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# Sorption processes in ion-exchange chromatography of viruses

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**Abstract**

Purified viruses are used in gene therapy and vaccine production. Ion-exchange chromatography (IEC) is the most common method for large-scale downstream purification of viruses and proteins. Published IEC protocols provide details for specific separations but not general methods for selecting operating parameters. To make the selection more systematic, we study adenovirus type 5 (Ad5) as a model virus and develop batch uptake and light scattering methods for optimizing the ionic strength and pH of adsorption, as well as providing heuristics for resin geometry. The static capacity for Ad5 was found to go through a maximum with increasing ionic strength. Comparison to a protein–resin system shows that resin capacity for the virus is at least an order of magnitude lower, even on a wide-pore resin. Virus penetration into the wide-pore resin is only partial and the uptake rate is an order of magnitude slower than the uptake onto a narrow-pore resin.


*Keywords:* Ion-exchange chromatography; Virus; Adenovirus type 5; Pore size distribution; Aggregation; $\zeta$ Potential; Isoelectric point; Binding capacity; Virus adsorption

## 1. Introduction

Although early applications of virus purification by chromatography date back almost half a century [1–3], the technique did not begin to advance until the 1990s, when gene therapy clinical trials created a need for large quantities of purified viruses. In addition to gene therapy, virus purification is also necessary for production of some vaccines and in cellular and molecular biology research. While some viruses can be oncogenic, they are also used for treatment of cancers [4–6].

Virus purification has traditionally been carried out by CsCl density gradient ultracentrifugation, but scale-up of this method is not practical, so it cannot meet the rising demand for purified viruses [7,8]. Additionally, density gradient centrifugation often gives variable degrees of purity, poor yields, it is not serotype specific [7], and it can require subsequent removal of CsCl [9].

The large size of viruses suggests the use of filtration as an alternative for purification. However, filtration through pores that are smaller than viruses is used primarily to remove viruses as opposed to purifying them because it often leads to aggregation of the highly concentrated particles near the filter [10,11]. Filtration through pores that are larger than viruses is frequently used to remove large impurities that remain in the supernatant after centrifugation of cell lysate [11–15].

Following the removal of large impurities, further purification is typically done by ion-exchange chromatography (IEC), which is also used in a majority of protein purification processes. Unlike ultracentrifugation, IEC is scalable, which makes it suitable for commercial production. Chromatography is also substantially faster, more consistent, and allows for system automation [13]. The purity achieved by an IEC step can match that of double CsCl gradient centrifugation [8,16]. One disadvantage of IEC, however, is that it cannot remove empty capsids [15]. Although purification of viruses by column chromatography is thought by many to be a prerequisite for large-scale production, the technique is still problematic and poorly understood.

In virus purification, IEC is usually employed as the capture step. Even though single-step purification has been reported [9,17], most processes require further polishing. Size-exclusion chromatography (SEC) is a typical polishing step [11,18,19], but SEC by itself cannot remove large contaminants. Chromatographic techniques other than IEC generally give lower purity and yield [8]; hydrophobic-interaction chromatography, for example, often results in low recovery and viral degradation [10].

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The investigation here focuses on IEC in packed beds, the most widely used format for chromatography of both viruses and proteins, particularly on a large scale. Monoliths are becoming increasingly popular for separation of viruses because they allow rapid convective mass transfer [20,21], but it is difficult to maintain uniform properties in bigger monoliths, so the largest ones are still two orders of magnitude smaller in volume than some industrial packed beds. The advantages of membranes, a special case of the monoliths, have also been demonstrated for purification of large macromolecules [22], but the capacity limitations are even greater.

The model virus chosen for this study is adenovirus type 5 (Ad5). The virus is composed of DNA enclosed in an icosahedral protein capsid (approximate diameter 80 nm [23,24]) with 37 nm fibers extending from each of its 12 vertices [25]. Adenoviruses are the most common vectors for gene therapy [26] and Ad5 is the most widely used serotype [7]. Ad5-based vaccines have also been developed [27,28].

The extensive use of Ad5 has created a substantial amount of literature on the virus and its purification by chromatography. Table 1 lists select details of successful IEC protocols for Ad5 capture, one of which uses a monolithic column [14], while the rest use conventional packed beds [7–9,12,13,15,17–19,29,30]. In addition to the usual low-salt loading and high-salt elution conditions, sucrose, magnesium, and/or glycerol are sometimes added to the base buffer to help stabilize the virus, particularly during freezing and thawing. The additives generally do not interfere with the chromatography [13].

