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.

SUMMARY

[0007] The disclosure 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, there is provided a method for inactivating virus or adventitious agents in cell culture media while the media maintains suitability for cell culture, said method comprising (a) adjusting one or more parameters in the cell culture media selected from the group consisting of pH, calcium level and phosphate level prior to or during high temperature short time (HTST) treatment, and (b) subjecting the cell culture media to HTST treatment to inactivate the virus or adventitious agents, wherein following adjustment:

(i) the phosphate concentration is 0 mM to 1 mM and the calcium concentration is 0.5 mM to 3 mM;

(ii) the pH is 5.0 to 6.9 and the total amount of calcium and phosphate is less than 10 mM;

(iii) the pH is 6.4 to 7.4 and the phosphate concentration is 0 mM to 0.5 mM; or

(iv) the pH is 6.65 to 7.4 and the calcium concentration is 0 mM to 1 mM; and,

wherein the temperature of the media during the high temperature short time (HTST) treatment is raised to no less than 85°C and no more than 150°C, for no less than 1 second and no more than 11 minutes.

[0009] In other embodiments, the 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, the disclosure provides for methods for inactivating virus in cell culture media comprising subjecting the cell culture media to high

CLAIMS

What is claimed is:

1. A method for inactivating virus or adventitious agents in cell culture media while the media maintains suitability for cell culture, said method comprising (a) adjusting one or more parameters in the cell culture media selected from the group consisting of pH, calcium level and phosphate level prior to or during high temperature short time (HTST) treatment, and (b) subjecting the cell culture media to HTST treatment to inactivate the virus or adventitious agents, wherein following adjustment:

   (i) the phosphate concentration is 0 mM to 1 mM and the calcium concentration is 0.5 mM to 3 mM;

   (ii) the pH is 5.0 to 6.9 and the total amount of calcium and phosphate is less than 10 mM;

   (iii) the pH is 6.4 to 7.4 and the phosphate concentration is 0 mM to 0.5 mM; or

   (iv) the pH is 6.65 to 7.4 and the calcium concentration is 0 mM to 1 mM; and,

   wherein the temperature of the media during the high temperature short time (HTST) treatment is raised to no less than 85°C and no more than 150°C, for no less than 1 second and no more than 11 minutes.

2. The method of claim 1 further comprising adjusting trace metal concentrations.

3. The method of claim 2, wherein the trace metal is selected from the group consisting of iron and copper.

4. The method of claim 3, wherein iron and/or copper concentrations are adjusted in the media prior to HTST treatment.

5. The method of claim 4, wherein iron and/or copper is removed from the media prior to HTST treatment.

6. The method of claim 4 further comprising supplementing iron and/or copper to the media following HTST treatment to a suitable level for cell culture.

7. The method of any one of claims 1-6, wherein the pH is adjusted when the media comprises calcium and phosphate.

8. The method of claim 7, wherein the pH is adjusted in preparing the media prior to HTST treatment to a low level suitable for cell culture.

9. The method of claim 7, wherein the pH is adjusted by lowering to a suitable level for cell culture.

10. The method of claim 7, wherein the pH is adjusted to less than about 7.2.

11. The method of claim 7, wherein the pH is adjusted to about 5.0 – 7.2.

12. The method of claim 7, further comprising adjusting the pH following HTST treatment to a suitable level for cell culture.

13. The method of claim 12, wherein the pH is adjusted to about 6.9 – 7.2.

14. The method of any one of claims 1-6, wherein the calcium level is adjusted when the media comprises phosphate.

15. The method of claim 14, wherein the calcium level is reduced.

16. The method of claim 15, wherein the calcium level is reduced such that formation of complexes comprised of calcium and phosphate is suppressed.

17. The method of claim 15, wherein calcium is removed from the media prior to HTST treatment.

18. The method of claim 14 further comprising adjusting the calcium level following HTST treatment to a suitable level for cell culture.

19. The method of any one of claims 1-6, wherein the phosphate level is adjusted when the media comprises calcium.

20. The method of claim 19, wherein the phosphate level is reduced.

21. The method of claim 19, wherein phosphate is removed from the media prior to HTST treatment.

22. The method of claim 19 further comprising adjusting the phosphate level following HTST treatment to a suitable level for cell culture.

23. The method of any one of claims 1-22, wherein the total phosphate and calcium concentration in the media is less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 mM during HTST treatment.

24. The method of any one of claims 1-6, wherein the calcium and phosphate levels are adjusted.

25. The method of any one of claims 1-6, wherein the pH, calcium, and phosphate levels are adjusted.

26. The method of any one of claims 1-25, wherein fouling of equipment used for HTST treatment is reduced.

27. The method of any one of claims 1-26, wherein filter fouling is suppressed.

28. The method of any one of claims 1-27, wherein the HTST treatment comprises raising the temperature of the media to no less than 85°C to no more 120 °C for no less than 1 second and no more than 60 seconds.

29. The method of claim 28, wherein the temperature of the media is raised to at least about 93, 95, 97, 99, 101, 102, or 103 degrees Celsius for a sufficient amount of time to inactivate the virus in the media.

30. The method of claim 28 or 29, wherein the temperature is raised for at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 seconds.

