Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Performance study of a sterilization box using a combination of heat and ultraviolet light irradiation for the prevention of COVID-19

Nilkamal Mahanta a, Varun Saxena b, Lalit M. Pandey b, Priyanka Batra c, U.S. Dixit a,⁎

a Department of Mechanical Engineering, Indian Institute of Technology Guwahati, India
b Bio-Interface and Environmental Engineering Laboratory, Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, India
c North East Center for Biological Sciences and Health Care Engineering (NECBH), Indian Institute of Technology Guwahati, India

ARTICLE INFO

Keywords:
SARS-CoV-2
COVID-19
Sterilization
UV radiation
Heat treatment
Bacteria

ABSTRACT

SARS-CoV-2 virus and other pathogenic microbes are transmitted to the environment through contacting surfaces, which need to be sterilized for the prevention of COVID-19 and related diseases. In this study, a prototype of a cost-effective sterilization box is developed to disinfect small items. The box utilizes ultra violet (UV) radiation with heat. For performance assessment, two studies were performed. First, IgG (glycoprotein, a model protein similar to that of spike glycoprotein of SARS-COV-2) was incubated under UV and heat sterilization. An incubation with UV at 70 °C for 15 min was found to be effective in unfolding and aggregation of the protein. At optimized condition, the hydrodynamic size of the protein increased to ~171 nm from ~5 nm of the native protein. Similarly, the OD280 values also increased from 0.17 to 0.78 indicating the exposure of more aromatic moieties and unfolding of the protein. The unfolding and aggregation of the protein were further confirmed by the intrinsic fluorescence measurement and FTIR studies, showing a 70% increase in the β-sheets and a 22% decrease in the α-helices of the protein. The designed box was effective in damaging the protein's native structure indicating the effective inactivation of the SARS-COV-2. Furthermore, the incubation at 70 °C for 15 min inside the chamber resulted in 100% antibacterial efficacy for the clinically relevant E.coli bacteria as well as for bacteria collected from daily use items. It is the first detailed performance study on the efficacy of using UV irradiation and heat together for disinfection from virus and bacteria.

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) caused a global pandemic resulting in disruption of normal life all over the world through a very infectious disease called coronavirus disease 2019 (COVID-19). The virus is being spread from aerosols and contacting surfaces (Pandey, 2020b). It is essential to use personal protective equipment (PPE) for checking the spread of the disease. In developing countries like India, the sanitization of PPE kits and other personal accessories is in high demand as the throwaway kits/accessories require a huge amount of money. Further, the disposal of PPE kits and other routine items to the environment poses a serious threat. Recent studies have confirmed the presence of SARS-CoV-2 in wastewater (Conde-Cid et al., 2021), which are prone to be transmitted in the soils (Conde-Cid et al., 2020; Núñez-Delgado, 2020) and further back to the ecosystem (Cela-Dablanca et al., 2021). Hence it is essential to sterilize the contacting surfaces before their disposal to the environment. The design of a cost-effective sanitization device for day-to-day life accessories appear to be one of the promising approaches.

The major stability of SARS-CoV-2 is due to the presence of the spike-glycoprotein, which is a three-domain protein with an isoelectric point (pI) of ~5.9 (Pandey, 2020b). The virus can be inactivated by altering the stability of this protein. Various physical (e.g., temperature and adsorption) and chemical (e.g., pH, disinfectants and surface modification) methods have been explored to test the stability of the spike protein (Narita et al., 2021; Pandey, 2020a, 2020b). It has been reported that SARS-CoV-2 remains stable on different surfaces but can be inactivated by heating at a temperature greater than 65 °C for more than 5 min of duration (Abraham et al., 2020). Sterilizers have been a topic of importance among researchers for the disinfection (Boudam and Moisan, 2010; Darmady et al., 1961; Hugo, 1991). Further, the COVID-19 pandemic has emphasized the need to design and develop a sterilization chamber equipped with an appropriate technique (Liao

⁎ Corresponding author. Department of Mechanical Engineering, Indian Institute of Technology Guwahati, Guwahati, 781039, Assam, India.
E-mail address: uday@iitg.ac.in (U.S. Dixit).

https://doi.org/10.1016/j.envres.2021.111309
Received 20 January 2021; Received in revised form 6 May 2021; Accepted 6 May 2021
Available online 10 May 2021
0013-9351/© 2021 Elsevier Inc. All rights reserved.
Liao et al. (2020) studied the influence of five different types of sterilization techniques on the filtration efficiency of face masks (Liao et al., 2020). The five studied techniques were as follows: (1) heat with humidity, (2) steam, (3) 75% alcohol, (4) household diluted chlorine-based solution and (5) ultraviolet germicidal irradiation. Ludwig-Begall et al. (2020) studied three techniques of decontaminating face masks: (1) ultraviolet germicidal irradiation, (2) vaporized hydrogen peroxide and (3) dry heat (Ludwig-Begall et al., 2020). Sarada et al. (2020) described the technologies of the International Advanced Research Center for Powder Metallurgy and New Materials (ARCI), India, in developing various disinfection systems to combat COVID-19 (Sarada et al., 2020). ARCI used physical, chemical and dry heat processes to disinfect surfaces effectively.

