FLOCCULATION METHOD

FIELD OF INVENTION

The present invention relates to a method for harvesting recombinant proteins from mammalian cell culture broth. The method makes use of cationic polymers, non-ionic polymers and non-ionic surfactants.

BACKGROUND OF INVENTION

Clinical manufacture of therapeutic proteins is an expensive, large scale endeavor. Demand for greater quantities of therapeutic recombinant proteins has driven advances in cell culture processing which have resulted in dramatically increased product titer. High titer cell culture processes are typically produced by maintaining high viable cell densities over longer culture durations. A corresponding increase in the biomass solids (viable and non-viable cells) and the submicron cellular debris particles are also observed. The higher burden of solids and submicron cellular debris particles can challenge mammalian cell culture harvest processes, making the harvest process less effective at removing the debris without a substantial loss of product capacity.

Cationic polymer flocculents are used for many applications ranging from potable water purification, waste water treatment, uses in the petroleum, mining and paper making industries, cosmetics and medical uses, and have also been used to encapsulate mammalian cells and enzymes and to flocculate microbial cell cultures. However, for use in a commercial scale mammalian cell harvest process, lengthy flocculation settling time can be problematic, resulting in a harvest process that is time consuming and less efficient than standard harvest practices.

There is a continuing need to improve mammalian cell culture harvest methods, particularly commercial scale methods. Any improvements that allow for quicker recovery times and/or greater recovery can lead to reduced costs associated with manufacturing protein therapeutics. The invention fulfills this need by providing a quick and efficient method of cell culture harvest.

SUMMARY OF THE INVENTION

~~The present invention provides~~In a first aspect, there is provided a mammalian cell culture harvest method comprising culturing mammalian cells expressing a recombinant protein in a cell culture medium for a predetermined time or until a desired cell density and/or packed cell volume is achieved,adding a cationic polymer and a non-ionic polymer to the cell culture medium initiating flocculation,

mixing the cell culture medium during flocculation, allowing the flocculent to settle for a primary settle, and recovering the primary clarified supernatant.

The present ~~invention~~ disclosure also provides a mammalian cell culture harvest method comprising culturing mammalian cells expressing a recombinant protein in a cell culture medium for a predetermined time or until a desired cell density and/or packed cell volume is achieved, adding poly diallyldimethyammonium chloride and PEG 3,000 to the cell culture medium initiating flocculation, mixing the cell culture medium during flocculation, allowing the flocculent to settle, and recovering the clarified supernatant.

The present ~~invention~~ disclosure also provides a mammalian cell culture harvest method comprising culturing mammalian cells expressing a recombinant protein in a cell culture medium for a predetermined time or until a desired cell density and/or packed cell volume is achieved, adding poly diallyldimethyammonium chloride, PEG 3,000 and Triton X-100 to the cell culture medium initiating flocculation, mixing the cell culture medium during flocculation, allowing the flocculent to settle, and recovering the clarified supernatant.

The present disclosure~~invention~~ also provides a mammalian cell culture harvest method comprising culturing mammalian cells expressing a recombinant protein in a cell culture medium for a predetermined time or until a desired cell density and/or packed cell volume is achieved, adding a cationic polymer and a non-ionic polymer to the cell culture medium initiating flocculation, mixing the cell culture medium during flocculation, allowing the flocculent to settle for a primary settle, recovering the primary clarified supernatant, washing the primary settle flocculent, allowing the washed flocculent to settle for a secondary settle, and recovering the secondary clarified supernatant.

The present ~~invention~~ disclosure also provides a mammalian cell culture harvest method comprising culturing mammalian cells expressing a recombinant protein in a cell culture medium for a predetermined time or until a desired cell density and/or packed cell volume is achieved, adding a cationic polymer and a non-ionic polymer to the cell culture medium initiating flocculation, mixing the cell culture medium during flocculation, allowing the flocculent to settle for a primary settle, recovering the primary clarified supernatant, washing the primary settle flocculent if product recovery in the primary clarified supernatant is less than 80%, allowing the washed flocculent to settle for a secondary settle, and recovering the secondary clarified supernatant.

The present ~~invention~~ disclosure also provides a mammalian cell culture harvest method comprising culturing mammalian cells expressing a recombinant protein in a cell culture medium for a predetermined time or until a desired cell density and/or packed cell volume is achieved, adding poly diallyldimethyammonium chloride and PEG 3,000 to the cell culture medium initiating

flocculation, mixing the cell culture medium during flocculation, allowing the flocculent to settle for a primary settle, recovering the primary clarified supernatant, washing the primary settle flocculent, allowing the washed flocculent to settle for a secondary settle, and recovering the secondary clarified supernatant.

