Inactivation of Viruses by UVC Treatment

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INTRODUCTION

Biopharmaceutical purification processes frequently include a step that is intended to inactivate any viruses that could potentially contaminate the process during production. Most commonly used inactivation steps have limited effectiveness against non-enveloped viruses. Recent improvements in the technology for UV inactivation have made it practical to include this technology in purification processes. UV treatment is known to inactivate parvoviruses; however, there is limited data on the effectiveness of UVC inactivation for different viruses. This study was undertaken to evaluate the sensitivity of a number of different viruses to UVC inactivation under a variety of conditions.

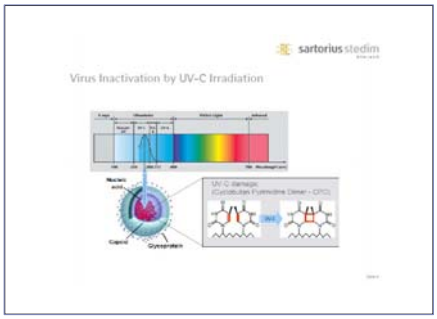
METHODS

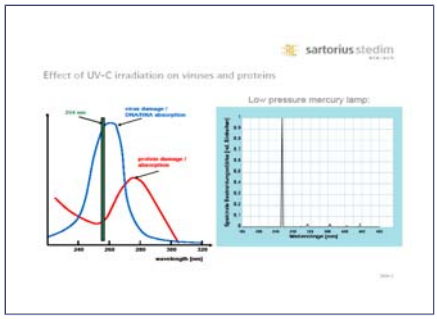
Viruses from seven different families were tested for their sensitivity to UVC inactivation. BSA was used as a model protein. The effects of protein concentration and buffer conditions were also evaluated.

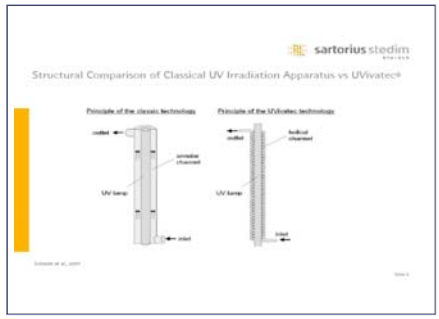
Virus	Family	Size (nm)	Genome	Envelope	Strand	Genome size (kb)
Minute Mouse Virus (MMV)	Parvo	18-24	DNA	No	SS	4-6
Porcine Parvovirus (PPV)	Parvo	18-24	DNA	No	SS	4-6
Polio	Picorna	25-32	RNA	No	SS	7-8
Bovine viral diarrhea virus (BVDV)	Flavi	50-70	RNA	Yes	SS	10-11
Reovirus type 3 (Reo 3)	Reo	60-80	RNA	No	DS	18-30
Adenovirus type 5 (Ad5)	Adeno	90-100	DNA	No	DS	26-46
Xenotropic Murine Leukemia Virus (XMuLV)	Retro	80-110	RNA	Yes	SS	10-11
Pseudorabies virus (PRV)	Herpes	120-200	DNA	Yes	DS	150-200

The UVC inactivation studies were performed using the UVivatec® from Sartorius-Stedim Biotech. The UV lamp provides monochromatic light at 254 nm, which targets primarily nucleic acids rather than protein. Mechanism of action is through the formation of pyrimidine dimers. The reactor provides efficient mixing by pumping through a helical flow cell that generates Dean vortices, ensuring a consistent UV dose through the entire volume of material. UV dose is controlled by the lamp power and the flow rate.

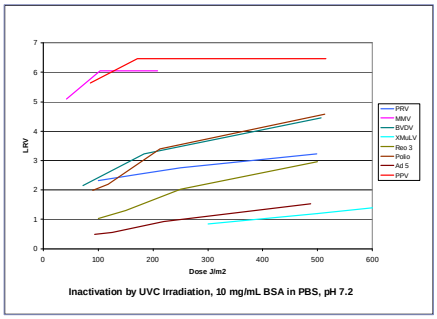
Slides courtesy of Sartorius-Stedim Biotech

