[0005] Methods for reducing the content of chromatin from cell culture harvests containing monoclonal antibodies have been described (H. Gan et al, J. Chromatogr. A, 1291 (2013) 33-40. Chromatin is known to include DNA and histone proteins in stable associations known as nucleosomes. Gan et al reported that chromatin and its catabolites also form associations with antibodies that limit the efficacy of subsequent fractionation methods. They reported methods that achieved chromatin reduction of 99%, and further reported that such reduction improved the quality of purification obtained by cation exchange chromatography. A variation of a technique reported by Gan et al has been reported by Gagnon et al (2013, supra) to improve recovery and purity of IgG fractionated by steric exclusion chromatography on fluidized hydrophilic particles.

SUMMARY

[0006] A method of purifying a desired protein from a preparation comprising (a) providing the preparation in a form having less than about 5% of chromatin residing in an original production medium, (b) contacting the preparation with a nonionic organic polymer and a salt, wherein a concentration of nonionic organic polymer is sufficient to precipitate the desired protein or cause its accretion on a hydrophilic surface, or maintain it in a precipitated state or accreted on the hydrophilic surface, the salt concentration being sufficient to produce greater than physiological conductivity, wherein the nonionic organic polymer is polyethylene glycol (PEG), and (c) contacting the preparation with at least one electropositive surface, optionally in the presence of a salt concentration sufficient to produce greater than physiological conductivity, whereby the desired protein does not substantially adsorb to the at least one electropositive surface while not preventing adsorption of acidic contaminants to the at least one electropositive surface.

DETAILED DESCRIPTION

[0007] It has been surprisingly discovered that PEG-mediated fractionation methods can achieve a higher degree of protein purification than the known high-functioning method of biological affinity chromatography when the PEG-mediated method is performed on an impure preparation that has been conditioned to remove at least 95% of the chromatin, and the PEG-mediated method comprises at least one stage where the precipitation mixture contains an elevated concentration of salt, and at least one stage where at least one electropositive surface is present, and at least one stage where the mixture of PEG and the desired protein is in contact with at least one electropositive surface in a relative absence

CLAIMS

What is claimed is:

1. A method of purifying a desired protein from a preparation comprising:

(a) providing the preparation in a form having less than about 5% of chromatin residing in an original production medium;

(b) contacting the preparation with a nonionic organic polymer and a salt, wherein a concentration of nonionic organic polymer is sufficient to precipitate the desired protein or cause its accretion on a hydrophilic surface, or maintain it in a precipitated state or accreted on the hydrophilic surface, the salt concentration being sufficient to produce greater than physiological conductivity; and

(c) contacting the preparation with at least one electropositive surface, optionally in the presence of a salt concentration sufficient to produce greater than physiological conductivity, whereby the desired protein does not substantially adsorb to the at least one electropositive surface while not preventing adsorption of acidic contaminants to the at least one electropositive surface.

2. The method of claim 1, further comprising (d) contacting the preparation with at least one nonionic hydrophilic surface.

3. The method of claim 1, wherein the salt concentration during step (a) and (b) are the same.

4. The method of claim 1, further comprising retaining the precipitated desired protein on a porous membrane while soluble contaminants are eliminated by passage therethrough.

5. The method of claim 1, further comprising retaining the precipitated desired protein on a microporous membrane that is substantially inert while soluble contaminants are eliminated by passage there through, independently from the salt concentration.

6. The method of claim 1, further comprising retaining the precipitated desired protein on a microporous membrane that is electropositive while soluble contaminants pass there through when the conductivity is greater than physiological conductivity.

7. The method of claim 1, wherein the method is conducted in a single integrated apparatus.

8. The method of claim 1, wherein the nonionic organic polymer is polyethylene glycol (PEG).

9. The method of claim 1, wherein the average polymer size of the nonionic organic polymer is in the range of one from the group consisting of (a) from about 1,500 Daltons to about 15,000 Daltons, (b) from about 2,000 Daltons to about 12,000 Daltons (c) from about 3,000 Daltons to about 10,000 Daltons, (d) from about 4,000 Daltons to about 8,000 Daltons, and (e) from about 5,000 Daltons to about 6,000 Daltons.

10. The method of claim 1, wherein the conductivity is at least 1 mS/cm greater than physiological conductivity.

11. The method of claim 1, wherein the salt is selected from the group consisting of sodium chloride, potassium chloride, sodium acetate, potassium acetate, sodium thiocyanate, potassium thiocyanate, guanidinium hydrochloride, and combinations thereof.

12. The method of claim 11, wherein the salt is sodium chloride at a concentration selected from the group consisting of (a) from about 0.5 M to about 1.5 M, (b) from about 2.0 M to about 3.0 M, and intermediate ranges thereof.

13. The method of claim 1, wherein the at least one electropositive surface is membrane with pores of an average size selected from the group consisting of (a) about 100 nm, (b) about 220 nm, (c) about 450 nm, (d) about 1 micron, (e) about 2 microns, and intermediate values thereof.

14. The method of claim 1, wherein the at least one electropositive solid is a plurality of particles.

15. The method of claim 1, wherein the at least one electropositive solid is part of a chromatography device selected from the group comprising, a monolith-based chromatography device, a membrane-based chromatography device, a particle-based chromatography device, a macroreticulate skeleton supporting a hydrogel-based device, and combinations thereof.

16. The method of claim 1, wherein the salt concentration is selected from the group comprising a zero amount, 1 mM, 2 mM, 5 mM, 10 mM, 25 mM, 50 mM, 100 mM, and intermediate concentrations thereof.