The present ~~invention~~disclosure also provides a mammalian cell culture harvest method comprising culturing mammalian cells expressing a recombinant protein in a cell culture medium for a predetermined time or until a desired cell density and/or packed cell volume is achieved, adding poly diallyldimethyammonium chloride, PEG 3,000 and Triton X-100 to the cell culture medium initiating flocculation, mixing the cell culture medium during flocculation, allowing the flocculent to settle for a primary settle, recovering the primary clarified supernatant, washing the primary settle flocculent, allowing the washed flocculent to settle for a secondary settle, and recovering the secondary clarified supernatant.

In one ~~embodiment~~ example the cationic polymer is poly diallyldimethyammonium chloride.

In another ~~embodiment~~example the non-ionic polymer is selected from poly ethylene glycol and dextran.

In another ~~embodiment~~example the non-ionic polymer is selected from PEG 3,000 and PEG 6,000.

In another ~~embodiment~~example, the mammalian cell culture harvest methods provided above further comprise adding a non-ionic surfactant to the cell culture medium. In a related ~~embodiment~~example the non-ionic surfactant is Triton X-100.

In another ~~embodiment~~example, the cationic polymer and the non-ionic polymer are added simultaneously.

In another ~~embodiment~~example, the cationic polymer, the non-ionic polymer and the non-ionic surfactant are added simultaneously.

In another ~~embodiment~~example, the cationic polymer is added first and mixed for at least 30 seconds followed by addition of the non-ionic polymer.

In another ~~embodiment~~example, the cationic polymer is added first and mixed for at least 30 seconds followed by addition of the non-ionic polymer and a non-ionic surfactant.

In another ~~embodiment~~example, the cationic polymer is a polymer of diallyldimethylammonium chloride, polydiallyldimethyammonium chloride, polyethyleneimine, polyacrylamide or chitosan.

In another ~~embodiment~~example, the non-ionic surfactant is Sapoin or Triton X100.

In another ~~embodiment~~example, the poly diallyldimethyammonium chloride is added at a concentration of at or about 20 to at or about 90 pg/total cell density.

In another ~~embodiment~~example the poly diallyldimethyammonium chloride is added at a concentration of at or about 25 pg/total cell density wherein the mammalian cells originate from a diploid cell line.

In another ~~embodiment~~example, the poly diallyldimethyammonium chloride is added between 43 pg/total cell density and 57 pg/total cell density wherein the mammalian cells originate from a tetraploid cell line.

In another ~~embodiment~~example, the concentration of PEG 3,000 is at or about 3% to at or about 4.5%.

In another ~~embodiment~~example, the concentration of PEG 6,000 is at or about 2.5% to at or about 3.5%.

In another ~~embodiment~~example, the concentration of Triton X100 is 0.05% (w/v).

In another ~~embodiment~~example, the mammalian cell culture broth is between 36°C and 20°C.

In another ~~embodiment~~example, the mammalian cell culture broth is at or above 20°C.

In another ~~embodiment~~example, the flocculent from the primary settle is washed in a 9% sucrose solution.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 provides the structure of PDADMAC.

Figure 2A shows the difference in settling times achieved by PDADMAC at various molecular weights. The concentration of PDADMAC at each molecular weight was 57 pg/Total cell density. The closed diamond/dashed line represents PDADMAC molecular weight 100,000 – 200,000. The closed square with dashed line represents PDADMAC molecular weight of 200,000 – 300,000. The closed triangle and solid line represents PDADMAC molecular weight of 400,000 – 500,000.

Figure 2B shows the supernatant clarity achieved when flocculating cell culture broth with PDADMAC at various molecular weights. From left to right the bars represent PDADMAC molecular weight of < 100,000; 100,000 – 200,000; 200,000 -350,000; and 400,000 – 500,000.

Figure 3A shows the impact of high molecular weight PDADMAC concentration on flocculation of 15-20 μm cells. The closed diamond dashed line represents PDADMAC at 11pg/Total cell density. The closed square with dashed line represents PDADMAC at 18 pg/Total cell density.

What is claimed is:

1. A mammalian cell culture harvest method comprising

culturing mammalian cells expressing a recombinant protein in a cell culture medium for a predetermined time or until a desired cell density and/or packed cell volume is achieved,

adding a cationic polymer and a non-ionic polymer to the cell culture medium initiating flocculation,

mixing the cell culture medium during flocculation,

allowing the flocculent to settle for a primary settle, and

recovering the primary clarified supernatant.

2. The method according to claim 1, wherein the cationic polymer is a polymer of diallyldimethylammonium chloride, polydiallyldimethyammonium chloride, polyethyleneimine, polyacrylamide or chitosan.

3. The mammalian cell culture harvest method according to claim 1, wherein the cationic polymer is polydiallyldimethyammonium chloride.

