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Inactivation of coronaviruses under irradiation by UVA-range light-emitting diodes

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Abstract. **We report the results of the development of an experimental stand based on UVA light-emitting diodes (UVA LEDs) with radiation wavelengths of 385 and 395 nm for studying experimentally the inactivation of viruses of the coronavirus family, including SARS-CoV-2. Methodological grounds are presented for determining the inactivation dose that provides a predetermined decrease in the virus titre under the impact of UVA radiation. The effect of the diode radiation divergence on the virus photoinactivation process is investigated. It is shown that UVA LEDs can be used to reduce the virus titre by 4 orders of magnitude.**

Keywords: *ultraviolet radiation, LED, coronaviruses, bovine coronavirus.*

1. Introduction

The COVID-19 pandemic, which continues for the second year, caused by the SARS-CoV-2 coronavirus, has intensified the search for effective mechanisms for inactivation and eradication of SARS-CoV-2 and its equivalent pathogens in order to develop new treatment technologies, as well as effective means of preventing the spread of infections in the environment [1–5].

The SARS-CoV-2 virus is highly contagious; its transmission occurs by airborne droplets through water and surfaces, which contributes to its ubiquitous spread. Van Doremalen et al. [6] presented the results of studying the stability of SARS-CoV-2 in aerosols and on nonliving surfaces (for example, glass, metal, plastic, and cardboard), which can act as transmission vectors. They showed that the virus can remain viable and infectious for up to several hours in aerosols and up to several days on the surface. This is consistent with the results of studies of resistance on nonliving surfaces of a wide group of human coronaviruses – severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS) and endemic human coronaviruses (HCoV) [7]; viruses can remain infectious from 2 hours to 9 days. The incubation temperature is crucial, as some viruses remain viable at a temperature of 4 °C for up to 28 days, while at 30–40°C the viability of viruses decreases.

The task of developing technologies for the safe treatment of contact surfaces for humans in order to reduce the number of pathogenic microorganisms and reduce the risks of human-to-human virus transmission becomes urgent.

In the last decade, the effectiveness of inactivation of a number of pathogenic agents under the impact of UV and visible radiation in various wavelength ranges from 220 to 480 nm has been demonstrated [3, 4, 8–17]. Radiation in the visible spectrum can initiate photodynamic inactivation of microorganisms. Individual monochromatic peaks can cause excitation of photosensitising molecules, in particular porphyrins. Porphyrins photosensitise the oxidation of organic compounds with oxygen, which, reacting with intracellular components, damages DNA and the plasma membrane [18]. Search for and studies of photocatalysts capable of generating active radicals (hydroxide ion and reactive oxygen species) are underway. For example, Tuchina et al. [4] considered gypsum-titanium nanocomposites as photocatalysts, which can be used as coatings and enhance the effect of exposure to ultraviolet (UV) radiation.

Ultraviolet irradiation is an environmentally friendly method for destroying viruses, bacteria and fungi without the use of harmful chemicals or heat. According to ISO-DIS-21348, UV radiation is divided into three ranges: UVA (315–400 nm), UVB (280–315 nm), and UVC (100–280 nm).

Exposure to UVC radiation is an effective method for inactivating viruses and bacteria, since this radiation is well absorbed by RNA and DNA molecules [9, 12]. UVC radiation is effectively used to sterilise rooms and water, but only in the absence of a man (because of its harmful effects on health).

The most harmless to humans is UVA radiation. The nature and mechanisms of the effect of this radiation on pathogens have not been fully studied. UVA radiation is poorly absorbed by DNA and RNA and is much less effective than UVC in inducing pyrimidine dimers, but it can cause additional genetic damage, for example, due to the formation of reactive oxygen species, which result in base oxidation and strand breaks [19].

