METHODS OF CLEANING CHROMATOGRAPHY MATRICES

BACKGROUND

[001] Production of recombinant Fc-containing proteins for therapeutic use typically involves expression of the proteins in mammalian cells and subsequent purification of these proteins from host cell contaminants. Protein A affinity chromatography is commonly used as part of the purification process for Fc-containing proteins, because of the ability of protein A to selectively bind to the Fc region of the Fc-containing proteins. To reduce the production costs associated with protein A affinity chromatography, protein A chromatography matrices are typically cleaned and reused multiple times. However, the cleaning of protein A matrices is challenging because the chemicals that are effective at cleaning protein A matrices can also damage the matrices, thereby reducing their useful life.

[002] Accordingly, there is a need for improved methods of cleaning protein A chromatography matrices that prolong the useful life of the matrices.

SUMMARY

[003] The present disclosure provides improved methods for cleaning a protein A chromatography matrix. The methods generally involve washing the protein A chromatography matrix with a series of three solutions, the first containing an acid, the second containing Tris, and the third containing NaOH (e.g., 0.001-0.075 M NaOH). The methods disclosed herein are particularly advantageous in that they can significantly increase the useful life of a protein A chromatography matrix, and thereby reduce the cost of purifying Fc-containing proteins.

[004] In an aspect, provided herein is a method for cleaning a protein A chromatography matrix that has previously been used to purify dulaglutide, the method comprising sequentially washing a chromatography column comprising the protein A chromatography matrix with: a) a first solution comprising one or more acid; b) a second solution comprising Tris; and c) a third solution comprising 0.001-0.075 M NaOH.

[005] In an example, the first solution comprises acetic acid. In an example, the first solution comprises phosphoric acid. In an example, the first solution comprises acetic acid and phosphoric acid. In an example, the first solution comprises 0.5-5% acetic acid. In an example, the first solution comprises 0.5-1.5% acetic acid. In an example, the first

solution comprises about 1% acetic acid. In an example, the first solution comprises 0.5-5% phosphoric acid. In an example, the first solution comprises 0.5-1.5% phosphoric acid. In an example, the first solution comprises about 1% phosphoric acid. In an example, the first solution comprises about 1% acetic acid and about 1% phosphoric acid.

[006] In an example, the second solution has a pH of 7-9. In an example, the second solution has a pH of about 8. In an example, the second solution comprises 10-100 mM Tris. In an example, the second solution comprises 25-75 mM Tris. In an example, the second solution comprises about 50 mM Tris.

[007] In an example, the third solution comprises 0.005-0.05 M NaOH. In an example, the third solution comprises 0.009-0.015 M NaOH. In an example, the third solution comprises about 0.01 M NaOH.

[008] In an example, the protein A chromatography matrix is washed with 1-10 column volumes of the first solution. In an example, the protein A chromatography matrix is washed with 2–3 column volumes of the first solution. In an example, the protein A chromatography matrix is washed with 2-10 column volumes of the second solution. In an example, the protein A chromatography matrix is washed with 1-2 column volumes of the second solution. In an example, the protein A chromatography matrix is washed with 2-10 column volumes of the third solution. In an example, the protein A chromatography matrix is washed with 2-3 column volumes of the third solution.

[009] In an example, the protein A chromatography matrix is washed with the first solution at a flow rate of about 270 cm/hr. In an example, the protein A chromatography matrix is washed with the second solution at a flow rate of about 270 cm/hr. In an example, the protein A chromatography matrix is washed with the third solution at a flow rate of about 270 cm/hr.

[0010] In an example, the chromatography column has a diameter of 75-150 cm. In an example, the chromatography column has a diameter of about 100 cm. In an example, the chromatography column has a diameter of about 140 cm.

[0011] In an example, the protein A chromatography matrix has an average particle size of 80-90 µm. In an example, the protein A chromatography matrix has an average particle size of about 85 µm.

