than without the wash (Gagnon *et al. supra*). Finally, hydroxyapatite with pre-elution washes of urea and/or salt have also reduced hetero-aggregates more effectively than without such washes (Gagnon *supra*). Despite these observations, in general, the use of dissociating agents in pre-elution washes of antibodies bound to chromatography columns has been only moderately successful.

[0006] Organic multivalent cations have been indicated for the precipitation of acidic proteins (Farhner et al., U.S. Patent Application No. 20080193981; Ma *et al.*, *J. Chromatogr. B* (2010) 878:798-806; Peram *et al.*, *Biotechnol. Progr.*, (2010) 26:1322-1326; Glynn, in U. Gottschalk (ed.), *Process Scale Purification of Antibodies*, J. T. Wiley and Sons, (2009) Hoboken, 309-324), as well as for precipitation of DNA and endotoxins (Glynn *supra*; Cordes *et al.*, Biotechnol. Progr., (1990) 6:283-285; Dissing *et al.*, *Bioseparation*, (1999) 7 221:9-11) and inactivation of virus (Bernhardt, U.S. Patent. 5,559,250). Multivalent metal cations have also been indicated to remove DNA and endotoxin from some protein preparations (Akcasu *et al.*, *Nature*, (1960) 187:323-324; Matsuzawa *et al.*, *Nucl. Acids Res.*, (2003) 3(3):163-164; Christensen *et al.*, *Prot. Expr. Purif.*, (2004) 37:468-471; Kejnovsky *et al.*, *Nucl. Acids Res.*, (1997) 25:1870-1871; Ongkudon *et al.*, *Anal. Chem.*, (2011) 83 391:13-17).

SUMMARY OF THE INVENTION

[0007] In ~~certain embodiments~~one aspect, the invention provides methods for reducing the aggregate content of a sample such as a protein preparation containing a desired protein; certain methods include the steps of: (i) providing a first component which is a first solid substrate having an electronegative surface: (ii) providing a second component which is a second solid substrate having an electropositive surface; (iii) contacting the sample with the first and second components, where the first and second components are configured such that the sample may contact both components simultaneously under operating conditions that substantially prevent binding of the desired protein to the first or second component; and (iv) separating a resulting sample containing the desired protein with a reduced aggregate content from the first and second components; wherein the electronegativity of the surface of the first component is conferred in part by a moiety from the group consisting of iminodiacetic acid, ethylene glycol(aminoethylether)diacetic acid, nitriloacetic acid, aspartic acid, glutamic acid, a carboxylic acid, sulfurous acid, sulfonate, and phosphoric acid and the electropositivity of the surface of the second component is conferred in part by a moiety from the group consisting of tris(2-aminoethyl)amine, diethylenetriamine, triethylenetetramine, tetraethylenepentamine, polypropylenimine tetramine, PAMAM dendrimer (ethylenediamine core), deferoxamine, a primary amino group, a secondary amino group, a tertiary amino group, and a quaternary amino group. In another aspect there is provided a kit suitable for reducing the aggregate content of a sample

containing a desired protein as per the method as described herein, comprising a first component which is a first solid substrate having an electronegative surface and a second component which is a second solid substrate having an electropositive surface.

BRIEF DESCRIPTION OF THE FIGURES

[0008] Figure 1 shows a time course plot of the size exchange chromatography (SEC) profiles sampled at indicated intervals in the purification of IgM-529 which was subjected to allantoin-ethacridine batch particle treatment. Aggr: aggregate. HCP: host cell protein. Solid line: 280 nm. Broken line: 254 nm. All time scales same as center panel.

Claims.

1. A method of reducing the aggregate content of a sample containing a desired protein, wherein the method comprises the steps of: (i) providing a first component which is a first solid substrate having an electronegative surface: (ii) providing a second component which is a second solid substrate having an electropositive surface; (iii) contacting the sample with the first and second components, where the first and second components are configured such that the sample may contact both components simultaneously, where the operating conditions substantially prevent the binding of the desired protein to the first or second components; and (iv) separating a resulting sample containing the desired protein with a reduced aggregate content from the first and second components; wherein the electronegativity of the surface of the first component is conferred in part by a moiety from the group consisting of iminodiacetic acid, ethylene glycol(aminoethylether)diacetic acid, nitriloacetic acid, aspartic acid, glutamic acid, a carboxylic acid, sulfurous acid, sulfonate, and phosphoric acid and the electropositivity of the surface of the second component is conferred in part by a moiety from the group consisting of tris(2-aminoethyl)amine, diethylenetriamine, triethylenetetramine, tetraethylenepentamine, polypropylenimine tetramine, PAMAM dendrimer (ethylenediamine core), deferoxamine, a primary amino group, a secondary amino group, a tertiary amino group, and a quaternary amino group.

