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Adenovirus purification by two-column, size-exclusion, simulated countercurrent chromatography

Piergiuseppe Nestola[a,b], Ricardo J.S. Silva[a], Cristina Peixoto[a], Paula M. Alves[a,b], Manuel J.T. Carrondo[a,b,c], José P.B. Mota[d,a,*]

[a] Instituto de Biologia Experimental e Tecnológica, Apartado 12, 2781-901 Oeiras, Portugal
[b] Instituto de Tecnologia Química e Biológica, Universidade Nova de Lisboa, 2780-157 Oeiras, Portugal
[c] Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal
[d] Requimte/CQFB, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal

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ABSTRACT

Adenovirus serotype 5 (Ad5) was successfully separated by size-exclusion chromatography (SEC) using a simple, yet efficient, two-column, quasi-continuous, simulated moving-bed process operated in an open-loop configuration. The operating cycle is divided into two identical half-cycles, each of them consisting of the following sequence of sub-steps: (i) elution of the upstream column and direction of the effluent of the downstream column to waste; (ii) elution of the upstream column and redirection of its effluent to waste while the downstream column is fed with the clarified bioreaction bulk and its effluent collected as purified product; (iii) operation of the system as in step (i) but collecting the effluent of the downstream column as product; (iv) elution of the upstream column and direction of its effluent to waste while the flow through the downstream column is temporarily halted. Clearance of impurities, namely DNA and host cell protein (HCP), were experimentally assessed. The pilot-scale run yielded a virus recovery of 86%, and a clearance of 90% and 89% for DNA and HCP, respectively, without any fine tunning of the predetermined operating parameters. These figures compare very favorably against single-column batch chromatography for the same volume of size-exclusion resin. However, and most importantly, the virus yield was increased from 57% for the batch system to 86% for the two-column SEC process because of internal recycling of the mixed fractions of contaminated Ad5, even though the two-column process was operated strictly in an open-loop configuration. And last, but not least, the productivity was increased by 6-fold with the two-column process. In conclusion, the main drawbacks of size-exclusion chromatography, namely low productivity and low product titer, were overcome to a considerable extent by an innovative two-column configuration that keeps the mixed fractions inside the system at all times.

1. Introduction

Viral vectors are playing an increasingly important role in the vaccine and gene therapy fields. Adenoviruses (Ads), in particular, are considered one of the most suitable platforms for production of viral vaccines and gene therapy vectors. They are medium-sized (90–100 nm), nonenveloped, icosahedral viruses composed of a nucleocapsid and linear, non-segmented double stranded DNA genome that is about 36 kb long. Their broad tissue tropism and large transgene packing capacity make them attractive candidates for innovative virotherapies [1]. Adenoviruses can be produced in a complementary cell line in both adherent and suspension culture systems, such as HEK-293 or PER-C6 cells, or A549 for oncolytic therapies [2,3].

The use of recombinant adenoviruses for vaccination and gene therapy requires fast and highly efficient purification protocols that yield high recoveries of infectious particles, maintain viral infectivity, and effectively remove contaminating DNA and host cell proteins (HCPs), while also concentrating the viral samples for final delivery. The concentration of adenoviruses is critical not only to obtain high titer vector stocks, but also to reduce the handled volume; the latter accelerates the downstream processing and keeps the scalability of the purification train at a manageable level [4].

* Corresponding author at: Requimte/CQFB, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal. Tel.: +351 21 2948300; fax: +351 21 2948550.

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The downstream biopurification train has been extensively developed in the past years by combining different chromatography steps, namely ion-exchange (IEX) and size-exclusion chromatography (SEC), and, less frequently, affinity chromatography, intermingled with concentration and ultra/diafiltration steps [1,5–9].

To be more specific, the classical approach for adenovirus purification consists of three major steps—clarification, concentration/purification, and polishing—applied sequentially: (i) clarification of the harvested bioreaction bulk to remove cell and cell debris; (ii) ultra/diafiltration; (iii) anion exchange; (iv) ultra/diafiltration to concentrate the product; and (v) SEC as a final polishing step. The SEC step is usually carried out last, mainly because of its low productivity and low product titer. Usually in this final step the amount processed is reduced by 50–100-fold.

The purpose of the clarification step is to efficiently remove cell debris and large product- or process-related aggregates, while maintaining and protecting the product quality in the flow-through stream. The concentration step reduces the volume of clarified bulk by effectively removing low molecular weight HCP, fragmented HC DNA, and possibly fragmented, product-related impurities, e.g., viral proteins. This step is a key factor for decreasing the upfront investment in downstream equipment and materials. If detergent was used to disrupt the cells, the diafiltration removes the detergent and associated lipids from the cells [10].

