Efficacy of UV and UV-LEDs irradiation models for microbial inactivation applicable to automated sterile drug compounding

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Conflict of interest

None to declare

Abstract

LEDs development has attracted attention over conventional mercury lamps for the tiny size, high efficiency, long lifetime, low operating temperature. The antimicrobial effectiveness of traditional UV-lamps radiation (wavelength of 254 nm) compared to UV-C LEDs (LED1 wavelength range 275-286 nm and LED2 range 260-270 nm) was carried out, for possible applications to automated sterile drug compounding. The UV lamp and the tested UV-LED devices remarkably reduced microbial load, following a time-dose response, but the best performance was evidenced by LED1, which guaranteed the complete inactivation of high concentrations of bacteria, yeasts, and spores at doses between 200 and 2000 J/m^2.

Keywords: Microbial inactivation, Ultraviolet radiation, UV-LED, Aseptic compounding, Sanitization

INTRODUCTION

In hospital settings, a consistent portion of the prescribed medications is administered parenterally in a patient, as individual or standard doses, to ensure bioavailability and rapid delivery of the maximally tolerated dose. Generally, the aseptic preparation of the parenteral dosage forms takes place in a controlled environment under the responsibility of the pharmacists. Aseptically compounded products must fulfill the well-established pharmacopoeia standards in terms of content, sterility, purity, and bacterial endotoxins contamination [1-3]. Concerning the relevant guidelines for the aseptic preparation of parenteral drugs in hospital pharmacies, the direct working environment must meet the requirements set for cleanroom grade A zones [2,3]. In the last decade, many pharmacy-based aseptic units implemented robotic systems to increase the preparation of parenteral products, while reducing the risk of occurrence of human errors, exposure to hazardous drugs, and the risk of musculoskeletal disorders due to repetitive motions. Several studies showed that the aseptic procedures during automated preparation are well controlled, and the working areas of the robotic systems meet the requirements set by the EU-cGMP guidelines [4]. The sanitization through UV irradiation of the surfaces inside the robots demonstrated to be highly effective in reducing the microbiological burden [4,5]. UV radiation is confirmed, therefore, as promising strategy to minimize the microbiological contamination.

Conventionally, UV light is generated from low- or medium-pressure mercury lamps. Over the last decade UV-light emitting diodes (UV-LEDs), an emerging semiconductor technology, have been developed as a new source of UV disinfection [6]. The fundamental structure of the LEDs is based on the junction of two-terminal semiconductors, called the p-n junction that converts direct current into radiation. Due to the variation of the available semiconductor material properties, LEDs can emit light at selected wavelengths or various wavelengths simultaneously. This flexibility opens the possibility to combine different LEDs wavelengths delivering a synergistic effect for bacterial inactivation [7]. Overall, the performance of UV-LEDs reached impressive efficiency regarding LED input power, LED life, and cost-effectiveness [8], making this system a valid alternative to conventional UV lamps.

The use of LEDs offers a safe and efficient application of UV treatment for decontamination processes without the risk of mercury contamination. UV-LEDs are widely used for disinfection due to their high germicidal action [8] inducing DNA damage by pyrimidine dimer formation as well as by the production of reactive oxygen species (ROS). Indeed, this technology has been successfully applied to liquid beverages and solid food

products for the reduction of foodborne pathogens load [9]. However, while the use of UV-LED in hospital settings was reported for room disinfection [10,11], their application in sterile drug preparation units of hospital pharmacies is not very common. The aim of our study is to evaluate the microbial inactivation of UV radiation by comparing the effectiveness of UV radiation emitted by traditional UV lamps and two types of UV-C LEDs, for possible applications to automated sterile drug compounding, such as APOTECA.