2. The method of claim 1 wherein the first and second components are combined prior to contacting the sample.

3. The methods of claims 1-2 wherein the sample is contacted with a soluble organic multivalent cation of mixed chemical character prior to contacting the first substrate having an electronegative surface.

4. The method of claim 3 wherein the sample is incubated with the soluble organic multivalent cation of mixed chemical character for an appropriate period of time prior to contacting the first and second components.

5. The methods of claims 1-4 wherein the sample is incubated with the first and second components for an appropriate period of time.

6. The methods of claims 3-5 wherein the organic multivalent cation having mixed chemical character is removed from the sample in the step of separating the sample with a reduced aggregate content from the first and second components to the extent that the organic multivalent cation having mixed chemical character is associated with a substrate of the first or second component.

7. The method of claims 1-6 wherein the first substrate is particulate.

8. The method of claims 1-6 wherein the second substrate is particulate.

9. The method of claims 7-8 wherein the particulate substrate or substrates is each provided as a plurality of particles.

10. The method of claim 7-9 wherein the particles of the first substrate, the second substrate or both are non-porous.

11. The method of claim 7-9 wherein the particles of the first substrate, the second substrate or both are porous.

12. The method of claim 11 wherein the pore size of the porous particles permits entry of a protein in a protein preparation.

13. The method of claim 11 wherein the pore size of the porous particles restricts entry of a protein in a protein preparation.

14. The method of claim 11 wherein the average pore size of the porous particles is between about 10nm and about 100nm.

15. The methods of claims 7-14 wherein the particles are sandwiched between porous membranes or monoliths.

16. The methods of claims 7-14 wherein the particles are sandwiched between woven or amorphous fibrous filters.

17. The methods of claims 7-14 wherein the particles are sandwiched between crystalline frits.

18. The methods of claims 7-14 wherein the particles are embedded in a reticular polymer network.

19. The method of claims 3-18 wherein the conductivity of the sample is at a level to avoid precipitation of the desired protein from the sample while contacting the soluble organic multivalent cation of mixed chemical character.

20. The method of claim 19 wherein the conductivity of the sample is greater than 20mS/cm.

21. The method of claim 20 wherein the conductivity of the sample is greater than 30 mS/cm.

22. The method of claim 20 wherein the conductivity of the sample is greater than about 40 mS/cm.

23. The method of claim 20 wherein the conductivity of the sample is greater than about 100 mS/cm.

24. The method of claim 19 wherein the conductivity of the sample is at least 5%, 10%, 20%, 50%, 100% or 200% higher than the conductivity to avoid precipitation of the desired

protein from the sample while contacting the soluble organic multivalent cation of mixed chemical character.

25. The method of claims 1-24 wherein the aggregates comprise homo-aggregates of the desired protein.

26. The method of claim 25 wherein the presence of homo-aggregates of the desired protein is substantially eliminated.

27. The method of claims 1-26 wherein the aggregates comprise hetero-aggregates of the desired protein and a contaminant.

28 The method of claim 27 wherein the hetero-aggregates are of substantially the same hydrodynamic size as the desired protein.

29. The method of claim 27 wherein the contaminant is a nucleic acid, nucleotide, endotoxin, metal ion, protein, lipid, or cell culture media component.

30. The method of claims 27-29 wherein the presence of hetero-aggregates of the desired protein and a contaminant is substantially eliminated.

31. The method of claims 1-30 wherein the desired protein is an antibody or antibody fragment.

32. The method of claims 1-30 wherein the desired protein is a recombinant protein.

33. The method of claims 1-30 wherein the desired protein is a clotting factor.

34. The method of claim 33 wherein the clotting factor is Factor VIII.

35. The method of claim 32 wherein the desired protein is a growth hormone.

36. The method of claim 31 wherein the sample is a cell culture harvest, a cell culture supernatant, an antibody-containing solution derived from a cell culture, or an antibody-containing solution from a previous stage of protein purification.

