CLAIMS

We claim:

1. A method of purifying an rAAV vector by AEX, the method comprising a step of:

i) loading a solution comprising the rAAV vector to be purified onto an AEX stationary phase in a column;

ii) performing gradient elution of a material from the stationary phase in the column wherein a percentage of a first gradient elution buffer is varied in a manner inversely proportional to variation in a percentage of a second gradient elution buffer; and

iii) collecting at least one fraction of eluate from the column during the gradient elution beginning when the absorbance of a column flow-through reaches an absorbance threshold, and wherein the at least one fraction of eluate comprises the rAAV vector to be purified.

2. The method of purifying a rAAV vector by AEX of claim 1, wherein the method further comprises measuring an absorbance of the at least one fraction of eluate collected from the column and determining an A260/A280 ratio.

3. The method of purifying a rAAV vector by AEX of claim 1 or 2, wherein the solution comprising the rAAV vector to be purified is diluted about 2-fold to 25-fold (e.g., 15-fold) with a dilution solution comprising histidine, Tris and P188, and optionally filtered prior to application to the stationary phase.

4. The method of purifying a rAAV vector by AEX of any one of claims 1-3, wherein the solution is an affinity eluate.

5. The method of purifying a rAAV vector by AEX of claim 5, wherein a pH of the diluted, and optionally filtered affinity eluate is increased as compared to a pH of the solution; and wherein a conductivity of the diluted, and optionally filtered affinity eluate is decreased as compared to a conductivity of the solution.

6. The method of purifying a rAAV vector by AEX of any one of claims 1-5, wherein the first gradient elution buffer comprises about 50 mM to about 150 mM Tris, about 0.005% to about 0.015% P188 and has a pH of about pH 8.5 to 9.5; wherein the second gradient elution buffer comprises about 400 mM to about 600 mM sodium acetate, about 50 mM to about 150 mM Tris, about 0.005% to about 0.015% P188 and has a pH of about pH 8.5 to 9.5; and wherein 10 to 60 column volumes (CV) (e.g., about 20 CV, about 37.5 CV) of the first gradient elution buffer, the second gradient elution buffer or a mixture of both are applied to the stationary phase during the gradient elution.

7. The method of purifying a rAAV vector by AEX of any one of claims 1-6, wherein at a start of the gradient elution the percentage of the first gradient elution buffer is 50% -100% and at an end of the gradient elution the percentage of the second gradient elution buffer is 50% - 100% and wherein the percentage of the second elution buffer increases at a rate of about 2% to 5% per CV over the gradient elution.

8. The method of purifying a rAAV vector by AEX of any one of claims 1-7, wherein a concentration of sodium acetate of the first gradient elution buffer, the second gradient elution buffer or the mixture of both increases continuously during the gradient elution; and wherein the concentration of the sodium acetate increases at a rate of about 10 mM/CV to 50 mM/CV (e.g., about 10 mM/CV, about 25 mM/CV) over the gradient elution.

9. The method of purifying a rAAV vector by AEX of any one of claims 1-8, wherein full capsids are eluted from the stationary phase in a first elution peak and/or in a first portion of a second elution peak during the gradient elution.

10. The method of purifying a rAAV vector by AEX of any one of claims 1-9, wherein empty capsids are recovered in the column flow-through, in a first elution peak and/or in a last portion of a second elution peak during the gradient elution.

11. The method of purifying a rAAV vector by AEX of any one of claims 1-10, wherein an absorbance of the at least one fraction of eluate is measured at 280 nm,

and wherein optionally, an absorbance threshold is ≥ 0.5 mAU/mm path length measured at 280 nm.

12. The method of purifying a rAAV vector by AEX of any one of claims 1-11, wherein a volume of the at least one fraction of eluate is equivalent to 1/8 of a CV to 10 CV, e.g., 1/8 of a CV, 1/4 of a CV, 1/3 of a CV, 1/2 of a CV, 3/4 of a CV, 1 CV, 2 CV, 3 CV, 4 CV, 5 CV, 6 CV, 7 CV, 8 CV, 9 CV, 10 CV or more of a CV, and optionally, wherein an A260/A280 ratio of the at least one fraction of eluate is ≥ to 1.25.