[0012] In an example, the protein A chromatography matrix a comprises a protein A ligand that has increased stability under alkali conditions relative to wild type Staphylococcus aureus protein A.

[0013] In an example, the first solution comprises about 1% acetic acid and about 1% phosphoric acid; the second solution comprises about 50 mM Tris at a pH of about 8; and the third solution comprises about 0.01 M NaOH.

[0014] In an example, the protein A chromatography matrix is washed with: 2-3 column volumes of the first solution; b) 1-2 column volumes of the second solution; and c) 2-3 column volumes of the third solution. In an example, the protein A chromatography matrix is washed with the first, second, and third solutions at a flow rate of about 270 cm/hr.

[0015] In an example, the method further comprises washing the protein A chromatography matrix with about 2 column volumes of the second solution after the protein A chromatography matrix is washed with the third solution. In an example, the method further comprises washing the protein A chromatography matrix with a fourth solution comprising about 0.1 M NaOH.

[0016] In an example, the protein A chromatography matrix is washed with about 2 column volumes of the fourth solution. In an example, the protein A chromatography matrix is washed with the fourth solution at a flow rate of 100-140 cm/hr. In an example, the protein A chromatography matrix is washed with the fourth solution at a flow rate of about 130 cm/hr. In an example, the protein A chromatography matrix is washed with the fourth solution at a flow rate of 100-110 cm/hr. In an example, the protein A chromatography matrix is washed with the fourth solution for about 25 minutes.

[0017] In an example, the protein A chromatography matrix is washed with the first solution, second solution, and/or third solution in an upflow or downflow direction.

[0018] In an example, the protein A chromatography matrix has less than 1% carryover from the previous use after the protein A chromatography matrix is cleaned. In an example, the protein A chromatography matrix has less than 0.1% carryover from the previous use after the protein A chromatography matrix is cleaned.

[0019] In an example, the protein A chromatography matrix is used for 200-500 cycles of dulaglutide purification. In an example, the protein A chromatography matrix is used for

300-400 cycles of dulaglutide purification. In an example, the protein A chromatography matrix is used for about 304 cycles of dulaglutide purification.

[0019a] In another aspect, provided herein is dulaglutide produced by the method as described herein.

[0019b] In another aspect, provided herein is a composition comprising dulaglutide produced by the method as described herein.

[0020] Statements of the present disclosure are described below:

1. A method for cleaning a protein A chromatography matrix, the method comprising sequentially washing a chromatography column comprising the protein A chromatography matrix with:

a) a first solution comprising one or more acid;

b) a second solution comprising Tris; and

c) a third solution comprising 0.001-0.075 M NaOH.

2. The method of statement 1, wherein the first solution comprises acetic acid.

3. The method of statement 1 or 2, wherein the first solution comprises phosphoric acid.

4. The method of any one of statements 1-3, wherein the first solution comprises acetic acid and phosphoric acid.

5. The method of any one of statements 1-4, wherein the first solution comprises 0.5%-5% acetic acid.

6. The method of any one of statements 1-5, wherein the first solution comprises 0.5%-1.5% acetic acid.

7. The method of any one of statements 1-6, wherein the first solution comprises about 1% acetic acid.

8. The method of any one of statements 1-7, wherein the first solution comprises 0.5%-5% phosphoric acid.

9. The method of any one of statements 1-8, wherein the first solution comprises 0.5%-1.5% phosphoric acid.

10. The method of any one of statements 1-9, wherein the first solution comprises about 1% phosphoric acid.

11. The method of any one of statements 1-10, wherein the first solution comprises about 1% acetic acid and about 1% phosphoric acid.

