Nature Biotechnology, 32(10), 992-1000, 2014; Ecker et al., mAbs 7(1), 9-14, 2015; Spiess et al., Mol Immunol 67, 95-106, 2015; Fan et al., J Hematol & Oncology 8:130-143, 2015; Williams et al., Process Design for Bispecific Antibodies, 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 product-related 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.

Product-related impurities having similar charge (isoelectric point, pI) 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 wash conditions for removal of these low pI product-related impurities during cation exchange chromatography.

BRIEF SUMMARY ~~OF THE INVENTION~~

In one aspect, t~~T~~he ~~invention~~disclosure provides a method for purifying a multispecific protein comprising loading a sample comprising a multispecific protein onto a cation exchange chromatography medium; washing the cation exchange medium with at least one wash buffer comprising 100 – 147 mM sodium chloride; and eluting the multispecific protein from the cation exchange chromatography resin. In one ~~embodiment~~example, at least one wash buffer comprises 100 – 125 mM sodium chloride. In one ~~embodiment~~example at least one wash buffer comprises 100 – 105 mM sodium chloride. In one ~~embodiment~~example at least one wash buffer comprises 105 – 147 mM sodium chloride. In one ~~embodiment~~example at least one wash buffer comprises 105 – 125 mM sodium chloride. In one ~~embodiment~~example at least one wash buffer comprises 125 – 147 mM sodium chloride. In one ~~embodiment~~example at least one wash buffer comprises acetate. In a related ~~embodiment~~example at least one wash buffer comprises acetate, pH 5.0 ± 0.05% to pH 5.0 ±0.1%. In a related ~~embodiment~~example at least one wash buffer comprises acetate, pH 4.91-5.1. In a related ~~embodiment~~example at least one wash buffer comprises acetate, pH 4.9, 5.0, or 5.1. In another related ~~embodiment~~example the wash buffer comprises 100 mM acetate. In one ~~embodiment~~example at least one wash buffer comprises acetate, 100–125 mM sodium chloride. In one ~~embodiment~~example at least one wash buffer comprises acetate, 100–105 mM sodium chloride. In one ~~embodiment~~example at least one wash buffer comprises acetate, 105–147 mM sodium chloride. In one ~~embodiment~~example at least one wash buffer comprises acetate, 105–125 mM sodium chloride. In one ~~embodiment~~example at least one wash buffer comprises acetate, 125–147 mM sodium chloride.

In one ~~embodiment~~example the cation exchange medium is washed with at least two wash buffers. In one ~~embodiment~~example, the cation exchange medium is washed with at least three wash buffers.

In one ~~embodiment~~example the cation exchange medium is washed with at least two wash buffers, at least one of the wash buffers comprising 0-147 mM sodium chloride. In a related ~~embodiment~~example the cation exchange medium is washed with at least two wash buffers, at least one of the wash buffers comprising 0-70 mM sodium chloride. In one ~~embodiment~~example the cation exchange medium is washed with at least two wash buffers, at least one wash buffer comprising acetate, 0 mM sodium chloride, followed by a wash buffer comprising acetate, 100–147 mM sodium chloride. In a related ~~embodiment~~example the cation exchange medium is washed with a wash buffer comprising acetate, 0 mM sodium chloride, followed by a wash buffer selected from the group consisting of a wash buffer comprising acetate, 100 mM sodium chloride, a wash buffer comprising acetate, 105 mM sodium chloride, or a wash buffer comprising acetate, 125 mM sodium chloride. In one ~~embodiment~~example the cation exchange medium is washed with a wash buffer comprising acetate, 100–147 mM sodium chloride, followed by a wash buffer comprising acetate, 0-70 mM sodium chloride. In a related ~~embodiment~~example the cation exchange medium is washed with a wash buffer comprising acetate, 100–147 mM sodium chloride, followed by a wash buffer comprising acetate, 0 mM sodium chloride. In another related ~~embodiment~~example the cation exchange medium is washed a wash buffer comprising acetate, 100 – 147 mM sodium chloride, followed by a wash buffer comprising 70 mM sodium chloride.