The duration and temperature required for sterilization of SARS-CoV-2 are not sufficient for inactivating the potentially harmful bacteria (e.g., *E. coli*), which are commonly present on the surfaces of objects (Bang et al., 2011; Bari et al., 2009; Choi et al., 2016). In this context, an effective ultraviolet (UV) type C based killing of bacteria have been reported (Gayán et al., 2012; Unluturk et al., 2008), which was also efficient for decontamination from SARS-CoV-2 (Cadnum et al., 2020; Maris et al., 2020). Heilingloh et al. (2020) carried out a comparative study between UV-A and UV-C irradiation for complete inactivation of SARS-CoV-2 and found that UV-C is more effective in inactivating viruses (Heilingloh et al., 2020). Complete inactivation of SARS-CoV-2 after 9 min of UV-C exposure was also mentioned (Heilingloh et al., 2020). Gayán et al. (2014) demonstrated the combined effect of UV and heat treatment in liquid foods viz., apple juice, orange juice and vegetable and chicken broth from *S. aureus* disinfection (Gayán et al., 2014). Cheon et al. (2015) studied the degree of deactivation of foodborne pathogens on powdered red pepper under the combined treatment of UV-C irradiation and mild heat (Cheon et al., 2015). It was concluded that the combination of UV radiation and mild heat treatment is more effective than UV radiation alone for deactivating *E. coli* O157:H7 and *S. typhimurium*. The existence of the synergistic effect of UV and heat treatment process was studied by Gouma et al. (2015) for microbial inactivation of apple juice (Gouma et al., 2015). To predict the effect of temperature on UV inactivation kinetics of microorganisms viz., *E. coli*, *Salmonella typhimurium*, *L. monocytogenes* and *S. aureus*, mathematical models were also developed (Gouma et al., 2015). Although these techniques are sufficient alone or in combination for the effective killing of bacteria, the synergistic effect on the microbiome over surfaces is still under scrutiny.

The disinfection capacity of UV radiation is limited to smooth surfaces only. Abdussamad et al. (2016) reported that the efficacy of UV based sterilization is affected by the surface properties of the items being sterilized (Abdussamad et al., 2016). UV radiation is more effective on fruits and vegetables with smooth surfaces, e.g., tomatoes and apples. On the other hand, pathogens in unexposed areas and pores can be killed by a heating process. Thus, it is necessary to design a cost-effective sterilization chamber with both UV radiation and heating facilities. UV radiation is more effective in killing the pathogens in the surfaces and heat is effective in sterilizing the unexposed areas and pores. The combination of UV radiation and heat can be used for disinfecting small items like mask, wallet, currency note, wristwatch and other routine items for their safe reuse or disposal to the environment.

It is clear from the aforementioned discussion that temperature based sanitization has been a promising candidate for various applications. Similarly, UV based sanitization has also been found to be very effective for various surfaces. However, a synergistic effect of UV and temperature is still under extensive research. In this study, a sterilization box was designed and tested for its effectiveness for denaturation of a glycoprotein and inactivation of bacteria. The glycoprotein Immunglobulin G (IgG) was used as a model material in lieu of SARS-CoV-2 (Fischer et al., 2020; Schmidt et al., 2020) (Salazar et al., 2020). The effects of heat and ultraviolet type C (UV–C), individually and in combination, were examined. The required temperature and duration without and with UV–C were optimized to destabilize the IgG and
disinfect bacterial strain. In order to analyze the actual effect of the optimized conditions on day-to-day life accessories, the real samples were also sanitized at the optimized conditions. The main focus of the present work was to provide an inexpensive alternative to sterilization boxes available in the market.

2. Materials and methods

A low cost UV and heat based sterilization box was fabricated. Lyophilized Immunoglobulin G (IgG) from bovine serum was procured from Sigma Aldrich (L5506). NaCl (GRM 853); NaOH (TC460) and HCl (AS003) were purchased from HiMedia India. KCl (104936), Na$_2$HPO$_4$ (137036) and KH$_2$PO$_4$ (104873) were procured from Merck India. Milli-Q water (resistivity: 18 MΩ cm at 25°C) was utilized throughout the work.

