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 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, 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 substrates selected from the group consisting of solid substrates, soluble substrates, and combinations thereof, wherein the one or more functionalized substrates comprise a cationic functional group, a metal binding functional group, or both; wherein the metal binding functional group comprises 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 (c) separating solid materials after contacting the mixture with the one or more functionalized substrates, 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: contacting a cell culture harvest or protein preparation with at least one fatty acid having 8 to 10 carbon atoms 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 1%; contacting the mixture with allantoin and at least one chemically functionalized solid or soluble substrate comprising a polyamine; and separating solid materials after contacting the mixture with allantoin and the at least one chemically functionalized solid or soluble substrate to provide a solution comprising the antibody. ~~In some aspects, embodiments~~Also disclosed herein ~~relate~~

~~to~~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 ~~aspects, embodiments~~examples disclosed herein, ~~relate to~~ 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 ~~aspects, embodiments~~examples disclosed herein, ~~relate to~~ 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

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 antibody. The processed liquid may optionally, in addition, be passed through a device with an internal contact surface contact that embodies positive charges before being processed by other purification methods, if desired.

[0008] In some ~~aspects, embodiments~~examples disclosed herein, ~~relate to~~ methods are described for 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 allantoin, and separating solid materials after contacting the mixture with allantoin to provide a solution comprising the antibody.

DETAILED DESCRIPTION

[0009] It has been discovered that a combination of materials that are chemically antagonistic to one another has the unexpected effect of providing target proteins, including antibodies, at a higher level of purity and a lower level of turbidity than purification methods relying on any one of the materials individually. Combinations of antagonistic materials would normally be expected to cancel each others' individual effects and result in inferior protein purification, inferior protein recovery, or both. Instead, the present embodiments provide defined windows within which the materials work synergistically to achieve levels of protein purity and recovery that are substantially beyond the ability of any of the individual components to provide. These observations are particularly applicable to antibodies, such as IgG and IgM antibodies. In addition, experimental results indicate that reactivity curves for each of the components in the combination is distinct from their reactivity curves when used individually. This highlights that the utility of the embodiments disclosed herein could not have been predicted by the known properties or applications of the individual components. The components to be combined may include saturated fatty acids containing 7 or 8 or 9 or 10 carbon atoms, or unsaturated fatty acids containing 6 or 7 or 8 or 9 carbon atoms with one double bond, and soluble and/or solid materials bearing a positive charge. They may further include one or more solid or soluble materials that comprise a metal binding functional group. They may further include allantoin. In the methods disclosed herein, the materials are combined in a liquid preparation containing a target protein, such as a species of antibody, then after a suitable period of incubation, solids are

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 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, 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 substrates selected from the group consisting of solid substrates, soluble substrates, and combinations thereof, wherein the one or more functionalized substrates comprise a cationic functional group, a metal binding functional group, or both; wherein the metal binding functional group comprises 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

(c) separating solid materials after contacting the mixture with the one or more functionalized substrates, 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 about 0.6 to about 30%, (b) from about 1 to about 10%, and (c) from about 1 to about 2%.

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

4. The method of claim 1, further comprising, prior to (c), contacting the cell culture harvest with allantoin at a concentration in a range from a non-zero amount up to about 0.6%.

5. The method of claim 1 , wherein a total amount of the one or more functionalized substrates is a volumetric proportion of the total volume of the preparation of about 0.01%, 0.05%, 0.1 %, 0.25%, 0.5%, 1 %, 2%, 5%, 10%, 20%, or intermediate values in between.

6. The method of claim 1, wherein the at least one fatty acid and the one or more functionalized substrates are disposed in a single vessel.

7. 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.

8. The method of claim 7, 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.

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

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

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

12. The method of claim 11, wherein filtration comprises membrane filtration or depth filtration.

13. The method of claim 12, wherein the membrane filtration or depth filtration comprises a contact surface that is functionalized.

14. The method of any one of claims 1 to 13, wherein the step (a) is preceded by partial purification of the antibody.

15. The method of any one of claims 1 to 14, wherein the cell culture harvest or protein preparation contains cells, and optionally resides in a bioreactor within which the cell culture harvest was generated.

16. The method of any one of claims 1 to 14, wherein the cell culture harvest or protein preparation does not contain cells.