In one ~~embodiment~~example the cation exchange medium is washed with at least three wash buffers, a first wash buffer comprising acetate, 0 mM sodium chloride, followed by a second wash buffer comprising acetate, 100–147 mM sodium chloride, followed by a third wash buffer comprising acetate, 0 mM sodium chloride or a wash buffer comprising acetate, 70 mM sodium chloride. In a related ~~embodiment~~example the cation exchange medium is washed with a first wash buffer comprising acetate, 0 mM sodium chloride, followed by a second wash buffer selected from the group consisting of a wash buffer comprising acetate, 100 mM sodium chloride, a wash buffer comprising acetate, 105 mM sodium chloride, followed by a wash buffer comprising acetate, or a wash buffer comprising acetate, 125 mM sodium chloride, followed by a third wash buffer comprising acetate, 0 mM sodium chloride. In a related ~~embodiment~~example the cation exchange medium is washed with a first wash buffer comprising acetate, 0 mM sodium chloride, followed by a second wash buffer comprising acetate, 147 mM sodium chloride, followed by a wash buffer comprising acetate, followed by a third wash buffer comprising acetate, 70 mM sodium chloride.

In one ~~embodiment~~example the cation exchange medium is washed with 2.5 mM/CV of a wash buffer comprising 147 mM sodium chloride.

In one ~~embodiment~~example the multispecific protein is eluted from the cation exchange medium by a gradient. In a related ~~embodiment~~example the gradient is a linear or step gradient. In a related ~~embodiment~~example the gradient is a salt gradient. In a related ~~embodiment~~example the buffers used to form the elution gradient comprises 0–1M sodium chloride. In another related ~~embodiment~~example at least one of the buffers used to form the elution

gradient comprises 70–500 mM sodium chloride. In another related ~~embodiment~~example at least one of the buffers used to form the elution gradient comprises 125 mM sodium chloride. In a related ~~embodiment~~example at least one wash buffer and one elution buffer comprise 125 mM sodium chloride.

In one ~~embodiment~~example the cation exchange medium is loaded with at least 10 g/L of the multispecific protein. In one ~~embodiment~~example the cation exchange medium is loaded with 10 g/L to 40 g/L of the multispecific protein. In a related ~~embodiment~~example the cation exchange medium is loaded with 15 g/L to 30 g/L. In a related ~~embodiment~~example the cation exchange medium is loaded with 25 g/L – 40 g/L. In one ~~embodiment~~example the cation exchange medium is loaded with 10 g/L of the multispecific protein, washed with a wash buffer comprising 105 mM sodium chloride and eluted in a salt gradient at 8 mM/CV. In one ~~embodiment~~example the cation exchange medium is loaded with 15g/L to 30 g/L of the multispecific protein, washed with a wash buffer comprising 147 mM sodium chloride. In one ~~embodiment~~example the cation exchange medium is loaded with 25g/L to 40 g/L of the multispecific protein, wherein at least one wash buffer and one elution buffer comprise 125 mM sodium chloride.

In one ~~embodiment~~example at least one product-related impurity is a homodimer, high molecular weight species, half antibody, aggregate, low molecular weight species, antibody fragment, or a light chain mis-assembly. In another ~~one embodiment~~aspect, the present disclosure provides a purification process that includes a unit operation comprising cation exchange chromatography performed according to the method described above.

In one ~~embodiment~~example the method above further comprises, before and/or after the cation exchange chromatography step, one or more unit operations for purifying the multispecific protein, comprising affinity chromatography, ion exchange chromatography, hydrophobic interaction chromatography column, and/or mixed-mode chromatography column.

In one ~~embodiment~~example the multispecific protein is a bispecific protein. In one ~~embodiment~~example the multispecific protein is a bispecific antibody. In ~~one embodiment~~ a further aspect, the present disclosure provides a purified, multispecific protein produced according to the method described above. In one ~~embodiment~~example the cation exchange chromatography medium is a resin.

The ~~invention~~disclosure provides a method for reducing low pI product-related impurities in the eluate from cation exchange chromatography, the method comprising loading a composition comprising a multispecific protein and at least one product-related impurity having a pI lower than the multispecific protein, onto a cation exchange chromatography medium; washing the cation exchange medium with a first wash buffer, washing the cation exchange medium with a second wash buffer comprises 100 – 147 mM sodium chloride; eluting the multispecific protein from the cation exchange chromatography resin; wherein the cation exchange chromatography eluate has reduced low pI product-related impurities compared to the cation exchange chromatography eluate recovered in a corresponding method in which no sodium chloride is included in a wash buffer formulation. In one ~~embodiment~~example the cation exchange medium is washed with a third wash buffer.