MATERIALS AND METHODS

Conventional UV low-pressure mercury vapor lamps (wavelength of 254 nm) (HNS S 9 W G23, OSRAM) and UV light-emitting diodes (LEDs) were utilized. For LEDs, mid-range power output LEDs (45 mW, LST1-01G07-UV01-01, Luminus) with a wavelength ranging between 275-286 nm (LED1) and high-power output LEDs (70 mW, KL265-50V-SM-WD, Crystal IS) with a wavelength ranging between 260-270 nm (LED2) were employed. The experiments were conducted in three test chambers (Chamber 1: UV lamp, Chamber 2: LED1, Chamber 3: LED2) designed to simulate ideal conditions ensuring a uniform radiation distribution and consisted of a polyethylene hollow cylinder (30 cm wide, 50 cm high) with an aluminium closing cap and an aluminium base plate (**Fig. 1A**). The UV sources were embedded in the upper part of the chamber. Seven 45 mW UV LEDs (LED1) and four 70 mW UV LEDs (LED2) were used in Chamber 2 and Chamber 3, respectively. Four plate housings and one irradiance probe housing, tailored using a 3D printer (ARGO 500, Roboze), were embedded in the base plate. The irradiance probe (LP471 UVC, Delta OHM) was placed at the centre of the base plate during each sampling cycle and irradiance was measured continuously with a radiometer (HD2102.2, Portable Luxmeter Data Logger, DeltaOHM).

Four reference strains (American Type Culture Collection, ATCC, Rockville, Maryland, USA) were used in this study: *Pseudomonas aeruginosa* ATCC 9027, *Staphylococcus aureus* ATCC 6538, *Bacillus subtilis* ATCC 6633 and *Candida albicans* ATCC 10231. Bacterial strains were routinely grown in Tryptic Soy Agar (TSA, VWR, Milan, Italy) at 37 °C for 24 h, while *C. albicans* ATCC 10231 was grown on Sabouraud Dextrose agar (SDA, VWR) at 37 °C for 48 h. All strains were stored at – 80 °C in Nutrient Broth no. 2 (VWR) supplemented with 15% glycerol.

For all the experiments, one colony of each strain was inoculated in 5 mL of Tryptic Soy Broth (TSB, VWR) and incubated overnight at 37 °C. Densities of microbial suspensions were spectrophotometrically adjusted to

obtain a final concentration (OD_{600nm} *ca.* 0.13–0.15) corresponding to *ca.*1.5×10^8 cfu/mL for bacteria and 5×10^6 cfu/mL for *C. albicans*. *B. subtilis* sporulation was performed as described in Bruscolini et al. [5]. A titer of 5×10^8 cfu/mL is considered adequate to achieve the test inoculum. The spore suspension was stored at 4°C in the dark, until use.

The irradiation assay was performed three times for each UV device (UV lamp, LED1, LED2). TSA plates containing 0.1 mL of each microbial suspension dilution (10^6 cfu/mL for *S. aureus* and *P. aeruginosa*, 10^4 cfu/mL for *C. albicans*) were placed into the test chambers and exposed at pre-defined UV doses (**Table 1**). In the case of *B. subtilis*, 1 mL of spore suspension (10^4 spore/mL) was distributed into a 35 mm sterile petri dish (without agar) and then irradiated as described above. After irradiation, the content of each treated sample was transferred into a 90 mm petri dish, covered with 20 mL of sterile poured Nutrient agar (NA), and left to solidify for about 10 minutes. For each test cycle, controls were added keeping a plate of each microorganism (at the same dilutions as the irradiated ones) in a non-exposed location for the whole irradiation time and then incubated under the same conditions of the UV-irradiated plates. All plates were incubated at 36 ± 1 °C for 24–48 h and cfu/mL were enumerated. Microbial inactivation was expressed as absolute counts (log cfu/mL). During each test cycle, the UV irradiance was measured continuously with a radiometer. The duration of each test cycle ranged from 0.1 to 206.4 minutes depending on the dose to be delivered (**Fig. 1 B**). The exposure dose (J/m^2) was given by the product of UV irradiance (W/m^2) and exposure time. Indeed, the UV irradiance is the amount of energy received by microorganisms or a surface, defined as the power of incident electromagnetic radiation on a surface.

