Biopharmaceutical Processing, Development, Design and Implementation of Processes, Jagschies et al., editors, Elsevier Ltd, pages 837-855, 2018).

Development of these multispecific proteins brings new biomanufacturing challenges, particularly with regard to product instability and low expression yields. In particular, purification of multispecific proteins is complicated by the formation of product-related variants, such as homodimers, half-antibodies, aggregates, high and low molecular weight species and the like. These variants share similar structural and physical properties, such as charge, with the multispecific protein of interest, making them difficult to separate from during purification. These product-related impurities lower the yield and activity of the multispecific drug product and impact the robustness of manufacturing processes.

Product-related impurities having similar charge (isoelectric point) to a multispecific protein of interest may co-elute with the multispecific protein during cation exchange chromatography unit operations, complicating purification and lowering yield. It would be beneficial to separate the low pI product-related impurities prior to elution. The invention described herein meets this need by providing high salt load conditioning for removal of these low pI impurities during cation exchange chromatography.

BRIEF SUMMARY

In one aspect, the disclosure provides a method of performing cation exchange chromatography under high salt loading conditions to reduce product-related impurities, the method comprising equilibrating a cation exchange chromatography medium with an equilibration buffer of pH 4.9 to 5.1; loading a bispecific protein on to the cation exchange chromatography medium in a load buffer of pH 4.9 to 5.1; washing the column with a first and a second wash buffer of pH 4.9 to 5.1; and eluting the bispecific protein from the cation exchange chromatography medium; wherein the equilibration buffer, loading buffer and first wash buffer comprise 94-105 mM sodium chloride.

In another aspect, the disclosure provides a method of producing an isolated, purified, recombinant bispecific protein, the method comprising establishing a cell culture in a bioreactor with a host cell expressing the bispecific protein; culturing the host cells to express the bispecific protein; harvesting the recombinant bispecific protein; affinity purifying the harvested recombinant bispecific protein; inactivating virus by lowering the pH in an eluate pool from the affinity purification and neutralizing the pool at a higher pH compatible with the following method of performing cation exchange chromatography, wherein performing cation exchange chromatography comprises; equilibrating a cation exchange chromatography medium with an equilibration buffer of pH 4.9 to 5.1 comprising 94-105 mM sodium chloride; loading the neutralized affinity purified recombinant bispecific protein on to the equilibrated cation exchange chromatography medium in a load buffer of pH 4.9 to 5.1 comprising 94-105 mM sodium chloride; washing the cation exchange medium with a wash buffer of pH 4.9 to 5.1 comprising 94-105 mM sodium chloride, followed by a second wash buffer of pH 4.9 to 5.1 comprising 0-26mM sodium chloride; eluting the bispecific protein from the cation exchange chromatography medium; loading the cation exchange

chromatography eluate comprising the recombinant bispecific protein onto a second chromatography resin in flow through mode; and concentrating the purified recombinant bispecific protein in a formulation buffer.

The disclosure provides a method of purifying a multispecific protein from a composition comprising the multispecific protein and at least one product-related impurity, the method comprising equilibrating a cation exchange chromatography medium with an equilibration buffer comprising 94-105 mM Sodium chloride; loading the composition on to the cation exchange medium in a load buffer comprises 94-105 mM Sodium chloride; washing the column with at least one wash buffer comprising 94-105 mM Sodium chloride; and eluting the multispecific protein from the cation exchange chromatography medium. In one example the load buffer comprises 94-96 mM Sodium chloride. In a related example the load buffer comprises 96-105 mM Sodium chloride. In a related example the load buffer comprises 94 mM sodium chloride. In a related example the load buffer comprises 96 mM sodium chloride. In a related example the load buffer comprises 105 mM sodium chloride. In one example the load buffer comprises acetate. In a related example the load buffer comprises acetate, pH 4.9-5.1. In a related example the load buffer comprises acetate, pH 5.0 ± 0.05 to 5.0 ± 0.1. In a related example the load buffer comprises 100 mM acetate. In one example the load buffer comprises acetate and 94 mM–105 mM sodium chloride. In one example at least one wash buffer comprises 94-96 mM sodium chloride. In a related example at least one wash buffer comprises 96-105 mM sodium chloride. In a related example at least one wash buffer comprises 94 mM sodium chloride. In a related example at least one wash buffer comprises 96 mM sodium chloride. In a related example at least one wash buffer comprises 105

What is claimed is

1. A method of performing cation exchange chromatography under high salt loading conditions to reduce product-related impurities, the method comprising

equilibrating a cation exchange chromatography medium with an equilibration buffer of pH 4.9 to 5.1;

loading a bispecific protein on to the cation exchange chromatography medium in a load buffer of pH 4.9 to 5.1;

washing the column with a first and a second wash buffer of pH 4.9 to 5.1; and

eluting the bispecific protein from the cation exchange chromatography medium;

wherein the equilibration buffer, loading buffer, and first wash buffer comprise 94-105 mM sodium chloride.

