

Potential use of DBD based 222 nm Exciplex Light Source for Milk Sterilization and its Comparative Analysis with 253 nm UV-light and Conventional Pasteurization

4.1 Introduction

Raw milk, which does not undergo any processing such as pasteurization and sterilization, is rich in nutrients and bioactive compounds [Rumbold et al., 2022]. Milk contains water, lipids, non-fat solids, lactose, vitamins, proteins, and minerals. However, they can also harbor pathogens such as *Campylobacter*, *Listeria monocytogenes*, *Escherichia coli*, and *Staphylococcus aureus* [Sharma et al., 2014], leading to the outbreaks of dairy-related diseases in humans, including brucellosis, tuberculosis (TB), and listeriosis [Comerford et al., 2021] [Pokala et al., 2024] [Chelladurai et al., 2023]. Traditionally, pasteurization, a heat exchanger, has been used to reduce the number of bacterial contaminants in milk [Deeth, 2017]. Although heat treatments work well in reducing bacterial contaminants, they can negatively affect the flavor, texture, and color, as well as denature many vital vitamins in milk [Barba et al., 2012] [Abrahamsen et al., 2022]. Bezie [Bezie, 2019] reviewed and reported the thermal degradation of essential vitamins and the alteration of sensory properties in pasteurized milk. Also, pasteurization can only be performed at a consolidated industrial level and requires a large amount of energy. Removal of bacteria from milk without altering its nutritional properties has long been a challenge [Franzoi et al., 2022].

To address these issues, non-thermal methods such as high hydrostatic pressure, pulsed electric fields, ultrasonication, and ultraviolet (UV) radiation have been explored [Cameron et al., 2010] [Mahmoud et al., 2022] [Krishnamurthy et al., 2007a]. While promising, these methods face challenges in terms of efficiency, treatment duration, and cost when scaled up to an industrial level. Gurol et al. [Gurol et al., 2012] demonstrated that corona discharge could drastically lower *E. coli* colony-forming units in milk without negatively impacting pH and color tests. Lu et al. [Lu et al., 2011] designed an apparatus using an electrodeless UV lamp (EUL) with a radio frequency of 2.65 MHz and found that EUL worked more efficiently in the inactivation of milk-related bacteria than the conventional low-pressure high-intensity mercury lamp. They showed that, at a flow rate of 28.8 L/h, the total number of bacteria was reduced by more than 6 log with a UV dose of 21.3 mJ/cm².

Researchers have recently shown great interest in a new UV-C technology called far UV-C (200–230 nm), specifically known as the KrCl* exciplex emitting 222 nm for food sterilization, because of its better bacterial inactivation effectiveness and controlled and safe exposure to human skin and eyes. Various studies have been conducted to investigate the effects of 222 nm on the inactivation of bacteria and viruses in aqueous suspensions [Ahlawat et al., 2024b], as well as on the degradation of micro-pollutants in wastewater [Jangra et al., 2023a] [Ahlawat et al., 2024d] [Mishra et al., 2024]. Ha et al. [Ha et al., 2017] studied the application of 222 nm to control the foodborne pathogens on sliced cheese surfaces. Kang et al. [Kang et al., 2019] studied the decontamination effect of 222 nm against pathogens on Apples and Bell peppers and reported a bacterial reduction of approximately 5 log in just 7 min of treatment. Recently, a 222 nm exciplex source has been used to study various food matrices, however, to the best of our knowledge, there is currently no research available on the use of safer far UV-C (222 nm) for the sterilization of raw milk and non-thermal reactor development using 222 nm for small-scale sterilization purposes.

In this chapter, two exciplex UV sources, KrCl* (222 nm) and XeI* (253 nm), that are mercury-free and can be easily submerged in milk without additional sleeves have been used for the milk sterilization. The exciplex UV sources are fine-tuned to maximize their effectiveness in treating whole milk by optimizing the UV irradiation, discharge power, and operational temperature, along with the reactor design. The impact of different UV dosages on the physicochemical properties of milk, such as pH, conductivity, temperature, color, titratable acidity, and fat/SNF, has also been analysed. Microbiological analysis of whole milk was conducted using the methylene blue reduction test (MBRT) before and after treatment with exciplex UV sources. Additional research has been conducted on the inactivation of two specific foodborne pathogens, *Escherichia coli* and *Staphylococcus aureus*, in milk samples. Finally, to assess the designed reactor more effectively, the obtained findings are compared with the pasteurization method.

4.2 Experimental Setup and Characterization

An already explained exciplex sources (KrCl*, 222 nm; and XeI*, 253 nm) in section 2.2.1 has been used for the sterilization of raw milk. For the exciplex source design, a cylindrical tube made of quartz (170 mm × 10 mm/7 mm, length × outer/inner diameter) was fused co-axially inside another quartz tube (180 mm × 16 mm/13 mm, length × outer/inner diameter) to make a discharge/gas gap of 1.5 mm. A helical-shaped copper wire was used as the high-voltage electrode inside a smaller quartz tube. A food-grade stainless steel sheet having of 0.3 mm with equidistant circular holes of 5 mm was used as the grounded electrode and tightly fitted on the larger quartz tube in a co-axial DBD configuration. The actual view of the developed DBD-based exciplex sources (KrCl*, 222 nm; and XeI*, 253 nm) are shown in Figure 4.1. A bipolar pulsed power source was used to provide the electric potential between the electrodes to ignite the gas discharge to obtain uniform exciplex UV irradiation intensity. The exciplex UV sources were operated at an applied voltage of 7 – 9 kV, a pulse repetition frequency of 15 – 25 kHz, and a pulse width of 2 μm. The gas pressure inside the discharge gap was first optimized based on absolute UV irradiance and kept constant (140 mbar for the KrCl* exciplex and 200 mbar for the XeI* exciplex) throughout the milk treatment experiments. Electrodes were designed such that the lamps could operate with minimum heating.

Based on electrical and optical characterization, different parameters, that is, gas pressure and discharge power, were optimized, and the developed exciplex sources were sealed off for further use in milk quality assessment.

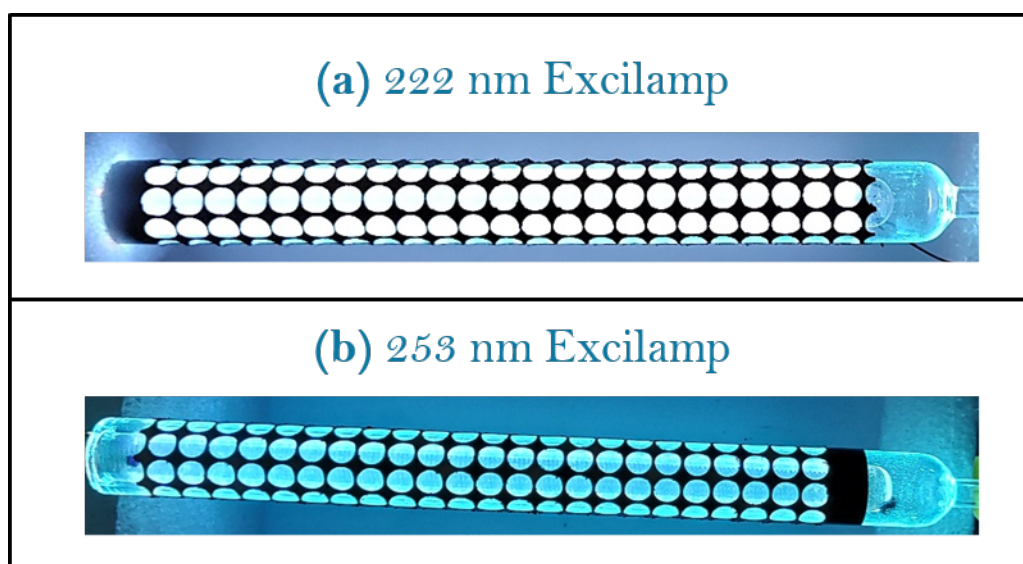


Figure 4.1: Original view of the exciplex UV lamp (a) KrCl* (222 nm), and (b) XeI* (253 nm).

Raw milk used for the treatment was procured from a local milk vendor in Jodhpur, Rajasthan, India. To ensure no homogenization or standardization of milk during the experiment, unprocessed milk was utilized. These processes alter the fat content and other constituents of milk, potentially affecting the pathogen inactivation and reducing the MBRT value. This is because fats can envelop pathogens, thereby providing protection. Unprocessed milk was exposed to different doses of 222 nm and 253 nm exciplex UV light. To ensure that the UV light could pass through the milk, potassium iodide was dissolved in DI water, and the solution was passed through the container while the UV lamp was on. The white solution became yellow, and the UV-vis absorption spectra confirmed the UV dose when compared with the calibration curve. The radiometry method (as described in Section 4.3.1) is utilized for the measurement of absolute UV radiation with and without milk. To prevent contamination before and after exciplex UV treatment, all milk handling and sample collection procedures were carried out within a Biological Safety Cabinet (BSC). This controlled environment ensured that the milk samples remained free from external contaminants. Additionally, the transfer and filling of UV-treated milk into test tubes were conducted in the BSC to maintain strict hygiene standards and avoid any potential cross-contamination. By utilizing the BSC for all steps, the integrity of the samples was preserved, ensuring accurate and reliable results during the UV treatment process.