37. The method of claim 31 wherein the cultured cells are mammalian cells, a bacterial cells, or a yeast cells.

38. The method of claim 35 wherein the sample is an antibody-containing solution from a previous stage of protein purification.

39. The method of claim 38 wherein the sample is an eluate from a chromatography column.

40. The method of claims 1-39, wherein the sample may be unpurified, at an intermediate level of purity, or highly purified.

41. The method of claims 1-40, wherein the sample is contacted with the first and second components by flowing the preparation through the first and second components.

42. The method of claims 1-41 wherein the electronegativity of the surface of the first

component is conferred through one or more kinds of complex chemical moieties that embody more than one chemical functionality, or through a combination of simple and complex chemical groups mixed on the surface, or a combination of surfaces of differing chemical composition.

43. The method of claims 1-41 wherein the electropositivity of the surface of the second component is conferred through one or more kinds of complex chemical groups that embody more than one chemical functionality, or through a combination of simple and complex chemical groups mixed on the surface, or a combination of surfaces of differing chemical composition.

44. The method of claims 1-43 wherein the electronegativity of the surface of the first component is conferred in part by iminodiacetic acid and the electropositivity of the surface of the second component is conferred in part by tris(2-aminoethyl)amine.

45. The method of claims 1-44 wherein the first component has a surface-bound chemical moiety possessing metal affinity functionality.

46. The method of claims 1-44 wherein the electropositive surface of the second component includes a surface-bound chemical moiety possessing metal affinity functionality.

47. The method of claims 45-46 wherein at least one of the substrates has one or more chemical moieties in addition to the surface-bound chemical moiety possessing metal affinity functionality wherein such additional chemical moieties enhance the capacity of one or more of the components to participate in hydrogen bonding, hydrophobic interactions, or pi-pi binding with a protein of the protein preparation.

48. The method of claims 45-46 wherein the surface-bound chemical moiety possessing metal affinity functionality is a multidentate metal chelating moiety.

49. The method of claims 1-48 wherein the electropositive surface of the first component includes a surface-bound chemical moiety possessing metal affinity functionality, the electronegative surface of the second component includes a surface-bound chemical moiety possessing metal affinity functionality, and another surface includes a surface-bound chemical moiety possessing an elevated hydrophobic functionality.

50. The method of claims 3-48 wherein the organic multivalent cation of mixed chemical character is selected from the group consisting of ethacridine, 9-aminoacridine (aminacrine), 3,6 acridinediamine (proflavin), acrisorcin, acrizane (phenacridane), acridine orange, quinacrine, acricide, acridone, acridine-9-carboxylic acid, acranil (1-[(6-chloro-2-methoxy-9-

acridinyl)amino]-3-(diethylamino)-2-propanol dihydrochloride), phenosafranin, phenoxazine, phenothiazine, acriflavine (3,6-diamino-10-methylacridinium, chloride and 3,6-acridineidiamine), polyethyleneimine, chlorhexidine, and poly-amino acids.

51. The method of claim 49 wherein the organic multivalent cation of mixed chemical character is ethacridine, polyethylenimine or chlorhexidine or a salt thereof.

52. The method of claim 50 wherein the organic multivalent cation of mixed chemical character is ethacridine or a salt thereof.

53. The method of claims 49-51 wherein the organic multivalent cation of mixed chemical character is present in an amount between approximately 0.01% and approximately 0.05%.

54. The method of claims 49-51 wherein the organic multivalent cation of mixed chemical character is present in an amount less than approximately 0.01%.

55. The method of claims 49-51 wherein the organic multivalent cation of mixed chemical character is present in an amount less than approximately 0.005%.

56. The method of claims 49-51 wherein the organic multivalent cation of mixed chemical character is present in an amount less than approximately 0.001%.

57. The method of claims 49-51 wherein the organic multivalent cation of mixed chemical character is present in an amount between approximately 0.020 and approximately 0.025%.