12. The method of any one of statements 1-11, wherein the second solution has a pH of 7-9.

13. The method of any one of statements 1-12, wherein the second solution has a pH of about 8.

14. The method of any one of statements 1-13, wherein the second solution comprises 10-100 mM Tris.

15. The method of any one of statements 1-14, wherein the second solution comprises 25-75 mM Tris.

16. The method of any one of statements 1-15, wherein the second solution comprises about 50 mM Tris.

17. The method of any one of statements 1-16, wherein the third solution comprises 0.005-0.05 M NaOH.

18. The method of any one of statements 1-17, wherein the third solution comprises 0.009-0.015 M NaOH.

19. The method of any one of statements 1-18, wherein the third solution comprises about 0.01 M NaOH.

20. The method of any one of statements 1-19, wherein the protein A chromatography matrix is washed with 1-10 column volumes of the first solution.

21. The method of any one of statements 1-20, wherein the protein A chromatography matrix is washed with 2–3 column volumes of the first solution.

22. The method of any one of statements 1-21, wherein the protein A chromatography matrix is washed with 2 - 10 column volumes of the second solution.

23. The method of any one of statements 1-22, wherein the protein A chromatography matrix is washed with 1 - 2 column volumes of the second solution.

24. The method of any one of statements 1-23, wherein the protein A chromatography matrix is washed with 2 - 10 column volumes of the third solution.

25. The method of any one of statements 1-24, wherein the protein A chromatography matrix is washed with 2 - 3 column volumes of the third solution.

26. The method of any one of statements 1-25, wherein the protein A chromatography matrix is washed with the first solution at a flow rate of about 270 cm/hr.

27. The method of any one of statements 1-26, wherein the protein A chromatography matrix is washed with the second solution at a flow rate of about 270 cm/hr.

28. The method of any one of statements 1-27, wherein the protein A chromatography matrix is washed with the third solution at a flow rate of about 270 cm/hr.

29. The method of any one of statements 1-28, wherein the chromatography column has a diameter of 75-150 cm.

30. The method of any one of statements 1-29, wherein the chromatography column has a diameter of about 100 cm.

31. The method of any one of statements 1-29, wherein the chromatography column has a diameter of about 140 cm.

32. The method of any one of statements 1-31, wherein the Protein A chromatography matrix has an average particle size of 80-90 µm.

33. The method of any one of statements 1-32, wherein the Protein A chromatography matrix has an average particle size of about 85 µm.

34. The method of any one of statements 1-33, wherein the protein A chromatography matrix a comprises a protein A ligand that has increased stability under alkali conditions relative to wild type Staphylococcus aureus protein A.

35. The method of any one of statements 1-34, wherein

a) the first solution comprises about 1% acetic acid and about 1% phosphoric acid;

b) the second solution comprises about 50 mM Tris at a pH of about 8; and

c) the third solution comprises about 0.01 M NaOH.

36. The method of statement 35, wherein the protein A chromatography matrix is washed with:

a) 2-3 column volumes of the first solution;

b) 1-2 column volumes of the second solution; and

c) 2-3 column volumes of the third solution.

37. The method of statement 35 or 36, wherein the protein A chromatography matrix is washed with the first, second, and third solutions at a flow rate of about 270 cm/hr.

38. The method of any one of statements 1-37, further comprising washing the protein A chromatography matrix with about 2 column volumes of the second solution after the protein A chromatography matrix is washed with the third solution.

39. The method of any one of statements 1-38, further comprising washing the protein A chromatography matrix with a fourth solution comprising about 0.1 M NaOH.

40. The method of statement 39, wherein the protein A chromatography matrix is washed with about 2 column volumes of the fourth solution.

41. The method of statement 39 or 40, wherein the protein A chromatography matrix is washed with the fourth solution at a flow rate of 100-110 cm/hr.

42. The method of statement 39 or 40, wherein the protein A chromatography matrix is washed with the fourth solution at a flow rate of about 130 cm/hr.

43. The method of any one of statements 39-42, wherein the protein A chromatography matrix is washed with the fourth solution for about 25 minutes.

44. The method of any one of statements 1-43, wherein the protein A chromatography matrix is washed with the first solution, second solution, and/or third solution in an upflow or downflow direction.

45. The method of any one of statements 1-44, wherein the protein A chromatography matrix has previously been used to purify an Fc-containing protein.

