Optimising Viral Vector Purification Strategies with Multimodal Chromatography

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The global vaccine market is expected to surpass $85 billion USD by 2027, with an annual growth rate of c. 9% [1]. At the same time, the global gene therapy market is expected to surpass $5 billion USD, with an even faster annual growth rate of approximately 34% [2]. The successful use of virus preparations as nucleic acid delivery vectors or vaccines has been one of the most significant contributors to these rapidly expanding biomedical sectors. As the diversity and clinical application of viral-based products continues to grow, so do challenges during downstream processing. Taking clinical application, manufacturing scalability and inherent viral properties into consideration, chromatography can be utilised to streamline downstream processes, thus helping meet current needs.

Challenges in Downstream Virus Purification

Viruses are employed in numerous clinical applications ranging from gene therapy to vaccine development and cancer treatment. Contemporary downstream processes for viral particle purification have mostly been adapted from protocols originally developed for the purification of recombinant proteins and biologics, such as monoclonal antibodies (mAbs). Similar to protein isolation, downstream processes for virus purification must also ensure that essential virus aspects such as activity and stability remain intact throughout the entire production process [3]. However, the complex biophysical properties of viral particles pose some serious challenges that conventional protein purification protocols cannot effectively address. Unlike individual proteins, viral particles are much larger and are composed of a complex matrix of multiple proteins arranged in unique three-dimensional structures, known as capsids. Additionally, they are susceptible to damage by fluid shear stress conditions, non-physiological pH, surfactants and interfacial stress (Figure 1). Enveloped viral particles such as viral-like particles, retro- and lentiviruses are particularly susceptible to damage and possess additional surface characteristics (e.g., a lipid bilayer) and/or have undergone post-translational surface modifications (e.g., glycosylation) which can further complicate downstream processes [4].

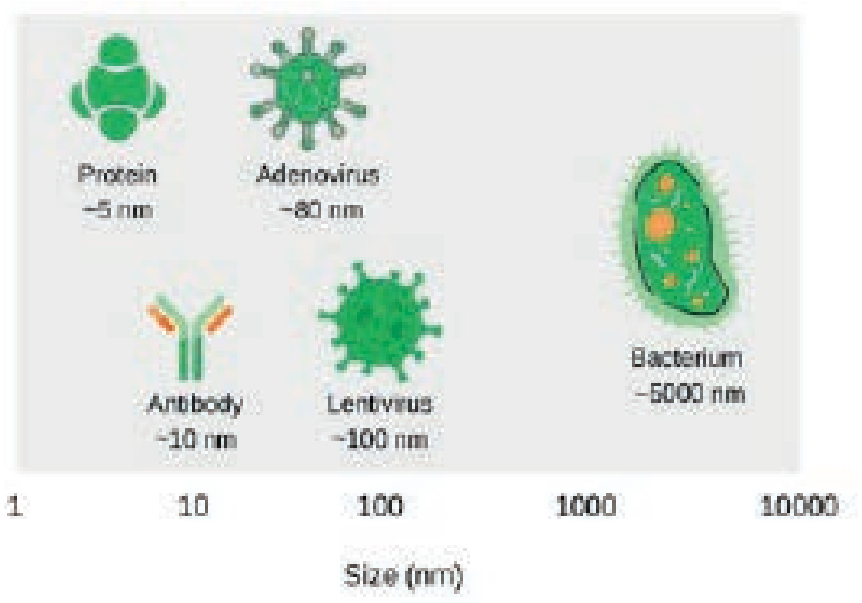

Figure 1: Average size comparison between biomolecules and cells

Typical cell culture impurities resulting from upstream processes should also be taken into consideration when determining which purification approach to follow. For example, adenoviruses and adeno-associated viruses (AAV), some of the most extensively researched vectors, are harvested from lysed cell cultures.

This inevitably introduces cell debris and other impurities into the virus preparation that need to be removed prior to product isolation, such as host cell DNA and proteins, endotoxins, foreign viral contaminants, and virus-DNA aggregates [5], which may compromise safety and efficacy. Upstream processes can also result in the production of damaged or empty viral particles that lack therapeutic payload or activity and viruses that encapsulate the wrong genetic material. Downstream purification processes have to be tailored to the needs of a particular product, taking into consideration the properties of specific viruses and impurities resulting from upstream virus production and harvesting approaches.

Traditionally, downstream viral purification protocols often involved laborious steps such as density gradient ultracentrifugation - while sufficient for proof-of-principle experiments, such techniques can be difficult to scale and may not meet the stringent purity standards of therapeutic products [6]. Consequently, the biopharmaceutical industry is leveraging the capabilities of chromatography in downstream processes to help meet large-scale virus purification requirements and achieve high viral recovery yields [5, 6].

Scaling-Up Downstream Processes Using Chromatography

Often, downstream processes for protein purification are comprised of an initial protein capture step, which for mAbs includes affinity chromatography (AC) using Protein A resin, followed by two polishing steps that take into consideration the physicochemical properties of the protein of interest, and help remove any remaining impurities prior to product isolation. Mirroring protein purification approaches, optimal virus purification could be obtained by combining steps based on distinct interaction modes between the stationary (resin) and mobile (solute) phase - for example, one step based on viral particle charge and another step based on its hydrophobicity.