17. The method of any one of claims 1 to 14, wherein the protein preparation is a naturally occurring biological fluid.

18. The method of any one of claims 1 to 17, wherein the at least one fatty acid comprises caprylic (octanoic) acid.

19. The method of any one of claims 1 to 18, wherein the at least one fatty acid is nonanoic acid.

20. The method of any one of claims 1 to 19, wherein the at least one fatty acid is present at a concentration in a range selected from the group consisting of: (a) from about 0.1 to about 1.0%, (b) from about 0.2 to about 0.4%, and (c) from about 0.1 to 0.2%.

21. The method of any one of claims 1 to 20, wherein the mixture comprises a surfactant.

22. The method of claim 21, wherein the surfactant is nonionic, or zwitterionic, or cationic.

23. The method of claim 22, wherein the cationic surfactant is cetyltrimethylammonium bromide.

24. The method of claim 23, wherein cetyltrimethylammonium bromide is present at a concentration ranging from about 0.001% to 0.05%, or from about 0.005% to 0.025%, or from about 0.0075% to about 0.01 %.

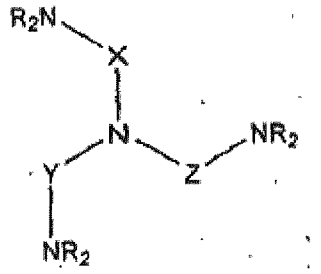
25. The method of any one of claims 1 to 24, wherein the one or more functionalized substrates comprise a cationic functional group and a metal binding functional group.

26. The method of any one of claims 1 to 17, or 19 to 26, wherein the metal binding functional group and the cationic functional group are on separate substrates.

27. The method of any one of claims 1 to 26, wherein the metal binding functional group is also cationic.

28. The method of any one of claims 1 to 27, 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, deferoxamine (desferioxamine), arginine, histidine, histamine, imidazole, and combinations thereof.

29. The method of any one of claims 1 to 28, wherein the metal binding functional group is a compound of formula I:



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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.

30. The method of any one of claims 1 to 29, wherein the metal binding functional group is anionic.

31. The method of claim 30, wherein the metal binding functional group is selected from the group consisting of iminodiacetic acid, nitriloacetic acid, glutamic acid, aspartic acid, aminophenyl phosphate, and combinations thereof.

32. The method of claim 31. wherein the anionic metal binding functional groupies iminodiacetic acid.

33. The method of any one of claims 1 to 32, wherein the antibody is an IgG or IgM antibody.

34. A method for purifying an antibody comprising: contacting a cell culture harvest or protein preparation with at least one fatty acid having 8 to 10 carbon atoms 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 1%; contacting the mixture with allantoin and at least one chemically functionalized solid or soluble substrate comprising a polyamine; and separating solid materials after contacting the mixture with allantoin and the at least one chemically functionalized solid or soluble substrate to provide a solution comprising the antibody.

35. The method of claim 34, comprising contacting the solution with at least one chemically functionalized solid.

36. The method of claim 35, wherein the at least one chemically functionalized solid comprises tris(2-aminoethyl)amine.

37. The method of any one of claim 34 to 36, wherein allantoin is present at a concentration in a range selected from the group consisting of: (a) from about 0.6 to about 30%, (b) from about 1 to about 10%, (c) from about 1 to about 5%, and (d) from about 1 to about 2%.

38. The method of any one of claims 34 to 36, wherein allantoin is in a range from a non-zero amount up to about 0.6%.

39. The method of any one of claims 34 to 38, wherein the antibody is an IgG or IgM antibody.

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 with at least one fatty acid having 7 to 10 carbon atoms to form a mixture;

(b) contacting the mixture with one or more functionalized substrates selected from the group consisting of solid substrates, soluble substrates, and combinations thereof, wherein the one or more functionalized substrates comprise a cationic functional group, a metal binding functional group, or both;

wherein the metal binding functional group comprises 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

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

2. The method of claim 1, further comprising (d) contacting the cell culture harvest with allantoin.

3. The method of claim 2, wherein allantoin is present at a concentration in a range selected from the group consisting of: (a) from about 0.6 to about 30%, (b) from about 1 to about 10%, and (c) from about 1 to about 2%.

4. The method of claim 2, wherein allantoin is in a range from a non-zero amount up to about 0.6%.

5. The method of claim 1, wherein a total amount of the one or more functionalized substrates is a volumetric proportion of the total volume of the preparation of about 0.01%, 0.05%, 0.1%, 0.25%, 0.5%, 1%, 2%, 5%, 10%, 20%, or intermediate values in between.

6. The method of claim 1, wherein the at least one fatty acid and the one or more functionalized substrates are disposed in a single vessel.

7. 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.

