

WHAT IS CLAIMED:

1. A method of producing an aqueous formulation of an antigen-binding protein comprising

(a) contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter to form a filtrate; and

(b) incubating the filtrate for at least four hours, wherein the percentage of reduced antigen-binding protein molecules in the filtrate after the incubating step is decreased by 20% when compared to the percentage of reduced antigen-binding protein molecules observed in the aqueous solution prior to the contacting step.

2. A method of enhancing re-oxidization of an antigen-binding protein comprising

(a) contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter to form a filtrate; and

(b) incubating the filtrate for at least four hours, wherein the re-oxidation of the antigen binding protein molecules in the filtrate is increased at least two-fold after the contacting step when compared to a level of re-oxidized antigen-binding molecules in the aqueous solution prior to the contacting step.

3. The method according to claim 1, wherein step (a) or step (b) is followed by subjecting the solution of antigen-binding protein molecules to Protein A chromatography.

4. The method according to claim 1, wherein step (a) is preceded by subjecting the solution of antigen-binding protein molecules to Protein A chromatography.

5. The method according to claim 1, further comprising a step of inactivating one or more viruses in said solution of antigen-binding protein molecules.

6. The method according to claim 1, wherein the amount of reduced disulfide bonds in the antigen-binding protein molecules is decreased by at least 15% following the charged depth filtration compared to before the contacting step and/or wherein the percentage of reduced antigen-binding protein molecules continues to decrease for at least 1 hour, at least 2 hours, at least 3 hours, or at least 4 hours following step (a).

7. The method according to claim 6, wherein the amount of reduced disulfide bonds in the antigen-binding protein molecules is decreased by at least 20% following the charged depth filtration when compared to an amount of reduced disulfide bonds in the antigen-binding protein molecules in the aqueous solution before the contacting step and/or wherein the percentage reduced antigen-binding protein molecules continues to decrease for at least 1 hour, at least 2 hours, at least 3 hours or at least 4 hours following step (a).

8. The method according to claim 1, wherein the contacting occurs at room temperature or at a temperature of 2 degrees to 8 degrees Celsius.

9. The method according to claim 1, further comprising a step of sparging air or oxygen through the solution of antigen-binding protein molecules.

10. The method according to claim 1, further comprising contacting the solution of antigen-binding protein molecules with a positive ion selected from the group consisting of sodium, calcium, magnesium, mercury, molybdenum, chromium, cadmium, aluminum, potassium, cobalt, iron, manganese, titanium, zinc, nickel, copper, and combinations thereof.

11. The method according to claim 1, wherein the solution of antigen-binding protein molecules is contacted with more than one charged depth filter.

12. The method according to claim 1, wherein the charged depth filter comprises a diatomaceous earth layer.

13. The method according to claim 12, wherein the charged depth filter further comprises a cellulose layer and an inorganic layer.

14. The method according to claim 14, wherein the inorganic layer comprises a polyamine resin.

15. The method according to claim 1, wherein the charged depth filter comprises a positively charged ion selected from the group consisting of sodium, calcium, magnesium, mercury, chromium, cadmium, aluminum, potassium, lead, arsenic, cobalt, iron, manganese, titanium, zinc, nickel, copper, and combinations thereof.

16. The method according to claim 1, wherein the contacting occurs at a throughput of between 250 L/m² and 850 L/m².

17. The method according to claim 1, wherein the antigen-binding protein binds an antigen selected from the group consisting of RANKL, tumor necrosis factor alpha, epidermal growth factor receptor, CD20, calcitonin gene-related peptide, sclerostin, and platelet glycoprotein IIb/IIIa.

18. The method according to claim 1, wherein the antigen-binding protein is selected from the group consisting of abciximab, adalimumab, alemtuzumab, basiliximab, belimumab, bevacizumab, brentuximab vedotin, canakinumab, cetuximab, certolizumab pegol, daclizumab, denosumab, eculizumab, efalizumab, gemtuzumab, golimumab, ibritumomab tiuxetan, infliximab, ipilimumab, muromonab-CD3, natalizumab, nivolumab, ofatumumab, omalizumab, palivizumab, panitumumab, ranibizumab, rituximab, tocilizumab, tositumomab, trastuzumab, ustekinumab, vedolizumab, and a biosimilar of any of the foregoing.

19. The method according to claim 1, wherein the antigen-binding protein comprises an antigen-binding region comprising an amino acid sequence selected from the group consisting of SEQ ID NOS: 1-8.

20. The method according to claim 1, wherein the amount of reduced antigen-binding protein molecules is measured using non-reduced capillary electrophoresis with sodium dodecyl sulfate.

21. The method according to claim 1, further comprising a step of cation exchange chromatography.

22. The method of claim 1, comprising (1) a Protein A chromatography step; (2) a viral inactivation step; (3) a charged depth filtration step; and (4) an incubation step for at least four hours.

23. The method of claim 22, further comprising (5) a cation exchange chromatography step, and one or more of (6) a salt-intolerant interaction chromatography step; (7) a virus filtration step; and (8) ultrafiltration and/or diafiltration.

24. The method of claim 1, wherein the amount or relative amount of reduced antigen-binding protein molecules is determined using non-reduced capillary electrophoresis with sodium dodecyl sulfate (nrCE-SDS).

25. A method of producing an aqueous formulation of an antigen-binding protein comprising

(a) contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter to form a filtrate,

(b) incubating the filtrate for at least four hours, wherein the percentage of reduced antigen-binding protein molecules in the filtrate after the incubating step when compared to the percentage of reduced antigen-binding protein molecules observed in the aqueous solution prior to the contacting step is decreased by at least 20% decrease as determined using non-reduced capillary electrophoresis with sodium dodecyl sulfate (nrCE-SDS); and

(c) contacting the filtrate after the incubating step with a protein A column.

26. A method of enhancing re-oxidization of an antigen-binding protein comprising

(a) contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter to form a filtrate;

(b) incubating the filtrate for at least four hours, wherein the re-oxidation of the antigen-binding protein molecules in the filtrate when compared to a level of re-oxidized antigen-binding molecule in the aqueous solution prior to the contacting step is increased two-fold as determined using non-reduced capillary electrophoresis with sodium dodecyl sulfate (nrCE-SDS); and

(c) contacting the filtrate after the incubating step with a protein A column.

27. A method of producing an aqueous formulation of an antigen-binding protein comprising

(a) contacting an aqueous solution comprising antigen binding protein molecules with a charged depth filter comprising a copper ion to form a filtrate, and

(b) incubating the filtrate for at least four hours, wherein the percentage of reduced antigen-binding protein molecules in the filtrate after the incubating step is decreased by at least 20% when compared to the percentage of reduced antigen-binding protein molecules observed in the aqueous solution prior to the contacting step.

28. A method of enhancing re-oxidation of an antigen-binding protein comprising

(a) contacting an aqueous solution comprising antigen-binding protein molecules with a charged depth filter comprising a copper ion to form a filtrate; and

(b) incubating the filtrate for at least four hours, wherein the re-oxidation of the antigen-binding protein molecules in the filtrate is increased at least two-fold of re-oxidized antigen-binding molecules in the aqueous solution prior to the contacting step.

29. The method according to any one of claims 1, 7, 25, 26, 27 and 28, wherein the antigen-binding protein is an IgG antibody.

30. The method according to claim 29, wherein the antibody is an IgG1 antibody with Kappa light chain, or an IgG1 antibody with a Lambda light chain.