4. The mammalian cell culture harvest method according to claim 1, wherein the non-ionic polymer is selected from polyethyleneglycol and dextran.

5. The mammalian cell culture harvest method according to claim 1, wherein the non-ionic polymer is selected from PEG 3,000 and PEG 6,000.

6. The method according to claim 5, wherein the concentration of PEG 3,000 is at or about 3% to at or about 4.5%.

7. The method according to claim 5, wherein the concentration of PEG 6,000 is at or about 2.5% to at or about 3.5%.

8. The mammalian cell culture harvest method according to claim 1, further comprising adding a non-ionic surfactant to the cell culture medium.

9. The method according to claim 8, wherein the non-ionic surfactant is Sapoin or Triton X100.

10. The mammalian cell culture harvest method according to claim 8, wherein the non-ionic surfactant is Triton X-100.

11. The method according to claim 10, wherein the concentration of Triton X100 is 0.05% (w/v).

12. The mammalian cell culture harvest method according to claim 1, further comprising,

washing the primary settle flocculent,

allowing the washed flocculent to settle for a secondary settle, and

recovering the secondary clarified supernatant.

13. The method according to claim 12, wherein the flocculent from the primary settle is washed in a 9% sucrose solution.

14. The method according to claim 1, where the cationic polymer and the non-ionic polymer are added simultaneously.

15. The method according to claim 1, where the cationic polymer is added first and mixed for at least 30 seconds followed by addition of the non-ionic polymer.

16. The method according to claim 1, where the cationic polymer, the non-ionic polymer and a non-ionic surfactant are added simultaneously.

17. The method according to claim 1, where the cationic polymer is added first and mixed for at least 30 seconds followed by addition of the non-ionic polymer and a non-ionic surfactant.

18. The method according to claim 1, wherein the polydiallyldimethyammonium chloride is added at a concentration of at or about 20 to at or about 90 pg/total cell density.

19. The method according to claim 1, wherein the polydiallyldimethyammonium chloride is added at a concentration of at or about 25 pg/total cell density wherein the mammalian cells originate from a diploid cell line.

20. The method according to claim 1, wherein the polydiallyldimethyammonium chloride is added between 43 pg/total cell density and 57 pg/total cell density wherein the mammalian cells originate from a tetraploid cell line.

21. The method according to claim 1, wherein the mammalian cell culture medium is between 36°C and 20°C.

22. The method according to claim 1, wherein the mammalian cell culture medium is at or above 20°C.

What is claimed is:

1. A mammalian cell culture harvest method comprising

culturing mammalian cells expressing a recombinant protein in a cell culture medium for a predetermined time or until a desired cell density and/or packed cell volume is achieved,

adding a cationic polymer and a non-ionic polymer to the cell culture medium initiating flocculation,

mixing the cell culture medium during flocculation,

allowing the flocculent to settle, and

recovering the clarified supernatant.

2. A mammalian cell culture harvest method according to claim 1, wherein the cationic polymer is poly diallyldimethyammonium chloride.

3. A mammalian cell culture harvest method according to claim 1, wherein the non-ionic polymer is selected from poly ethyleneglycol and dextran.

4. A mammalian cell culture harvest method according to claim 1, wherein the non-ionic polymer is selected from PEG 3,000 and PEG 6,000.

5. A mammalian cell culture harvest method according to claim 1, further comprising adding a non-ionic surfactant to the cell culture medium.

6. A mammalian cell culture harvest method according to claim 5, wherein the non-ionic surfactant is Triton X-100.

7. A mammalian cell culture harvest method comprising

culturing mammalian cells expressing a recombinant protein in a cell culture medium for a predetermined time or until a desired cell density and/or packed cell volume is achieved,

adding poly diallyldimethyammonium chloride and PEG 3,000 to the cell culture medium initiating flocculation,

mixing the cell culture medium during flocculation,

allowing the flocculent to settle, and

recovering the clarified supernatant.

8. A mammalian cell culture harvest method comprising

culturing mammalian cells expressing a recombinant protein in a cell culture medium for a predetermined time or until a desired cell density and/or packed cell volume is achieved,

adding poly diallyldimethyammonium chloride, PEG 3,000 and Triton X-100 to the cell culture medium initiating flocculation,

mixing the cell culture medium during flocculation,

allowing the flocculent to settle, and

recovering the clarified supernatant.

9. A mammalian cell culture harvest method comprising

culturing mammalian cells expressing a recombinant protein in a cell culture medium for a predetermined time or until a desired cell density and/or packed cell volume is achieved,

adding a cationic polymer and a non-ionic polymer to the cell culture medium initiating flocculation,

mixing the cell culture medium during flocculation,

allowing the flocculent to settle for a primary settle,

recovering the primary clarified supernatant,

washing the primary settle flocculent,

allowing the washed flocculent to settle for a secondary settle, and

recovering the secondary clarified supernatant.

