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Evaluating ultraviolet sensitivity of adventitious agents in biopharmaceutical manufacturing

Sarah M. Meunier[1,2] · Michael R. Sasges[1] · Marc G. Aucoin[2]

Abstract Incidents of contamination in biopharmaceutical production have highlighted the need to apply alternative or supplementary disinfection techniques. Ultraviolet (UV) irradiation is a well-established method for inactivating a broad range of microorganisms, and is therefore a good candidate as an orthogonal technique for disinfection. To apply UV as a safeguard against adventitious agents, the UV sensitivity of these target agents must be known so that the appropriate dose of UV may be applied to achieve the desired level of inactivation. This document compiles and reviews experimentally derived 254 nm sensitivities of organisms relevant to biopharmaceutical production. In general, different researchers have found similar sensitivity values despite a lack of uniformity in experimental design or standardized quantification techniques. Still, the lack of consistent methodologies has led to suspicious UV susceptibilities in certain instances, justifying the need to create a robust collection of sensitivity values that can be used in the design and sizing of UV systems for the inactivation of adventitious agents.

Keywords Biopharmaceutical · Adventitious agent · Ultraviolet · Irradiation · Disinfection · Viral clearance

✉ Michael R. Sasges
msasges@trojanuv.com

1 Trojan Technologies, 3020 Gore Rd., London, ON N5V 4T7, Canada

2 Department of Chemical Engineering, Waterloo Institute for Nanotechnology, University of Waterloo, Waterloo, ON N2L 3G1, Canada

Introduction

There have been a number of publicized incidents in which biopharmaceutical production has experienced contamination with a virus or bacterial agent [6, 10, 24, 33, 72]. These contamination events are highly disruptive, requiring that production be halted, inventory be quarantined, and extensive testing be conducted to identify the nature and source of the contamination. The cost of a contamination event can be significant. For example, costs associated with a vesivirus 2117 contamination event at Genzyme in 2009 were estimated at more than $200 million including a fine of $175 million from the US FDA [3]. In addition, there is the direct or indirect cost to patients whose medications become unavailable.

Ultraviolet (UV) disinfection has been applied in drinking water for more than a century, with the first installation in Marseille in 1910, and the first US installation in Henderson KY in 1916 [66]. Many millions of people rely on drinking water disinfected with ultraviolet light, including the residents of New York City, USA, and Vancouver, Canada. Benefits of UV disinfection include the avoidance of chemical addition, as well as simple control and maintenance. The unique mode of action of UV, directly affecting the nucleic acids of an organism or virus, also makes it a desirable orthogonal methodology to complement technologies such as filtration or chemical inactivation. As a result, it is often considered a treatment method for small organisms or viruses that cannot be readily filtered or that are not susceptible to chemical disruption.

Biopharmaceutical manufacturers Genzyme and Sanofi Pasteur consider UV to be a promising prospective adventitious agent clearance technology to mitigate the risk of contamination from raw materials [44]. However, there is reluctance to implement it in processes

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because of the lack of available literature on the topic. Recently, an effort to correct this has been undertaken and our group has published research considering the effect of UV fluence on the composition and function of cell culture media [70, 117].

UV inactivation is generally achieved using lamps emitting at 254 nm. This wavelength, in the UVC range, is produced very efficiently using mercury vapor lamps, and it nearly corresponds to the nucleic acid absorption peak of about 265 nm. The absorbed energy can result in pyrimidine cyclobutane dimer formation; if the dimers are formed in a critical location and cannot be overcome by repair mechanisms, the organism cannot reproduce and is thereby inactivated. The amount of UV relevant to inactivation is properly denoted as fluence, which is the direction-independent irradiance-analog multiplied by the exposure time. The units of fluence are energy per unit area.

There have been attempts to predict the UV sensitivity of organisms by correlations with genome size, single-stranded vs. double-stranded, DNA vs. RNA, or detailed genomic analysis [57, 56, 64]. The model predictions have good general agreement with experiment, allowing the estimation of UV sensitivity for newly discovered organisms or in cases where experimental results are not available. However, even the most detailed mechanistic predictions by Kowalski [56] differ by up to a factor of 2 or 0.5 from specific experimental results. For that reason, this review considers only experimentally measured inactivation rates; model predictions are not included here.

Several review articles identify adventitious agents that are concerns for CHO cell processes based on known contamination events or known susceptibilities [12, 54, 74]. The objective of this study is to provide a critical review of the available UV inactivation literature for organisms that are crucial to the biopharmaceutical industry, and to provide a comprehensive list of the organisms studied along with their UV sensitivities. This work will hopefully be a valuable source of information in the design of UV disinfection equipment and experiments, as well as provide researchers a foundation to evaluate and analyze UV inactivation studies.

Methodology

Notes:

1. In the present work, definition and terminology are based on the IUPAC 2006 recommendations [18] (Table 1).
2. All plots were created in R, a programming language that facilitates statistical computing and graphic visualization (https://www.r-project.org/), using the ggplot2 package (http://docs.ggplot2.org/). Box-plot whiskers extend above the upper edge of the box to the highest value point (or below the lower edge of the box to the lowest value point) that is within 1.5 times the inter-quartile range.

Data selection and errors in UV inactivation studies

UV sensitivity is generally measured by applying a well-quantified, single-valued UV fluence to a fluid containing virus, and determining the resulting degree of inactivation of the virus. The process is repeated for a sequence of fluence values, and the resulting inactivation is plotted to determine the shape of the inactivation curve (the kinetics) and to minimize the impact of error in any one data point. For first-order kinetics, the D_{10} value (the fluence required for a single log reduction in infectivity) is readily determined from the slope of the resulting plot.

Table 1 Symbols and definitions

Symbol	Name	Definition
F_o	Fluence	The integral of fluence rate and time. Units: energy/area, e.g. mJ/cm^2. For UV disinfection, this is commonly referred to as "UV dose"
E_o	Fluence Rate	The radiant energy flux (power) from all directions passing through an infinitesimal spherical volume, divided by the cross-sectional area of the sphere. Units: power/area, e.g. mW/cm^2 This is the spherical analog of irradiance, and is independent of direction
E (see note)	Irradiance	The radiant energy flux incident on a surface element, divided by the area of the element. Units: power/Area, e.g. mW/cm^2. This quantity depends on the angle of incidence
D_{10}		For an organism with first-order UV inactivation kinetics, the Fluence required to decrease the number of viable organisms to 1/10 of the original number
A		The optical absorption coefficient, in base 10. Used in the Beer–Lambert–Bouguere law.
A_e		The optical absorption coefficient, in base e
L	Length	The fluid path length through which UV radiation passes

The same symbol is often used for both irradiance and fluence rate

Quality assurance

The quality of data in a UV inactivation study should be checked by a simple plot of survival vs. UV fluence. For most organisms, the UV inactivation is well modeled by a first-order equation [66]. That is, the number of infective virus particles, N, is an exponential function of the applied fluence, F_o multiplied by the original amount of infective virus, N_0:

$$N = N_0 10^{\frac{-F_o}{D_{10}}}. \tag{1}$$

When plotted as the log of the number of viable virus vs. the fluence, the data will form a straight line for many viruses. A simple check of data quality can be done by examining this log plot of the data. If the data deviates from the typical straight line, it may indicate that the experiment was flawed. In Fig. 1, we plotted data from two different investigators for the UV inactivation of feline calicivirus (FCV). It can be seen that the results from Park et al. [81] show first-order kinetics to about 5 log inactivation, while the results from Nuanualsuwan et al. [77] show tailing with a maximum inactivation of about 3 log. The work of Nuanualsuwan et al. [77] also shows tailing for φX174 phage at about 3 log, in contrast to the first-order kinetics reported by Battigelli et al. [9] even beyond 6 log inactivation (Fig. 1).

Although some organisms may not exhibit first-order kinetics for UV inactivation, first-order kinetics are very common in response to UV irradiation. Any data set should be compared against other literature results to determine
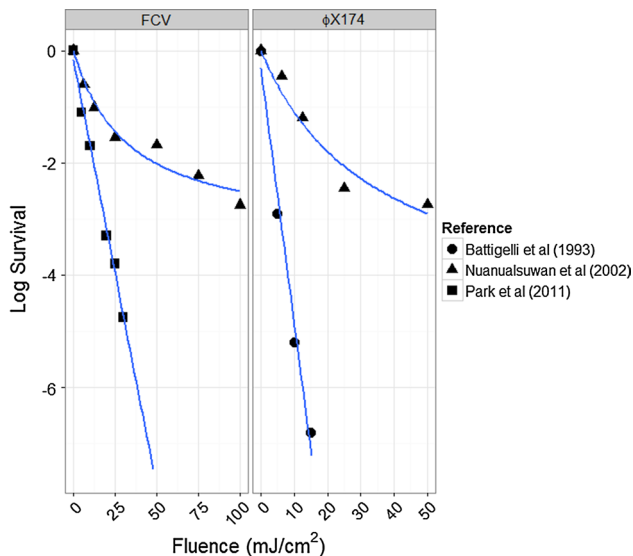
the expected inactivation kinetics for the virus, or for other viruses of the same family or genus. Deviation from expected kinetics may be the result of a number of factors, discussed below.

