Claims

1. A method of performing chromatography with a gamma-irradiated chromatography resin, comprising:

(a) providing a chromatography column containing a gamma-irradiated chromatography resin;

(b) performing a first cycle of chromatography through the column, wherein the first cycle of chromatography comprises:

capturing a recombinant protein by exposing the resin to a liquid comprising the recombinant protein;

washing the resin by exposing the resin to a wash buffer;

eluting the recombinant protein by exposing the chromatography resin to an elution buffer; and

recovering binding capacity of the resin by exposing the resin to a denaturing buffer; and

(c) performing at least one additional cycle of chromatography through the column, wherein each of the at least one additional cycle of chromatography comprises:

capturing the recombinant protein by exposing the resin to the liquid comprising the recombinant protein;

washing the resin by exposing the resin to the wash buffer;

eluting the recombinant protein by exposing the chromatography resin to the elution buffer; and

recovering binding capacity of the resin by exposing the resin to the denaturing buffer;

wherein the denaturing buffer comprises one or more of urea, guanidine hydrochloride, and Triton™ X-100.

2. The method of claim 1, wherein the liquid containing a recombinant protein is a liquid culture medium.

3. The method of claim 1, wherein the cycles in (b) and (c) are performed using a closed and integrated system.

4. The method of claim 3, wherein the denaturing buffer in (b) and (c) has a sterility assurance level of between 1 x 10^{-3} and 1 x 10^{-12}.

5. The method of claim 4, wherein the denaturing buffer in (b) and (c) is prepared by filtration.

6. The method of claim 1, wherein the denaturing buffer comprises 6 M to 9 M urea.

7. The method of claim 1, wherein the denaturing buffer comprises 5 M to 7 M guanidine hydrochloride.

8. The method of claim 1, wherein the denaturing buffer comprises Triton™ X-100.

9. The process of claim 1, wherein the denaturing buffer is selected from the group consisting of:

8 M urea, 1 M NaCl, 0.1 M citric acid, pH 2.5;

6 M guanidine hydrochloride, pH 2.5; and

0.5% Triton™ X-100 in 0.1 M acetic acid, pH 2.5.

10. The method of claim 1, wherein the first cycle of chromatography in (b) and each of the at least one additional cycle of chromatography in (c) further comprise exposing the chromatography resin to wash buffer comprising 0.5 M to 1.5 M sodium hydroxide following exposure to the denaturing buffer.

11. The method of claim 1, wherein the column is part of a multi-column chromatography system (MCCS).

12. The method of claim 11, wherein the MCCS is a periodic counter current chromatography system (PCCS).

13. The method of claim 1, wherein the chromatography resin is anionic exchange chromatography resin, cationic exchange chromatography resin, size exclusion chromatography resin, hydrophobic interaction chromatography resin, affinity chromatography resin, or any combination thereof.

14. The method of claim 13, wherein the chromatography resin is anionic exchange chromatography resin.

15. The method of claim 1, wherein the chromatography resin has been treated with a dose of gamma-irradiation between 10 kGy to 40 kGy.

16. The method of claim 15, wherein the chromatography resin has been treated with a dose of gamma-irradiation between 15 kGy to 35 kGy.

17. The method of claim 16, wherein the chromatography resin has been treated with a dose of gamma-irradiation between 20 kGy to 30 kGy.

18. The method of claim 1, wherein four or more additional cycles of chromatography are performed.

19. The method of claim 18, wherein nine or more additional cycles of chromatography are performed.

20. The method of claim 19, wherein fourteen or more additional cycles of chromatography are performed.

21. The method of claim 20, wherein nineteen or more additional cycles of chromatography are performed.

22. The method of claim 21, wherein twenty-four or more additional cycles of chromatography are performed.

23. The method of claim 22, wherein twenty-nine or more additional cycles of chromatography are performed.

24. The method of claim 23, wherein thirty-nine or more additional cycles of chromatography are performed.

25. The method of claim 1, wherein (c) is performed continuously over a period of at least 4 days.

26. The method of claim 25, wherein (c) is performed continuously over a period of at least 7 days.

27. The method of claim 26, wherein (c) is performed continuously over a period of at least 14 days.

28. The method of claim 27, wherein (c) is performed continuously over a period of at least 28 days.

29. The method of claim 1, wherein the recombinant protein is a recombinant therapeutic protein.

30. An integrated, closed, and continuous process for manufacturing of a purified recombinant protein comprising:

(a) providing a liquid culture medium comprising a recombinant protein that is substantially free of cells; and

(b) continuously feeding the liquid culture medium into a multi-column chromatography system (MCCS) comprising at least one chromatography column containing gamma-irradiated chromatography resin and performing at least two cycles of chromatography with the at least one chromatography column, wherein each of the at least two cycles of chromatography comprises:

capturing the recombinant protein by exposing the resin to the liquid culture medium;

washing the resin by exposing the resin to a wash buffer;

eluting the recombinant protein by exposing the chromatography resin to an elution buffer; and

recovering binding capacity of the resin by exposing the resin to a denaturing buffer;

wherein the denaturing buffer comprises one or more of urea, guanidine hydrochloride, and Triton^{TM} X-100;

wherein the process utilizes a buffer with a sterility assurance level of between 1 x 10^{-3} and 1 x 10^{-12}, is integrated, and runs continuously from the liquid culture medium to an eluate from the MCCS that is the purified recombinant protein.

