the UF/DF-purified antibody composition to anion exchange chromatography (AEX) to form a pharmaceutically pure antibody composition. The polishing step is often cation exchange chromatography and optionally followed by anion exchange chromatography.

**[0008]** A further aspect of the invention relates to a method of purifying an antibody composition, which comprises subjecting an antibody composition having an antibody concentration of at least 1 mg/ml, such as at least 5 mg/ml, at least 10 mg/ml, 5 to 250 mg/ml, 10 to 150 mg/ml, 15 to 100 mg/ml, 20 to 50 mg/ml, and 30-35 mg/ml, and having a parenterally-acceptable buffer and pH, to anion exchange chromatography (AEX) sufficient to reduce the amount of host cell proteins in said antibody composition.

[0008a] Another aspect of the invention relates to a method of purifying an antibody composition, which comprises subjecting an ultrafiltration-diafiltration (UF/DF)-purified antibody composition, wherein the antibody is produced in eukaryotic cells, to anion exchange chromatography (AEX) to form a pharmaceutically-pure antibody composition.

[0008b] Another aspect of the invention relates to a method of purifying an antibody composition, which comprises:

subjecting an antibody eukaryotic cell culture harvest to affinity chromatography, such as Protein A chromatography, to form a crude antibody composition;

subjecting the crude antibody composition to at least one polishing step to form a partially-purified antibody composition;

subjecting the partially-purified antibody composition to a UF/DF step to form a UF/DF-purified antibody composition; and

subjecting the UF/DF-purified antibody composition to anion exchange chromatography (AEX) to form a pharmaceutically-pure antibody composition

## DETAILED DESCRIPTION OF THE INVENTION

**[0009]** The present invention relates to a method of purifying an antibody using AEX late in the purification, relative to the previously known purification techniques. Conventional mAb purification processes, which use a capture step followed by IEX polishing steps and ending on a final UF/DF step before bulk filling, were thought to be proficient in achieving desirably-low levels of HCP. Common ELISA-based HCP detection kits often showed suitably low amounts of HCP after the final UF/DF step. But after switching to a newer, more sensitive ELISA-based HCP detection kit, the present inventors discovered that the amount of HCP was actually much higher than previously thought and

beyond the desired purity limit. Based on this discovery, the inventors sought to find a purification process that would provide a higher purity antibody composition.

**[0010]** The inventors found that AEX can be performed after the final UF/DF step with improved purification effects. As shown in the Examples below, moving AEX from the pre-UF/DF polishing stage to post-UF/DF provided superior HCP removal without any unwanted rise in other measured impurities. Locating the AEX step after UF/DF increased the purification effect as compared to conventional processes. Performing AEX after the final ultrafiltration is also advantageous from an economical and/or time perspective. The AEX step can generally be performed faster after UF/DF than before (i.e., during the polishing phase) because the antibody composition is more concentrated after the final UF/DF (e.g. less volume). Another benefit of the invention is enhanced virus control; AEX is known to remove some viruses. When AEX is performed in as a polishing step (pre-UF/DF), subsequent processes and handling risk re-introduction of viruses. That risk can be minimized by performing AEX later in the purification scheme.

## CLAIMS

1. A method of purifying an antibody composition, which comprises subjecting a UF/DF-purified antibody composition to anion exchange chromatography (AEX) to form a pharmaceutically-pure antibody composition.
2. The method of claim 1, wherein said UF/DF-purified antibody composition has an antibody concentration of at least 1 mg/ml, such as at least 5 mg/ml and at least 10 mg/ml.
3. The method of claim 2, wherein said UF/DF-purified antibody composition has an antibody concentration of 5 to 250 mg/ml, such as 10 to 150 mg/ml and 15 to 100 mg/ml.
4. The method of claim 3, wherein said UF/DF-purified antibody composition has an antibody concentration of not greater than 250 mg/ml.
5. The method of claim 4, wherein said UF/DF-purified antibody composition has an antibody concentration of 20 to 50 mg/ml, such as 30-35 mg/ml.
6. The method of any one of claims 1-5, which further comprises filling said pharmaceutically-pure antibody composition into vials.
7. The method according to claim 6, which further comprises aseptically filtering said pharmaceutically-pure antibody composition prior to said filling step.
8. The method according to claim 7, which further comprises at least one of the following steps prior to said filling step: (a) adding an excipient to said pharmaceutically-pure antibody composition; (b) concentrating said pharmaceutically-pure antibody composition; (c) diluting said pharmaceutically-pure antibody composition; and/or (d) adjusting the pH of said pharmaceutically-pure antibody composition.
9. The method of any one of claims 1-8, which further comprises subjecting a partially-purified antibody composition to UF/DF to form said UF/DF-purified antibody composition.
10. The method of claim 9, which further comprises subjecting an antibody cell culture harvest to an antibody capture step and optionally at least one polishing steps to form said partially-purified antibody composition.
11. The method of claim 10, wherein said capture step utilizes affinity chromatography, such as Protein A chromatography.