46. The method of statement 45, wherein the Fc-containing protein is an antibody.

47. The method of statement 45, wherein the Fc-containing protein is not an antibody.

48. The method of statement 45, wherein the Fc-containing protein comprises a glucagon-like peptide 1 (GLP-1) analog comprising one or more modifications compared to a wild type GLP-1 amino acid sequence (SEQ ID NO: 1).

49. The method of statement 45 or 48, wherein the Fc-containing protein comprises a GLP-1 analog comprising the amino acid sequence of SEQ ID NO: 2.

50. The method of any one of statements 45, 48, or 49, wherein the Fc-containing protein comprises a peptide linker.

51. The method of statement 50, wherein the peptide linker comprises 1 to 10 G_4S units (SEQ ID NO: 6).

52. The method of any one of statements 44 or 47-51, wherein the Fc-containing protein comprises:

a) a GLP-1 analog comprising the amino acid sequence of SEQ ID NO: 2;

b) a peptide linker comprising 1 to 10 G_4S units (SEQ ID NO: 6); and

c) an Fc portion of an immunoglobulin.

53. The method of statement 52, wherein the N-terminal residue of the peptide linker is directly fused to the C-terminal residue of the GLP-1 analog, and the C-terminal residue of the peptide linker is directly fused to the N-terminal residue of the Fc portion.

54. The method of any one of statements 44 or 47-53, wherein the Fc-containing protein comprises the amino acid sequence of SEQ ID NO: 5.

55. The method of any one of statements 44 or 47-54, wherein the Fc-containing protein is a homodimer comprising two identical amino acid chains each comprising the amino acid sequence of SEQ ID NO: 5.

56. The method of any one of statements 44 or 47-55, wherein the Fc-containing protein is dulaglutide.

57. The method of any one of statements 44-56, wherein the protein A chromatography matrix has less than 1% carryover from the previous use.

58. The method of any one of statements 44-57, wherein the protein A chromatography matrix has less than 0.1% carryover from the previous use.

59. The method of any one of statements 1-58, wherein the protein A chromatography matrix is used for 200-500 cycles.

60. The method of any one of statements 1-59, wherein the protein A chromatography matrix is used for more than 250-400 cycles of dulaglutide purification.

61. The method of any one of statements 1-60, wherein the protein A chromatography matrix is used for more than 300-400 cycles of dulaglutide purification.

62. The method of any one of statements 1-61, wherein the protein A chromatography matrix is used for about 304 cycles.

63. The method of any one of the preceding statements, wherein the method does not include a static hold of the first solution.

64. The method of any one of the preceding statements, wherein the method does not include a static hold of the second solution.

65. The method of any one of the preceding statements, wherein the method does not include a static hold of the third solution.

BRIEF DESCRIPTION OF DRAWINGS

[0021] **FIG. 1A-1B** are plots showing the neutralized low pH viral inactivation (LpHVI) intermediate pH (**FIG. 1A**) and conductivity (**FIG. 1B**) for each of the 304 cycles over the course of the study.

[0022] **FIG. 2** is a graph that depicts the % yield and dynamic binding capacity (DBC) response for the protein A chromatography matrix throughout the 304 cycles of the study. The % yield for high loads (18 g/L) is shown in open squares and low loads (10 g/L) is shown in open triangles. The horizontal black dashed lines indicate minimum and maximum process validation acceptance criteria (PVAC) of 70% and 105%, respectively.

[0023] **FIG. 3** is a graph that depicts Height Equivalent to Theoretical Plate (HETP) and Asymmetry assessments for the packed protein A chromatography matrix bed as metrics to measure column pack quality. The black dotted line indicates the minimum acceptance criterion of at least 1100 plates/m for HETP and the red dotted lines indicate the minimum and maximum acceptance criteria asymmetry values of 0.7 and 1.8, respectively.

[0024] **FIG. 4** is a plot that depicts the percent carryover for after mock elution of the protein A chromatography matrix throughout the 304 cycles of the study.

