METHODS OF PRODUCING FC-CONTAINING PROTEINS

BACKGROUND

[001] Production of recombinant Fc-containing proteins for therapeutic use typically involves expression of the proteins in cultured mammalian cells. Cell culture conditions can affect the yield and/or quality of Fc-containing proteins produced by the cultured cells. In particular, sub-optimal cell culture conditions can result in an increase in the amount of protease clipping of Fc-containing proteins, such as dulaglutide, produced, which can impact yield and/or product quality.

[002] Accordingly, there is a need for improved cell culture methods for producing recombinant Fc-containing proteins that maximize protein yield and quality.

SUMMARY

[003] The present disclosure provides improved methods for producing Fc-containing proteins. The methods generally involve culturing a mammalian cell that expresses the Fc-containing protein at a first pH setpoint for a first period of time followed by culturing the cell at a second pH setpoint that is higher than the first pH set point for a second period of time. The methods disclosed herein are particularly advantageous in that they can reduce protease clipping of Fc-containing proteins, such as dulaglutide.

[004] Provided herein is a method for producing dulaglutide, the method comprising the steps of a) culturing a mammalian cell that expresses the dulaglutide in a cell culture medium at a first pH setpoint for a first period of time; followed by b) culturing the mammalian cell in the cell culture medium at a second pH setpoint for a second period of time, wherein the second pH setpoint is higher than the first pH setpoint, such that the dulaglutide is produced by the mammalian cell.

[005] In an example, the first pH setpoint has a deadband of 0.01-0.10. In an example, the first pH setpoint has a deadband of 0.07-0.10. In an example, the first pH setpoint has a deadband of about 0.09.

[006] In an example, the second pH setpoint has a deadband of 0.01-0.10. In an example, the second pH setpoint has a deadband of 0.03-0.06. In an example, the second pH setpoint has a deadband of about 0.05.

[007] In an example, the deadband of the second pH setpoint is narrower than the deadband of the first pH setpoint. In an example, the first pH setpoint has a deadband of about 0.09 and the second pH setpoint has a deadband of about 0.05.

[008] In an example, the second pH setpoint is 0.01-1.0 pH units higher than the first pH setpoint. In an example, the first pH setpoint is 6.0-7.0. In an example, the first pH setpoint is 6.5-6.9. In an example, the first pH setpoint is 6.8-6.9. In an example, the first pH setpoint is about 6.86.

[009] In an example, the second pH setpoint is 6.5-7.5. In an example, the second pH setpoint is 6.9-7.5. In an example, the second pH setpoint is 7.0-7.1. In an example, the second pH setpoint is about 7.0.

[0010] In an example, the first pH setpoint is about 6.86 with a deadband of about 0.09 and the second pH setpoint is about 7.0 with a deadband of about 0.05.

[0011] In an example, the first period of time is longer than the second period of time. In an example, the first period of time is 7-12 days. In an example, the first period of time is 9-11 days. In an example, the first period of time is about 10 days.

[0012] In an example, the second period of time is at least 1 day. In an example, the second period of time is 4-7 days. In an example, the second period of time is 4-6 days. In an example, the second period of time is about 5 days.

[0012a] In an aspect, provided herein is a method for producing dulaglutide, the method comprising the steps of: a) culturing a mammalian cell that expresses the dulaglutide in a cell culture medium at a first pH setpoint for a first period of time; followed by b) culturing the mammalian cell in the cell culture medium at a second pH setpoint for a second period of time, wherein the second pH setpoint is higher than the first pH setpoint, such that the dulaglutide is produced by the mammalian cell, wherein: the first pH set point is 6.0-7.0 and has a deadband of 0.07-0.10, the first period of time is 7-12 days, the second pH set point is 6.5-7.5 and has a deadband of 0.03-0.06, and the second period of time is 4-7 days.

[0013] In an example, the first period of time is about 10 days and the second period of time is about 5 days.

