Review

Everything You Need to Know Before Trying Pulsed Light Treatment for Your Applications

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Everything You Need to Know Before Trying Pulsed Light Treatment for Your Applications

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Abstract: Pulsed light (PL) is a non-thermal technology which leads to different applications (food, pharmaceutical and cosmetic packaging, medical devices) by inactivating microorganisms, knowing that the properties of PL technology are cumulative treatment, especially for bacteria. PL uses intense flashes of white light (200 nm–1100 nm wavelengths), rich in UV (200 nm–400 nm) and produced by xenon flash lamps. Localized photothermal and photophysical effects, but also the capability of its UV light component to modify the structure of biomolecules (photochemical effect), such as DNA and proteins, are the main mechanisms involved to explain the inactivation of microorganisms by PL. As compared to other sterilization methods, such as heat or chemical disinfectant treatment, PL treatment has several advantages; it is faster, induces low operational cost and leaves no residues. Nevertheless, no exhaustive data on exposure conditions are available, which would make it possible to repeat, interpret and evaluate the data needed for large-scale industrial deployment of PL in pharmaceutical and cosmetic packaging and medical devices. Research should also focus on understanding the inactivation mechanisms of different bacterial strains, states, and morphologies when exposed to PL.

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1. Introduction

The aim of every sterilization system is to eliminate all viable microorganisms that could induce health risks to patients (in healthcare) or consumers (food, beverages) or reduce product quality and stability. To achieve these objectives, the sterilization process must be appropriately selected and tailored for the intended targets. Several factors influence the design and effectiveness of the process, including the type of biocontaminants, the location of the bioburden, the surrounding conditions, the exposure time or sterilant dose, and the packaging material itself. Biocontaminants refer to cells or biological entities, which can be found on packaging surfaces or within the product before filling, potentially causing health issues for consumers or damaging the product. Such cells or microbial life include viruses, bacteria, bacterial spores, fungi, protozoa, multicellular parasites, and contaminating eukaryotic cells. Biological entities encompass aberrant proteins (prions), endotoxins, or active DNA or RNA. Besides sterilization processes that are used to render the product free from viable microorganisms [1], there is disinfection using to inactivate viable microorganisms to a level previously specified as being appropriate for a defined purpose. Pre-disinfection or (decontamination) is the first treatment to be performed on soiled equipment, surfaces and objects. The aim of pre-disinfection is to reduce the population of microorganisms and to make the environment or the object to be treated safe and harmless. Both disinfection and sterilization methods are a part of the biodecontamination process and are used for a variety of purposes in different industrial fields (medical devices, pharmaceuticals, cosmetic, food industry [2]. To call a product "sterile" does not imply absolute sterility, as proving the absence of all microorganisms is impossible

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[3]. Therefore, regulations and industries define two key terms: spore log reduction (SLR) and sterility assurance level (SAL). SLR measures the effectiveness of the sterilization process, expressed logarithmically to base 10, where each 1-log reduction signifies a 90% decrease in the number of spores. Regulations and standards set predefined SLR targets, validated through microbiological challenge tests. Typically, a 6-log reduction for indicator microorganisms is considered sufficient to achieve sterility. SAL represents the likelihood that a microorganism remains on an item after sterilization (i.e., the probability of non-sterility) and is expressed as a negative exponent of 10. This target is set by the industry to ensure the safety of the product for consumers. An SAL of better than 10^{-6} means there is a one in a million chance of having a contaminated product, providing a high level of assurance for product safety.

