Purification of adenoviral vectors using anion exchange chromatography

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Introduction

Modified viruses are becoming the most commonly used vectors in gene therapy. The viral vector can deliver the genetic material to the target cell in the patient but it cannot replicate in the patient. Adeno and retrovirus are the two vectors most frequently used as delivery vehicles because of a high transduction yield.

Traditionally, purification of virus/viral vectors to high purities is done by cesium chloride density gradient centrifugation. The work presented in this poster illustrates that one-step anion exchange chromatography purification on SOURCE™ 15Q is a very attractive alternative to gradient centrifugation for the production of adenoviral vectors. Purification of adenoviral vectors with anion exchange chromatography is also more efficient with respect to time and costs than preparative cesium chloride density gradient centrifugation.

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Experimental and Results

The adenoviral vector was expressed in a mammalian cell culture system. The cells were lysed by three freeze-thaw cycles. After centrifugation to remove cell debris, the supernatant was applied directly to the chromatography column.

The chromatography protocol was developed at small-scale using RESOURCE™ Q, a 1 ml column pre-packed with SOURCE 15Q media. To confirm the scalability of both the purification protocol and the SOURCE media used, the run was scaled up 16-fold using a hydraulically packed FineLINE™ Pilot 35 column (i.d 35 mm).

An ÄKTA™*explorer* 100 automated chromatography system was used for the chromatography experiments. Anion exchange chromatography was performed in a 50 mM Tris™/HCl buffer, pH 8.0. Elution was performed with a linear gradient from 0 to 0.75 M NaCl in 50 mM Tris/HCl, pH 8.0, for 15 column volumes, followed by a step to 1.0 M NaCl. In all experiments the flow rate was 20 cm/h. Detection was set at 260 and 280 nm. Between each run the column was cleaned with two column volumes of 1.0 M NaOH. An alternative cleaning procedure included a step with 1.0 M NaCl in a weak phosphoric acid solution, which is highly effective for removing residual nucleic acids from the chromatography media.

To illustrate the performance of SOURCE 15Q media, an adenoviral vector purified by cesium chloride density gradient was run on a 1 ml RESOURCE Q column. The chromatogram is shown in Figure 1.

Sample volume and type:	100 µl virusprep., Ad-CMV-46-ßgal (2-25-99) LY
Column:	RESOURCE Q 1 ml
Eluent A:	50 mM Tris/HCl, pH 8.0
Eluent B:	A + 1.0 M NaCl
Flow:	0.2 ml/min

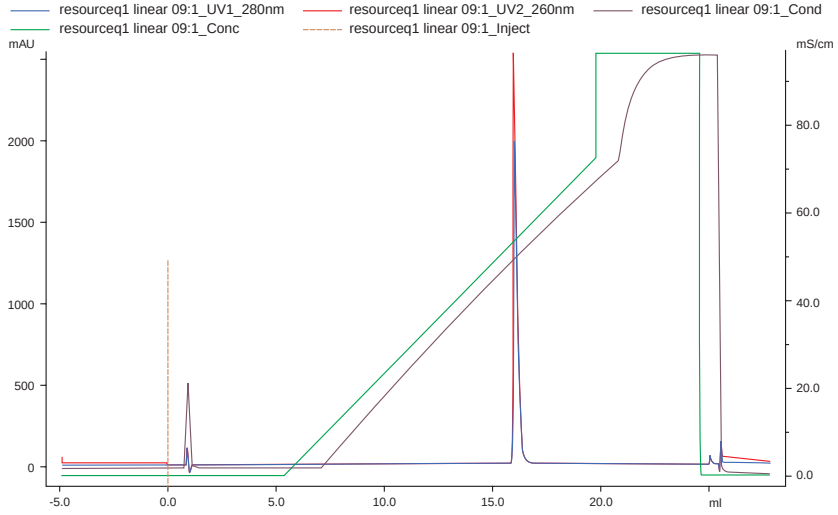


Figure 1.
Adenoviral vector on SOURCE 15Q.

