METHODS FOR REDUCING CHROMATIN CONTENT IN PROTEIN PREPARATIONS BY TREATMENT WITH ALKYL CATIONS

BACKGROUND

[0001] Embodiments disclosed herein relate to methods for enhancing purification of proteins, including antibodies. They particularly relate to methods for reducing the level of aggregates and can be combined with methods of cell culture harvest clarification. They further relate to integration of these capabilities with other purification methods to achieve a desired level of protein purity.

[0002] Aggregate removal is an important aspect of protein purification. It has been shown that low concentrations of the yellow fluorescent heterocyclic dye ethacridine reduces aggregate content of antibody preparations, and that this result may be due, in part, to chromatin removal (Gan *et al* J. Chromatography A 1291 (2013) 33-40). Ethacridine has a long history as a protein precipitating agent.

[0003] The surfactant cetrimonium bromide is used as a topical antiseptic. It is also used in the production of gold nanoparticles, and widely in hair-care products. It is used in the field of protein analysis where it improves resolution of complex glycoproteins in polyacrylamide gel electrophoresis, and in DNA extraction. It is not known to have applications in the field of protein fractionation.

SUMMARY

[0004] Described herein is a method of reducing aggregate content in a preparation comprising a target protein, the method comprising contacting the preparation with an alkyl cation to form a mixture and contacting the mixture with at least one functionalized solid to remove excess alkyl cation. In one aspect, the present invention refers to a method of reducing aggregate content in a preparation comprising a target protein, wherein the target protein comprises an antibody of the class IgA, IgD, IgE, IgG or IgM, the method comprising: contacting the preparation with an alkyl cation to form a mixture, wherein the alkyl cation is selected from a bromide or chloride salt of a quaternary ammonium cation selected from the group consisting of cetyl trimethyl ammonium; N,N,N-Trimethyl-1-heptadecanammonium; N,N,N-Trimethyl-1-octadecanammonium; N,N,N-Trimethyl-1-pentadecanammonium; and N,N,N-Trimethyl-1-tetradecanammonium, and the alkyl cation is present in the mixture at a concentration of 0.001% to 1% (w/v); and contacting the mixture with at least one functionalized solid to remove excess alkyl cation.

DETAILED DESCRIPTION OF THE DISCLOSED METHODS

[0005] The methods disclosed herein provide a means of reducing protein aggregates in a sample containing a desired protein by treating the sample with alkyl cations. In some embodiments, the reduction of complexes and/or aggregates may be achieved with ultralow levels of alkyl cations. In some embodiments, the sample may be treated at elevated conductivity values (using elevated salt concentration). In some embodiments, the treated sample may be subsequently exposed to solid materials bearing chemical moieties that selectively remove alkyl cations and aggregates from the protein preparation.

[0025] In some embodiments, methods may further comprise contacting the mixture with an aryl cation. In some such embodiments, the aryl cation may be ethacridine or methylene blue. In some embodiments, the combined concentration of the alkyl and aryl cations may be the same as the concentration as when the alkyl cation is used alone. In such embodiments, the alkyl cation is present in a non-zero amount.

[0026] In some embodiments, methods disclosed herein may further comprise contacting the mixture, simultaneously or sequentially, with at least one electropositive solid to further reduce aggregate content of the preparation.

[0027] In some embodiments, the alkyl cation comprises a bromide or chloride salt of a quaternary ammonium cation selected from the group consisting of cetrimonium, cetyl trimethyl ammonium, N,N,N-Trimethyl-1-hexadecanammonium, N,N,N-trimethyl-1-heptadecanammonium, N,N,N-Trimethyl-1-octadecanammonium, N,N,N-Trimethyl-1-pentadecanammonium, and N,N,N-Trimethyl-1-tetradecanammonium.

[0028] In some embodiments, the alkyl cation is present in a concentration range selected from the group consisting of: (a) from about 0.001% to about 1%; (b) from about 0.01 to about 1%, and (c) from about 0.02 to about 0.03%.

[0029] In some embodiments, allantoin is present in a concentration range selected from the group consisting of: (a) from about 0.6 to about 50%; (b) from about 1 to about 10%; and (c) from about 1 to about 2%.

