but can also involve cation exchange. Following the train of one or more chromatographic fractionations in the downstream polishing steps of a harvest/purification methodology, there is often a filtration step such as an ultrafiltration step, which may involve diafiltration, a form of ultrafiltration with solvent replenishment. Following filtration, the purified protein is typically formulated for use such as administration to subjects in need.

[0005] The use of cell-culture-based expression systems to produce therapeutic biologics and biosimilars has required these multi-step purification protocols to ensure sufficient purity of products destined for administration to humans. To ensure sufficient purity, governmental regulators throughout the world have developed stringent standards that must be met, including the requirement in the US to meet Quality Target Protein Profiles, which are rigorous standards for the purification of products to be administered to humans. The requirement to obtain governmental approval to administer biologics and biosimilars has resulted in continuing demand for greater purity of products expressed in culture. Thus, a need continues to exist in the art for improvements to protein harvest/purification methodologies to yield protein target products with reduced levels and types of culture-based contaminants.

SUMMARY

[0006] The disclosure provides methodologies that improve the purity of target proteins, such as biologics and biosimilars, that are produced in cell culture. The disclosure takes an approach at odds with conventional technologies in the downstream example of protein purification from cell culture. More particularly, in the downstream polishing phase of protein purification from cell culture, the disclosure relies on one or more chromatography steps prior to an ultrafiltration/diafiltration (UF/DF) step, consistent with conventional purification protocols, but then takes the unusual step of returning to chromatographic fractionation involving at least a mixed-mode chromatography or an ion exchange chromatography (e.g., cation exchange chromatography) step. The purification methodology of the disclosure results in a significant reduction of undesired high molecular weight (HMW) material contaminating the purified target protein material while minimizing reductions in yield of that target protein. In adding this unusual step late in the polishing phase of target harvest/purification, the method provides improved removal of all forms of high molecular weight compounds, including nucleic acids, proteins, lipids, and other forms of high molecular weight compounds found in cell-based target protein production methods. The result is a target protein of greater purity that exhibits an improved profile conforming to Quality Target Protein Profiles.

[0007] In one aspect, the disclosure provides a method of harvesting a target protein from a host cell culture fluid comprising a chromatography step after an ultrafiltration/diafiltration (UF/DF) step, wherein the high molecular weight species in the eluate from the chromatography step are reduced by at least 10% compared to the level of high molecular weight species in the UF/DF filtrate. In some examples, the method further comprises at least one chromatography step preceding the ultrafiltration/diafiltration step. In some examples, the at least one chromatography step comprises protein A chromatography. In some examples, the at least one chromatography step further comprises ion exchange chromatography, mixed-mode chromatography, or both ion exchange chromatography and mixed-mode chromatography. In some examples, the method further comprises upstream bulk harvest steps of centrifuging, depth filtering, or both centrifuging and depth filtering the host cell culture fluid followed by downstream polishing steps to purify the target protein, wherein the polishing steps comprise a protein A chromatography step, a low pH viral inactivation step, a cation exchange step, a mixed-mode anion exchange chromatography step, a viral filtration step, an ultrafiltration/diafiltration step, the chromatography step after the ultrafiltration/diafiltration step, a polysorbate 80 addition step, and a final filtration step.

[0008] Still other examples of the method are provided wherein the target protein subjected to the chromatography step is present in a formulation buffer comprising 10 mM glutamic acid, 250 mM threonine, pH 5.5. In some examples, the output from the chromatography step is an eluate wherein the high molecular weight compounds are reduced by at least 9% compared to the level in the UF/DF filtrate. In some examples, the high molecular weight compounds in the eluate are reduced by at least 20%, at least 25%, at least 30%, or at least 75% compared to the level in the UF/DF filtrate. In some examples, the output from the chromatography step is an eluate comprising 0.3-1.9% high molecular weight compounds. In some examples, the yield of target protein from the chromatography step is at least 58%, 60%, 65%, 70%, 75%, 80%, 86%, at least 90%, at least 95%, or at least 98%.