4.3 Results and Discussion

4.3.1 Electrical and Optical Characterization of Exciplex UV Sources

The aforementioned DBD exciplex lamps were powered using a microsecond pulsed bipolar power supply. Gas pressure optimization was performed at different applied voltages and frequencies for a narrow spectral band and maximum absolute irradiation intensity output. For the KrCl* exciplex source, the optimized pressure of the KrCl gas mixture was 140 mbar and the maximum intensity was recorded at 27.5 kHz. In the case of the XeI* exciplex source, the maximum intensity was obtained at a gas pressure of 200 mbar and frequency of 27.5 kHz. Figure 4.2 (a) and 4.2 (b) show the typical current-voltage waveforms of the exciplex far UV-C (222 nm) and UV (253 nm) sources, respectively. As the gas pressure inside the exciplex source increased, there was a proportional increase in the quantity of gas molecules accessible for collisions in the gas gap. Because of the higher gas pressure in the case of the XeI* exciplex, more molecules are available for collision, which results in reduced current peaks and the corresponding discharge power. Figure 4.2 (c) depicts the power dissipation at various applied voltages and frequency of 27.5 kHz. The average discharge power was calculated using the V-I characteristics of the respective lamps. The calculated average discharge power for KrCl* and XeI* exciplex lamps at optimized parameters are 24.72 W and 22.67 W, respectively. To determine the efficacy of the developed exciplex sources in milk sterilization, the input parameters were chosen based on the absolute irradiation intensity and lamp heating. KrCl* exciplex source at 8 kV and 27.5 kHz produces the same output intensity (as measured by the UV power meter) as XeI* exciplex source at 8.5 kV and 27.5 kHz. Therefore, these two parameters were selected for the milk sterilization experiments.

The OES of the developed exciplex lamps were recorded, as shown in Figure 4.2 (d). The primary emission lines at 222 and 253 nm correspond to the characteristic emission of the B-X transitions of the KrCl* and XeI* exciplexes. These emission lines are attributed to various excitation and relaxation processes of the excited species within the exciplex lamps shown as [Ahlawat et al., 2024b] [Prakash et al., 2017];



Both wavelengths are well known for their germicidal and surface modification capabilities. Furthermore, 222 nm is extensively utilized in many applications that require safe and efficient UV-C radiation [Blatchley et al., 2023] [Payne et al., 2022]. For more information regarding the design and development of 222 nm and 253 nm excilamps, please refer to Chapter 3.

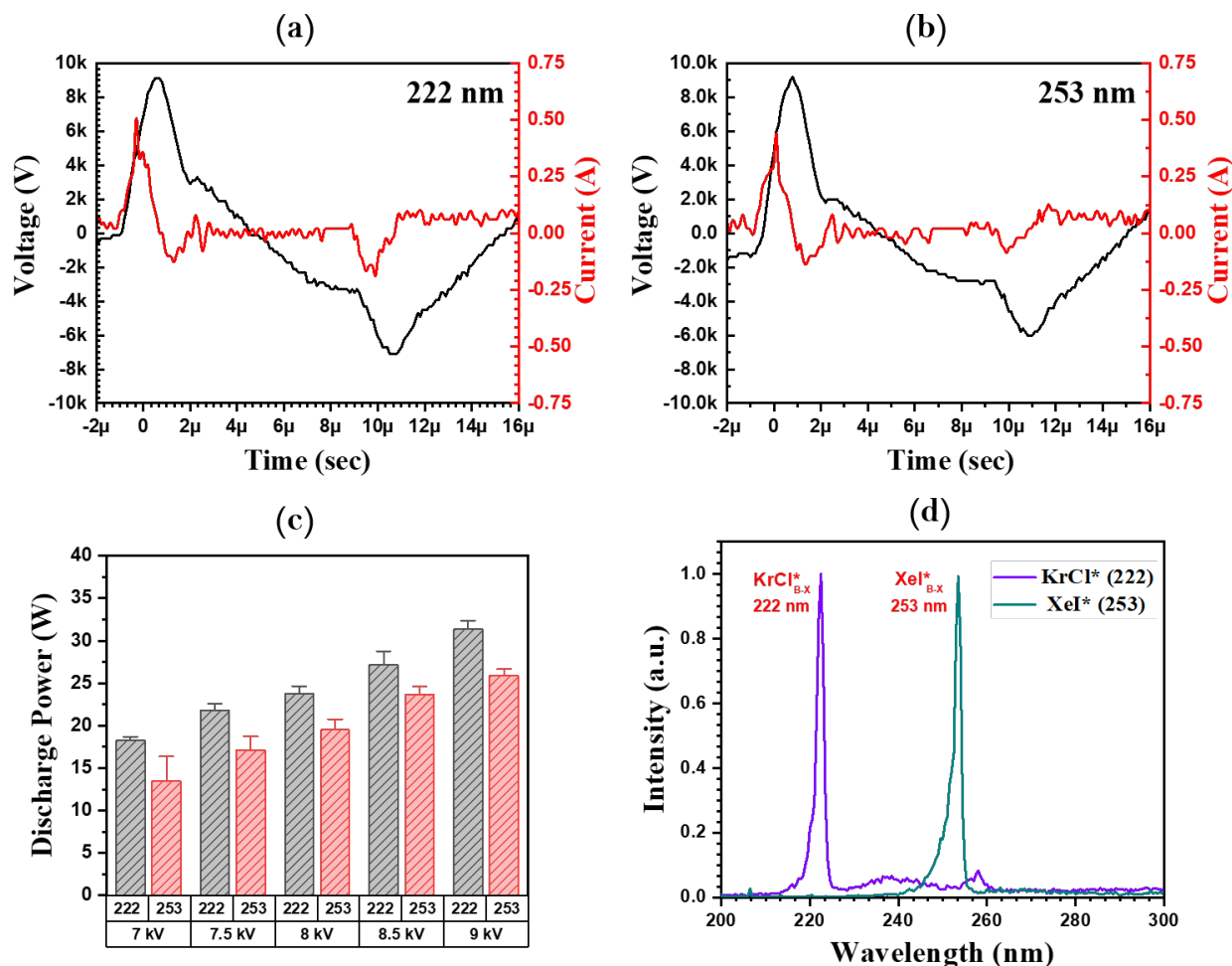


Figure 4.2: Typical V-I characteristics of exciplex lamps (a) KrCl* (222 nm), and (b) XeI* (253 nm), (c) discharge power of both the excilamp at different applied voltages and at a fixed frequency, (d) optical emission spectra of KrCl* and XeI* excilamps.

The absolute UV irradiation intensity of both excimer sources was examined using radiometric techniques with and without whole milk outside the container. Because the container used was made up of quartz, the penetration of UV outside the container was also possible. It was observed that the developed exciplex sources have an absolute UV intensity of $\sim 2.24 \text{ mW/cm}^2$ in air, and when measured with a quartz container without milk, this absolute UV intensity is decreased to $\sim 1.83 \text{ mW/cm}^2$. Figure 4.3 shows the absolute UV intensity outside the container, with and without whole milk. In the case of whole milk, the UV 253 nm radiation penetrating the container was somewhat higher ($\sim 0.215 \text{ mW/cm}^2$) than the UV 222 nm light ($\sim 0.048 \text{ mW/cm}^2$). This is because of the higher absorbance of UV 222 nm light in milk, as can be seen from the UV-Vis spectra [see Figure 4.4 (b)].

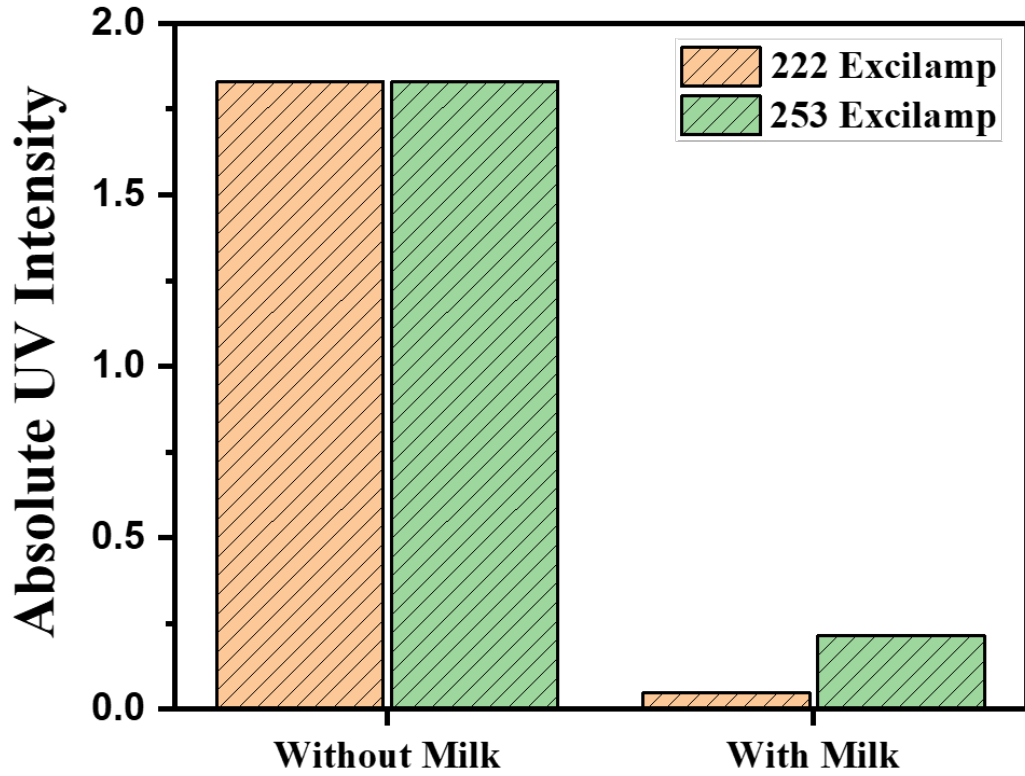


Figure 4.3: Measured UV intensity with and without whole milk outside the container.

