(2021) 2000015; Kaludov, N., et al., Hum. Gene Ther. 13 (2002) 1235–1243; Lock, M, et al., Hum. Gene Ther. Methods. 23 (2012) 56–64).

In AEX the rAAV particles follow an "on/off" retention behavior (Snyder, L.R., et al., Anal. Chem. 55 (1983) 1412A-1430A; Fekete, S., et al., Anal. Chem. 93 (2021) 1277–1284).

WO 2021/158915 discloses recombinant adeno-associated virus compositions and methods for producing and using the same.

US 2021/355503 discloses compositions and methods for manufacturing gene therapy vectors.

Khatwani Santoshkumar et al. disclose an anion-exchange HPLC assay for separation and quantification of empty and full capsids in multiple adeno-associated virus serotypes (Mol. Ther. Meth. Clin. Dev. 21 (2021) 548-558).

US 2021/009964 discloses the separation and quantification of empty and full viral capsid particles.

Dickerson, R., et al. reported the separation of empty and full recombinant adeno-associated virus particles using isocratic anion exchange chromatography (Biotechnol J 16 (2021) 2000015.

Summary

Herein is reported an anion-exchange chromatography (AEX) method comprising a novel elution scheme. Without being bound by this theory, it is assumed that the method takes advantage of the fact that viral capsids follow an "on/off" retention behavior. Based thereon an elution method has been found that allows the unprecedented separation of full rAAV particles from empty rAAV particles. The advantages of the method according to the disclosure is, e.g., a resolution gain of 3.7 as compared to the resolution obtainable with a linear gradient. With the method according to the current disclosure a precise and accurate baseline separation and quantification of full and empty rAAV particles is possible, allowing the application amongst others, e.g., as quality control (QC) method.

Thus, the current disclosure encompasses the at least following examples:

wherein the second buffered solution has a conductivity that is increased with respect to the first buffered solution by about 8 mS/cm to 9.5 mS/cm

wherein in step c) or/and d) the empty rAAV particle is eluted,

wherein the applying in step d) is for at least 2 minutes,

wherein in step e) the full rAAV particle is eluted,

wherein the conductivity is increased in step e) by at least about 20 mS/cm.

2. The method according to example 1, wherein the rAAV particles are of the serotype 8.

3. The method according to any one of examples 1 to 2, wherein the anion exchange chromatography is a strong anion exchange chromatography.

4. The method according to any one of examples 1 to 3, wherein the anion exchange chromatography material is a stationary phase consisting of microporous ethylvinylbenzene cross-linked with 55 % divinylbenzene polymer substrate with quaternary ammonium groups providing for the anion exchange functionality.

5. The method according to any one of examples 1 to 4, wherein the AEX chromatography column has the dimension of about 50 mm length and about 4-5 mm diameter.

6. The method according to any one of examples 1 to 5, wherein the AEX chromatography material has a particle size of about 10 µm.

7. The method according to any one of examples 1 to 6, wherein the method is for/provides baseline separation of full and empty rAAV particles.

8. The method according to any one of examples 1 to 7, wherein the method is for quantification of full and empty rAAV particles.

9. The method according to any one of examples 1 to 8, wherein the method is a high-throughput method for quantification of full and empty rAAV particles rAAV.

10. The method according to any one of examples 1 to 9, wherein the method is a quality control (QC) method for rAAV preparations.

11. The method according to any one of examples 1 to 10, wherein the solution comprising one or both of an empty rAAV particle and a full rAAV particle is a buffered solution comprising about 0.001 % (w/v) of a non-ionic detergent.

12. The method according to any one of examples 1 to 11, wherein the solution comprising one or both of an empty rAAV particle and a full rAAV particle is a phosphate buffered saline solution (comprising 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4) comprising about 0.001 % (w/v) of a non-ionic detergent.

13. The method according to any one of examples 1 to 12, wherein the solution comprising one or both of an empty rAAV particle and a full rAAV particle, is a buffered solution comprising about 0.001 % (w/v) of poloxamer 188 or polysorbate 20.