58. The method of claims 3-48 wherein the sample is treated with more than one organic multivalent cation from the group consisting of polyethyleneimine, polyallyamine, ethacridine, and chlorhexidine and salts thereof prior to the step of contacting the sample with the first and second components.

59. The method of claim 58 wherein the organic multivalent cations used to treat the sample prior to the step of contacting the sample with the first and second components are provided in a concentration of less than 1%.

60. The method of claim 58 wherein the organic multivalent cations used to treat the sample prior to the step of contacting the sample with the first and second components are provided in a concentration between approximately 0.01% and approximately 0.05%.

61. The method of claim 58 wherein the organic multivalent cations used to treat the sample prior to the step of contacting the sample with the first and second components are provided in a concentration less than approximately 0.01%.

62. The method of claim 58 wherein the organic multivalent cations used to treat the

sample prior to the step of contacting the sample with the first and second components are provided in a concentration less than approximately 0.005%.

63. The method of claim 58 wherein the organic multivalent cations used to treat the sample prior to the step of contacting the sample with the first and second components are provided in a concentration less than approximately 0.001%.

64. The method of claim 58 wherein the organic multivalent cations used to treat the sample prior to the step of contacting the sample with the first and second components are provided in a concentration between approximately 0.020 and approximately 0.025%.

65. The method of claims 1-64, wherein the sample is additionally contacted with a soluble organic modulator selected from the group consisting of nonionic organic polymers, organic solvents, surfactants, and ureides, prior to the step of contacting the sample with the first and second components.

66. The method of claims 1-65 wherein the sample is additionally contacted with an antiviral agent, prior to the step of contacting the sample with the first and second components.

67. The method of claim 66 wherein the antiviral agent is a non-multivalent organic cation.

68. The method of claim 66 wherein the antiviral agent is selected from the group consisting of benzalkonium chloride, methylene blue and tri (n-butyl) phosphate.

69. The method of claim 63-68 wherein the antiviral agent is present in an amount less than approximately 1% (w/v).

70. The method of claim 66 wherein the antiviral agent is present in an amount less than approximately 0.1% (w/v).

71. The method of claim 70 wherein the antiviral agent is present in an amount less than approximately 0.01% (w/v).

72. The method of claim 71 wherein the antiviral agent is present in an amount less than approximately 0.001% (w/v).

73. The method of Claims 1-72 comprising the additional steps of, prior to the step of contacting the sample with the first and second components, contacting the sample with a ureide in an amount sufficient for the ureide to be supersaturated in the protein preparation, and separating the supernatant containing the desired protein from the solid or undissolved portions of the sample.

74. The method of claim 73 wherein the step of contacting the sample with the ureide occurs prior to the step of contacting the sample with the soluble organic multivalent cation

of mixed chemical character.

75. The method of claim 73 wherein the step of contacting the sample with the ureide occurs substantially simultaneously with the step of contacting the sample with the soluble organic multivalent cation of mixed chemical character.

76. The method of claim 73 wherein the step of contacting the sample with the ureide occurs after the step of contacting the sample with the soluble organic multivalent cation of mixed chemical character.

77. The method of claims 73-76 wherein the ureide is selected from the group consisting of urea, uric acid, hydantoin, allantoin, alcloxa, aldioxa, hemocane, ureidohydantoin, 5-ureidohydantoin, glyoxylureide, glyoxylic acid diureide, 2,5-dioxo-4-imidazolidinyl urea, and purines.

78. The method of claim 77 wherein the ureide is allantoin.

79. The method of claim 77 wherein the ureide is uric acid.

80. The method of claim 78 wherein the allantoin is present in an amount greater than 0.5% (w/v).

81. The method of claim of claim 80 wherein the allantoin is present in an amount greater than approximately 1% (w/v).

82. The method of claim 79 wherein the uric acid is present in an amount greater than 0.0025% (w/v).

83. The method of claim 81 wherein the allantoin is present in an amount greater than approximately 2% (w/v).

84. The method of claim 81 wherein the allantoin is present in an amount greater than approximately 5% (w/v).

85. The method of claim 81 wherein the allantoin is present in an amount greater than approximately 10% (w/v).

86. The method of claim 65 wherein the step of contacting the sample with the organic modulator occurs prior to the step of contacting the sample with the soluble organic multivalent cation of mixed chemical character.