[0014] In an example, the first pH setpoint is about 6.86 with a deadband of about 0.09 and the first period of time is 9-10 days; and the second pH setpoint is about 7.0 with a deadband of about 0.05 and the second period of time is 4-5 days.

[0014a] In another aspect, provided herein is a method for producing dulaglutide, the method comprising the steps of: a) culturing a mammalian cell that expresses the dulaglutide in a cell culture medium at a pH setpoint of 6.86 with a deadband of 0.09 for a first period of time comprising 9-10 days; followed by b) culturing the mammalian cell in the cell culture medium at a pH setpoint of 7.0 with a deadband of 0.05 for a second period of time comprising 4-5 days, such that the dulaglutide is produced by the mammalian cell.

[0015] In an example, the method further comprises culturing the mammalian cell at a first temperature for 1-5 days followed by culturing the mammalian cell at a second temperature for 9-14 days. In an example, the second temperature is lower than the first temperature.

[0016] In an example, the first temperature is 34°C- 40°C. In an example, the first temperature is about 36°C. In an example, the second temperature is 30°C-36°C. In an example, the second temperature is about 33°C.

[0017] In an example, the mammalian cell is cultured at the first temperature for 3-4 days. In an example, the mammalian cell is cultured at the first temperature for about 3 days. In an example, the mammalian cell is cultured at the second temperature for 11-12 days. In an example, the mammalian cell is cultured at the second temperature for about 11 days.

[0018] In an example, the mammalian cell is cultured in a bioreactor. In an example, the mammalian cell is cultured in fed batch mode.

[0019] In an example, the method further comprises adding a base source to the cell culture medium if the pH goes below the deadband or adding an acid source to the cell culture medium if the pH goes above the deadband during the first period of time. In an example, the method further comprises adding a base source to the cell culture medium if the pH goes below the deadband or adding an acid source to the cell culture medium if the pH goes above the deadband during the second period of time. In an example, the acid source is CO2. In an example, the base source is a solution comprising NaOH.

[0020] In an example, the mammalian cell culture medium is sparged with air during the first period of time. In an example, the mammalian cell culture medium is sparged with air during the second period of time. In an example, the mammalian cell culture medium is sparged with air during the first and second period of time.

[0021] In an example, the mammalian cell is selected from the group consisting of a COS cell, a CHO cell, a BHK cell, an MDCK cell, a HEK293 cell, a HEK293T cell, a HeLa cell, an NS0 cell, a PER.C6 cell, a VERO cell, a CRL7O3O cell, an HsS78Bst cell, an NIH 3T3 cell, a HepG2 cell, an SP210 cell, an R1.1 cell, a B-W cell, an L-M cell, a BSC1 cell, a BSC40 cell, a YB/20 cell, and a BMT10 cell. In an example, the mammalian cell is a CHO cell.

[0022] In an example, one or more protease has reduced activity within the second pH deadband compared to the first pH deadband. In an example, the one or more protease is in the cell or the cell culture medium. In an example, one or more protease has reduced activity at the second pH setpoint compared to the first pH setpoint. In an example, the one or more

protease clips dulaglutide at the N-terminus. In an example, the protease is cathepsin D. In an example, less than about 4% of the dulaglutide produced is N-terminally clipped.

[0022a] In another aspect, provided herein is a method for producing dulaglutide in a CHO cell, the method comprising the steps of: a) culturing the CHO cell in a cell culture medium maintained at a first pH setpoint of 6.68 having a deadband of 0.09 for about 10 days; followed by b) culturing the CHO cell in the cell culture medium maintained at a second pH setpoint of 7.0 having a deadband of 0.05 for about 5 days; and wherein the culturing is initiated at a temperature of about 36°C and then decreased to about 33°C after about 3 days; such that the dulaglutide is produced by the CHO cell.

[0023] Also provided herein is dulaglutide produced by a method disclosed herein.