The ~~invention~~disclosure provides a method for performing cation exchange chromatography under high salt wash conditions to reduce product-related impurities, the method comprising loading a composition comprising a multispecific protein and at least one product-related impurity onto an equilibrated cation exchange column; washing the cation exchange medium with at least two wash buffers, wherein one wash buffer comprising 100 – 147 mM sodium chloride; and eluting the bound multispecific protein from the cation exchange chromatography resin. In one ~~embodiment~~example prior to loading the composition, the cation exchange medium is equilibrated with a buffer that contains no sodium chloride.

The ~~invention~~disclosure provides a method for producing an isolated, purified, recombinant multispecific protein, the method comprising establishing a cell culture in a bioreactor with a host cell expressing the multispecific protein; culturing the host cells to express the multispecific protein; harvesting the recombinant multispecific protein from the cell culture; affinity purifying the recombinant multispecific protein; loading the affinity purified recombinant multispecific protein onto a cation exchange chromatography resin; washing the cation exchange resin with at least one wash comprising 100 – 147 mM sodium chloride; eluting the multispecific protein from the cation exchange chromatography resin; and loading the cation exchange chromatography eluate comprising the recombinant multispecific protein onto an additional chromatography medium in flow through mode. In one ~~embodiment~~example the additional chromatography medium is selected from cation exchange chromatography medium, multi-modal chromatography medium, hydrophobic interaction chromatography medium, and hydroxyapatite chromatography medium. In one ~~embodiment~~example the affinity purified multispecific protein is in an eluate pool and is subjected to low pH viral inactivation, followed by neutralization, prior to loading onto the cation exchange medium. In one ~~embodiment~~example the flow through from the third chromatography medium is subjected to an ultrafiltration and diafiltration unit operation. In one ~~embodiment~~example an isolated, purified, recombinant multispecific protein is made according to the method described above. In one ~~embodiment~~example a pharmaceutical composition comprising the isolated, purified, recombinant multispecific protein produced with the method described above.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 Shows product-related impurities (homodimer, NCG) eluting with the main product, Bi-specific #1.

Figure 2 Shows that following high salt wash conditions, all the product-related impurities that were lower in pI than the main product were washed off the column between the second and third wash steps. The baseline returned to zero before the elution. The elution peak was reduced to one peak.

Figure 3 Shows that multiple impurities remained on the column and were eluted with the main product. These impurities included half antibodies (fractions 1-4 with ~ 50% half antibodies), 2X light chain-mis-assemblies (Fractions 5 and 6), and high molecular weight (fractions 12-21).

What is claimed is:

1. A method for purifying a multispecific protein comprising

loading a sample comprising a multispecific protein onto a cation exchange chromatography medium;

washing the cation exchange medium with at least one wash buffer comprising 100 – 147 mM sodium chloride; and

eluting the multispecific protein from the cation exchange chromatography resin.

2. The method according to claim 1, wherein at least one wash buffer comprises 100 – 125 mM sodium chloride; ~~.~~

~~3. The method according to claim 1, wherein at least one wash buffer~~ comprises 100 – 105 mM sodium chloride~~.~~; or

~~4. The method according to claim 1, wherein at least one wash buffer~~ comprises 105 – 147 mM sodium chloride~~.~~; or

~~5. The method according to claim 1, wherein at least one wash buffer~~ comprises 105 – 125 mM sodium chloride;~~.~~ or

~~6. The method according to claim 1, wherein at least one wash buffer~~ comprises 125 – 147 mM sodium chloride.

~~7~~3. The method according to claim 1, wherein at least one wash buffer comprises acetate.

~~8~~4. The method according to claim ~~7~~3, wherein at least one wash buffer comprises acetate, pH 5.0 ± 0.05% to pH 5.0 ±0.1%~~.~~; or

~~9. The method according to claim 7,~~ wherein at least one wash buffer comprises acetate, pH 4.91-5.1~~.~~; or

~~10. The method according to claim 7,~~ wherein at least one wash buffer comprises acetate, pH 4.9, 5.0, or 5.1; or~~.~~

~~11. The method according to claim 7,~~ wherein the wash buffer comprises 100 mM acetate.

~~12~~5. The method according to claim 1, wherein at least one wash buffer comprises acetate, 100–125 mM sodium chloride~~.~~; or

~~13. The method according to claim 1, wherein at least one wash buffer~~ comprises acetate, 100–105 mM sodium chloride~~.~~; or

~~14. The method according to claim 1, wherein at least one wash buffer~~ comprises acetate, 105–147 mM sodium chloride~~.~~; or

~~15. The method according to claim 1, wherein at least one wash buffer~~ comprises acetate, 105–125 mM sodium chloride~~.~~; or

~~16. The method according to claim 1, wherein at least one wash buffer~~ comprises acetate, 125–147 mM sodium chloride.