87. The method of claim 65 wherein the step of contacting the sample with the organic modulator occurs substantially simultaneously with the step of contacting the sample with the soluble organic multivalent cation of mixed chemical character.

88. The method of claim 65 wherein the step of contacting the sample with the organic modulator occurs after the step of contacting the sample with the soluble organic multivalent cation of mixed chemical character.

89. The method of claims 86-88 wherein the organic modulator is a nonionic organic polymer selected from the group consisting of glycerol, polyethylene glycol, polypropylene glycol and polybutylene glycol.

90. The method of claim 89 wherein the nonionic organic polymer has an average molecular weight of approximately 500 D or less.

91. The method of claims 86-88 wherein the organic modulator is an organic solvent selected from the group consisting of ethylene glycol, propylene glycol, butylene glycol, dimethylsulfoxide, ethanol, isopropanol, and phenoxyethanol.

92. The method of claim 86-91 wherein the organic modulator is provided at a concentration of approximately 1% (w/v) or less.

93. The method of claims 86-88 wherein the organic modulator is a surfactant selected from the group consisting of Tween, triton, CHAPS, CHAPSO and octyl glucoside.

94. The method of claim 93 wherein the surfactant is provided at a concentration of approximately 1% (w/v) or less.

95. The method of claim 93 wherein the surfactant is provided at a concentration of approximately 0.1% (w/v) or less.

96. The method of claim 89-91 wherein the organic modulator is a ureide provided in a subsaturating amount.

97. The method of claim 96 wherein the ureide is selected from the group consisting of urea, hydantoin, and allantoin.

98. A kit suitable for reducing the aggregate content of a sample containing a desired protein as per the method of any one of claims 1 to 97, comprising a first component which is a first solid substrate having an electronegative surface and a second component which is a second solid substrate having an electropositive surface.

Claims.

1. A method of reducing the aggregate content of a sample containing a desired protein, wherein the method comprises the steps of: (i) providing a first component which is a first solid substrate having an electronegative surface: (ii) providing a second component which is a second solid substrate having an electropositive surface; (iii) contacting the sample with the first and second components, where the first and second components are configured such that the sample may contact both components simultaneously, where the operating conditions substantially prevent the binding of the desired protein to the first or second components; and (iv) separating a resulting sample containing the desired protein with a reduced aggregate content from the first and second components.

2. The method of claim 1 wherein the first and second components are combined prior to contacting the sample.

3. The methods of claims 1-2 wherein the sample is contacted with a soluble organic multivalent cation of mixed chemical character prior to contacting the first substrate having an electronegative surface.

4. The method of claim 3 wherein the sample is incubated with the soluble organic multivalent cation of mixed chemical character for an appropriate period of time prior to contacting the first and second components.

5. The methods of claims 1-4 wherein the sample is incubated with the first and second components for an appropriate period of time.

6. The methods of claims 3-5 wherein the organic multivalent cation having mixed chemical character is removed from the sample in the step of separating the sample with a reduced aggregate content from the first and second components to the extent that the organic multivalent cation having mixed chemical character is associated with a substrate of the first or second component.

7. The method of claims 1-6 wherein the first substrate is particulate.

8. The method of claims 1-6 wherein the second substrate is particulate.

9. The method of claims 7-8 wherein the particulate substrate or substrates is each provided as a plurality of particles.

10. The method of claim 7-9 wherein the particles of the first substrate, the second substrate or both are non-porous.

11. The method of claim 7-9 wherein the particles of the first substrate, the second substrate or both are porous.

12. The method of claim 11 wherein the pore size of the porous particles is large enough to permit entry of a protein in a protein preparation.

13. The method of claim 11 wherein the pore size of the porous particles is too small to permit entry of a protein in a protein preparation.

14. The method of claim 11 wherein the average pore size of the porous particles is between about 10nm and about 100nm.

15. The methods of claims 7-14 wherein the particles are sandwiched between porous membranes or monoliths.

16. The methods of claims 7-14 wherein the particles are sandwiched between woven or amorphous fibrous filters.

17. The methods of claims 7-14 wherein the particles are sandwiched between crystalline frits.

18. The methods of claims 7-14 wherein the particles are embedded in a reticular polymer network.

19. The method of claims 3-18 wherein the conductivity of the sample is at a sufficiently high level to substantially avoid precipitation of the desired protein from the sample while contacting the soluble organic multivalent cation of mixed chemical character.

