CLAIMS

What is claimed is:

1. A method for preparing monoclonal antibodies in crystal form, the method comprising:
   a) providing a cell-free cell culture supernatant comprising monoclonal antibodies,
   b) introducing a low ionic strength buffer to the cell free cell culture supernatant in an amount sufficient to promote the crystallization of said antibody,
   c) adjusting the pH of said pre-crystallization solution to produce crystals, and
   d) isolating the crystals formed in step c),

   wherein at least 50% of the antibody contained in the cell-free cell culture supernatant is isolated in step d.

2. A method for preparing purified monoclonal antibodies, the method comprising:
   a) dialyzing a composition comprising monoclonal antibodies against a low ionic strength buffer wherein impurities precipitate from the composition;
   b) removing the precipitate to produce a first clarified composition;
   c) optionally dialyzing the clarified composition against a low ionic strength buffer wherein impurities precipitate from the composition to produce a second clarified composition;
   d) removing the precipitate from the composition of step c);
   e) adjusting the pH of the first or the second clarified composition to about the pI of the monoclonal antibody and optionally introducing one or more additives to produce crystals; and,
   f) isolating the crystals formed in step e).

3. A method for preparing monoclonal antibodies in crystal form directly from cell culture supernatant, the method comprising:
   a) dialyzing cell-free cell culture supernatant comprising monoclonal antibody against a low ionic strength buffer;
   b) removing precipitate formed in step a) from the supernatant, if present therein, to produce a clarified supernatant;
   c) optionally concentrating the clarified supernatant;

d) optionally dialyzing the clarified supernatant of b) or c) against a low ionic strength buffer to produce a pretreated solution;
e) removing precipitate from the pretreated solution of step d), if present therein;
f) adjusting the pH of the pretreated solution of step d) or e) to about the pI of the monoclonal antibody and optionally introducing one or more additives to produce crystals; and,
g) isolating the crystals formed in step f).

4. The method of claim 3 comprising concentrating the cell-free cell culture supernatant before step b).

5. The method of any preceding claim wherein the low ionic strength buffer provides a conductivity of less than or equal to about 12 mS/cm^{-1}.

6. The method of any preceding claim wherein the pH of the low ionic strength buffer prior to the adjusting step is at about a pH where the antibody is soluble and does not crystallize or precipitate.

7. The method of any preceding claim wherein the low ionic strength buffer is a histidine buffer.

8. The method of any preceding claim wherein the low ionic strength buffer comprises at least one or more salts.

9. The method of any preceding claim wherein the low ionic strength buffer comprises at least one or more sugars.

10. The method of any preceding claim wherein the pH is adjusted using a Tris buffer.

11. The method of any preceding claim wherein the pH is adjusted using a buffer comprising one or more additives selected from the group consisting of sodium chloride, polyethylene glycol, and a sugar.

12. The method of any preceding claim wherein at least about 50% of the antibody contained in the cell-free culture supernatant is isolated in the isolating step.

13. The method of any preceding claim wherein the purity of the crystallized antibody is at least about 90%.

14. The method of any preceding claim further comprising dissolving the isolated crystals in a solution.

15. The method of any preceding claim further comprising re-crystallizing the monoclonal antibody by adjusting the pH of the solution to about the pI of the monoclonal antibody.

16. The method of any preceding claim further comprising controlling crystal size by adjusting the starting protein concentration of the cell culture supernatant.

17. The method of any preceding claim further comprising controlling crystal size by stirring the substrate at a particular speed.

18. The method of any preceding claim wherein crystallization occurs with stirring at a power input per volume of less than 1 W L^{-1}.

19. The method of claim 17 wherein the maximum local energy dissipation (ε_{max}) is between about 0.009 W kg^{-1} and about 1.3 W kg^{-1}.

20. The method of claim 19 wherein the the maximum local energy dissipation (ε_{max}) is between about 0.1 to about 0.4 W kg^{-1}.

21. The method of any one of claims 17-20 wherein a three-bladed segment impeller is used for stirring.

Claims

1. A method for preparing monoclonal antibodies in crystal form, the method comprising:
   a) providing a cell-free cell culture supernatant comprising monoclonal antibodies,
   b) introducing a low ionic strength buffer to the cell free cell culture supernatant in an amount sufficient to promote the crystallization of said antibody, wherein the pH of the low ionic strength buffer is at a pH where the antibody is soluble and does not crystallize or precipitate,
   c) adjusting the pH of said pre-crystallization solution to produce crystals, and
   d) isolating the crystals formed in step c),

   wherein at least 50% of the antibody contained in the cell-free cell culture supernatant is isolated in step d.

2. A method for preparing purified monoclonal antibodies, the method comprising:
   a) introducing a low ionic strength buffer into a composition comprising monoclonal antibodies, wherein impurities precipitate from the composition, and wherein the pH of the low ionic strength buffer is at a pH where the antibody is soluble and does not crystallize or precipitate;
   b) removing the precipitate to produce a first clarified composition;
   c) optionally introducing a low ionic strength buffer into the clarified composition, wherein impurities precipitate from the composition to produce a second clarified composition, and wherein the pH of the low ionic strength buffer is at a pH where the antibody is soluble and does not crystallize or precipitate;
   d) removing the precipitate from the composition of step c);
   e) adjusting the pH of the first or the second clarified composition to about the pI of the monoclonal antibody and optionally introducing one or more additives to produce crystals; and,
   f) isolating the crystals formed in step e).

3. The method according to claim 2, wherein the composition comprising monoclonal antibodies is a cell-free cell culture supernatant comprising monoclonal antibody, and wherein steps a) and c) include dialyzing the cell cell-free culture supernatant or the clarified supernatant, respectively, against the low ionic strength buffer, and wherein between steps a) and b) and/or between steps b) and c) the supernatant may optionally be concentrated.

4. The method of any preceding claim wherein the low ionic strength buffer provides a conductivity of less than or equal to about 12 mS cm^{-1}.

5. The method of any preceding claim wherein the low ionic strength buffer provides a conductivity of about 4 mS cm-1 or less.

6. The method of any preceding claim wherein the low ionic strength buffer provides a conductivity of about 2 mS cm-1 or less.

7. The method of any preceding claim wherein the low ionic strength buffer
   (i) is a histidine buffer,
   (ii) comprises at least one or more salts, and/or
   (iii) comprises at least one or more sugars.

8. The method of any preceding claim wherein the pH is adjusted using
   (i) a Tris buffer, and/or
   (ii) a buffer comprising one or more additives selected from the group consisting of sodium chloride, polyethylene glycol, and a sugar.

9. The method of any preceding claim wherein at least about 50% of the antibody contained in the cell-free culture supernatant is isolated in the isolating step.

10. The method of any preceding claim wherein the purity of the crystallized antibody is at least about 90%.

11. The method of any preceding claim further comprising
   (i) dissolving the isolated crystals in a solution,
   (ii) re-crystallizing the monoclonal antibody by adjusting the pH of the solution to about the pI of the monoclonal antibody, and/or
   (iii) controlling crystal size by adjusting the starting protein concentration of the cell culture supernatant.

12. The method of any preceding claim further comprising controlling crystal size by stirring the substrate at a particular speed.

13. The method of any preceding claim wherein crystallization occurs with stirring at a power input per volume of less than 1 W L^{-1}.

14. The method of claim 12 wherein the maximum local energy dissipation (ϵ_{max}) is between 0.009 W kg^{-1} and 1.3 W kg^{-1}, in particular between 0.1 to 0.4 W kg^{-1}.

15. The method of any one of claims 12-14 wherein a three-bladed segment impeller is used for stirring.