UV measurement errors

Examining the outliers in this literature review, it is apparent that some authors did not precisely apply single fluence values, but instead applied fluence distributions including higher and lower values than that quoted. Applying a well-quantified, mono-valued fluence requires careful design of the irradiation apparatus, careful fluid characterization, and usually careful mixing.

Fluence detectors are very difficult to design, construct, and calibrate. An ideal fluence sensor would have a spherical detector that was equally sensitive to radiation arriving from all directions (4π steradians). Some investigators have used small spherical containers of chemical actinometry solutions [83], or small coated plastic spheres that change fluorescence or color with UV fluence [13], but this approach does not permit real-time measurement. Creating a fluence detector with an electronic readout is nearly impossible. The solution to this dilemma is to use an irradiation geometry in which an irradiance sensor will suffice, since these are available from vendors, e.g. International Light Technologies and Solar Light, along with calibration traceable to U.S. National Institute of Standards (NIST) or other national laboratories.

The UV irradiance must be accurately known so that fluence can be calculated. In their guideline for applying UV fluence, Bolton and Linden [15] recommend using a collimated beam apparatus. While this device does not actually collimate the UV radiation field, it effectively uses an aperture to ensure that UV only reaches the fluid from a single direction, directly above the fluid. This is important for two reasons: it ensures that the incident irradiance can be measured accurately, and it ensures that the attenuated radiation field in the fluid sample can be calculated accurately.

If the UV flux reaches the fluid sample from a variety of directions, most UV sensors cannot quantify the fluence rate. Most sensors will, by design, have a response that drops off as the angle of incidence deviates from the normal. A well-designed irradiance sensor will have a response that varies with the cosine of the angle of incidence. However, viruses do not respond differently to off-axis radiation or on-axis, so the fluence rate, not irradiance, is the appropriate measurement quantity. The solution is to have the UV reaching the sample only from a single angle, and to orient the UV sensor normal to this radiation. Under these conditions, irradiance and fluence rate are identical, so the irradiance reading will be correct. The attenuated

Fig. 1 Inactivation data for feline calicivirus and φX174 phage from different research groups

radiation field in the fluid sample can then be readily calculated, since the geometry is simple.

It is also important to correct for the attenuation of UV fluence in the fluid sample in which the organism or virus is suspended. The radiation field in the fluid sample must be calculated from the fluid depth and absorbance. This is done using the measured irradiation at the fluid surface and the measured optical absorbance of the fluid at the relevant wavelength, usually 254 nm. The intensity in a non-scattering fluid may be calculated from the Beer–Lambert–Bouguer law [71]. Assuming that the fluid is well mixed and the irradiation time is long compared with the mixing time, the expression for the volume-average intensity can readily be found by integration. The equations governing average fluence in a well-mixed, semi-transparent media were published by Morowitz [73] expressing the average fluence rate, F_{avg}, as:

$$F_{avg} = F_0 \left(\frac{1 - e^{-A_e L}}{A_e L} \right), \tag{2}$$

where F_0 is the incident fluence rate, A_e is the absorption coefficient (base e) at the wavelength of interest, and L is the fluid depth in the direction of the radiation field. Expressed as a function of the more common base-10 absorption coefficient, A, this equation becomes:

$$F_{avg} = F_0 \left(\frac{1 - 10^{-AL}}{AL \ln (10)} \right). \tag{3}$$

There are a number of other correction factors highlighted in the Bolton and Linden guideline, but this absorption factor is the most significant in many configurations used for inactivation studies, since suspensions of organisms and viruses often have strong absorbance in the UV range.

Other errors

Clumping can result in apparent deviations from the true inactivation kinetics. If organisms or viral particles are not fully dispersed, some will be present in clumps. This may result in an inactivation plot with a plateau as a result of shielding of the central virus or bacterium by those surrounding it, which will allow the central virus or bacterium to survive [106]. For example, if 1 in 1000 virus particles is fully shielded, the effect will be tailing above 3 log reductions, while fully dispersed virus will demonstrate intrinsic kinetics through higher inactivation levels. These effects were recognized and addressed by Furness [37] and by Das [30] in studying the UV inactivation kinetics of mycoplasma, in which they used sonication and filtration to ensure single-cell suspensions. As Furness [37]

noted, "That the suspension contained only single cells was confirmed by demonstrating that the colony-forming units (CFU) were inactivated exponentially by ultraviolet irradiation".

Fluid optics and poor mixing can also lead to tailing in dose–response curves. If the fluid sample is not adequately mixed, so that not all components of the sample receive the same integrated UV exposure (fluence), the less-irradiated fluid will dominate the results and can lead to a plateau in inactivation. As an extreme example, if one part in 1000 of the fluid receives zero fluence, then the inactivation cannot exceed 3 logs, even as the exposure time is increased. Similar, though less extreme, behavior can result from poor dose distribution: survival is dominated by the low-dose fluid regions. For this reason, it is preferable to conduct inactivation studies in relatively clear solutions to minimize intensity gradients and lessen the importance of mixing. Likewise, the irradiation device (collimated beam) should also be set up to minimize the UV intensity gradients across the surface of the fluid sample. This deviation from uniformity is denoted by the Petri factor in the method published by Bolton and Linden [15], and methods are provided to quantify the magnitude of the effect as well as recommendations for an acceptable range.

It is important to utilize a relevant assay to determine whether infectious virus remains after irradiation. Assays, such as tissue culture 50% infectious dose ($TCID_{50}$), or plaque assays, will correctly assess infectivity; morphological changes to detector cells due to viral infection are the basis of the $TCID_{50}$ assay and are responsible for plaque formation.

PCR methods have significant drawbacks in measuring the degree of disinfection, as they may be unable to distinguish between viable and inactivated organisms. The detection of specific sequences does not indicate the presence of an intact, infectious virion. Even the presence of a viral sequence in a host cell does not indicate infectious virus, since the virus might enter the host cell but still not be able to replicate. Care must be taken to ensure that the assay is quantifying infectious virus.

Randazzo et al. [82] and Ju et al. [51] have investigated the ability of PCR-based techniques to distinguish between viable and inactivated norovirus and *E. coli*, respectively. In both papers, the authors examined the ability of RT-qPCR (reverse-transcriptase, quantitative PCR), augmented by photoactivatable dye methods (propidium monoazide and ethidium monoazide), to discriminate between viable and inactivated organisms. RT-PCR amplifies RNA, and is thought therefore to detect only viable organisms that are expressing RNA (or an RNA virus). The dyes are furthermore intended to prevent amplification of nucleic acids from organisms with compromised capsids, since the dyes can penetrate to the nucleus and prevent amplification.

Table 2 Average dose per $\log_{10}$ inactivation of adventitious agents

Family	Genome	Adventitious agent	D_{10} (mJ/cm^2)
Enterobacteriaceae	dsDNA	*E. coli*	1.9
Mycoplasmataceae	dsDNA	Mycoplasma	1.9
Acholeplasmataceae	dsDNA	Acholeplasma	2.5
Leptospiraceae	dsDNA	Leptospira	1.9
Adenoviridae	dsDNA	Adenovirus	42.3
Arenaviridae	ssRNA	Junin virus	3.0
Arenaviridae	ssRNA	Lassa virus	3.0
Bunyaviridae	ssRNA	Cache Valley virus (CVV)	20.8
Bunyaviridae	ssRNA	Hanta virus	2.8
Bunyaviridae	ssRNA	Rift Valley fever virus	2.8
Caliciviridae	ssRNA	Canine calicivirus (CaCV)	6.6
Caliciviridae	ssRNA	Feline calicivirus (FCV)	5.3
Caliciviridae	ssRNA	Murine norovirus (MNV)	8.3
Circoviridae	Circular ssDNA	Porcine circovirus (PCV)	17.8
Filoviridae	ssRNA	Ebola virus	1.7
Filoviridae	ssRNA	Marburg virus	1.7
Flaviviridae	ssRNA	Bovine viral diarrhea virus	12.2
Flaviviridae	ssRNA	West Nile virus	5.5
Herpesviridae	dsDNA	Bovine herpesvirus 1	16.7
Herpesviridae	dsDNA	Cytomegalovirus (CMV)	4.8
Herpesviridae	dsDNA	Pseudorabies virus	8
Leviviridae	ssRNA	Bacteriophage MS2	19.3
Microviridae	Circular ssDNA	φX174 phage	2.3
Orthomyxoviridae	ssRNA	Influenza A virus	4.5
Paramyxoviridae	ssRNA	Parainfluenza Virus	14.3
Parvoviridae	ssDNA	Bovine parvovirus	12.5
Parvoviridae	ssDNA	Mouse minute virus (MMV)	1.8
Parvoviridae	ssDNA	Parvovirus H1	23.0
Parvoviridae	ssDNA	Porcine parvovirus (PPV)	2.0
Picornaviridae	ssRNA	Cardiovirus A—encephalomyocarditis virus	5.2
Picornaviridae	ssRNA	Coxsackievirus	10.4
Picornaviridae	ssRNA	Echovirus	9.1
Picornaviridae	ssRNA	Foot and mouth disease virus	22.2
Picornaviridae	ssRNA	Hepatitis A virus (HAV)	6.3
Picornaviridae	ssRNA	Poliovirus (PV)	8.0
Polyomaviridae	Circular ssDNA	Mouse polyoma virus	57.0
Polyomaviridae	Circular ssDNA	Simian vacuolating virus (SV40)	92.8
Poxviridae	dsDNA	Variola virus	2.5
Reoviridae	dsRNA	Reovirus	22.4
Retroviridae	ssRNA	Murine leukemia and sarcoma viruses	24.9
Rhabdoviridae	ssRNA	Vesicular stomatitis virus	2.3
Togaviridae	ssRNA	Semliki forest virus	11.1
Togaviridae	ssRNA	Sindbis virus	7.1
Togaviridae	ssRNA	Equine encephalitis virus	4.9

