Amended Claim Set

WHAT IS CLAIMED IS:

1. (Currently Amended) A method of reducing bioburden of [[a]] an affinity chromatography resin comprising:

exposing a container comprising a composition comprising (i) [[a]] the affinity chromatography resin and (ii) a liquid comprising at least one antioxidant agent ~~and/or chelator~~ to a dose of gamma-irradiation sufficient to reduce the bioburden of the container and the affinity chromatography resin, wherein the at least one antioxidant agent ~~and/or chelator~~ is present in an amount sufficient to ameliorate the loss of binding capacity of the affinity chromatography resin after exposure to the dose of gamma-irradiation.

2. The method of claim 1, further comprising, prior to exposing, disposing the composition into the container.

3. The method of claim 1, wherein the container is a storage vessel.

4. The method of claim 1, wherein the container is a chromatography column.

5. The method of claim 1, wherein the container is a packed chromatography column.

6. (Currently Amended) The method of claim 1, wherein the composition is a slurry of sedimented affinity chromatography resin in [[a]] the liquid comprising the at least one antioxidant agent ~~and/or chelator~~.

~~7. The method of claim 1, wherein the composition is a solid mixture.~~

7.~~8.~~ (Currently Amended) The method of claim 1, wherein the liquid ~~composition~~ comprises at least one antioxidant agent selected from the group consisting of: reduced glutathione, reduced thioredoxin, reduced cysteine, a carotenoid, melatonin, lycopene, tocopherol, reduced ubiquinone, ascorbate, bilirubin, uric acid, lipoic acid, a flavonoid, a phenolpropanoid acid, lidocaine, naringenin, fullerene, glucose, mannitol, 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl, and dimethylmethoxy chromanol.

8.~~9.~~ (Currently Amended) The method of claim 1, wherein the liquid ~~composition~~ comprises at least one antioxidant agent selected from the group consisting of: mannitol, sodium ascorbate, histidine, and methionine.

9.~~10.~~ (Currently Amended) The method of claim 1, wherein the liquid ~~composition~~ comprises mannitol, sodium ascorbate, histidine, and methionine.

10.~~11.~~ (Currently Amended) The method of claim 6, wherein the liquid comprises:

(i) between 75 mM and ~~about~~ 125 mM mannitol;

(ii) between 75 mM and ~~about~~ 125 mM methionine;

(iii) between 75 mM and ~~about~~ 125 mM sodium ascorbate;

(iv) between 75 mM and ~~about~~ 125 mM histidine;

(v) between 30 mM and ~~about~~ 70 mM methionine and between ~~about~~ 30 mM and ~~about~~ 70 mM histidine;

(vi) between ~~about~~ 10 mM and ~~about~~ 50 mM methionine, between ~~about~~ 10 mM and ~~about~~ 50 mM histidine, and between ~~about~~ 10 mM and ~~about~~ 50 mM sodium ascorbate; or

(vii) between ~~about~~ 5 mM to ~~about~~ 45 mM sodium ascorbate, between ~~about~~ 5 mM and ~~about~~ 45 mM methionine, between ~~about~~ 5 mM and ~~about~~ 45 mM mannitol, and between ~~about~~ 5 mM to ~~about~~ 45 mM histidine.

11.~~12.~~ (Currently Amended) The method of claim 6 or 10[[11]], wherein the liquid is a buffered solution.

~~13. The method of claim 1, wherein the composition comprises at least one chelator selected from the group consisting of: ethylenediaminetetraacetic acid (EDTA), 2,3-dimercapto-1-propanesulfonic acid sodium (DMPS), dimercaptosuccinic acid (DMSA), metallothionin, and desferroxamine.~~

~~14. The method of claim 1, wherein the composition comprises at least one chromatography resin selected from the group consisting of: anionic exchange chromatography resin, cationic exchange chromatography resin, affinity chromatography resin, hydrophobic interaction chromatography resin, and size exclusion chromatography resin.~~

12.~~15.~~ (Currently Amended) The method of claim 1[[14]], wherein the composition comprises affinity chromatography resin comprising a protein ligand.

13.~~16.~~ (Currently Amended) The method of claim 12[[15]], wherein the protein ligand is protein A.