[0024] Items of the present disclosure are described below:

1. A method for producing an Fc-containing protein, the method comprising the steps of:
a) culturing a mammalian cell that expresses the Fc-containing protein in a cell culture medium at a first pH setpoint for a first period of time; followed by
b) culturing the mammalian cell in the cell culture medium at a second pH setpoint for a second period of time, wherein the second pH setpoint is higher than the first pH setpoint,
such that the Fc-containing protein is produced by the mammalian cell.

2. The method of item 1, wherein the first pH setpoint has a deadband of 0.01-0.10.

3. The method of item 1 or 2, wherein the first pH setpoint has a deadband of 0.07-0.10.

4. The method of any one of the preceding items, wherein the first pH setpoint has a deadband of about 0.09.

5. The method of any one of the preceding items, wherein the second pH setpoint has a deadband of 0.01-0.10.

6. The method of any one of the preceding items, wherein the second pH setpoint has a deadband of 0.03-0.06.

7. The method of any one of the preceding items, wherein the second pH setpoint has a deadband of about 0.05.

8. The method of any one of the preceding items, wherein the deadband of the second pH setpoint is narrower than the deadband of the first pH setpoint.

9. The method of any one of the preceding items, wherein the first pH setpoint has a deadband of about 0.09 and the second pH setpoint has a deadband of about 0.05.

10. The method of any one of the preceding items, wherein the second pH setpoint is 0.01-1.0 pH units higher than the first pH setpoint.

11. The method of any one of the preceding items, wherein the first pH setpoint is 6.0-7.0.

12. The method of any one of the preceding items, wherein the first pH setpoint is 6.5-6.9.

13. The method of any one of the preceding items, wherein the first pH setpoint is 6.8-6.9.

14. The method of any one of the preceding items, wherein the first pH setpoint is about 6.86.

15. The method of any one of the preceding items, wherein the second pH setpoint is 6.5-7.5.

16. The method of any one of the preceding items, wherein the second pH setpoint is 6.9-7.5.

17. The method of any one of the preceding items, wherein the second pH setpoint is 7.0-7.1.

18. The method of any one of the preceding items, wherein the second pH setpoint is about 7.0.

19. The method of any one of the preceding items, wherein the first pH setpoint is about 6.86 with a deadband of about 0.09 and the second pH setpoint is about 7.0 with a deadband of about 0.05.

20. The method of any one of the preceding items, wherein the first period of time is longer than the second period of time.

21. The method of any one of the preceding items, wherein the first period of time is 7-12 days.

22. The method of any one of the preceding items, wherein the first period of time is 9-11 days.

23. The method of any one of the preceding items, wherein the first period of time is about 10 days.

24. The method of any one of the preceding items, wherein the second period of time is at least 1 day.

25. The method of any one of the preceding items, wherein the second period of time is 4-7 days.

26. The method of any one of the preceding items, wherein the second period of time is 4-6 days.

27. The method of any one of the preceding items, wherein the second period of time is about 5 days.

28. The method of any one of the preceding items, wherein the first period of time is about 10 days and the second period of time is about 5 days.

29. The method of any one of the preceding items, wherein:

a) the first pH setpoint is about 6.86 with a deadband of about 0.09 and the first period of time is 9-10 days; and

b) the second pH setpoint is about 7.0 with a deadband of about 0.05 and the second period of time is 4-5 days.

30. The method of any one of items 1-22, further comprising culturing the mammalian cell at a first temperature for the first 1-5 days of the first period of time followed by culturing the mammalian cell at a second temperature for 9-14 days.

31. The method of item 30, wherein the second temperature is lower than the first temperature.

32. The method of item 30 or 31, wherein the first temperature is 34°C- 40°C.

33. The method of any one of items 30-32, wherein the first temperature is about 36°C.

34. The method of any one of items 30-33, wherein the second temperature is 30°C-36°C.

35. The method of any one of items 30-34, wherein the second temperature is about 33°C.

36. The method of any one of items 30-35, wherein the mammalian cell is cultured at the first temperature for 3-4 days.

37. The method of any one of items 30-36, wherein the mammalian cell is cultured at the first temperature for about 3 days.