Randazzo found that even the best combination of these dyes was only able to suppress about 2 log of thermally inactivated norovirus. Ju et al. [51] found similar issues, with up to a 7-log discrepancy between culture and qPCR results for *E. coli* inactivated by desiccation. In disinfection applications where 3 log or greater inactivation is expected, these methods will not correct the inherent over-estimation of viable virus by qPCR methods. Furthermore, UV
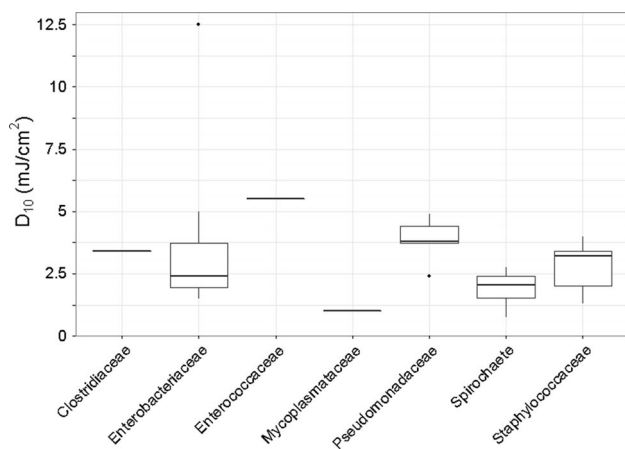
Fig. 2 D_{10} values for bacterial families. *Points* plotted *beyond the box-plot whiskers* are outliers [104, 105]
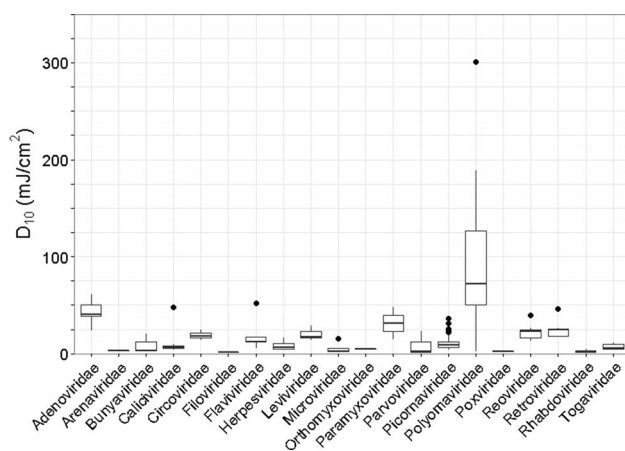

Fig. 3 D_{10} values for viral families. *Points* plotted *beyond the box-plot whiskers* are outliers [104, 105]

disinfection will leave any capsid virtually intact, which will further limit the performance of these dye techniques. The authors of the present review do not recommend PCR methods for assessing UV disinfection.

UV sensitivities of bacteria and viruses

UV inactivation kinetics are often first order, and are commonly described by the D_{10} (the dose required to reduce the counts by one $\log_{10}$), defined above. A larger D_{10} value indicates that an organism is more resistant to UV inactivation. Considering pitfalls in methodology and overall consistency within multiple research labs, average D_{10} values based on experimental results are proposed (Table 2) based on a thorough review of the literature D_{10} values (Fig. 2, Fig. 3; Appendix Table 3). Unless addressed in the text below, the reported values in Table 2 are simply an

arithmetic average of the available literature listed in the appendix.

In general, the experimentally determined UV sensitivity values are consistent across multiple laboratories (Figs. 2, 3 and Table S1), which gives confidence in the reported values. Inconsistencies in the experimental data reviewed are discussed below (Fig. 4).

Bacteria

This review focused only on bacteria that are of concern as adventitious agents in biopharmaceutical production. More extensive reviews are available for bacteria and other organisms, including that of Hijnen [48]. Two organisms of particular interest as adventitious agents are mycoplasma and leptospira.

Mycoplasma and acholeplasma contaminations are relatively common, and since these bacteria lack a cell wall they are unaffected by many common antibiotics. They are approximately 0.1 μm in diameter, making them difficult to remove via typical sterilizing filtration. Several strains of mycoplasma and acholeplasma have been evaluated for sensitivity to UV irradiation. The one outlier in this data set is the result of Kurth [57], who reported only full clearance of mycoplasma. Therefore, the precise UV sensitivity cannot be determined from the data set of Kurth. Most experimental results show D_{10} values between 1 and 5 mJ/cm^2, indicating that mycoplasma and acholeplasma are highly susceptible to UV inactivation.

Leptospira are a concern in biopharmaceutical production since their minor dimension of about 0.1 micron can allow them to penetrate many filters, especially those designed for bacteria, where the pore size is often about 0.2 micron. The D_{10} values for leptospira reported by Stamm and Charon [97] and by Fonseca [36] range from 1 to 3 mJ/cm^2, indicating high susceptibility to UV disinfection. Stamm and Charon [97] provide only minimal information on the methodology and no discussion of the fluid optics, so it is difficult to determine whether the leptospira data are reliable. The authors of this review could not find any other quantitative data on the UV sensitivity of leptospira. This represents an area for additional research.

Escherichia coli has been studied by many investigators, and is readily inactivated by UV. Some strains have been found to have a shoulder in the inactivation curve, in which a low UV dose has a small impact below 1- or 2-log inactivation, with increasing impact at higher doses. For this reason, the D_{10} value in the summary table is based on the 4-log inactivation data, simply dividing the dose for 4-log inactivation by a factor of 4. This will be conservative for higher levels of inactivation. Since UV as a broad-spectrum inactivation method is likely to be

Fig. 4 D_{10} values for adventitious agents that show inconsistencies in the data from different research laboratories. *Points* plotted *beyond the box-plot whiskers* are outliers [104, 105]
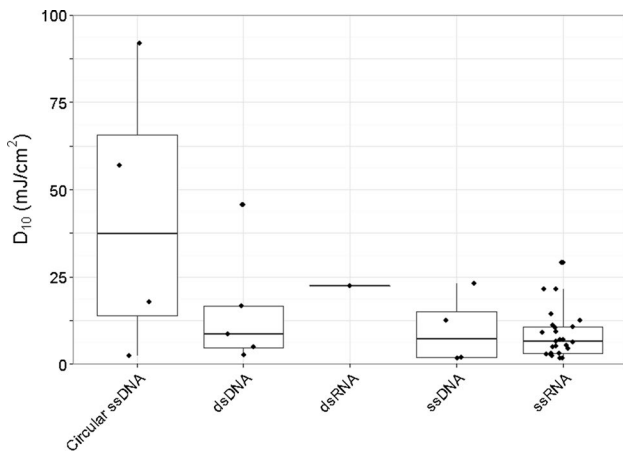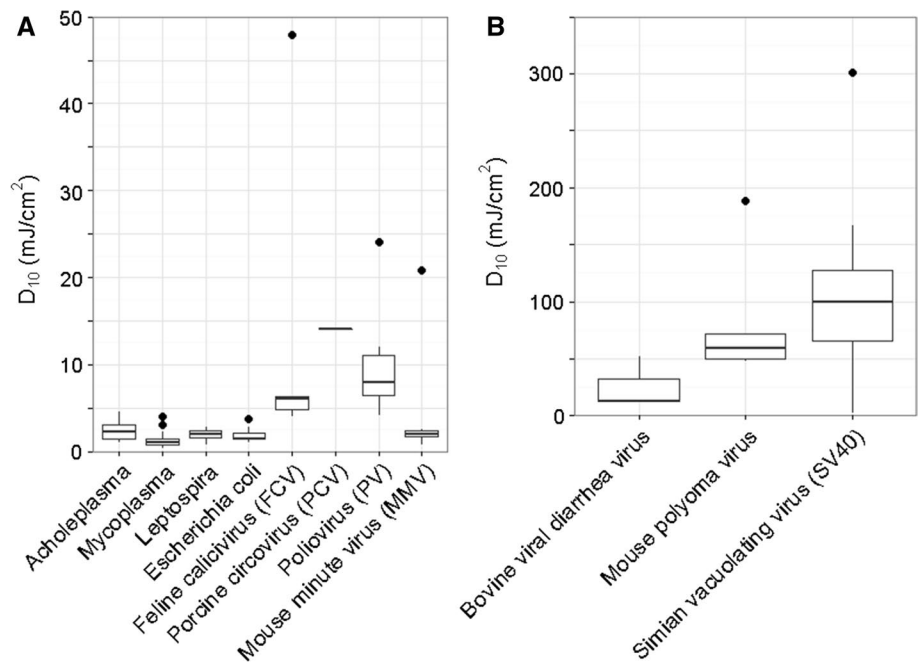

