CLAIMS

What is claimed is:

1. A method for producing a low acidic species composition comprising an antibody that comprises the heavy and light chains of adalimumab, or an antigen-binding portion thereof, the method comprising:

(a) contacting a first sample comprising the antibody, or antigen-binding portion thereof, with a chromatography media, wherein the first sample comprises more than 10% acidic species of the antibody,

wherein the acidic species of the antibody have a net negative charge relative to adalimumab main species,

wherein the acidic species of the antibody comprise species selected from the group consisting of charge variants, structure variants, fragmentation variants, and any combinations thereof,

wherein the acidic species of the antibody do not include process-related impurities selected from the group consisting of host cell proteins, host cell nucleic acids, chromatographic materials and media components, and

wherein the antibody, or antigen-binding portion thereof, binds to the chromatography media;

(b) displacing the antibody, or antigen-binding portion thereof, bound to the chromatography media with a displacing buffer comprising at least one displacer molecule; and

(c) collecting a second sample comprising the displaced antibody, or antigen-binding portion thereof, wherein the second sample comprises

less than 10% acidic species of antibody, or antigen-binding portion thereof,

thereby producing a low acidic species composition comprising an antibody that comprises the heavy and light chains of adalimumab, or an antigen-binding portion thereof.

2. The method of claim 1, wherein the pH of the displacing buffer is lower than the isoelectric point of the antibody, or antigen-binding portion thereof.

3. The method of claim 1, wherein the displacing buffer carries positive charge and wherein the concentration of the displacer in the displacing buffer is at least about 0.1 mM.

4. The method of claim 1, wherein the conductivity of the displacing buffer is about 2 mS/cm to about 20 mS/cm.

5. The method of claim 1, wherein one displacing buffer is used.

6. The method of claim 1, wherein a first displacing buffer and a second displacing buffer are used, and wherein the first and second displacing buffers comprise different concentrations of displacer.

7. The method of claim 1, wherein the method is run in a linear gradient displacement chromatography mode, a two-step displacement chromatography mode, or a multiple-step displacement chromatography mode.

8. The method of claim 1, wherein the second sample comprises a reduced level of host cell proteins as compared to the first sample.

9. The method of claim 1, wherein the second sample comprises a reduced level of one or more of charge variants, structure variants or fragmentation variants as compared to the first sample.

10. The method of claim 9, wherein the chromatography sample comprises a reduced level of basic species as compared to the first sample.

11. The method of claim 10, wherein the reduced level of basic species comprise a reduced level of a lysine species Lys 0 as compared to the first sample.

12. The method of claim 1, wherein the second sample comprises a reduced level of aggregates as compared to the first sample.

13. The method of claim 1, wherein the second sample comprises a reduced level of antibody fragments as compared to the first sample.

14. The method of claim, wherein the acidic species comprise a first acidic region (AR1) and a second acidic region (AR2).

15. The method of claim, wherein the composition comprises about 5% or less acidic species (AR).

16. The method of claim 1, wherein the composition comprises about 3% or less acidic species (AR).

17. The method of claim 14, wherein the composition comprises about 0.1% or less AR1 and about 3% or less AR2.

18. The method of claim 15, wherein the composition comprises about 1.4% or less acidic species (AR).

19. The method of claim 14, wherein the composition comprises about 1.4% AR2 and about 0.0% AR1.

20. The method of claim 1, wherein the antibody, or antigen-binding portion thereof, comprises a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 1 and a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2.

21. The method of claim 1, wherein the anti-TNFα antibody, or antigen-binding portion thereof, is adalimumab, or an antigen binding-portion thereof.

22. The method of claim 1, wherein the acidic species are product preparation-derived acidic species, cell-culture-derived acidic species or storage-derived acidic species.

23. Use of a composition as defined in claim 1 in the manufacture of a medicament for treating a subject having a disorder in which TNFα is detrimental, wherein the disorder in which TNFα is detrimental is rheumatoid arthritis (RA), psoriasis, psoriatic arthritis, ankylosing spondylitis, Crohn's Disease, Ulcerative Colitis, juvenile idiopathic arthritis, active axial spondyloarthritis or non-radiographic axial spondyloarthritis.