20. The method of claim 19 wherein the conductivity of the sample is greater than 20mS/cm.

21. The method of claim 20 wherein the conductivity of the sample is greater than 30 mS/cm.

22. The method of claim 20 wherein the conductivity of the sample is greater than about 40 mS/cm.

23. The method of claim 20 wherein the conductivity of the sample is greater than about 100 mS/cm.

24. The method of claim 19 wherein the conductivity of the sample is at least 5%, 10%, 20%, 50%, 100% or 200% higher than the conductivity sufficient to substantially avoid precipitation of the desired protein from the sample while contacting the soluble organic multivalent cation of mixed chemical character.

25. The method of claims 1-24 wherein the aggregates comprise homo-aggregates of the desired protein.

26. The method of claim 25 wherein the presence of homo-aggregates of the desired protein is substantially eliminated.

27. The method of claims 1-26 wherein the aggregates comprise hetero-aggregates of the desired protein and a contaminant.

28 The method of claim 27 wherein the hetero-aggregates are of substantially the same hydrodynamic size as the desired protein.

29. The method of claim 27 wherein the contaminant is a nucleic acid, nucleotide, endotoxin, metal ion, protein, lipid, or cell culture media component.

30. The method of claims 27-29 wherein the presence of hetero-aggregates of the desired protein and a contaminant is substantially eliminated.

31. The method of claims 1-30 wherein the desired protein is an antibody or antibody fragment.

32. The method of claims 1-30 wherein the desired protein is a recombinant protein.

33. The method of claims 1-30 wherein the desired protein is a clotting factor.

34. The method of claim 33 wherein the clotting factor is Factor VIII.

35. The method of claim 32 wherein the desired protein is a growth hormone.

36. The method of claim 31 wherein the sample is a cell culture harvest, a cell culture supernatant, an antibody-containing solution derived from a cell culture, or an antibody-containing solution from a previous stage of protein purification.

37. The method of claim 31 wherein the cultured cells are mammalian cells, a bacterial cells, or a yeast cells.

38. The method of claim 35 wherein the sample is an antibody-containing solution from a previous stage of protein purification.

39. The method of claim 38 wherein the sample is an eluate from a chromatography column.

40. The method of claims 1-39, wherein the sample may be unpurified, at an intermediate level of purity, or highly purified.

41. The method of claims 1-40, wherein the sample is contacted with the first and second components by flowing the preparation through the first and second components.

42. The method of claims 1-41 wherein the electronegativity of the surface of the first component is conferred through one or more kinds of complex chemical moieties that embody more than one chemical functionality, or through a combination of simple and complex chemical groups mixed on the surface, or a combination of surfaces of differing chemical composition.

43. The method of claims 1-42, wherein the electronegativity of the surface of the first

component is conferred in part by a moiety from the group consisting of iminodiacetic acid, ethylene glycol(aminoethylether)diacetic acid, nitriloacetic acid, aspartic acid, glutamic acid, a carboxylic acid, sulfurous acid, sulfonate, and phosphoric acid.

44. The method of claims 1-41 wherein the electropositivity of the surface of the second component is conferred through one or more kinds of complex chemical groups that embody more than one chemical functionality, or through a combination of simple and complex chemical groups mixed on the surface, or a combination of surfaces of differing chemical composition.

45. The method of claims 1-44, wherein the electropositivity of the surface of the second component is conferred in part by a moiety from the group consisting of tris(2-aminoethyl)amine, diethylenetriamine, triethylenetetramine, tetraethylenepentamine, polypropylenimine tetramine, PAMAM dendrimer (ethylenediamine core), deferoxamine, a primary amino group, a secondary amino group, a tertiary amino group, and a quaternary amino group.

46. The method of claims 1-45 wherein the electronegativity of the surface of the first component is conferred in part by iminodiacetic acid and the electropositivity of the surface of the second component is conferred in part by tris(2-aminoethyl)amine.

47. The method of claims 1-46 wherein the first component has a surface-bound chemical moiety possessing metal affinity functionality.

