preparations. In certain embodiments, the compositions particularly reduce the content of aggregates that include chromatin remnants, such as nucleosomes, and/or histones, and/or DNA. In a first aspect embodiments, a composition of matter for reducing the aggregate content of a protein preparation is provided where the composition includes a first surface-bound ligand possessing metal affinity functionality and a second surface-bound possessing a metal affinity functionality, wherein the second surface-bound ligand is ligand having an aggregate charge opposite to that of the metal affinity functionality of the first surface and where the first surface-bound ligand and the second surface-bound ligand are positioned such that a protein preparation may contact both the first surface-bound ligand and the second surface-bound ligand simultaneously, wherein the first surface-bound ligand is electronegative and selected from the group consisting of iminodiacetic acid (2-(carboxymethylamino)acetic acid), ethylene glycol(aminoethylether)diacetic acid, and nitriloacetic acid (2,2',2''-nitrilotriacetic acid), and wherein the second surface-bound ligand is tris(2-aminoethyl)amine, diethylenetriamine, triethylenetetramine, tetraethylenepentamine, polypropylenimine tetraamine, or desferroxamine.

In a second aspect, the present invention provides an apparatus comprising the composition of the present invention, wherein the apparatus is optionally configured for chromatography.

BRIEF DESCRIPTION OF THE DRAWINGS

[0007] 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.

[0008] Figure 2A shows an SEC profile of IgM-84 filtered cell culture supernatant (CCS). Aggr: aggregates. HMW: high molecular weight aggregates. HCP: host cell protein. LMW: low molecular weight cell culture media components. Solid line: 280 nm. Broken line: 254 nm.

[0009] Figure 2B shows an SEC profile of the IgM-84 filtered CCS of Figure 2A after allantoin-ethacridine and column flow through treatment. Aggr: aggregate. HCP: host cell protein. Solid line: 280 nm. Broken line: 254 nm.

[0010] Figure 3 shows an SEC profile of monoclonal IgG HER2 CCS before (left panel) and after (right panel) treatment with allantoin-ethacridine and column flow through treatment. Aggr: aggregate. HCP: host cell protein. LMW: low molecular weight cell culture media components. LC: light chain. Solid line: 280 nm. Broken line: 254 nm.

DETAILED DESCRIPTION

It has been surprisingly discovered that in certain embodiments of the invention a composition of matter comprising a combination an electronegative surface-bound metal affinity ligand (first surface-bound ligand) with an electropositive surface-bound ligand (second surface-bound ligand), or alternatively comprising a combination an electropositive surface-bound metal affinty ligand (first surface-bound ligand) and an electronegative surface-bound ligand (second-surface-bound ligand), has the ability to substantially reduce the content of high molecular weight (HMW) aggregates and aggregates of smaller size in protein preparations. In certain embodiments, the first surface-bound ligand and the second surface-bound ligand may reside on the same surface. In certain embodiments the first surface-bound ligand and the second surface-bound ligand may

Claims

1. A composition of matter for reducing the aggregate content of a protein preparation, comprising a first substrate comprising a first surface-bound ligand possessing a metal affinity functionality and a second surface-bound ligand possessing a metal affinity functionality, wherein the second surface-bound ligand is optionally provided on a second substrate and having an aggregate charge opposite to that of the metal affinity functionality of the first substrate, wherein the first surface-bound ligand and the second surface-bound ligand are positioned such that the protein preparation may contact both the first surface-bound ligand and the second surface-bound ligand simultaneously, wherein the first surface-bound ligand is electronegative and selected from the group consisting of iminodiacetic acid (2-(carboxymethylamino)acetic acid), ethylene glycol(aminoethylether)diacetic acid, and nitriloacetic acid (2,2',2"-nitrilotriacetic acid), and wherein the second surface-bound ligand is tris(2-aminoethyl)amine, diethylenetriamine, triethylenetetramine, tetraethylenepentamine, polypropylenimine tetraamine, or desferroxamine.

2. The composition of claim 1, wherein the first surface-bound ligand, the second surface-bound ligand, or both comprise a multidentate metal chelating moiety.

3. The composition of claim 1, wherein the first surface-bound ligand has an aggregate charge which is electronegative.

4. The composition of claim 3, wherein the first substrate has additional chemical moieties bound to it, provided that an aggregate charge of the first substrate is electronegative.

5. The composition of claim 1, wherein the first surface-bound ligand is ethylene glycol(aminoethylether)diacetic acid, or nitriloacetic acid (2,2',2"-nitrilotriacetic acid).

6. The composition of claim 1, wherein the second surface-bound ligand is tris(2-aminoethyl)amine.

7. The composition of claim 1, wherein the first surface-bound ligand is iminodiacetic acid (2-(carboxymethylamino)acetic acid).