2.1. Low cost UV and heat based sterilization box

The sterilization box comprised a wooden box of size 58 cm × 25 cm × 19 cm, two 100 W incandescent bulbs, two 11 W UV-C lamps, one Digital Temperature Controller (DTC) and one timer. A small part of the wooden box was used as a compartment for electrical equipment and the other part was used as a sterilization chamber. Incandescent bulbs manufactured by Crompton were used for heating whereas UV-C lamps manufactured by Philips were used for radiation. Two-sided linear lamp holders (manufactured by EVERLITES in China) were used for holding the UV-C lamp in the wooden sterilization box. Aluminium lamp holders were used in the UV-C lamps because of their lightweight. Two switches were used for turning on/off the incandescent bulbs and UV-C lamps. Incandescent bulbs were connected with a DTC (manufactured by Robocraze) which measured the inside temperature of the sterilization chamber with the help of a thermocouple and showed it in the LED display. It also maintained the temperature inside the sterilization chamber. Once the temperature inside the box crossed the set value in DTC, it would automatically turn off the heating source i.e. incandescent bulb. DTC would turn on the incandescent bulb again, when the temperature lowered by 2°C with respect to the set value. A timer manufactured by Selec 800XA was also placed inside the chamber of electrical equipment. It was connected with the main supply to turn off after sterilizing an item for a pre-decided time. A limit switch controlled top cover was used to prevent the direct exposure of UV light to the human eye and skin. Incandescent bulbs were covered all around with a fencing net to safeguard against burning due to direct contact. To reduce the heat loss through the walls of the box, the inner surfaces were covered by a high reflecting galvanized iron (GI) sheet; the thin layer of air between the surfaces of the wooden box and GI sheet acted as a thermal insulator. Further, to increase the reflectivity of the GI sheet, it was covered through aluminium foil adhesive tape (of about 0.1 mm thickness) on the inner surfaces. Fig. 1 and Fig. 2 show the photograph and a schematic diagram of the box, respectively. Due to efficient design, it takes only about 150 s for an empty box to achieve a temperature of 70°C from an ambient temperature of 24°C.

Table 1 lists the items that were used in fabricating the sterilization box. A total amount of US $34.16 was spent on material for fabricating one unit of sterilization box. The labour cost for fabricating a sterilization box was estimated as $13.58. This was based on considering 8 man-hours for fabrication and assuming a monthly emolument of $339.38 for a skilled technician based on the average Indian scenario. Thus, the total fabrication cost was estimated as $47.74 per unit of sterilization box. In mass production and scale-up, the cost will further reduce. Online shopping portals provided a typical price of $176 for a UV-C based disinfection system of similar size. Compared to it, the proposed sterilization box is cost-effective. Moreover, it has both heat and UV-C radiation facilities.

Table 1

| S. No. | Item Description | Quantity | Unit Cost ($) | Total Cost ($) |
|--------|-----------------|----------|---------------|---------------|
| 1      | 11-W UV-C Lamp  | 2        | 3.12          | 6.24          |
| 2      | UV-C Lamp holder| 2        | 2.99          | 5.98          |
| 3      | 100-W Incandescent bulb | 2 | 0.20 | 0.4 |
| 4      | Incandescent bulb holder | 2 | 0.20 | 0.4 |
| 5      | Electrical switches | 2 | 0.14 | 0.28 |
| 6      | Digital Temperature Controller (DTC) | 1 | 2.72 | 2.72 |
| 7      | Timer           | 1        | 9.37          | 9.37          |
| 8      | Limit switch    | 1        | 0.41          | 0.41          |
| 9      | 8 mm thick plywood of surface area 6026.5 cm$^2$ | 1 | | 3.99 |
| 10     | 0.8 mm thick GI sheet of surface area 4073.94 cm$^2$ | 1 | | 2.33 |
| 11     | Miscellaneous   |          | 2.04          |               |
| Total  |                 |          | $34.16        |               |
PBS (1X) solution was prepared by mixing the appropriate amount of IgG (1 mg/mL) was prepared in phosphate buffer saline (PBS) at pH 7.4.

### 2.2. Preparation of protein solution

To analyze the effect of heat on the IgG protein, the stock solution of IgG (1 mg/mL) was prepared in phosphate buffer saline (PBS) at pH 7.4. PBS (1X) solution was prepared by mixing the appropriate amount of NaCl (8 g), KCl (0.2 g), Na₂HPO₄ (1.44 g) and KH₂PO₄ (0.24 g) in Milli-Q (800 mL) water, followed by mixing until a transparent and clear solution was obtained (Hasan et al., 2018). The final volume was made up to 1000 mL by adding Milli-Q water. The pH of the as-prepared PBS solution was maintained at 7.4 through 0.1 M of NaOH and HCl solution. The protein solution was then filtered using a 0.22 μm syringe filter. To estimate the final concentration of the stock solution, the absorbance values were measured using a UV spectrophotometer (Thermo Scientific; Evolution 201) at 280 nm. The stock concentration was calculated using Beer Lambert’s law with the extinction coefficient value of 210, 000 M⁻¹ cm⁻¹ (Reader et al., 2019). A final concentration of 0.1 mg/mL was prepared as a working solution for further usage.

### 2.3. Design of experimental conditions

The effect of temperature and the UV incubation with respect to time was analyzed on the glycoprotein (IgG) and the bacterial cells. First, the expected time and temperature ranges were determined by the Response Surface Methodology (RSM) using the Design-Expert software (columns 2 and 3 of Table 2). The temperature range was varied from 40 to 70 °C and the time ranged from 5 to 15 min based on the previous reports (Pandey, 2020a, 2020b). The software resulted in 13 different combinations of input variables (time and temperature). The hydrodynamic sizes obtained through dynamic light scattering (DLS), optical density at 280 nm (OD₂₈₀) and intrinsic fluorescence were considered as response variables, which reflect the unfolding of the protein (Sharma and Pandey, 2021; Sharma et al., 2020).