The range of sterilization and decontamination processes available has expanded, including not only steam, ethylene oxide, irradiation, ozone, hydrogen peroxide…, but also other emerging processes such as pulsed light (LP). LP is a technology used primarily for surface microbial decontamination by emitting broad-spectrum electromagnetic radiation in a series of short, but high-intensity pulses [4]. The number of published research on PL technology has substantially increased as attested by 6075 citations in Pubmed between 1967 and 2024 with 1802 citations over the last three years. It is a rapid non-thermal treatment method that also has the advantage of leaving no residue [5]. Also known as pulsed ultraviolet light (PUV), intense pulsed light (IPL), high intensity pulsed light (HILP) and broad-spectrum pulsed light (BSPL), this technology covers a broad spectrum including ultraviolet light, visible light and part of the near infrared (NIR) spectrum [6]. The growing interest in PL can be explained by its non-aqueous, non-thermal (over short periods), non-chemical and non-ionizing characteristics, while ensuring a rapid antimicrobial response. Indeed, PL is currently used to quickly deactivate microorganisms on various surfaces in the food industry, including food products, equipment, and packaging materials [7]. It is generated by flashlamps that contain inert gases like xenon, which emit electromagnetic radiation. PL offers several advantages over continuous UV light, such as better microbial inactivation efficiency, greater penetrating power, and enhanced lamp safety. It is considered that PL disinfection efficiency is higher compared with continuous-wave low-pressure UV irradiation due to its high peak power along with the ability to deliver its stored energy over short durations, typically 1 to 10 pulses per second [8]. Nevertheless, a specific UV range is proven to be more effective and varies with the target organism. It is important to note that PL treatments were primarily used for aesthetic purposes, such as cosmetic procedures, skin and hair care. However, after receiving FDA approval in 1996, there has been significant progress in PL-based disinfection technologies, which have since expanded into the food industry, pharmaceuticals process, drinkable water, and process water for chemistry as well. PL seems also very effective for treating the surfaces of food products, even its activity is different for solids and liquid food products [6]. This review therefore intended to give an overview in pulsed light principles and applications.

2. An Overview of Pulse Light Technology

Pulsed light decontamination technology has been around for many years, but its transfer to industrial applications is recent. It is a non-thermal method using pulse energy technology. PL delivers a polychromatic spectrum of white light comprising UV wavelengths from 200 to 400 nm (UV-C: 200–280 nm, UV-B: 280–315 nm, UV-A: 315–400 nm), visible spectrum wavelengths from 400 to 700 nm, and near-infrared region (IR) wavelengths from 700 to 1,100 nm [9], with flash durations ranging from microseconds to milliseconds. Although pulsed light equipment varies from system to system, the way it works remains the same. In fact, PL equipment contains flash lamps (xenon or UV lamps), a high voltage power supply and a storage capacitor. PL is produced by a lamp that converts electricity into pulses of light with a broad, intense spectrum [10]. Several PL equipments have been developed for the food and pharmaceutical industries. These include the PureBright™ system (PurePulse Technologies, Inc., San Diego, CA, USA) designed for biopharmaceutical manufacturers, the Robotic Pulsed Light Sterilizer (RPLS1) and Robotic Tub Decontamination System (RTDS2) utilizing pulsed light technology from CLARANOR (France) for the pharmaceutical sector, Claranor

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Pulsed Light Sterilization Systems (CLARANOR, France) for industrial applications in various fields : food, pharmaceutical, cosmetics, and the SteriPulse™ XLR Pulsed Light Systems (Xenon Corp., Wilmington, USA), available in different series (S-, RC-, Z-, X-), primarily for disinfection in the food industry. Although these devices differ in various factors such as radiant energy density per pulse, pulse characteristics, pulse rate, pulse width, in general the pulses are released rapidly to achieve a peak energy that can be utilized to produce PL. Light bulbs emitting PL are usually filled with inert gases, such as xenon and krypton, because these gases efficiently convert electrical energy into optical energy. When a high-energy pulsed electrical current passes through these bulbs, the gases are ionized, producing intense light. The resulting LP is short but of high intensity, reaching approximately twenty thousand times the intensity of sunlight at sea level [11]. Approximately 25% of the wavelengths of this light fall within the UV spectrum, 45% within the visible spectrum, and 30% within the infrared spectrum. The dose received by the product is generally described by the fluence ($J.cm^{-2}$), which represents the energy received per unit area of the target (a unit also used in the case of continuous UV treatments), knowing that the fluence is considered a crucial factor in PL treatments [12]. However, the efficiency of PL is related to the voltage applied to the lamp terminals, which not only affects the fluence (the higher the voltage, the higher the flash fluence), but also the quality of the emitted spectrum (% UV). In addition, the number, duration and frequency of flashes emitted, the distance between the light source and the sample, the treatment time and the peak illuminance ($W.cm^{-2}$), which represents the maximum power on $1cm^2$ of surface for the duration of a flash, all influence the PL treatment. Product area, thickness, colour, opacity, type of microorganism are also factors to be considered to obtain the highest effectiveness of the PL treatment [13]. Finally, Chamontin [14] reported that in addition to the physical parameters that have a direct influence on the level of decontamination, the density of germs per unit area is also crucial in assessing the effectiveness of bacterial reduction. For the same surface area and inoculation drop, PL treatment effectively eliminates bacterial suspensions at concentrations of 3, 4 and 5 logs. However, at a concentration of 6 logs, decontamination is only 66%. This can be explained by the formation of multilayers of microorganisms due to the high spore density. As PL is a surface treatment, it has difficulty reaching and destroying the organisms in these dense, aggregated layers. To overcome this problem, Raguse et al. [15] reported an automated spray deposition method that was successfully used to produce B. subtilis spore monolayers. Compared to other sample preparation methods, such as spot deposition, this automated spray provides a more reliable assessment of sterilization efficiency, which can be widely applied to various traditional and innovative decontamination methods, with a particular focus on non-penetrating surface agents.