~~17~~6. The method according to claim 1, wherein the cation exchange medium is washed with at least two wash buffers.

~~18~~7. The method according to claim 1, wherein the cation exchange medium is washed with at least three wash buffers.

~~19~~8. The method according to claim ~~18~~7, wherein the cation exchange medium is washed with at least two wash buffers, at least one of the wash buffers comprising 0-147 mM sodium chloride~~.~~; or

~~20. The method according to claim 19,~~ wherein the cation exchange medium is washed with at least two wash buffers, at least one of the wash buffers comprising 0-70 mM sodium chloride.

~~21~~9. The method according to claim 1 wherein the cation exchange medium is a) washed with at least two wash buffers, at least one wash buffer comprising acetate, 0 mM sodium chloride, followed by a wash buffer comprising acetate, 100–147 mM sodium chloride~~.~~; or

~~22. The method according to claim 21~~ wherein the cation exchange medium is b) washed with a wash buffer comprising acetate, 0 mM sodium chloride, followed by a wash buffer selected from the group consisting of a wash buffer comprising acetate, 100 mM sodium chloride, a wash buffer comprising acetate, 105 mM sodium chloride, or a wash buffer comprising acetate, 125 mM sodium chloride~~.~~; or

~~23. The method according to claim 21,~~ wherein the cation exchange medium is c) washed with a wash buffer comprising acetate, 100–147 mM sodium chloride, followed by a wash buffer comprising acetate, 0-70 mM sodium chloride.

~~24~~10. The method according to claim ~~23~~9 c), wherein the cation exchange medium is washed with a wash buffer comprising acetate, 100–147 mM sodium chloride, followed by a wash buffer comprising acetate, 0 mM sodium chloride~~.~~; or

~~25. The method according to claim 23,~~ wherein the cation exchange medium is washed a wash buffer comprising acetate, 100 – 147 mM sodium chloride, followed by a wash buffer comprising 70 mM sodium chloride.

~~26~~11. The method according to claim ~~18~~7, wherein the cation exchange medium is washed with at least three wash buffers,

a first wash buffer comprising acetate, 0 mM sodium chloride, followed by

a second wash buffer comprising acetate, 100–147 mM sodium chloride, followed by

a third wash buffer comprising acetate, 0 mM sodium chloride or a wash buffer comprising acetate, 70 mM sodium chloride.; or

27. The method according to claim 26, wherein the cation exchange medium is washed with

a first wash buffer comprising acetate, 0 mM sodium chloride, followed by

a second wash buffer selected from the group consisting of a wash buffer comprising acetate, 100 mM sodium chloride, a wash buffer comprising acetate, 105 mM sodium chloride, or a wash buffer comprising acetate, 125 mM sodium chloride, followed by

a third a wash buffer comprising acetate, 0 mM sodium chloride.; or

28. The method according to claim 26, wherein the cation exchange medium is washed with

a first wash buffer comprising acetate, 0 mM sodium chloride, followed by

a second wash buffer comprising acetate, 147 mM sodium chloride, followed by

a third a wash buffer comprising acetate, 70 mM sodium chloride.

2912. The method according to claim 1, wherein the cation exchange medium is washed with 2.5 mM/CV of a wash buffer comprising 147 mM sodium chloride.

3013. The method according to claim 1, wherein the multispecific protein is eluted from the cation exchange medium by a gradient.

3114. The method according to claim 3013, wherein the gradient is a linear or step gradient.; or

32. The method according to claim 30, wherein the gradient is a salt gradient.

3315. The method according to claim 3013, wherein at least one of the buffers used to form the elution gradient comprises 0–1M sodium chloride.; or

34. The method according to claim 33, wherein at least one of the buffers used to form the elution gradient comprises 70–500 mM sodium chloride.; or

35. The method according to claim 33, wherein at least one of the buffers used to form the elution gradient comprises 125 mM sodium chloride.

3616. The method according to claim 1, wherein at least one wash buffer and one elution buffer comprise 125 mM sodium chloride.

3717. The method according to claim 1, wherein the cation exchange medium is loaded a) with at least 10 g/L of the multispecific protein.; or

38. The method according to claim 1, wherein the cation exchange medium is b) loaded with 10 g/L to 40 g/L of the multispecific protein.