~~17. The method of claim 14, wherein the composition comprises an anionic exchange chromatography resin.~~

~~18. The method of claim 17, wherein the anionic exchange chromatography resin comprises N-benzyl-N-methyl-ethanolamine groups.~~

14.~~19.~~ (Currently Amended) The method of claim 1, wherein the dose is between ~~about~~ 15 kGy to ~~about~~ 45 kGy.

15.~~20.~~ (Currently Amended) The method of claim 14[[19]], wherein the dose is between ~~about~~ 20 kGy to ~~about~~ 30 kGy.

16.~~21.~~ (Currently Amended) The method of claim 15[[20]], wherein the dose is between ~~about~~ 23 kGy and ~~about~~ 27 kGy.

17.~~22.~~ (Currently Amended) The method of claim 1, wherein exposing is performed at a temperature between ~~about~~ -25 °C and ~~about~~ 0 °C, inclusive.

18.~~23.~~ (Currently Amended) The method of claim 1, wherein exposing is performed at a temperature between ~~about~~ 0 °C and ~~about~~ 25 °C, inclusive.

~~24. A reduced bioburden~~ affinity ~~chromatography resin produced by the method of claim 3.~~

~~25. The reduced bioburden chromatography resin of claim 24, wherein the resin has a sterility assurance level (SAL) of between about 1 x 10^{-8} to about 1 x 10^{-5}.~~

~~26. The reduced bioburden chromatography resin of claim 25, wherein the resin has a sterility assurance level (SAL) of between about 1 x 10^{-7} to about 1 x 10^{-6}.~~

~~27. The reduced bioburden chromatography resin of claim 24, wherein the chromatography resin comprises at least one resin selected from the group consisting of: anionic exchange chromatography resin, cationic exchange chromatography resin, affinity chromatography resin, hydrophobic interaction chromatography resin, and size exclusion chromatography resin.~~

~~28. The reduced bioburden chromatography resin of claim 27, wherein the chromatography resin comprises affinity chromatography resin comprising a protein ligand.~~

~~29. The reduced bioburden chromatography resin of claim 28, wherein the protein ligand is protein A.~~

~~30.The reduced bioburden chromatography resin of claim 27, wherein the chromatography resin comprises anionic exchange chromatography resin.~~

~~31. The reduced bioburden chromatography resin of claim 30, wherein the anionic exchange chromatography resin comprises N-benzyl-N-methyl-ethanolamine groups.~~

~~32. A method of making a reduced bioburden packed chromatography column comprising:~~

~~providing the reduced bioburden chromatography resin of claim 24; and~~

~~packing the chromatography resin into a reduced bioburden column in an aseptic environment.~~

~~33. A reduced bioburden packed chromatography column produced by the method of claim 32.~~

~~34. A reduced bioburden packed chromatography column produced by the method of~~

claim 5.

35. The reduced bioburden packed chromatography column of claim 34, wherein the resin in the packed column has a sterility assurance level (SAL) of between about 1 x 10^{-8} to about 1 x 10^{-5}.

36. The reduced bioburden packed chromatography column of claim 35, wherein the resin has a sterility assurance level (SAL) of between about 1 x 10^{-7} to about 1 x 10^{-6}.

37. The reduced bioburden packed chromatography column of claim 34, wherein the resin in the packed column comprises at least one resin selected from the group consisting of: anionic exchange chromatography resin, cationic exchange chromatography resin, affinity chromatography resin, hydrophobic interaction chromatography resin, and size exclusion chromatography resin.

38. The reduced bioburden packed chromatography column of claim 37, wherein the resin comprises affinity or pseudo-affinity chromatography resin comprising a protein ligand.

39. The reduced bioburden packed chromatography column of claim 38, wherein the ligand is protein A.

40. The reduced bioburden packed chromatography column of claim 37, wherein the resin comprises anionic exchange chromatography resin.

41. The reduced bioburden packed chromatography column of claim 40, wherein the anionic exchange chromatography resin comprises N-benzyl-N-methyl-ethanolamine groups.

42. A composition comprising a chromatography resin and at least one antioxidant agent and/or chelator, wherein the at least one antioxidant agent and/or chelator is present in an amount sufficient to ameliorate the loss of binding capacity of the chromatography resin upon treatment with a dose of gamma-irradiation sufficient to reduce bioburden of the composition.