#### Table 2

| S. No | Input/independent variables | Time (min.) | Temperature (°C) |
|-------|-----------------------------|-------------|-----------------|
| 1     | 2.93                        | 55          |                 |
| 2     | 10                          | 55          |                 |
| 3     | 5                           | 40          |                 |
| 4     | 5                           | 70          |                 |
| 5     | 15                          | 70          |                 |
| 6     | 10                          | 76.21       |                 |
| 7     | 17.07                       | 55          |                 |
| 8     | 15                          | 40          |                 |
| 9     | 10                          | 33.79       |                 |
| 10    | 10                          | 55          |                 |
| 11    | 10                          | 55          |                 |
| 12    | 10                          | 55          |                 |
| 13    | 10                          | 55          |                 |

### 2.4. Analysis of the temperature and UV incubation on the stability of the protein

The protein samples were transferred to a 5 mL glass test tube, and the protein samples were incubated under heat and UV along with heat as listed in Table 2. After treatment, the samples were investigated via calculating the absorbance at 280 nm (Absorbance₂₈₀) wavelength for the protein estimation. Similarly, the hydrodynamic sizes of the treated protein under heat and combined (UV + heat) incubation were calculated through DLS analysis using Litesizer 500 (Anton Paar) at room temperature. Further, to analyze the protein unfolding, the intrinsic fluorescence of the protein was measured using a fluorimeter (Fluoromax 4). The native protein at room temperature without any incubation was considered as the control sample.

### 2.5. Effect of temperature and UV incubation over bacterial inactivation

Similar to that of protein, the effect of temperature and UV incubation was analyzed over the bacterial cells. For this purpose, two separate experiments were performed—(1) experiments on clinically relevant bacteria and (2) experiments on real samples. The methodology is described in the sequel.

#### 2.5.1. Effect of temperature and UV incubation over clinically relevant bacteria

To analyze the effect of temperature and UV incubation over bacterial cells, Escherichia coli (E.coli), a Gram-negative, MTCC 1610) was first incubated in autoclaved Luria Bertani (LB) Medium (GM1245; Himedia, India) for overnight. After incubation, the bacteria were subcultured for 6 h to obtain the log phase. The bacterial cells were centrifuged at 8000 revolutions per minute (RPM) for 5 min, and the pallets were incubated into sterile Milli-Q water at pH 7.4. The optical density at 600 nm (OD₆₀₀) of the culture was adjusted to 0.1 corresponding to an approximate concentration of 10⁷ CFU/mL (control cells). The bacterial cells were then treated at 70 °C, under UV-C incubation (~0.14 W/cm² at source) (1 mL bacterial suspension in 5 mL of a glass test tube) as well as under simultaneous heat and UV incubation up to 15 min and the OD₆₀₀ values were measured after incubation. The 4th dilution of the control and undiluted treated samples were spread over LB agar plate overnight to confirm the bacterial inactivation. In addition, the death rate (kₑ) of the E.coli was estimated under all the given incubations using:

\[ \text{OD}_{600t} = \text{OD}_{600t_0} e^{-k_e t} \]

where, kₑ values were calculated from the (OD₆₀₀t - OD₆₀₀t₀) versus time graph, where OD₆₀₀t₀ is the OD₆₀₀ value at time zero.

#### 2.5.2. Effect of temperature and UV incubation over real samples

To estimate the effect of temperature and UV incubation over real samples, the bacterial cells were isolated from the 1 × 1 cm² area of the leather belt, leather wallet, and metallic wristwatch using a sterile cotton swab and incubated into 1 mL of the autoclaved Milli-Q water. Next, these samples (leather belt, leather wallet, and metallic wristwatch) were treated at 70 °C under UV incubation for 15 min. After the treatment, again samples were isolated from the 1 × 1 cm² area using a sterile cotton swab and incubated into 1 mL of the autoclaved Milli-Q water. Similar to E.coli, the isolated bacteria from the given real samples before and after treatment were spread over the LB agar plate for overnight at 4th dilution and undiluted, respectively. The bacterial growth (colony) after the incubation was examined.

All the experiments were performed in triplicates. The results shown in the manuscript are the average values with their respective standard deviations.

### 3. Results and discussion

In this study, a cost-effective sterilization box is developed and the synergistic effect of UV and temperature over sanitization was examined. For this purpose, the effect of UV and heat sanitization was performed, and its effect on glycoprotein viz., IgG and bacterial cells were observed. The detailed results are described in the sequel.

#### 3.1. Effect of heat and UV treatment over IgG

The effects of temperature alone and UV incubation along with temperature were analyzed on IgG model protein. The hydrodynamic size of the native IgG was found to be ~5 nm, in agreement with the literature (Howe et al., 2011). The absorbance of native IgG was 0.172, which corresponded to 0.1 mg/mL of IgG using Beer Lambert’s law (Reader et al., 2019). The effects of varying temperature and combined UV and temperature with time were evaluated at the conditions suggested by Design-Expert software (Table 2). For this purpose, the
Absorbance values at 280 nm and hydrodynamic sizes were measured after treating the protein at different temperatures with and without UV as discussed in Section 2.3. The responses (hydrodynamic size and absorbance) at these conditions are shown in Fig. 3. In the figure, unfilled symbols pertain to heat only and filled symbol correspond to heat with UV-C.