[0030] In some embodiments an operating conductivity is within a range selected from the group consisting of: (a) from about 0.1 to about 50 mS/cm; (b) from about 1 to about 30 mS/cm; and (c) from about 5 to about 15 mS/cm.

[0031] In some embodiments, an operating pH is in a range selected from the group consisting of: (a) from about 4 to about 10; (b) from about 5 to about 9; and from about 6 to about 8.

[0032] In some embodiments, the mixture further comprises an antiviral agent that is not the alkyl cation, the antiviral agent being selected from the group consisting of chlorhexidine, benzalkonium chloride, methylene blue, ethacridine, and tri (n-butyl) phosphate.

[0033] In some embodiments, a surface of the at least one functionalized solid promotes a chemical interaction selected from the group consisting of: electrostatic interactions, hydrophobic interactions, hydrogen bonding, and metal affinity.

[0034] In some embodiments, the at least one functionalized solid is particulate.

CLAIMS

1. A method of reducing aggregate content in a preparation comprising a target protein, wherein the target protein comprises an antibody of the class IgA, IgD, IgE, IgG or IgM, the method comprising:

contacting the preparation with an alkyl cation to form a mixture, wherein the alkyl cation is selected from a bromide or chloride salt of a quaternary ammonium cation selected from the group consisting of cetyl trimethyl ammonium; N,N,N-Trimethyl-1-heptadecanammonium; N,N,N-Trimethyl-1-octadecanammonium; N,N,N-Trimethyl-1-pentadecanammonium; and N,N,N-Trimethyl-1-tetradecanammonium, and the alkyl cation is present in the mixture at a concentration of 0.001% to 1% (w/v) ; and

contacting the mixture with at least one functionalized solid to remove excess alkyl cation.

2. The method of claim 1, further comprising contacting the mixture with an aryl cation selected from ethacridine and methylene blue.

3. The method of claim 2, wherein the aryl cation is ethacridine.

4. The method of an one of claims 1 to 3, further comprising contacting the mixture, simultaneously or sequentially, with at least one electropositive solid to further reduce aggregate content of the preparation.

5. The method of any one of claims 1 to 4, wherein the alkyl cation comprises a bromide or chloride salt of cetyl trimethyl ammonium.

6. The method of any one of claims 1 to 5, wherein the alkyl cation is present in the mixture at a concentration range of from 0.01 to 1%, or from 0.02 to 0.03% (w/v).

7. The method of any one of claims 1 to 6, wherein allantoin is present in the mixture at a concentration range selected from the group consisting of: (a) from 0.6 to 50%; (b) from 1 to 10%; and (c) from 1 to 2% (w/v).

8. The method of any one of claims 1 to 7, wherein a conductivity of the mixture is within a range selected from the group consisting of: (a) from 0.1 to 50 mS/cm; (b) from 1 to 30 mS/cm; and (c) from 5 to 15 mS/cm.

9. The method of any one of claims 1 to 8, wherein a pH of the mixture is in a range selected from the group consisting of: (a) from 4 to 10; (b) from 5 to 9; and (c) from 6 to 8.

10. The method of any one of claims 1 to 9, wherein the mixture further comprises an antiviral agent that is not the alkyl cation, the antiviral agent being selected from the group consisting of chlorhexidine, benzalkonium chloride, and tri(n-butyl)phosphate.

11. The method of any one of claims 1 to 10, wherein a surface of the at least one functionalized solid promotes a chemical interaction selected from the group consisting of: electrostatic interactions, hydrophobic interactions, hydrogen bonding, and metal affinity.

12. The method of any one of claims 1 to 11, wherein the at least one functionalized solid is particulate.

13. The method of any one of claims 1 to 12, wherein the antibody is a recombinant antibody.

14. The method of any one of claims 1 to 13, wherein the protein preparation is one selected from the group consisting of a cell-containing cell culture harvest, a substantially cell-free cell culture harvest, and a partially purified protein.

15. The method of any one of claims 1 to 14, wherein the at least one functionalized solid comprises tris(2-ethylaminoethyl)amine (TREN).