8. The composition of claim 1, wherein the second surface-bound ligand has an aggregate charge which is electropositive.

9. The composition of claim 1, wherein the second substrate has additional chemical moieties bound to it, and an aggregate charge of the second substrate is electropositive.

10. The composition of claim 1, wherein the second surface-bound ligand is t diethylenetriamine, triethylenetetramine, tetraethylenepentamine, or polypropylenimine tetraamine.

11. The composition of claim 1, wherein at least one of the first substrate or optional second substrate has one or more further surface-bound ligands in addition to the first surface-bound ligand and the second surface-bound ligand, wherein the one or more further surface-bound ligands enhance

the capacity of the composition to participate in hydrogen bonding, hydrophobic interactions, or pi-pi binding with a component of the protein preparation.

12. The composition of claim 1, wherein the first substrate, the second substrate, or both comprise more than one negatively charged metal chelating group in combination with one or more oppositely charged groups.

13. The composition of claim 1, wherein the first substrate, the second substrate, or both comprise more than one positively charged metal chelating group in combination with one or more oppositely charged groups.

14. The composition of claim 1, wherein the first surface-bound ligand and the second surface-bound ligand are covalently bound to the first substrate.

15. The composition of claim 1, wherein the first surface-bound ligand is covalently bound to the first substrate and the second surface-bound ligand covalently bound to the second substrate which is different from the first substrate.

16. The composition of claim 1 wherein the first substrate, second substrate, or both comprise particles.

17. The composition of claim 16, wherein the particles are non-porous.

18. The composition of claim 16, wherein the particles are porous.

19. The composition of claim 16, wherein the particles have a pore size large enough to permit entry of a protein in a protein preparation.

20. The composition of claim 16, wherein the particles have a pore size too small to permit entry of a protein in a protein preparation.

21. The composition of claim 18, wherein the particles have an average pore size between about 10 nm and about 100 nm.

22. The composition of claim 18, wherein the particles have an average pore size less than about 10 nm.

23. The composition of claim 18, wherein the particles have an average pore size more than about 100 nm.

24. The composition of claim 1, wherein the first substrate, second substrate, or both, comprise a membrane, or a monolith.

25. The composition of any one of claims 1 to 11, wherein the first substrate, second substrate, or both comprise a solid or porous walled hollow fiber.

26. An apparatus comprising the composition of any one of claims 1 to 25, wherein the apparatus is optionally configured for chromatography.

27. The apparatus of claim 26, further configured to allow the protein preparation to subsequently contact, in any order, the composition according to any one of claims 1 to 25 and one or more further substrates comprising one or more further surface-bound ligands comprising a charge selected from the group consisting of electronegative, electropositive, neutral, and combinations thereof.

28. The apparatus of any one of claims 26 or 27, wherein the apparatus is a chromatographic device packed with the first substrate, optional second substrate, one or more further substrates, and combinations thereof.

29. The apparatus of any one of claims 26 to 28, wherein a composition at an entrance of the apparatus is different from a composition at the exit of the apparatus.

30. The apparatus of any one of claims 26 to 29, wherein a chemical surface of one or more components of the apparatus is substantially inert or of sufficiently low surface area as to make no significant contribution to the chemical functionality of the apparatus.

31. The apparatus of any one of claims 26 to 30, wherein the chemical surface of one or more components is configured to allow one or more of (1) create structural integrity, (2) direct flow of liquids therethrough, and (3) physically block, entrap, or entrain insoluble materials in a protein preparation to prevent them from interfering with the effective use of the device.

32. The apparatus of any one of claims 26 to 31, wherein the apparatus comprises one or more porous membranes and at least one of the one or more porous membranes is the first substrate to which the first surface-bound ligand or the second surface-bound ligand is bound.

33. The apparatus of any one of claims 26 to 31, wherein the first substrate, optional second substrate, or both comprise a permeable chopped fiber mat.

34. The apparatus of any one of claims 26 to 31, wherein the first substrate, optional second substrate, or both comprise an ordered wound, woven, or non-woven fiber structure.

35. The apparatus of any one of claims 26 to 31, wherein the first substrate, optional second substrate, or both comprise a combination of a permeable chopped fiber mat and an ordered wound, woven, or non-woven fiber structure.

36. The apparatus of any one of claims 26 to 31, wherein the apparatus comprises non-porous fibers or porous reticular fibers, wherein the porous reticular fibers are hollow porous-walled fibers, and wherein the porous reticular fibers are the first substrate to which the first surface-bound ligand and the second surface-bound ligand are bound.

