SUMMARY

[0006] In one aspect, the present disclosure refers to a method of removing contaminants from a sample comprising a desired protein, the method comprising: (a) contacting the sample with (i) an amount of allantoin in excess of saturation, (ii) a cationic polymer selected from chitosan, polyallylamine, polydiallyldimethylammonium chloride (pDADMAC), DEAE-cellulose and DEAE-dextran and (iii) a fatty acid selected from caprylic acid, heptanoic acid, heptenoic acid, octenoic acid, nonanoic acid, nonenoic acid, and decanoic acid , thereby forming a mixture; and (b) removing solids from the mixture, wherein the desired protein comprises an antibody or an antigen-binding fragment thereof. In some examples, presented herein is a method of removing contaminants from a sample comprising a desired protein where the method comprises (a) contacting the sample with (i) an amount of allantoin in excess of saturation, (ii) a cationic polymer and (iii) a fatty acid comprising 6 to 10 carbon atoms, thereby forming a mixture, and (b) removing solids from the mixture. In some examples, the desired protein comprises an antibody, a binding fragment thereof or portion thereof. In certain examples, the sample comprises a cell culture harvest which can include cells. In some examples, the sample comprises one or more contaminants selected from host cell proteins, DNA, chromatin, and aggregates. In some examples, the mixture, after removing the solids, comprises less than 20% of the one or more contaminants that were present in the sample prior to (b). In certain examples, the mixture, after removing the solids, comprises at least 90% of the desired protein that was present in the sample prior to (b).

[0007] In some examples, method is conducted at a pH in a range of 5.0 to 5.5.

[0008] In certain examples, the cationic polymer is selected from a polyallylamine, chitosan, polydiallyldimethylammonium chloride (pDADMAC), DEAE-cellulose and DEAE-dextran. In some examples, the amount of the cationic polymer is in a range of 0.001% (w/v) to 2% (w/v).

[0009] In certain examples, the fatty acid is selected from the group consisting of caprylic acid, heptanoic acid, heptenoic acid, octenoic acid, nonanoic acid, nonenoic acid, and decanoic acid. In some examples, the fatty acid is at a concentration of about 0.2 % (v/v) to about 0.6% (v/v).

[0010] In certain examples, the amount of allantoin is at least 0.9% (w/v).

[0011] In some examples, the solids are removed in (b) by a process comprising filtration or sedimentation. In some examples, the solids are removed in (b) by contacting the mixture with a chemically reactive surface comprising positive charges. In certain examples, the chemically reactive surface comprises silica.

[0012] In some examples the method further comprising (c) further purifying the mixture by a use of at least one suitable chromatography process. In some examples, a chromatography process comprises one or more of protein affinity chromatography, cation exchange chromatography, electronegative multimodal chromatography, anion exchange

chromatography, hydrophobic interaction chromatography, and immobilized metal affinity chromatography.

[0013] In another aspect, the present disclosure refers to an aqueous composition comprising (a) an amount of allantoin in excess of saturation, (b) a cationic polymer at a concentration of in a range of 0.001% w/v to 0.2% w/v, wherein the cationic polymer is selected from polyallylamine, chitosan, polydiallyldimethylammonium chloride (pDADMAC), DEAE-cellulose and DEAE-dextran, (c) a fatty acid at a concentration of 0.25 % (vol/vol) to about 0.55% (vol/vol), wherein the fatty acid is selected from caprylic acid, heptanoic acid, heptenoic acid, octenoic acid, nonanoic acid, nonenoic acid, and decanoic acid, and (d) an antibody, a binding fragment thereof or a portion thereof. In some examples, presented herein is a composition, for example an aqueous composition, comprising (a) an amount of allantoin in excess of saturation, (b) a cationic polymer at a concentration of in a range of 0.001% w/v to 0.2% w/v, wherein the cationic polymer is selected from polyallylamine, chitosan, polydiallyldimethylammonium chloride (pDADMAC), DEAE-cellulose and DEAE-dextran, and (c) a fatty acid at a concentration of 0.25 % (vol/vol) to about 0.55% (vol/vol), wherein the fatty acid is selected from caprylic acid, heptanoic acid, heptenoic acid, octenoic acid, nonanoic acid, nonenoic acid, and decanoic acid. In some examples, the composition further comprises a desired protein, for example an antibody, a binding fragment thereof or a portion thereof.

[0014] In some aspects, presented herein is a kit comprising (a) allantoin, (b) a cationic polymer selected from polyallylamine, chitosan, polydiallyldimethylammonium chloride (pDADMAC), DEAE-cellulose and DEAE-dextran, (c) a fatty acid selected from caprylic acid, heptanoic acid, heptenoic acid, octenoic acid, nonanoic acid, nonenoic acid, and decanoic acid, and (d) one or more inserts or labels comprising instructions for carrying out a method described herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] The invention will be better understood with reference to the detailed description when considered in conjunction with the non-limiting examples and the accompanying drawings, in which:

[0016] **Figure 1** compares analytical size exclusion chromatography (SEC) profiles illustrating the size distribution of contaminants in cell culture harvest before and after treatment with a composition comprising either A) chitosan or B) pDADMAC in

combination with allantoin and caprylic acid. Impurity peaks are smaller on SEC after treatment.