Sample volume and type:	0.5 ml, Ad-RSV-ßgal
Column:	RESOURCE Q 1 ml
Eluent A:	50 mM Tris/HCl, pH 8.0
Eluent B:	A + 1.0 M NaCl
Gradient:	0 to 75% B in 15 CV
Flow:	20 cm/h

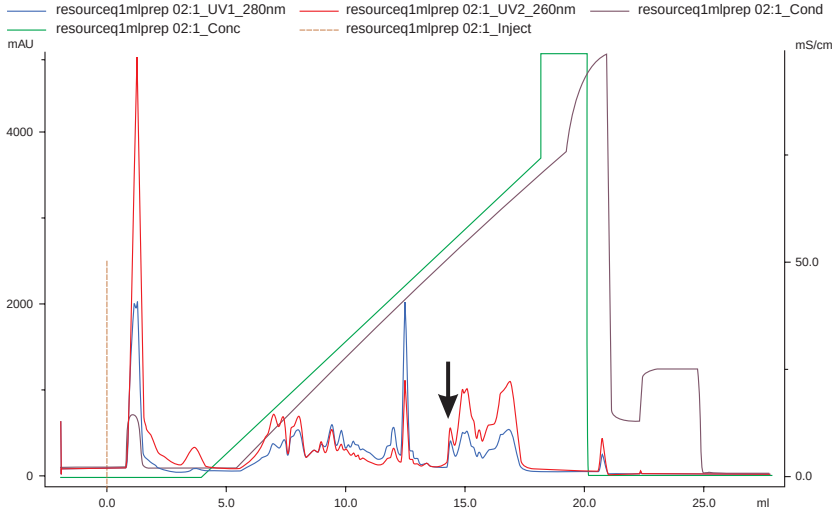


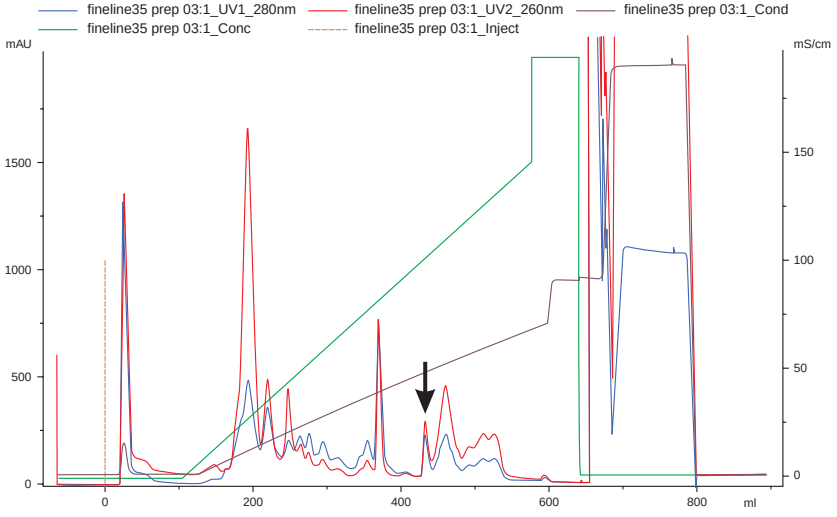
Figure 2.
Purification of an adenoviral vector on a 1 ml RESOURCE Q column. Adenoviral containing peak indicated by arrow.

Method development was carried out on a 1 ml RESOURCE Q column. In each run 0.5 ml of clarified lysate was used; pH and gradient shape and slope were observed. The best results were obtained with the conditions described above (i.e. a 15 column volume linear gradient from 0 to 0.75 M NaCl in a 50 mM Tris/HCl buffer, pH 8.0). Figure 2 shows a purification run at this scale using the optimized conditions.

To test the scalability of SOURCE 15Q media and the purification protocol, the process was scaled up 16-fold using a FineLINE Pilot 35 column. The 35 mm diameter column was packed to a bed height of 33 mm. The bed height of the 1 ml RESOURCE column is 30 mm. The binding and elution conditions were kept the same as in the small-scale experiment. Linear flow rate was also kept constant at 20 cm/h. Figure 3 shows the resulting chromatogram from a run with a sample of 8.0 ml clarified adenoviral vector lysate. Figure 4 shows an exploded portion of the chromatogram. In other experiments (data not shown), sample volumes of up to 15.5 ml of the virus preparation were purified on FineLINE 35 Pilot column, corresponding to a 31-fold scale up. The overall results were consistent with the smaller scale. Collected fractions were analyzed for purity and yield. On a 50 flask preparation scale (175 cm^2 per flask), chromatography purification of the adenoviral vector gave 25 to 50% greater yield than the centrifugation method. The purity of the viral vector was confirmed by gel electrophoresis, with both Coomassie™ Blue and Silver Staining.