13. The method of purifying a rAAV vector by AEX of any one of claims 1-12, wherein at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19, at least 20, at least 21, at least 22, at least 23, at least 24, at least 25, or more, fractions of eluate are collected.

14. The method of purifying a rAAV vector by AEX of any one of claims 1-13, wherein the method further comprises combining at least two fractions of eluate collected from the column, each having an A260/A280 ratio of ≥ 0.98 or ≥ 1.0 to form a pooled eluate comprising the rAAV vector.

15. The method of purifying a rAAV vector by AEX of claim 14, wherein the pooled eluate has a % VG column yield of 20% to 100% (e.g., 63 +/- 26%), a % VG step yield of 31% to 66% (e.g., 47 +/- 11%) and/or an A260/A280 ratio of ≥ 1.0.

16. The method of purifying a rAAV vector by AEX of claim 14 or 15, wherein the pooled eluate is enriched for full capsids, and/or depleted of empty capsids, as compared to the solution loaded onto the column.

17. The method of purifying a rAAV vector by AEX of any one of claims 1-16, wherein a purified rAAV vector is produced.

18. The method of purifying a rAAV vector by AEX of any one of claims 14-17, further comprising filtering the pooled eluate by a method selected from the group

consisting of viral filtration, ultrafiltration/diafiltration (UF/DF), filtration through a 0.2 µm filter and a combination thereof, to produce a drug substance.

19. The method of purifying a rAAV vector by AEX of claim 18, wherein the drug substance comprises: i) 45% to 65% (e.g., 52 +/- 7%) full capsids of total capsids; ii) 19% to 37% (e.g., 28 +/- 5%) intermediate capsids of total capsids; and/or iii) 10% to 37% (e.g., 20 +/- 7%) empty capsids of total capsids.

20. The method of purifying a rAAV vector by AEX of claim 18 or 19, wherein the drug substance is enriched for full capsids, and/or depleted of empty capsids, as compared to the solution loaded onto the column.

21. The method of purifying a rAAV vector by AEX of any one of claims 1-20, wherein the rAAV vector comprises an AAV capsid protein from an AAV serotype selected from the group consisting of AAV1, AAV2, AAV3, AAV3A, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAVrh10, AAVrh74, AAV12, AAV2i8, NP4, NP22, NP66, AAVDJ, AAVDJ/8, AAVDJ/9, AAVLK03, AAV1.1, AAV2.5, AAV6.1, AAV6.3.1, AAV9.45, RHM4-1 (SEQ ID NO:5 of WO 2015/013313), RHM15-1, RHM15-2, RHM15-3/RHM15-5, RHM15-4, RHM15-6, AAV hu.26, AAV1.1, AAV2.5, AAV6.1, AAV6.3.1, AAV9,45, AAV2i8, AAV29G, AAV2,8G9, AVV-LK03, AAV2-TT, AAV2-TT-S312N, AAV3B-S312N, AAVHSC1, AAVHSC2, AAVHSC3, AAVHSC4, AAVHSC5, AAVHSC6, AAVHSC7, AAVHSC8, AAVHSC9, AAVHSC10, AAVHSC11, AAVHSC12, AAVHSC13, AAVHSC14 and AAVHSC15.

22. The method of purifying a rAAV vector by AEX of any one of claims 1-21, wherein the rAAV vector comprises an AAV9 capsid protein and a transgene comprising the nucleic acid of SEQ ID NO:1.

23. The method of purifying a rAAV vector by AEX of any one of claims 1-21, wherein the rAAV vector comprises an AAV3B capsid protein and a transgene comprising a nucleic acid encoding the amino acid sequence of SEQ ID NO:15.

24. A method of preparing a solution comprising a rAAV vector for purification by AEX, the method comprising a step of:

i) diluting a first solution 2 to 25-fold (e.g., 15-fold) with a dilution solution comprising histidine, Tris and P188; and optionally

ii) filtering the first solution from step i) through a filter to produce a diluted, and optionally filtered solution;

wherein the pH of the diluted, and optionally filtered solution is increased as compared to the pH of the first solution; and wherein the conductivity of the diluted, and optionally filtered solution is decreased as compared to the conductivity of the first solution.