[0017] **Figure 2** compares analytical size exclusion chromatography (SEC) profiles illustrating relative aggregate content after protein A affinity chromatography where the profile at left illustrates results when protein A is loaded with cell culture harvest not treated by the composition comprising either A) chitosan or B) pDADMAC in combination with allantoin and caprylic acid, and the profile on the right shows the result from protein A loaded with cell culture harvest that has been treated by the composition comprising either

CLAIMS

1. A method of removing contaminants from a sample comprising a desired protein, the method comprising:

(a) contacting the sample with (i) an amount of allantoin in excess of saturation, (ii) a cationic polymer selected from chitosan, polyallylamine, polydiallyldimethylammonium chloride (pDADMAC), DEAE-cellulose and DEAE-dextran and (iii) a fatty acid selected from caprylic acid, heptanoic acid, heptenoic acid, octenoic acid, nonanoic acid, nonenoic acid, and decanoic acid , thereby forming a mixture; and

(b) removing solids from the mixture,

wherein the desired protein comprises an antibody or an antigen-binding fragment thereof.

2. The method of claim 1, wherein the desired protein comprises an antibody.

3. The method of claim 1 or 2, wherein the sample comprises a cell culture harvest.

4. The method of claim 3, wherein the cell culture harvest comprises cells.

5. The method of any one of claims 1 to 4, wherein prior to (b), the mixture is adjusted to, or maintained at, a pH in a range of 5.0 to 5.5.

6. The method of any one of claims 1 to 5, wherein the cationic polymer is a polyallylamine or polydiallyldimethylammonium chloride (pDADMAC) .

7. The method of any one of claims 1 to 6, wherein the fatty acid comprises octenoic acid.

8. The method of any one of claims 1 to 6, wherein the fatty acid comprises caprylic acid.

9. The method of any one of claims 1 to 8, wherein an amount of the cationic polymer is in a range of 0.001% (w/v) to 2% (w/v).

10. The method of claim 9, wherein an amount of the cationic polymer is in a range of 0.02% (w/v) to 0.1% (w/v).

11. The method of any one of claims 1 to 10, wherein the fatty acid is at a concentration of about 0.2 % (v/v) to about 0.6% (v/v).

12. The method of claim 11, wherein the fatty acid is at a concentration of about 0.40% (v/v) to about 0.50% (v/v).

13. The method of any one of claims 1 to 12, wherein the amount of allantoin is at least 0.9% (w/v).

14. The method of any one of claims 1 to 13, wherein the solids are removed in (b) by a process comprising filtration or sedimentation.

15. The method of any one of claims 1 to 14, wherein the solids are removed in (b) by contacting the mixture with a chemically reactive surface comprising positive charges.

16. The method of claim 15, wherein the chemically reactive surface comprises silica.

17. The method of any one of claims 1 to 16, further comprising (c) purifying the mixture by a process comprising protein affinity chromatography, cation exchange chromatography, electronegative multimodal chromatography, anion exchange chromatography, hydrophobic interaction chromatography, or immobilized metal affinity chromatography.

18. The method of any one of claims 1 to 17, wherein the sample comprises one or more contaminants selected from host cell proteins, DNA, chromatin, and aggregates.

19. The method of claim 18, wherein the mixture, after (b), comprises less than 20% of the one or more contaminants that were present in the sample prior to (b).

20. The method of any one of claims 1 to 19, wherein the mixture, after (b), comprises at least 90% of the desired protein that was present in the sample prior to (b).

21. An aqueous composition comprising (a) an amount of allantoin in excess of saturation, (b) a cationic polymer at a concentration of in a range of 0.001% w/v to 0.2% w/v, wherein the cationic polymer is selected from polyallylamine, chitosan, polydiallyldimethylammonium chloride (pDADMAC), DEAE-cellulose and DEAE-dextran, (c) a fatty acid at a concentration of 0.25 % (vol/vol) to about 0.55% (vol/vol), wherein the fatty acid is selected from caprylic acid, heptanoic acid, heptenoic acid, octenoic acid, nonanoic acid, nonenoic acid, and decanoic acid, and (d) an antibody, a binding fragment thereof or a portion thereof.

22. A kit comprising (a) allantoin, (b) a cationic polymer selected from polyallylamine, chitosan, polydiallyldimethylammonium chloride (pDADMAC), DEAE-cellulose and DEAE-dextran, (c) a fatty acid selected from caprylic acid, heptanoic acid, heptenoic acid, octenoic acid, nonanoic acid, nonenoic acid, and decanoic acid, and (d) one or more inserts or labels comprising instructions for carrying out the method of any one of claims 1 to 20.