The sources of radiation in the UVC, UVB and UVA ranges are UV lamps and LEDs, as well as sunlight. Despite

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the fact that sunlight is the most affordable and environmentally friendly source of UV radiation, it is impossible to talk about the stability and reproducibility of the result of its exposure for bactericidal purposes, since UV radiation is attenuated by air masses, clouds, and atmospheric pollution. In addition, the intensity of solar radiation strongly depends on the time of year and varies throughout the day.

Currently, quartz and bactericidal lamps are used to fight infections, the action of which is based on a plasma discharge in mercury vapour. The quartz lamp spectrum contains radiation with a wavelength of 254 nm, which destroys the RNA/DNA of viruses and bacteria, but it is also extremely harmful to humans. In addition, the lamp spectrum contains radiation with a wavelength of 185 nm, under the impact of which oxygen from the air is converted into ozone, which negatively affects the skin and lungs of a person. In this regard, the World Health Organization does not recommend the use of quartz lamps to combat the SARS-CoV-2 coronavirus indoors in the presence of a person.

For disinfection, bactericidal lamps are currently used, the bulb of which is made of special glass that transmits radiation with a wavelength of 254 nm and blocks radiation with a wavelength of 185 nm. As a rule, disinfection is performed in a room where there are no people at the moment. In rooms with people, it is possible to use quartz lamps if the process of disinfecting the premises is based on the circulation of air flows through a housing closed for the free output of the lamp radiation.

A promising direction is the use of laser diodes and LEDs for UV disinfection. Due to their small size, it is possible to focus the radiation more precisely on the object to be disinfected. It is possible to develop a radiation source with the required wavelength and the radiation power varied over a wide range. The use of LEDs with high mechanical strength and small size will make it possible to obtain a compact disinfection device that will have fewer restrictions in comparison with the currently used installations based on bactericidal lamps, while the use of UVA LEDs will allow one, without damage to health, to install disinfectant in rooms where people are constantly present.

Below are the results of work on the development of an experimental stand based on UVA LEDs with wavelengths of 385 and 395 nm for conducting experimental studies on the inactivation of viruses of the coronavirus family, including SARS-CoV-2, as well as methodological grounds for determining the dose of inactivation that provides a given decrease in the titre of a virus exposed to UVA radiation.

2. Experimental

2.1. Setup based on UVA LEDs

In order to perform experiments on the determination of the virus inactivation dose as a function of wavelength, we designed an experimental stand based on UVA LEDs. We used commercially available LEDs with wavelengths of 385 and 395 nm and a divergence of 70 deg. The emitting aperture of UVA LEDs was 3.5×3.5 mm. The external view of the LED chip with a lens is shown in Fig. 1. To ensure efficient heat dissipation (up to 3 W of thermal energy), LED chips were soldered onto a star-type board, which, in turn, was installed on an aluminium heatsink.

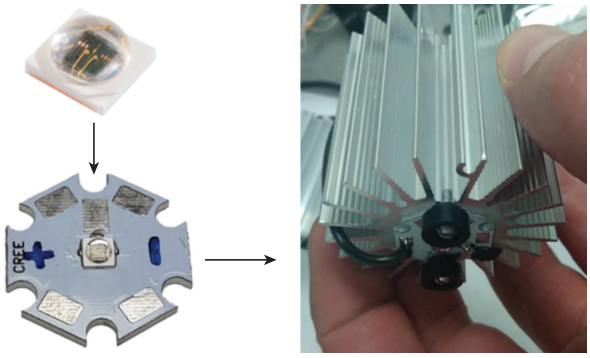

Figure 1. (Colour online) LED chip with a lens, mounted on a star-type board and placed on a heatsink.

To power the LEDs, DC sources were manufactured, individual for each LED, with their own characteristics. The LEDs were fixed in a tripod for subsequent irradiation of biological samples. Figure 2 shows a LED with a heatsink mounted on a tripod. The mounting scheme made it possible to adjust the distance from the LED to the irradiated surface of the object.

