development, narrow operating window, and high concentration of salt required in the buffer.

[0010] Weak partitioning chromatography (WPC) is another mode of chromatographic operation, in which the product binds weaker than in the case of bind-elute chromatography but stronger than in the case of flow-through chromatography (See, *e.g*. U.S Patent No. 8,067,182); however, WPC also has certain draw back associated with it including, a narrow operating window and lower binding capacity for impurity removal compared to bind and elute methods.

[0011] **Prior Art Documents**

- **Patent Documents**
  1. WO 2005/044856
  2. US 6,620,918 B
  3. US 7,427,659 B
  4. US 8,067,182 B
- **Non-Patent Documents**
  1. Giovannini, Biotechnology and Bioengineering, vol. 73, pp. 522-529 (2000)
  2. Jungbauer, J.Chromatography, vol. 476, pp. 257-268 (1989)
  3. Aldington, et. al., J. Chrom. B, vol 848, pp. 64-78 (2007)

While, some of the flow-through methods described in the prior art have been reported to bind aggregates, the specificity for binding aggregates relative to the product of interest appears to be low. Further, there appear to be no known methods in the prior art which exhibit a high specificity for binding lower order protein aggregates such as, *e.g*., dimers, trimers and tetramers.

**Summary of the Invention**

[0012] The present invention provides novel and improved compositions as well as flow-through methods which use such compositions for separating a product of interest, *e.g*., a therapeutic antibody or a monomeric protein from protein aggregates in a biopharmaceutical composition. The compositions and methods described herein are especially useful for separating a monomeric protein of interest

from lower order protein aggregates, such as, *e.g.*, dimers, trimers and tetramers, which are generally difficult to separate from the monomeric protein.

[0013] The present invention is based, at least in part, on increasing selectivity of binding of protein aggregates compared to a product of interest (*i.e.*, monomeric molecule) to a surface in flow-through mode, thereby to separate the protein aggregates from the product of interest. The present invention is able to accomplish this by the unique design of a surface having a certain density of cation exchange binding groups, thereby facilitating a greater number of protein aggregates to bind to the surface, as compared to the monomeric molecules.

[0014] In some embodiments according to the present invention, a flow-through chromatography method of separating a monomeric protein of interest from protein aggregates in a sample is provided, where the method comprises contacting the sample with a solid support comprising one or more cation exchange binding groups attached thereto, at a density of 1 to 30 mM, where the solid support

aromatic organic residues, and may contain heteroatoms such as O, N, S, P, F, Cl, and others.

[00136] In yet other embodiments, a representative structure of a binding group containing polymer is depicted in Figure 2H.

[00137] The sulfonic acid group in Figures 2B-2D and 2F-2H can be in the protonated form as depicted, as well as in the salt form, containing a suitable counterion such as sodium, potassium, ammonium, and the like.

## ~~I~~V. Devices incorporating the compositions described herein

[00138] In some embodiments, solid supports having binding groups attached thereto, as described herein, are incorporated into devices. Suitable devices for solid supports, such as microporous membranes, include filtration cartridges, capsules, and pods. Exemplary devices also include stacked-plate filtration cartridges disclosed in the U.S. Publication Nos. US20100288690 A1 and US20080257814 A1, incorporated by reference herein. In case of these devices, a solid support is permanently bonded to the polymeric housing and the devices have a liquid inlet, an outlet, and a vent opening, and further minimize the volume of retained liquid. Other exemplary devices include pleated filter cartridges and spiral-wound filter cartridges. Yet other exemplary devices are chromatography columns. Chromatography columns can be produced from a number of suitable materials, such as glass, metal, ceramic, and plastic. These columns can be packed with solid support by the end user, or can also be pre-packed by a manufacturer and shipped to the end user in a packed state.

## VI. Methods of using the compositions and devices described herein

[00139] The devices containing solid supports having binding groups attached thereto (*e.g.*, cation exchange binding groups) can be used for removal of protein aggregates in a flow-through mode. Prior to application for preparative scale separation, the process must be developed and validated for proper solution conditions such as pH and conductivity, and the range of protein loading on the device must be determined. The methods for process development and validation are widely known and routinely practiced in the industry. They usually involve Design of Experiments (DoE) approaches that are illustrated in the Examples herein.

## Claims

**What is claimed is:**

1. A flow-through chromatography method of separating a monomeric protein of interest from protein aggregates in a sample, the method comprising contacting the sample with a solid support comprising one or more cation exchange binding groups attached thereto, at a density of 1 to 30 mM, wherein the solid support selectively binds protein aggregates, thereby to separate the monomeric protein of interest from protein aggregates.

2. The method of claim 1, wherein the solid support is selected from a chromatographic resin, a membrane, a porous bead, a porous monolith, a winged fiber, a woven fabric and a non-woven fabric.

3. The method of claim 1, wherein the solid support is a porous polyvinylether polymeric bead or a porous crosslinked polymethacrylate polymer bead.