14. The method according to any one of examples 1 to 13, wherein the solution comprising one or both of an empty rAAV particle and a full rAAV particle is a phosphate buffered saline solution (comprising 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 1.8 mM KH_2PO_4) comprising about 0.001 % (w/v) of poloxamer 188 or polysorbate 20.

15. The method according to any one of examples 1 to 14, wherein the first and second buffered solution as well as the first and the second intermediate buffered solutions comprise a buffer substance with a pKa value of about 9.

16. The method according to any one of examples 1 to 15, wherein the first and second buffered solution as well as the first and the second intermediate buffered solutions comprise a buffer substance selected from 1,3-Bis[tris(hydroxymethyl)methylamino]propane and N-(1,1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropane sulfonic acid.

17. The method according to any one of examples 1 to 16, wherein the first and second buffered solution as well as the first and the second intermediate buffered solutions comprise have a pH value of 9.0 to 10.0.

18. The method according to any one of examples 1 to 17, wherein the first and second buffered solution as well as the first and the second intermediate buffered solutions comprise have a pH value of 9.2 to 9.6.

19. The method according to any one of examples 1 to 18, wherein the first and second buffered solution as well as the first and the second intermediate buffered solutions comprise have a pH value of about 9.4.

20. The method according to any one of examples 1 to 19, wherein the first and second buffered solution as well as the first and the second intermediate buffered solutions comprise an organic salt as elution salt.

21. The method according to any one of examples 1 to 20, wherein the first and second buffered solution as well as the first and the second intermediate buffered solutions comprise an organic chloride as elution salt.

22. The method according to any one of examples 1 to 21, wherein the first and second buffered solution as well as the first and the second intermediate buffered solutions comprise an organic chloride with a molecular weight below 170 g/mol as elution salt.

23. The method according to any one of examples 1 to 22, wherein the first and second buffered solution as well as the first and the second intermediate buffered solutions comprise tetraethyl ammonium chloride as elution salt.

24. The method according to any one of examples 1 to 22, wherein the first and second buffered solution as well as the first and the second intermediate buffered solutions comprise tetramethyl ammonium chloride as elution salt.

25. The method according to any one of examples 1 to 23, wherein the method is performed at a flow rate of about 0.3 mL/min.

26. The method according to any one of examples 1 to 25, wherein the first buffered solution comprises about 65 mM of the buffer substance, about 10 mM of the elution salt, about 2 mM of the salt and has a pH value of about 9.4.

27. The method according to any one of examples 1 to 26, wherein the second buffered solution comprises about 65 mM of the buffer substance, about 90 mM of the elution salt, about 2 mM of the salt and has a pH value of about 9.4.

28. The method according to any one of examples 1 to 27, wherein the buffer substance is N-(1,1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropane sulfonic acid, the elution salt is tetramethyl ammonium chloride, and the salt is magnesium chloride.

29. The method according to any one of examples 1 to 28, wherein step b) has a length of about 1 minute, step c) has a length of about 5 minutes, step d) has a length of at least 2 minutes and at most 5 minutes, and step e) has a length of about 5 minutes.

30. A method for separating full and empty recombinant adeno-associated virus particles using an anion exchange chromatography step,

wherein the method comprises a sequence of a applying a solution comprising empty and/or full rAAV particles to an anion exchange chromatography material inside a chromatography column, a first isocratic step, a first linear gradient, a second isocratic step and a second linear gradient,

wherein the empty recombinant adeno-associated virus particles are eluted during the first linear gradient and the full recombinant adeno-associated virus particles are eluted during the second linear gradient,

wherein

i) the rAAV particle is of the serotype 8, or/and

ii) the anion exchange chromatography material is a strong anion exchange material, or/and

iii) the anion exchange chromatography material has a stationary phase consisting of microporous ethylvinylbenzene cross-linked with 55 % divinylbenzene polymer substrate with quaternary ammonium groups providing for the anion exchange functionality, or/and

iv) the AEX chromatography column has the dimension of about 50 mm length and about 4-5 mm diameter, or/and

