CLAIMS

1. A method for isolating a population of recombinant adeno-associated virus (rAAV) particles from in-process impurities in a feedstream, comprising the steps of:

(a) contacting a feedstream containing the rAAV particles with an apatite chromatography medium in the presence of polyethylene glycol (PEG), wherein the rAAV particles bind to the apatite chromatography medium; and

(b) eluting the rAAV particles bound to the apatite chromatography medium with an elution buffer containing less than 3% (w/v) PEG.

2. The method of claim 1, wherein the apatite chromatography medium is ceramic hydroxyapatite (CHT).

3. The method of claim 1, wherein the apatite chromatography medium is ceramic fluoroapatite (CFT).

4. The method of claim 1, wherein the specific binding of the apatite chromatography medium to the rAAV particles is between 10^{14} and 10^{16} DNase-resistant particles per milliliter (DRP/mL).

5. The method of claim 1, further comprising a step of binding the rAAV particles in the feedstream eluted from the apatite chromatography medium to an anionic chromatography medium.

6. The method of claim 1, wherein the feedstream containing the rAAV particles in step (a) is contacted with an apatite chromatography medium in the presence of polyethylene glycol (PEG) and a basic buffer.

7. The method of claim 6, wherein the basic buffer is between pH 7.6 and 10.

8. The method of claim 6, wherein the basic buffer comprises borate.

9. The method of claim 1, wherein the PEG has an average molecular weight between about 5,000 (PEG5000) grams per mole and about 15,000 (PEG15000) grams per mole.

10. The method of claim 1, wherein the feedstream containing the rAAV particles in step (a) is contacted with the apatite chromatography medium in the presence of between about 3% (w/v) and about 10% (w/v) PEG.

11. The method of claim 1, further comprising a step of washing the apatite chromatography medium with a wash buffer after the feedstream is contacted with the apatite chromatography medium but before eluting the rAAV particles from the apatite chromatography medium.

12. The method of claim 11, wherein the apatite chromatography medium is washed one or more times with a wash buffer containing about 7.5% (w/v) PEG and/or a wash buffer containing about 5% (w/v) PEG.

13. The method of claim 12, wherein the apatite chromatography medium is further washed with a wash buffer containing less than about 3% (w/v) PEG and/or a wash buffer containing no PEG.

14. The method of claim 11, wherein the wash buffer comprises a buffer selected from the group consisting of borate, N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), and Tris-HCl.

15. The method of claim 11, wherein the wash buffer has a basic pH.

16. The method of claim 15, wherein the wash buffer further comprises between 100 and 500 mM of a phosphate.

17. The method of claim 15, wherein the wash buffer further comprises between 50 and 250 mM NaCl.

18. The method of claim 1, wherein the rAAV particles bound to the apatite chromatography medium are eluted with an elution buffer containing low concentrations of PEG or in the absence of PEG.

19. The method of claim 18, wherein the elution buffer comprises a buffer selected from the group consisting of borate, N-2-Hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), and Tris-HCl at neutral pH.

20. The method of claim 18, wherein the elution buffer contains less than about 3% (w/v) PEG6000.

21. The method of claim 20, wherein the elution buffer further comprises less than 100 mM phosphate.

22. The method of claim 21, wherein the elution buffer further comprises 50 mM phosphate.

23. The method of claim 22, wherein the elution buffer further comprises between 50 and 250 mM NaCl.

24. A method for isolating a population of recombinant adeno-associated virus (rAAV) particles from in-process impurities in a feedstream, comprising the steps of:

(a) contacting a feedstream containing the rAAV particles with a hydrophobic interaction chromatography (HIC) medium in a high salt buffer comprising citrate, wherein the rAAV particles and the in-process impurities bind to the HIC medium; and

(b) eluting the rAAV particles bound to the HIC medium with a medium salt buffer comprising citrate.

25. The method of claim 1, wherein the rAAV particles comprise an AAV capsid protein from an AAV capsid serotype selected from the group consisting of AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12, AAV-13, AAV-14, AAV-15 and AAV-16.

26. The method of claim 24, wherein the HIC medium is selected from the group consisting of Tosoh Butyl 650M, Tosoh SuperButyl 650C, Tosoh Phenyl 650C, and Tosoh Has(butyl) resin.

27. The method of claim 24, wherein the high salt buffer comprises between about 0.5 M and about 2.0 M citrate.

28. The method of claim 27, wherein the high salt buffer further comprises between about 1 and about 100 mM phosphate.

29. The method of claim 24, wherein the medium salt buffer comprises less than 0.5 M citrate.

30. The method of claim 29, wherein the medium salt buffer further comprises between about 1 and about 100 mM phosphate.

31. The method of claim 29, wherein the medium salt buffer comprises 0.2 M to 0.5 M citrate.

32. The method of claim 31, wherein a population of rAAV particles with empty capsids, partially denatured capsids, and/or partially full capsids are bound to the HIC medium after the elution with the medium salt buffer.

33. The method of claim 24, wherein the rAAV particles comprise an AAV capsid protein from an AAV capsid serotype selected from the group consisting of AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12, AAV-13, AAV-14, AAV-15 and AAV-16.

34. The method of claim 33, wherein the rAAV particles comprise an AAV capsid protein from an AAV capsid serotype selected from the group consisting of AAV-1, AAV-4, AAV-5, and AAV-8.