AC could be applied in downstream virus purification processes by leveraging the ability of viral surface protein motifs to bind to specific cellular ligands, thus helping to isolate the viral particle of interest. A significant limitation of this approach is that a ligand used for initial AC capture is directed toward existing serotypes, resulting in time-consuming ligand design every time a new viral serotype or platform emerges - making such an approach unsuitable for large-scale viral vector and vaccine manufacturing [3].

Ion Exchange Chromatography

Ion exchange chromatography (IEX) is an effective alternative to AC and is commonly used for initial mass virus capture across the biopharmaceutical industry [4,5,6]. An ion-exchanger exploits the differences in net surface charge between the viral particle of interest and other biomolecules present in the solution. Nucleic acids for example, will always be negatively charged due to the presence of ionised phosphate groups in the backbone, while virus net charge depends on the proportion of surface charged amino acids at a particular pH [5].

In the case of negatively charged retroviruses and adenoviruses, nuclease treatment of the feedstock followed by single-step chromatography utilising anion exchange chromatography (AEX) resins (e.g., Nuvia HP-Q) has been shown to be an effective mass capture step well-suited for the production of clinical-grade viral vectors [4, 9]. Unlike cation-exchangers, anion-exchangers typically bind viruses under pH and ionic strength (salt concentration) conditions consistent with those in upstream bioreactor feed-streams improving both process economics and efficiency. AEX can also be employed as a polishing step, to help separate empty and full viral capsids based on subtle charge differences and remove negatively charged impurities from the feedstock [8, 9]. Cation exchange chromatography (CEX) resins (e.g., Nuvia S) can be used to capture viruses, and are also commonly used in polishing steps, to successfully remove the majority of process- and product-related impurities [5, 10].

Considering that viral particle integrity is susceptible to environmental fluctuations which includes pH changes, different buffer composition, temperature alterations and shear stress, it is crucial to design a downstream processing platform capable of achieving the highest levels of purity with the minimal number of steps, to maximise quality, efficiency and safety.

Improving Downstream Process Efficiency with Multimodal Chromatography

The emergence of multimodal (or mixed-mode) chromatography has given rise to highly selective media designed to simplify the purification process, reduce downstream processing time and improve overall process economics. Mixed-mode resins combine ligands capable of at least two modes of interaction, such as affinity, size exclusion, ion exchange, and hydrophobic interactions in a single purification step. Multimodal chromatography allows for the optimal purification of viral particles that would be ineffectively purified by sequential single-mode approaches.

For example, combining the properties of hydrophobic interaction and cation exchange in a single hydrophobic cation exchange resin allowed for optimised capture of recombinant adenovirus [7]. Specifically, a hydrophobic cation exchange resin (e.g., Nuvia cPrime) was used in a capture step as it offered the best clearance from negatively charged feed-stream contaminants (e.g., animal serum) and resulted in a tenfold reduction in processing volume. Eluate from this resin could then be loaded into the second and final anion exchange column (e.g., Nuvia Q) which resulted in an additional two-fold reduction of product volume and optimised product purity comparable to clinical grade products [7]. Leveraging multiple modes of interaction, such two-step approaches enhance separation of viral particles from impurities in a more cost- and time-effective manner.

Similarly, hydroxyapatite chromatography (HAC) mixed-mode media, with the ability to bind a wide range of viral particles, can be used effectively for intermediate or final polishing. The mixed-mode capabilities of HAC can separate molecules based on calcium affinity and cation exchange interactions. Single-step HAC has successfully been used for the separation of both enveloped (e.g., dengue virus, Japanese encephalitis virus, influenza, and mouse hepatitis virus) and non-enveloped (e.g., adenovirus, feline calicivirus and poliovirus) mammalian viruses from impurities, resulting in concentrated preparation of highly active virus [11, 12].

Conclusions

As the application of viral vectors in gene therapy and vaccine development continues to grow, so does the demand for faster product development and manufacturing. Complexities associated with the physicochemical properties of viral particles present unique purification challenges, especially in a large-scale manufacturing setting. Chromatography has emerged as a highly selective and yet robust scalable method for virus purification - specifically, mixed-mode media innovations can be the driving force behind faster and more cost-effective product development and manufacturing. Unlike single-mode chromatography resins, mixed-mode resins allow for (i) effective separation of molecules that appear homogeneous using other chromatographic methods, (ii) selective single-step removal of impurities, (iii) establishment of optimal binding and elution conditions due to large design space, (iv) minimal feedstock processing due to robust salt tolerance, and (v) large capacity high-titre feed-streams [13].

Application of multimodal chromatography in downstream purification processes can significantly improve the manufacturing process of such therapeutic modalities to help meet the ever-increasing demands for safe and effective gene therapies and viral vector vaccines.

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