Sample volume and type:	8.0 ml of Ad-RSV-ßgal (3-31-99)
Column:	SOURCE 15Q, FineLINE Pilot 35, bed heigh; 3.3 cm
Eluent A:	50 mM Tris/HCl, pH 8.0
Eluent B:	A + 1.0 M NaCl
Gradient:	0 to 75% B in 15 CV
Flow:	20 cm/h

Figure 3.
Purification of an adenoviral vector on a FineLINE Pilot 35 column packed with SOURCE 15Q. The adenoviral vector containing peak indicated by arrow.



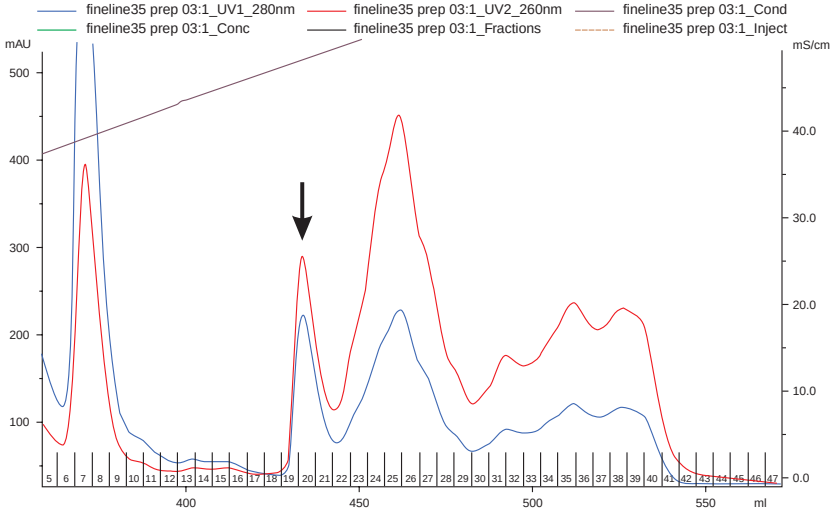


Figure 4.
Magnification of part of chromatogram shown in Figure 3.

Adenoviral vector containing peak indicated by arrow.

Discussion

Because of the large size of the vector, approximate diameter 20 nm, only the surface of the chromatography medium is utilized for binding. High binding capacity for viral particles is best achieved with small particle diameter beads. With SOURCE 15Q, the small 15 µm diameter particles give a large surface area per volume of packed bed and are ideal for the purification of such vectors.

The purity of the adenoviral vector purified by chromatography corresponds with the purity obtained with preparative cesium chloride density gradient centrifugation. However, the yield of vector is well above that achieved with centrifugation.

The chromatographic purification of an adenoviral vector on SOURCE 15Q media was shown to be easily scalable up to 31-fold. The process went from a small 1 ml column to pilot scale without any modification in the purification procedure. This purification method should be scalable even further as numerous other applications on SOURCE 15Q media have been scaled up considerably more.

The different chromatograms shown in Figures 1-4 illustrate that the purification of adenoviral vectors can be achieved with high efficiency and high resolution, which are retained when the method is scaled to pilot-scale.

Chromatographic purification of adenoviral vectors is a very attractive alternative to preparative cesium chloride density gradient centrifugation. It is easily scaled to industrial production scale and at a lower cost compared with centrifugation. Chromatographic purification is also considerably faster: 5 hours compared with about 24 hours for comparable runs with traditional cesium chloride density centrifugation separation. Chromatographic purification thus allows more vectors to be purified per time unit. Furthermore, only relatively inexpensive buffers and salts are used in chromatography, and removal of cesium chloride from the vector is not be an issue as it is for adenoviral vectors purified by centrifugation.

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