31. The process of claim 30, wherein the MCCS performs at least two different unit operations.

32. The process of claim 31, wherein the use of the MCCS involves column switching.

33. The process of claim 30, wherein all of the columns in the MCCS contain gamma-irradiated chromatography resin.

34. The process of claim 30, wherein the MCCS performs the unit operations of capturing the recombinant protein and inactivating viruses.

35. The process of claim 30, wherein the MCCS performs the unit operations of capturing and purifying the recombinant protein.

36. The process of claim 30, wherein the MCCS is a periodic counter current chromatography system.

37. The process of claim 30, wherein the MCCS includes a plurality of columns for affinity chromatography, cation exchange chromatography, anion exchange chromatography, size exclusion chromatography, or hydrophobic interaction chromatography, or any combination thereof.

38. The process of claim 37, wherein the MCCS includes a column for affinity chromatography, and 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.

39. The process of claim 38, wherein the affinity chromatography is performed with a protein-A binding capture mechanism, and the recombinant protein is an antibody or an antibody fragment.

40. The process of claim 30, wherein the recombinant protein is a therapeutic recombinant protein.

41. The process of claim 40, further comprising formulating the purified recombinant protein into a pharmaceutical composition.

42. The method of claim 30, wherein the chromatography resin is exposed to wash buffer comprising 0.5 M to 1.5 M sodium hydroxide following exposure to denaturing buffer in each cycle.

43. An integrated, closed, and continuous process for manufacturing of a recombinant protein comprising:

(a) providing a liquid culture medium comprising a recombinant protein that is substantially free of cells;

(b) continuously feeding the liquid culture medium into a first multi-column chromatography system (MCCS1);

(c) producing an eluate from the MCCS1 that comprises the recombinant protein; and

(d) continuously feeding the recombinant protein from the eluate in (c) into a second multi-column chromatography system (MCCS2) and subsequently eluting the recombinant protein from the MCCS2 to thereby produce the purified recombinant protein,

wherein:

the process utilizes a buffer with a sterility assurance level of between 1×10^{-3} and 1×10^{-12}, is integrated, and runs continuously from the liquid culture medium to the purified recombinant protein, and

at least one chromatography column in the MCCS1 and/or MCCS2 is a chromatography column containing gamma-irradiated chromatography resin, and

the process includes performing at least two cycles of chromatography with the at least one chromatography column, wherein each of the at least two cycles of chromatography comprises:

capturing the recombinant protein by exposing the resin to the liquid culture medium;

washing the resin by exposing the resin to a wash buffer;

eluting the recombinant protein by exposing the chromatography resin to an elution buffer; and

recovering binding capacity of the resin by exposing the resin to a denaturing buffer, and wherein the denaturing buffer comprises one or more of urea, guanidine hydrochloride, and Triton™ X-100.

44. The process of claim 43, wherein the MCCS1 and/or the MCCS2 performs at least two different unit operations.

45. The process of claim 43, wherein the use of the MCCS1 or the MCCS2, or both, involves column switching.

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

47. The process of claim 43, wherein the MCCS2 performs the unit operations of purifying and polishing the recombinant protein.

48. The process of claim 43, wherein the MCCS1 and/or MCCS2 utilizes at least two chromatography columns.

49. The process of claim 43, wherein all of the chromatography column(s) in MCCS1 and MCCS2 are chromatography columns containing gamma-irradiated chromatography resin.

50. The process of claim 43, wherein the MCCS1 is a first periodic counter current chromatography system (PCCS1).

51. The process of claim 43, wherein the capturing is performed using affinity chromatography, cation exchange chromatography, anion exchange chromatography, or size exclusion chromatography, hydrophobic interaction chromatography, or any combination thereof.

52. The process of claim 51, wherein capturing is performed using affinity chromatography 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.

53. The process of claim 52, wherein the affinity chromatography is performed with a protein A-binding capture mechanism, and the recombinant protein is an antibody or an antibody fragment.

54. The process of claim 43, wherein the MCCS2 is a second periodic counter current (PCCS2) chromatography system.

55. The process of claim 43, wherein the recombinant protein is a therapeutic recombinant protein.

56. The process of claim 55, further comprising formulating the purified recombinant protein into a pharmaceutical composition.

57. The process of claim 30 or 43, wherein the process is performed continuously for a period of at least 4 days.

58. The process of claim 57, wherein the process is performed continuously for a period of at least 7 days.

59. The process of claim 58, wherein the process is performed continuously for a period of at least 14 days.

60. The process of claim 59, wherein the process is performed continuously for a period of at least 28 days.

61. The process of claim 30 or 43, wherein the chromatography resin is anionic exchange chromatography resin, cationic exchange chromatography resin, size exclusion chromatography resin, hydrophobic interaction chromatography resin, affinity chromatography resin, or any combination thereof.

62. The process of claim 61, wherein the chromatography resin is anionic exchange chromatography resin.

63. The process of claim 30 or 43, wherein the chromatography resin has been treated with a dose of gamma-irradiation between 10 kGy to 40 kGy.

64. The process of claim 63, wherein the chromatography resin has been treated with a dose of gamma-irradiation between 15 kGy to 35 kGy.

65. The process of claim 64, wherein the chromatography resin has been treated with a dose of gamma-irradiation between 20 kGy to 30 kGy.

66. The method of claim 43, wherein the chromatography resin is exposed to a wash buffer comprising 0.5 M to 1.5 M sodium hydroxide following exposure to the denaturing buffer.