Fig. 5 D_{10} values grouped by nucleic acid type. For a better appreciation of the number of studies contributing to the conclusions around the relationship between D_{10} values and nucleic acid type, all data points have been included in this figure using the geom_jitter (http://docs.ggplot2.org/current/geom_jitter.html) command in ggplot2

sized for doses that would achieve more than 4-log inactivation of *E. coli* (a fluence of only 8 mJ/cm^2) the authors feel that this is inherently conservative. Where an individual author reported values for multiple strains, only a single average value was used. This was intended to avoid giving extra emphasis to any individual author and their own systematic approach. The average D_{10} value for *E. coli* is 1.9 mJ/cm^2, which confirms that this organism is highly susceptible to UV inactivation.

Feline calicivirus

Feline calicivirus has been studied by a number of investigators, and most find that it is relatively sensitive to UV inactivation, with an average D_{10} of about 5.3 mJ/cm^2 (Fig. 4a). The outlier in this case is the result from Nuanualsuwan et al. [77], who report a value that is 9 × higher. Their D_{10} values reported for φX174, hepatitis A virus, and poliovirus are all significantly higher than those of other researchers. There are several concerns with the methodology reported by Nuanualsuwan et al. [77] that would cause erroneous results. First, they use a long lamp (91 cm) very close (12.5 cm) to the petri dish to be irradiated. This geometry can make it very difficult to obtain accurate flux measurements, and can cause shadowing near the edges of the dish. Combining the geometry and the lack of agitation, poor fluence uniformity is likely. The data presented display significant tailing at high fluences (Fig. 1), consistent with a lack of uniformity. Additionally, to determine the D_{10} values, the researchers used a trend line over the entire range of fluences, but a single log reduction in the virus actually occurs at a much lower UV fluence than presented. Therefore, we do not consider the D_{10} values reported by Nuanualsuwan et al. [77] to be accurate.

Porcine circovirus

Another organism of concern is porcine circovirus (PCV), which was detected in a commercial rotavirus vaccine

(Rotarix®, GSK) [10], and can enter the biopharmaceutical production process through trypsin used in cell line development. Recent guidance from the European Medicines Agency [35] recommends the use of two complementary virus reduction steps for trypsin, including a final inactivation step of gamma, e-beam, or UV irradiation. UV sensitivity of PCV has been measured by Lackner et al. [58] and Remington [85], with a D_{10} range of 14–25 mJ/cm^2. Although this range is large as a percentage, the moderate sensitivity reported by all investigators indicates that PCV can be readily inactivated by UV.

Bovine viral diarrhea virus

There is very little consistency in the D_{10} values reported for bovine viral diarrhea virus (BVDV), with a range of D_{10} values from 11.8 to 366 mJ/cm^2 (Fig. 4b). Three of the five studies report a D_{10} value less than 20 mJ/cm^2. The value of 366 mJ/cm^2 reported by Daryany et al. [29] is remarkably high. Their experimental apparatus involved a UV lamp placed very close (10 cm) to the sample, and their methodology does not indicate the absorbance of the fluid or the presence of agitation, suggesting that the fluid was not stirred and that optical absorbance was not considered. These factors would lead to a lack of uniformity in fluence delivery and cause portions of the fluid to evade irradiation, increasing the apparent D_{10}. The true D_{10} would be lower, but cannot be determined from the published information. A D_{10} value of 52 mJ/cm^2 was reported by Steinmann et al. [98]. The fluid in this case was a platelet concentrate, which would have higher absorbance compared to the typical phosphate buffer. Minimal experimental information was provided for this study, and there was no indication that the fluid absorbance was considered in the calculated fluence delivery. Again, this would falsely increase the apparent resistance to UV disinfection, and the method would not correctly measure the inherent UV sensitivity. Based on these uncertainties, a consensus D_{10} value of 12.2 mJ/cm^2 is recommended, but additional studies are warranted.

Mouse minute virus

One particularly important adventitious agent is mouse minute virus (MMV), which is a potential contaminant in biopharmaceutical production, and is known to be resistant to physico-chemical treatment [16]. It is very sensitive to UV inactivation, with a consensus D_{10} of about 1.8 mJ/cm^2 (Fig. 4a). The outlier in this data is the report of Schleh et al. [90]. In this publication, full viral clearance was achieved, so the actual D_{10} value is lower than the calculated value. Also, minimal information about the experiments was provided, making it difficult to critically assess

the methodology (the same is true for the D_{10} value of Cache Valley virus determined from the same publication).

Mouse polyoma virus

For mouse polyoma virus (MPyV), there are four publications that report similar results, with a consensus D_{10} value of 57 mJ/cm^2, but Heberman and Ting [47] report a value roughly three times higher (Fig. 4b). Heberman and Ting [47] report the surface energy as the fluence and do not consider the absorbance of the fluid, both of which could lead to erroneous D_{10} values that are likely too high.

Simian vacuolating virus

The results for simian vacuolating virus type 40 (SV40) represent an interesting case since there have been numerous UV irradiation studies with a wide range of D_{10} values reported (Fig. 4b). There is an outlier on both the high- and low-sensitivity side. The average D_{10} without these two values is 92.8 mJ/cm^2, compared with the low of only 1.8 mJ/cm^2 from Wang et al. [110] and a high value of 301 mJ/cm^2 for Bourre et al. [17]. The authors of the present review have measured the D_{10} for SV40 to be approximately 100 mJ/cm^2 [89], consistent with the consensus literature value. SV40 may represent a worst case design organism for UV inactivation, with the highest resistance to UV inactivation of the organisms considered.

Correlations with nucleic acid

In contrast to previous assumptions by other authors about UV irradiation, such as single-stranded genomes being more sensitive to UV than double-stranded genomes [68], it is evident (Fig. 5) that UV resistance does not simply correlate with the overall nature of the genome. The most resistant viruses have a range of genome types: SV40 (ssDNA, 92.8 mJ/cm^2), mouse polyoma virus (ssDNA, 57.0 mJ/cm^2), and adenovirus (dsDNA, 42.3 mJ/cm^2). Therefore, the UV sensitivity cannot be simply correlated to nucleic acid conformation. More successful models, such as the proprietary model from Kowalski [56], take into account more details including locations and frequency of pyrimidines.

Discussion and conclusions

Although UV disinfection has been broadly applied industrially for both drinking water (high UV transparency) and municipal wastewater (lower UV transparency), it is not widely accepted for biopharmaceutical applications. The only commercially available UV system designed for biopharmaceuticals, Sartorius UVivatec, is too large for most

cell line development and too small for most full-scale batch production.

Compared to drinking water and wastewater, UV treatment of cell culture media is complicated by the opacity of the fluid as well as the requisite to minimize the UV dose delivery to avoid damaging essential components of the media. Therefore, biopharmaceutical applications would necessitate custom-designed UV equipment. Similarly designed systems for fluids with high optical absorbance and stringent regulations are those for apple cider (the FDA-approved Cidersure by Headwater Foods, Rochester NY) and sugar solutions (Aquafine LS HX by Aquafine Corporation, Valencia CA).

