recombinantly, for example in mammalian cells. In such examples, the Fc region-containing proteins can be purified from cell culture supernatants or cell lysates, such as those produced as a result of harvest operations from a bioreactor.

[0016] The disclosure is also based, in part, on the discovery that size exclusion chromatography, when used with a buffer comprising a chaotropic agent, a sugar alcohol, and at least one amino acid as the mobile phase, can effectively separate fully assembled antibodies from half antibody contaminants. Accordingly, in one aspect, the present disclosure also provides a method for separating antibodies from half antibody forms thereof, comprising: contacting a solution comprising antibodies and half antibody forms thereof with a gel filtration matrix using a mobile phase having a pH of 6.5 to 7.5 and comprising a chaotropic agent, a sugar alcohol, an apolar amino acid, and a basic amino acid; and collecting elution fractions from the gel filtration matrix, wherein the antibodies are eluted in one set of elution fractions and the half antibody forms thereof are eluted in another set of elution fractions, thereby separating the antibodies from the half antibody forms thereof, wherein the chaotropic agent is present at a concentration of 0.4 M to 5 M, wherein the sugar alcohol present at a concentration of 1 M to 4.5 M, wherein the basic amino acid is present at a concentration of 0.25 M to 1 M, wherein the apolar amino acid is present at a concentration of 0.25 M to 1 M, wherein the salt is present at a concentration of 0.25 M to 0.8 M.. In one example, the method comprises contacting a solution comprising antibodies and half antibody forms thereof with a gel filtration matrix using a mobile phase having a pH of about 6.5 to about 7.5 and comprising a chaotropic agent, a sugar alcohol, an apolar amino acid, and a basic amino acid; and collecting elution fractions from the gel filtration matrix, wherein the antibodies are eluted in one set of elution fractions and the half antibody forms thereof are eluted in another set of elution fractions, thereby separating the antibodies from the half antibody forms thereof. In certain examples of the method, the antibodies are multispecific (e.g. bispecific) heterodimeric antibodies.

[0017] Any of the elution buffers described herein for eluting Fc region-containing proteins from a temperature-responsive protein A resin at neutral pH and constant temperature can be used as a mobile phase in size exclusion chromatography for separating antibodies from half antibody forms thereof. In some examples, the mobile phase comprises about 2 M to about 4.5 M of a chaotropic agent, about 1 M to about 4.5 M of a sugar alcohol, about 0.25 M to about 1 M of a basic amino acid, and about 0.25 M to about 1 M of an apolar amino acid. In these and other examples, the mobile phase comprises urea, sorbitol, arginine, and proline. In certain examples, the mobile phase comprises about 2 M to about 4.5 M urea, about 1 M to about 4.5 M sorbitol, about 0.25 M to about 1 M arginine, about 0.25 M to about 1 M proline, and 0.25 M to about 0.8 M sodium chloride. In some examples, the mobile phase comprises about 4 M urea, about 2.2 M

CLAIMS

What is claimed:

1. A method for separating antibodies from half antibody forms thereof, comprising:

contacting a solution comprising antibodies and half antibody forms thereof with a gel filtration matrix using a mobile phase having a pH of 6.5 to 7.5 and comprising a chaotropic agent, a sugar alcohol, an apolar amino acid, ~~and~~ a basic amino acid and a salt; and

collecting elution fractions from the gel filtration matrix, wherein the antibodies are eluted in one set of elution fractions and the half antibody forms thereof are eluted in another set of elution fractions, thereby separating the antibodies from the half antibody forms thereof~~.~~,

wherein the chaotropic agent is present at a concentration of 0.4 M to 5 M, wherein the sugar alcohol present at a concentration of 1 M to 4.5 M, wherein the basic amino acid is present at a concentration of 0.25 M to 1 M, wherein the apolar amino acid is present at a concentration of 0.25 M to 1 M, wherein the salt is present at a concentration of 0.25 M to 0.8 M.

2. The method of claim 1, wherein the chaotropic agent is urea, guanidinium chloride, sodium thiocyanate, potassium thiocyanate, or ammonium thiocyanate.

3. The method of claim 2, wherein the chaotropic agent is urea.

4. The method of claim 3, wherein the urea is present at a concentration of 2 M to 4.5 M.

5. The method of claim 4, wherein the urea is present at a concentration of about 4 M.

6. The method of clam 1, wherein the sugar alcohol is sorbitol, mannitol, xylitol, or glycerol.

7. The method of claim 1, wherein the sugar alcohol is sorbitol.

~~8. The method of claim 1, wherein the sugar alcohol is present at a concentration of 1 M to 4.5 M.~~

~~9.~~8. The method of claim ~~8~~1, wherein the sugar alcohol is present at a concentration of 2 M to 2.5 M.

~~10.~~9. The method of claim 1, wherein the basic amino acid is histidine, lysine, ornithine, or arginine.

~~11.~~10. The method of claim ~~10~~9, wherein the basic amino acid is arginine.

~~12.~~11. The method of claim 1, wherein the apolar amino acid is alanine, cysteine, glycine, isoleucine, leucine, methionine, phenylalanine, proline, tryptophan, or valine.

~~13.~~12. The method of claim ~~12~~11, wherein the apolar amino acid is proline.

~~14. The method of claim 1, wherein the basic amino acid and/or the apolar amino acid is present at a concentration of 0.25 M to 1 M.~~

~~15.~~13. The method of claim 1, wherein the basic amino acid and/or the apolar amino acid is present at a concentration of about 0.5 M.

~~16. The method of claim 1, wherein the mobile phase further comprises a salt.~~

~~17. The method of claim 16, wherein the salt is present at a concentration of 0.25 M to 0.8 M.~~

~~18.~~14. The method of claim ~~16~~1, wherein the salt is sodium chloride.

~~19.~~15. The method of claim ~~18~~14, wherein the mobile phase comprises 2 M to 4.5 M urea, 1 M to 4.5 M sorbitol, 0.25 M to 1 M arginine, 0.25 M to 1 M proline, and 0.25 M to 0.8 M sodium chloride.

~~20.~~16. The method of claim ~~19~~15, wherein the mobile phase comprises about 4 M urea, about 2.2 M sorbitol, about 0.5 M arginine, about 0.5 M proline, and about 0.75 M sodium chloride.

~~21.~~17. The method of claim 1, wherein the mobile phase has a pH of 7.0 to 7.4.

~~22.~~18. The method of claim 1, wherein the mobile phase is applied to the gel filtration matrix at a flow rate of 0.01 ml/min to 0.2 ml/min.

~~23.~~19. The method of claim 1, wherein the mobile phase is applied to the gel filtration matrix at a flow rate of 0.02 ml/min to 0.06 ml/min.

~~24.~~20. The method of claim 1, wherein the gel filtration matrix has a fractionation range of 10 kDa to 600 kDa.

~~25.~~21. The method of claim 1, wherein the gel filtration matrix comprises cross-linked agarose and dextran.

~~26.~~22. The method of claim 1, wherein the antibodies are recombinantly produced in mammalian cells.

~~27.~~23. The method of claim ~~26~~22, wherein the mammalian cells are Chinese Hamster Ovary cells.

~~28.~~24. The method of claim 1, wherein the antibodies are multi-specific heterodimeric antibodies.

~~29.~~25. The method of claim 1, wherein the solution comprising antibodies and half antibody forms thereof is an eluate pool or effluent stream from a protein A chromatography.

~~30.~~26. The method of claim 1, wherein the solution comprising antibodies and half antibody forms thereof is a cell culture supernatant or a cell lysate.