2. The method according to claim 1, wherein the second wash buffer comprises 0–26 mM sodium chloride.

3. The method according to claim 1, wherein the load buffer comprises 94-96 mM sodium chloride; or wherein the load buffer comprises 96-105 mM sodium chloride; or wherein the load buffer comprises 94 mM sodium chloride; or wherein the load buffer comprises 96 mM sodium chloride; or wherein the load buffer comprises 98 mM sodium chloride or wherein the load buffer comprises 105 mM sodium chloride.

4. The method according to claim 1, wherein the wash buffer comprises 94-105 mM sodium chloride; or wherein the wash buffer comprises 94-96 mM sodium chloride; or wherein the wash buffer comprises 96-105 mM sodium chloride; or wherein the wash buffer comprises 94 mM sodium chloride; or wherein the wash buffer comprises 96 mM sodium chloride; or wherein the wash buffer comprises 98 mM sodium chloride; or wherein the wash buffer comprises 105 mM sodium chloride.

5. The method according to claim 1, wherein the equilibration buffer comprises 94-105mM sodium chloride; or wherein the equilibration buffer comprises 94-96 mM sodium chloride; or wherein the equilibration buffer comprises 96-105 mM sodium chloride; or wherein the equilibration buffer comprises 94 mM sodium chloride; or wherein the equilibration buffer comprises 96 mM sodium chloride; or wherein the equilibration buffer comprises 98 mM sodium chloride; or wherein the equilibration buffer comprises 105 mM sodium chloride.

6. A method of producing an isolated, purified, recombinant bispecific protein, the method comprising

establishing a cell culture in a bioreactor with a host cell expressing the bispecific protein;

culturing the host cells to express the bispecific protein;

harvesting the recombinant bispecific protein;

affinity purifying the harvested recombinant bispecific protein;

inactivating virus by lowering the pH in an eluate pool from the affinity purification and neutralizing the pool at a higher pH compatible with the following method of performing cation exchange chromatography, wherein performing cation exchange chromatography comprises:

equilibrating a cation exchange chromatography medium with an equilibration buffer of pH 4.9 to 5.1 comprising 94-105 mM sodium chloride;

loading the neutralized affinity purified recombinant bispecific protein on to the equilibrated cation exchange chromatography medium in a load buffer of pH 4.9 to 5.1 comprising 94-105 mM sodium chloride;

washing the cation exchange chromatography medium with a wash buffer of pH 4.9 to 5.1 comprising 94-105 mM sodium chloride, followed by a second wash buffer of pH 4.9 to 5.1 comprising 0-26mM sodium chloride; and

eluting the bispecific protein from the cation exchange chromatography medium;

loading the cation exchange chromatography eluate comprising the recombinant bispecific protein onto a second chromatography resin in flow through mode; and

concentrating the purified recombinant bispecific protein in a formulation buffer.

7. The method according to claim 6, wherein the load buffer comprises 94-96 mM sodium chloride; or wherein the load buffer comprises 96-105 mM sodium chloride; or wherein the load buffer comprises 94 mM sodium chloride; or wherein the load buffer comprises 96 mM sodium chloride; or wherein the load buffer comprises 98 mM sodium chloride or wherein the load buffer comprises 105 mM sodium chloride.

8. The method according to claim 6, wherein the wash buffer comprises 94-105 mM sodium chloride; or wherein the wash buffer comprises 94-96 mM sodium chloride; or wherein the wash buffer comprises 96-105 mM sodium chloride; or wherein the wash buffer comprises 94 mM sodium chloride; or wherein the wash buffer comprises 96 mM sodium chloride; or wherein the wash buffer comprises 98 mM sodium chloride; or wherein the wash buffer comprises 105 mM sodium chloride.

9. The method according to claim 6, wherein the equilibration buffer comprises 94-105mM sodium chloride; or wherein the equilibration buffer comprises 94-96 mM sodium chloride; or wherein the equilibration buffer comprises 96-105 mM sodium chloride; or wherein the equilibration buffer comprises 94 mM sodium chloride; or wherein the equilibration buffer comprises 96 mM sodium

chloride; or wherein the equilibration buffer comprises 98 mM sodium chloride; or wherein the equilibration buffer comprises 105 mM sodium chloride.

10. The method according to claim 6 wherein the second chromatography resin is selected from an anion exchange chromatography resin, cation exchange chromatography resin, multi-modal chromatography resin, hydrophobic interaction chromatography resin, and hydroxyapatite chromatography resin.