Absorbance values at 280 nm were found to increase with the increase in temperature above 70°C. Protein solution incubated at 76.21°C exhibited the maximum absorbance value of 0.94 within 10 min followed by absorbance at 70°C. The increase in absorbance is related to the exposure of aromatic amino acids present in IgG because of the conformational changes (Sharma and Pandey, 2021). The denaturation temperatures of IgG is reported as 61 and 71°C (Vermeer and Norde, 2000). Moreover, the melting point, Tm of the IgG was found to be 69°C (Martin et al., 2014); hence, the observed increase in the absorbance values indicated the deformation in the IgG’s native conformation. However, the effect of time was found not to be significant below the temperature of 70°C.

Similar to the absorbance values at a higher temperature, the hydrodynamic sizes of the IgG protein was found to increase with the increase in temperature and time of exposure. The increase in the hydrodynamic size at a higher temperature indicated the unfolding (increase in the size) of IgG at elevated temperatures (Martin et al., 2014; Zhang and Topp, 2012). IgG incubated at 76.21°C under UV incubation resulted in the maximum hydrodynamic size of 309.02 nm (Fig. 3); at the same temperature without UV the hydrodynamic size was found to be lower i.e., 293.93 nm (Fig. 3). It indicates the significant role of UV incubation on the unfolding/denaturation of the IgG at elevated temperatures (Zhang and Topp, 2012). It has been observed that a 4 W UV-C lamp under 0.930–0.932 mW/cm² and 65°C dry heat for 20 min was sufficient to inactivate the entire swine coronavirus from N95 mask surfaces (Chotiprasitsakul et al., 2020). In the present study, two 11 W UV-C lamps resulted in the unfolding of IgG protein. The effect of temperature below a critical time was found not to be significant at the conditions selected in this study. Similarly, the effect of time up to 15 min was also ineffective below a critical temperature. Hence, model equations relating input variables with the responses as predicted by Design-Expert software were not significant. However, the experimental data were fitted to a sigmoidal expression as shown in Fig. 3A (dotted line). The data fitted very well with R² value of 0.99. The critical point (lag time) was estimated from the sigmoidal expression and was found to be 70 ± 1°C. This temperature also agreed to the denaturation temperatures of IgG (Vermeer et al., 1998). Therefore, a temperature of 70°C appears to be optimal for the unfolding of this model glycoprotein. It has been reported that unfolding/conformation changes of the surface glycoprotein of a virus leads to its disintegration.
and finally its inactivation (Hsu et al., 2011). The loss of viral surface protein due to external factors like a higher temperature and the strong surface-protein interactions disintegrates the virus assembly (Liu et al., 2015; Pandey, 2020b). It is also observed that UV-C light enhances the hydrodynamic size, but not drastically. Heat alone can perform well in the denaturation of IgG. (Fig. 3B Absorbance$_{280}$ of IgG at various conditions.)

To examine the combined effect of heat and UV treatments on the conformational changes of IgG, the intrinsic fluorescence values were recorded by exciting the protein at 290 nm and the emission values were scanned from 300 nm to 450 nm. The intrinsic fluorescence is produced due to the presence of aromatic amino acids (monomers) of the protein. The native IgG exhibited the maxima at 327 nm, whereas a peak-shift was observed at 332 nm and 331 nm at 76.21 °C for 10 min and 70 °C for 15 min, respectively (Fig. 4A). This indicates the unfolding and deformation in the native structure of the IgG at temperatures above a critical temperature of 70 °C (Arfat et al., 2014; Pandey, 2020b). Further, in the case of combined UV and heat exposure, the maximum fluorescence intensity was observed at 70 °C for 15 min (Fig. 4B). This indicates the synergistic effective role of UV in the unfolding and conformational changes in the IgG structure (Arfat et al., 2014). All these results suggested that the incubation of heat and UV together at 70 °C for 15 min unfolds the glycoprotein to a required extent and can be useful for the inactivation of viruses for sanitization purposes.

Further, the conformation changes of the combined heat and UV treated IgG at the above optimized condition (70 °C for 15 min) were analyzed as compared to the native IgG using Fourier Transform Infrared Spectroscopy (FTIR) analysis. The FTIR spectra of native and heat treated IgG are shown in Fig. 5, which were de-convoluted and the area of de-convoluted peaks have been used to deduce the contents of the secondary structure (Sharma et al., 2020). The contents of α-helix, β-sheet and β-turn in native IgG were found to be 32, 55 and 13%, respectively, which agreed with the reported data (Hasan et al., 2018). In the case of heat treated IgG, the contents of β-sheet increased to 70% and α-helix decreased to 22%. This complemented the DLS, absorbance and fluorescence data.

3.2. Effect of temperature and UV incubation over bacterial inactivation

The effect of temperature and UV incubation was scrutinized over clinically relevant Gram-negative bacteria, E.coli as discussed in Section 2.5.1. OD$_{600}$ values were found to decrease with an increase in time (Fig. 6). At 15 min of incubation, the OD$_{600}$ values followed the order of 0.09 ± 0.01 (UV only) > heat only at 70 °C (0.682 ± 0.007) > 0.077 ± 0.007 (combined UV and heat). The result indicated that UV incubation along with heat treatment is more effective in bacterial destruction than either heat or UV treatment. The death rate ($k_d$) of the E.coli samples were calculated from the exponential fitting of OD$_{600}$ versus time graph. The $k_d$ values were found to be 0.008, 0.014 and 0.019 min$^{-1}$ for UV-C, heat and simultaneous heat and UV-C incubation treatments, respectively. The highest death rate during the simultaneous heat and UV-C incubation reflected the effectiveness of the designed chamber for bacterial sanitizing purposes.