Figure 4.4 (a) shows the UV dose delivered to the milk samples at different treatment times. The absolute UV intensity of both exciplex sources in air was measured using radiometry (i.e., a UV power meter), and the operating parameters for both excilamps were adjusted to deliver the same UV doses for the milk treatment experiments. The UV dose (J/L) delivered to the milk samples was calculated using the following equation [Müller et al., 2017]:

$$UV\ Dose = \frac{A_{352\ nm} \times P_{222/253\ nm}}{pl \times \emptyset \times \epsilon_{352\ nm}} \quad (4.3)$$

The UV dosages were measured using iodine-iodate actinometry and are found to be ~ 4.57 J/ml, ~ 6.83 J/ml, ~ 8.53 J/ml, and ~ 10.9 J/ml at various treatment times, i.e. 1 minute, 1.5 minutes, 2 minutes, and 2.5 minutes, respectively. Figure 4.4 (b) shows the absorption spectrum of milk, indicating that milk absorbs 222 nm more than 253 nm. This suggests that 222 nm is more effective in reducing microbial load, but has a lower penetration ability in milk. The system was designed to enable both 222 nm and 253 nm wavelengths to penetrate whole milk.

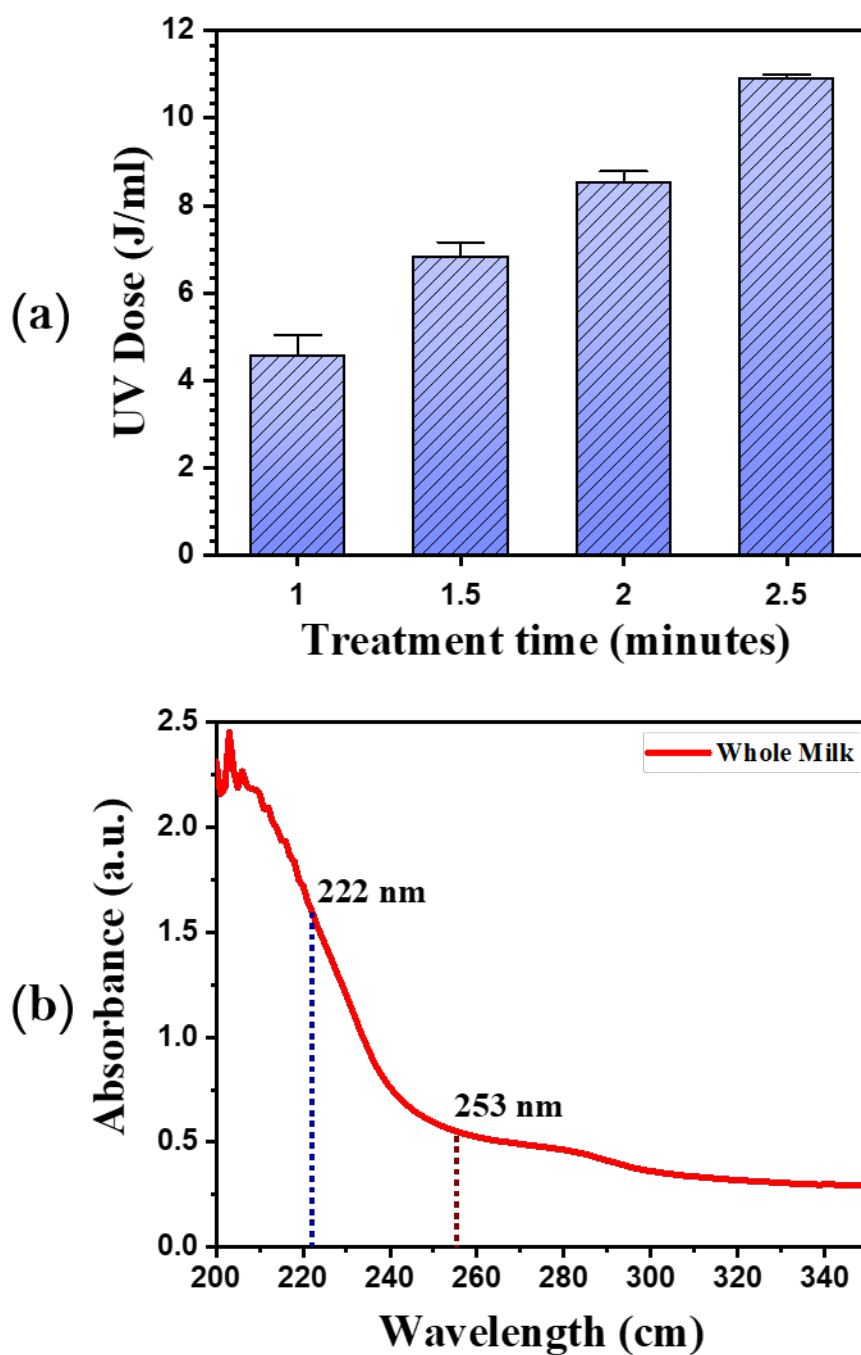


Figure 4.4: (a) UV dose delivered to milk samples at different treatment times, and (b) absorbance of whole milk (3 mm quartz cuvette).

4.3.2 Physicochemical Properties Analysis

Physicochemical characteristics are important indicators for quality assessment and preservation of milk. Heat treatment (pasteurization) is a widely used technique for milk processing. If we are seeking a different and efficient technique for milk processing, it is essential that we carefully examine the physicochemical parameters of milk, especially the temperature. The instantaneous temperature of the milk sample after different exciplex UV treatment times is shown in Figure 4.5 (a), and the same is compared with that before treatment. Untreated milk served as 0 min treated or control milk. Extending the duration of exciplex UV treatment leads to a significant increase in milk temperature. This was due to joule heating in the treated milk during exciplex UV treatment.

The initial temperature of the milk was $\sim 26^{\circ}\text{C}$, and after the exciplex UV 222 nm and 253 nm treatments, the maximum temperature increase was $\sim 32.5^{\circ}\text{C}$ and $\sim 34^{\circ}\text{C}$, respectively.

However, the obtained temperature was significantly lower than the temperature at which milk was pasteurized (63 °C for 30 min). According to a study conducted by Ponraj et al. [Ponraj et al., 2017], the application of plasma treatment resulted in a considerable increase in the temperature of milk, reaching up to 65 °C, primarily owing to joule heating. To control the temperature, the authors employed a water-cooling jacket and successfully maintained the temperature at 42 °C. Nevertheless, the present study was able to maintain a temperature well below 35 °C without the need for additional water-cooling owing to the design of our source. As shown in Figure 4.5 (b), treatment of milk with different exciplex UV sources did not significantly change the temperature of the milk.

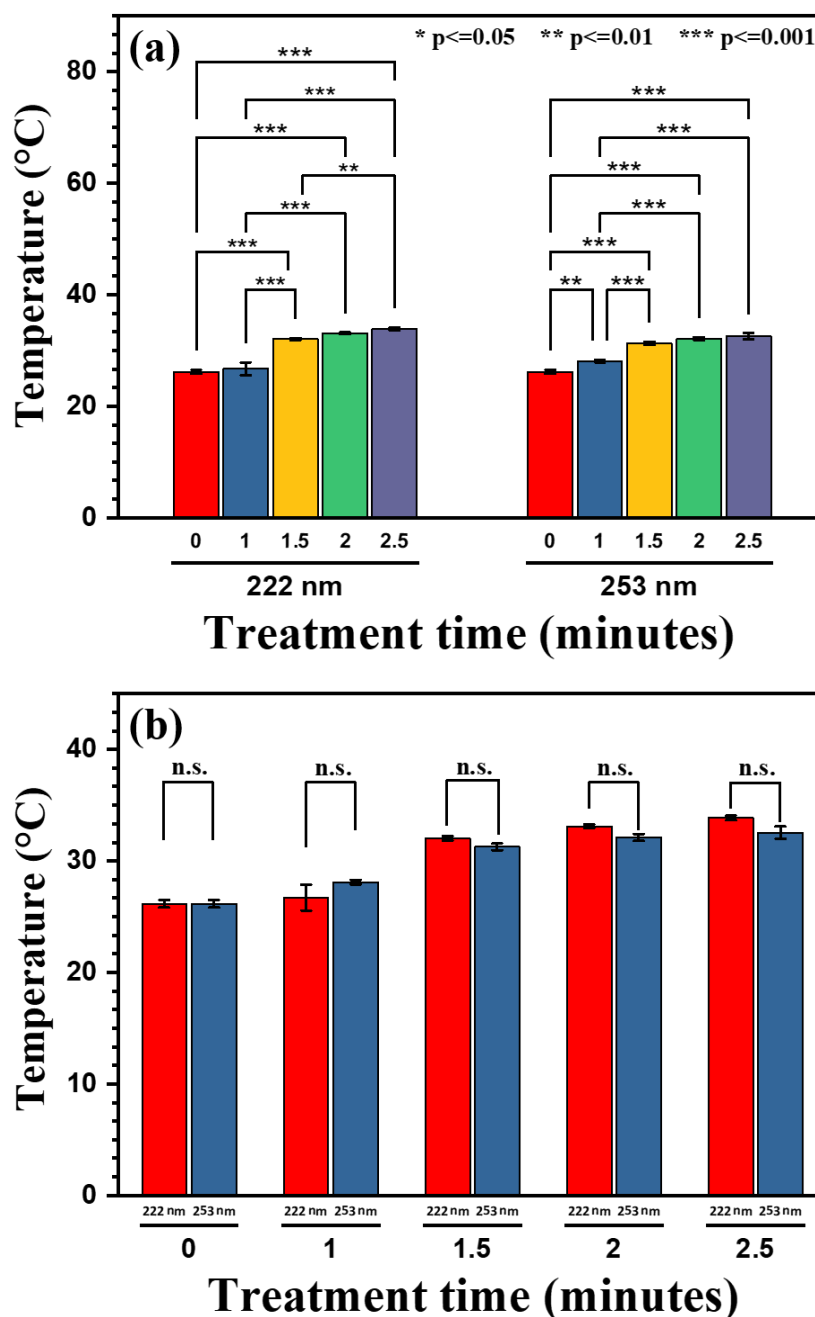


Figure 4.5: (a) Temporal behaviour of temperature of milk before and after exciplex treatment and (b) Temperature profile for the exciplex UV 222 and UV 253 nm treated milk at different treatment times. The error bars indicate the mean $\pm$ SD of samples and the statistical significance (*, **, and *** indicates $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively) was calculated between the untreated and exciplex 222 nm and 253 nm treated samples at different treatment times/UV dosages.

