CLAIMS

1. A method of processing a fluid comprising a recombinant therapeutic protein, the method comprising:

(a) providing a first circuit system comprising (i) a tangential flow filtration (TFF) unit having first and second inlets, and (ii) a first conduit in fluid communication between the first and second inlets of the TFF unit, comprising at least one port for flowing fluid into or out of, or both, of the first circuit system,

wherein the first circuit system is configured such that fluid can be flowed through the first conduit and the TFF unit, and filtrate not comprising the recombinant therapeutic protein can be collected from the TFF unit;

(b) providing a second circuit system comprising (i) a tangential flow virus filtration (TFVF) unit having first and second inlets, and (ii) a second conduit in fluid communication between the first and second inlets of the TFVF unit, comprising at least one port for flowing fluid into or out of, or both, of the second circuit system,

wherein the second circuit system is configured such that fluid can be flowed through the second conduit and the TFVF unit, and filtrate comprising the recombinant therapeutic protein can be collected from the TFVF unit;

(c) continuously flowing a fluid comprising a recombinant therapeutic protein into the first circuit system through one of the at least one port, and discarding filtrate not comprising the recombinant therapeutic protein for a first period of time;

(d) continuously flowing a diafiltration medium into the first circuit system through one of the at least one port, and discarding filtrate not comprising the recombinant therapeutic protein for a second period of time;

(e) collecting a fluid comprising the recombinant therapeutic protein that is present in the first circuit system after the first and second time periods through one of the at least one port; and

(f) flowing the collected fluid of step (e) into the second circuit system using a connecting conduit in fluid communication between a port in the first circuit system and a port in the second circuit system, recirculating retentate in the second circuit system, and collecting filtrate comprising the recombinant therapeutic protein from the TFVF unit for a third period of time,

wherein rate of recirculating the retentate in the second circuit system and rate of flowing the collected fluid of step (e) into the second circuit system are independently controlled.

2. The method of claim 1, wherein step (c) comprises continuously flowing the fluid into the first circuit system at a rate of about 0.1 mL/minute to about 100 L/minute.

3. The method of claim 2, wherein step (c) comprises continuously flowing the fluid into the first circuit system at a rate of about 0.5 mL/minute to about 200 mL/minute.

4. The method of any one of claims 1-3, wherein the first period of time is about 0.1 minute to about 12 hours.

5. The method of claim 4, wherein the first period of time is about 10 minutes to about 4 hours.

6. The method of any one of claims 1-5, wherein the flowing of the fluid into the first circuit system in step (c) occurs unidirectionally.

7. The method of any one of claims 1-6, wherein step (d) comprises continuously flowing the diafiltration medium into the first circuit system at a rate of about 0.1 mL/minute to about 100 L/minute.

8. The method of claim 7, wherein step (d) comprises continuously flowing the diafiltration medium into the first circuit system at a rate of about 0.5 mL/minute to about 200 mL/minute.

9. The method of any one of claims 1-8, wherein the second period of time is about 5 minutes to about 12 hours.

10. The method of claim 9, wherein the second period of time is about 10 minutes to about 4 hours.

11. The method of any one of claims 1-10, wherein the flowing of the diafiltration medium into the first circuit system in step (d) occurs unidirectionally.

12. The method of any one of claims 1-11, wherein the first conduit has an inner diameter of about 5 mm to about 5 cm.

13. The method of claim 12, wherein the first conduit has an inner diameter of about 10 mm to about 3 cm.

14. The method of any one of claims 1-13, wherein the TFF unit comprises one or more tangential filter(s) having a surface area of about 0.1 cm^2 to about 50 cm^2.

15. The method of claim 14, wherein the TFF unit comprises one or more tangential filter(s) having a surface area of about 0.5 cm^2 to about 20 cm^2.

16. The method of any one of claims 1-15, wherein the first circuit system further comprises a pump disposed in the first conduit.

17. The method of claim 16, wherein the pump is a peristaltic pump.

18. The method of claim 1, wherein step (f) comprising flowing the fluid into the second circuit system at a rate of about 0.1 mL/minute to about 100 L/minute.

19. The method of claim 18, wherein step (f) comprises flowing the fluid into the second circuit system at a rate of about 0.5 mL/minute to about 200 mL/minute.

