CLAIMS

1. A method of producing a viral vaccine formulated in a sub-virion form, the method comprising the steps:

    a. purifying viral particles from harvested cell culture;

    b. inactivating and splitting the viral particles to produce sub-virions; and

    c. purifying the sub-virions;

    wherein inactivating and splitting the viral particles of step b comprises treating the viral particles with a reagent comprising a non-ionic surfactant and an ionic surfactant, and wherein the at non-ionic surfactant and the ionic surfactant is present in an amount effective to reduce particle size of the viral particles.

2. The method of claim 1, wherein the reagent further comprises a salt, wherein the salt is present in an amount effective to reduce particle size of the viral particles.

3. The method of any one of claims 1-2, wherein the average hydrodynamic radius of the sub-virion ranges from 150 nm to 350 nm.

4. The method of any one of claim 1-3, wherein the non-ionic surfactant is polysorbate 80.

5. The method of any one of claims 1-4, wherein the non-ionic surfactant comprises at least about 1.0 g/L polysorbate 80, e.g., at least about 1.5 g/L, at least about 2.0 g/L, or at least about 2.5 g/L.

6. The method of any one of claims 1-5, wherein the ionic surfactant comprises cetrimonium bromide (CTAB).

7. The method of any one of claims 1-6, wherein the ionic surfactant comprises about 1.25 g/L -3.0 g/L CTAB, e.g., about 1.5 g/L, about 2.0 g/L, about 2.5 g/L, or about 3.0 g/L.

8. The method of any one of claims 1-7, wherein the salt comprises sodium chloride (NaCl).

9. The method of any one of claims 1-8, wherein the salt comprises 0 - 200 mM NaCl, e.g., about 25 mM, about 50 mM, about 75 mM, about 100 mM, about 125 mM, about 150 mM, or about 175 mM.

10. The method of any one of claims 1-9, wherein the non-ionic surfactant comprises polysorbate 80, the ionic surfactant comprises CTAB, and the salt comprises sodium chloride NaCl.

11. The method of any one of claims 1-10, wherein purifying in step c comprises adsorption filtration.

12. The method of claim 11, wherein adsorption filtration throughput is at least about 50 L/m^2, e.g., at least about 100 L/m^2, at least about 150 L/m^2, at least about 200 L/m^2, at least about 200 L/m^2, at least about 300 L/m^2, or at least about 350 L/m^2.

13. The method of any one of claims 1-12, wherein the particle size of the sub-virion is less than about 500 nm, e.g., less than about 400 nm, less than about 300 nm, less than about 200 nm, or less than about 150 nm.

14. The method of claim 1-13, wherein the particle size of the sub-virion is the hydrodynamic radius.

15. The method of any one of claims 1-14, wherein the size of the sub-virion is measured by dynamic light scattering (DLS).

16. The method of any one of claims 1-15, wherein the viral particles are from the influenza virus.

17. The method of any one of claims 1-16, wherein the viral particles are from the influenza virus A strain.

18. The method of any one of claims 4-17, wherein the concentration of polysorbate 80 is 0.3 g/L prior to splitting.

19. The method of any one of claims 4-18, wherein the concentration of polysorbate 80 increases at a range of 0 - 2.2 g/L during splitting.

20. The method of any one of claims 4-19, wherein the concentration of polysorbate 80 is 0.3 g/L, 1.4 g/L, or 2.5 g/L during splitting.

21. The method of any one of claims 8-20, wherein a concentration of NaCl ranging from 0 - 25 mM is associated with increased filtration throughput.

22. A method of reducing viral particle size, the method comprising treating the viral particles with a reagent comprising a non-ionic surfactant and an ionic surfactant, and wherein the non-ionic surfactant and the ionic surfactant are present in an amount effective to reduce particle size of the viral particles.

23. The method of claim 22 wherein the reagent further comprises a salt, wherein the salt is present in an amount effective to reduce particle size of the viral particles.