The incubation time of 15 min is not adequate to observe the actual decrease in cell numbers through the measurement of optical density. Thus, in order to confirm the bacterial destruction, the treated and non-treated E.coli cells were spread over LB agar plate to determine the live and dead cells. Untreated bacterial cells were used as control samples as discussed in Section 2.5.1. As expected, the E.coli control samples reflected colonies of live cells and concentration was calculated to be 10$^7$.
CFU/mL (10⁻⁴ dilutions), which agrees with literature (Shivaprasad et al., 2021; Singh et al., 2021). As compared to the control samples, a significant decrease in the bacterial colonies was observed after the incubation as depicted in Fig. 7. No colony could be seen in the treated sample spread over the agar plate without any dilution. This indicates the efficacy of the heat and UV treatment for the complete and effective disinfection of bacterial pathogens.

Heat treatment is the most basic method for reducing the populations of pathogenic microorganisms. It has been reported that the heat treatment alone at 60 °C did not show a required sterilization effect, but was found to be effective at simultaneous UV-C and heat treatment of Salmonella and Escherichia species (Park et al., 2019). Moreover, a single treatment with either heat or UV radiation may not kill or inactivate the pathogenic microorganisms, but can injure the cells. However, these injured cells may exhibit a revival and hence, can be quite harmful (Back et al., 2012). This effect stems from the fact that heat increases the fluidity of the cell membrane making the affected cells more sensitive to UV exposure (Gayán et al., 2013; Zhang et al., 2015). Thus, it can be

Fig. 7. The number of E.coli bacteria colonies in case of (A) control (OD₆₀₀ = 0.1) at spread at 10⁻⁴ dilutions, and (B) treated at 70 °C under UV-C incubation for 15 min spread at 10⁰ dilution (undiluted). No colonies are observed after the treatment as compared to the control cells.

Fig. 8. Bacterial inactivation at UV and heat treatment. The samples were taken from (A) belt, (B) watch and (C) wallet and at spread at 10⁻⁴ dilutions. (D–F) are treated samples spread at 10⁰ dilution (undiluted). No colonies as compared to control samples were observed after treatment at 70 °C under UV-C incubation for 15 min.
concluded that the combination of both UV-C and heat treatments is much more effective than the individual use of either of these. It has been reported that UV-C light was found to be effective for decontamination up to a distance of 1 m (González, 2021). In another study, Candida auris were effectively killed by the UV-C light in 30 min up to a distance of 2 m (de Groot et al., 2019). In this study, the dimensions of the designed box are 0.17 × 0.43 × 0.20 m (H × L × W), which are adequate for the UV-C sterilization. To further analyze the synergistic effect of UV-C and heat over real samples, the bacterial cells were collected from various sources as given in Section 2.5.2. Samples collected from given surfaces reflected > 10^7 colonies from belt, watch and wallet surfaces at 10^-2 dilutions as shown in Fig. 8. Interesting results are obtained after the treatment. After treatment, no colonies were observed in all the samples without any dilution as shown in Fig. 8 (D–F). This reflects the effective sterilization of real samples in the designed chamber. These results suggest that UV-C irradiation combined with mild heating can be utilized for sterilization of personal accessories. Hence, combined UV-C and heat treatment can be utilized for sanitizing the bacteria and viruses from various day-to-day surfaces.

4. Conclusions

Considering the crisis created by COVID-19 pandemic, a sterilization box (prototype) was designed and fabricated for disinfecting the items of daily use such as face masks, wallets, belt and wristwatch. The box comprised two 100 W incandescent bulbs and two 11 W UV-C lamps daily use such as face masks, wallets, belt and wristwatch. The box chamber for sanitizing the items from bacteria comprised two 100 W incandescent bulbs and two 11 W UV-C lamps designed chamber. These results suggest that UV-C irradiation combined with mild heating can be utilized for sterilization of personal accessories. Hence, combined UV-C and heat treatment can be utilized for sanitizing the bacteria and viruses from various day-to-day surfaces.