37. The apparatus of any one of claims 26 to 31, wherein the apparatus comprises porous or non-porous particles sandwiched between porous membranes, or monoliths.

38. The apparatus of any one of claims 37, wherein porous or non-porous particles are the first substrate to which either the first surface-bound ligand or the second surface-bound ligand is bound and a membrane or monolith is a second substrate to which the other of the first surface-bound ligand and the second surface-bound ligand is bound.

39. The apparatus of any one of claims 26 to 31, wherein the apparatus comprises porous or non-porous particles sandwiched between a woven or amorphous fibrous filter, or embedded within a fibrous matrix.

40. The apparatus of any one of claims 39, wherein particles comprise the first substrate or second substrate to which either the first surface-bound ligand or the second surface-bound ligand is bound and fibrous filters comprise the second substrate to which the other of the first surface-bound ligand and the second surface-bound ligand is bound.

41. The apparatus of any one of claims 26 to 31, wherein the apparatus comprises porous or non-porous particles sandwiched between woven or crystalline frits.

42. The apparatus of any one of claims 41, wherein the porous or non-porous particles are the first substrate to which either the first surface-bound ligand or the second surface-bound ligand is bound and the woven or crystalline frits are the second substrate to which the other of the first surface-bound ligand and the second surface-bound ligand is bound.

43. The apparatus of any one of claims 26 to 31 wherein the apparatus comprises porous or non-porous particles embedded in a reticular polymer network.

44. The apparatus of any one of claims 26 to 43, wherein the first substrate and second substrate comprise any combination of configurations as recited in claims 26-43.

Claims

1. A composition of matter for reducing the aggregate content of a protein preparation, comprising a first substrate comprising a first surface-bound ligand possessing a metal affinity functionality and a second surface-bound ligand optionally provided on a second substrate and having an aggregate charge opposite to that of the metal affinity functionality of the first substrate, wherein the first surface-bound ligand and the second surface-bound ligand are positioned such that the protein preparation may contact both the first surface-bound ligand and the second surface-bound ligand simultaneously.

2. The composition of claim 1, wherein the first surface-bound ligand, the second surface-bound ligand, or both comprise a multidentate metal chelating moiety.

3. The composition of claim 1, wherein the second surface-bound ligand possesses metal affinity functionality.

4. The composition of claim 1, wherein the first surface-bound ligand has an aggregate charge which is electronegative.

5. The composition of claim 4, wherein the first substrate has additional chemical moieties bound to it, provided that an aggregate charge of the first substrate remains electronegative.

6. The composition of claim 1, wherein the first surface-bound ligand is electronegative and selected from the group consisting of iminodiacetic acid (2-(carboxymethylamino)acetic acid), ethylene glycol(aminoethylether)diacetic acid, nitriloacetic acid (2,2',2"-nitrilotriacetic acid), aspartic acid, and glutamic acid.

7. The composition of claim 1, wherein the second surface-bound ligand is tris(2-aminoethyl)amine, diethylenetriamine, triethylenetetramine, tetraethylenepentamine, polypropylenimine tetraamine, or desferroxamine.

8. The composition of claim 1, wherein the first surface-bound ligand is iminodiacetic acid (2-(carboxymethylamino)acetic acid).

9. The composition of claim 1, wherein the first surface-bound ligand has an aggregate charge which is electropositive.

10. The composition of claim 1, wherein the first substrate has additional chemical moieties bound to it, provided that an aggregate charge of first substrate remains electropositive.

11. The composition of claim 1, wherein the first surface-bound ligand is tris(2-aminoethyl)amine, diethylenetriamine, triethylenetetramine, tetraethylenepentamine, polypropylenimine tetraamine, or desferroxamine.

12. The composition of claim 1, wherein at least one of the first substrate or optional second

substrate has one or more further surface-bound ligands in addition to the first surface-bound ligand and the second surface-bound ligand, wherein the one or more further surface-bound ligands enhance the capacity of the composition to participate in hydrogen bonding, hydrophobic interactions, or pi-pi binding with a component of the protein preparation.

13. The composition of claim 1, wherein the first substrate, the second substrate, or both comprise more than one negatively charged metal chelating group in combination with one or more oppositely charged groups.

14. The composition of claim 1, wherein the first substrate, the second substrate, or both comprise more than one positively charged metal chelating group in combination with one or more oppositely charged groups.

15. The composition of claim 1, wherein the first surface-bound ligand and the second surface-bound ligand are covalently bound to the first substrate.

16. The composition of claim 1, wherein the first surface-bound ligand is covalently bound to the first substrate and the second surface-bound ligand covalently bound to the second substrate which is different from the first substrate.

