samples for subsequent purification by other means (Gagnon *supra*; Y. Yigsaw *et al*, 2008, Improving upstream feed stock to downstream operations, Recovery of Biologics Conference XIII, Quebec; Arunakumari, A. *et al*, US Patent Application 20120101262 A1), but has also been applied as a polishing step following antibody capture by protein A affinity chromatography (Y. Brodsky *et al* Biotechnol. Bioeng. 109 (2012) 2589-2598).

SUMMARY

[0005] In one aspect, the present invention refers to a method of purifying an antibody comprising: (a) contacting a cell culture harvest or a protein preparation comprising at least one antibody of the class IgG or IgM with at least one fatty acid to form a mixture comprising a precipitate, wherein the at least one fatty acid is selected from enanthic (heptanoic) acid, caprylic (octanoic) acid, octenoic acid, pelargonic (nonanoic) acid, nonenoic acid, and capric (decanoic) acid and the at least one fatty acid is present at a concentration in a range of 0.05 to 5%; (b) contacting the mixture with one or more functionalized solid substrates comprising a cationic metal binding functional group; wherein the metal binding functional group comprises a polyamine; and (c) separating solid materials after contacting the mixture with the one or more functionalized substrates, and removing solid materials from the mixture to provide a solution comprising the antibody. In another aspect, the present invention refers to a method for purifying an antibody comprising: (a) contacting a cell culture harvest or protein preparation comprising at least one antibody of the class IgG or IgM with at least one fatty acid to form a mixture comprising a precipitate, wherein the at least one fatty acid is selected from enanthic (heptanoic) acid, caprylic (octanoic) acid, octenoic acid, pelargonic (nonanoic) acid, nonenoic acid, and capric (decanoic) acid and the at least one fatty acid is present at a concentration in a range of 0.1% to 2%; (b) contacting the mixture with allantoin; and (c) separating solid materials after contacting the mixture with the at least one fatty acid and allantoin to provide a solution comprising the antibody. In still another aspect, the present invention refers to a method of purifying an antibody comprising: (a) contacting a cell culture harvest or a protein preparation comprising at least one antibody of the class IgG or IgM with at least one fatty acid having 7 to 10 carbon atoms to form a mixture comprising a precipitate, wherein the at least one fatty acid is selected from enanthic (heptanoic) acid, octenoic acid, pelargonic (nonanoic) acid, nonenoic acid, and capric (decanoic) acid and the at least one fatty acid is present at a concentration in a

range of 0.05 to 5%; (b) contacting the mixture with allantoin at a concentration in a range from 0.6% to 30%; and (c) removing solid materials from the mixture to provide a solution comprising the antibody. Also disclosed herein are methods of purifying a target protein comprising contacting a cell culture harvest with at least one fatty acid having 7 to 10 carbon atoms to form a mixture, contacting the mixture with one or more solid or soluble materials to form a mixture, wherein the one or more solid or soluble materials comprise a cationic functional group and a metal binding functional group, the metal binding functional group comprising a nitrogen-containing moiety selected from the group consisting of (1) a polyamine, (2) an imine, (3) an N-heterocycle, (4) an amino acid, (5) an N-hydroxyamide, (6), an arylamine, and combinations thereof, and separating solid materials after contacting the mixture with the one or more electrostatically charged solids to provide a solution comprising the target protein. The processed liquid may optionally, be further passed through a device with an internal contact surface contact that comprises positive charges before being processed by other purification methods, if desired.

[0006] In some examples disclosed herein, methods are described for purifying a target protein comprising contacting a cell culture harvest with at least one fatty acid having 7 to 10 carbon atoms to form a mixture, contacting the mixture with allantoin, and separating solid materials after contacting the mixture with allantoin to provide a solution comprising the target protein.

[0007] In some examples disclosed herein, methods are described of purifying an antibody comprising contacting a cell culture harvest with at least one fatty acid having 7 to 10 carbon atoms to form a mixture, contacting the mixture with one or more solid or soluble materials to form a mixture, wherein the one or more solid or soluble materials comprise a cationic functional group and a metal binding functional group, the metal binding functional group comprising a nitrogen-containing moiety selected from the group consisting of (1) a polyamine, (2) an imine, (3) an N-heterocycle, (4) an amino acid, (5) an N-hydroxyamide, (6), an

WHAT IS CLAIMED IS:

1. A method of purifying an antibody comprising:

(a) contacting a cell culture harvest or a protein preparation comprising at least one antibody of the class IgG or IgM with at least one fatty acid to form a mixture comprising a precipitate, wherein the at least one fatty acid is selected from enanthic (heptanoic) acid, caprylic (octanoic) acid, octenoic acid, pelargonic (nonanoic) acid, nonenoic acid, and capric (decanoic) acid and the at least one fatty acid is present at a concentration in a range of 0.05 to 5%;

(b) contacting the mixture with one or more functionalized solid substrates comprising a cationic metal binding functional group; wherein the metal binding functional group comprises a polyamine; and

(c) separating solid materials after contacting the mixture with the one or more functionalized substrates, and removing solid materials from the mixture to provide a solution comprising the antibody.