As the biopharmaceutical industry progresses to disposable production equipment, UV technology could be well disposed to provide a sterile, disposable disinfection system. The authors of the present review have been using a family of prototype reactors in which the wetted part consists of disposable Teflon, and the design has been scaled from internal volumes of 27–500 mL [70] with corresponding increases in flow. The same design could be readily scaled further, achieving a range of disposable UV reactors suitable for perfusion, or for parallel batch production. Knowledge of the UV sensitivity of adventitious agents is crucial for efficient sizing and operation of UV reactors for biopharmaceutical applications.

High log reductions are readily achievable with UV disinfection. In water treatment, phages are commonly used for verifying UV fluence [106]. Phage stocks such as MS2 or T1UV are available at titres of up to 10^{11} PFU/ml and are inoculated into test fluids that are then treated with UV equipment. Typical titres of 10^8 PFU/ml may be fully inactivated, or more commonly the test is designed to have a countable titre after treatment, so that the fluence can be calculated from the log inactivation and known UV sensitivity of the phage [65]. Particle-association is one of the primary causes of deviation from first-order kinetics with phage and other virus. If the fluid to be treated, such as a cell culture medium, does not contain particles that could shelter viruses, then first-order kinetics can be expected up to 8 $\log_{10}$ inactivation or more.

The present review has found that experimentally determined 254 nm sensitivity values for many adventitious agents in the biopharmaceutical industry have been reported and are fairly consistent between different research groups. Based on the experimental data available in the literature, outliers and explanations for the inconsistent results have been highlighted. Reviewing the available data, average D_{10} sensitivity values have been established that can be used to design and size UV disinfection systems to inactivate specific agents. Additionally, some of the common pitfalls in experimental design of UV treatment have been identified, such as neglecting to consider the fluid absorbance, not having a proper light distribution on the sample surface, and considering only the fluence intensity at the surface of the sample rather than within the liquid.

Most of the adventitious agents that have been identified as concerns for CHO cell processes are included in this review, but reliable UV sensitivity data for the following agents were not readily available and merit further study: bluetongue virus, bovine coronavirus, bovine polyoma virus, bovine respiratory syncytial virus, epizootic hemorrhagic disease virus, monkey pox virus, mycobacteria, and vesivirus 2117.

Appendix

See Table 3

Table 3 Reported dose per $\log_{10}$ inactivation (D_{10}) of adventitious agents

Family	Adventitious agent	Serotype/strain	D_{10} (mJ/cm^2)	References
Bacteria—Acholeplasmataceae	*Acholeplasma*		<14.3	[57]
		Laidlawii, late logarithmic	1	[30]
		Laidlawii, stationary	1.5	[30]
		Laidlawii	4.5	[41]
		Laidlawii JA1	3	[76]
		Laidlawii JA1 REP-	2.25	[76]
Bacteria—Clostridiaceae	*Clostridium pasteurianum*		3.4	[26]
	Clostridium perfringens		≤42	[92]
Bacteria—Enterobacteriaceae	*Escherichia coli*		1.5	[103]
			2.1	[23]
			1.25	[45]
			1.9	[121]
			2.8	[50]
			2	[95]
			1.4	[96]
			2.5	[115]
			1.4	[80]
			3.7	[100]
			1	[116]
			1.4	[114]
			1.5	[102]
	Salmonella anatum		5	[102]
	Salmonella derby		3.8	[102]
	Salmonella eteritidis		2.5	[102]
	Salmonella infantis		2	[102]
	Salmonella spp.	5 strains	1.8	[116]
	Salmonella typhi	ATCC6539	1.8	[23]
	Salmonella typhi	ATCC19430	2.1	[114]
	Salmonella typhi		2.3	[102]
	Salmonella typhi		12.5	[67]
	Streptococcus faecalis		5.5	[45]
Bacteria—Mycoplasmataceae	Mycoplasma		1	[38]
			<14.3	[57]
		Arthritidis	0.7	[38]
		Fermentans	0.9	[38]
		Hominis type 1	0.7	[38]
		Orale type 1	1.1	[38]
		Orale type 2	0.6	[38]
		Pneumoniae	0.8	[38]
		Salivarium	1.1	[38]
		Orale	1.1	[37]
		T210	0.3	[39]
		T-Pi	0.4	[39]
		T960	0.6	[39]
		Orale	2.2	[5]
		Buccale	0.75	[5]
		Hominis	1.2	[5]
		Fermentans	2.1	[5]

Table 3 continued

Family	Adventitious agent	Serotype/strain	D_{10} (mJ/cm^2)	References
		Salivarium	1.8	[5]
		Gallisepticum A5969	3	[42]
		Gallisepticum A5969	4	[41]
Bacteria—Pseudomonadaceae	*Pseudomonas aeruginosa*	PAO-1	2.4	[21]
		ATCC14207	3.7	[2]
		ATCC15442	3.8	[2]
		ATCC9027	3.8	[2]
		ATCC10145	4.6	[2]
		ATCC27853	4.9	[2]
Bacteria—Spirochaete	*Leptospira biflexa* Patoc		2.3	[97]
	Leptospira illini 3055		2.75	[97]
	Leptospira interrogans		0.75	[97]
	Leptospira biflexa Copenhagenii		1.8	[36]
Bacteria—Staphylococcaceae	*Staphylococcus aureus*	112	1.3	[43]
		112	3.4	[43]
		RN1349	2	[8]
			3.2	[100]
		RN1349	4	[8]
Prion	Bovine spongiform encephalopathy		Resistant	[55]
Adenoviridae	Adenovirus	41	23.6	[69]
		40	30	[69]
		1	34.5	[79]
		6	38.5	[79]
		2	39.7	[40]
		2	40	[79]
		5 and 2	40	[34]
		2	42	[62]
		5 and 7	42.5	[22]
		40	50	[101]
			54	[110]
		40	54.3	[79]
		5	61	[52]
	Junin virus		3	[64]
	Lassa virus		3	[64]
Bunyaviridae	Cache Valley virus (CVV)		20.8	[90]
	Hanta virus		2.8	[64]
	Rift Valley fever virus		2.8	[64]
Caliciviridae	Canine calicivirus (CaCV)	48	6.6	[31]
	Feline calicivirus (FCV)		4	[31]
			4.8	[103]
			6	[101]
			6.3	[81]
			47.9	[77]
	Murine norovirus (MNV)		7.3	[81]
			9.3	[60]
	Porcine circovirus (PCV)	1	14	[58]
		2	18 to 25	[85]

Table 3 continued

Family	Adventitious agent	Serotype/strain	D_{10} (mJ/cm^2)	References
Filoviridae	Ebola virus		1.7	[64]
	Marburg virus		1.7	[64]
Flaviviridae	Bovine viral diarrhea virus		≤17	[7]
			11.8	[100]
			12.5	[57]
			52	[98]
			366	[29]
	West Nile virus		5.5	[64]
Herpesviridae	Bovine herpesvirus 1		16.7	[57]
	Cytomegalovirus (CMV)	Murine	4.6	[93]
		C87 and AD169	5	[4]
	Pseudorabies virus		8	[100]
Leviviridae	Bacteriophage MS2		13.6	[103]
			14	[69]
			15.9	[60]
			16.4	[84]
			17.5	[81]
			21.7	[31]
			23	[78]
			23	[101]
			28.9	[79]
Microviridae	φX174 phage		2.1	[84]
			2.3	[9]
			2.5	[95]
			15.5	[77]
Orthomyxoviridae	Influenza A virus	H5N1	4.5	[61]
Paramyxoviridae	Bovine parainfluenza virus	Type III	≤48.2	[7]
	Mumps virus		Weak	[25]
	Parainfluenza Virus	3	14.3	[57]
Parvoviridae	Bovine parvovirus		12.5	[57]
	Mouse minute virus (MMV)		0.8	[46]
			1.7	[11]
			1.7	[87]
			1.7	[86]
			2.2	[108]
			2.3	[58]
			2.5	[111]
			20.8	[90]
	Parvovirus H1		23	[27], [28]
	Porcine parvovirus (PPV)		<18.2	[57]
			1.8	[58]
			2.3	[110]
Picornaviridae	Cardiovirus A—Encephalomyocarditis virus (EMCV)		4	[20]
			5.1	[84]
			6.6	[120]

