Mammalian Virus Purification Using Ceramic Hydroxyapatite

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Tech Note

Process Separations

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Abstract

Conventional techniques for mammalian virus purification produce variable quality, quantity, and significant loss of particle infectivity. Here, we propose the chromatographic separation of viral particles of diverse sizes and from different families, such as dengue, Japanese encephalitis, influenza, mouse hepatitis, adenovirus, poliovirus, and feline calicivirus, using CHT™ Ceramic Hydroxyapatite Media (Resin). The separation of viral particles from impurities in at least one case was best observed on CHT Ceramic Hydroxyapatite Type II Media when compared to three other apatite media, suggesting the importance of determining which media works best for a specific virus. Additionally, factors such as adjusting the flow rate and gradient slope showed variable differences in the separation of viral particles on ceramic hydroxyapatite media.

Introduction

Viruses can infect mammalian cells and cause diseases such as influenza, hepatitis, yellow fever, smallpox, and AIDS. Since some biotherapeutic products are produced using mammalian cell lines or plasma, the risk of viral contamination in these products is a concern and guidelines have been enforced to alleviate this risk. Chromatographic separation of viral particles from process intermediates is a key part of ensuring viral safety in biotherapeutics (ICH Expert Working Group 1999, Möritz 2005). Additionally, purification of viral particles is used extensively in the study and characterization of these infectious agents. Understanding aspects of a virus, such as how it infects host cells, uses the host cells for reproduction, and evades the host immune system, aids scientists in determining how to use viruses for research and therapy.

In order to study a virus, a pure, high-quality, infectious population is required. Conventional techniques for mammalian virus purification, for uses such as vaccine production or biological studies, can produce material of variable quality and quantity, often with significant loss of particle infectivity.

In this paper, we report the use of ceramic hydroxyapatite media for purification of a wide variety of mammalian viruses. Chromatography using ceramic hydroxyapatite media is simple, easily scalable, and results in a concentrated preparation of highly active virus.

Materials and Methods

The viral particles used in this study are shown in Table 1.

Table 1. Viral type and size.

Virus	Family	Genus	Genome	Envelope	Size, nm
Dengue	Flaviviridae	Flavivirus	ssRNA	+	50
Japanese encephalitis	Flaviviridae	Flavivirus	ssRNA	+	50
Influenza	Orthomyxoviridae	Influenzavirus	ssRNA	+	80–120
Mouse hepatitis	Coronaviridae	Coronavirus	ssRNA	+	100–150
Adenovirus	Adenoviridae	Mastadenovirus	dsDNA	–	90
Poliovirus	Picornaviridae	Enterovirus	ssRNA	–	30
Feline calicivirus	Caliciviridae	Vesivirus	ssRNA	–	30–38

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Viral Activity Assays

Viral activity was determined using the assays shown in Table 2. Protein contaminants were detected by UV absorbance at 280 nm and SDS-PAGE analysis. DNA derived from host cells was quantified using the Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies Corporation, USA).

Table 2. Detection methods used for viral activity.

Detection Method	Virus
Hemagglutination (HA) test	Dengue, influenza, adenovirus
Plaque assay	Japanese encephalitis
50% tissue culture infective dose ($TCID_{50}$)	Poliovirus, feline calicivirus, mouse hepatitis

Standard Chromatography Protocol

Chromatography was performed using Bio-Rad's BioLogic DuoFlow™ System. Columns (4.6 x 35 mm, Sugiyama Shoji Co., Ltd., Japan) with a 10-µm frit were packed with 40-µm CHT Ceramic Hydroxyapatite Type II Media. Frit pore size was important, as smaller porosities significantly reduced virus recovery (Y. Kurosawa, unpublished data). The flow rate was 1 ml/min. The purification protocol is outlined in Table 3, unless otherwise noted.

Table 3. Standard purification protocol.