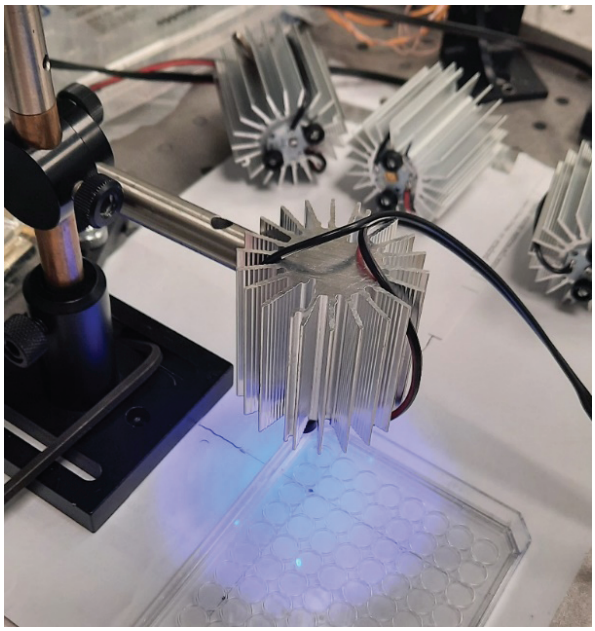

Figure 2. (Colour online) LED with a heatsink mounted on a tripod.

The emission spectra of LEDs were measured using an Avesta ASP-150 spectrometer (Fig. 3). The radiation power of UVA LEDs was measured by a portable THORLABS power meter with a PM160T measuring head. A plate with samples of the viral culture was placed directly under the LED. The distance between the LED and the culture medium surface with the virus was 44 mm. The radiation intensity of the LEDs on the well surface in the case of irradiation of four wells was 65.3 mW cm^{-2} for the UVA-385 emitter and 72.6 mW cm^{-2} for the UVA-395 emitter. When irradiating nine wells, the radiation intensity decreases according to the distribution shown in Fig. 4b.

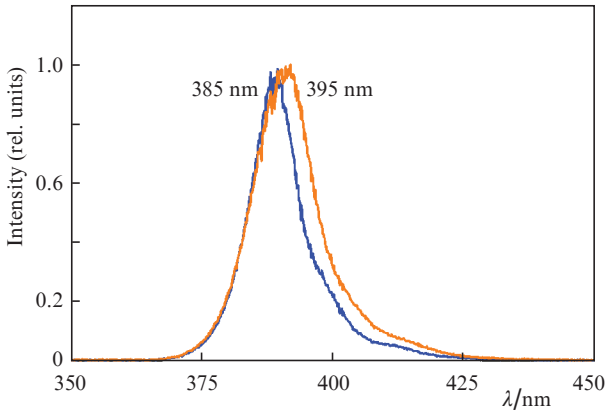

Figure 3. (Colour online) Emission spectra of LEDs.

2.2. Experiments on irradiation of virus-containing fluid

Studies on viral-cellular systems were conducted using proven protocols based on the reference centre for coronavirus infection at Gamaleya National Centre of Epidemiology and Microbiology. We used bovine coronavirus as a model environment for the study of the SARS-CoV-2 coronavirus [20, 21].

The virus-containing fluid (VCF) was a DMEM culture medium with bovine coronavirus in a titre of 10^5 $TCID_{50}$/mL [5]. The VCF was placed in the plate wells (a sterile flat-bottom plate with 96 cylindrical wells). The same amount of the VCF was poured into each irradiated well, which ensured the same thickness of the irradiated layer. The plate with filled wells was placed on a blackened surface below the radiation source. The irradiation dose was determined as the product of the radiation intensity measured on the VCF surface in the well and the exposure time. The experimental scheme was similar to that used in [5].

The wells of the plate with the VCF were labelled. Several wells with the VCF were irradiated simultaneously (four or nine on a 96-well plate), as shown in Fig. 4.