RESULTS AND DISCUSSION

In sterile compounding performed in a Class-A environment, the GMP guidelines recommended the following limits for microbial contamination: <1 cfu/4 hours in settle plates (diameter 90 mm) and <1 cfu/plate in contact plates (diameter 55 mm) [**11**]. In our experiments, after exposure to UV-lamps at 50 J/m^2 dose, the viable counts of *S. aureus* and *P. aeruginosa* resulted to be 2.82 and 1.62 log cfu/mL (Logarithmic Reduction, LR 3.26 and 5.17 respectively), reaching 1.16 log cfu/mL (LR 4.92) for *S. aureus* and the complete growth inhibition for *P. aeruginosa* (LR 6.79) at 200 J/m^2. In the case of LED1, the reduction of bacterial growth was evident in *S. aureus* from 50 J/m^2 dose with 1.18 log cfu/mL (LR 4.9), reaching the complete inactivation (LR

5.43) at 200 J/m^2 dose. For *P. aeruginosa*, a LR 5.96 was obtained with a 50 J/m^2 dose, up to the complete inactivation at 200 J/m^2 (LR 6.79). As regards to LED2, LRs of *S. aureus* and *P. aeruginosa* reached 2.78 and 1.88 log cfu/mL (LR 3.3 and 4.91, respectively) applying 50 J/m^2 dose, up to the complete inactivation of *P. aeruginosa* at 200 J/m^2 dose (LR 6.79) (**Fig. 2 A-B**).

The tested LED1 device was equipped with a diode emitting in the range of 275-286 nm wavelength, the most used for UV-LED treatment [3]. In the literature, many studies reported that this range of longer LED wavelengths was more efficient compared to the shorter wavelength LEDs, as also observed in our experiments. The investigation of Murashita et al. [12] on the potential of UVC-LEDs from 270 to 280 nm against different types of microorganisms, revealed that UVC-LED at 152 J/m^2 dose led to a 4.45 log cfu/mL reduction of *E. coli* ATCC 25922, while *Listeria monocytogenes* was completely inactivated at 400 J/m^2. Similarly, Green et al. [13] confirmed that the nearer wavelength LED to 280 nm was the best choice for effective UV-LED performance. The UVC inactivation mechanism is due to the absorption of UV photons from the genetic material with the following production of dimers that can inhibit the transcription and replication of genes [8]. It must also be considered that some microorganisms can activate a mechanism of DNA repair, known as photoreactivation, during exposure to visible/blue light (330 to 480 nm), which is one of the most significant drawbacks of UV treatment. Noteworthy, the 280 nm wavelength or wavelengths close to 275 nm help in repressing photoreactivation. In water treatment, the photoreactivation of *E. coli* was significantly repressed after exposure to UVA/UVC-LEDs (310/275 nm) and UVC/UVC-LEDs (267/275 nm) wavelengths combination compared to that of 267 nm UV-LED [14,15]. In this direction, the technological characteristics of LED1 (275-286 nm wavelength), besides the highest germicidal activity, can avoid the possible photoreactivation of the treated microorganisms.

In the case of *C. albicans*, the complete inactivation was reached with high UV doses, specifically starting from UV-lamps 500 J/m^2 dose (LR 4.93), while the spores of *B. subtilis* resulted to be more resistant to the tested UV radiations, showing a remarkable growth reduction at 4,000 J/m^2 dose (LR 4.20). Using LED1*, C. albicans* was completely inactivated starting from 250 J/m^2 dose (LR 4.93), while for *B. subtilis* a dose of 4,000 J/m^2 was necessary to obtain the same result (LR 4.51). As regards to LED2, the complete inactivation was reached with 500 J/m^2 dose for *C. albicans* (LR 4.93) as well as for the spores of *B. subtilis* (LR 4.21); using 2,000 and 4,000 J/m^2 doses the highest LRs (4.93 and 4.51 for *C. albicans* and *B. subtilis,* respectively)

