bacteria, from the medium. Such filter fouling can lead to inability to complete the medium processing step prior to the cell culture process. In some instances the precipitate may also impact the performance of the cell culture media and prevent efficient production of biologic drugs from the cultured cell lines. To prevent precipitation, the temperature can be lowered but successful viral inactivation may be negatively affected. Furthermore, precipitate formation during HTST cell media treatment can result in frequent cleaning or repair of equipment used for HTST treatment during the manufacturing process which contributes significantly to the cost of processing. Therefore, there is a need for methods to prevent precipitate formation during HTST treatment without adversely affecting the efficacy of this treatment in the removal or inactivation of viral contaminants.

[0005] The invention described herein addresses these needs by providing methods to effectively inactivate viral contaminants in cell culture media using HTST treatment with adjusted processing parameters that results in the reduction or prevention of precipitate formation.

[0006] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

~~BRIEF~~ SUMMARY ~~OF THE INVENTION~~

[0007] The disclosure~~invention~~ provides for methods, processes, systems and compositions for inactivating viral contamination and/or other contaminants in cell culture media by using high temperature short time (HTST) treatment in combination with adjustments of various parameters, such as pH and/or calcium and/or phosphate concentration in the media. Furthermore, methods, processes, systems and compositions reducing the fouling of equipment and filters used for HTST treatment are provided as well.

[0008] Accordingly, in one aspect, ~~the invention~~there is provided~~s~~ ~~for~~a method~~s~~ for inactivating virus or adventitious agents in cell culture media while the media maintains suitability for cell culture, said method comprising (a) subjecting the cell culture media to high temperature short time (HTST) treatment; and (b) adjusting one or more parameters selected from the group consisting of pH, calcium level and phosphate level.

[0009] In other ~~aspects~~embodiments, the ~~invention~~ disclosure provides for methods for inactivating virus in cell culture media comprising subjecting the cell culture media to high temperature short time (HTST) treatment wherein the media has a pH of between about pH 5.0 to about pH 6.9 during HTST treatment. In another embodiment~~aspect~~, the disclosure~~invention~~ provides for methods for inactivating virus in cell culture media comprising subjecting the cell culture media to high

temperature short time (HTST) treatment wherein the media has a pH of between about pH 5.0 to about pH 7.2 during HTST treatment. In some embodiments, the media has a pH of between about pH 5.3 to about pH 6.3 during HTST treatment. In other embodiments, the media has a pH of about pH 6.0 during HTST treatment. In any of the embodiments, the HTST treatment comprises raising the temperature of the media to at least about 85 degrees Celsius for a sufficient amount of time to inactivate the virus or potential virus in the media. In some embodiments, the temperature of the media is raised to at least about 93 degrees Celsius for a sufficient amount of time to inactivate the virus or potential virus in the media. In some embodiments, the temperature of the media is raised to at least about 95, 97, 99, 101 or 103 degrees Celsius for a sufficient amount of time to inactivate the virus or potential virus in the media. In some embodiments, the pH of the media is lowered to between about pH 5.0 to about pH 6.9 during HTST treatment prior to polypeptide production phase. In some embodiments, the pH of the media is then brought to between about 6.9-7.2 for the polypeptide production phase.

[0010] In another embodiment~~aspect~~, the disclosure~~invention~~ provides methods for inactivating virus in cell culture media comprising limiting the total amount of phosphate and calcium in the media to less than about 10 mM during HTST treatment. In some embodiments, the total phosphate and calcium concentration in the media is limited to less than about 9, 8, 7, 6, 5, 4, 3, 2, or 1 mM during HTST treatment. In some embodiments, the total amount of phosphate and calcium in the media is limited to less than about 10 mM during HTST treatment prior to polypeptide production phase. In some embodiments, the total amount of phosphate and calcium in the media is then raised to a level sufficient for the polypeptide production during the protein production phase.

[0011] In another embodiment~~aspect~~, the disclosure~~invention~~ provides methods for reducing fouling of equipment used for HTST treatment to inactivate virus, the method comprising subjecting cell culture media used in the equipment to high temperature short time (HTST) treatment wherein the media has a pH of between about pH 5.0 to about pH 6.9 during HTST treatment. In some embodiments, the media has a pH of between about pH 5.3 to about pH 6.3 during HTST treatment. In some embodiments, the media has a pH of about pH 6.0 during HTST treatment. In some embodiments, the fouling comprises precipitation on equipment used for HTST treatment. In any of the embodiments, the HTST treatment comprises raising the temperature of the media to at least about 85 degrees Celsius for a sufficient amount of time to inactivate the virus in the media. In some embodiments, the temperature of the media is raised to at least about 93 degrees Celsius for a sufficient amount of time to inactivate the

virus in the media. In some embodiments, the temperature of the media is raised to at least about 95, 97, 99, 101 or 103 degrees Celsius for a sufficient amount of time to inactivate the virus in the media.

[0012] In another embodimentaspect, the disclosureinvention provides methods for reducing fouling of equipment used for HTST treatment to inactivate virus, the method comprising limiting the total amount of phosphate and calcium in cell culture media used in the equipment to less than about 10 mM during HTST treatment. In some embodiments, the total phosphate and calcium concentration in the media is limited to less than about 9, 8, 7, 6, 5, 4, 3, 2, or 1 mM during HTST treatment. In some embodiments, the fouling comprises precipitation on equipment used for HTST treatment. In any of the embodiments, the virus is selected from the group consisting of parvoviridae, paramyoxviridae, orthomyxoviridae, bunyaviridae, rhabdoviridae, reoviridae, togaviridae, caliciviridae, and picornaviridae. In any of the embodiments, the virus is an enveloped virus. In any of the embodiments, the virus is a non-enveloped virus.

BRIEF DESCRIPTION OF THE DRAWINGS

[0013] Figure 1 is a schematic of a representative HTST skid used in manufacturing.

[0014] Figure 2 is a graph depicting heating profiles from the sand-bath method and HTST treatment to the 102°C target set point. The traces show the heating profiles in minutes where the end-point of each trace was described by the time and temperature at the end-point (e.g. end-point value of "6.2, 102" = end-point value at 6.2 minutes was at 102 °C).

[0015] Figure 3 is a picture of media samples known to precipitate during HTST treatment after heat treatment by the sand-bath method. **A)** Uncentrifuged samples of Media 1 at pH 7.0 or pH 6.4, and Media 2 at pH 7.0 or pH 6.7 in heat treated pressure vessels. **B)** Centrifuged aliquots of Media 1 at pH 7.0 or pH 6.4, and Media 2 at pH 7.0 or pH 6.7 from the treated pressure vessels.

[0016] Figure 4 depicts sorted parameter estimates from Media 4 based media formulations for parameter terms that correlated with a greater level of sand-bath treated sample turbidity.

[0017] Figure 5 is a series of graphs depicting precipitation response surface as measured by turbidity (NTU) from 3 perspectives for Media 4 based formulations with varied calcium, phosphate, and pH levels during sand-bath heat treatment. Portions of each perspective-plane where the grid is visible show a region where turbidity was lower than 5 NTU, correlated with no visible precipitation in media samples tested and represented a safe-operating regime. **A)** Side view of graph showing turbidity measurements in media with varying concentrations of phosphate and calcium. Top view of graph showing turbidity measurements in media with