10. A mammalian cell culture harvest method comprising

culturing mammalian cells expressing a recombinant protein in a cell culture medium for a predetermined time or until a desired cell density and/or packed cell volume is achieved,

adding a cationic polymer and a non-ionic polymer to the cell culture medium initiating flocculation,

mixing the cell culture medium during flocculation,

allowing the flocculent to settle for a primary settle,

recovering the primary clarified supernatant,

washing the primary settle flocculent if product recovery in the primary clarified supernatant is less than 80%,

allowing the washed flocculent to settle for a secondary settle, and

recovering the secondary clarified supernatant.

11. A mammalian cell culture harvest method according to claims 9 or 10, wherein the cationic polymer is poly diallyldimethyammonium chloride.

12. A mammalian cell culture harvest method according to claims 9 or 10, wherein the non-ionic polymer is selected from poly ethyleneglycol and dextran.

13. A mammalian cell culture harvest method according to claims 9 or 10, wherein the non-ionic polymer is selected from PEG 3,000 and PEG 6,000.

14. A mammalian cell culture harvest method according to claims 9 or 10, further comprising adding a non-ionic surfactant to the cell culture medium.

15. A mammalian cell culture harvest method according to claim 14, wherein the non-ionic surfactant is Triton X-100.

16. A mammalian cell culture harvest method comprising

culturing mammalian cells expressing a recombinant protein in a cell culture medium for a predetermined time or until a desired cell density and/or packed cell volume is achieved,

adding poly diallyldimethyammonium chloride and PEG 3,000 to the cell culture medium initiating flocculation,

mixing the cell culture medium during flocculation,

allowing the flocculent to settle for a primary settle,

recovering the primary clarified supernatant,

washing the primary settle flocculent,

allowing the washed flocculent to settle for a secondary settle, and

recovering the secondary clarified supernatant.

17. A mammalian cell culture harvest method comprising

culturing mammalian cells expressing a recombinant protein in a cell culture medium for a predetermined time or until a desired cell density and/or packed cell volume is achieved,

adding poly diallyldimethyammonium chloride, PEG 3,000 and Triton X-100 to the cell culture medium initiating flocculation,

mixing the cell culture medium during flocculation,

allowing the flocculent to settle for a primary settle,

recovering the primary clarified supernatant,

washing the primary settle flocculent,

allowing the washed flocculent to settle for a secondary settle, and

recovering the secondary clarified supernatant.

18. A method according to any of the above claims, where the cationic polymer and the non-ionic polymer are added simultaneously.

19. A method according to any of the above claims, where the cationic polymer is added first and mixed for at least 30 seconds followed by addition of the non-ionic polymer.

20. A method according to any of the above claims, where the cationic polymer, the non-ionic polymer and a non-ionic surfactant are added simultaneously.

21. A method according to any of the above claims, where the cationic polymer is added first and mixed for at least 30 seconds followed by addition of the non-ionic polymer and a non-ionic surfactant.

22. A method according to any of the above claims, wherein the cationic polymer is a polymer of diallyldimethylammonium chloride, polydiallyldimethyammonium chloride, polyethyleneimine, polyacrylamide or chitosan.

23. A method according to any of the above claims, wherein the non-ionic polymer is polyethylene glycol or a dextran.

24. A method according to any of the above claims, wherein the non-ionic surfactant is Sapoin or Triton X100.

25. A method according to any of the above claims, wherein the poly diallyldimethyammonium chloride is added at a concentration of at or about 20 to at or about 90 pg/total cell density.

26. A method according to any of the above claims, wherein the poly diallyldimethyammonium chloride is added at a concentration of at or about 25 pg/total cell density wherein the mammalian cells originate from a diploid cell line.

27. A method according to any of the above claims, wherein the poly diallyldimethyammonium chloride is added between 43 pg/total cell density and 57 pg/total cell density wherein the mammalian cells originate from a tetraploid cell line.

28. A method according to any of the above claims, wherein the concentration of PEG 3,000 is at or about 3% to at or about 4.5%.

29. A method according to any of the above claims, wherein the concentration of PEG 6,000 is at or about 2.5% to at or about 3.5%.

30. A method according to any of the above claims, wherein the concentration of Triton X100 is 0.05% (w/v).

31. A method according to any of the above claims, wherein the mammalian cell culture broth is between 36°C and 20°C.

32. A method according to any of the above claims, wherein the mammalian cell culture broth is at or above 20°C.

33. A method according to any of the above claims, wherein the flocculent from the primary settle is washed in a 9% sucrose solution.