Step	Mobile Phase	pH	Volume, ml
Wash	600 mM sodium phosphate	7.2	5
Equilibration	10 mM sodium phosphate	7.2	10
Sample loading	10 mM sodium phosphate	7.2	10
Wash	10 mM sodium phosphate	7.2	10
Elution	Gradient elution from 10–600 mM sodium phosphate	7.2	15
Wash	600 mM sodium phosphate	7.2	5

Results

Dengue Virus

Figure 1A shows the recovery of dengue virus type 2 from cell culture fluid. HA activity was recovered near the end of the gradient, separated from the bulk of A_{280}-absorbing material and from dsDNA (Kurosawa et al. 2012b). Figure 1B demonstrates that decreasing the flow rate by tenfold improves the sharpness of the elution peaks and, hence, separation. In both cases, recovery of HA activity was greater than 95%. Recent studies have indicated that adsorption of dengue virus particles to the surface of CHT Type II Media is similar to their adsorption to cells (Saito et al. 2013).

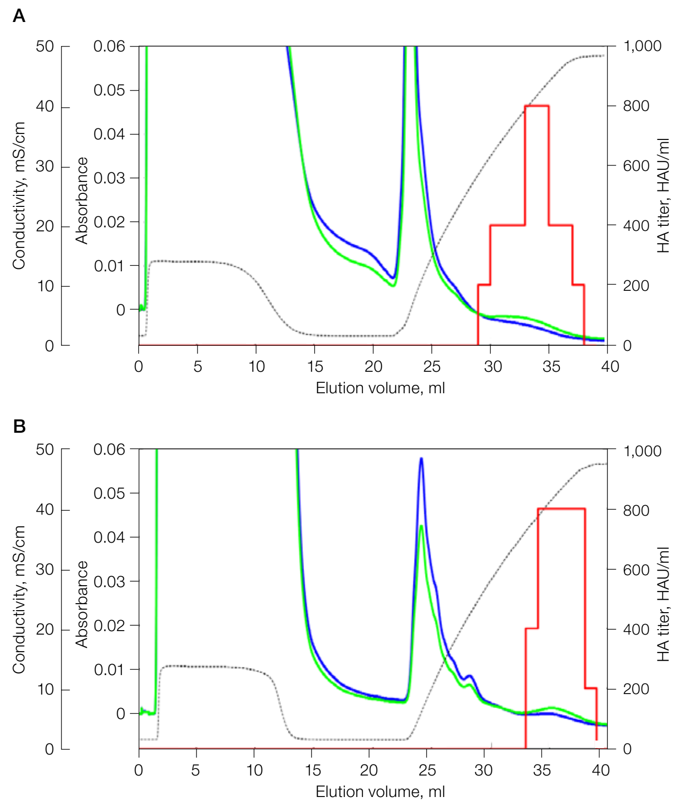

Fig. 1. Chromatograms of the separation of dengue virus type 2 by CHT Type II Media. A, flow rate at 1.0 ml/min; **B**, flow rate at 0.1 ml/min. UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity (- -); viral activity in HA test (—).

Other serotypes of dengue virus also bound to and eluted from CHT Type II Media. The approximate elution points in the sodium phosphate gradient for each serotype are shown in Table 4. Types 2 and 4 eluted at roughly the same position.

Table 4. Elution points of dengue serotypes in a sodium phosphate gradient.

Virus Serotype	Approximate Elution Point, mM
1	250
2	450
4	425

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Influenza Virus

Chromatography of influenza virus A/Beijing/262/95 and A/Panama/2007/99 (Schickli et al. 2001) cultured in the presence of 0.02% and 0.20% BSA, respectively, is shown in Figure 2.