Several studies have evaluated the effects of non-thermal plasma technology on the physicochemical properties of milk [Nikmaram et al., 2022a] [Wu et al., 2021]. There are several explanations for these physicochemical changes, such as enzymatic hydrolysis, oxidative processes, and the generation of microbial metabolites. Bacteria that metabolize milk to produce undesirable compounds produce microbial metabolites. Lactic acid is produced when lactic acid bacteria break down lactose, which lowers pH and causes proteins to precipitate [Lu et al., 2017]. In this study, several physicochemical properties of milk, including pH, conductivity, titratable acidity, and fat/SNF, were evaluated. Table 4.1 shows the pH and titratable acidity measurements before treatment at 0, 5, 10, 15, and 20 min post-treatment. The pH and titratable acidity values were within 6.67 ± 0.04 and 0.133 ± 0.005 (% lactic acid), respectively, and no significant change was observed after the exciplex UV treatment. According to Sepulveda et al. [Sepulveda et al., 2005], sensory changes occur when the lactic acid percentage increases above 0.2. Therefore, exciplex treatment showed no significant differences in the sensory properties and pH value of the milk. These results are in good agreement with those of Orłowska et al. [Orłowska et al., 2013], who reported that after UV treatment, the pH of the milk remained unaffected.

Table 4.1: pH and titratable acidity measurements after 0, 5, 10, 15, and 20 minutes of post-treatment.

Post treatment time (minutes)	pH		Titratable acidity (% lactic acid)	
	UV 222	UV 253	UV 222	UV 253
	2.5 minutes	2.5 minutes	2.5 minutes	2.5 minutes
Untreated	6.70 ± 0.02	6.70 ± 0.02	0.131 ± 0.004	0.131 ± 0.004
0	6.64 ± 0.04	6.60 ± 0.03	0.130 ± 0.007	0.130 ± 0.006
5	6.68 ± 0.03	6.69 ± 0.07	0.129 ± 0.007	0.134 ± 0.001
10	6.69 ± 0.04	6.68 ± 0.04	0.127 ± 0.007	0.130 ± 0.006
15	6.63 ± 0.07	6.64 ± 0.08	0.131 ± 0.007	0.134 ± 0.009
20	6.67 ± 0.05	6.66 ± 0.04	0.127 ± 0.006	0.132 ± 0.008

Note: Values are shown as the mean $\pm$ SD of three readings, and no statistically significant difference was observed.

Another crucial factor related to bacterial inactivation/MBRT analysis is the electrical conductivity of the milk. Figure 4.6 shows the conductivity measurement of the milk for the exciplex UV 222 and UV 253 nm treatments (after 2.5 min of treatment) at 0, 5, 10, 15, and 20 min post-treatment. It was observed that conductivity increases rapidly up to 6.54 mS/cm and 6.47 mS/cm just after the 222 nm and 253 nm treatment, respectively, and then decreases up to its original value (4.87 mS/cm) during a 15-minutes time frame. The observed increase in conductivity for the initial 15 min was attributed to the increase in milk temperature. Once the milk temperature returns to its original value, its conductivity also returns to its original value. Goncalves et al. [Gonçalves et al., 2017] studied the effect of temperature on the conductivity of dairy products and concluded that with increasing temperature, the conductivity of dairy products increased because of the presence of different salts. To further extricate the sensory properties of the milk, the fat and SNF were measured for the untreated and treated milk, and no significant difference in the fat and SNF was recorded after the exciplex UV treatment, as shown in Table 4.2, because of the low UV dose used for the milk treatment.

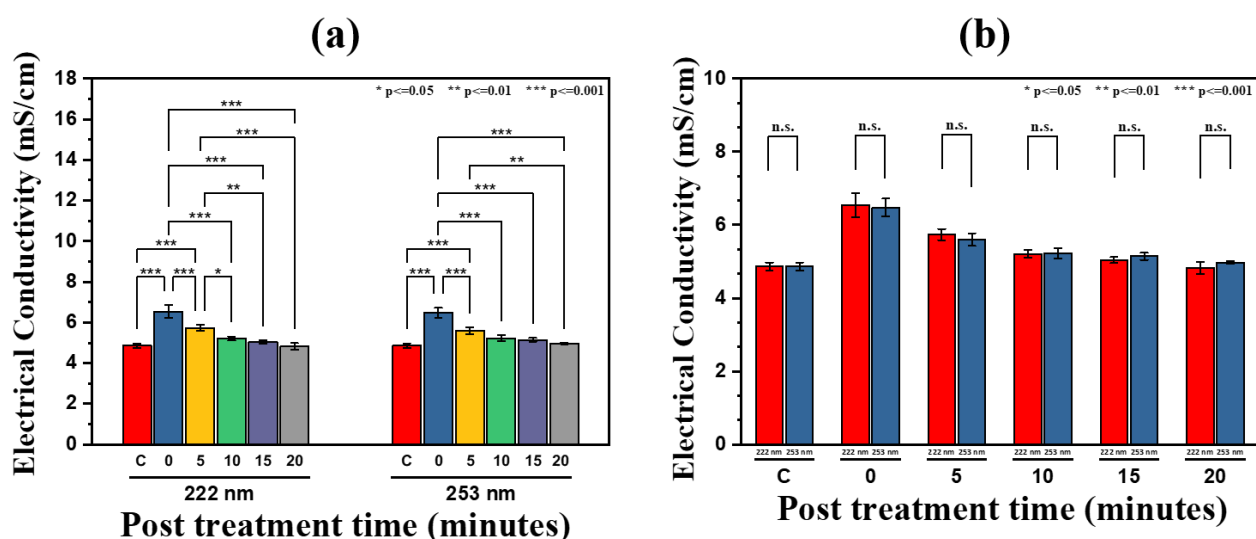


Figure 4.6: (a) Temporal behaviour of electrical conductivity of milk before and after exciplex treatment and (b) Electrical conductivity profile for the exciplex UV 222 and UV 253 nm treated milk at different treatment times. The error bars indicate the mean $\pm$ SD of samples and the statistical significance (*, **, and *** indicates $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively) was calculated between the untreated and exciplex 222 nm and 253 nm treated samples at different treatment times/UV dosages.

Table 4.2: Fat (%) and SNF (%) measurements after 0, 5, 10, 15, and 20 min of post-treatment

Post treatment	Fat (%)		SNF (%)	
time (min)	UV 222	UV 253	UV 222	UV 253
	2.5 min	2.5 min	2.5 min	2.5 min
Untreated	4.31 $\pm$ 0.19	4.31 $\pm$ 0.19	8.74 $\pm$ 0.29	8.74 $\pm$ 0.29
0	4.48 $\pm$ 0.22	4.43 $\pm$ 0.19	8.47 $\pm$ 0.29	8.52 $\pm$ 0.14
5	4.29 $\pm$ 0.16	4.38 $\pm$ 0.21	8.46 $\pm$ 0.11	8.51 $\pm$ 0.12
10	4.46 $\pm$ 0.23	4.3 $\pm$ 0.21	8.63 $\pm$ 0.11	8.71 $\pm$ 0.56
15	4.32 $\pm$ 0.17	4.26 $\pm$ 0.05	8.63 $\pm$ 0.45	8.41 $\pm$ 0.16
20	4.29 $\pm$ 0.09	4.45 $\pm$ 0.09	8.52 $\pm$ 0.10	8.43 $\pm$ 0.17

Note: Values are shown as mean $\pm$ SD of three readings, and no statistical significance difference has been observed

The change in milk color during preservation is thought to be significant and affects how people perceive it. The color changes following exciplex UV 222/253 treatment were measured and compared to the color of raw milk. During exciplex UV treatment of milk, L^* , a^* , and b^* values decreased slightly for all samples compared with the control. The total color difference (ΔE^*) in the range of 0 – 0.05 corresponds to non-noticeable, while the range 1 – 3 corresponds to a noticeable difference [Manoharan et al., 2021]. If ΔE^* exceeds 6, it corresponds to a significant change in color. In our study, the ΔE^* values for all samples are found to be between 0.01 – 0.17. Table 4.3 shows the color difference of UV 222 and UV 253 treated milk samples at different

exposure times using the CIE color system. These physicochemical properties are consistent with the results of previous studies on milk treated with non-thermal treatment. However, the use of UV light treatment (222 nm mercury-free) provides a more practical solution because of its environmentally friendly nature.