3. Microbial Decontamination by PL

The ability of PL to kill microorganisms is well documented [16], depending on several technical parameters as previously reported (i.e., PL fluence and the relative amount of UV light, product characteristics such as surface roughness, presence of packaging but also on biological factors such as type of microorganism, spore/vegetative form) [17]. The PL treatment can provide decontamination with a 3 to 4 log reduction in the reference microorganisms (fungal spores and bacterial spore) without the use of additional chemical agents [18]. PL can also be effective against a wide range of micro-organisms, including highly heat-resistant species such as vegetative bacteria, mold spores, yeasts, viruses or parasites [19–21]. Focusing on the inactivation of spores using PL, Hwang et al. [5] summarized that distance, pulse width, charging voltage, and processing time all crucially affected the inactivation of spores, pulse width having the greatest effect on spore inactivation, followed by distance, and charging voltage. Furthermore, despite a lack of consensus, it seems that Gram-negative bacteria are more sensitive than Gram-positive bacteria [22,23]. Yeast also appears more resistance compared to vegetative bacteria [16]. Farkas [24] reported a link between the resistance of certain molds to UV rays and their color (black pigment), the survival of fungal spores being due to the melanin they contain [25]. For Cassar et al. [4], the microbial sensitivity to PL treatment is predominantly attributed to the ultraviolet portion of the spectrum. In line with these authors, Dorbani et al. [26] suggested that the resistance of spore-forming bacteria could be predicted

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from the knowledge of their UVC resistance. Kramer et al. [23] sum up these concepts by suggesting that bacterial sensitivity to PL depends on the species and strain level with a general trend of increasing PL resistance for pigmented conidiospores > bacterial endospores or spores > vegetative bacteria. Finally, the ability of the surface to absorb light also affects the decontamination effectiveness of PL.

3.1. Mechanism of Inactivation

PL, using very intense, broad-spectrum and high peak power pulsed light, induces microbiological inactivation attributed to high UV emission causing DNA damages and preventing cell proliferation [27]. In fact, both UVB and UVA can indirectly inactivate microorganisms via oxidative damage. This occurs through the formation of single-strand breaks and pyrimidine dimers. Recently, Dorbani et al. [26] found that per UVC fluence base, PL has a similar capacity to UVC to inactivate spore-forming bacteria. However, the exposure times required to achieve a given reduction are shorter with PL than with continuous UVC. In addition, photo-thermal and photo-physical effects contribute to microbial inactivation. Indeed, a stronger infrared light component produces a photo-thermal effect, which causes localized overheating, cell damage, and cell rupture [28,29]. Micro-vibrations resulting from the repeated short-duration (μs-ms) pulses cause membrane damage, leakage of cell organelles, changes in cell shape and cell lysis [30].

The characterization of inactivation kinetics has been the subject of several reports [31]. A "typical" PL inactivation curve is generally considered to be non-linear with a sigmoid shape, consisting of three phases: (i) an initial plateau or shoulder effect indicative of cell injury, (ii) a rapid increase in inactivation, and (iii) a tailing phase due to survival factors and strain-specific effects [22]. It is important to note that the application of kinetic models requires specific conditions, such as sufficient data points and a minimum log reduction.

4. Applications

4.1. Microbial Decontamination in Food Industry

PL is one of the emerging technologies that can be used to inactivate various pathogenic and spoilage microorganisms both in vitro and in different foods, with minimal impact on quality attributes [32]. Indeed, a range of molds, viruses and bacteria can be inactivated both in solid foods, in water, in liquid foods and on packaging materials [33]. More specifically, decontamination data of solid and liquid foods, fresh fruits and vegetables have been reported for many microbial species such as 1) bacteria (*B. subtilis*, *E. coli*, *S. aureus*...), 2) yeasts (*S. cerevisiae*, *C. albicans*…), 3) molds (*A. niger*, *B. cinerea*,…) [6,34,35]. This technology has, moreover, been approved by the FDA in the production, processing and handling of foods since 1996 up to cumulative UV dose or fluence of 12 J cm^{-2} where emission spectra to be kept between 200 and 1100 nm and pulse duration at ≤ 2 ms [36].