31. The method of any one of claims 1-30, wherein the virus is selected from the group consisting of parvoviradae, paramyoxviradae, orthomyxoviradae, bunyaviridae, rhabdoviridae, reoviridae, togaviridae, calciviridae, and picornaviridae.

32. The method of any one of claims 1-30, wherein the virus is an enveloped virus.

33. The method of any one of claims 1-30, wherein the virus is a non-enveloped virus.

34. The method of any one of claims 1-30, wherein the adventitious agent is bacteria.

35. The method of any one of claims 1-34, wherein the cell culture media is mammalian cell culture media.

CLAIMS

What is claimed is:

1. A method for inactivating virus or adventitious agents in cell culture media while the media maintains suitability for cell culture, said method comprising (a) adjusting one or more parameters in the cell culture media selected from the group consisting of pH, calcium level and phosphate level prior to or during high temperature short time (HTST) treatment, and (b) subjecting the cell culture media to HTST treatment to inactivate the virus or adventitious agents, wherein following adjustment:

   (i) the phosphate concentration is 0 mM to 1 mM and the calcium concentration is 0.5 mM to 3 mM;

   (ii) the pH is 5.0 to 6.9 and the total amount of calcium and phosphate is less than 10 mM;

   (iii) the pH is 6.4 to 7.4 and the phosphate concentration is 0 mM to 0.5 mM; or

   (iv) the pH is 6.65 to 7.4 and the calcium concentration is 0 mM to 1 mM.

2. The method of claim 1 further comprising adjusting trace metal concentrations.

3. The method of claim 2, wherein the trace metal is selected from the group consisting of iron and copper.

4. The method of claim 3, wherein iron and/or copper concentrations are adjusted in the media prior to HTST treatment.

5. The method of claim 4, wherein iron and/or copper is removed from the media prior to HTST treatment.

6. The method of claim 4 further comprising supplementing iron and/or copper to the media following HTST treatment to a suitable level for cell culture.

7. The method of any one of claims 1-6, wherein the pH is adjusted when the media comprises calcium and phosphate.

8. The method of claim 7, wherein the pH is adjusted in preparing the media prior to HTST treatment to a low level suitable for cell culture.

9. The method of claim 7, wherein the pH is adjusted by lowering to a suitable level for cell culture.

10. The method of claim 7, wherein the pH is adjusted to less than about 7.2.

11. The method of claim 7, wherein the pH is adjusted to about 5.0 – 7.2.

12. The method of claim 7, further comprising adjusting the pH following HTST treatment to a suitable level for cell culture.

13. The method of claim 12, wherein the pH is adjusted to about 6.9 – 7.2.

14. The method of any one of claims 1-6, wherein the calcium level is adjusted when the media comprises phosphate.

15. The method of claim 14, wherein the calcium level is reduced.

16. The method of claim 15, wherein the calcium level is reduced such that formation of complexes comprised of calcium and phosphate is suppressed.

17. The method of claim 15, wherein calcium is removed from the media prior to HTST treatment.

18. The method of claim 15, wherein the pH is adjusted such that formation of complexes comprised of calcium and phosphate is suppressed.

19. The method of claim 14 further comprising adjusting the calcium level following HTST treatment to a suitable level for cell culture.

20. The method of any one of claims 1-6, wherein the phosphate level is adjusted when the media comprises calcium.

21. The method of claim 20, wherein the phosphate level is reduced.

22. The method of claim 21, wherein the phosphate level is reduced such that formation of complexes comprised of calcium and phosphate is suppressed.

23. The method of claim 20, wherein phosphate is removed from the media prior to HTST treatment.

24. The method of claim 20, wherein the pH is adjusted such that formation of complexes comprised of calcium and phosphate is suppressed.

25. The method of claim 20 further comprising adjusting the phosphate level following HTST treatment to a suitable level for cell culture.

26. The method of any one of claims 1-25, wherein the total phosphate and calcium concentration in the media is less than about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 mM during HTST treatment.

27. The method of any one of claims 1-6, wherein the calcium and phosphate levels are adjusted.

28. The method of any one of claims 1-6, wherein the pH, calcium, and phosphate levels are adjusted.

29. The method of any one of claims 1-28, wherein precipitate formation is suppressed.

30. The method of any one of claims 1-29, wherein fouling of equipment used for HTST treatment is reduced.

31. The method of any one of claims 1-30, wherein filter fouling is suppressed.

32. The method of any one of claims 1-31, wherein 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 virus or adventitious agents in the media.

33. The method of claim 32, wherein the temperature of the media is raised to at least about 93, 95, 97, 99, 101, 102, or 103 degrees Celsius for a sufficient amount of time to inactivate the virus in the media.

34. The method of claim 32 or 33, wherein the temperature is raised for at least about 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 seconds.

35. The method of any one of claims 1-34, wherein the virus is selected from the group consisting of parvoviradae, paramyoxviradae, orthomyxoviradae, bunyaviridae, rhabdoviridae, reoviridae, togaviridae, calciviridae, and picornaviridae.

36. The method of any one of claims 1-34, wherein the virus is an enveloped virus.

37. The method of any one of claims 1-34, wherein the virus is a non-enveloped virus.

38. The method of any one of claims 1-34, wherein the adventitious agent is bacteria.

39. The method of any one of claims 1-38, wherein the cell culture media is mammalian cell culture media.