

compositions.

## Summary

**[0004]** Provided herein are methods for making stable protein compositions. The present application is directed to the surprising findings that post UF DF stable protein compositions (e.g., protein compositions with reduced aggregates) can be obtained if conditions for reducing instability are present before the UF DF operation. In particular, the present application is directed to the surprising findings that adjusting the pH of a protein preparation obtained from a purification process to a target pH before a final UF DF operation results in a stable composition comprising the protein after the UF DF operation. Protein compositions prepared by the methods disclosed herein have increased stability compared to protein compositions prepared by a similar method without the pH adjusting step.

**[0005]** In one aspect, disclosed herein is a method for preparing a composition comprising a protein, the method comprises providing a preparation comprising a protein of interest, the preparation is obtained after subjecting a sample comprising the protein and one or more impurities to a purification process, adjusting the pH of the preparation to a target pH prior to a final ultrafiltration and diafiltration operation, and obtaining a composition comprising the protein from the final ultrafiltration and diafiltration operation, wherein the target pH is at or about the pH of the composition comprising the protein obtained from the final ultrafiltration and diafiltration operation. In one example, the pH is adjusted using a pH adjusting agent. In one example, the pH adjusting agent is an acid, a base, or a buffer. In one example, the target pH is from pH 4.0 to about pH 7.0 or from pH 4.0 to about pH 6.0.

**[0006]** In one example, the acid used for adjusting the pH of the preparation is acetic acid, adipic acid, alginic acid, ascorbic acid, aspartic acid, benzene sulfonic acid, citric acid, caprylic acid, formic acid, glutamic acid, hydrochloride acid, hydrobromic acid, hydroxy acids, hyaluronic acid, lactic acid, malic acid, methanesulfonic acid, nitric acid, phosphoric acid, propionic acid, sulfuric acid, or tartaric acid. In another example, the base used for adjusting the pH of the preparation is ammonia solution, ammonium carbonate, diethanolamine, calcium hydroxide, ethanolamine, lysine, poly-lysine, meglumine, potassium hydroxide, sodium bicarbonate, sodium borate, sodium carbonate, sodium hydroxide, or triethanolamine. In one example, the buffer used for adjusting the pH of the preparation is acetate buffer, aspartate buffer, ascorbate buffer, citrate buffer, phosphate buffer, succinate buffer, glycine buffer, carbonate buffer, lactate buffer, Tris buffer, Bis-tris buffer, histidine buffer, HEPES buffer, MOPS buffer, MES buffer, ADA buffer, borate

buffer, tartrate buffer, or benzoate buffer.

**[0007]** In one example, the method further comprises concentrating the protein in the preparation to a target concentration. In one example, the concentrating step is carried out after the pH adjusting step and before the final UF DF operation. In another example, the concentrating step is carried out before the pH adjusting step. In one example, the target concentration is from about 1 mg/mL to about 50 mg/mL, in another example, the target concentration is from about 1 mg/mL to about 200 mg/mL, in another example, the target concentration is from about 1 mg/mL to about 250 mg/mL, in another example, the target concentration is from about 50 mg/mL to about 200 mg/mL, in another example, the target concentration is from about 50 mg/mL to about 250 mg/mL.

**[0008]** In one example, the final ultrafiltration and diafiltration operation is carried out using a medium that comprises a buffer, in one example, the final ultrafiltration and diafiltration operation is carried out using a medium that comprises a buffer at a concentration of from 2 mM to 10 mM, from 2 mM to 50 mM, or from 2 mM to 300 mM. In another example, the final ultrafiltration and diafiltration operation is carried out using a medium that comprises essentially no buffer.

**[0009]** In one example, the composition obtained from the final ultrafiltration and diafiltration operation comprises essentially no buffer and comprises the protein at a concentration of from about 40 mg/mL to about 200 mg/mL. In one example, the composition obtained from the final ultrafiltration and diafiltration operation comprises a buffer and the protein at a concentration of from about 1 mg/mL to about 200 mg/mL. In one example, the composition obtained from the final ultrafiltration and diafiltration operation comprises a buffer at a concentration of from 2 mM to about 10 mM and comprises the protein at a concentration of from about 1 mg/mL to about 50 mg/mL. In one example, the composition obtained from the final ultrafiltration and diafiltration operation comprises a buffer at a pH outside the buffer capacity range of the bugger and comprises the protein at a concentration of from about 1 mg/mL to about 50 mg/mL. In one example, the composition obtained from the final ultrafiltration and diafiltration operation is a pharmaceutical formulation comprising the protein. In one example, the composition obtained from the final ultrafiltration and diafiltration operation is a drug substance comprising the protein. In one example, the composition obtained from the final ultrafiltration and diafiltration operation is more stable compared to a composition prepared by the same method without the pH adjusting step.