Table 4.3: Colour difference of treated milk samples at different exposure times using the CIE colour system.

Exciplex treatment time (minutes)	Colour							
	UV 222				UV 253			
	L*	a*	b*	ΔE^*	L*	a*	b*	ΔE^*
Untreated	94.83±0.03	-1.58±0.09	10.09±0.08	--	94.83±0.03	-1.58±0.09	10.09±0.08	--
1	94.80±0.02	-1.56±0.07	10.11±0.05	0.09±0.04	94.89±0.05	-1.54±0.06	10.11±0.05	0.13±0.04
1.5	94.79±0.01	-1.53±0.03	10.14±0.06	0.08±0.07	94.81±0.06	-1.51±0.06	10.06±0.03	0.08±0.06
2	94.75±0.04	-1.54±0.09	10.17±0.04	0.12±0.04	94.89±0.04	-1.54±0.09	10.14±0.02	0.09±0.06
2.5	94.73±0.07	-1.60±0.05	10.09±0.05	0.12±0.06	94.79±0.08	-1.52±0.09	10.13±0.05	0.08±0.05

Note: Values are shown as the mean±SD of three readings, and no statistically significant difference was observed.

4.4 MBRT and Bacterial Reduction Testing

Qualitative analysis of untreated and treated milk was performed using the methylene blue reduction test (MBRT) [Jenitta et al., 2014]. MBRT is especially relevant from an industrial viewpoint, as it is the standard test conducted at the time of milk collection across India. Figure 4.7 and 4.8 depict the visual representation of the MBRT analysis conducted on raw milk using the developed DBD-based UV 222/253 exciplex light source at different treatment times and UV dosages.

Figure 4.9 compares the MBRT data of raw milk when treated with different times/UV dosage of 222 nm and 253 nm exciplex light sources. The MBRT of raw milk was approximately 2 h 40 min, and it was increased to more than 5 h and 30 min, and 4 h and 30 min by exposing a 10.9 J/ml dosages of 222 nm and 253 nm of UV light, respectively. It should be emphasized that the increase in MBRT was significant when the raw milk was treated with exciplex 222 nm light as compared to exciplex 253 nm light at every treatment time [see Figure 4.9 (b)]. This improvement in MBRT may be due to the higher photonic energy of 222 nm light than 253 nm light [Amano et al., 2020] and the more absorption of 222 nm by milk compared to 253 nm. Ma et al. also explained that UV at 222 nm greatly enhances the disinfection of UV-resistant bacteria and viruses [Ma et al., 2021].

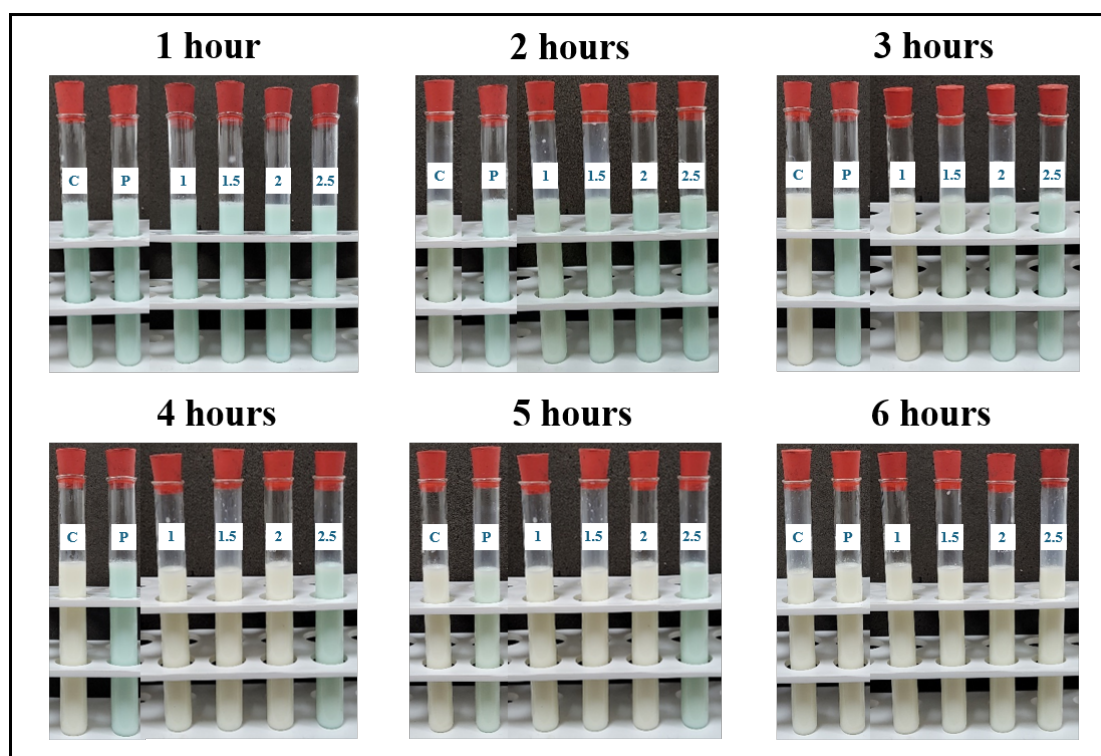


Figure 4.7: Pictorial representation of MBRT analysis of raw milk from the developed DBD based 222 exciplex light source at different treatment times/UV dosages.

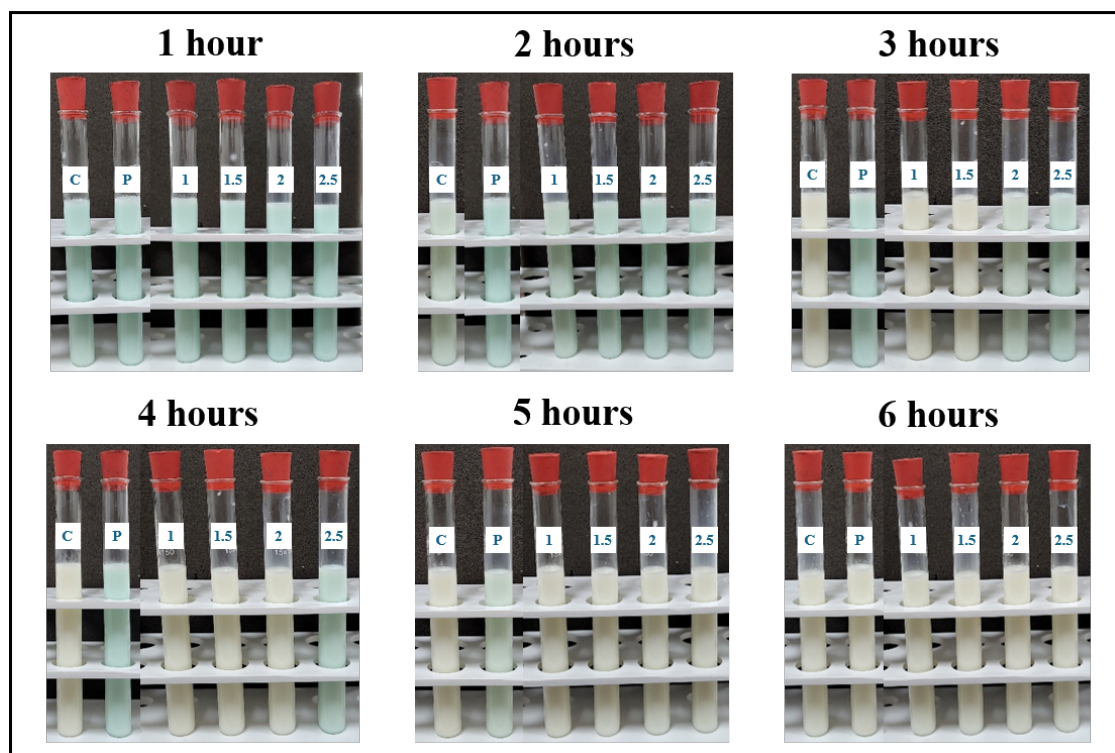


Figure 4.8: Pictorial representation of MBRT analysis of raw milk from the developed DBD based 253 exciplex light source at different treatment times/UV dosages.