2. The method of claim 1, further comprising, prior to (c), contacting the cell culture harvest with allantoin at a concentration in a range selected from the group consisting of: (a) from 0.6 to 30%, (b) from 1 to 10%, and (c) from 1 to 2%.

3. The method of claim 2, wherein allantoin is present at a concentration in a range from 1 to 10%.

4. The method of claim 1, wherein a total amount of the one or more functionalized substrates is 0.01%, 0.05%, 0.1 %, 0.25%, 0.5%, 1 %, 2%, 5%, 10%, 20% (vol/vol), or intermediate values in between, of a total volume of the mixture.

5. The method of claim 1, wherein the one or more functionalized substrates are disposed in a device that permits the passage of fluid while preventing the passage of solid materials therethrough.

6. The method of claim 5, wherein the device comprises a porous material selected from the group consisting of a membrane, a monolith, a woven material, a crystalline material, a gelatinous material, a column of packed particles, and combinations thereof.

7. The method claim 1, wherein step (b) is carried out with the one or more functionalized substrates disposed in a device.

8. The method of claim 1, wherein solid materials are removed by sedimentation or sedimentation following centrifugation.

9. The method of claim 1, wherein solid materials are removed by filtration.

10. The method of claim 9, wherein filtration comprises membrane filtration or depth filtration.

11. The method of claim 10, wherein the membrane filtration or depth filtration comprises a contact surface that is functionalized.

12. The method of any one of claims 1 to 11, wherein the at least one fatty acid comprises caprylic (octanoic) acid.

13. The method of any one of claims 1 to 12, wherein the at least one fatty acid is nonanoic acid.

14. The method of any one of claims 1 to 13, wherein the at least one fatty acid is present at a concentration in a range selected from the group consisting of: (a) from 0.1 to 1.0%, (b) from 0.2 to 0.4%, and (c) from 0.1 to 0.2%.

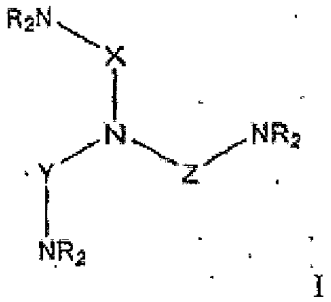
15. The method of any one of claims 1 to 14, wherein the mixture comprises a surfactant, and the surfactant is a nonionic, zwitterionic or a cationic surfactant.

16. The method of claim 15, wherein the cationic surfactant is cetyltrimethylammonium bromide.

17. The method of claim 16, wherein cetyltrimethylammonium bromide is present at a concentration ranging from 0.001% to 0.05%, or from 0.005% to 0.025%, or from 0.0075% to 0.01 %.

18. The method of any one of claims 1 to 17, wherein the metal binding functional group comprises one selected from the group consisting of tris(2-aminoethyl)amine, diethylenetriamine, triethylenetriamine, tetraethylenepentamine, polypropylenimine tetramine, poly(amidoamine) (PAMAM) dendrimer, and deferoxamine (desferioxamine).

19. The method of any one of claims 1 to 18, wherein the metal binding functional group is a compound of formula I:



I

wherein each incidence of R is independently hydrogen or C_1-C_4 alkyl, with the proviso that at least one R is the site of attachment to a solid support, optionally via a linker; and

each of X, Y, and Z are independently $(CH_2)_n$, where n is an integer from 2 to 6, wherein a CH_2 group is optionally replaced by O, or NH.

20. A method for purifying an antibody comprising: (a) contacting a cell culture harvest or protein preparation comprising an antibody of the class IgG or IgM with at least one fatty acid to form a mixture comprising a precipitate, wherein the at least one fatty acid is selected from enanthic (heptanoic) acid, caprylic (octanoic) acid, octenoic acid, pelargonic (nonanoic) acid, nonenoic acid, and capric (decanoic) acid and the at least one fatty acid is present at a concentration in a range of 0.1% to 2%; (b) contacting the mixture with allantoin; and (c)

separating solid materials after contacting the mixture with the at least one fatty acid and allantoin to provide a solution comprising the antibody.

21. The method of claim 20, comprising contacting the solution with at least one chemically functionalized solid.

22. The method of claim 20, wherein the at least one chemically functionalized solid comprises tris(2-aminoethyl)amine.

23. The method of any one of claim 20 to 22, wherein allantoin is present at a concentration in a range selected from the group consisting of: (a) from 0.6 to 30%, (b) from 1 to 10%, (c) from 1 to 5%, and (d) from 1 to 2%.

24. The method of any one of claims 20 to 23, wherein allantoin is in a range from a non-zero amount up to 0.6%.

25. A method of purifying an antibody comprising:

(a) contacting a cell culture harvest or a protein preparation comprising at least one antibody of the class IgG or IgM with at least one fatty acid having 7 to 10 carbon atoms to form a mixture comprising a precipitate, wherein the at least one fatty acid is selected from enanthic (heptanoic) acid, octenoic acid, pelargonic (nonanoic) acid, nonenoic acid, and capric (decanoic) acid and the at least one fatty acid is present at a concentration in a range of 0.05 to 5%;

(b) contacting the mixture with allantoin at a concentration in a range from 0.6% to 30%; and

(c) removing solid materials from the mixture to provide a solution comprising the antibody.