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Ultraviolet-C irradiation for inactivation of viruses in foetal bovine serum

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ABSTRACT

Foetal Bovine Serum (FBS) and porcine trypsin are one of the essential raw materials used in the manufacturing of cell culture based viral vaccines. Being from animal origin, these raw materials can potentially contaminate the final product by known or unknown adventitious agents. The issue is more serious in case of live attenuated viral vaccines, where there is no inactivation step which can take care of such adventitious agents. It is essential to design production processes which can offer maximum viral clearance potential for animal origin products. Ultraviolet-C irradiation is known to inactivate various adventitious viral agents; however there are limited studies on ultraviolet inactivation of viruses in liquid media. We obtained a recently developed UVivatec ultraviolet-C (UV-C) irradiation based viral clearance system for evaluating its efficacy to inactivate selected model viruses. This system has a unique design with spiral path of liquid allowing maximum exposure to UV-C light of a short wavelength of 254 nm. Five live attenuated vaccine viruses and four other model viruses were spiked in tissue culture media and exposed to UV-C irradiation. The pre and post UV-C irradiation samples were analyzed for virus content to find out the extent of inactivation of various viruses. These experiments showed substantial log reduction for the majority of the viruses with few exceptions based on the characteristics of these viruses. Having known the effect of UV irradiation on protein structure, we also evaluated the post irradiation samples of culture media for growth promoting properties using one of the most fastidious human diploid cells (MRC-5). UV-C exposure did not show any notable impact on the nutritional properties of culture media. The use of an UV-C irradiation based system is considered to be promising approach to mitigate the risk of adventitious agents in cell culture media arising through animal derived products.

1. Introduction

It is highly desirable to eliminate potential contaminating adventitious agents from the biopharmaceutical products derived from cell culture based processes employing animal originated materials [1]. Introduction of adventitious agents can occur at any step in complex biological manufacturing processes where cells are propagated using animal derived raw materials such as fetal bovine serum (FBS) and trypsin [2]. Bovine Viral Diarrhea virus (BVDV) has been predominantly found to be present in most, if not all, commercial scale batches of FBS [3–6]. The use of trypsin has been found to pose a risk of contamination with parvoviruses and circoviruses. The outcome of such evidences is that the overall viral safety of bovine serum should be subject to a risk analysis as the infectious virus will invariably be present in some batches of serum but remain undetected; thus, implementation of virus inactivation steps should be taken into consideration. Viral contamination of biopharmaceutical manufacturing, especially human medicinal products such as vaccines, has led to the requirements by regulatory authorities for manufacturers to develop risk mitigation strategies [7–10].

It is widely acknowledged that testing alone is not sufficient to assure safety from any adventitious agents contamination and additional measures are always recommended. Due to the recognized risk associated with use of animal sera in the production of biological products, the European guideline on the use of bovine serum in the manufacture of human biological medicinal products strongly recommends inactivation of viral contaminants in serum using a validated and efficacious treatment [11]. For this purpose, a short wavelength Ultraviolet light (UV-C) irradiation looks an attractive option to treat media for cell culture processes to inacti-

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vate any potential viruses and to substantially reduce risks of viral contamination [12–21]. UV-C irradiation has been demonstrated to be an effective means of inactivating also small non-enveloped viruses [13,14]. UV-C has been known as an effective virus inactivating method since 1940s [17]. Significant log reductions have been reported for many viruses (4–8 logs) and various *Mycoplasma* species (>7 logs) upon UV-C treatment of virus or *Mycoplasma* spiked fetal calf serum [16]. UV-C treatment is commonly used in the water treatment industry [18,19], however for biotech and vaccine industries, UV-C media inactivation can be particularly attractive [20,21].

Historically the use of UV-C was limited to medical sanitation and sterile work facilities. In recent years, due to advancements in technologies, the use of UV-C has been made possible for inactivation of microorganisms in liquids also. Recently, a specially designed UV-C irradiation system was developed which enables the use of UV-C irradiation with a defined consistent dose size with reliable microbial inactivation properties. A bench scale model of such UV-C irradiation system was evaluated for inactivation of various viruses spiked in FBS, which is an essential raw material for cell culture based vaccines.

Initially, UV-C irradiation was not used in biologic manufacturing due to the concern for damage to the products or inability of UV-C light to penetrate deeply into bulk, viscous liquids [22]. However, developmental research have overcome these difficulties by designing spiral tube based continuous flow UV-C reactors which are able to inactivate adventitious agents in dense liquids like sera or plasma while mitigating damage to proteins [23–26]. Due to long spiral tubes, Dean vortices ensure consistent mixing but also consistent liquid exposure time [25].