38. The method of any one of items 30-37, wherein the mammalian cell is cultured at the second temperature for 11-12 days.

39. The method of any one of items 30-38, wherein the mammalian cell is cultured at the second temperature for about 11 days.

40. The method of any one of the preceding items, wherein the mammalian cell is cultured in a bioreactor.

41. The method of item 40, wherein the mammalian cell is cultured in fed batch mode.

42. The method of any one of the preceding items, wherein the mammalian cell is selected from the group consisting of a COS cell, a CHO cell, a BHK cell, an MDCK cell, a HEK293 cell, a HEK293T cell, a HeLa cell, an NS0 cell, a PER.C6 cell, a VERO cell, a CRL7O3O cell, an HsS78Bst cell, an NIH 3T3 cell, a HepG2 cell, an SP210 cell, an R1.1 cell, a B-W cell, an L-M cell, a BSC1 cell, a BSC40 cell, a YB/20 cell, and a BMT10 cell.

43. The method of any one of the preceding items, wherein the mammalian cell is a CHO cell.

44. The method of any one of the preceding items, further comprising adding a base source to the cell culture medium if the pH goes below the deadband or adding an acid source to the cell culture medium if the pH goes above the deadband during the first period of time.

45. The method of any one of the preceding items, further comprising adding a base source to the cell culture medium if the pH goes below the deadband or adding an acid source to the cell culture medium if the pH goes above the deadband during the second period of time.

46. The method of item 44 or 45, wherein the acid source is CO_2.

47. The method of item 44 or 45, wherein the base source is a solution comprising NaOH.

48. The method of any one of the preceding items, wherein the cell culture medium is sparged with air during the first and/or second period of time.

49. The method of any one of the preceding items, wherein one or more protease has reduced activity within the second pH deadband compared to the first pH deadband.

50. The method of any one of the preceding items, wherein one or more protease has reduced activity at the second pH setpoint compared to the first pH setpoint.

51. The method of item 49 or 50, wherein the one or more protease clips the Fc-containing protein at the N-terminus, optionally wherein the protease is cathepsin D.

52. The method of any one of the preceding items, wherein the mammalian cell culture comprises less than about 4% of a clipped form of the Fc-containing protein at the end of the second period of time.

53. The method of any one of the preceding items, wherein the Fc-containing protein comprises a glucagon-like peptide 1 (GLP-1) analog comprising one or more modifications compared to a wild type GLP-1 amino acid sequence (SEQ ID NO: 1).

54. The method of any one of the preceding items, wherein the Fc-containing protein comprises a GLP-1 analog comprising the amino acid sequence of SEQ ID NO: 2.

55. The method of any one of the preceding items, wherein the Fc-containing protein comprises a peptide linker.

56. The method of item 55, wherein the peptide linker comprises 1-10 G_4S units (SEQ ID NO: 3).

57. The method of item 55 or 56, wherein the peptide linker comprises the amino acid sequence set forth in SEQ ID NO: 3.

58. The method of any one of the preceding items, wherein the Fc-containing protein comprises:

a) a GLP-1 analog comprising the amino acid sequence of SEQ ID NO: 2;

b) a peptide linker comprising the amino acid sequence of SEQ ID NO: 3; and

c) an Fc portion of an immunoglobulin.

59. The method of item 58, wherein the N-terminal residue of the peptide linker is directly fused to the C-terminal residue of the GLP-1 analog, and the C-terminal residue of the peptide linker is directly fused to the N-terminal residue of the Fc portion.

60. The method of any one of the preceding items, wherein the Fc-containing protein comprises the amino acid sequence of SEQ ID NO: 4.

61. The method of any one of the preceding items, wherein the wherein the Fc-containing protein is a homodimer comprising two identical amino acid chains each comprising the amino acid sequence of SEQ ID NO: 4.