17. The method of claim 1, wherein a pH during step (a) is in a range selected from the group consisting of (a) from about 8 to about 9, (b) from about 8 to about 8.5, (c) from about 7.5 to about 8.5, (d) from about 7.25 to about 8.25, (e) from about 7.0 to about 8.0, (f) from about 6 to about 7, and intermediate pH ranges thereof.

18. The method of claim 1, wherein a pH during step (b) is in a range selected from the

group consisting of (a) from about 5 to about 9, (b) from about 6 to about 8, (c) from about 6.5 to about 7.5, (d) from about 7.5 to about 8.5, and intermediate pH ranges thereof.

19. The method of claim 1, wherein the at least one hydrophilic surface comprises one selected from the group consisting of a membrane, a monolith, and a plurality of particles, wherein the plurality particles are optionally magnetic.

20. The method of claim 1, wherein the at least one electropositive surface comprises one from the group consisting of a membrane, a monolith, and a plurality of particles, wherein the plurality of particles are optionally magnetic.

21. The method of claim 1, wherein the preparation is one selected from the group consisting of a cell culture medium, an extract from cultured organisms, and a bodily fluid.

CLAIMS

What is claimed is:

1. A method of purifying a desired protein from a preparation comprising:

(a) providing the preparation in a form having less than about 5% of chromatin residing in an original production medium;

(b) contacting the preparation with a nonionic organic polymer and a salt, wherein a concentration of nonionic organic polymer is sufficient to precipitate the desired protein or cause its accretion on a hydrophilic surface, or maintain it in a precipitated state or accreted on the hydrophilic surface, the salt concentration being sufficient to produce greater than physiological conductivity, wherein the nonionic organic polymer is polyethylene glycol (PEG); and

(c) contacting the preparation with at least one electropositive surface, optionally in the presence of a salt concentration sufficient to produce greater than physiological conductivity, whereby the desired protein does not substantially adsorb to the at least one electropositive surface while not preventing adsorption of acidic contaminants to the at least one electropositive surface.

2. The method of claim 1, further comprising (d) contacting the preparation with at least one nonionic hydrophilic surface.

3. The method of claim 1, wherein the salt concentration during step (a) and (b) are the same.

4. The method of claim 1, further comprising retaining the precipitated desired protein on a porous membrane while soluble contaminants are eliminated by passage therethrough.

5. The method of claim 1, further comprising retaining the precipitated desired protein on a microporous membrane that is substantially inert while soluble contaminants are eliminated by passage there through, independently from the salt concentration.

6. The method of claim 1, further comprising retaining the precipitated desired protein on a microporous membrane that is electropositive while soluble contaminants pass there through when the conductivity is greater than physiological conductivity.

7. The method of claim 1, wherein the method is conducted in a single integrated apparatus.

8. The method of claim 1, wherein the average polymer size of the nonionic organic polymer is in the range of one from the group consisting of (a) from about 1,500 Daltons to about 15,000 Daltons, (b) from about 2,000 Daltons to about 12,000 Daltons (c) from about 3,000 Daltons to about 10,000 Daltons, (d) from about 4,000 Daltons to about 8,000 Daltons, and (e) from about 5,000 Daltons to about 6,000 Daltons.

9. The method of claim 1, wherein the conductivity is at least 1 mS/cm greater than physiological conductivity.

10. The method of claim 1, wherein the salt is selected from the group consisting of sodium chloride, potassium chloride, sodium acetate, potassium acetate, sodium thiocyanate, potassium thiocyanate, guanidinium hydrochloride, and combinations thereof.

11. The method of claim 10, wherein the salt is sodium chloride at a concentration selected from the group consisting of (a) from about 0.5 M to about 1.5 M, (b) from about 2.0 M to about 3.0 M, and intermediate ranges thereof.

12. The method of claim 1, wherein the at least one electropositive surface is membrane with pores of an average size selected from the group consisting of (a) about 100 nm, (b) about 220 nm, (c) about 450 nm, (d) about 1 micron, (e) about 2 microns, and intermediate values thereof.

13. The method of claim 1, wherein the at least one electropositive solid is a plurality of particles.

14. The method of claim 1, wherein the at least one electropositive solid is part of a chromatography device selected from the group comprising, a monolith-based chromatography device, a membrane-based chromatography device, a particle-based chromatography device, a macroreticulate skeleton supporting a hydrogel-based device, and combinations thereof.

15. The method of claim 1, wherein the salt concentration is selected from the group comprising a zero amount, 1 mM, 2 mM, 5 mM, 10 mM, 25 mM, 50 mM, 100 mM, and intermediate concentrations thereof.

16. The method of claim 1, wherein a pH during step (a) is in a range selected from the group consisting of (a) from about 8 to about 9, (b) from about 8 to about 8.5, (c) from about 7.5 to about 8.5, (d) from about 7.25 to about 8.25, (e) from about 7.0 to about 8.0, (f) from about 6 to about 7, and intermediate pH ranges thereof.

17. The method of claim 1, wherein a pH during step (b) is in a range selected from the

group consisting of (a) from about 5 to about 9, (b) from about 6 to about 8, (c) from about 6.5 to about 7.5, (d) from about 7.5 to about 8.5, and intermediate pH ranges thereof.

18. The method of claim 1, wherein the at least one hydrophilic surface comprises one selected from the group consisting of a membrane, a monolith, and a plurality of particles, wherein the plurality particles are optionally magnetic.

19. The method of claim 1, wherein the at least one electropositive surface comprises one from the group consisting of a membrane, a monolith, and a plurality of particles, wherein the plurality of particles are optionally magnetic.

20. The method of claim 1, wherein the preparation is one selected from the group consisting of a cell culture medium, an extract from cultured organisms, and a bodily fluid.