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

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 selected from the group consisting of: a polymer of diallyldimethyammonium chloride and a polymer of polydiallyldimethyammonium chloride, and a non-ionic polymer selected from the group consisting of: polyethyleneglycol and dextran to the cell culture medium initiating flocculation,

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 selected from the group consisting of: a polymer of diallyldimethyammonium chloride and a polymer of polydiallyldimethyammonium chloride, and a non-ionic polymer selected from the group consisting of: polyethyleneglycol and dextran 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 mammalian cell culture harvest method according to claim 1, wherein the polyethyleneglycol is selected from PEG 3,000 and PEG 6,000.

3. The method according to claim 2, wherein the concentration of PEG 3,000 is at or about 3% (w/v) to at or about 4.5% (w/v).

4. The method according to claim 2, wherein the concentration of PEG 6,000 is at or about 2.5% (w/v) to at or about 3.5% (w/v).

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

6. The method according to claim 5, wherein the non-ionic surfactant is Sapoin or Triton X100.

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

8. The method according to claim 7, wherein the concentration of Triton X100 is 0.05% (w/v).

9. 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.

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

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

12. 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.

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

14. 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.

15. 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.

16. 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.

17. 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.

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

19. 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 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.