25. The method of preparing a solution comprising a rAAV vector for purification by AEX of claim 24, wherein the first solution comprising the rAAV vector is selected from the group consisting of an affinity eluate, a supernatant from a cell lysate and a post-harvest solution.

26. The method of preparing a solution comprising a rAAV vector for purification by AEX of claim 24 or 25, wherein the dilution solution comprises about 100 mM to 300 mM (e.g., about 200 mM) histidine, 100 mM to 300 mM (e.g., about 200 mM) Tris, 0.1% to 1.0% (e.g., about 0.5%) P188 and has a pH of pH 8.5 to 9.5.

27. The method of preparing a solution comprising a rAAV vector for purification by AEX of any one of claims 24-26, wherein i) a pH of the diluted, and optionally filtered solution is 8.5 to 9.5; ii) a conductivity of the diluted, and optionally filtered solution is 1.7 to 3.3 mS/cm; and/or iii) a % VG dilution yield of the diluted solution is 35% to 100%.

28. The method of preparing a solution comprising a rAAV vector for purification by AEX of any one of claims 24-27, wherein the rAAV vector comprises an AAV9 capsid protein or an AAV3B capsid protein.

29. A purified rAAV vector prepared by a method comprising a step of:

i) loading a solution comprising the rAAV vector to be purified onto an AEX stationary phase in a column;

ii) performing gradient elution of a material from the stationary phase in the column wherein a first gradient elution buffer, a second gradient elution buffer or a

mixture of both are applied to the stationary phase and a concentration of a salt is varied from 0 mM to 500 mM such that the rate of increase in concentration of the salt over the course of the gradient elution is about 10 mM/CV to 50 mM/CV (e.g., about 25 mM/CV);

iii) collecting at least one fraction of eluate from the column during gradient elution beginning when absorbance of a column flow-through reaches an absorbance threshold; and/or

vi) measuring an absorbance of the at least one fraction of eluate collected from the column and determining an A260/A280 ratio.

30. The purified rAAV vector prepared by the method of claim 29, wherein the method further comprises combining at least two fractions of eluate collected from the column when the A260/A280 ratio is ≥1.0 to form a pooled eluate comprising the purified rAAV vector.

31. The purified rAAV vector prepared by the method of claim 29 or 30, wherein the salt is sodium acetate.

32. The purified rAAV vector prepared by the method of any one of claims 29-31, wherein the rAAV vector comprises an AAV9 capsid protein or an AAV3B capsid protein.

33. The purified rAAV vector prepared by the method of any one of claims 29-32, wherein the solution comprising the rAAV vector is an affinity eluate that has been diluted and optionally filtered prior to loading onto the stationary phase.

34. The purified rAAV vector prepared by the method of any one of claims 29-33, wherein the material eluted from the stationary phase comprises the rAAV vector.

35. The purified rAAV vector prepared by the method of any one of claims claim 30-34, wherein the method further comprises filtering the pooled eluate by a method selected from the group consisting of viral filtration, ultrafiltration/diafiltration (UF/DF), filtration through a 0.2 µm filter and a combination thereof, to produce a drug substance.

36. The purified rAAV vector prepared by the method of claim 35, wherein the drug substance is used to make a drug product suitable for administration to a human subject to treat a disease, disorder or condition.

37. The purified rAAV vector prepared by the method of claim 36, wherein the disease, disorder or condition is DMD or Wilson's disease, and optionally wherein the rAAV vector comprises a nucleic acid encoding the amino acid sequence of SEQ ID NO:2 or SEQ ID NO:15.

38. An solution comprising a rAAV vector for purification by AEX prepared by a method comprising a step of:

i) diluting an affinity eluate 2 to 25-fold (e.g., 15-fold) with a dilution solution comprising histidine, Tris, and P188; and optionally

ii) filtering the affinity eluate from step i) through a 0.2 µm filter to produce a diluted, and optionally filtered affinity eluate;

wherein a pH of the diluted, and optionally filtered affinity eluate is increased as compared to a pH of the affinity eluate; and wherein a conductivity of the diluted, and optionally filtered affinity eluate is decreased as compared to a conductivity of the affinity eluate.