62. The method of any one of the preceding items, wherein the Fc-containing protein is dulaglutide.

63. A method for producing dulaglutide in a CHO cell, the method comprising the steps of:

a) culturing the CHO cell in a cell culture medium maintained at a first pH setpoint of about 6.68 having a deadband of about 0.09 for about 10 days; followed by

b) culturing the CHO cell in the cell culture medium maintained at a second pH setpoint of about 7.0 having a deadband of about 0.05 for about 5 days; and

wherein the culturing is initiated at a temperature of about 36°C and then decreased to about 33°C after about 3 days;

such that the dulaglutide is produced by the CHO cell.

64. The method of item 63 wherein the CHO cell is cultured in a bioreactor in fed batch mode.

65. The method of either of items 63-64 wherein NaOH is added to the cell culture medium if the pH goes below the deadband of either the first or second pH setpoint, and CO_2 is added to the cell culture medium if the pH goes above the deadband of either the first or second pH setpoint.

66. The method of any one of items 63-65 wherein the cell culture medium is sparged with air one or more times.

67. The method of any one of items 63-67 wherein less than about 4% of the dulaglutide produced by the CHO cell is N-terminally clipped.

64. An Fc-containing protein produced by the method of any one of the preceding items.

BRIEF DESCRIPTION OF DRAWINGS

[0025] **FIG. 1** is a graph showing pCO_2 levels in the production bioreactor over a 14 day culture for two different runs at pH 6.80 ± 0.03 or two different runs at pH 6.86 ± 0.09.

[0026] **FIG. 2** is a plot showing day 14 pH levels and des H/HG levels in 3L and 5L production bioreactors.

DETAILED DESCRIPTION

[0027] The present disclosure provides improved methods for producing Fc-containing proteins. The methods generally involve culturing a mammalian cell that expresses the Fc-containing protein at a first pH setpoint for a first period of time followed by culturing the cell at a second pH setpoint that is higher than the first pH set point for a second period of time. The

CLAIMS

1. A method for producing dulaglutide, the method comprising the steps of:

a) culturing a mammalian cell that expresses the dulaglutide in a cell culture medium at a first pH setpoint for a first period of time; followed by

b) culturing the mammalian cell in the cell culture medium at a second pH setpoint for a second period of time, wherein the second pH setpoint is higher than the first pH setpoint,

such that the dulaglutide is produced by the mammalian cell,

wherein:

the first pH set point is 6.0-7.0 and has a deadband of 0.07-0.10,

the first period of time is 7-12 days,

the second pH set point is 6.5-7.5 and has a deadband of 0.03-0.06, and

the second period of time is 4-7 days.

2. The method of claim 1, wherein the first pH setpoint has a deadband of 0.09.

3. The method of claim 1, wherein the second pH setpoint has a deadband of 0.05.

4. The method of claim 1, wherein the first pH setpoint has a deadband of 0.09 and the second pH setpoint has a deadband of 0.05.

5. The method of claim 1, wherein the first pH setpoint is 6.5-6.9.

6. The method of claim 1, wherein the first pH setpoint is 6.8-6.9.

7. The method of claim 1, wherein the first pH setpoint is 6.86.

8. The method of claim 1, wherein the second pH setpoint is 6.9-7.5.

9. The method of claim 1, wherein the second pH setpoint is 7.0-7.1.

10. The method of claim 1, wherein the second pH setpoint is 7.0.

11. The method of claim 1, wherein the first pH setpoint is 6.86 with a deadband of 0.09 and the second pH setpoint is 7.0 with a deadband of 0.05.

12. The method of claim 1, wherein the first period of time is 9-11 days.

13. The method of claim 1, wherein the first period of time is about 10 days.

14. The method of claim 1, wherein the second period of time is 4-6 days.

15. The method of claim 1, wherein the second period of time is about 5 days.

16. The method of claim 1, wherein the first period of time is about 10 days and the second period of time is about 5 days.

17. A method for producing dulaglutide, the method comprising the steps of:

a) culturing a mammalian cell that expresses the dulaglutide in a cell culture medium at a pH setpoint of 6.86 with a deadband of 0.09 for a first period of time comprising 9-10 days; followed by

b) culturing the mammalian cell in the cell culture medium at a pH setpoint of 7.0 with a deadband of 0.05 for a second period of time comprising 4-5 days,

such that the dulaglutide is produced by the mammalian cell.