24. The method of any one of claims 22-23, wherein the non-ionic surfactant comprises polysorbate 80.

25. The method of any one of claims 22-24, wherein the non-ionic surfactant comprises at least about 1.0 g/L polysorbate 80, e.g., at least about 1.5 g/L, at least about 2.0 g/L, or at least about 2.5 g/L.

26. The method of any one of claims 22-25, wherein the ionic surfactant comprises cetrimonium bromide (CTAB).

27. The method of any one of claims 22-26, wherein the ionic surfactant comprises about 1.25 g/L -3.0 g/L CTAB, e.g., about 1.5 g/L, about 2.0 g/L, about 2.5 g/L, or about 3.0 g/L.

28. The method of any one of claims 22-27, wherein the salt comprises sodium chloride (NaCl).

29. The method of any one of claims 22-28, wherein the salt comprises 25 - 200 mM NaCl, e.g., about 50 mM, about 75 mM, about 100 mM, about 125 mM, about 150 mM, or about 175 mM.

30. The method of any one of claims 22-29, wherein the non-ionic surfactant comprises polysorbate 80, the ionic surfactant comprises CTAB, and the salt comprises sodium chloride NaCl.

31. The method of any one of claims 22-30, further comprising filtering the treated viral particles through an adsorption filter.

32. The method of claim 31, wherein the throughput of the adsorption filter is at least about 50 L/m^2, e.g., at least about 100 L/m^2, at least about 150 L/m^2, at least about 200 L/m^2, at least about 250 L/m^2, at least about 300 L/m^2, or at least about 350 L/m^2.

33. The method of any one of claims 22-32, wherein the viral particle size is less than about 500 nm, e.g., less than about 400 nm, less than about 300 nm, less than about 200 nm, or less than about 150 nm.

34. The method of claim 22-33, wherein the viral particle size is the hydrodynamic radius of the viral particle.

35. The method of any one of claims 22-34, wherein the viral particle size is measured by dynamic light scattering (DLS).

36. The method of any one of claims 22-35, wherein the viral particles are from the influenza virus.

37. The method of any one of claims 22-36, wherein the viral particles are from the influenza virus A strain.

38. The method of any one of claims 24-37, wherein the concentration of polysorbate 80 is 0.3 g/L prior to splitting.

39. The method of any one of claims 24-38, wherein the concentration of polysorbate 80 increases at a range of 0 - 2.2 g/L during splitting.

40. The method of any one of claims 24-39, wherein the concentration of polysorbate 80 is 0.3 g/L, 1.4 g/L, or 2.5 g/L during splitting.

41. The method of any one of claims 28-40, wherein a concentration of NaCl ranging from 0 - 25 mM is associated with increased filtration throughput.

42. A method of reducing viral particle size in an influenza virus purification process, the method comprising

a. purifying viral particles harvested from cell culture;

b. inactivating and splitting the viral particles to produce sub-virions; and

c. purifying the sub-virions;

wherein activating and splitting the viral particles of step b comprises treating the viral particles with a reagent comprising a non-ionic surfactant and an ionic surfactant, and wherein the non-ionic surfactant and the ionic surfactant are present in an amount effective to reduce particle size of the viral particles.

43. A method of producing a viral vaccine formulated in a sub-virion form, the method comprising the steps:

a. purifying viral particles from harvested cell culture;

b. inactivating and splitting the viral particles to produce sub-virions; and

c. purifying the sub-virions;

wherein activating and splitting the viral particles of step b comprises treating the viral particles with a reagent comprising a non-ionic surfactant and an ionic

surfactant, and wherein the non-ionic surfactant and the ionic surfactant are present in an amount effective to reduce particle size of the viral particles.

44. A method of producing an influenza viral vaccine formulated in a sub-virion form, the method comprising the steps:

    a. purifying viral particles from a harvested cell culture of MDCK cells;

    b. inactivating and splitting the viral particles to produce sub-virions; and

    c. purifying the sub-virions;

    wherein inactivating and splitting the viral particles of step b comprises treating the viral particles with a reagent comprising a non-ionic surfactant and an ionic surfactant, and wherein the non-ionic surfactant and the ionic surfactant are present in an amount effective to reduce particle size of the viral particles.

