[0004] Treating antibody preparations with agents that might be expected to dissociate hetero-aggregates has generally proven ineffective. For example, employing high concentrations of urea, salts, or combinations of the two does not substantially dissociate IgM-contaminant hetero-aggregates (Gagnon *et al.*, *supra*). Protein A affinity chromatography with pre-elution washes of urea, alcohol, and surfactants has been indicated to reduce hetero-aggregate levels more effectively than without washes (Shukla *et al.*, *supra*), as did pre-elution washes combining urea, salt, and EDTA with protein G affinity chromatography (Mechetner *et al.*, *supra*). Anion exchange chromatography with a pre-elution wash of urea has been indicated to reduce hetero-aggregates more effectively than in the absence of a urea wash (Gagnon *et al.*, *supra*). Cation exchange chromatography has also been indicated to reduce hetero-aggregates more effectively with a pre-elution EDTA wash than without the wash (Gagnon *et al.*, *supra*). Finally, hydroxyapatite with pre-elution washes of urea and/or salt have also reduced hetero-aggregates more effectively than without such washes (Gagnon, *supra*). Despite these observations, in general, the use of dissociating agents in pre-elution washes of antibodies bound to chromatography columns has been only moderately successful.

[0005] Electropositive organic additives have been indicated for the precipitation of acidic proteins (Farhner et al., U.S. Patent Application No. 20080193981; Ma et al., J. Chromatogr. B (2010) 878:798-806; Peram et al., Biotechnol. Progr., (2010) 26:1322-1326; Glynn, in U. Gottschalk (ed.), Process Scale Purification of Antibodies, J. T. Wiley and Sons, (2009) Hoboken, 309-324), as well as for precipitation of DNA and endotoxins (Glynn supra; Cordes et al., Biotechnol. Progr., (1990) 6:283-285; Dissing et al., Bioseparation, (1999) 7 221:9-11) and inactivation of virus (Bernhardt, U.S. Patent. 5,559,250). Multivalent metal cations have also been indicated to remove DNA and endotoxin from some protein preparations (Akcasu et al., Nature, (1960) 187:323-324; Matsuzawa et al., Nucl. Acids Res., (2003) 3(3):163-164; Christensen et al., Prot. Expr. Purif., (2004) 37:468-471; Kejnovsky et al., Nucl. Acids Res., (1997) 25:1870-1871; Ongkudon et al., Anal. Chem., (2011) 83 391:13-17).

SUMMARY OF THE INVENTION

[0006] In one aspect, the present invention refers to a method of reducing the aggregate content of a sample comprising a desired protein at a conductivity value of 15 mS/cm or higher, the method comprising adding an electropositive organic additive at a non-zero concentration of 0.1% or less (weight/volume), wherein the electropositive organic additive is selected from ethacridine, chlorhexidine and polyethyleneimine, and wherein the desired protein is an antibody. This disclosure provides in certain examples methods for reducing the amount of product-contaminant complexes and aggregates in a sample containing a desired protein species, such as a protein preparation, by treating the sample with electropositive organic additives. In certain examples, the reduction of complexes and/or aggregates is achieved with ultralow levels of electropositive organic additives. In certain examples, the invention particularly reduces the

CLAIMS

1. A method of reducing the aggregate content of a sample comprising a desired protein at a conductivity value of 15 mS/cm or higher, the method comprising adding an electropositive organic additive at a non-zero concentration of 0.1% or less (weight/volume), wherein the electropositive organic additive is selected from ethacridine, chlorhexidine and polyethyleneimine, and wherein the desired protein is an antibody.

2. The method of claim 1, wherein the electropositive organic additive is ethacridine.

3. The method of any one of claims 1 to 2, wherein a salt is added to a level to substantially prevent precipitation of the desired protein, wherein the conductivity value is adjusted to 20 mS/cm or higher.

4. The method of any one of claims 1 to 3, wherein the desired protein is a recombinant protein.

5. The method of any one of claims 1 to 4, wherein more than one electropositive organic additive is present, wherein a total concentration of the electropositive organic additives is 0.1% or less (weight/volume).

6. The method of any one of claims 1 to 5, wherein allantoin is present at a concentration in range of from about 0.56% weight/volume to about 2% weight/volume.

7. The method of claim 6, wherein the allantoin is added to the sample before addition of the electropositive organic additive.

8. The method of any one of claims 1 to 7, wherein the sample comprises a conductivity that is adjusted to a sufficiently high level to counteract substantial loss of the desired protein through precipitation, wherein the conductivity value is adjusted to 30 mS/cm or higher.

9. The method of any one of claims 1 to 8, wherein the conductivity of the sample is adjusted before addition of the electropositive organic additive.

10. The method of any one of claims 1 to 9, wherein an organic solvent is present, and the organic solvent comprises one selected from the group consisting of ethanol, ethylene glycol, and propylene glycol.

11. The method of claim 10, wherein the organic solvent comprises a concentration in a range of from about 1% or greater (weight/volume).

12. The method of any one of claims 1 to 11, wherein a surfactant is present, wherein the surfactant comprises a nonionic surfactant or a zwitterionic surfactant.

13. The method of claim 13, wherein the nonionic surfactant comprises one selected from the group consisting of Tween and Triton.

14. The method of claim 13, wherein the zwitterionic surfactant comprises one selected from the group consisting of CHAPS, CHAPSO, and octaglucoside.

15. The method of any one of claims 12 to 14, wherein the surfactant is present at a non-zero concentration less than 1%.

16. The method of any one of claims 1 to 15, wherein a chelating agent is present.

17. The method of any one of claims 1 to 16, wherein more than one of the following is present: (a) an organic solvent, (b) an organic polymer, (c) a ureide, (d) a surfactant, (e) a chelating agent, and (f) a salt.

18. The method of any one of claims 1 to 17, wherein the contacted sample is exposed to one or more solids to remove at least one electropositive organic additive before subsequent processing steps to fractionate the desired protein from remaining contaminants.

19. The method of claim 1, wherein the electropositive organic additive is present at a concentration ranging from 0.02% to 0.03%.