**[0010]** In one example, the ultrafiltration and diafiltration operation is carried out at a temperature of from about 25 °C to about 50 °C.

**[0011]** In one example, the protein is obtained from a purification process comprises one or

more steps selected from centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, ultrafiltration, diafiltration, SPTFF, depth filtration, and mixed-mode chromatography. In one example, the purification process comprises a virus filtration step and one or more steps selected from centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, SPTFF, and depth filtration, and the preparation is obtained from the virus filtration step.

**[0012]** In one example, the purification process comprises a cation exchange chromatography step, and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, anion exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, SPTFF, virus filtration, and depth filtration, and the preparation is obtained from the cation exchange chromatography step. In one example, the purification process comprises an anion exchange chromatography step, and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, hydrophobic interaction chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, SPTFF, virus filtration, and depth filtration, and the preparation is obtained from the anion exchange chromatography step. In one example, the purification process comprises hydrophobic interaction chromatography step, and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, mixed-mode chromatography, ultrafiltration, diafiltration, SPTFF, virus filtration, and depth filtration, and the preparation is obtained from the hydrophobic interaction chromatography step. In one example, the purification process comprises a mixed-mode chromatography step, and one or more of the following steps: centrifugation, microfiltration, TFF, virus inactivation, affinity chromatography, cation exchange chromatography, anion exchange chromatography, hydrophobic interaction chromatography, ultrafiltration, diafiltration, SPTFF, virus filtration, and depth filtration, and the preparation is obtained from the mixed-mode chromatography step.

**[0013]** In one example, the method disclosed herein further comprises adding a stabilizer to the preparation. In one example, the stabilizer is one or more selected from an amino acid, a sugar, a polyol, an anti-oxidant, a chelating agent, a lipid or a lipid derivative, a salt, a polymer, an inert protein, a surfactant, and a water-miscible co-solvent.

[0014] In one example, the protein that can be used in the method disclosed herein is a therapeutic protein. In one example, the protein is any one of the following proteins: etanercept, aflibercept, adalimumab, epoetin alfa, darbepoetin alfa, filgrastim, pegfilgrastim, bevacizumab, cetuximab, infliximab, rituximab, eculizumab, trastuzumab, evolocumab, denosumab, romosozumab, erenumab, blinatumomab, anti-CD33 and anti-CD3 BiTE, anti-EGFRvIII and anti-CD3 BiTE, anti-DLL3 and anti-CD3 BiTE, and anti-BCMA and anti-CD3 BiTE.

### **Brief Description of the Drawings**

[0015] Figure 1 shows the pH stability of the conditioned AEX intermediate pool at controlled room temperature (CRT) in the assay of Example 1.

[0016] Figure 2 shows the UF DF pool pH (A) and conductivity (B) stability at CRT in the assay of Example 1.

[0017] Figure 3 shows the pH (A) and conductivity (B) stability of etanercept formulated in SAS solution in the assay of Example 1.

[0018] Figure 4 shows the amount of HMW post UF DF operation in buffered and buffer-free formulations upon pH adjustment of the VF pool.

[0019] Figure 5 shows four manufacturing options (Options A-D) for pH adjusting and concentrating steps.

[0020] Figure 6 shows %HMW of the aflibercept compositions prepared using the four manufacturing options.

[0021] Figure 7 shows the % acidic peak of the adalimumab formulations at 4 °C without transport measured by CEX-HPLC in Example 7.

[0022] Figure 8 shows the % acidic peak of the adalimumab formulations at 4 °C with transport measured by CEX-HPLC in Example 7.

[0023] Figure 9 shows the % acidic peak of the adalimumab formulations at 25 °C without