43. The composition of claim 42, wherein the composition is a slurry of sedimented chromatography resin in a liquid comprising the at least one antioxidant agent and/or chelator.

44. The composition of claim 42, wherein the composition is a solid mixture.

45. The composition of claim 42, wherein the composition comprises at least one antioxidant agent selected from the group consisting of: reduced glutathione, reduced thioredoxin, reduced cysteine, a carotenoid, melatonin, lycopene, tocopherol, reduced ubiquinone, ascorbate, bilirubin, uric acid, lipoic acid, a flavonoid, a phenolpropanoid acid, lidocaine, naringenin, fullerene, glucose, mannitol, 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl, and dimethylmethoxy chromanol.

46. The composition of claim 42, wherein the composition comprises at least one

antioxidant agent selected from the group consisting of: mannitol, sodium ascorbate, histidine, and methionine.

47. The composition of claim 42, wherein the composition comprises mannitol, sodium ascorbate, histidine, and methionine.

48. The composition of claim 43, wherein the liquid comprises:
(i) between 75 mM and about 125 mM mannitol;
(ii) between 75 mM and about 125 mM methionine;
(iii) between 75 mM and about 125 mM sodium ascorbate;
(iv) between 75 mM and about 125 mM histidine;
(v) between 30 mM and about 70 mM methionine and between about 30 mM and about 70 mM histidine;
(vi) between about 10 mM and about 50 mM methionine, between about 10 mM and about 50 mM histidine, and between about 10 mM and about 50 mM sodium ascorbate; or
(vii) between about 5 mM to about 45 mM sodium ascorbate, between about 5 mM and about 45 mM methionine, between about 5 mM and about 45 mM mannitol, and between about 5 mM and about 45 mM histidine.

49. The composition of claim 43 or 48, wherein the liquid is a buffered solution.

50. The composition of claim 42, wherein the composition comprises at least one chelator selected from the group consisting of: ethylenediaminetetraacetic acid (EDTA), 2,3-dimercapto-1-propanesulfonic acid sodium (DMPS), dimercaptosuccinic acid (DMSA), metallothionin, and desferroxamine.

51. The composition of claim 42, wherein the chromatography resin comprises at least one resin selected from the group consisting of: anionic exchange chromatography resin, cationic exchange chromatography resin, affinity chromatography resin, hydrophobic interaction chromatography resin, and size exclusion chromatography resin.

52. The composition of claim 51, wherein the resin comprises affinity chromatography resin comprising a protein ligand.

53. The composition of claim 52, wherein the protein ligand is protein A.

54. A method of performing reduced bioburden column chromatography comprising:
(a) providing a reduced bioburden packed chromatography column of claim 33 or 34; and
(b) performing column chromatography using the reduced bioburden packed chromatography column and reduced bioburden buffer in a closed system.

55. The method of claim 54, wherein reduced bioburden column chromatography using the reduced bioburden packed chromatography column is performed continuously for a period of at least 4 days.

56. The method of claim 55, wherein reduced bioburden column chromatography using the reduced bioburden packed chromatography column is performed continuously for a period of at least 5 days.

57. The method of claim 56, wherein the reduced bioburden column chromatography using the reduced bioburden packed chromatography column is performed continuously for a period of at least 7 days.

58. The method of claim 57, wherein the reduced bioburden column chromatography using the reduced bioburden packed chromatography column is performed continuously for a period of at least 14 days.

59. The method of claim 58, wherein the reduced bioburden column chromatography using the reduced bioburden packed chromatography column is performed continuously for a period of at least 28 days.

60. The method of claim 54, wherein the resin in the reduced bioburden packed chromatography column in (a) has a percentage binding capacity of between about 75% and about 100% as compared to the same resin not treated with gamma-irradiation.

61. The method of claim 54, wherein the resin in the reduced bioburden packed chromatography column comprises at least one resin selected from the group consisting of: anionic exchange chromatography resin, cationic exchange chromatography resin, affinity chromatography resin, hydrophobic interaction chromatography resin, and size exclusion chromatography resin.

