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**FLOCCULATION METHOD****CROSS REFERENCE TO RELATED APPLICATIONS**

This application is a national stage application under 35 U.S.C. §371 of International Application No. PCT/US2012/069916, having an international filing date of Dec. 14, 2012; which claims priority to U.S. Provisional Patent Application No. 61/576,303, filed Dec. 15, 2011, which is incorporated herein by reference.

**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 sub-micron 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 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.

The present 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

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packed cell volume is achieved, adding poly diallyldimethylammonium 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 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 diallyldimethylammonium 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 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 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 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 diallyldimethylammonium 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 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 diallyldimethylammonium 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 the cationic polymer is poly diallyldimethylammonium chloride.