Table 3 continued

Family	Adventitious agent	Serotype/strain	D_{10} (mJ/cm^2)	References
	Coxsackievirus	B5	7.3	[9]
		B3	8.2	[40]
		B5	9	[40]
		A9	11.9	[49]
		B1	15.6	[49]
	Echovirus	2	6.8	[40]
		12	7.4	[81]
		1	8.3	[40]
		1	10.8	[49]
		11	12.2	[49]
	Foot and mouth disease virus		12.5	[57]
		A132	19.7	[78]
		A-Sakol	22.1	[78]
		O189	25.2	[78]
		AS1	31.3	[78]
	Hepatitis A virus (HAV)	HM-175	4	[9]
			4.5	[110]
			7.5	[112]
			9.2	[109]
			36.5	[77]
	Poliovirus (PV)	1	4.1	[69]
			4.7	[103]
			6.5	[45]
		1	7.7	[40]
		1	8	[79]
		3	10.3	[49]
		1	11	[49]
		2	12	[49]
		1	24.1	[77]
Polyomaviridae	Mouse polyoma virus		47.6	[107]
			50	[59]
			58.8	[32]
			71.4	[84]
			188.7	[47]
	Simian vacuolating virus (SV40)		1.8	[110]
			21.3	[22]
			43.2	[14]
			65	[88]
			72	[1]
			80	[32]
			100	[100]
			112	[52]
			127	[19]
			140	[28]
			167	[91]
			301	[17]
			100	[89]

Table 3 continued

Family	Adventitious agent	Serotype/strain	D_{10} (mJ/cm^2)	References
Poxviridae	Variola Virus		2.5	[64]
Reoviridae	Reovirus	3	13.4	[119]
			15.4	[49]
			16.5	[45]
			18.5	[110]
		2	22.7	[68]
		1	24	[68]
		3	25	[57]
		3	26.3	[68]
			39.6	[94]
Retroviridae	Murine leukemia virus	Moloney	16.9	[75]
		Rauscher	17.3	[99]
		Friend	25	[118]
		Rauscher	46	[63]
		Moloney	18	[53]
			24.9	[71]
			26	[110]
Rhabdoviridae	Vesicular stomatitis virus		1	[52]
			1.2	[84]
			4.8	[100]
Togaviridae	Semliki forest virus		11.1	[113]
	Sindbis virus		4.2	[119]
			10	[110]
	Venezuelan equine encephalitis virus (VEEV)		5.3	[64]
	Western equine encephalitis virus (WEEV)		4.4	[64]

References

1. Aaronson SA (1970) Effect of ultraviolet irradiation on the survival of simian virus 40 functions in human and mouse cells. J Virol 6(4):393–399
2. Abshire RL, Dunton H (1981) Resistance of selected strains of *Pseudomonas aeruginosa* to low-intensity ultraviolet radiation. Appl Environ Microbiol 41:1419–1423
3. Ailworth E, Weisman R (2009) Virus shuts Genzyme plant, holds up drugs for 8000. Boston Globe. http://www.boston.com/business/healthcare/articles/2009/06/17/genzyme_temporarily_halts_production_on_2_key_drugs/. Accessed Nov 2015
4. Albrecht T, St. Jeor SC, Funk FD, Rapp F (1974) Multiplicity reactivation of human cytomegalovirus inactivated by ultra-violet light. Int J Radiat Biol Relat Stud Phys Chem Med 26(5):445–454
5. Aoki S, Ito S, Watanabe T (1979) UV survival of human mycoplasmas: evidence of dark reactivation in mycoplasma buccale. Microbiol Immunol 23(3):147–158
6. Aranha H (2011) Virus safety of biopharmaceuticals: absence of evidence is not evidence of absence. Contract Pharma 13:82–87
7. Bae JE, Jeong EK, Lee JI, Kim IS, Kim JS (2009) Evaluation of viral inactivation efficacy of a continuous flow ultraviolet-C reactor (UVivatec). Korean J Microbiol Biotechnol 37(4):377–382
8. Barreto HM, Siqueira-Junior JP (2006) Protective effect of furocoumarins against 254-nm ultraviolet in *Staphylococcus aureus*. Curr Microbiol 52(1):40–44
9. Battigelli D, Sobsey M, Lobe D (1993) The inactivation of hepatitis A virus and other model viruses by UV irradiation. Wat Sci Technol 27(3–4):339–342
10. Baylis SA, Finsterbusch T, Bannert N, Blümel J, Mankertz A (2011) Analysis of porcine circovirus type 1 detected in Rotarix vaccine. Vaccine 29(4):690–697
11. Bergmann K (2014) UV-C irradiation: a new viral inactivation method for biopharmaceuticals. Amer Pharm Rev 17(6)
12. Berting A, Farcet MR, Kreil TR (2010) Virus susceptibility of Chinese hamster ovary (CHO) cells and detection of viral contaminations by adventitious agent testing. Biotechnol Bioeng 106(4):598–607
13. Blatchley ER III, Chan P, Lee CC, Mofidi A, Scheible OK, Shen C (2008) Validation of medium-pressure UV disinfection reactors by Lagrangian actinometry using dyed microspheres. Water Res 43(5):1370–1380
14. Bockstahler LE, Lytle CD (1977) Radiation enhanced reactivation of nuclear replicating mammalian viruses. Photochem Photobiol 25(5):477–482
15. Bolton JR, Linden KG (2003) Standardization of methods for fluence (UV dose) determination in bench-scale UV experiments. J Environ Eng 129(3):209–215
16. Boschetti N, Wyss K, Mischler A, Hostettler T, Kempf C (2003) Stability of minute virus of mice against temperature and sodium hydroxide. Biologicals 31(3):181–185
17. Bourre F, Benoit A, Sarasin A (1989) Respective roles of pyrimidine dimer and pyrimidine (6-4) pyrimidone photoproducts in

UV mutagenesis of simian virus 40 DNA in mammalian cells. J Virol 63(11):4520–4524

18. Braslavsky SE (2007) Glossary of terms used in photochemistry 3rd edition. J Pure Appl Chem 79(3):293–465

19. Brown TC, Cerutti PA (1986) Ultraviolet radiation inactivates SV40 by disrupting at least four genetic functions. EMBO J 5(1):197–203

20. Caillet-Fauquet P, Di Giambattista M, Draps ML, Sandras F, Branckaert T, De Launoit Y et al (2004) Continuous-flow UVC irradiation: a new, effective, protein activity-preserving system for inactivating bacteria and viruses, including erythrovirus B19. J Virol Methods 118(2):131–139

21. Cairns G, Kerr KG, Beggs CB, Sleigh PA, Mooney L, Keig P et al (2001) Susceptibility of *Burkholderia cepacia* and other pathogens of importance in cystic fibrosis to UV light. Lett Appl Microbiol 32(3):135–138

22. Cameron KR, Tomkins LM, Eglin RP, Ross LJN, Wildy P, Russell WC (1979) The effects of ultraviolet and ionizing radiation on herpesviruses, SV40 and adenoviruses in relation to the small-plaque effect. Arch Virol 62(1):31–40

23. Chang JCH, Osoff SF, Lobe DC, Dorfman MH, Dumais CM, Qualls RG et al (1985) UV inactivation of pathogenic and indicator microorganisms. Appl Environ Microbiol 49(6):1361–1365

24. Chen J, Bergevin J, Kiss R, Walker G, Battistoni T, Lufburrow P et al (2012) Case study: a novel bacterial contamination in cell culture production—*Leptospira licerasiae*. PDA J Pharm Sci Technol 66(6):580–591

25. Chu LW, Morgan HR (1950) Studies of the hemolysis of red blood cells by mumps virus I. The development of mumps virus hemolysin and its inactivation by certain physical and chemical agents. J Exp Med 91(4):393–402

26. Clauß M (2006) Higher effectiveness of photoinactivation of bacterial spores, UV resistant vegetative bacteria and mold spores with 222 nm compared to 254 nm wavelength. Acta Hydroch Hydrob 34(6):525–532

27. Cornelis JJ, Rommelaere J (1982) Direct and indirect effects of ultraviolet light on the mutagenesis of parvovirus H-1 in human cells. EMBO J 1(6):693–699

28. Cornelis JJ, Su ZZ, Ward DC, Rommelaere J (1981) Indirect induction of mutagenesis of intact parvovirus H-1 in mammalian cells treated with UV light or with UV-irradiated H-1 or simian virus 40. Proc Natl Acad Sci 78(7):4480–4484

29. Daryany MKA, Hosseini SM, Raie M, Fakharie J, Zareh A (2009) Study on continuous (254 nm) and pulsed UV (266 and 355 nm) lights on BVD virus inactivation and its effects on biological properties of fetal bovine serum. J Photoch Photobio B 94(2):120–124

30. Das J, Maniloff J, Bhattacharjee S (1972) Dark and light repair in ultraviolet irradiated acholeplasma laidlawii. Biochim Biophys Acta 259(2):189–197

31. de Roda Husman AM, Bijkerk P, Lodder W, Van Den Berg H, Pribil W, Cabaj A et al (2004) Calicivirus inactivation by nonionizing (253.7-nanometer-wavelength [UV]) and ionizing (gamma) radiation. Appl Environ Microbiol 70(9):5089–5093

32. Defendi V, Jensen F (1967) Oncogenicity by DNA tumor viruses. Science 157:703–705

33. Drexler HG, Uphoff CC (2002) Mycoplasma contamination of cell cultures: incidence, sources, effects, detection, elimination, prevention. Cytotechnology 39(2):75–90