As with many bioactive therapies, there is a clear trend towards liquid chromatography as the core technique for vector purification, and its use is often integrated vertically within the DSP strategy, as it easily fits into the early capture stage as well as into the final purification phase. The use of high-performance liquid chromatography (HPLC) for large-scale adenoviral purification was first described by Huyghe et al. [11], and several approaches have been applied since then, including IEX, SEC, hydrophobic interaction, and immobilized metal affinity chromatography [12–14]. Unlike traditional processes based on CsCl-gradient purification, HPLC offers a straightforward linear scale-up path, and procedures for purifying up to around 10^{14} input particles have been reported.

IEX chromatography of virus particles is typically operated in positive (bind-and-elute) mode: most of the impurities are collected in the flow-through pool, while the virus particles and some of the impurities are retained in the resin. Due to the differences in charge of the different components, it is possible to use this process with high-resolution elution gradients, separating the adsorbed materials into fractionated cuts, even though they are closely related.

SEC and ultra/diafiltration (usually by tangential flow filtration) are two other widely used processes at the very latest stage for formulating the product [15,10,16]. However, purification schemes where SEC is followed by IEX have also been reported in the literature; two prominent examples are the works of Kalbfuss et al. [17] and Eglon et al. [1] for influenza and adenovirus, respectively. These schemes, when operated in batch mode, do not appear to be very cost efficient because they apply a low-productivity unit operation (SEC) at an early stage of the downstream train.

The increasing interest in vaccines and gene therapy, together with the need to decrease the cost per dose, has led to the development of new chromatographic tools or their conversion from other application areas [18–21]. One process-based way to reduce the overall cost of the downstream chromatographic steps is by changing to a continuous processing mode, which, in principle, yields higher throughput, lower buffer consumption, higher capacity utilization and reduced column volume, hence increased productivity. In particular, simulating moving bed (SMB) chromatography, which is the best practical implementation of continuous countercurrent solid-fluid chromatography [22,23], is now widely applied for the binary separation of small molecules, in particular chiral compounds under isocratic elution conditions [24–26].

Studies concerning continuous downstream bioprocessing have targeted mostly proteins and monoclonal antibodies (mAb) [28,29,27,30,31]; this is primarily because the biopharmaceutical industry is currently dominated by these bioproducts. To date, the continuous purification of large biomolecules, such as viruses, has rarely been explored [32]. Krober et al. [33] have recently implemented a classical three-zone, open-loop SMB for influenza virus purification. These authors have successfully increased the productivity by switching to simulated countercurrent operation, although the DNA and HCP clearances were not properly evaluated. In particular, since the bulk was not pretreated with benzonase, DNA co-eluted with the virus; also, HCP contaminants were not properly assessed due to the lack of an adequate ELISA kit for the utilized cell line (MDCK).

Despite the clear advantages of continuous chromatography, as demonstrated by the works cited above and others, the biopharmaceutical industry is somewhat skeptical about switching to continuous or quasi-continuous, multi-column chromatography. This is, in part, due to the fact that innovation in this industry has traditionally been more product- than process-oriented [27], but also due to the increased complexity in terms of process design and validation. However, the implementation of single-use and ready-to-process technologies are mitigating these issues. Therefore, there seems to be room for exploring compact and efficient, simulated countercurrent, multi-column chromatographic processes for biopurification.

The present work reports on the design and experimental validation of a simple quasi-continuous, open-loop, two-column countercurrent chromatographic process for size-exclusion purification of adenovirus serotype 5 (Ad5). As mentioned above, SEC has been used in the past for both polishing and intermediate purification of adenoviruses [1,34,13,35]. However, it is often claimed that the main drawbacks of SEC, namely low productivity and high product dilution, make it a costly purification step. Here, it is shown that these drawbacks can be eliminated to a large extent by switching from single-column batch operation to two-column SMB-type operation.

Because of the anticipated boost in performance obtained by switching to multi-column (quasi-)continuous operation, the Ad5 purification train employed for assessing the effectiveness of the enhanced SEC step was streamlined. Fig. 1 shows schematics of the standard and streamlined purification trains; in the latter case, the SMB–SEC step is applied after the first ultra/diafiltration step and replaces the last three steps of the standard purification train. The main reason for working with the streamlined purification train was to challenge the SMB–SEC step with a less purified bulk in order to prove that, once the classical limitations of SEC are alleviated, the standard purification train can be changed to meet specific needs in terms of cost reduction or purity requirements. Moreover, running the process with a less clean bulk gives more confidence on the performance assessment of the SMB–SEC step.

The paper is organized as follows. The materials and methods, including the physical realization of the two-column SMB, are first described. Then, the design of the SEC step and the numerical tools employed to predict and optimize its operation are discussed. We then proceed with the experimental validation of the two-column process, demonstrating the stability of its cyclic steady behavior and operating robustness. Impurity clearance, namely DNA and HCP, were experimentally assessed on a cycle-to-cycle basis. Finally, the performance of the streamlined purification train, evaluated with respect to impurity clearance, productivity, and yield, is compared to that of a similar train but operated with a standard