17. The composition of claim 1 wherein the first substrate, second substrate, or both comprise particles.

18. The composition of claim 17, wherein the particles are non-porous.

19. The composition of claim 17, wherein the particles are porous.

20. The composition of claim 17, wherein the particles have a pore size large enough to permit entry of a protein in a protein preparation.

21. The composition of claim 17, wherein the particles have a pore size too small to permit entry of a protein in a protein preparation.

22. The composition of claim 19, wherein the particles have an average pore size between about 10 nm and about 100 nm.

23. The composition of claim 19, wherein the particles have an average pore size less than about 10 nm.

24. The composition of claim 19, wherein the particles have an average pore size more than about 100 nm.

25. The composition of claim 1, wherein the first substrate, second substrate, or both, comprise a membrane, or a monolith.

26. The composition of any one of claims 1 to 12, wherein the first substrate, second substrate, or both comprise a solid or porous walled hollow fiber.

27. An apparatus comprising the composition of any one of claims 1 to 26, wherein the apparatus is optionally configured for chromatography.

28. The apparatus of claim 27, further configured to allow the protein preparation to subsequently contact, in any order, the composition according to any one of claims 1 to 26 and one or more further substrates comprising one or more further surface-bound ligands comprising a charge selected from the group consisting of electronegative, electropositive, neutral, and combinations thereof.

29. The apparatus of any one of claims 27 or 28, wherein the apparatus is a chromatographic device packed with the first substrate, optional second substrate, one or more further substrates, and combinations thereof.

30. The apparatus of any one of claims 27 to 29, wherein a composition at an entrance of the apparatus is different from a composition at the exit of the apparatus.

31. The apparatus of any one of claims 27 to 30, wherein a chemical surface of one or more components of the apparatus is substantially inert or of sufficiently low surface area as to make no significant contribution to the chemical functionality of the apparatus.

32. The apparatus of any one of claims 27 to 31, wherein the chemical surface of one or more components is configured to allow one or more of (1) create structural integrity, (2) direct flow of liquids therethrough, and (3) physically block, entrap, or entrain insoluble materials in a protein preparation to prevent them from interfering with the effective use of the device.

33. The apparatus of any one of claims 27 to 32, wherein the apparatus comprises one or more porous membranes and at least one of the one or more porous membranes is the first substrate to which the first surface-bound ligand or the second surface-bound ligand is bound.

34. The composition of any one of claims 27 to 32, wherein the first substrate, optional second substrate, or both comprise a permeable chopped fiber mat.

35. The composition of any one of claims 27 to 32, wherein the first substrate, optional second substrate, or both comprise an ordered wound, woven, or non-woven fiber structure.

36. The composition of any one of claims 27 to 32, wherein the first substrate, optional second substrate, or both comprise a combination of a permeable chopped fiber mat and an ordered wound, woven, or non-woven fiber structure.

37. The apparatus of any one of claims 27 to 32, wherein the apparatus comprises porous reticular fibers, wherein the porous reticular fibers are hollow porous-walled fibers or non-porous fibers, and wherein the porous reticular fibers are the first substrate to which the first surface-bound ligand and the second surface-bound ligand are bound.

38. The apparatus of any one of claims 27 to 32, wherein the apparatus comprises porous or non-porous particles sandwiched between porous membranes, or monoliths.

39. The apparatus of any one of claims 27 to 32, wherein porous or non-porous particles are the first substrate to which either the first surface-bound ligand or the second surface-bound ligand is bound and a membrane or monolith is a second substrate to which the other of the first surface-bound ligand and the second surface-bound ligand is bound.

40. The apparatus of any one of claims 27 to 32, wherein the apparatus comprises porous or non-porous particles sandwiched between a woven or amorphous fibrous filter, or embedded within a fibrous matrix.

41. The apparatus of any one of claims 27 to 32, wherein particles comprise the first substrate or second substrate to which either the first surface-bound ligand or the second surface-bound ligand is bound and fibrous filters comprise the second substrate to which the other of the first surface-bound ligand and the second surface-bound ligand is bound.

42. The apparatus of any one of claims 27 to 32, wherein the apparatus comprises porous or non-porous particles sandwiched between woven or crystalline frits.

43. The apparatus of any one of claims 27 to 32, wherein the porous or non-porous particles are the first substrate to which either the first surface-bound ligand or the second surface-bound ligand is bound and the woven or crystalline frits are the second substrate to which the other of the first surface-bound ligand and the second surface-bound ligand is bound.

44. The apparatus of any one of claims 27 to 32 wherein the apparatus comprises porous or non-porous particles embedded in a reticular polymer network.

45. The apparatus of any one of claims 27 to 44, wherein the first substrate and second substrate comprise any combination of configurations as recited in claims 27-44.