As a control item, we used the original unirradiated material (VCF), which was kept in the environment without irradiation for a certain time equal to the irradiation time of the samples for each source and the exposure time. The system for evaluating the antiviral effect consisted in obtaining a quantitative estimate of the suppression of virus reproduction determined on the cell line. As a criterion for the antiviral effectiveness of UV radiation, the difference between the virus titres in the control (without irradiation) and irradiated groups, expressed as $\Delta\lg TCID_{50}$/mL, is presented. In virologic testing, the antiviral effect of a drug with $\Delta\lg TCID_{50}/mL \geqslant 2.0$ is considered as satisfactory [22].

The antiviral effect of UV radiation was evaluated *in vitro* as follows. A decrease in the level of virus accumulation under the radiation exposure ($\Delta\lg TCID_{50}$/mL) can be represented by the value A determined by the formula $A = A_{cult} - A_{ir}$, where A_{cult} is the level of virus accumulation during cultivation without irradiation ($\Delta\lg TCID_{50}$/mL), and A_{ir} is the level of virus accumulation during cultivation with irradiation ($\Delta\lg TCID_{50}$/mL). In fact, the virus sensitivity to UV radiation was defined as the effect of reducing the virus titre by two orders of magnitude or more.

It is important to note that, according to the experimental conditions, the VCF from all 4 or 9 wells was mixed after irradiation for further processing. From the data obtained, it follows that a decrease in the virus titre is observed when irradiating 9 wells by radiation with wavelengths of 385 and 395 nm at low doses, and with a further increase in the radiation dose, a step is observed in the virus titre value and no decline occurs. A decrease in the virus titre by three orders of magnitude for the experiment with simultaneous irradiation of 9 wells was achieved at a dose of ~80 J cm^{-2}, while for the experiment with irradiation of 4 wells, at a dose of 40 J cm^{-2} (Fig. 5).

This difference is due to the diode radiation divergence up to 70 deg. In the case of irradiation of 9 wells, the wells located at the periphery receive a noticeably lower dose, which, when averaged over all wells, leads to an overestimation of the required radiation dose for a given decrease in the virus titre. This indicates that at small angles of divergence of LED radiation, the radiation intensity at the periphery is noticeably reduced and the required dose of virus inactivation is not achieved within the specified irradiation time. At the same time, when irradiating 4 wells, it is obvious that the radiation intensity distribution over the sample surface is more uniform. Consequently, the required degree of the virus titre reduction is observed at lower doses. In order to avoid errors

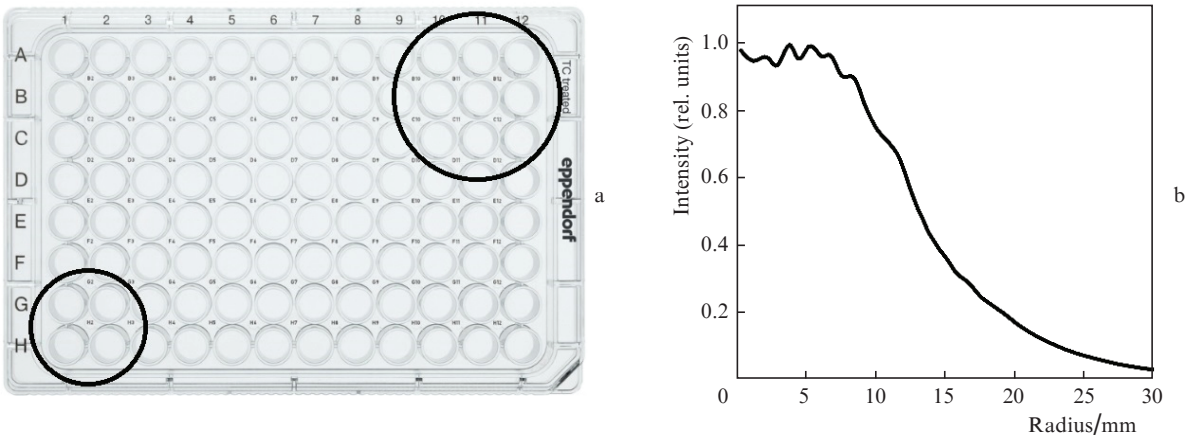

Figure 4. (Colour online) (a) VCF layout in the plate (circles indicate the irradiation areas when 4 and 9 wells are illuminated) and (b) radiation intensity distribution inside the irradiated areas. The irradiation area radius of 4 and 9 wells is 10 and 16 mm, respectively.