SUMMARY

The present disclosure is based on the optimization of displacement chromatography process technologies for protein production, *e.g.*, production of antibodies or antigen-binding portions thereof, resulting in the production of compositions comprising proteins that comprise low percentages of acidic species. These low acidic species compositions have improved therapeutic efficacy and improved biological properties, for example, increased cartilage tissue penetration, reduced cartilage destruction, reduced synovial proliferation, reduced bone erosion, increased protection against the development of arthritis as measured by arthritic scores and/or histopathology scores, reduced cell infiltration, reduced proteoglycan loss, reduced chondrocyte death, and/or increased TNFα affinity, as compared to a non-low acidic species composition.

Displacement chromatography is a chromatographic separation technology that involves the use of a displacer molecule to aid in the separation of a mixture, *e.g.*, an antibody-containing solution derived from cell culture harvest. The displacer molecule is conventionally selected to have a higher affinity for the stationary phase (*i.e.*, the chromatographic support) as compared to the components present in the material to be separated. Due to its higher affinity, the displacer molecule competes with protein mixture components for the binding sites on the stationary phase. Under appropriate conditions, the displacer induces the components of the mixture to develop into consecutive zones of concentrated and purified species in the order of decreasing binding affinity ahead of the displacer front. This ordered displacement of the components of the mixture results in the formation of a so-called "displacement train." In contrast to traditional elution mode chromatography, the displacement process takes advantage of the nonlinearity of the adsorption isotherm, allowing for higher column loading levels without compromising the purity and recovery of the component of interest. Finally, washing of the displacement train with the displacing buffer from the column allows for the component of interest to be isolated by collecting (and pooling if necessary) the proper fraction(s) of the displaced eluate.

In one aspect, there is provided a method for producing a low acidic species composition comprising an antibody that comprises the heavy and light chains of adalimumab, or an antigen-binding portion thereof, the method comprising:

(a) contacting a first sample comprising the antibody, or antigen-binding portion thereof, with a chromatography media, wherein the first sample comprises more than 10% acidic species of the antibody,

wherein the acidic species of the antibody have a net negative charge relative to adalimumab main species,

wherein the acidic species of the antibody comprise species selected from the group consisting of charge variants, structure variants, fragmentation variants, and any combinations thereof,

wherein the acidic species of the antibody do not include process-related impurities selected from the group consisting of host cell proteins, host cell nucleic acids, chromatographic materials and media components, and

wherein the antibody, or antigen-binding portion thereof, binds to the chromatography media;

(b) displacing the antibody, or antigen-binding portion thereof, bound to the chromatography media with a displacing buffer comprising at least one displacer molecule; and

(c) collecting a second sample comprising the displaced antibody, or antigen-binding portion thereof, wherein the second sample comprises

less than 10% acidic species of antibody, or antigen-binding portion thereof,

thereby producing a low acidic species composition comprising an antibody that comprises the heavy and light chains of adalimumab, or an antigen-binding portion thereof.

In one aspect, there is provided the use of a composition as disclosed herein in the manufacture of a medicament for treating a subject having a disorder in which TNFα is detrimental, wherein the disorder in which TNFα is detrimental is rheumatoid arthritis (RA), psoriasis, psoriatic arthritis, ankylosing spondylitis, Crohn's Disease, Ulcerative Colitis, juvenile idiopathic arthritis, active axial spondyloarthritis or non-radiographic axial spondyloarthritis.

Accordingly, in one aspect, the disclosure provides a method for producing a low acidic species composition comprising an antibody, or antigen-binding portion thereof, by contacting a first sample comprising the antibody, or antigen-binding portion thereof, with a chromatography media, wherein the antibody, or antigen-binding portion thereof, binds to the chromatography media; displacing the antibody, or antigen-binding portion thereof, bound to