48. The method of claims 1-46 wherein the electropositive surface of the second component includes a surface-bound chemical moiety possessing metal affinity functionality.

49. The method of claims 47-48 wherein at least one of the substrates has one or more chemical moieties in addition to the surface-bound chemical moiety possessing metal affinity functionality wherein such additional chemical moieties enhance the capacity of one or more of the components to participate in hydrogen bonding, hydrophobic interactions, or pi-pi binding with a protein of the protein preparation.

50. The method of claims 47-48 wherein the surface-bound chemical moiety possessing metal affinity functionality is a multidentate metal chelating moiety.

51. The method of claims 1-50 wherein the electropositive surface of the first component includes a surface-bound chemical moiety possessing metal affinity functionality, the electronegative surface of the second component includes a surface-bound chemical moiety possessing metal affinity functionality, and another surface includes a surface-bound chemical moiety possessing an elevated hydrophobic functionality.

52. The method of claims 3-50 wherein the organic multivalent cation of mixed chemical character is selected from the group consisting of ethacridine, 9-aminoacridine (aminacrine), 3,6 acridinediamine (proflavin), acrisorcin, acrizane (phenacridane), acridine orange, quinacrine, acricide, acridone, acridine-9-carboxylic acid, acranil (1-[(6-chloro-2-methoxy-9-acridinyl)amino]-3-(diethylamino)-2-propanol dihydrochloride), phenosafranin, phenoxazine, phenothiazine, acriflavine (3,6-diamino-10-methylacridinium, chloride and 3,6-acridineidiamine), polyethyleneimine, chlorhexidine, and poly-amino acids.

53. The method of claim 51 wherein the organic multivalent cation of mixed chemical character is ethacridine, polyethylenimine or chlorhexidine or a salt thereof.

54. The method of claim 52 wherein the organic multivalent cation of mixed chemical character is ethacridine or a salt thereof.

55. The method of claims 51-53 wherein the organic multivalent cation of mixed chemical character is present in an amount between approximately 0.01% and approximately 0.05%.

56. The method of claims 51-53 wherein the organic multivalent cation of mixed chemical character is present in an amount less than approximately 0.01%.

57. The method of claims 51-53 wherein the organic multivalent cation of mixed chemical character is present in an amount less than approximately 0.005%.

58. The method of claims 51-53 wherein the organic multivalent cation of mixed chemical character is present in an amount less than approximately 0.001%.

59. The method of claims 51-53 wherein the organic multivalent cation of mixed chemical character is present in an amount between approximately 0.020 and approximately 0.025%.

60. The method of claims 3-50 wherein the sample is treated with more than one organic multivalent cation from the group consisting of polyethyleneimine, polyallyamine, ethacridine, and chlorhexidine and salts thereof prior to the step of contacting the sample with the first and second components.

61. The method of claim 60 wherein the organic multivalent cations used to treat the sample prior to the step of contacting the sample with the first and second components are provided in a concentration of less than 1%.

62. The method of claim 60 wherein the organic multivalent cations used to treat the sample prior to the step of contacting the sample with the first and second components are provided in a concentration between approximately 0.01% and approximately 0.05%.

63. The method of claim 60 wherein the organic multivalent cations used to treat the sample prior to the step of contacting the sample with the first and second components are provided in a concentration less than approximately 0.01%.

64. The method of claim 60 wherein the organic multivalent cations used to treat the sample prior to the step of contacting the sample with the first and second components are provided in a concentration less than approximately 0.005%.

65. The method of claim 60 wherein the organic multivalent cations used to treat the sample prior to the step of contacting the sample with the first and second components are provided in a concentration less than approximately 0.001%.

66. The method of claim 60 wherein the organic multivalent cations used to treat the sample prior to the step of contacting the sample with the first and second components are provided in a concentration between approximately 0.020 and approximately 0.025%.

67. The method of claims 1-66, wherein the sample is additionally contacted with a soluble organic modulator selected from the group consisting of nonionic organic polymers, organic solvents, surfactants, and ureides, prior to the step of contacting the sample with the first and second components.

68. The method of claims 1-67 wherein the sample is additionally contacted with an antiviral agent, prior to the step of contacting the sample with the first and second components.