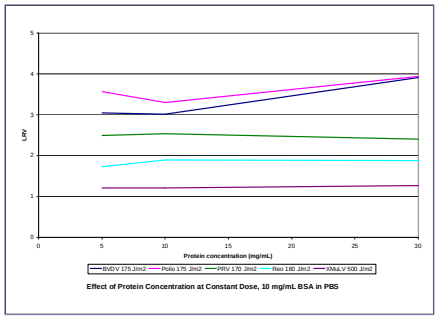


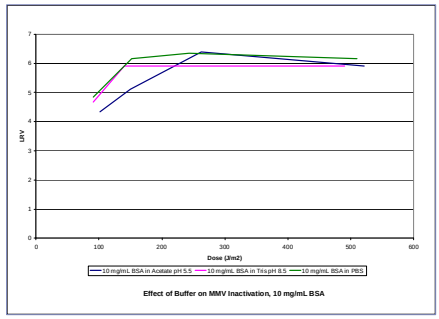


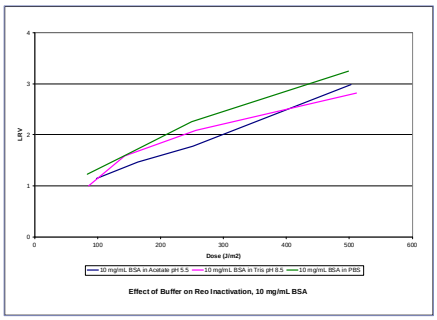


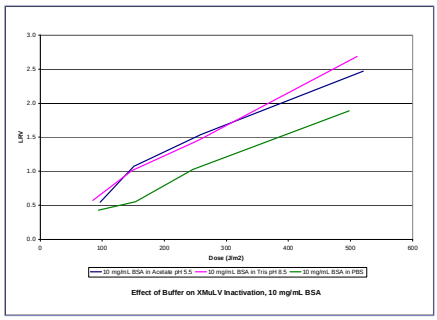
RESULTS

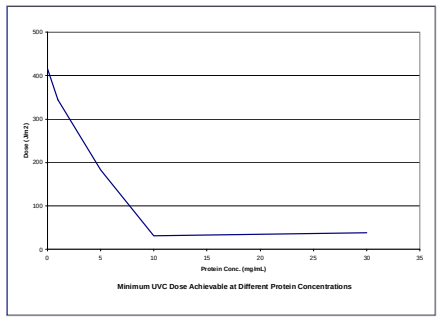












CONCLUSIONS

- Parvoviruses are extremely sensitive to inactivation by UVC treatment. This is important, since it is difficult to inactivate these viruses by any other means.
- Other viruses are also inactivated effectively; however, there is significant variation in sensitivity to UVC inactivation. The degree of sensitivity does not correlate with any obvious property of the virus, including size, envelope, genome type or size. The sensitivity may be in part dependent on the frequency of sequential pyrimidines.
- There is no significant effect of protein concentration or buffer composition on sensitivity to UVC inactivation. Therefore, UVC inactivation is a highly robust technique that can be performed under a variety of process conditions.
- UVC dosage is dependent upon the Absorbance of the material at 254 nm. A lower Absorbance results in a higher dose. As a result, there is a minimum dosage that can be achieved based upon the protein concentration. Also, at very high Absorbance there is a limit of the maximum dose that can be achieved.
- Since proteins absorb to some extent at 254 nm, protein damage does occur. Product aggregation has been seen, although other effects may also occur. Damage is dose-dependent, and doses of 200 J/m2 can usually be tolerated with little damage.
- UVC inactivation can be extremely useful for challenging products where it is difficult to obtain sufficient viral clearance (such as IgM or VLP products).
- UVC inactivation may also be useful for other products as well, where it may be used to obtain additional clearance of parvoviruses or to replace a more complex process step.
- UVC treatment is a flow-through process, and is therefore relatively easy to add to a purification process. It has very few critical process parameters, and can be used under a wide range of conditions.
- The optimal placement of this step in a purification process would be downstream of the capture step, as a high Absorbance may limit the dosage that may be obtained. UVC should be placed upstream of polishing steps, so that any altered product may be removed.
- UVC treatment can also be used for treatment of raw materials. Medium is not likely to be affected by UVC irradiation, which allows the use of a high dose. Since it is a flow-through operation, it is easily compatible with medium addition.