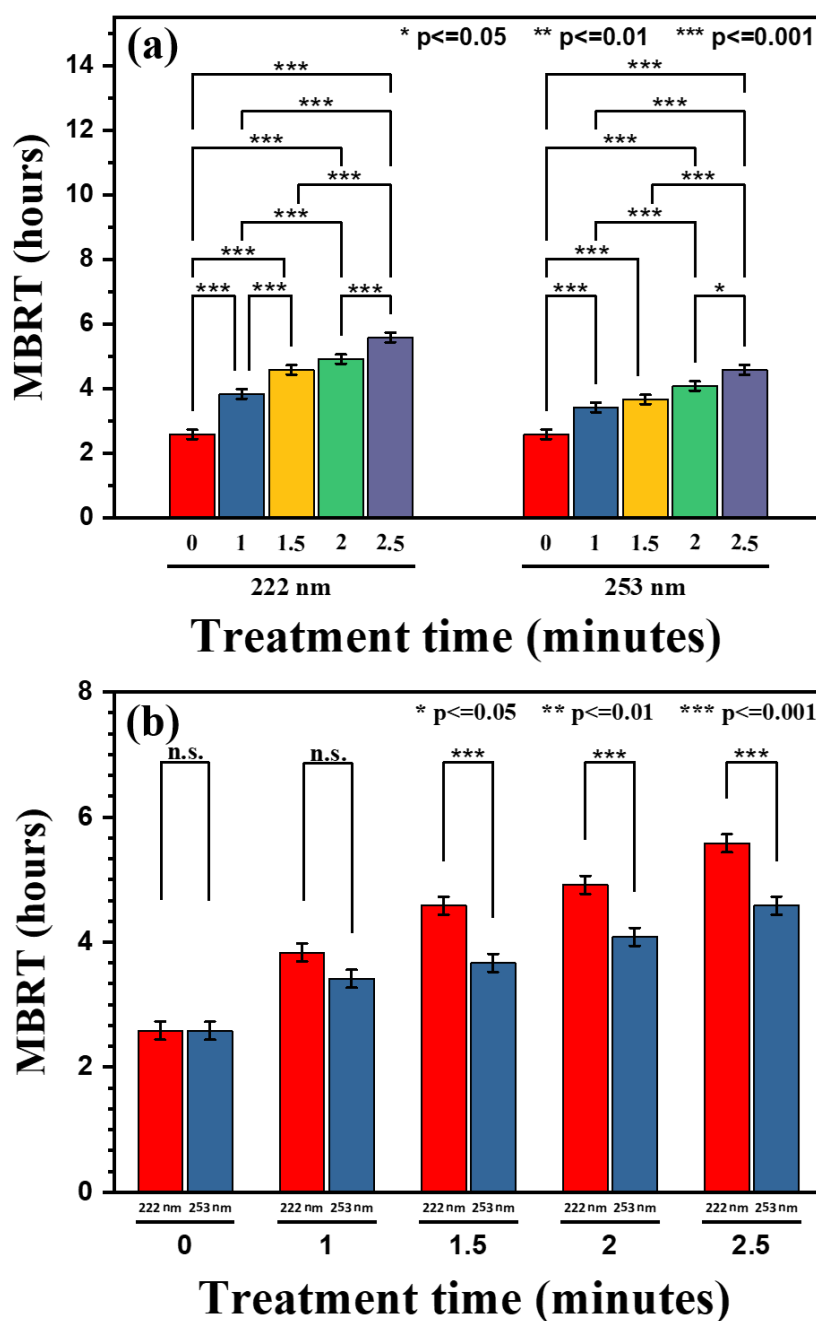


Figure 4.9: (a) MBRT results of raw milk following exciplex treatment at 8 kV/ 27.5 kHz for 222 nm and 8.5 kV/27.5 kHz for 253 nm at different treatment times, and (b) efficiency comparison of UV 222 and UV 253 for MBRT of raw milk. The error bars indicate the mean $\pm$ SD of samples and the statistical significance (*, **, and *** indicates $P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively) was calculated between the untreated and exciplex 222 nm and 253 nm treated samples at different treatment times/UV dosages.

Additional bacterial analyses were performed on the specific bacteria present in the milk, and gram-positive (*S. aureus*) and gram-negative (*E. coli*) bacteria were selected. In this study, we checked the efficiency of UV 222 nm on the aforementioned bacteria in milk, and compared the results with the UV 253 nm treatment, keeping all the experimental conditions identical. The total bacterial population for *S. aureus* was found to be 5.07 log/ml in the untreated raw milk. Figure 4.10 (a) shows that the initial load of *S. aureus* decreased significantly at different treatment times for both exciplex sources. Comparing the efficiency of both exciplex sources in *S. aureus* reduction [Figure 4.10 (b)], it is evident that the 222 nm exciplex is more effective than the 253 nm. A notable disparity of approximately 2.11 log and 2.58 log has been seen in the decrease in initial *S. aureus* when employing a 222 nm exciplex and 253 nm exciplex, respectively.

Furthermore, the total bacterial population for *E. coli* was found to be 4.85 log/ml in the untreated raw milk. Figure 4.10 (c) shows that the initial load of *E. coli* decreased significantly at different treatment times for both exciplex sources. The 222 nm exciplex shows a significant difference at every treatment time but the 253 nm exciplex was only significant at 2.5 min of treatment time if we compare it with the initial load. Furthermore, the 222 nm exciplex was more effective than the 253 nm exciplex for 2.5 min of treatment in *E.coli* reduction, as shown in Figure 4.10 (d). Atik and Gumus [Atik et al., 2021b] treated bovine milk with different doses of UV-C light and obtained 2.5 log and 3 log reductions in *S. aureus* and *E. coli*, respectively, with a UV dosage of 90 J/ml. These results are in good agreement with our previous study, which suggests that the far UV-C wavelength is more effective on gram-positive bacteria than gram-negative bacteria [Ahlawat et al., 2024b].

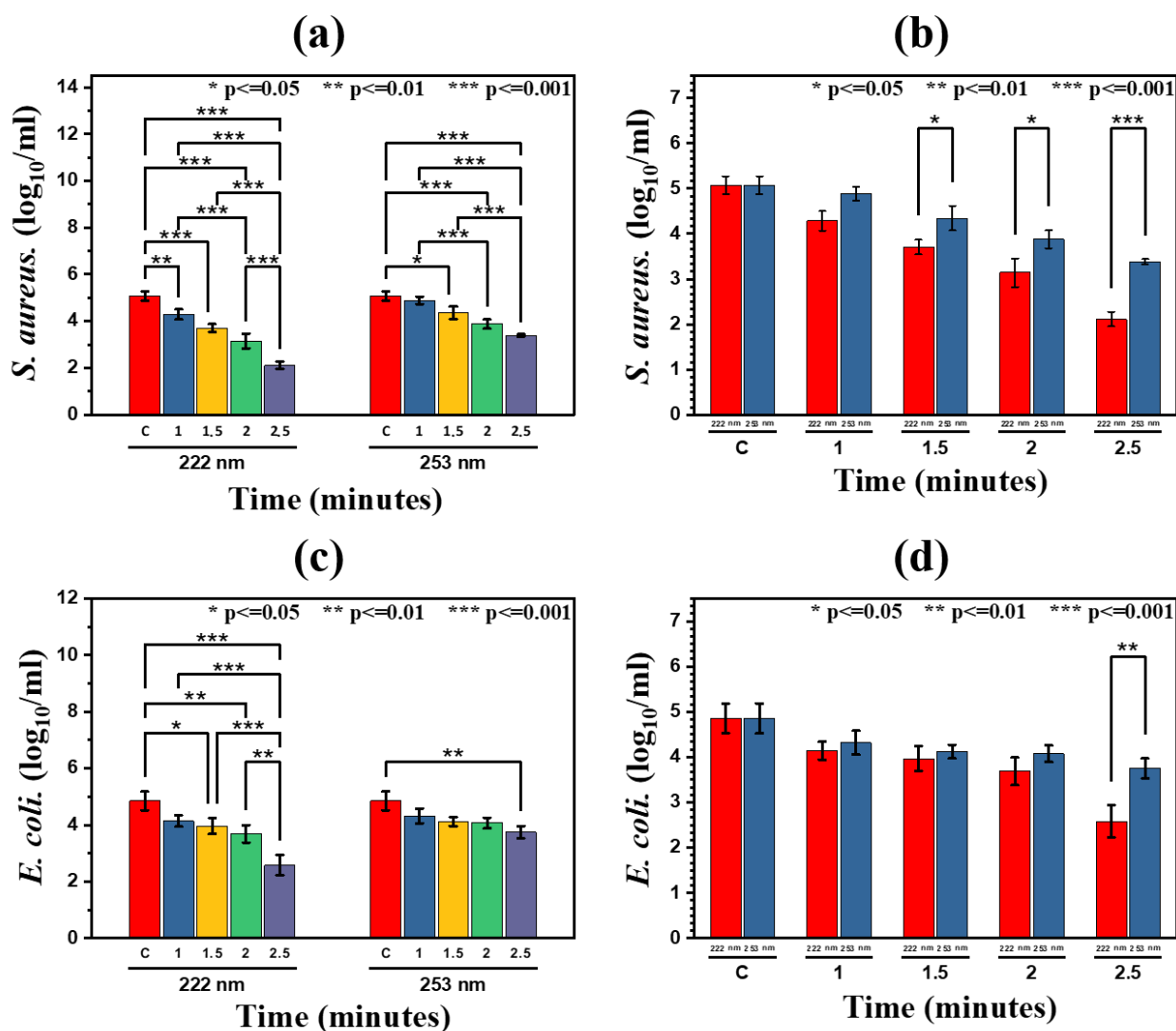


Figure 4.10: (a) *S. aureus* counts in milk following UV 222 and UV 253 exciplex treatment at different treatment times, (b) efficiency comparison of UV 222 and UV 253 for *S. aureus* reduction, (c) *E. coli* counts in milk following UV 222 and UV 253 exciplex treatment at different treatment times, and (d) efficiency comparison of UV 222 and UV 253 for *E. coli* reduction. The error bars indicate the mean±SD of samples and the statistical significance (*, **, and *** indicates $P \leq 0.05$, $P \leq 0.01$ and $P \leq 0.001$, respectively) was calculated between the untreated and exciplex 222 nm and 253 nm treated samples at different treatment times/UV dosages.

To validate the effectiveness of the non-thermal exciplex UV treatment as an alternative technology for heat-based pasteurization, all physicochemical and microbial results were compared with those of the pasteurization method. Ponraj et al. [Ponraj et al., 2017] compared the

non-thermal plasma processing of milk with the traditional pasteurization method and concluded that plasma-based sterilization shows better efficiency. In this study, the pH, titratable acidity, fat, and SNF did not show significant differences after exciplex UV treatment and pasteurization, as shown in **Fig. 8 (b)**, **8 (c)**, **8 (e)**, and **8 (f)**. It is obvious that the pasteurization of milk increases its temperature to a significant value compared to exciplex UV treatment; consequently, the electrical conductivity (which is directly related to temperature) increased, as shown in **Fig. 8 (a)** and **8 (d)**. However, the increase in temperature in the case of exciplex UV treatment was well below 35 °C, which indicates the non-thermal nature of the developed reactor.