It is important to note the consistency of certain parameters among the different protocols in Table 1: the operating pH range is $7.5 \leq \text{pH} \leq 9$, the range of NaCl concentration during loading is $0 \leq C_{\text{NaCl}} \leq 0.3$ M, and, in order to elute the virus, $C_{\text{NaCl}}$ is raised to $\geq 0.6$ M. With the exception of the monolith, the average resin pore diameter is too small for Ad5 to enter, so only the external surface area of these resins is utilized.

Although published protocols provide useful ways to purify specific viruses, little rationale is provided for selecting particular operating conditions. These independent variables, such as buffer and stationary phase characteristics, are selected so as to optimize the resulting dependent variables, such as dynamic binding capacity. This work seeks to develop approaches for making such selection more systematic by focusing on understanding the underlying mechanisms. Instead of creating a specific purification process, the goal is to develop general strategies for purifying viruses by IEC. We use batch contacting of the model virus and chromatographic resins to study the effects of various system parameters on virus uptake. A major goal is to determine relationships between the independent and dependent variables and to find parameters that are most influential in affecting particular figures of merit. The insights gained will aid in finding methods and heuristics that ease development and optimization of chromatographic processes.

## 2. Materials and methods

### 2.1. Adsorbates

Recombinant adenovirus type 5 (Ad5-CMV-GFP, further abbreviated here as Ad5) was purchased from Baylor College of Medicine/Vector Development Laboratory (Houston, TX, USA). The replication-deficient virus was supplied at an approximate concentration of $5 \times 10^{12}$ p/mL in 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.15 M NaCl, pH 7.8, and 10% glycerol. This stock solution was stored at −80 °C prior to use. Storage at such low temperatures usually eliminates loss of infectivity [27]. Ovalbumin (Sigma A5503, lot 032K7029) was used as a model protein for comparison with Ad5. The molecular weight of ovalbumin is 44.3 kDa and its isoelectric point (p*I*) is about 4.5, which is approximately the same as the p*I* of Ad5 found in this study.

### 2.2. Light scattering measurements of particle size and ζ potential

The ζ potential and aggregation state of Ad5 were determined by light scattering on a Zetasizer Nano ZS particle analyzer (Malvern Instruments ZEN3600) using folded capillary cells (Malvern DTS1060). The particle radius was found from the diffusivity using the Stokes–Einstein equation and the ζ poten-

Table 1
Details of IEC steps in published protocols for Ad5 purification

| References | Intended use | pH | Base buffer | $I_b$ (mM) | $C_{\text{NaCl}}$ (M) | | Stationary phase | $d_{\text{pore}}$ (nm) |
|---|---|---|---|---|---|---|---|---|
| | | | | | Load | Elute | | |
| [13] | Preparative | 7.5 | 50 mM HEPES | 21 | 0.3 | 0.6 | Fractogel DEAE-650M | 80 |
| [15,19] | Preparative | 7.5 | 50 mM PBS | 132 | 0.26 | 0.6 | | |
| [12] | Preparative | 8 | 50 mM Tris/HCl | 30 | 0.25 | 0.7 | | |
| [12–15,30] | Analytical | 7.5 | 50 mM HEPES | 21 | 0.3 | 0.6 | SOURCE 15Q | 65 |
| [29] | Preparative | 8 | 50 mM Tris/HCl | 30 | 0 | 0.75 | | |
| [7] | Analytical | 7.5 | 50 mM HEPES | 21 | 0.2 | 0.6 | | |
| [14] | Analytical | 7.5 | 5 mM HEPES | 2 | 0.1 | 0.6 | UNO Q monolith | 1000 |
| [18] | Both | 8 | 20 mM Tris/HCl | 11 | 0.25 | 1 | Q Sepharose XL | 12 |
| [9,17] | Preparative | 8 | 50 mM Tris/HCl | 30 | 0 | 1 | | |
| [8] | Preparative | 9 | 20 mM Tris/HCl | 2 | 0.25 | 2 | Toyopearl Super Q 650M | 100 |

$I_b$ is the ionic strength due to the base buffer and $d_{\text{pore}}$ is the average resin pore diameter.