

shows the retention time of Ad11 virus and Ad5 virus when analysed by anion exchange chromatography. These viruses have very different retention times of about 10 vs 15 on the x-axis. Figure IB shows that the Ad11-type viruses such as EnAd, elute with the host cell proteins using anion-exchange chromatography. Thus, although ion-exchange chromatography is currently the gold standard for adenovirus purification, it is not effective for group B viruses, for example Ad11-type viruses, such as EnAd because these viruses behave differently from group C viruses, such as Ad5.

The state of the art for work in the field of GMP manufacture of adenoviruses has primarily been performed on Ad5, i.e. a group C adenovirus.

The present inventors have found optimal conditions and processes for the purification of adenoviruses differs depending on the adenovirus group. Adenoviruses are grouped based on DNA homology and/or their hexon, fibre and capsid properties in chromatographic analysis.

Developing a successful recombinant adenoviral purification process requires a detailed understanding of the recombinant virus, such as the interaction between the host cell line and the virus. Essentially the process requires adaptation depending on the particular group of viruses.

Surprisingly the present inventors have found that group B adenoviruses, for example Ad11-type adenoviruses, such as EnAd can purified away from host cell proteins using essentially a one diafiltration step employing a high concentration of salt in the buffer. This has not been possible using the standard prior art processes. In embodiments it is possible to completely omit ion-exchange chromatography from the process.

Accordingly, there is a need for an improved purification process specifically tailored for the production of Group B adenoviruses.

Summary

The present disclosure describes that group B adenoviral vectors can be purified by a process that significantly reduces the levels of contaminating host cell proteins in the final product. In one aspect, the present disclosure refers to a method for purifying a replication competent group B adenovirus from host cell proteins, comprising a purification step of: subjecting a composition comprising said group B adenovirus to diafiltration employing a diafiltration-buffer with a high salt concentration, wherein said salt concentration is at least 2M. In some examples, the present disclosure refers to the following:

1. A method for purifying a replication competent group B adenovirus from host cell proteins, comprising a purification step of:
subjecting a composition comprising said group B adenovirus to diafiltration employing a diafiltration-buffer with a conductivity of at least 180 mScm^{-1} , for example a conductivity of 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, or 290 mScm^{-1} .
2. A method according to paragraph 1, wherein the conductivity is provided by a strong electrolyte.

3. A method according to paragraph 2, wherein the electrolyte is a salt, such as an ionic salt (in particular a salt that is fully soluble and highly dissociated in water).
4. A method for purifying a replication competent group B adenovirus from host cell proteins, comprising a purification step of:
subjecting a composition comprising said group B adenovirus to diafiltration employing a diafiltration-buffer with a high salt concentration, wherein said salt concentration is at least

Claims

1. A method for purifying a replication competent group B adenovirus from host cell proteins, comprising a purification step of:
subjecting a composition comprising said group B adenovirus to diafiltration employing a diafiltration-buffer with a high salt concentration, wherein said salt concentration is at least 2M.
2. The method according to claim 1, wherein the salt concentration is in the range 2.5M to 5.5M.
3. The method according to claim 1 or 2, wherein the salt concentration is 4.3M.
4. The method according to any one of claims 1 to 4, wherein the diafiltration-buffer comprises a salt selected from a chloride salt, a sulfate salt, any salt fully soluble and dissociated in water, and combinations thereof.
5. The method according to any one of claims 1 to 4, wherein the diafiltration-buffer comprises one or more of the following: an alkaline earth metal salt, sodium acetate, Tris, Bis-Tris, NaH_2PO_4 , NaCl, and KCl.
6. The method according to any one of claims 1 to 5, wherein the diafiltration-buffer is selected from: meglumine buffer, glycine buffer, and TRIS buffer.
7. The method according to claim 6, wherein the diafiltration-buffer comprises HEPES.
8. The method according to claim 7, wherein the diafiltration-buffer comprises 50 mM HEPES.
9. The method according to any one of claims 1 to 8, wherein the diafiltration-buffer is at a pH in the range 7 to 9.8.
10. The method according to claim 9, wherein the pH is 7.5.
11. The method according to any one of claims 1 to 10, wherein the diafiltration employs an ultrafiltration membrane at least 300KDa or greater, MWCO.

12. The method according to any one of claims 1 to 11, wherein the diafiltration has a flow rate of 1 to 3m²/s.
13. The method according to any one of claims 1 to 12, wherein the diafiltration is carried out employing a hollow fibre cartridge or flat membrane cassette filter.
14. The method according to claim 13, wherein the diafiltration is performed using a consistent volume method.
15. The method according to any one of claims 1 to 14, wherein the diafiltration is performed using at least 8 diavolumes of high salt diafiltration-buffer.
16. The method according to claim 15, wherein the diafiltration is performed using 12 diavolumes of high salt diafiltration-buffer.
17. The method according to any one of claims 1 to 16, wherein the diafiltration process comprises two steps (i.e. a first step and a second step).
18. The method according to claim 17, wherein the first step of the diafiltration process is diafiltration with the high salt diafiltration-buffer.
19. The method according to claim 17 or 18, wherein the second step of the diafiltration process is diafiltration with the final formulation buffer.
20. The method according to any one of claims 1 to 19, wherein only one type of diafiltration-buffer is employed.
21. The method according to any one of claims 1 to 20, comprising a further purification step comprising subjecting the composition of adenovirus to a chromatography purification.
22. The method according to claim 21, wherein the chromatography purification comprises two chromatography steps.
23. The method according to claim 21 or 22, wherein the chromatography purification employs ion-exchange chromatography.
24. The method according to claim 23, wherein the chromatography purification employs anion exchange chromatography.

25. The method according to claim 24, wherein the anion exchange chromatography utilizes DEAE, TMAE, QAE, or PEI.
26. The method according to any one of claims 1 to 20, wherein the method does not employ chromatography.
27. The method according to any one of claims 1 to 26, wherein a crude cell lysate after addition of an endonuclease is filtered to clarify the adenovirus composition .
28. The method according to claim 27, wherein the crude cell lysate after addition of an endonuclease is filtered through a depth filter.
29. The method according to claim 28, wherein the depth filter has a specification of 4 to 2 μm .
30. The method according to any one of claims 27 to 29 wherein a second filter is employed in the clarification.
31. The method according to claim 30, wherein the second filter is a depth filter.
32. The method according to claim 31, wherein the second filter is a depth filter that has a specification of 1 to 0.4 μm
33. The method according to any one of claims 1 to 32, wherein the filtration step comprises passing the adenovirus composition through a 0.2 μm filter.
34. The method according to claim 33, wherein the filtration step is performed prior to the diafiltration step.