[0025] **FIG. 5A-5B** are plots that depict a product purity assessment as determined by size exclusion chromatography. **FIG. 5A** is a plot showing the percentage of dulaglutide monomer. The horizontal dashed red line represents the minimum acceptance criteria of 91.2 % monomer applied at low pH viral inactivation unit operation. **FIG. 5B** is a plot showing the percentage of total aggregates. Data points in yellow represent unincubated low pH viral inactivation samples.

[0026] **FIG. 6** is a plot showing a product purity assessment as determined by measuring residual host cell proteins (rHCPs) in neutralized low pH viral inactivation samples. The horizontal red dashed line represents the maximum acceptance criteria of 482 ppm applied at low pH viral inactivation unit operation.

[0027] **FIG. 7** is a plot showing a product purity assessment as determined by measuring residual protein A (rProA) leached from the column in neutralized low pH viral inactivation samples. The horizontal red dashed line represents the maximum acceptance criteria of 523 ppm applied at low pH viral inactivation unit operation.

[0028] **FIG. 8** is a plot showing a product purity assessment as determined by measuring residual DNA in neutralized low pH viral inactivation samples. The horizontal red dashed line represents the maximum acceptance criteria of 500 ppb applied at AEX unit operation.

[0029] **FIG. 9** is a plot showing a product purity assessment as determined by measuring residual Triton X-100 surfactant in neutralized low pH viral inactivation samples. The horizontal red dashed line represents the maximum acceptance criteria of 518,000 ppm.

Percent Yield (%Yield) and Dynamic Binding Capacity (DBC)

[00132] The cut strategy for dulaglutide protein A purification is based on a UV trigger and set collection volume. The frontside cut occurs at a UV signal of 4 AU/cm at 280 nm, and the backside cut in this study 1.5 CV after the frontside cut is made. **FIG. 2** shows the % yield trend and reduction in dynamic binding capacity across the protein A resin lifetime. The % yield was calculated based on the concentration of the neut. low pH viral inactivation intermediate and the response range observed in the protein A cycle lifetime study was 84.4 to 99.6%. The yield data show that this parameter remained consistent across the resin lifetime with the exception of a hardware pump issue (Runs 221 – 236) (**FIG. 2**, dashed box, a pump malfunction resulting in reduced charge material loaded onto column but had no impact on the study conclusions). A small downward trend when accounting for batch variation was observed in the % yield towards the end of the lifetime on runs targeting the upper loading limit of 18 g/L. This is likely due to degradation of the MSS LX protein A ligand which is caustic liable and/or peak broadening at the upper proven acceptable range loading. However, the % yield across the lifetime remained well within the 70-105 % critical in-process control (CIPC) range for the duration of the study.

[00133] The DBC observed in the protein A cycle lifetime study was 28.1 to 20.5 g/L. Based on these data, the protein A DBC decreased at a rate of 0.025 g/L per column cycle, most likely due to a combination of caustic sanitization exposure and binding site occlusion due to low level matrix fouling. Based on the equation of the slope of DBC values across the resin lifetime the DBC of the resin can be extrapolated to reach the upper proven acceptable range loading of 18 g/L after 408 product cycles. With a DBC value of 20.5 g/L at the end of this resin lifetime study and an anticipated routine manufacturing load of 14.4 g/L, this study demonstrates sufficient capacity of the resin to bind dulaglutide at the target load ratio over 304 product cycles.

Column HETP and Asymmetry

[00134] To detect changes in column pack quality, regular assessment of plates/meter and column asymmetry was performed across the column cycle lifetime study. The HETP observed in the protein A cycle lifetime study was 1782 to 3180 plates/meter. This range appears wider as the column repack performed post cycle 88 reduced the height of the column and the theoretical plate count but subsequently only a gentle decrease in plate number was observed which is anticipated due to repeat cycling. The plate count remained above the post-pack acceptance criteria of NLT 1100 plates/meter for the duration of the column lifetime study (**FIG. 3**).