34. Durance CS, Hoffman R, Andrews RC, Brown M (2005) Applications of ultraviolet light for inactivation of adenovirus. P Water Environ Fed 1:1–12

35. European Medicines Agency (2015) Guideline on the use of porcine trypsin used in the manufacture of human biological medicinal products. http://www.ema.europa.eu/docs/ en_GB/document_library/Scientific_guideline/2014/02/ WC500162147.pdf. Accessed Nov 2015

36. Fonseca LS, da Silva JB, Milanez JS, Monteiro-Vitorello CB, Momo L et al (2013) Leptospira interrogans serovar copenhageni harbors two lexA genes involved in SOS response. PLoS One 8(10):e76419

37. Furness G (1968) Analysis of the growth cycle of mycoplasma orale by synchronized division and by ultraviolet irradiation. J Infec Diseases 118(4):436–442

38. Furness G (1969) Differential responses of single cells and aggregates of mycoplasmas to ultraviolet irradiation. Appl Microbiol 18(3):360–364

39. Furness G (1975) T-Mycoplasmas: growth patterns and physical characteristics of some human strains. J Infect Dis 132(5):592–596

40. Gerba CP, Gramos DM, Nwachuku N (2002) Comparative inactivation of enteroviruses and adenovirus 2 by UV light. Appl Enviro Microbiol 168(10):5167–5169

41. Ghosh A, Das J, Manilof J (1977) Lack of repair of ultraviolet light damage in mycoplasms gallisepticum. J Mol Biol 116(2):337–344

42. Ghosh A, Das J, Manilof J (1978) Effect of acriliflavine on ultraviolet inactivation of *Acholeplasma laidlawii*. Biochim Biophys Acta 543(4):570–575

43. Goering RV, Pattee PA (1971) Mutants of *Staphylococcus aureus* with increased sensitivity to ultraviolet radiation. J Bacteriol 106(1):157–161

44. Goetschalckx S, Fabre V, Wynants M, Bertaux L, Plavsic M, Boussif O et al (2014) A holistic biosafety risk mitigation approach. Amer Pharm Rev 17(4):48–56

45. Harris GD, Adams VD, Sorenson DL, Curtis MS (1987) Ultraviolet inactivation of selected bacteria and viruses with photoreactivation of the bacteria. Water Res 21(6):687–692

46. Harris RE, Coleman PH, Morahan PS (1974) Stability of minute virus of mice to chemical and physical agents. Appl Microbiol 28(3):351–354

47. Herberman RB, Ting RC (1969) Dissociation of multiple polyoma virus functions by ultraviolet irradiation. Exp Biol Med 131(2):461–464

48. Hijnen WAM, Beerendonk EF, Medema GJ (2006) Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: a review. Water Res 40:3–22

49. Hill WF, Hamblet FE, Benton WH, Akin EW (1970) Ultraviolet devitalization of eight selected enteric viruses in estuarine water. Appl Microbiol 19(5):805–812

50. Hoyer O (1998) Testing performance and monitoring of UV systems for drinking water disinfection. Water Supply 16(1–2):424–429

51. Ju W, Moyne AL, Marco ML (2016) RNA-based detection does not accurately enumerate living *Escherichia coli* O157:H7 cells on plants. Front Microbiol 7:233

52. Kallenbach NR, Cornelius PA, Negus D, Montgomerie D, Englander S (1988) Inactivation of viruses by ultraviolet light. Curr St Hematol Blood Transfus 56:70–82

53. Kelloff G, Aaronson SA, Gilden RV (1970) Inactivation of murine sarcoma and leukemia viruses by ultra-violet irradiation. Virology 42:1133–1135

54. Kerr A, Nims R (2010) Adventitious viruses detected in biopharmaceutical bulk harvest samples over a 10 Year Period. PDA J Pharm Sci Tech 64(5):481–485

55. Kimberlin RH (1992) Bovine spongiform encephalopathy. Rev Sci Tech OIE 11(2):347–390

56. Kowalski W, Bahnfleth W, Hernandez M (2009) A genomic model for predicting the ultraviolet susceptibility of viruses. IUVA News 11(2):15–28

57. Kurth J, Waldmann R, Heith J, Mausbach K, Burian R (1998) Efficient inactivation of viruses and mycoplasma in animal sera using UVC irradiation. Devel Biol Stand 99:111–118

58. Lackner C, Leydold SM, Modrof J, Farcet MR, Grillberger L, Schäfer B et al (2014) Reduction of spiked porcine circovirus during the manufacture of a vero cell-derived vaccine. Vaccine 32(18):2056–2061

59. Latarjet R, Cramer R, Montagnier L (1967) Inactivation, by UV-, X-, and γ-radiations, of the infecting and transforming capacities of polyoma virus. Virology 33(1):104–111

60. Lee J, Zoh K, Ko G (2008) Inactivation and UV disinfection of murine norovirus with TiO$_2$ under various environmental conditions. Appl Environ Microbiol 74(7):2111–2117

61. Lénès D, Deboosere N, Ménard-Szczebara F, Jossent J, Alexandre V, Machinal C et al (2010) Assessment of the removal and inactivation of influenza viruses H5N1 and H1N1 by drinking water treatment. Water Res 44(8):2473–2486

62. Linden KG, Scheible K, Shen C, Shin GA, Lee JK, Posy P (2009) Demonstrating 4-log adenovirus inactivation in a medium pressure UV reactor. P Water Environ Fed 1:287–288

63. Lovinger GG, Ling HP, Gilden RV, Hatanaka M (1975) Effect of UV light on RNA directed DNA polymerase activity of murine oncornaviruses. J Virol 15(5):1273–1275

64. Lytle CD, Sagripanti JL (2005) Predicted inactivation of viruses of relevance to biodefense by solar radiation. J Virol 79(22):14244–14252

65. Mackey ED (2004) Bridging pilot-scale testing to full-scale design of UV disinfection systems. American Water Works Association

66. Masschelein WJ, Rice RG (2002) Ultraviolet light in water and wastewater sanitation. CRC Press, Boca Raton, FL, USA. ISBN 9781566706032

67. Maya C, Beltran N, Jimenez B, Bonilla P (2003) Evaluation of the UV disinfection process in bacteria and amphizoic amoebae inactivation. Water Supply 3(4):285–291

68. McClain ME, Spendlove RS (1966) Multiplicity reactivation of reovirus particles after exposure to ultraviolet light. J Bacteriol 92(5):1422–1429

69. Meng QS, Gerba CP (1996) Comparative inactivation of enteric adenoviruses, poliovirus and coliphages by ultraviolet irradiation. Water Res 30(11):2665–2668

70. Meunier SM, Todorovic B, Dare EV, Begum A, Guillemette S, Wenger A, Saxena P, Campbell JL, Sasges M, Aucoin MG (2016) Impact of dissolved oxygen during UV-irradiation on the chemical composition and function of CHO cell culture media. PLoS One 11(3):e0150957

71. Miller FP, Vandome AG, McBrewster J (2009) Beer-Lambert Law. VDM publishing, Saarbrucken, Germany. ISBN 6130200609, 9786130200602

72. Moody M, Alves W, Varghese J, Khan F (2011) Mouse minute virus (MMV) contamination—a case study: detection, root cause determination, and corrective actions. PDA J Pharm Sci Technol 65(6):580–588

73. Morowitz HJ (1950) Absorption effects in volume irradiation of microorganisms. Science 111(2879):229–230

74. Nims R, Presente E, Sofer G, Phillips C, Chang A (2005) Adventitious agents: concerns and testing for biopharmaceuticals. Biotech Bioprocess Ser 29:143

75. Nomura S, Bassin RH, Turner W, Haapala DK, Fischinger PJ (1972) Ultraviolet inactivation of moloney leukaemia virus: relative target size required for virus replication and rescue of "defective" murine sarcoma virus. J Gen Virol 14:213–217

76. Nowak J, Das J, Maniloff J (1976) Characterization of an *Acholeplasma laidlawii* variant with a REP- phenotype. J Bacteriol 127:832–836

77. Nuanualsuwan S, Mariam T, Himathongkham S, Cliver DO (2002) Ultraviolet inactivation of feline calicivirus, human enteric viruses, and coliphages. Photochem Photobiol 76(4):406–410

78. Nuanualsuwan S, Thongtha P, Kamolsiripichaiporn S, Subharat S (2008) UV inactivation and model of UV inactivation of foot-and-mouth disease viruses in suspension. Int J Food Microbiol 127(1):84–90

79. Nwachuku N, Gerba CP, Oswald A, Mashadi FD (2005) Comparative inactivation of adenovirus serotypes by UV light disinfection. Appl Environ Microbiol 71(9):5633–5636