v) the AEX chromatography material has a particle size of about 10 µm, or/and

xix) the solutions used in the isocratic steps and linear gradients comprise an organic chloride with a molecular weight below 170 g/mol, or/and

xx) the solutions used in the isocratic steps and linear gradients comprise tetraethyl ammonium chloride, or/and

xxi) the solutions used in the isocratic steps and linear gradients comprise tetramethyl ammonium chloride, or/and

xxii) the method is performed at a flow rate of about 0.3 mL/min, or/and

xxiii) the solution applied in the first isocratic step comprises about 65 mM of the buffer substance, about 10 mM of the elution salt, about 2 mM of the salt and has a pH value of about 9.4, or/and

xxiv) the solution applied in the second isocratic step comprises about 65 mM of the buffer substance, about 90 mM of the elution salt, about 2 mM of the salt and has a pH value of about 9.4, or/and

xxv) the buffer substance is N-(1,1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropane sulfonic acid, the elution salt is tetramethyl ammonium chloride, and the salt is magnesium chloride, or/and

xxvi) a first isocratic step has a length of about 1 minute, the first linear gradient has a length of about 5 minute, a second isocratic step has a length of about 2 to 5 minutes and the second linear gradient has a length of about 5 minutes.

31. The method according to example 30 comprising items i), iii), xi), xiii), xvi), xxi), xxii), xxiv), xxv), or/and xxvi).

32. The method according to example 30 comprising items i), iii), xi), xiii), xvi), xxi), xxii), xxiv), xxv), and xxvi).

33. A method for separating full and empty recombinant adeno-associated virus particles using an anion exchange chromatography step,

wherein the method comprises a sequence of a applying a solution comprising empty and/or full rAAV particles to an anion exchange chromatography

v) the solutions used in the isocratic steps and linear gradients comprise as buffer substance N-(1,1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropane sulfonic acid, and

vi) the solutions used in the isocratic steps and linear gradients have a pH value of about 9.4, and

vii) the solutions used in the isocratic steps and linear gradients comprise tetramethyl ammonium chloride, and

viii) the method is performed at a flow rate of about 0.3 mL/min, and

ix) the solution applied in the first isocratic step comprises about 65 mM of the buffer substance, about 10 mM of the elution salt, about 2 mM of the salt and has a pH value of about 9.4, and

x) the solution applied in the second isocratic step comprises about 65 mM of the buffer substance, about 90 mM of the elution salt, about 2 mM of the salt and has a pH value of about 9.4, and

xi) the buffer substance is N-(1,1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropane sulfonic acid, the elution salt is tetramethyl ammonium chloride, and the salt is magnesium chloride, and

xii) the first isocratic step has a length of about 1 minute, the first linear gradient has a length of about 5 minute, a second isocratic step has a length of about 2 to 5 minutes and the second linear gradient has a length of about 5 minutes.

In one aspect, the present disclosure provides a method for separating full and empty recombinant adeno-associated virus particles using an anion exchange chromatography step,

wherein the method comprises a sequence of steps as follows:

a) applying a solution comprising empty and full recombinant adeno-associated virus (rAAV) particles to an anion exchange chromatography material inside a chromatography column,

b) performing a first isocratic step,

c) applying a first linear gradient,

d) performing a second isocratic step, and

e) applying a second linear gradient,

wherein the empty recombinant adeno-associated virus particles are eluted during the first linear gradient and the full recombinant adeno-associated virus particles are eluted during the second linear gradient,

wherein the solution comprising empty and full rAAV particles is a phosphate buffered saline solution comprising about 0.001 % (w/v) of a non-ionic detergent.

In addition to the various examples depicted and claimed, the disclosed subject matter is also directed to other examples having other combinations of the features disclosed and claimed herein. As such, the particular features presented herein can be combined with each other in other manners within the scope of the disclosed subject matter such that the disclosed subject matter includes any suitable combination of the features disclosed herein. The foregoing description of specific examples of the disclosed subject matter has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the disclosed subject matter to those examples disclosed.