/mJ, based on a one-log reduction. In both works, no difference was observed between SARS-CoV-2 and HCoV-OC43, which causes the common cold. Another recent study using low-pressure mercury vapor lamps at 254 nm and a similar approach found rate constants of 1.70 cm^2/mJ for SARS-CoV-2, based on one-log reduction.[25] Overall, this suggests that the approach used in our work can produce results consistent with recent research done by others using a similar approach. The use of a containment vessel does not appear to affect the results.

Both the Omicron and Delta variants have smaller rate constants and lower overall UV sensitivity when compared to SARS-CoV-2/SB2 at the 95% confidence level, as shown in Table 1. Since the required dose is directly proportional to the rate constant, the Delta and Omicron variants require a 19 and 16% higher dose to achieve any given log reduction, respectively. Though both variants are significantly different from SARS-CoV-2/SB2, there was no statistically significant

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difference between Delta and Omicron ($P(T > t) = 0.53$), so the average difference for both variants is 17%.

Overall, SARS-CoV-2 and its variants remain relatively sensitive to UV_{254}, with rate constants approximately twice as large as the other common airborne disease, tuberculosis, and most other viruses, as shown in Table 2. It is worth noting that

Table 2. UV Disinfection Rate Constants for Selected Bacteria, Viruses, and Pathogens

organism	UV_{254} rate constant (cm^2/mJ)
MS2 phage[28]	0.13
Bacillus subtilis spores[28]	0.17
Mycobacterium tuberculosis[28]	0.48
Influenza A virus[28]	1.02
Phi6 Virus[25]	0.03
T1UV bacteriophage[24]	0.48
average of 96 tested viruses[28]	0.58

other sources of UV radiation are possible, with KrCl* excimer sources being reported as especially effective for SARS-CoV-2.[25] The use of Far UVC radiation with wavelengths between 200 and 230 nm has the advantage that it is unlikely to harm skin or eyes, making it potentially safer than traditional germicidal UV at 254 nm.[26] A range of radiation wavelengths from 222 to 488 nm have been tested using SARS-CoV-2, with UVC wavelengths (≤280 nm) being the most effective.[27] Table 2 is intended to provide context for the results presented in this study that used UV at 254 nm and shows the comparable disinfection rate constants for a selected viruses, bacteria, and airborne pathogens.

Differences in the genome of the Delta and Omicron variants could explain the reduced sensitivity to UV irradiation at 254 nm. The formation of dimers from adjacent pyrimidine bases in the genome that prevent replication has traditionally been seen as the main mode of action of UV irradiation at 254 nm. Analysis of the bacterial genomes after exposure at 254 nm revealed the presence of these dimers, principally of thymine[29] (T) and to a lesser extent, cytosine (C). In the RNA of viruses, thymine is replaced by uracil (U), another pyrimidine base. Recent research reports that UV disinfection of SARS-CoV-2 halts infectivity while preserving its morphology and antigenic properties.[30] Since the morphology and antigenic properties did not change, this may suggest that changes in the genome may play a key role. Furthermore, recent research has been able to predict UV sensitivity based on the genome alone. Linear regressions based on the number of the pyrimidines C (cytosine), U (uracil), UU, and UUU in the genome agreed within 7% of experimental UV sensitivities.[31] The authors do not provide details of the relative importance of each; however, previous research would suggest that adjacent UU doublets and UUU triplets are likely to be important.

There are fewer UU doublets and UUU triplets in the genome of the Delta and Omicron variants when compared to SARS-CoV-2/SB2, as shown in Table 3. There are also 1.9 and 4.9% fewer cytosines in the genomes of Delta and Omicron, respectively. The reason for this difference is that portions of the genome are deleted, along with the repeating uracil bases they contain, in both the Delta and Omicron variants. A sample of deleted sequences for Omicron is shown in Figure 3. Notably, a single mutation (uracil to guanine) in a string of nine repeating uracil bases in SARS-CoV-2 shortens this string to just six repeating bases in both the Delta and Omicron

Table 3. Number of UV-Sensitive Cytosine, Uracil, and Repeating Uracil Bases Missing in Delta and Omicron Variants When Compared to Ancestral SARS-CoV-2/SB2

	Delta		Omicron	
	missing	% change	missing	% change
C	102	−1.9%	225	−4.2%
U	228	−2.4%	439	−4.7%
UU	30	−1.3%	69	−2.9%
UUU	17	−2.5%	25	−3.7%
UUUU···.	6	−2.6%	14	−6.3%

SARS-CoV-2/SB2:

TAGATCAGGCA**TT**AGTGTCTGATG**TT**GGTGATAGTGCGGAAG**TT**

Omicron:

TAGAT----------Deleted Sequence----------

SARS-CoV-2/SB2:

GCCTGG**TT**GTGATGGTGGCAG**TTT**GTATGTAAATA

Delta:

GCCT----------Deleted Sequence----------

Figure 3. Sample RNA sequences for SARS-CoV-2/SB2 and Omicron and Delta with missing uracil (indicated by T) repeating bases underlined in bold.

variants (not shown). Overall, it is likely that the reduced number pyrimidine bases in the Delta and Omicron variants, perhaps in key locations, may contribute to their reduced UV sensitivity. The data presented in Table 3 may suggest that Omicron is even more UV-resistant than Delta; however, this was not supported by the UV_{254} dose−response data collected in this study.

SUMMARY AND CONCLUSIONS

Overall, SARS-CoV-2 is quite sensitive to UV disinfection at 254 nm with a rate constant that is approximately double that of most other viruses and the bacterial airborne disease tuberculosis. However, the Delta and Omicron variants will require an approximately 17% higher UV dose to achieve the same level of disinfection as the ancestral SARS-CoV-2 virus, based on the results presented in this work. It is suggested that the reason for this difference is the deletion of RNA sequences containing UV-sensitive pyrimidine bases in the genome of these two variants. This difference in UV sensitivity has significance to a range of applications including upper-room UV disinfection, hospital room sanitization, and others.

ASSOCIATED CONTENT

Data Availability Statement

The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsestengg.3c00019.

SARS-CoV-2/Delta, SARS-CoV-2/Omicron, Delta/Omicron, SARS-CoV-2/OC43, *T*-test, and significant virus SAR-CoV-2/SB2 (XLSX)

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Notes

The authors declare no competing financial interest.

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