Author contribution

Nilkamal Mahanta: Investigation; Methodology; Roles/Writing – original draft. Varun Saxena: Investigation; Writing – review & editing. Lalit M. Pandey: Conceptualization; Formal analysis; Resources; Software; Supervision; Roles/Writing – original draft; Writing – review & editing. Priyanka Batra: Investigation; Writing – review & editing. U.S. Dixit: Conceptualization; Formal analysis; Resources; Software; Supervision; Roles/Writing – original draft; Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Abdussamad, T.R., Rasoo, B.A., Sahlini, S.S., 2016. Ultraviolet-C light sanitization of English cucumber (Cucumis sativus) packaged in polyethylene film. J. Food Sci. 81 (6), E1419–E1430.
Abraham, J.P., Poularde, B.D., Cheng, L., 2020. Using heat to kill SARS-CoV-2. Rev. Med. Virol. 30 (5), e2115.
Afroz, M.Y., Ashraf, J.M., Arif, Z., Alam, K., 2014. Fine characterization of glucosylated human IgG by biochemical and biophysical methods. Int. J. Biomed. Macromol. 69, 408–415.
Back, K.H., Kim, S.-O., Park, K.-H., Chung, M.-S., Kang, D.-H., 2012. Spray method for recovery of heat-injured Salmonella Typhiurium and Listeria monocytogenes. J. Food Protect. 75 (10), 1867–1872.
Bang, J., Kim, H., Kim, H., Beuchat, L.R., Ryu, J.-H., 2011. Combined effects of chlorine dioxide, drying, and dry heat treatments in inactivating microorganisms on radish and cucumber. Food Microbiol. 28 (1), 114–118.
Bari, M.I., Nei, D., Enomoto, K., Todoroki, S., Kawamoto, S., 2009. Combination treatments for killing Escherichia coli O157:H7 on alfalfa, radish, broccoli, and mung bean seeds. J. Food Protect. 72 (3), 631–636.
Boudam, M., Moisan, M., 2010. Synergy effect of heat and UV photons on bacterial-spore inactivation in an N2–O2 plasma-afterglow sterilizer. J. Phys. Appl. Phys. 43 (29), 295202.
Calderón, J.L., Li, D.F., Jones, L.D., Redmond, S.N., Pearlmuter, B., Wilson, B.M., Donuky, C.J., 2020. Evaluation of ultraviolet-C Light for rapid decontamination of airport security bins in the era of SARS-CoV-2. Pathogens microinf 5 (1), 133.
Cela-Iribarren, R., Santas-Miguez, V., Fernández-Calvino, D., Arias-Estévez, M., Fernández-Sanjurjo, M.I., Álvarez-Rodríguez, E., Núñez-Delgado, A., 2021. SARS-CoV-2 and other main pathogenic microorganisms in the environment: situation in Galicia and Spain. Environ. Res. 197, 111049.
Cheon, H.-L., Shin, J.-Y., Park, K.-H., Chung, M.-S., Kang, D.-H., 2011. Inactivation of foodborne pathogens in powdered red pepper (Capsicum annuum L.) using combined UV-C irradiation and mild heat treatment. Food Contr. 50, 441–445.
Choi, S., Beuchat, L.R., Kim, H., Ryu, J.-H., 2016. Viability of sprout seeds as affected by treatment with aqueous chlorine dioxide and dry heat, and reduction of Escherichia coli O157:H7 and Salmonella enterica on pak choi seeds by sequential treatment with chlorine dioxide, drying, and dry heat. Food Microbiol. 54, 127–132.
Chotpratitsakul, D., Kittiyakara, T., Jongkaewwattana, A., Santaninand, P., Jiaraiakulwanchan, A., Prahsarn, C., Mommatrapoj, N., Wadwongsri, P., Jeeradum, N., Watcharanan, S., 2020. Approach of Using a Household Device in Decontaminating Respirators with Ultraviolet C during the Scarcity in the COVID-19 Pandemic. Research Square.
Conde-Cid, M., Arias-Estévez, M., Núñez-Delgado, A., 2020. How to study SARS-CoV-2 in soils? Environ. Res. 110464.
Conde-Cid, M., Arias-Estévez, M., Núñez-Delgado, A., 2021. SARS-CoV-2 and other pathogens could be determined in liquid samples from soils. Environ. Pollut. 273, 116454.
Darmady, E., Hughes, K., Jones, J., Prince, D., Tuke, W., 1961. Sterilization by dry heat. J. Appl. Bacteriol. 71 (1), 9–18.
Decontaminating Respirators with Ultraviolet C during the Scarcity in the COVID-19 Pandemic. Research Square.
González, C.M., 2021. Cleaning with UV light. Mech. Eng. 143 (1), 32–33.
Gouma, M., Alvarez, I., Condón, S., Gayán, E., Technologies, E., 2015. Modelling microbial inactivation kinetics of combined UV-H treatments in apple juice. Innovative Food Science 27, 111–120.
Hasan, A., Waihaw, G., Pandey, L.M., 2018. Conformational and organizational insights into serum proteins during competitive adsorption on self-assembled monolayers. Langmuir 34 (28), 8178–8194.
Haw, A., Huile, W.L., Jickoot, W., Forbes, R.T., 2011. Taylor dispersion analysis compared to dynamic light scattering for the size analysis of therapeutic peptides and proteins and their aggregates. Pharmaceut. Res. 28 (9), 2302–2310.
Heiligers, C., Auferhorst, U.W., Shipp, L., Dittmer, U., Witzke, O., Yang, D., Deng, X., Sutter, K., Trilling, M., 2012. Inactivation of Salmonella enterica by UV-C light at mild temperatures. Appl. Environ. Microbiol. 78 (23), 8353–8361.
González, C.M., 2021. Cleaning with UV light. Mech. Eng. 143 (1), 32–33.
Gouma, M., Alvarez, I., Condón, S., Gayán, E., Technologies, E., 2015. Modelling microbial inactivation kinetics of combined UV-H treatments in apple juice. Innovative Food Science 27, 111–120.
Ludwig-Begall, L.F., Wielick, C., Dams, L., Nauwynck, H., Demeuldre, P.-F., Napp, A., Lapierre, J., Haubrege, E., Thiry, E., 2020. The use of germicidal ultraviolet light, vaporized hydrogen peroxide and dry heat to decontaminate face masks and filtering respirators contaminated with a SARS-CoV-2 surrogate virus. J. Hosp. Infect. 106 (3), 577–584.