8. The method of claim 7, 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.

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

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

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

12. The method of claim 11, wherein filtration comprises membrane filtration or depth filtration.

13. The method of claim 12, wherein the membrane filtration or depth filtration comprises a contact surface that is functionalized.

14. The method of any one of claims 1 to 13, wherein the step (a) is preceded by partial purification of the antibody.

15. The method of any one of claims 1 to 14, wherein the cell culture harvest or protein preparation contains cells, and optionally resides in a bioreactor within which the cell culture harvest was generated.

16. The method of any one of claims 1 to 14, wherein the cell culture harvest or protein preparation does not contain cells.

17. The method of any one of claims 1 to14, wherein the protein preparation is a naturally occurring biological fluid.

18. The method of any one of claims 1 to 17, wherein the at least one fatty acid comprises a general structural formula of $CH_3(CH_2)_nCOOH$, wherein n is an integer from 5 to 8.

19. The method of any one of claims 1 to 18, wherein the at least one fatty acid comprises enanthic (heptanoic) acid, caprylic (octanoic) acid, octenoic acid, pelargonic (nonanoic) acid, nonenoic acid, or capric (decanoic) acid.

20. The method of any one of claims 1-19, wherein the at least one fatty acid is nonanoic acid.

21. The method of any one of claims 1 to 20, wherein the at least one fatty acid is present at a concentration in a range selected from the group consisting of: (a) from about 0.05 to about 5%, (b) from about 0.1 to about 1.0%, (c) from about 0.2 to about 0.4%, and (d) from about 0.1 to 0.2%.

22. The method of any one of claims 1 to 21, wherein the mixture comprises a surfactant.

23. The method of claim 22, wherein the surfactant is nonionic, or zwitterionic, or cationic.

24. The method of claim 23, wherein the cationic surfactant is cetyltrimethylammonium bromide.

25. The method of claim 24, wherein cetyltrimethylammonium bromide is present at a concentration ranging from about 0.001% to 0.05%, or from about 0.005% to 0.025%, or from about 0.0075% to about 0.01%.

26. The method of any one of claims 1 to 25, wherein the one or more functionalized substrates comprises at least one charge configuration selected from the group consisting of anionic, cationic, or zwitterionic.

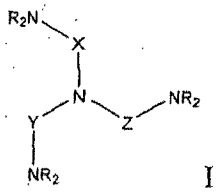
27. The method of claim any one of claims 1 to 26, wherein the metal binding functional group and the cationic functional group are on separate substrates.

28. The method of any one of claims 1 to 27, wherein the metal binding functional group is also cationic.

29. The method of any one of claims 1 to 28, 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, deferoxamine (desferioxamine), arginine, histidine, histamine, imidazole, and combinations thereof.

30. The method of any one of claims 1 to 28, wherein the metal binding functional group is a compound of formula I:



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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.

31. The method of claim 30, wherein the cationic chelating agent is tris(2-aminoethyl)amine.

32. The method of claim any one of claims 1 to 31, wherein the metal binding functional group and an anionic functional group are on separate substrates.

33. The method of any one of claims 1 to 31, wherein the metal binding functional group is anionic.

34. The method of claim 33, wherein the metal binding functional group is selected from the group consisting of iminodiacetic acid, nitriloacetic acid, glutamic acid, aspartic acid, aminophenyl phosphate, and combinations thereof.

35. The method of claim 34, wherein the anionic chelating agent is iminodiacetic acid.

36. The method of any one of claims 1 to 35, wherein the one or more functionalized substrates comprises one substrate that is cationic and a separate substrate that is anionic.

37. The method of any one of claims 1 to 36, wherein the antibody is an IgG or IgM antibody.

38. A method for purifying an antibody comprising:

contacting a cell culture harvest or protein preparation with at least one fatty acid having 8 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 antibody.

39. The method of claim 38, further comprising contacting the solution with at least one chemically functionalized solid or soluble substrate.

40. The method of claim 39, wherein the at least one chemically functionalized solid comprises tris(2-aminoethyl)amine.

41. The method of any one of claim 38 to 40, wherein allantoin is present at a concentration in a range selected from the group consisting of: (a) from about 0.6 to about 30%, (b) from about 1 to about 10%, (c) from about 1 to about 5%, and (d) from about 1 to about 2%.

42. The method of claim 38 to 40, wherein allantoin is in a range from a non-zero amount up to about 0.6%.

43. The method of any one of claims 38 to 42, wherein the antibody is an IgG or IgM antibody.

44. A kit to facilitate the practice of any one of the methods of claims 1 to 43.