39. The solution comprising a rAAV vector for purification by AEX prepared by a method of claim 38, wherein the dilution solution comprises about 100 mM to 300 mM (e.g., about 200 mM) histidine, 100 mM to 300 mM (about 200 mM) Tris, 0.1% to 1.0% (about 0.5%) P188, pH 8.5 to 9.5.

40. The solution comprising a rAAV vector for purification by AEX prepared by a method of claim 38 or 39, wherein the pH the affinity eluate is 3.0 to 4.4 prior to a step of diluting, and optionally filtering, and the pH of the affinity eluate after the step of diluting, and optionally filtering, is 8.5 to 9.5 or 8.7 to 9.0 (e.g., 8.8, 9.0).

41. The solution comprising a rAAV vector for purification by AEX prepared by a method of any one of claims 38-40, wherein the conductivity of the affinity eluate is 5.0 to 7.0 mS/cm (e.g., 5.5 to 6.5 mS/cm) prior to the step of diluting, and optionally

filtering, and the conductivity of the affinity eluate after the step of diluting, and optionally filtering, is 1.7 to 3.3 mS/cm, 1.8 to 2.8 mS/cm, and/or 2.2 to 2.6 mS/cm.

42. The solution comprising a rAAV vector for purification by AEX prepared by a method of any one of claims 38-41, wherein the % VG dilution yield of the diluted and optionally filtered affinity eluate is 35% to 100%.

43. The solution comprising a rAAV vector for purification by AEX prepared by a method of any one of claims 38-42, wherein the rAAV vector comprises an AAV9 or an AAV3B capsid protein.

44. The solution comprising a rAAV vector for purification by AEX prepared by a method of any one of claims 38-43, wherein the diluted, and optionally filtered affinity eluate is loaded on an AEX stationary phase.

45. A method of preparing a stationary phase for use in a method of purifying a rAAV vector by AEX of any one of claims 1-23, the method comprising at least one step of:

i) pre-use flushing comprising application of ≥4.5 CV (e.g., about 5 CV) of water for injection to an AEX stationary phase in a column;

ii) sanitizing comprising application of about 14.4 to 17.6 CV (e.g., about 16 CV) of a solution comprising about 0.1 M to 1.0 M NaOH to the AEX stationary phase in the column, optionally by upward flow; and/or

iii) regenerating comprising application of about 4.5 to 5.5 CV (e.g., about 5 CV) of a solution comprising about 1 M to 3 M NaCl, 50 mM to 150 mM Tris, pH 8.5 to 9.5 to the AEX stationary phase in the column.

46. A method of regenerating an AEX stationary phase, the method comprising a step of:

i) post-use sanitizing the stationary phase comprising application of 14.4 to 17.6 CV (e.g., about 16 CV) of a solution comprising about 0.1 M to 1.0 M NaOH to the stationary phase, optionally by upward flow;

ii) regenerating the stationary phase comprising application of 4.5 to 5.5 CV (e.g., about 5 CV) of a solution comprising about 1 M to 3 M NaCl, 50 mM to 150 mM Tris, pH 8.5 to 9.5 to the stationary phase;

iii) equilibrating the stationary phase comprising application of 4.5 to 5.5 CV (e.g., about 5 CV) of a solution comprising about 50 mM to 150 mM Tris, pH 8.5 to 9.5 to the stationary phase;

iv) post-use flushing the stationary phase comprising application of ≥4.5 (e.g., about 5 CV) of water for injection to the stationary phase; and/or

v) applying a storage solution to the stationary phase comprising application 2.7 to 3.3 CV (e.g., about 3 CV) of a solution comprising about 17% to 17.5% ethanol to the stationary phase.

47. The method of regenerating an AEX stationary phase of claim 46, wherein any one of steps i) – v) step follows a chromatography elution step of a method of purifying a rAAV vector by AEX.