Maris, A., Jacobs, J., Van Horn, G., Stratton, C., Schmitz, J., 2020. Microbiologic proof-of-concept: a novel device combining UV light and ozone for human skin antisepsis. Am. J. Clin. Pathol. 154 (Suppl. ment_1), S132–S133.

Martin, N., Ma, D., Herbet, A., Boquet, D., Winnik, F.o.M., Tribet, C., 2014. Prevention of thermally induced aggregation of IgG antibodies by noncovalent interaction with poly (acrylate) derivatives. Biomacromolecules 15 (8), 2952–2962.

Narita, F., Wang, Z., Kurita, H., Li, Z., Shi, Y., Jia, Y., Soutis, C., 2021. A review of piezoelectric and magnetostrictive biosensor materials for detection of COVID-19 and other viruses. Adv. Mater. 33 (1), 2005448.

Núñez-Delgado, A., 2020. SARS-CoV-2 in soils. Environ. Res. 190, 110045.

Pandey, L.M., 2020a. Design of engineered surfaces for prospective detection of SARS-CoV-2 using quartz crystal microbalance-based techniques. Expet Rev. Proteonomics 17 (6), 425–432.

Pandey, L.M., 2020b. Surface engineering of personal protective equipments (PPEs) to prevent the contagious infections of SARS-CoV-2. Surf. Eng. 36 (9), 901–907.

Park, M.-J., Kim, J.-H., Oh, S.-W., 2019. Inactivation effect of UV-C and mild heat treatment against Salmonella Typhimurium and Escherichia coli O157: H7 on black pepper powder. Food Sci Biotechnol 28 (2), 599–607.

Reader, P.P., Olkhov, R.V., Reeksting, S., Lubben, A., Hyde, C.J., Shaw, A.M., 2019. A rapid and quantitative technique for assessing IgG monomeric purity, calibrated with the NISTmAb reference material. Anal. Bioanal. Chem. 411 (24), 6487–6496.

Rubio-Romero, J.C., del Carmen Pardo-Ferreira, M., Torrecilla-García, J.A., Calero-Castro, S., 2020. Disposable masks: disinfection and sterilization for reuse, and non-certified manufacturing, in the face of shortages during the COVID-19 pandemic. Saf. Sci. 129, 104830.

Salazar, E., Kuchipudi, S.V., Christensen, P.A., Eagar, T., Yi, X., Zhao, P., Jin, Z., Long, S.W., Olsen, R.J., Chen, J., 2020. Convalescent plasma anti-SARS-CoV-2 spike protein ectodomain and receptor-binding domain IgG correlate with virus neutralization. J. Clin. Invest. 130 (12), 6728–6738.

Sarada, R., Vijay, R., Johnson, B., Rao, T.N., Padmanabham, G., 2020. Fight against COVID-19: ARCI’s technologies for disinfection. Transactions of the Indian National Academy of Engineering 5 (2), 349–354.

Schmidt, S.B., Grüter, L., Boltzmann, M., Rollnik, J.D., 2020. Prevalence of serum IgG antibodies against SARS-CoV-2 among clinic staff. PloS One 15 (6), e0235417.

Sharma, L.G., Pandey, L.M., 2021. Thermomechanical process induces unfolding and fibrillation of bovine serum albumin. Food Hydrocolloids 112, 106294.

Sivakumar, S., Naveen, R., Dhabliya, D., Shankar, B.M., Rajesh, B.N., 2021. Electronic currency note sterilizer machine. Mater. Today: Proceedings 37, 1442–1444.

Unluturk, S., Atılgan, M.R., Bayyal, A.H., Tarı, C., 2008. Use of UV-C radiation as a non-thermal process for liquid egg products (LEP). J. Food Eng. 85 (4), 561–568.

Vermeer, A.W.P., Bremer, M.G.E.G., Norde, W., 1998. Structural changes of IgG induced by heat treatment and by adsorption onto a hydrophobic Teflon surface studied by circular dichroism spectroscopy. Biochim. Biophys. Acta Gen. Subj. 1425 (1), 1–12.

Vermeer, A.W.P., Norde, W., 2000. The thermal stability of Immunoglobulin: unfolding and aggregation of a multi-domain protein. Biophys. J. 78 (1), 394–404.

Zhang, J., Topp, E.M., 2012. Protein G, protein A and protein A-derived peptides inhibit the agitation induced aggregation of IgG. Mol. Pharm. 9 (3), 622–628.

Zhang, S., Ye, C., Lin, H., Lv, L., Yu, X., 2015. UV disinfection induces a VBNC state in Escherichia coli and Pseudomonas aeruginosa. Environ. Sci. Technol. 49 (3), 1721–1728.