45. The method of any one of claims 42-44 wherein the reagent further comprises a salt, wherein the salt is present in an amount effective to reduce particle size of the viral particles.

46. The method of any one of claims 42-45, wherein the non-ionic surfactant comprises polysorbate 80.

47. The method of any one of claims 42-46, wherein the non-ionic surfactant comprises at least about 1.0 g/L polysorbate 80, e.g., at least about 1.5 g/L, at least about 2.0 g/L, or at least about 2.5 g/L.

48. The method of any one of claims 42-47, wherein the salt comprises sodium chloride (NaCl).

49. The method of any one of claims 42-48, wherein the salt comprises 25 - 200 mM NaCl, e.g., about 50 mM, about 75 mM, about 100 mM, about 125 mM, about 150 mM, or about 175 mM.

50. The method of any one of claims 46-49, wherein the concentration of polysorbate 80 is 0.3 g/L prior to splitting.

51. The method of any one of claims 46-50, wherein the concentration of polysorbate 80 increases at a range of 0 - 2.2 g/L during splitting.

52. The method of any one of claims 46-51, wherein the concentration of polysorbate 80 is 0.3 g/L, 1.4 g/L, or 2.5 g/L during splitting.

53. The method of any one of claims 48-52, wherein a concentration of NaCl ranging from 0 - 25 mM is associated with increased filtration throughput.

54. A method of producing an influenza viral vaccine formulated comprising purified viral proteins, the method comprising the steps:

a. purifying viral particles from a harvested cell culture of MDCK cells;

b. inactivating and splitting the viral particles to produce sub-virions; and

c. purifying the sub-virions to produce purified viral proteins;

wherein inactivating and splitting the viral particles of step b comprises treating the viral particles with a reagent comprising a non-ionic surfactant and a salt, and wherein the non-ionic surfactant and the salt are in an amount effective to reduce particle size of the viral particles.

55. A method of producing an influenza viral vaccine formulated comprising purified viral proteins, the method comprising the steps:

a. purifying viral particles from a harvested cell culture of MDCK cells;

b. inactivating and splitting the viral particles to produce sub-virions; and

c. purifying the sub-virions to produce purified influenza viral proteins;

wherein inactivating and splitting the viral particles of step b comprises treating the viral particles with a reagent comprising an ionic surfactant and a salt, and

~~wherein the ionic surfactant and the salt are in an amount effective to reduce particle size of the viral particles.~~

~~56. A method of producing an influenza viral vaccine formulated comprising purified viral proteins, the method comprising the steps:~~

~~a. purifying viral particles from a harvested cell culture of MDCK cells;~~

~~b. treating the purified viral particles with alkylating agent to inactivate the viral particles;~~

~~c. treating the inactivated viral particle with an ionic surfactant to split the inactivated viral particles to produce subvirions; and~~

~~d. purifying the sub-virions to produce purified influenza viral proteins;~~

~~wherein inactivating and splitting the viral particles of step b and/or c further comprises treating the viral particles with a reagent comprising a non-ionic surfactant and an ionic surfactant, and wherein the non-ionic surfactant and the ionic surfactant are in an amount effective to reduce particle size of the viral particles.~~

~~57. A method of producing an influenza viral vaccine formulated in a sub-virion form, the method comprising the steps:~~

~~a. purifying viral particles from a harvested cell culture of MDCK cells;~~

~~b. inactivating and splitting the viral particles to produce sub-virions; and~~

~~c. purifying the sub-virions;~~

~~wherein inactivating and splitting the viral particles of step b comprises treating the viral particles with a reagent comprising CTAB, NaCl, and polysorbate 80, in an amount effective to reduce particle size of the viral particles.~~

58. The method of claim 57, wherein the concentration of polysorbate 80 is 0.3 g/L prior to splitting.

59. The method of any one of claims 57-58, wherein the concentration of polysorbate 80 increases at a range of 0 - 2.2 g/L during splitting.