18. The method of any one of claims 1-17, further comprising culturing the mammalian cell at a first temperature for the first 1-5 days of the first period of time followed by culturing the mammalian cell at a second temperature for 9-14 days.

19. The method of claim 18, wherein the second temperature is lower than the first temperature.

20. The method of claim 19, wherein the first temperature is 34°C- 40°C.

21. The method of claim 19, wherein the first temperature is about 36°C.

22. The method of claim 19, wherein the second temperature is 30°C-36°C.

23. The method of claim 19, wherein the second temperature is about 33°C.

24. The method of claim 19, wherein the mammalian cell is cultured at the first temperature for 3-4 days.

25. The method of claim 19, wherein the mammalian cell is cultured at the first temperature for about 3 days.

26. The method of claim 19, wherein the mammalian cell is cultured at the second temperature for 11-12 days.

27. The method of claim 19, wherein the mammalian cell is cultured at the second temperature for about 11 days.

28. The method of any one of claims 1-17, wherein the mammalian cell is cultured in a bioreactor.

29. The method of claim 28, wherein the mammalian cell is cultured in fed batch mode.

30. The method of any one of claims 1-17, further comprising adding a base source to the cell culture medium if the pH goes below the deadband or adding an acid source to the cell culture medium if the pH goes above the deadband during the first period of time.

31. The method of claim 30, wherein the acid source is CO_2.

32. The method of claim 30, wherein the base source is a solution comprising NaOH.

33. The method of any one of claims 1-17, wherein the cell culture medium is sparged with air during the first and/or second period of time.

34. The method of any one of claims 1-17, wherein the mammalian cell is selected from the group consisting of a COS cell, a CHO cell, a BHK cell, an MDCK cell, a HEK293 cell, a HEK293T cell, a HeLa cell, an NS0 cell, a PER.C6 cell, a VERO cell, a CRL7O3O cell, an HsS78Bst cell, an NIH 3T3 cell, a HepG2 cell, an SP210 cell, an R1.1 cell, a B-W cell, an L-M cell, a BSC1 cell, a BSC40 cell, a YB/20 cell, and a BMT10 cell.

35. The method of any one of claims 1-17, wherein the mammalian cell is a CHO cell.

36. The method of any one of claims 1-17, wherein one or more protease has reduced activity within the second pH deadband compared to the first pH deadband.

37. The method of any one of claims 1-17, wherein one or more protease has reduced activity at the second pH setpoint compared to the first pH setpoint.

38. The method of claim 37, wherein the protease is cathepsin D.

39. The method of any one of claims 1-17, wherein less than about 4% of the dulaglutide produced is N-terminally clipped.

40. The method of claim 39, wherein the clipped form of dulaglutide does not have the HG residues at the N-terminus of dulaglutide.

41. A method for producing dulaglutide in a CHO cell, the method comprising the steps of:

a) culturing the CHO cell in a cell culture medium maintained at a first pH setpoint of 6.68 having a deadband of 0.09 for about 10 days; followed by
b) culturing the CHO cell in the cell culture medium maintained at a second pH setpoint of 7.0 having a deadband of 0.05 for about 5 days; and

wherein the culturing is initiated at a temperature of about 36°C and then decreased to about 33°C after about 3 days;

such that the dulaglutide is produced by the CHO cell.

42. The method of claim 41, wherein the CHO cell is cultured in a bioreactor in fed batch mode.

43. The method of claim 41, wherein NaOH is added to the cell culture medium if the pH goes below the deadband of either the first or second pH setpoint, and CO_2 is added to the cell culture medium if the pH goes above the deadband of either the first or second pH setpoint.

44. The method of claim 41, wherein the cell culture medium is sparged with air one or more times.

45. The method of claim 41, wherein less than about 4% of the dulaglutide produced by the CHO cell is N-terminally clipped.