METHODS OF REDUCING CONTAMINANTS IN PROTEIN PURIFICATION

BACKGROUND

[001] Production of recombinant Fc-containing proteins for therapeutic use typically involves expression of the proteins in mammalian cells and subsequent purification of these proteins from host cell contaminants. Host cell contaminants include host cell proteins (HCPs), such as proteases (e.g., cathepsin D). If these proteases are not removed, they can clip or fragment Fc-containing proteins, which reduces yield of intact protein.

[002] Accordingly, there is a need for improved methods of purifying Fc-containing proteins to remove HCPs, such as proteases (e.g., cathepsin D).

SUMMARY

[003] The present disclosure provides improved methods for purifying an Fc-containing protein (e.g., dulaglutide) from a mixture comprising the Fc-containing protein and a contaminant (e.g., an HCP). The methods generally comprise heating the mixture to produce a heat-treated mixture, and applying the heat-treated mixture to a depth filter to produce a filtrate comprising the Fc-containing protein (e.g., dulaglutide). The methods disclosed herein are particularly advantageous in that they reduce the level of HCPs, including cathepsin D, and significantly increase the amount of intact Fc-containing protein recovered during purification.

[004] In an aspect, provided herein is a method of purifying dulaglutide from a mixture of dulaglutide and a contaminant, the method comprising: (a) heating the mixture to produce a heat-treated mixture; and (b) applying the heat-treated mixture to a depth filter to produce a filtrate comprising dulaglutide.

[005] In an example, the mixture is heated to a temperature of 50°C to 55°C. In an example, the mixture is heated to a temperature of 51°C to 54°C.

[006] In an example, the mixture is heated for a period of time. In an example, the period of time is 120 to 180 minutes. In an example, the period of time is about 120 minutes.

[007] In an example, prior to step (a), dulaglutide was purified using affinity chromatography. In an example, prior to step (a), dulaglutide was purified using Protein A, Protein G, or Protein L affinity chromatography.

[008] In an example, the method further comprises purifying dulaglutide from the filtrate using ion exchange chromatography (IEX) to produce an IEX eluate. In an example, the IEX is anion exchange chromatography (AEX).

[009] In an example, the depth filter is a synthetic depth filter. In an example, the depth filter comprises silica filter aid and polyacrylic fiber.

[0010] In an example, the depth filter comprises a filter surface area of about 0.0023 m^2, about 0.0135 m^2, about 0.027 m^2, about 0.054 m^2, about 0.11 m^2, about 0.33 m^2, about 0.55 m^2, about 0.77 m^2, or about 1.1 m^2.

[0011] In an example, the depth filter is capable of product filtration at a flux of about 200 liters per meter squared per hour (LMH).

[0012] In an example, the depth filter is capable of flush filtration at a flux of about 300 LMH.

[0013] In an example, the heat-treated mixture is applied to the depth filter at a load of about 250 g/m^2 to about 2,000 g/m^2. In an example, the heat-treated mixture is applied to the depth filter at a load of about 500 g/m^2 to about 2,000 g/m^2.

[0014] In an example, the contaminant is one or more host cell proteins (HCPs). In an example, the one or more HCPs is selected from the group consisting of one or more of a serine protease, an aspartic protease, a cysteine protease, a metalloprotease, and an aminopeptidase. In an example, the aspartic protease is cathepsin D.

[0015] In an example, the filtrate comprises less than about 100 ng/mg HCPs. In an example, the filtrate comprises less than about 50 ng/mg HCPs, optionally less than about 10 ng/mg HCPs. In an example, the filtrate comprises less than 100 µU/mL cathepsin D activity.

[0016] In an example, the IEX eluate comprises less than 50 ng/mg HCPs, optionally less than 10 ng/mg HCPs. In an example, the IEX eluate comprises less than 100 µU/mL cathepsin D activity, optionally less than 50 µU/mL cathepsin D activity.

[0017] In an example, the concentration of the contaminant is measured by mass spectrometry or an enzyme-linked immunosorbent assay (ELISA).

[0018] In another aspect, provided herein is a composition produced by any of the methods described herein.

[0019] In another aspect, provided herein is dulaglutide produced by any of the methods described herein.

[0019a] In another aspect, provided herein is a composition comprising dulaglutide produced by any of the methods described herein.

Statements of the present disclosure are described below:

Statement 1. A method of purifying an Fc-containing protein from a mixture of the Fc-containing protein and a contaminant, the method comprising: (a) heating the mixture to 51°C to 54°C for a period of time to produce a heat-treated mixture; and (b) applying the heat-treated mixture to a depth filter to produce a filtrate comprising the Fc-containing protein.

Statement 2. The method of Statement 1, wherein the period of time is 120-180 minutes.

Statement 3. The method of Statement 1 or 2, wherein the period of time is about 120 minutes.

Statement 4. The method of any one of the preceding Statements, wherein prior to step (a), the Fc-containing protein was purified using Protein A, Protein G, or Protein L affinity chromatography.

Statement 5. The method of any one of the preceding Statements, further comprising purifying the Fc-containing protein from the filtrate using ion exchange chromatography (IEX) to produce an IEX eluate.