48. A regenerated AEX stationary phase prepared by a method comprising a step of:

i) post-use sanitizing of the stationary phase comprising application of 14.4 to 17.6 CV (e.g., about 16 CV) of a solution comprising about 0.1 M to 1.0 M NaOH to the stationary phase, optionally by upward flow ;

ii) regenerating the stationary phase comprising application of 4.5 to 5.5 CV (e.g., about 5 CV) of a solution comprising about 1 M to 3 M NaCl, 50 mM to 150 mM Tris, pH 8.5 to 9.5 to the stationary phase;

iii) equilibrating the stationary phase comprising application of 4.5 to 5.5 CV (e.g., about 5 CV) of a solution comprising about 50 mM to 150 mM, Tris, pH 8.5 to 9.5 to the stationary phase;

iv) post-use flushing of the stationary phase comprising application of ≥4.5 (e.g., about 5 CV) of water for injection to the stationary phase; and/or

v) applying a storage solution to the stationary phase comprising application 2.7 to 3.3 CV (e.g., about 3 CV) of a solution comprising about 17% to 17.5% ethanol to the stationary phase.

49. A regenerated AEX stationary phase of claim 48, wherein the regenerated AEX stationary phase is used for purification of a rAAV vector.

50. A method of purifying an rAAV vector by AEX, the method comprising a step of:

i) loading a solution comprising the rAAV vector to be purified onto an AEX stationary phase in a column;

ii) performing gradient elution of a material from the stationary phase in the column wherein a percentage of a first gradient elution buffer is varied in a manner inversely proportional to variation in a percentage of a second gradient elution buffer; wherein at the start of the gradient elution the percentage of the first gradient elution buffer is about 75% to about 100% and at the end of the gradient elution the percentage of the second gradient elution buffer is about 60% to about 100%; and wherein the percentage of the second elution buffer increases at a rate of about 2% to 5% per CV over the gradient elution;

iii) collecting at least one fraction of eluate from the column when performing the gradient elution beginning when the percentage of the second gradient elution buffer is about 30% to about 35%,

and wherein the at least one fraction of eluate comprises the rAAV vector to be purified.

51. The method of purifying an rAAV vector by AEX of claim 50, wherein the solution comprising the rAAV vector is an affinity elutate that has been diluted about 15 fold with a buffer comprising histidine, Tris and P188.

52. The method of purifying an rAAV vector by AEX of claim 50 or 51, wherein the first gradient elution buffer comprises 50 mM to 150 mM (e.g., about 100 mM) Tris, 0.005% to 0.015 % (e.g., about 0.01%) P188, pH 8.5 to 9.5 (e.g., 8.9) and/or the second gradient elution buffer comprises 400 mM to 600 mM (e.g., about 500 mM) sodium acetate, 50 mM to 150 mM (e.g., about 100 mM) Tris, 0.005% to 0.015% (e.g., about 0.01%) P188, pH 8.5 to 9.5 (e.g., pH 8.9).

53. The method of purifying an rAAV vector by AEX of any one of claims 50-52, wherein collecting at least one fraction of eluate from the column comprises

collecting the at least one fraction of eluate into a vessel comprising about 0.01 CV to 0.1 CV (e.g., about 0.066 CV) of a solution comprising 200 mM to 300 mM (e.g., about 250 mM) sodium citrate, pH 3.0 to 4.0 (e.g., about 3.5).

54. The method of purifying a rAAV vector by AEX of any one of claims 51-53, wherein the at least one fraction of eluate is enriched for full capsids, and/or depleted of empty capsids, as compared to the affinity eluate; optionally wherein the rAAV vector is a rAAV3B vector; and optionally wherein the AEX stationary phase is POROS™ 50 HQ.

55. The method of purifying a rAAV vector by AEX of any one of claims 50-54, wherein the collecting at least one fraction of eluate from the column when performing the gradient elution ends when the percentage of the second gradient elution buffer is about 50% to about 55%.

CLAIMS

1. A method of purifying a recombinant adeno-associated viral (rAAV) vector by anion exchange chromatography (AEX), comprising:

i) diluting a solution comprising a rAAV vector to be purified with a dilution solution comprising a buffering agent, an amino acid, and a detergent, such that the rAAV vector binds to an AEX stationary phase;

ii) loading the diluted solution onto said AEX stationary phase under conditions sufficient for rAAV vector in said diluted solution to bind to said AEX stationary phase; and

iii) eluting the rAAV vector from said AEX stationary phase with an elution solution that increases in conductivity as elution proceeds.