60. The method of any one of claims 57-59, wherein the concentration of polysorbate 80 is 0.3 g/L, 1.4 g/L, or 2.5 g/L during splitting.

61. The method of any one of claims 57-60, wherein a concentration of NaCl ranging from 0 - 25 mM is associated with increased filtration throughput.

62. A method of producing an influenza viral vaccine formulated in a sub-virion form, the method comprising the steps:

a. purifying viral particles from a harvested cell culture of MDCK cells;

b. inactivating and splitting the viral particles to produce sub-virions; and

c. purifying the sub-virions;

wherein inactivating and splitting the viral particles of step b comprises treating the viral particles with a reagent comprising 1.5 - 2.5 g/L CTAB, 0-150 mM NaCl, and 0 - 2.2 g/L polysorbate 80, in an amount effective to reduce particle size of the viral particles.

63. A method of producing an influenza viral vaccine formulated in a sub-virion form, the method comprising the steps:

a. purifying viral particles from a harvested cell culture of MDCK cells;

b. inactivating and splitting the viral particles to produce sub-virions; and

c. purifying the sub-virions;

wherein inactivating and splitting the viral particles of step b comprises treating the viral particles with a reagent comprising 1.5 - 2.5 g/L of an ionic surfactant, 0-

~~150 mM of a salt, and 0 - 2.2 g/L of a non-ionic surfactant, in an amount effective to reduce particle size of the viral particles.~~

~~64. The method of claim 63, wherein the ionic surfactant is CTAB.~~

~~65. The method of any one of claims 63-64, wherein the salt is NaCl.~~

~~66. The method of any one of claims 63-65, wherein the non-ionic surfactant is polysorbate 80.~~

~~67. The method of claim 66, wherein the concentration of polysorbate 80 is 0.3 g/L prior to splitting.~~

~~68. The method of any one of claims 66-67, wherein the concentration of polysorbate 80 increases at a range of 0 - 2.2 g/L during splitting.~~

~~69. The method of any one of claims 66-68, wherein the concentration of polysorbate 80 is 0.3 g/L, 1.4 g/L, or 2.5 g/L during splitting.~~

~~70. The method of any one of claims 65-69, wherein a concentration of NaCl ranging from 0 - 25 mM is associated with increased filtration throughput.~~

~~71. A method of producing an influenza viral vaccine formulated in a sub-virion form, the method comprising the steps:~~

~~a. purifying viral particles from a harvested cell culture of MDCK cells;~~

~~b. inactivating and splitting the viral particles to produce sub-virions; and~~

~~c. purifying the sub-virions;~~

~~wherein inactivating and splitting the viral particles of step b comprises treating the viral particles with a reagent comprising CTAB, NaCl, and polysorbate 80, in an amount selected from the group consisting of conditions 1 through 27 of Table 1.~~

1. A method of producing a viral vaccine, the method comprising the steps:

a. purifying viral particles from harvested cell culture;

b. inactivating and splitting the viral particles to produce sub-virions; and

c. purifying the sub-virions;

wherein inactivating and splitting the viral particles of step b comprises treating the viral particles with a reagent comprising at least one non-ionic surfactant and at least one ionic surfactant, and wherein the at least one non-ionic surfactant and the at least one ionic surfactant are present in an amount effective to reduce particle size of the viral particles.

2. The method of claim 1, wherein the reagent further comprises at least one salt, wherein the at least one salt is present in an amount effective to reduce particle size of the viral particles.