Statement 6. The method of Statement 5, wherein the IEX is anion exchange chromatography (AEX).

Statement 7. The method of any one of the preceding Statements, wherein the depth filter is a synthetic depth filter.

Statement 8. The method of any one of the preceding Statements, wherein the depth filter comprises silica filter aid and polyacrylic fiber.

Statement 9. The method of any one of the preceding Statements, wherein the depth filter comprises a filter surface area of about 0.0023 m^2, about 0.0135 m^2, about 0.027 m^2, about 0.054 m^2, about 0.11 m^2, about 0.33 m^2, about 0.55 m^2, about 0.77 m^2, or about 1.1 m^2.

Statement 10. The method of any one of the preceding Statements, wherein the depth filter is capable of product filtration at a flux of about 200 liters per meter squared per hour (LMH).

Statement 11. The method of any one of the preceding Statements, wherein the depth filter is capable of flush filtration at a flux of about 300 LMH.

Statement 12. The method of any one of the preceding Statements, wherein the heat-treated mixture is applied to the depth filter at a load of about 250 g/m^2 to about 2,000 g/m^2.

Statement 13. The method of any one of the preceding Statements, wherein the heat-treated mixture is applied to the depth filter at a load of about 500 g/m^2 to about 2,000 g/m^2.

Statement 14. The method of any one of the preceding Statements, wherein the contaminant is one or more host cell proteins (HCPs).

Statement 15. The method of Statement 14, wherein the one or more HCPs is selected from the group consisting of one or more of a serine protease, an aspartic protease, a cysteine protease, a metalloprotease, and an aminopeptidase.

Statement 16. The method of Statement 15, wherein the aspartic protease is cathepsin D.

Statement 17. The method of any one of the preceding Statements, wherein the filtrate comprises less than about 100 ng/mg HCPs.

Statement 18. The method of any one of the preceding Statements, wherein the filtrate comprises less than about 50 ng/mg HCPs, optionally less than about 10 ng/mg HCPs.

Statement 19. The method of any one of the preceding Statements, wherein the filtrate comprises less than 100 µU/mL cathepsin D activity.

Statement 20. The method of any one of Statements 5-19, wherein the IEX eluate comprises less than 50 ng/mg HCPs, optionally less than 10 ng/mg HCPs.

Statement 21. The method of any one of Statements 5-20, wherein the IEX eluate comprises less than 100 µU/mL cathepsin D activity, optionally less than 50 µU/mL cathepsin D activity.

Statement 22. The method of any one of Statements 17-21, wherein the concentration of the contaminant is measured by mass spectrometry or an enzyme-linked immunosorbent assay (ELISA).

Statement 23. The method of any one of the preceding Statements, wherein the Fc-containing protein is a glucagon-like peptide-1 (GLP-1) receptor agonist.

Statement 24. The method of Statement 23, wherein the GLP-1 receptor agonist comprises a GLP-1 analog comprising one, two, or three modifications compared to a wild type GLP-1 amino acid sequence (SEQ ID NO: 1).

Statement 25. The method of Statement 23 or 24, wherein the GLP-1 receptor agonist comprises a GLP-1 analog comprising the amino acid sequence of SEQ ID NO: 2.

Statement 26. The method of any one of Statements 23-25, wherein the GLP-1 receptor agonist comprises a peptide linker.

Statement 27. The method of Statement 26, wherein the peptide linker comprises 1 to 10 GGGGS units (SEQ ID NO: 3).

Statement 28. The method of any one of Statements 23-27, wherein the GLP-1 receptor agonist comprises: a) a GLP-1 analog comprising the amino acid sequence of SEQ ID NO: 2; b) a peptide linker comprising 1 to 10 GGGGS units (SEQ ID NO: 3); and c) a human Fc region.

Statement 29. The method of Statement 28, wherein the N-terminal glycine of the peptide linker is directly fused to the C-terminal residue of the GLP-1 analog, and the C-terminal serine of the peptide linker is directly fused to the N-terminal residue of the Fc region.

Statement 30. The method of any one of Statements 23-29, wherein the GLP-1 receptor agonist comprises the amino acid sequence of SEQ ID NO: 4.

Statement 31. The method of any one of Statements 23-30, wherein the Fc-containing protein comprises a homodimer of the amino acid sequence of SEQ ID NO: 4.

Statement 32. The method of Statement 23, wherein the Fc-containing protein is dulaglutide.

Statement 33. A composition produced by the method of any one of the preceding Statements.

Statement 34. Dulaglutide produced by the method of any one of the preceding Statements.

BRIEF DESCRIPTION OF DRAWINGS

[0020] **FIG. 1** is a flow diagram showing the various steps of three dulaglutide processing schemes as described in Example 2.

[0021] **FIG. 2** is a graph showing the HCP content in ppm as assessed by ELISA detected in samples from various points of the processing strands as described in FIG. 1. “HI” represents the HCP content detected in heat inactivated samples; “DF MS” represents the HCP content detected in depth filtrate samples; and “AEX MS” represents the HCP content detected in anion exchange chromatography eluate samples.

[0022] **FIG. 3** is a flow diagram showing the various steps of four dulaglutide processing schemes as described in Example 3.