4. The method of claim 1, wherein the protein aggregates are lower order protein aggregates.

5. The method of claim 4, wherein the lower order protein aggregates are selected from the group consisting of dimers, trimers, and tetramers.

6. The method of claim 1, wherein the protein aggregates are high molecular weight protein aggregates.

7. The method of claim 6, wherein the higher molecular weight aggregates are pentamers and higher.

8. The method of claim 1, wherein the one or more cation exchange group is selected from the group consisting of a sulfonic group, a sulfate group , a phosphonic group, a phosphoric group, and a carboxylic group.

9. The method of claim 1, wherein the monomeric protein of interest is an antibody.

10. The method of claim 9, wherein the antibody is a monoclonal antibody.

11. The method of claim 1, wherein the monomeric protein of interest is a recombinant protein.

12. The method of claim 1, wherein the sample, prior to aggregate removal, is purified by one or more of flow-through adsorbers selected from the group containing anion-exchange media and activated carbon.

13. The methods of claim 12, wherein the aggregate removal is connected directly to prior purification steps without an intermediate holding container.

14. A flow-through chromatography method of separating a monomeric protein of interest from protein aggregates in a sample, the method comprising contacting the sample with a solid support comprising one or more cation exchange binding groups attached thereto at a density of 1 to 30 mM, wherein the solid support binds protein aggregates relative to monomers at a selectivity greater than 10, thereby to separate the protein of interest from protein aggregates.

15. A flow-through chromatography method of reducing the concentration of protein aggregates in a sample, the method comprising

a) providing a sample comprising a protein of interest and protein aggregates;

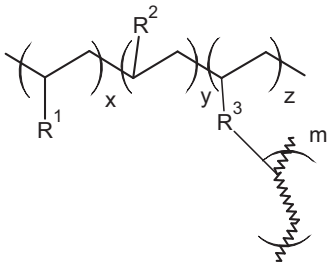
b) contacting the sample with a solid support comprising one or more cation exchange binding groups attached thereto, at a density of 1 to 30 mM; and

c) collecting a flow-through effluent of the sample,

wherein the concentration of protein aggregates in the effluent is reduced by at least 50% relative to the concentration of the aggregates in (a), thereby to reduce the concentration of the protein aggregates in the sample.

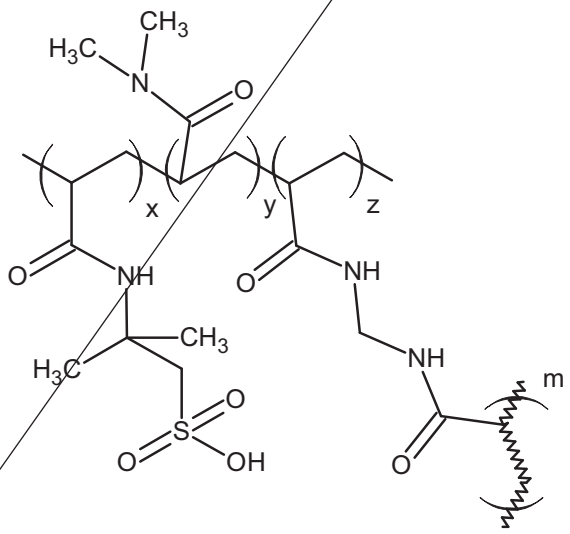
16. The flow-through chromatography method of claim 13, wherein concentration of the protein of interest in the effluent is at least 80% of the concentration of the protein of interest in (a).

17. A polymer comprising the following chemical structure:




wherein $R^1$ is a cation-exchange group; $R^2$ is any aliphatic or aromatic organic residue that does not contain a charged group; $R^3$ is any uncharged aliphatic or aromatic organic linker between any two or more polymeric chains; x, y, and z are average molar fractions of each monomer in the polymer, wherein y>x; and wherein l *m* denotes a similar polymer chain attached at the other end of the linker.

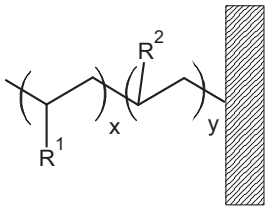
18. A polymer comprising the following chemical structure:




wherein x, y, and z are average molar fractions of each monomer in the polymer, wherein y>x; and wherein *m* represents a second polymer.

19. The polymer of claim 15 or 16, wherein the polymer is attached to a solid support.

20. A polymer comprising the following chemical structure:




wherein $R^1$ is a cation-exchange group; $R^2$ is any aliphatic or aromatic organic residue that does not contain a charged group; and x and y are average molar fractions of each monomer in the polymer, where y>x, and wherein the polymer is grafted via covalent linkage onto a solid support, shown as the rectangle.

21. A flow-through process for purifying a target molecule from a Protein A eluate comprising the steps of:

(a) contacting the eluate recovered from a Protein A chromatography column with activated carbon;
(b) contacting a flow-through sample from step (a) with an anion exchange chromatography media; and
(c) contacting a flow-through sample from step (b) with a solid support comprising one or more cation exchange binding groups attached thereto, at a density of 1 to 30 mM; and
(d) obtaining a flow-through sample from step (c) comprising the target molecule,

wherein the eluate flows continuously through steps(a)-(c) and wherein level of one or more impurities in the flow-through sample after step (c) is lower than the level in the eluate in step (a).