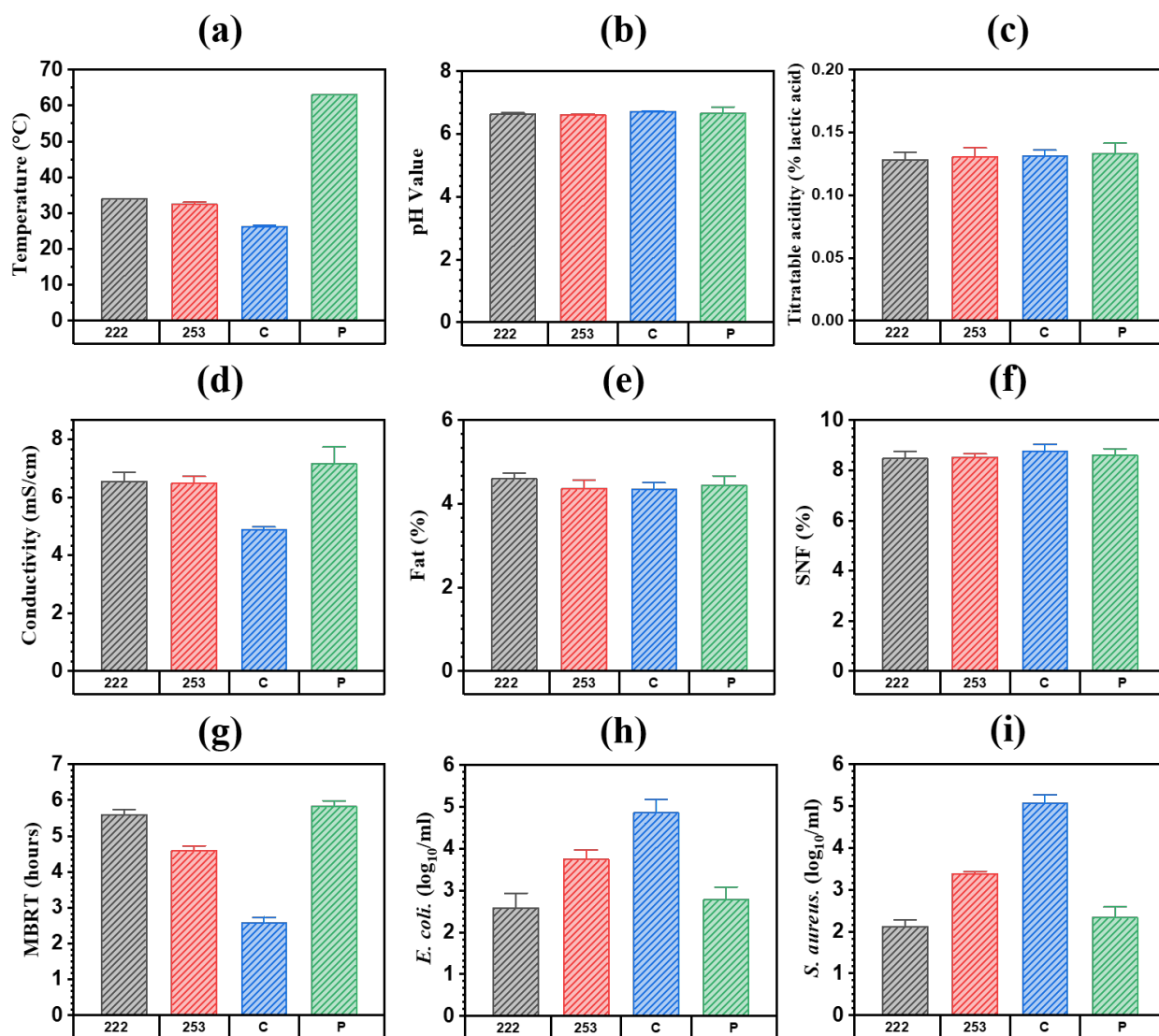


Figure 4.11: Comparison of physicochemical and microbial parameters of raw milk such as (a) temperature, (b) pH, (c) titratable acidity, (d) electrical conductivity, (e) fat, (f) SNF, (g) MBRT, (h) *E. coli* reduction, and (i) *S. aureus* reduction, before and after treatment with 222 nm exciplex, 253 nm exciplex, and pasteurization process of milk.

When the MBRT analysis is compared, the 222 nm exciplex shows a somewhat higher MBRT as compared to the 253 nm exciplex and pasteurization methods, as can be seen from **Fig. 8 (g)**. **Fig. 8 (h)** and **8 (i)** shows that the 222 nm exciplex treatment for 2.5 minutes significantly achieved a higher bacterial reduction (2.58 log in the case of *E. coli* and 2.11 log in the case of *S. aureus*) than the 253 nm exciplex (3.75 log in the case of *E. coli* and 3.38 log in the case of *S. aureus*) and pasteurization (2.78 log in the case of *E. coli* and 2.34 log in the case of *S. aureus*) for both gram-positive and gram-negative bacteria. Overall 222 nm exciplex outperforms the MBRT, gram-positive, and gram-negative bacterial load reduction when compared with the 253 nm exciplex and pasteurization methods. Furthermore, KrCl* exciplex lamps have an advantage over

conventional UV lamps because they do not contain mercury, and the far UV-C emission at 222 nm is not harmful to human tissues [Narita et al., 2020].

Upon doing a physicochemical and microbiological examination, it revealed that microbial and physicochemical gains persisted after 2.5 min of treatment. The physicochemical characteristics showed no change, which is in line with previous treatment durations. A maximum MBRT of 5 h and 30 min was obtained, and the MBRT saturation point was reached after 2.5 min of treatment. For this reason alone, even when it comes to microbial studies, the treatment was performed for up to 2.5 minutes only. In the present study, the treatment duration of 2–2.5 min was sufficient because the results obtained from excimer UV treatment were comparable or somewhat higher when compared with the pasteurization technique.

This study demonstrates a small-scale mercury-free exciplex UV lamp-based milk disinfection reactor that can be immediately utilized at the farmer level by reducing the bacterial load in the milk until it reaches the processing plants or to direct consumers without altering its physicochemical properties. This technology works at low capital and operating costs, making it affordable for farmers. The importance of food product safety indicates that there is great potential for the planned study of safe far UV-C light or non-thermal treatment of milk, which could have important real-world applications. Progress in this area for the plant testing and validation phase will be made possible by comprehending the effectiveness of exciplex UV sources in microbial reduction and optimizing test methods.

4.5 Cold Plasma Treatment of Raw Milk

Similar to the excimer UV lamp treatment of raw milk, we have also experimented with treating raw milk using a DBD plasma-based source and obtained some exciting results. These findings can be further explored in future studies for the treatment of larger quantities of milk.

4.5.1 Characterization of Discharge

All the experiments are conducted in ambient conditions, and no external gas is utilized for the discharge process. The overall discharge process can turn out to be very expensive if discharges are generated using any noble gases. Figure 4.12 (a) shows the typical V-I characteristic of the DBD plasma discharge at 4.5 kV/20 kHz.

The estimated discharge power at 4.5 kV/20 kHz is 17.64 W at 2 μ sec pulse width in a pulse power source. Figure 4.12 (b) shows the current and voltage mapping of the DBD plasma discharge at different applied voltages. The current increases with increasing the applied voltage, which means that the electric field that causes the process of ionization, excitation, and recombination of suspension's molecules will increase till heating effects are significantly visible.

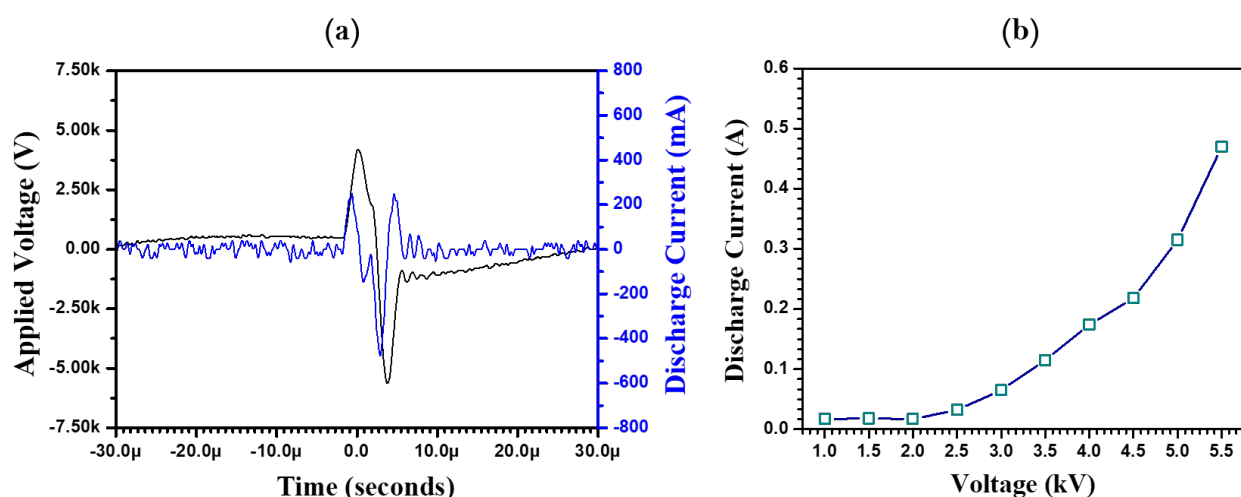


Figure 4.12: (a) Typical V-I characteristic of the DBD plasma discharge at 4.5 kV/20 kHz, and (b) current and voltage mapping of the DBD plasma discharge.