20. The method of any one of claims 1-19, wherein the third period of time is about 0.1 minute to about 12 hours.

21. The method of claim 20, wherein the third period of time is about 10 minutes to about 2 hours.

22. The method of any one of claims 1-21, wherein the flowing of the fluid into the second circuit system in step (f) occurs unidirectionally.

23. The method of any one of claims 1-22, wherein the second conduit in fluid communication between the first and second inlets of the TFVF unit has an inner diameter of about 5 mm to about 5 cm.

24. The method of claim 23, wherein the second conduit in fluid communication between the first and second inlets of the TFVF unit has an inner diameter of about 10 mm to about 3 cm.

25. The method of any one of claims 1-24, wherein the TFVF unit comprises one or more tangential virus filter(s) having a surface area of about 0.1 cm^2 to about 100 m^2.

26. The method of claim 25, wherein the TFVF unit comprises one or more tangential virus filter(s) having a surface area of about 0.5 cm^2 to about 200 cm^2.

27. The method of any one of claims 1-26, wherein the connecting conduit has an inner diameter of about 5 mm to about 10 cm.

28. The method of claim 27, wherein the connecting conduit has an inner diameter of about 10 mm to about 5 cm.

29. The method of any one of claims 1-28, wherein second circuit system further comprises a pump disposed in the second conduit in fluid communication between the first and second inlets of the TFVF unit.

30. The method of claim 29, wherein the pump disposed in the second conduit in fluid communication between the first and second inlets of the TFVF unit is a peristaltic pump.

31. A method of processing a fluid comprising a recombinant therapeutic protein, the method comprising:

(a) providing a circuit system comprising (i) a tangential flow virus filtration (TFVF) unit having first and second inlets, and (ii) a conduit in fluid communication between the first and second inlets of the TFVF unit, comprising at least one port for flowing fluid into or out of, or both, of the circuit system,

wherein the circuit system is configured such that fluid can be flowed through the conduit and the TFVF unit, and filtrate comprising the recombinant therapeutic protein can be collected from the TFVF unit; and

(b) flowing a fluid including a recombinant therapeutic protein into the circuit system through one of the at least one port at a rate of about 0.1 mL/minute to about 100 L/minute, recirculating retentate in the circuit system, and collecting filtrate comprising the recombinant therapeutic protein from the TFVF unit for a period of time,

wherein rate of recirculating the retentate in the circuit system and the rate of flowing the fluid into the circuit system are independently controlled.

32. The method of claim 31, wherein step (b) comprises flowing the fluid into the circuit system at a rate of about 0.5 mL/minute to about 200 mL/minute.

33. The method of claim 31 or 32, wherein the period of time is about 5 minutes to about 12 hours.

34. The method of claim 33, wherein the period of time is about 10 minutes to about 3 hours.

35. The method of any one of claims 31-34, wherein the flowing of the fluid into the circuit system in step (b) occurs unidirectionally.

36. The method of any one of claims 31-35, wherein the conduit has an inner diameter of about 5 mm to about 5 cm.

37. The method of claim 36, wherein the conduit has an inner diameter of about 10 mm to about 3 cm.

38. The method of any one of claims 31-37, wherein the TFVF unit comprises one or more tangential virus filter(s) having a surface area of about 0.1 cm^2 to about 100 m^2.

39. The method of claim 38, wherein the TFVF unit comprises one or more tangential virus filter(s) having a surface area of about 0.5 cm^2 to about 200 cm^2.

40. The method of any one of claims 31-39, wherein the circuit system further comprises a pump disposed in the conduit.

41. The method of claim 40, wherein the pump disposed in the conduit is a peristaltic pump.

42. An integrated and continuous process for manufacturing a therapeutic protein drug substance, the process comprising:

(a) providing a liquid culture medium comprising a recombinant therapeutic protein that is substantially free of cells, wherein the liquid culture medium is fed into a first multi-column chromatography system (MCCS1);

(b) capturing said recombinant therapeutic protein in the liquid culture medium using the MCCS1, wherein the eluate of the MCCS1 containing the recombinant therapeutic protein is continuously fed into a second multi-column chromatography system (MCCS2);

(c) purifying and polishing the recombinant therapeutic protein using the MCCS2, wherein the eluate from the MCCS2 is a fluid comprising the recombinant therapeutic protein; and

(d) processing the fluid comprising the recombinant therapeutic protein using a method of any one of claims 1-41,

wherein the process is integrated and runs continuously from the liquid culture medium to the collected fluid or filtrate comprising the recombinant therapeutic protein, wherein the collected fluid or filtrate is the therapeutic protein drug substance.