22. The flow-through process of claim 21, further comprising subjecting the flow-through sample from step (c) to virus filtration.

23. The flow-through process of claim 21, further comprising use of an in-line static mixer and/or a surge tank between steps (b) and (c) to change pH.

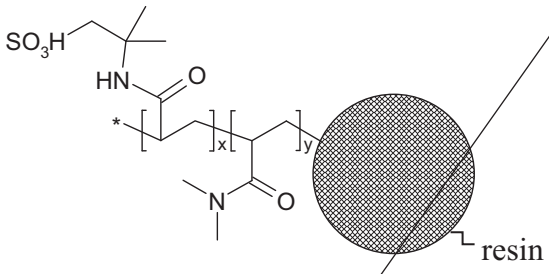
24. The flow-through process of claim 21 or 22 or 23, wherein the process employs a single skid.

25. The flow-through process of claim 21 wherein the eluate from the Protein A chromatography column is subjected to virus inactivation prior to contacting with activated carbon.

26. The process of claim 21, wherein steps (a)-(c) may be performed in any order.

27. A flow-through purification process for purifying a target molecule from a Protein A eluate, the process comprising contacting the eluate with a cation exchange media and at least one other media selected from the group consisting of activated carbon, anion exchange media and virus filtration media, wherein the flow of the eluate is continuous, and wherein the cation exchange media comprises one or more cation exchange binding groups at a density of 1 to 30 mM.

28. A polymer comprising the following chemical structure, wherein the polymer includes two or more monomers and the polymer is grafted via a linkage onto a chromatography resin:




wherein x and y are average molar fractions of each monomer in the polymer, wherein y > x.

29. A flow-through process for increasing the purity of a target molecule in a Protein A eluate comprising the steps of:

(a) contacting the eluate recovered from a Protein A chromatography column with a solid support comprising one or more cation exchange binding groups attached thereto, at a density of 1 to 30 mM; and

(b) obtaining a flow-through sample from step (a) comprising the target molecule,

wherein the level of aggregates in the flow-through sample is lower than the level of aggregates in the Protein A eluate, thereby increasing the purity of the target molecule

30. A flow-through process for purifying a target molecule from a Protein A eluate, wherein the process is performed at ionic conductivity less than or equal to 10 mS/cm.

31. The process of claim 29 or 30, wherein the target molecule is a monoclonal antibody.

32. The method claim 1, wherein the solid support is selected from a chromatographic resin or a porous bead.

33. The method of claim 31, wherein the chromatographic resin or the porous bead comprises a mean particle size of between 10 and 500 microns.

34. The method of claim 31, wherein the chromatographic resin or the porous bead comprises a mean particle size of between 20 and 140 microns.

35. The method of claim 31, wherein the chromatographic resin or the porous bead comprises a mean particle size of between 30 and 75 microns.

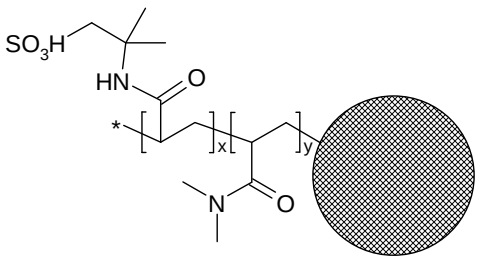
36. The method of claim 31, wherein the chromatographic resin or the porous bead comprises a mean particle size of 50 microns.

## Claims

**What is claimed is:**

1. A flow-through process for increasing the purity of a target molecule in a Protein A eluate comprising the steps of:

(a) contacting the eluate recovered from a Protein A chromatography with a polymer including two or more monomers, wherein the polymer is grafted via a linker onto a chromatography resin or a porous bead and the polymer comprises the following structure:



resin or bead

wherein x and y are average molar fractions of each monomer in the polymer, wherein y > x; and

(b) obtaining a flow-through sample from step (a) comprising the target molecule, wherein the level of aggregates in the flow-through sample is lower than the level of aggregates in the Protein A eluate, thereby increasing the purity of the target molecule

2. A flow-through process for purifying a target molecule from a Protein A eluate, wherein the process is performed at ionic conductivity less than or equal to 10 mS/cm.

3. The process of claim 1 or 2, wherein the target molecule is a monoclonal antibody.

4. The method of claim 1, wherein the chromatography resin or the porous bead comprises a mean particle size of between 10 and 500 microns.

5. The method of claim 1, wherein the chromatography resin or the porous bead comprises a mean particle size of between 20 and 140 microns.

6. The method of claim 1, wherein the chromatography resin or the porous bead comprises a mean particle size of between 30 and 75 microns.

7. The method of claim 1, wherein the chromatography resin or the porous bead comprises a mean particle size of 50 microns.