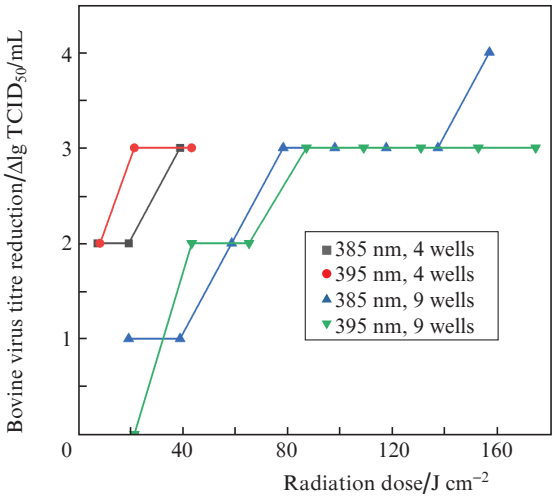

Figure 5. (Colour online) Dependence of the decrease in the titre of bovine coronavirus on the radiation dose for fibres with wavelengths of 385 and 395 nm.

in estimating the inactivation dose that provides the effect of a given decrease in the virus titre, it is required to use either a limited irradiation field in which the radiation intensity is sufficiently uniform, or several LEDs, carefully checking the radiation distribution uniformity over the surface of the samples under study.

To assess the human safety dose levels, we relied on the "Sanitary and Epidemiological Requirements for Physical Factors at Workplaces" [23]. Thus, the permissible irradiation intensity of workers in conditions that the unprotected areas of the skin surface are no more than 0.2 m^2, the irradiation period is no more than 5 min, the duration of pauses between irradiations is at least 30 min, and the total duration of exposure per shift is no more than 60 min should not exceed: for the UVA range, 50.0 W m^{-2}; for UVB, 0.05 W m^{-2}; and for UVC, 0.001 W m^{-2}. Accordingly, the permissible dose load for irradiation for 5 minutes is 15000 J m^{-2} UVA, 15 J m^{-2} UVB, and 0.3 J m^{-2} UVC. At the same time, the permissible intensity of UV irradiation of workers in conditions that the unprotected areas of the skin surface are no more than 0.2 m^2 (face, neck, hands, etc.), the total duration of exposure to radiation amounts to 50% of a working shift, and the duration of a single irradiation is over 5 min should not exceed 10.0 W m^{-2} UVA and 0.01 W m^{-2} UVB, while radiation in the UVC region at the specified duration is not allowed.

Taking into account the data on safe doses and the results obtained during inactivation, it can be deduced that LEDs with wavelengths of 385 and 395 nm in the UVA range are promising for practical use.

3. Conclusions

In accordance with the Recommendations of the Pharmacological Committee of the Russian Federation, with a decrease in the infectious virus titre that exceeds 2.0 lg $TCID_{50}$/mL, the method in question can be used as a remedy against viruses. Thus, UVA radiation at wavelengths of 385 and 395 nm is an effective antiviral agent.

To avoid errors in determining the virus inactivation dose, it is necessary to carefully monitor the uniformity of the distribution of the UV radiation intensity on the plate surface where the virus culture samples are installed.

It is shown that the radiation of LEDs with wavelengths of 385 and 395 nm in the UVA range can be used for effective inactivation of viruses while meeting human safety requirements. In the experiments carried out, the maximum values of reducing the virus titre – by 4 orders of magnitude at an irradiation dose of 157 J cm^{-2} – were achieved for a UVA LED at a wavelength of 385 nm.

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