It also appears that nutritional quality of food products can be improved by LP. For example, Rock and al. [37] reported that PL exposure increased the antioxidant capacity and total phenolic content of fresh blueberries by 50 and 48%, respectively, and significantly increased the total anthocyanin content, which may be due to the up-regulation of phenylalanine ammonium lyase (PAL) enzymes as a defense mechanism against PL-induced stress while maintaining other quality attributes. This is a phenomenon that has also been observed by other authors [38]. In addition to inactivating microorganisms, PL technology is also able to extend the shelf life of fruits and vegetables while preserving their nutritional value [39]. However, despite several publications on the use of PL on different food types with conclusive results, there is a lack of information on the parameters used, making it difficult to compare research results (e.g. lamp manufacturer, target matrix geometry, inoculation experiments, choice or selection of test organism per food in relation to the lack of consensus on an agreed list of test species and strains). In addition, the processing of LP foods may be questionable due to the difficulty in reproducing foods with a repeatable surface quality. Therefore, although this technique is very promising, further research into repeatability is required for large-scale industrial adoption of PL [8].

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4.2. Microbial Decontamination in Food Package Decontamination

The inactivation of micro-organisms on food packaging materials has been well documented. LP packaging technology is particularly interesting because it allows food to be treated directly in the packaging, thus avoiding undesirable recontamination after treatment. However, the development of appropriate packaging is necessary for the successful marketing of PL-treated foods. To achieve this, it is essential to consider not only the food matrix, micro-organisms and treatment parameters, but also the permeability and stability of the packaging throughout the process [32]. In this line, Mandal et al. [6] reported that packaging materials should be transparent to UV light, including polyethylene, polypropylene, nylon, ethyl vinyl acetate (EVA), polybutylene, ethyl vinyl alcohol (EVOH), and Aclar [40].

4.3. Microbial Decontamination in Pharmaceutical Package and Hospital Environment

It is essential that sterility is maintained throughout the manufacturing and filling of pharmaceutical packaging and in the hospital environment. PL could replace existing technologies for microbial decontamination of packaging before it enters sterile filling areas as the pharmaceutical industry and hospitals look for fast, effective, non-chemical means of in-line decontamination. For example, pharmaceutical packaging ensures the quality and safety of medicines and is critical to their correct use. It is therefore essential to control bio-contamination through disinfection, biodecontamination or sterilization. PL is one of the technologies that can help achieve this. This biodecontamination is validated using Biological Indicators (BIs) placed at strategic points on the packaging. The EN ISO 11138-1 [41] specifies general requirements for BIs used in the validation and routine monitoring of sterilization processes for healthcare products. It covers the production, labeling, and testing methods of these indicators, including inoculated carriers and suspensions. Therefore, BIs are a critical component of the sterilization validation process, as ensuring appropriate sterility assurance levels requires confidence in the D-value, which is defined as the exposure time required to achieve a one log reduction in the treated microbial population using a fixed dose of sterilant [42]. The higher the D-value, the more resistant the BI. Because the bacterial spore suspension is to be used for the manufacture of BIs, the final spore suspension should be homogeneous, and the spores should exhibit adequate resistance to the sterilization or decontamination method. Bacterial spores of the genera *Bacillus* and *Geobacillus* are frequently used as BIs of sterility, because these spores are more resistance than all other types of microorganisms. In our laboratory (data not shown), using LP technology, we reported that *Geobacillus stearothermophillus* was the most resistant spore.

5. Conclusions

Compared with processing methods such as heat treatment and chemical sterilization or decontamination, PL is a rapid non-thermal treatment method that also has the advantage of leaving no residue, and is an effective technology for killing bacterial spores, much simpler than e-beam or gamma irradiation. It is an environmentally friendly, innovative method with significant potential for decontaminating food, food-contact surfaces, and packaging materials in pharmaceutical and cosmetic fields. New areas of application for the LP are emerging, such as his potential use against SARS-CoV-2 in touching surfaces in health-care facilities [43,44]. Nevertheless, there is a lack of harmonized data on exposure conditions including pulse duration, lamp pressure, voltage.., which would make it possible to repeat, interpret and evaluate the data needed for large-scale industrial deployment of PL. Research should also focus on understanding the inactivation mechanisms of different bacterial strains, states, and morphologies, including Gram-positive and Gram-negative bacteria and various growth phases, when exposed to PL.

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