12. The method of claim 10, wherein said capture step utilizes cation exchange chromatography (CEX), hydrophobic charge induction chromatography (HCIC), or mixed mode chromatography.

13. The method of any one of claims 10-12, wherein at least one polishing step is carried out after said capture step.

14. The method according to claim 13, wherein said at least one polishing step is selected from the group consisting of ion exchange chromatography (IEX), hydrophobic interaction chromatography (HIC), and hydroxyapatitie chromatography.

15. A method of purifying an antibody composition, which comprises:
subjecting an antibody cell culture harvest to affinity chromatography, such as Protein A chromatography, to form a crude antibody composition;
subjecting the crude antibody composition to at least one polishing step to form a partially-purified antibody composition;
subjecting the partially-purified antibody composition to a UF/DF step to form a UF/DF-purified antibody composition; and
subjecting the UF/DF-purified antibody composition to anion exchange chromatography (AEX) to form a pharmaceutically-pure antibody composition.

16. The method of claim 15, which further comprises subjecting said pharmaceutically-pure antibody composition to a virus removal step such as nanofiltration.

17. The method of claim 15, wherein said at least one polishing step comprises subjecting said crude antibody composition to at least one ionic exchange chromatography (IEX) step.

18. The method of any one of claims 1-17, wherein said antibody is trastuzumab or pertuzumab.

19. The method of any one of claims 1-18, wherein said antibody was produced in CHO cells.

20. A method of purifying an antibody composition, which comprises subjecting an antibody composition having an antibody concentration of at least 10 mg/ml, such 20 to 50 mg/ml and 30-35 mg/ml, and having a parenterally-acceptable buffer and pH, to anion exchange chromatography (AEX) sufficient to reduce the amount of at least one impurity such as host cell proteins in said antibody composition.

## CLAIMS

1. A method of purifying an antibody composition, which comprises subjecting an ultrafiltration-diafiltration (UF/DF)-purified antibody composition, wherein the antibody is produced in eukaryotic cells, to anion exchange chromatography (AEX) to form a pharmaceutically-pure antibody composition.
2. The method according to claim 1, wherein said UF/DF-purified antibody composition has an antibody concentration of 5 to 250 mg/ml, such as 10 to 150 mg/ml and 15 to 100 mg/ml.
3. The method according to claim 1 or 2, which further comprises filling said pharmaceutically-pure antibody composition into vials.
4. The method according to claim 3, which further comprises aseptically filtering said pharmaceutically-pure antibody composition prior to said filling step.
5. The method according to claim 4, which further comprises at least one of the following steps prior to said filling step: (a) adding an excipient to said pharmaceutically-pure antibody composition; (b) concentrating said pharmaceutically-pure antibody composition; (c) diluting said pharmaceutically-pure antibody composition; and/or (d) adjusting the pH of said pharmaceutically-pure antibody composition.
6. The method according to any one of claims 1 to 5, which further comprises subjecting a partially-purified antibody composition to UF/DF to form said UF/DF-purified antibody composition.
7. The method according to claim 6, which further comprises subjecting an antibody cell culture harvest to an antibody capture step and optionally at least one polishing step to form said partially-purified antibody composition.
8. The method according to claim 7, wherein said capture step utilizes affinity chromatography, such as Protein A chromatography.
9. The method according to claim 7, wherein said capture step utilizes cation exchange chromatography (CEX), hydrophobic charge induction chromatography (HCIC), or mixed mode chromatography.
10. The method according to any one of claims 7 to 9, wherein at least one polishing step is carried out after said capture step.
11. The method according to claim 10, wherein said at least one polishing step is selected from the group consisting of ion exchange chromatography (IEX), hydrophobic interaction chromatography (HIC), and hydroxyapatite chromatography.

12. A method of purifying an antibody composition, which comprises:
subjecting an antibody eukaryotic cell culture harvest to affinity chromatography, such as Protein A chromatography, to form a crude antibody composition;
subjecting the crude antibody composition to at least one polishing step to form a partially-purified antibody composition;
subjecting the partially-purified antibody composition to a UF/DF step to form a UF/DF-purified antibody composition; and
subjecting the UF/DF-purified antibody composition to anion exchange chromatography (AEX) to form a pharmaceutically-pure antibody composition.

13. The method according to claim 12, which further comprises subjecting said pharmaceutically-pure antibody composition to a virus removal step such as nanofiltration.

14. The method according to claim 12, wherein said at least one polishing step comprises subjecting said crude antibody composition to at least one ionic exchange chromatography (IEX) step.

15. The method according to any one of claims 1 to 14, wherein said antibody is trastuzumab or pertuzumab.