62. The method of claim 61, wherein the resin comprises affinity chromatography resin comprising a protein ligand.

63. The method of claim 62, wherein the protein ligand is protein A.

64. The method of claim 61, wherein the resin comprises anionic exchange chromatography resin.

65. An integrated, closed, and continuous process for reduced bioburden 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 reduced bioburden packed chromatography column of claim 33 or 34;

wherein the process utilizes reduced bioburden buffer, is integrated, and runs continuously from the liquid culture medium to an eluate from the MCCS that is the purified recombinant protein.

66. The process of claim 65, wherein the MCCS performs at least two different unit operations.

67. The process of claim 65, wherein the process includes column switching.

68. The process of claim 65, wherein the MCCS performs the unit operations of capturing the recombinant protein and inactivating viruses.

69. The process of claim 65, wherein the MCCS performs the unit operations of capturing and purifying the recombinant protein.

70. The process of claim 65, wherein the MCCS comprises at least two reduced bioburden packed chromatography columns.

71. The process of claim 65, wherein the MCCS is a periodic counter current chromatography system.

72. The process of claim 65, wherein the MCCS includes a plurality of columns for affinity or pseudo-affinity chromatography, cation exchange chromatography, anion exchange chromatography, or size exclusion chromatography, or any combination thereof.

73. The process of claim 72, wherein the MCCS includes a column for affinity chromatography, and affinity chromatography is performed in the process 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.

74. The process of claim 73, wherein the affinity chromatography is performed in the process with a protein A-binding capture mechanism, and the recombinant protein is an antibody or an antibody fragment.

75. An integrated, closed, and continuous process for reduced bioburden manufacturing of a purified 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) capturing the recombinant protein in the liquid culture medium using the MCCS1;

(d) producing an eluate from the MCCS1 that comprises the recombinant protein and continuously feeding the eluate into a second multi-column chromatography system (MCCS2);

(e) continuously feeding the recombinant protein from the eluate into the MCCS2 and subsequently eluting the recombinant protein to thereby produce the purified recombinant protein, wherein:

~~the process utilizes reduced bioburden buffer, is integrated, and runs continuously from the liquid culture medium to the purified recombinant protein, and~~

~~at least one column in the MCCS1 and/or MCCS2 contains a reduced bioburden packed chromatography column of claim 33 or 34.~~

~~76. The process of claim 75, wherein the MCCS1 and/or the MCCS2 performs at least two different unit operations.~~

~~77. The process of claim 75, wherein the process involves column switching.~~

~~78. The process of claim 75, wherein the MCCS1 performs the unit operations of capturing the recombinant therapeutic protein and inactivating viruses.~~

~~79. The process of claim 75, wherein the MCCS2 performs the unit operations of purifying and polishing the recombinant protein.~~

~~80. The process of claim 75, wherein the MCCS1 and/or MCCS2 comprise at least two chromatography columns.~~

~~81. The process of claim 75, wherein the MCCS1 is a first periodic counter current chromatography system (PCCS1).~~

~~82. The process of claim 75, wherein the capturing is performed using affinity chromatography, cation exchange chromatography, anion exchange chromatography, or size exclusion chromatography, or any combination thereof.~~

~~83. The process of claim 82, 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.~~

~~84. The process of claim 83, wherein the affinity chromatography is performed with a protein-A binding capture mechanism, and the recombinant protein is an antibody or an antibody fragment.~~

~~85. The process of claim 75, wherein the MCCS2 is a second periodic counter current (PCCS2) chromatography system.~~

~~86. The process of claim 65 or 75, wherein the recombinant protein is a therapeutic recombinant protein.~~

~~87. The process of claim 86, further comprising formulating the purified therapeutic recombinant protein into a pharmaceutical composition.~~

~~88. The process of claim 65 or 75, wherein process is performed continuously for a~~

~~period of at least 4 days.~~

~~89. The process of claim 88, wherein the process is performed continuously for a period of at least 5 days.~~

~~90. The process of claim 89, wherein the process is performed continuously for a period of at least 7 days.~~

~~91. The process of claim 90, wherein the process is performed continuously for a period of at least 14 days.~~

~~92. The process of claim 91, wherein the process is performed continuously for a period of at least 28 days.~~