3. The method of claim 1, wherein the average hydrodynamic radius of the sub-virion ranges from 150 nm to 350 nm.

4. The method of claim 1, wherein the at least one non-ionic surfactant is polysorbate 80.

5. The method of claim 1, wherein the at least one non-ionic surfactant comprises at least about 1.0 g/L polysorbate 80.

6. The method of claim 1, wherein the at least one ionic surfactant comprises cetrimonium bromide (CTAB).

7. The method of claim 1, wherein the at least one ionic surfactant comprises about 1.25 g/L to 3.0 g/L CTAB.

8. The method of claim 2, wherein the at least one salt comprises sodium chloride (NaCl).

9. The method of claim 2, wherein the at least one salt comprises 0 - 200 mM NaCl.

10. The method of claim 1, wherein the at least one non-ionic surfactant comprises polysorbate 80, the at least one ionic surfactant comprises CTAB, and the at least one salt comprises sodium chloride NaCl.

11. The method of claim 1, wherein the particle size of the sub-virion is less than about 500 nm.

12. The method of claim 1, wherein the particle size of the sub-virion is the hydrodynamic radius.

13. The method of claim 1, wherein the viral particles are from influenza virus.

14. The method of claim 4, wherein the concentration of polysorbate 80 is 0.3 g/L prior to splitting.

15. The method of claim 4, wherein the concentration of polysorbate 80 increases in an amount that ranges from 0 - 2.2 g/L during splitting.

16. The method of claim 4, wherein the concentration of polysorbate 80 is 0.3 g/L, 1.4 g/L, or 2.5 g/L during splitting.

17. The method of claim 8, wherein a concentration of NaCl ranging from 0 - 25 mM is associated with increased filtration throughput.

18. A method of reducing viral particle size in a virus manufacturing process, the method comprising

a. purifying viral particles harvested from cell culture;
b. inactivating and splitting the viral particles to produce sub-virions; and
c. purifying the sub-virions;

wherein inactivating and splitting the viral particles of step b comprises treating the viral particles with a reagent comprising at least one non-ionic surfactant and at least one ionic surfactant, and wherein the at least one non-ionic surfactant and the at least one ionic surfactant are present in an amount effective to reduce particle size of the viral particles.

19. A method of producing an influenza viral vaccine formulation comprising purified viral proteins, the method comprising the steps:

a. purifying viral particles from a harvested cell culture of MDCK cells;

b. inactivating and splitting the viral particles to produce sub-virions; and

c. purifying the sub-virions to produce purified viral proteins;

wherein inactivating and splitting the viral particles of step b comprises treating the viral particles with a reagent comprising at least one non-ionic surfactant and at least one salt, and wherein the at least one non-ionic surfactant and the at least one salt are present in an amount effective to reduce particle size of the viral particles.

20. A method of producing an influenza viral vaccine formulation comprising purified viral proteins, the method comprising the steps:

a. purifying viral particles from a harvested cell culture of MDCK cells;

b. inactivating and splitting the viral particles to produce sub-virions; and

c. purifying the sub-virions to produce purified influenza viral proteins;

wherein inactivating and splitting the viral particles of step b comprises treating the viral particles with a reagent comprising at least one ionic surfactant and at least one salt, and wherein the at least one ionic surfactant and the at least one salt are present in an amount effective to reduce particle size of the viral particles.

21. A method of producing an influenza viral vaccine formulation comprising purified viral proteins, the method comprising the steps:

a. purifying viral particles from a harvested cell culture of MDCK cells;

b. treating the purified viral particles with at least one alkylating agent to inactivate the viral particles;

c. treating the inactivated viral particle with at least one ionic surfactant to split the inactivated viral particles to produce subvirions; and

d. purifying the sub-virions to produce purified influenza viral proteins;

wherein inactivating and splitting the viral particles of step b and/or c further comprises treating the viral particles with a reagent comprising at least one non-ionic surfactant and at least one ionic surfactant, and wherein the at least one non-ionic surfactant and the at least one ionic surfactant are present in an amount effective to reduce particle size of the viral particles.

22. The method of claim 1, wherein the viral vaccine is formulated in a sub-virion form.

23. The method of claim 1, wherein the particle size of the sub-virion ranges from greater than about 150 nm to less than about 500 nm.