~~39~~18. The method according to claim ~~38~~17 b), wherein the cation exchange medium is loaded with 15 g/L to 30 g/L~~.~~; or

~~40. The method according to claim 38,~~ wherein the cation exchange medium is loaded with 25 g/L – 40 g/L.

~~41~~19. The method according to claim 1, wherein the cation exchange medium is loaded with 10 g/L of the multispecific protein, washed with a wash buffer comprising 105 mM sodium chloride and eluted in a salt gradient at 8 mM/CV~~.~~; or

~~42. The method according to claim 1,~~ wherein the cation exchange medium is loaded with 15g/L to 30 g/L of the multispecific protein, washed with a wash buffer comprising 147 mM sodium chloride~~.~~; or

~~43. The method according to claim 1,~~ wherein the cation exchange medium is loaded with 25g/L to 40 g/L of the multispecific protein, wherein at least one wash buffer and one elution buffer comprise 125 mM sodium chloride.

~~44~~20. The method according to claim 1, wherein at least one product-related impurity is a homodimer, high molecular weight species, half antibody, aggregate, low molecular weight species, antibody fragment, or a light chain mis-assembly.

~~45~~21. A purification process that includes a unit operation comprising cation exchange chromatography preformed according to the method of claim 1.

~~46~~22. The method according to claim 1, further comprising, before and/or after the cation exchange chromatography step, one or more unit operations for purifying the multispecific protein, comprising affinity chromatography, ion exchange chromatography, hydrophobic interaction chromatography column, and/or mixed-mode chromatography column.

~~47~~23. The method according to claim 1, wherein the multispecific protein is a bispecific protein; or ~~.~~

~~48. The method according to claim 1,~~ wherein the multispecific protein is a bispecific antibody.

~~49~~24. A purified, multispecific protein produced according to the method of claim 1.

~~50~~25. The method according to claim 1, wherein the cation exchange chromatography medium is a resin.

~~51. A method for reducing low pI product-related impurities in the eluate from cation exchange chromatography, the method comprising~~

~~loading a composition comprising a multispecific protein and at least one product-related impurity having a pI lower than the multispecific protein, onto a cation exchange chromatography medium;~~

~~washing the cation exchange medium with a first wash buffer,~~

washing the cation exchange medium with a second wash buffer comprises 100 – 147 mM sodium chloride;

eluting the multispecific protein from the cation exchange chromatography resin;

wherein the cation exchange chromatography eluate has reduced low pI product-related impurities compared to the cation exchange chromatography eluate recovered in a corresponding method in which no sodium chloride is included in a wash buffer formulation.

52. The method according to claim 51, wherein the cation exchange medium is washed with a third wash buffer.

53. A method for performing cation exchange chromatography under high salt wash conditions to reduce product-related impurities, the method comprising

loading a composition comprising a multispecific protein and at least one product-related impurity onto an equilibrated cation exchange column;

washing the cation exchange medium with at least two wash buffers, wherein one wash buffer comprising 100 – 147 mM sodium chloride; and

eluting the bound multispecific protein from the cation exchange chromatography resin.

54. The method according to claim 53, wherein prior to loading the composition, the cation exchange medium is equilibrated with a buffer that contains no sodium chloride.

55. A method for producing an isolated, purified, recombinant multispecific protein, the method comprising

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

culturing the host cells to express the multispecific protein;

harvesting the recombinant multispecific protein from the cell culture;

affinity purifying the recombinant multispecific protein;

loading the affinity purified recombinant multispecific protein onto a cation exchange chromatography resin;

washing the cation exchange resin with at least one wash comprising 100 – 147 mM sodium chloride;

eluting the multispecific protein from the cation exchange chromatography resin; and

loading the cation exchange chromatography eluate comprising the recombinant multispecific protein onto an additional chromatography medium in flow through mode.

~~56. The method according to claim 55, wherein the additional chromatography medium is selected from cation exchange chromatography medium, multi modal chromatography medium, hydrophobic interaction chromatography medium, and hydroxyapatite chromatography medium.~~

~~57. The method according to claim 55, wherein the affinity purified multispecific protein is in an eluate pool and is subjected to low pH viral inactivation, followed by neutralization, prior to loading onto the cation exchange medium.~~

~~58. The method according to claim 56, wherein the flow through from the third chromatography medium is subjected to an ultrafiltration and diafiltration unit operation.~~

~~59. An isolated, purified, recombinant multispecific protein according to the method of claim 55.~~

~~60. A pharmaceutical composition comprising the isolated, purified, recombinant multispecific protein produced with the method of claim 55.~~