4.5.2 Physicochemical Properties and Microbial Analysis

Physicochemical properties are key indicators in assessing and maintaining the quality of milk. Numerous studies have investigated the impact of non-thermal plasma technology on these properties [Nikmaram et al., 2022a] [Wu et al., 2021]. For the cold plasma treatment of milk, various physicochemical properties of milk, such as pH, conductivity, and temperature, were evaluated. The milk was treated for durations of 10, 15, and 20 minutes. The results, shown in Table 4.4, indicate that pH, temperature, fat/SNF, and titratable acidity remained unchanged across all treatment times. However, conductivity initially increased before returning to its original value within 45 minutes. These findings align with previous research, which suggests that non-thermal plasma treatment does not significantly alter the physicochemical properties of milk [Nikmaram et al., 2022a] [Manoharan et al., 2021]. This increase in conductivity is generally due to the short and long-lived active species, in which short-lived active species instantly increase the conductivity up to a certain value and long-lived active species increase the time span of conductivity before it comes to its original mark. Figure 4.13 (a) shows the conductivity of raw milk treated with non-thermal plasma at different times. The deactivation time of milk is around 45 minutes in all cases.

Table 4.4: Effect of cold plasma on different physicochemical properties of milk.

Treatment time	pH	Temperature (°C)	Fat/SNF (%)	Titratable acidity (g lactic acid/L)
Control	6.60±0.05	26.5±0.10	5.0/8.41±0.00	0.126±0.00
10 min	6.58±0.04	29.0±0.40	5.0/8.41±0.00	0.126±0.00
15 min	6.56±0.04	28.5±0.25	5.0/8.41±0.00	0.126±0.00
20 min	6.50±0.04	28.0±0.01	5.0/8.41±0.00	0.126±0.00

The pH value of treated milk remains the same, but conductivity changes over a period of time, which clearly indicates that in the cold plasma treatment of milk, there are an equal number of generations of acidic and basic species in the milk, and after some time (15-20 minutes) they

neutralize. This increase in conductivity for a short period of time plays an important role in the microbial load reduction.

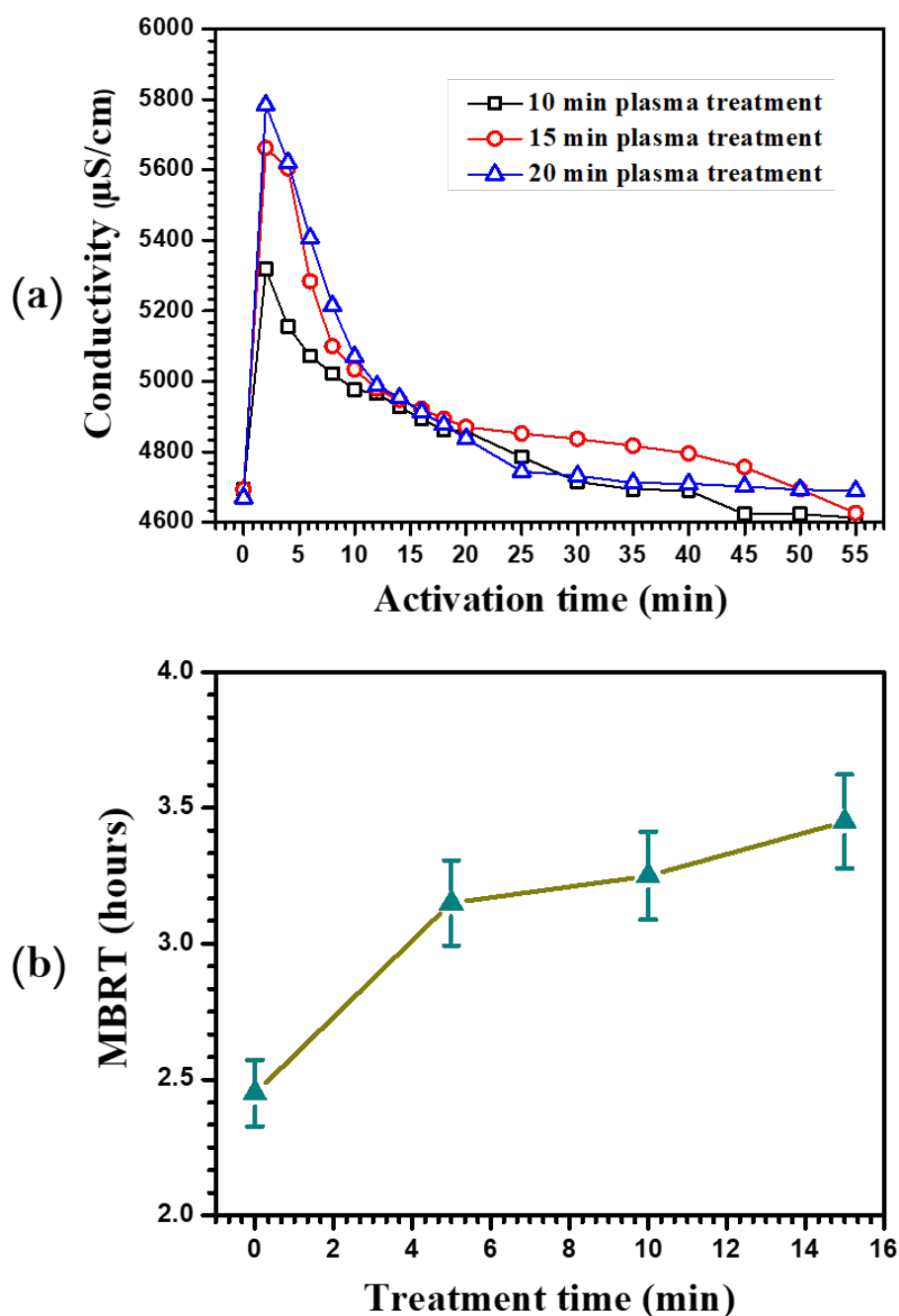


Figure 4.13: (a) MBRT analysis of raw milk from developed DBD-NTP source and (b) Raw milk conductivity analysis from developed DBD-NTP source.

Infectious diseases, such as salmonellosis and tuberculosis, resulting from the consumption of bacteria-laden contaminated milk, continue to pose significant health risks, particularly for children. Numerous studies have explored the effectiveness of non-thermal plasma technology in microbial inactivation. [Gadodia et al., 2024] [Wang et al., 2018], but to the best of our knowledge, no investigations are there on the MBRT analysis of milk treated with plasma. The MBRT holds significant relevance in the dairy industry, as it is the standard test performed during milk collection throughout India. Figure 4.13 (b) presents the MBRT analysis of raw milk treated with the developed DBD-based non-thermal plasma source. The untreated raw milk has an MBRT of around 2 hours and 40 minutes, which extends to 3 hours and 15

minutes following a 5-minute treatment with non-thermal plasma. No significant increment is seen between 5 minutes to 10 minutes. However, MBRT further improves to 3 hours 45 minutes when the treatment time is increased up to 15 minutes. So, a one-hour MBRT increase was achieved within just 15 minutes of operation of the cold plasma source, which is quite a significant improvement.

4.6 Conclusion

Two DBD-based mercury-free KrCl* (222 nm) and XeI* (253 nm) exciplex sources were designed, developed, and utilized for milk sterilization. From this study, we conclude that DBD-based exciplex UV sources, particularly 222 nm, can be used as a potential solution for milk sterilization to reduce bacterial load without significant or permanent alteration of the physicochemical characteristics of milk. The proposed method is environmentally friendly, efficient at low temperatures, and has minimal effect on the product matrix. The MBRT value of milk increased for more than 5 h, with just 2.5 min of 222 nm treatment. Approximately 2.58 log and 2.11 log decrease in the *E. coli* and *S. aureus* have been achieved after 2.5 minutes of far UV-C (222 nm) treatment of whole milk. The optimized UV dose utilized for the sterilization of whole milk has also been estimated using the chemical actinometry method and found to be 10.9 J/ml at a discharge power of ~ 24.5 W. This presents a method for the effective sterilization of milk at low temperatures while also minimizing power usage with a simple reactor design. The obtained results are significant at this level and show the utility of non-thermal exciplex UV sources to improve milk quality without altering its physicochemical properties.

Our study further presents compelling evidence supporting the potential use of cold plasma technology as an effective method for milk sterilization that has been demonstrated in additional work. This innovative approach is capable of significantly reducing bacterial load while preserving the physicochemical integrity of milk. One of the key advantages of cold plasma treatment is its environmentally friendly nature, operating efficiently at low temperatures and exerting minimal impact on the product matrix. While most of the physicochemical parameters remained largely unaffected by non-thermal plasma exposure, a notable increase in conductivity was observed. This change in conductivity was directly linked to the enhancement of bacterial reduction, as confirmed through the MBRT (Milk Bacillus Reduction Test). This finding underscores the effectiveness of cold plasma in microbial control without compromising the quality of the milk. Additionally, the study highlights the technique's low energy consumption and straightforward system design. Looking ahead, further research could focus on developing continuous treatment systems, exploring conjugate plasma technologies, and optimizing the MBRT process for broader industrial applications. The results of this study open up new avenues for utilizing cold plasma in the dairy industry.