2. The method of claim 1, wherein said solution comprising a rAAV vector to be purified is an affinity eluate.

3. The method of claim 1 or claim 2, wherein said solution comprising a rAAV vector to be purified is diluted at least 2-fold.

4. The method of any one of claims 1 to 3, wherein said dilution solution has pH 8 to 10, and comprises 10 mM to 500 mM of said buffering agent, 10 mM to 600 mM of said amino acid, and 0.005% to 1.5% of said detergent.

5. The method of any one of claims 1 to 4, wherein said buffering agent is Tris, BIS-Tris propane, diethanolamine, diethylamine, tricine, triethanolamine, or bicine.

6. The method of any one of claims 1 to 5, wherein said amino acid is histidine, arginine, glycine, or citrulline.

7. The method of any one of claims 1 to 6, wherein said detergent is poloxamer 188 (P188), Triton X-100, polysorbate 80 (PS80), Brij-35, or nonyl-phenoxypolyethoxylethanol (NP-40).

8. The method of any one of claims 1 to 7, wherein after the step of diluting, the pH and conductivity of said diluted solution is higher and lower, respectively,

compared to the pH and conductivity of said solution comprising a rAAV vector to be purified.

9. The method of any one of claims 1 to 8, wherein after the step of diluting, the pH of said diluted solution is 8.5 to 9.5

10. The method of any one of claims 1 to 9, wherein after the step of diluting, the conductivity of said diluted solution is 1.7 to 3.5 mS/cm.

11. The method of any one of claims 1 to 10, wherein said elution solution comprises a salt.

12. The method of claim 11, wherein said salt is sodium chloride, sodium acetate, or ammonium acetate.

13. The method of claim 11 or 12, wherein at the beginning of said step of eluting, said elution solution comprises 0 mM to 100 mM of said salt, and at the end of said step of eluting, said elution solution comprises 100 mM to 1 M of said salt.

14. The method of any one of claims 11 to 13, wherein at the beginning of said step of eluting, the conductivity of said elution solution is between 1.0 mS/cm and 2.5 mS/cm, and at the end of said step of eluting, the conductivity of said elution solution is between 20 mS/cm and 35 mS/cm.

15. The method of any one of claims 1 to 14, wherein said dilution solution has pH of about 8.5 to 9.5 and comprises about 100 mM to 300 mM Tris, about 100 mM to 300 mM histidine, and about 0.1% to 1.0% P188, and wherein said elution solution comprises sodium acetate.

16. The method of any one of claims 1 to 15, further comprising measuring the UV light absorbance of the eluate at 260 nm and 280 nm and collecting a fraction of said eluate when the A260/A280 ratio is at least 1.25.

17. The method of claim 16, wherein the proportion of full capsids in said eluate fraction is at least 50%.

18. The method of any one of claims 1 to 17, wherein said stationary phase is polystyrenedivinylbenzene particle with covalently bound quaternized polyethyleneimine disposed in a column.

19. The method of claim 18, wherein the concentration of rAAV vector in said diluted solution is 2.6×10^{12} to 6.8×10^{13} VG/mL of column volume.

20. The method of any one of claims 1 to 19, wherein said rAAV vector comprises an AAV capsid selected from the group consisting of AAV1, AAV2, AAV3, AAV3A, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAVrh10, AAVrh74, AAV12, AAV2i8, NP4, NP22, NP66, AAVDJ, AAVDJ/8, AAVDJ/9, AAVLK03, AAV1.1, AAV2.5, AAV6.1, AAV6.3.1, AAV9.45, RHM4-1 (SEQ ID NO:5 of WO 2015/013313), RHM15-1, RHM15-2, RHM15-3/RHM15-5, RHM15-4, RHM15-6, AAVhu.26, AAV1.1, AAV2.5, AAV6.1, AAV6.3.1, AAV9,45, AAV2i8, AAV29G, AAV2,8G9, AVV-LK03, AAV2-TT, AAV2-TT-S312N, AAV3B-S312N, AAVHSC1, AAVHSC2, AAVHSC3, AAVHSC4, AAVHSC5, AAVHSC6, AAVHSC7, AAVHSC8, AAVHSC9, AAVHSC10, AAVHSC11, AAVHSC12, AAVHSC13, AAVHSC14, and AAVHSC15.