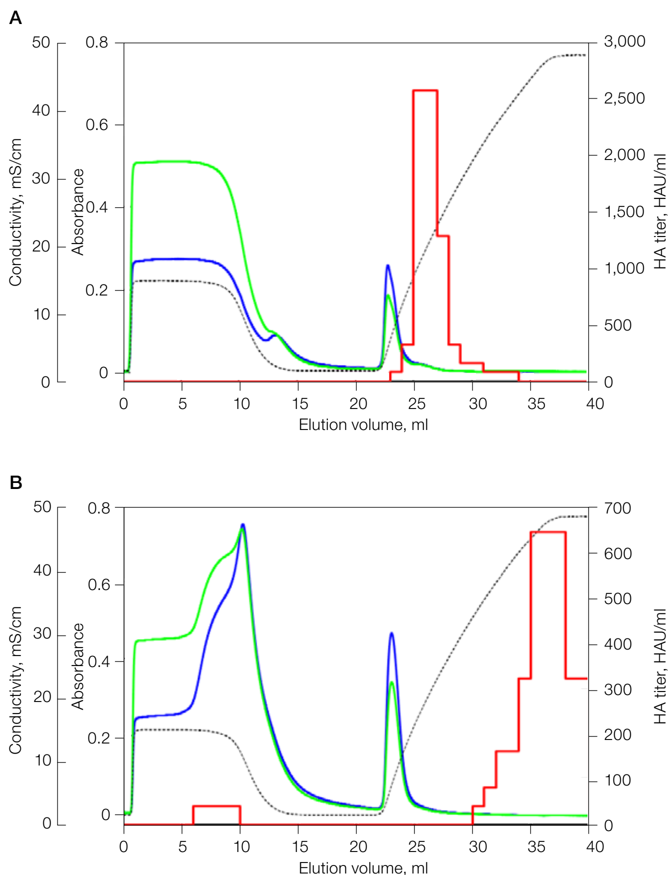

Fig. 2. Chromatography of influenza virus. A, A/Beijing/262/95; **B**, A/Panama/2007/99. UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity (—); viral activity in HA test (—).

HA activity is separated from a small BSA peak and a significant amount of material that did not bind to the column. Recovery, as measured by the HA assay, was 98% for the A/Beijing/262/95 virus. Higher concentrations of sodium phosphate are required to elute the A/Panama/2007/99 virus. In addition, the retention time was not affected by the source (allantoic fluid vs. cell culture; data not shown).

Mouse Hepatitis Virus

Mouse hepatitis virus (MHV) is a coronavirus (CoV), a genus that includes SARS-CoV. Two strains of MHV (MHV-NuU and MHV-S) (Hirano et al. 1981) were applied and bound to CHT Type II Media. Both were eluted at 26–28 minutes in the gradient (Figure 3).

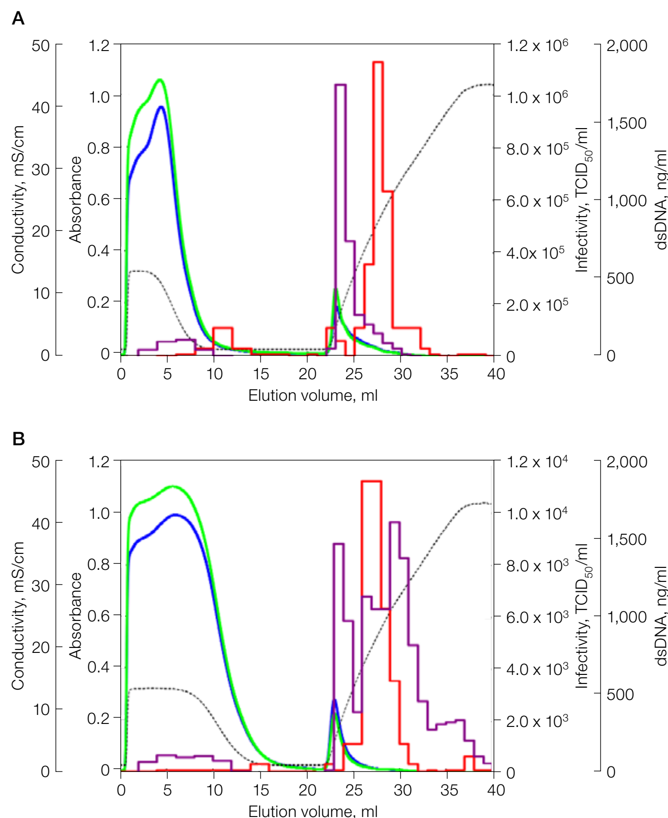

Fig. 3. Chromatography of two strains of MHV (A, MHV-NuU; B, MHV-S). UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity (- -); viral infectivity in $TCID_{50}$ (—); dsDNA (—). Culture fluid contained 10% fetal bovine serum (FBS).