43. The process of claim 42, wherein the MCCS1 performs at least two different unit operations.

44. The process of claim 43, wherein the MCCS1 performs the unit operations of capturing the recombinant therapeutic protein and inactivating viruses.

45. The process of any one of claims 42-44, wherein the use of the MCCS1 or the MCCS2, or both, involves column switching.

46. The process of any one of claims 42-45, wherein the MCCS1 and/or MCCS2 utilizes at least two chromatography columns.

47. The process of any one of claims 42-45, wherein the MCCS1 and/or MCCS2 utilizes at least two chromatographic membranes.

48. The process of any one of claims 42-45, wherein the MCCS1 and/or MCCS2 utilizes at least one chromatography column and at least one chromatographic membrane.

49. The process of any one of claims 42-48, wherein the MCCS1 is a first periodic counter current chromatography system (PCCS1).

50. The process of claim 49, wherein the PCCS1 comprises a four-column PCCS.

51. The process of claim 50, wherein three of the four columns in the four-column PCCS perform the unit operation of capturing the recombinant therapeutic protein from the liquid culture medium.

52. The process of claim 51, wherein the capturing is performed using affinity chromatography, cation exchange chromatography, anion exchange chromatography, or molecular sieve chromatography.

53. The process of claim 52, wherein the affinity chromatography is performed with a capture mechanism selected from the group consisting of: protein A-binding capture mechanism, substrate-binding capture mechanism, antibody- or antibody fragment-binding capture mechanism, aptamer-binding capture mechanism, and cofactor-binding capture mechanism.

54. The process of claim 53, wherein the affinity chromatography is performed with a protein A-binding capture mechanism, and the recombinant therapeutic protein is an antibody or an antibody fragment.

55. The process of any one of claims 51-54, wherein the eluate containing the recombinant therapeutic protein from three of the four columns in the four-column PCCS is fed into the fourth column of the four-column PCCS.

56. The process of claim 55, wherein the fourth column of the four-column PCCS performs the unit operation of inactivating viruses by holding the eluate containing recombinant therapeutic protein at a low pH for viral inactivation.

57. The process of claim 56, wherein the fourth column of the four-column PCCS holds the eluate containing the recombinant protein at a low pH for viral inactivation for a period of about 10 minutes to about 1.5 hours.

58. The process of claim 56, wherein the MCCS2 is a second periodic counter current chromatography system (PCCS2).

59. The process of claim 58, further comprising adjusting the pH of the eluate from the fourth column of the four-column PCCS using an in-line buffer adjustment reservoir before the eluate from the fourth column of the four-column PCCS is fed into the PCCS2.

60. The process of claim 59, wherein the PCCS2 comprises three chromatography columns and a chromatographic membrane.

61. The process of claim 60, wherein the three chromatographic columns in the PCCS2 perform the unit operation of purifying the recombinant therapeutic protein from the eluate of the PCCS1 through cation or anion exchange chromatography.

62. The process of claim 61, wherein the eluate from the three chromatography columns in the PCCS2 is fed into the chromatographic membrane in the PCCS2.

63. The process of claim 62, wherein the chromatographic membrane in the PCCS2 performs the unit operation of polishing the recombinant therapeutic protein present in the eluate from the three chromatographic columns in the PCCS2 through cation or anion exchange chromatography.

64. The process of claim 63, wherein the chromatographic membrane in the PCCS2 performs the unit operation of polishing through cation exchange chromatography.

65. The process of claim 64, wherein the flow through and wash of the chromatographic membrane comprises the recombinant therapeutic protein.

66. The process of claim 55, further comprising adjusting the ionic concentration of the eluate from the three columns in the PCCS2 using in-line buffer adjustment before the eluate from the three columns in the PCCS2 is fed into the chromatographic membrane in the PCCS2.

67. The process of claim 58, further comprising the use of a break tank between the PCCS1 and the PCCS2.

68. The process of claim 58, further comprising filtering the eluate from the PCCS1 before it is fed into the PCCS2.

69. The process of any one of claims 42-68, further comprising formulating the therapeutic protein drug substance into a pharmaceutical composition.

70. The method of any one of claims 42-53 and 55-69, wherein the recombinant therapeutic protein is an antibody or antibody fragment, an enzyme, an engineered protein, or an immunogenic protein or protein fragment.