[0009] Some examples of the method are disclosed wherein the chromatography media is a mixed-mode resin, a mixed-mode membrane, an ion exchange resin or an ion exchange membrane. In some examples, the chromatography media is Ca++Pure-HA, Capto MMC, Capto MMC ImpRes, Capto SP ImpRes, Capto Adhere, CIMultus PrimaS, CIMultus Hbond, CMM Hypercel, Eshmuno CP-FT, Eshmuno HCX, Fibro MMC, Fibro Adhere, Fractogel COO- (M), Fractogel SO3- (M), Mustang XT S, Nuvia S, Nuvia HR-S, Nuvia cPrime, Sartobind Phenyl, ToyoPearl MX-Trp, ToyoPearl Sulfate 650M, or UNOsphere S. In some examples, the chromatography media is Ca++ Pure HA, Capto

MMC, Capto MMC ImpRes, Capto Adhere, CMM Hypercel, Eshmuno HCX, Fibro MMC, Fibro Adhere, Nuvia cPrime, ToyoPearl mX-Trp, or ToyoPearl Sulfate. In some examples, the chromatography media is Capto MMC ImpRes, Eshmuno HCX, Eshmuno CP-FT, Fibro MMC, or Nuvia cPrime. In some examples, the chromatography media is Fibro MMC or Nuvia cPrime. In some examples, the chromatography media is a membrane. In some examples, the fluid comprising the target protein is applied to the chromatography media with a load factor of at least 400 grams/Liter-resin, including examples wherein the load factor is between 400-800 grams/Liter-resin and examples wherein the load factor is at least 800 grams/Liter-resin.

[0010] The method also provides examples wherein the load %HMW is at least 0.5%. In some examples, the load %HMW is at least 0.9%, is at least 1.2%, is between 0/5-2.6%, or is at least 2.6%. In some examples of the method, the chromatography step yields a %HMW clearance of at least 5%, at least 8%, at least 24%, at least 30%, at least 33%, at least 40%, at least 45%, at least 55%, at least 65%, at least 70%, or at least 75%.

[0011] The method also provides examples wherein the fluid comprising the target protein is applied to the chromatography media at a load concentration of no more than 50 g/L. In some examples, the load concentration is no more than 20 g/L. In some examples of the method, the chromatography media comprises a ligand at a ligand density of at least 98 mmol/mL chromatography media. In some examples, the ligand density is between 98-157 mmol/mL, is between 98-140 mmol/mL, or is between 127-157 mmol/mL. In some examples, the eluate from the chromatography step has a Quality Target Protein Profile of 0.3% or less, wherein the eluate comprises the target protein.

[0012] The disclosure will be better understood upon consideration of the following detailed description of the disclosure, including a consideration of the figures.

BRIEF DESCRIPTION OF THE DRAWING

[0013] **Figure 1.** Bar graphs demonstrating the cross-molecule applicability of adding a post-UF/DF chromatography step to a purification protocol for a large protein such as an antibody biologic/biosimilar. A worst-case load material level of 3.4% HMW was used in assessing the performance of the post-UF/DF chromatography step when purifying mAb4 from culture fluid. Performance was assessed by determining %HMW as a measure of HMW clearance and by determining %step yield. The tested resins were CaptoMMC ImpRes, Nuvia cPrime, CMM Hypercel, ToyoPearl Sulfate, and Capto Adhere. Nuvia cPrime resin provided 24% HMW clearance with a %step yield greater than 90%. The results demonstrate that addition of a post-UF/DF chromatography step in biologic/biosimilar purification protocols has wide applicability in showing significant reduction of HMW and

CLAIMS

What is claimed is:

1. A method of harvesting a target protein from a host cell culture fluid comprising a chromatography step after an ultrafiltration/diafiltration (UF/DF) step, wherein the high molecular weight species in the eluate from the chromatography step are reduced by at least 10% compared to the level of high molecular weight species in the UF/DF filtrate.

2. The method of claim 1 further comprising at least one chromatography step preceding the ultrafiltration/diafiltration step.

3. The method of claim 2 wherein the at least one chromatography step preceding the ultrafiltration/diafiltration step comprises protein A chromatography.