69. The method of claim 68 wherein the antiviral agent is a non-multivalent organic cation.

70. The method of claim 68 wherein the antiviral agent is selected from the group consisting of benzalkonium chloride, methylene blue and tri (n-butyl) phosphate.

71. The method of claim 65-70 wherein the antiviral agent is present in an amount less than approximately 1% (w/v).

72. The method of claim 68 wherein the antiviral agent is present in an amount less than approximately 0.1% (w/v).

73. The method of claim 72 wherein the antiviral agent is present in an amount less than approximately 0.01% (w/v).

74. The method of claim 73 wherein the antiviral agent is present in an amount less than approximately 0.001% (w/v).

75. The method of Claims 1-74 comprising the additional steps of, prior to the step of contacting the sample with the first and second components, contacting the sample with a

ureide in an amount sufficient for the ureide to be supersaturated in the protein preparation, and separating the supernatant containing the desired protein from the solid or undissolved portions of the sample.

76. The method of claim 75 wherein the step of contacting the sample with the ureide occurs prior to the step of contacting the sample with the soluble organic multivalent cation of mixed chemical character.

77. The method of claim 75 wherein the step of contacting the sample with the ureide occurs substantially simultaneously with the step of contacting the sample with the soluble organic multivalent cation of mixed chemical character.

78. The method of claim 75 wherein the step of contacting the sample with the ureide occurs after the step of contacting the sample with the soluble organic multivalent cation of mixed chemical character.

79. The method of claims 75-78 wherein the ureide is selected from the group consisting of urea, uric acid, hydantoin, allantoin, alcloxa, aldioxa, hemocane, ureidohydantoin, 5-ureidohydantoin, glyoxylureide, glyoxylic acid diureide, 2,5-dioxo-4-imidazolidinyl urea, and purines.

80. The method of claim 79 wherein the ureide is allantoin.

81. The method of claim 79 wherein the ureide is uric acid.

82. The method of claim 80 wherein the allantoin is present in an amount greater than 0.5% (w/v).

83. The method of claim of claim 82 wherein the allantoin is present in an amount greater than approximately 1% (w/v).

84. The method of claim 81 wherein the uric acid is present in an amount greater than 0.0025% (w/v).

85. The method of claim 83 wherein the allantoin is present in an amount greater than approximately 2% (w/v).

86. The method of claim 83 wherein the allantoin is present in an amount greater than approximately 5% (w/v).

87. The method of claim 83 wherein the allantoin is present in an amount greater than approximately 10% (w/v).

88. The method of claim 67 wherein the step of contacting the sample with the organic modulator occurs prior to the step of contacting the sample with the soluble organic multivalent cation of mixed chemical character.

89. The method of claim 67 wherein the step of contacting the sample with the organic modulator occurs substantially simultaneously with the step of contacting the sample with the soluble organic multivalent cation of mixed chemical character.

90. The method of claim 67 wherein the step of contacting the sample with the organic modulator occurs after the step of contacting the sample with the soluble organic multivalent cation of mixed chemical character.

91. The method of claims 88-90 wherein the organic modulator is a nonionic organic polymer selected from the group consisting of glycerol, polyethylene glycol, polypropylene glycol and polybutylene glycol.

92. The method of claim 91 wherein the nonionic organic polymer has an average molecular weight of approximately 500 D or less.

93. The method of claims 88-90 wherein the organic modulator is an organic solvent selected from the group consisting of ethylene glycol, propylene glycol, butylene glycol, dimethylsulfoxide, ethanol, isopropanol, and phenoxyethanol.

94. The method of claim 88-93 wherein the organic modulator is provided at a concentration of approximately 1% (w/v) or greater

95. The method of claims 88-90 wherein the organic modulator is a surfactant selected from the group consisting of Tween, triton, CHAPS, CHAPSO and octyl glucoside.

96. The method of claim 95 wherein the surfactant is provided at a concentration of approximately 1% (w/v) or less.

97. The method of claim 95 wherein the surfactant is provided at a concentration of approximately 0.1% (w/v) or less.

98. The method of claim 91-93 wherein the organic modulator is a ureide provided in a subsaturating amount.

99. The method of claim 98 wherein the ureide is selected from the group consisting of urea, hydantoin, and allantoin.

100. A kit providing for the convenient practice of a method of any of claims 1-99.