Special lamps are used to generate UV radiation, and are enclosed in a reaction chamber made of stainless steel or, less commonly, plastics. Low pressure mercury lamps are used to Generate UV radiation and their wavelength is in the optimum germicidal range of 250–265 nm. Typically the life of UV lamp is 10–12 month, after which lamp needs to be replaced. The dose of UV radiation is expressed as an energy flux, in units of J/m^2 (Joules per square meter), which is calculated from mean intensity (watts per square meter) and mean residence time of liquid in the reactor. Minimum dose required for disinfection depends on several factors, including susceptibility of micro-organisms. Adequate residence time and UV intensity are important factors to ensure effective disinfection. Colour and turbidity also affect radiation intensity in the reactor and turbidity may protect microorganisms from the effect of radiation.

1.1. Aim of work

There are limited studies on UV-C inactivation of viruses in liquid medium. The extent of inactivation also varies with the composition of medium e.g. liquids with high protein or solid content, tend to reduce the efficiency of UV-C irradiation. As such, it was necessary to evaluate UV-C virus inactivation properties for viruses when present in FBS, which is routinely used in the manufacturing of biological products. Similarly, having known the effect of UV irradiation on proteins, its impact on biological properties of trypsin and FBS was also assessed. The aim of study was to evaluate laboratory scale equipment for development of a higher scale machine for use in large scale commercial vaccine manufacturing process.

2. Material and methods

2.1. Materials

UVivatec system (Sartorius-Stedim)

Media Components:
FBS (Moregate Biotech)
CM, Cell Medium (MEM, Hank's salts (Make Invitrogen), supplemented with 10% FBS)
Trypsin (Pangaea)
Viruses:
Measles Edmonston-Zagreb virus strain
Mumps L-Zagreb virus strain
Rubella RA-27/3 virus strain
Influenza A/17/California virus strain
Poliomyelitis Pol 14211, Type I virus strain
Simian virus type 40 (SV40): ATCC VR-305 strain
Porcine parvovirus (PPV): ATCC VR-742 strain
Reovirus 3 (Reo 3): ATCC VR-232 strain
Bovine viral diarrhea virus (BVDV): ATCC VR-1422 strain
Cell substrates:
Human Diploid Cells (MRC-5)
VERO cells
RK-13 cells
Hep-2 cells
MDBK cells
HEK 293
ST cells
Embryonated Chicken Eggs

2.2. UV-C irradiation system

We obtained a proprietary machine from the manufacturer along with necessary software for operation of machine for precise ultraviolet (UV-C) dosing based on the intensity of UV-C light source and flow rate of medium through the system. The design of system consisted of a helical channel tube formed with the semicircular outer side consisting of teflon and the straight inner site of quartz glass. The helical channel could be irradiated from inside to the outside by a rod shaped UV light source placed inside the quartz glass. This light source was a low-pressure mercury lamp which generates UV at a specific wavelength of 254 nm. Novel hydraulic spiral flow around an irradiation source induces Dean vortices in a fluid stream. These vortices provide highly efficient mixing in a fluid steam and optimized virus exposure to the light source. As a consequence, high doses of UV-C irradiation can be delivered evenly and uniformly throughout the solution. Thus, the required residence times in irradiation chamber are extremely short and the UV-C treatment can be accurately controlled.

2.3. Selection of UV-C dose sizes

The software of the system calculates the UV-C dosage based on the intensity of UV-C light and flow rate of liquid through the system. It also takes values of absorbance of liquid at 254 nm wavelength for calculation of dose. The available UV intensity range for the machine was 7–20 mA, while the available flow rate range was 6–20 L/hr. Table 1 provides achievable dose range obtained through the software of machine based on the absorbance of liquids. A lowest UV-C light intensity and highest flow rate gives lowest dose size, while a highest UV-C light intensity and lowest flow rate gives highest dose size

2.4. Phase-1 studies

For Phase-1 virus inactivation studies, to cover up a wide dose range, we decided to use 100 and 700 J/m^2 doses for viruses spiked in FBS and 300 and 2000 J/m^2 doses for viruses spiked in CM. Trypsin, being dissolved in simple salt solution, was not used for spiking of viruses during UV-C inactivation studies, as FBS & CM with

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