4. The method of claim 3 wherein the at least one chromatography step preceding the ultrafiltration/diafiltration step further comprises ion exchange chromatography, mixed-mode chromatography, or both ion exchange chromatography and mixed-mode chromatography.

5. The method of claim 1 further comprising upstream bulk harvest steps of centrifuging, depth filtering, or both centrifuging and depth filtering the host cell culture fluid followed by downstream polishing steps to purify the target protein, wherein the polishing steps comprise a protein A chromatography step, a low pH viral inactivation step, a cation exchange step, a mixed-mode anion exchange chromatography step, a viral filtration step, an ultrafiltration/diafiltration step, the chromatography step after the ultrafiltration/diafiltration step, a polysorbate 80 addition step, and a final filtration step.

6. The method of claim 1, wherein the chromatography step after the ultrafiltration/diafiltration step comprises mixed-mode chromatography.

7. The method of claim 1 wherein the high molecular weight compounds in the eluate are reduced by at least 20% compared to the level in the UF/DF filtrate.

8. The method of claim 7 wherein the high molecular weight compounds in the eluate are reduced by at least 30% compared to the level in the UF/DF filtrate.

9. The method of claim 7 wherein the high molecular weight compounds in the eluate are reduced by 75% compared to the level in the UF/DF filtrate.

10. The method of claim 1 wherein the output from the chromatography step is an eluate comprising 0.3-1.9% high molecular weight compounds.

11. The method of claim 1 wherein the yield of target protein from the chromatography step is at least 58%.

12. The method of claim 11 wherein the yield of target protein from the chromatography step is at least 86%.

13. The method of claim 11 wherein the yield of target protein from the chromatography step is at least 90%.

14. The method of claim 1 wherein the chromatography step after the ultrafiltration/diafiltration step comprises using a chromatography media, wherein the chromatography media is a mixed-mode resin, a mixed-mode membrane, an ion exchange resin or an ion exchange membrane.

15. The method of claim 14 wherein the chromatography media is Ca++Pure-HA, Capto MMC, Capto MMC ImpRes, Capto SP ImpRes, Capto Adhere, CIMultus PrimaS, CIMultus Hbond, CMM Hypercel, Eshmuno CP-FT, Eshmuno HCX, Fibro MMC, Fibro Adhere, Fractogel COO- (M), Fractogel SO3- (M), Mustang XT S, Nuvia S, Nuvia HR-S, Nuvia cPrime, Sartobind Phenyl, ToyoPearl MX-Trp, ToyoPearl Sulfate 650M, or UNOsphere S.

16. The method of claim 14 wherein the chromatography media is Ca++ Pure HA, Capto MMC, Capto MMC ImpRes, Capto Adhere, CMM Hypercel, Eshmuno HCX, Fibro MMC, Fibro Adhere, Nuvia cPrime, ToyoPearl mX-Trp, or ToyoPearl Sulfate.

17. The method of claim 16 wherein the chromatography media is Capto MMC ImpRes, Eshmuno HCX, Eshmuno GP-FT, Fibro MMC, or Nuvia cPrime.

18. The method of claim 14 wherein the chromatography media is Fibro MMC or Nuvia cPrime.

19. The method of claim 1 wherein the chromatography media is a membrane.

20. The method of claim 1 wherein the load factor is between 400-800 grams/Liter-resin.

21. The method of claim 1 wherein the load factor is at least 800 grams/Liter-resin.

22. The method of claim 1 wherein the load %HMW is at least 0.9%.

23. The method of claim 1 wherein the load %HMW is at least 1.2%.

24. The method of claim 1 wherein the load %HMW is between 0.5-2.6%.

25. The method of claim 1 wherein the load %HMW is at least 2.6%.

26. The method of claim 1 wherein the fluid comprising the target protein is applied to the chromatography media at a load concentration of no more than 50 g/L.

27. The method of claim 26 wherein the load concentration is no more than 20 g/L.

28. The method of claim 1 wherein the chromatography media comprises a ligand at a ligand density of at least 98 mmol/ml chromatography media.

29. The method of claim 1 wherein the eluate from the chromatography step has a Quality Target Protein Profile of 0.3% or less, wherein the eluate comprises the target protein.