were observed (**Figure 2 C-D**). Indeed, *B. subtilis* possesses an arsenal of preventive and repair mechanisms to counteract the mutagenic effects related to different chemical and physical insults [16]. For this reason, *B. subtilis* spores were 10 to 20 times more resistant to the killing effects of UV radiation than the related vegetative cells, exhibiting two mechanisms for spore UV resistance. First, the chromosomal DNA of *B. subtilis* spores acquires an A-type conformation with altered DNA photochemistry interacting with a group of small-acid-soluble spore proteins (SASP). Thus, the UV-C radiation induces the formation of a special type of pyridine dimers (PD), termed spore photoproduct (SP), between adjacent thymidine residues. Second, the accumulated SP is processed during spore germination with two repair routes involving the SplB dimer, which specifically recognizes and splits SP back into two thymidine residues, and incision and excision by the nucleotide excision repair (NER) pathway [16]. From our experiments, we can hypothesize that LED1 and LED2 at the applied doses induced DNA cell damage not repairable by the described systems, contrarily to the irradiation with a conventional UV lamp that, also at the highest intensity, was unable to completely inactivate *B. subtilis* spores.

Most of the UV-LED applications are in the state of R&D and the available protocols were developed and approved for UV mercury lamps measurement, which is not suitable for UV-LEDs. Moreover, several factors, such as the condition of the test suspension, the fluence calculation, and the differences in the LED construction apparatus, might influence the results. Considering the wide application of UV-LEDs, a standard protocol should be established to accurately control and monitor the output of UV-LEDs with a reliable and adaptable method for different experiments and devices.

Our results evidenced the effectiveness of UV-LED irradiation for microbial inactivation and its potential use inside APOTECA. Indeed, both LED1 and LED2 can be considered valid alternatives to UV for the best-performing germicidal activity, including spore inactivation, quantifiable in less than 1 h of exposure time rather than the longer time needed with UV lamp [4]. The reduced exposure time has direct positive consequences in terms of cost production and environmental impact and the longer the LED wavelengths, the more energy-efficient they are in comparison to the shorter wavelength LEDs, with a related increased lifetime [17].

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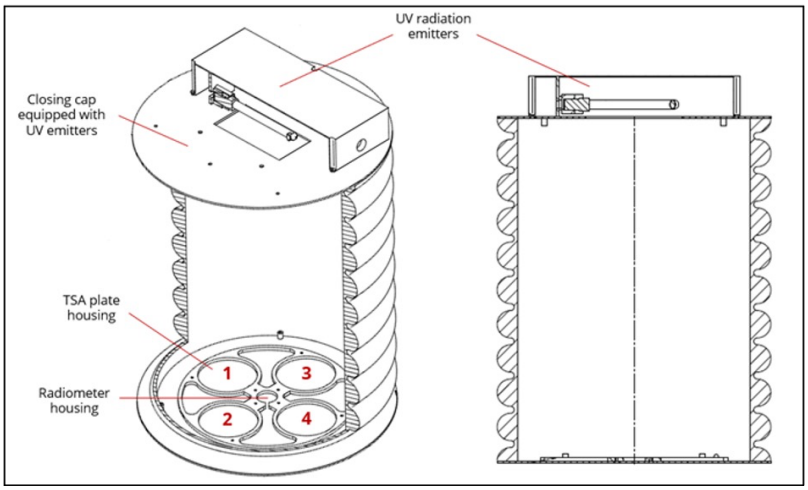
Figure Captions

Figure 1 Test chamber with three different UV-radiation sources (UV lamps, LED1, LED2) (a). Related doses of UV irradiation and the corresponding irradiation times (min) used in this study (b).



Dose (J/m²)	Irradiation time (min)		
	UV lamps	LED1	LED2
10	0.1	0.2	0.5
20	0.2	0.4	1.0
50	0.5	1.1	2.6
100	1.1	2.1	5.2
200	2.2	4.3	10.3
250	2.7	5.4	12.9
500	5.4	10.7	25.8
1000	10.9	21.5	51.6
2000	21.8	43.0	103.2
4000	43.6	85.9	206.4

Figure 2 Microbial inactivation in *S. aureus* (a), *P. aeruginosa* (b), *C. albicans* (c) and *B. subtilis* (d) after irradiation with each UV device (UV lamp, LED1, LED2).