80. Otaki M, Okuda A, Tajima K, Iwasaki T, Kinoshita S, Ohgaki S (2003) Inactivation differences of microorganisms by low pressure UV and pulsed xenon lamps. Wat Sci Technol 47(3):185–190

81. Park GW, Linden KG, Sobsey MD (2011) Inactivation of murine norovirus, feline calicivirus and echovirus 12 as surrogates for human norovirus (NoV) and coliphage (F+) MS2 by ultraviolet light (254 nm) and the effect of cell association on UV inactivation. Lett Appl Microbiol 52(2):162–167

82. Randazzo W, Lopez-Galvez F, Allende A, Aznar R, Sanchez G (2016) Evaluation of viability PCR performance for assessing norovirus infectivity in fresh-cut vegetables and irrigation water. Int J Food Microb 229:1–6

83. Rahn RO, Bolton J, Stefan MI (2006) The iodide/iodate actinometer in UV disinfection determination of the fluence rate distribution in UV reactors. Photochem Photobiol 82(2):611–615

84. Rauth AM (1965) The physical state of viral nucleic acid and the sensitivity of viruses to ultraviolet light. Biophys J 5(3):257–273

85. Remington K (2014) Use of porcine circovirus as a challenge for filtration and other virus risk mitigation steps. 17th Planova Workshop, Washington, DC, 12–13 Jun 2014

86. Rommelaere J, Ward DC (1982) Effect of UV-irradiation on DNA replication of the parvovirus minute-virus-of-mice in mouse fibroblasts. Nucleic Acids Res 10(8):2577–2596

87. Rommelaere J, Vos J-M, Cornelis JJ, Ward DC (1981) UV-enhanced reactivation of minute-virus-of-mice: stimulation of a late step in the viral life cycle. Photochem Photobiol 33:845–854

88. Sarasin AR, Hanawalt PC (1978) Carcinogens enhance survival of UV-irradiated simian virus 40 in treated monkey kidney cells: induction of a recovery pathway? Proc Natl Acad Sci 75(1):346–350

89. Sasges M, Remington K (2015) Clearance of adventitious agents from cell culture media in a flow-through UV-C reactor, presented at International Society for Bioprocess Technology Fall meeting Sept 2015

90. Schleh M, Lawrence B, Park T, Rosenthal S, Hart R, Dehghani H (2010) Effectiveness of upstream barrier technologies for inactivation of adventitious contaminants of cell culture. Amer Pharm Rev 13(7):72

91. Seemayer NH, Defendi V (1973) Analysis of minimal functions of simian virus 40 II. Enhancement of oncogenic transformation in vitro by UV irradiation. J Virol 12(6):1265–1271

92. Seltsam A, Müller TH (2011) UVC irradiation for pathogen reduction of platelet concentrates and plasma. Transfus Med Hemother 38(1):43–54

93. Shanley JD (1982) Ultraviolet irradiation of murine cytomegalovirus. J Gen Virol 63:251–254

94. Shaw JE, Cox DC (1973) Early inhibition of cellular DNA synthesis by high multiplicities of infectious and UV-inactivated reovirus. J Virol 12(4):704–710

95. Sommer R, Haider T, Cabaj A, Pribil W, Lhotsky M (1998) Time dose reciprocity in UV disinfection of water. Water Sci Technol 38(12):145–150

96. Sommer R, Lhotsky M, Haider T, Cabaj A (2000) UV inactivation, liquid-holding recovery, and photoreactivation of *E. coli* O157 and other pathogenic *E.coli* strains in water. J Food Prot 63(8):1015–1020

97. Stamm L, Charon N (1988) Sensitivity of pathogenic and free-living *Leptospira* to UV radiation and mitomycin C. Appl Environ Microbiol 54(3):728–733

98. Steinmann E, Gravemann U, Friesland M, Doerrbecker J, Müller TH, Pietschmann T, Seltsam A (2013) Two pathogen reduction technologies—methylene blue plus light and short-wave ultraviolet light—effectively inactivate hepatitis C virus in blood products. Transfusion 53(5):1010–1018

99. Stull H, Gazdar A (1976) Stability of Rauscher leukemia virus under certain laboratory conditions. P Soc Exp Biol Med 152:554–556

100. Terpstra FG, Van't Wout AB, Schuitemaker H, Van Engelenburg FA, Dekkers DW, Verhaar R et al (2008) Potential and limitation of UVC irradiation for the inactivation of pathogens in platelet concentrates. Transfusion 48(2):304–313

101. Thurston-Enriquez JA, Haas CN, Jacangelo J, Riley K, Gerba CP (2003) Inactivation of feline calicivirus and adenovirus type 40 by UV radiation. Appl Environ Microbiol 69(1):577–582

102. Tosa K, Hirata T (1998) Photoreactivation of *Salmonella* following UV disinfection. In: Proc IAWQ 19th Biennial Int Conf 10

103. Tree J, Adams M, Lees D (2005) Disinfection of feline calicivirus (a surrogate for norovirus) in wastewaters. J Appl Microbiol 98:155–162

104. Tukey JW (1970) Plots of Relationship. In: Exploratory data analysis (limited preliminary edition), vol. 1, Ch. 5. Addison-Wesley Publishing Co, Reading

105. Tukey JW (1977) Exploratory Data Analysis, 1st edn. Addison-Wesley Publishing Co, Reading

106. United States Environmental Protection Agency (2006) Ultraviolet disinfection guidance manual for the final long term 2 enhanced surface water treatment rule. EPA 815-R-06-007 Nov 2006

107. van der Eb AJ, Cohen JA (1967) The effect of UV-irradiation on the plaque-forming ability of single-and double-stranded polyoma virus DNA. Biochem Bioph Res Com 28(2):284–288

108. Vos JM, Cornelis JJ, Limbosch S, Zampetti-Bosseler F, Rommelaere J (1981) UV-irradiation of related mouse hybrid cells: similar increase in capacity to replicate intact minute-virus-of-mice but differential enhancement of survival of UV-irradiated virus. Mutat Res 83(2):171–178

109. Wang C-H, Tschen S-Y, Flehmig B (1995) Antigenicity of hepatitis A virus after ultra-violet irradiation. Vaccine 13(9):835–840

110. Wang J, Mauser A, Chao SF, Remington K, Treckmann R, Kaiser K, Pifat D, Hotta J (2004) Virus inactivation and protein recovery in a novel ultraviolet-C reactor. Vox Sang 86(4):230–238

111. Weaver B, Rosenthal S (2010) Viral risk mitigation for mammalian cell culture media. PDA J Pharm Sci Tech 64(5):436–439

112. Weidenmann A, Fischer B, Straub U, Wang C-H, Flehmig B, Schoenen D (1993) Disinfection of hepatitis A virus and MS-2 coliphage in water by ultraviolet irradiation: comparison of UV-susceptibility. Water Sci Technol 27(3–4):335–338

113. White J, Matlin K, Helenius A (1981) Cell fusion by Semliki Forest, influenza, and vesicular stomatitis viruses. J Cell Biol 89(3):674–679

114. Wilson BR, Roessler PF, Van Dellen E, Abbaszadegan M, Gerba CP (1992) Coliphage MS-2 as a UV water disinfection efficacy test surrogate for bacterial and viral pathogens. In: Proceedings of the water quality technol ogy conference. American Water Works Association, Toronto, Ontario, Canada 15–19 Nov 1992

115. Wu Y, Clevenger T, Deng B (2005) Impacts of goethite particles on UV disinfection of drinking water. Appl Environ Microbiol 71(7):4140–4143

116. Yaun BR, Sumner SS, Eifert JD, Marcy JE (2003) Response of *Salmonella* and *E. coli* O157: H7 to UV energy. J Food Protect 66(6):1071–1073

117. Yen S, Sokolenko S, Manocha B, Patras A, Daynouri-Pancino F, Blondeel EJM, Sasges M, Aucoin MG (2014) Treating cell culture media with UV irradiation against adventitious agents: minimal impact on CHO performance. Biotechnol Prog 30(5):1190–1195

118. Yoshikura H (1971) Ultraviolet inactivation of murine leukemia and sarcoma viruses. Int J Cancer 7:131–140

119. Zavadova Z, Libikova H (1975) Comparison of the sensitivity to ultraviolet irradiation of reovirus 3 and some viruses of the Kemerovo group. Acta Virol 19:88–90

120. Zavadova Z, Gresland L, Rosenbergova M (1968) Inactivation of single- and double-stranded ribonucleic acid of encephalomyocarditis virus by ultraviolet light. Acta Virol 12(6):515–522

121. Zimmer JL, Slawson RM (2002) Potential repair of E. coli DNA following exposure to UV radiation from both medium- and low-pressure UV sources used in drinking water treatment. Appl Environ Microbiol 68(7):3293–3299