Nonenveloped Viral Particles

Nonenveloped viral particles can be purified by ceramic hydroxyapatite chromatography in the same way as enveloped viruses. Adenovirus (AdV) type 27, feline calicivirus (FCV) A391 (Hirano et al. 1986), and poliovirus (PV) Sabin type 2 all adsorbed to CHT Type II Media (Figure 4), although they showed different elution times.

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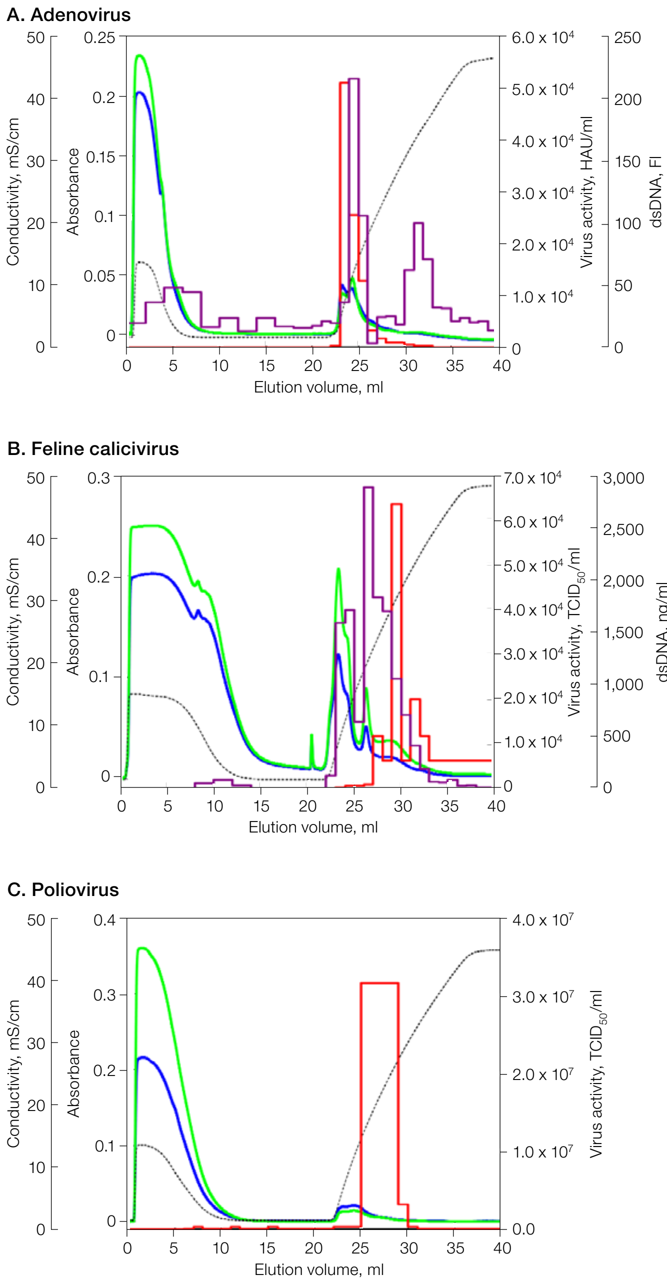

Fig. 4. Chromatograms of the separation of cell lysate (A) or culture fluid (B, C) containing nonenveloped viral particles by CHT Ceramic Hydroxyapatite Type II Media. A, AdV type 27; **B**, FCV A391; **C**, PV Sabin type 2. UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity (**—**); viral activity (AdV in HA test, FCV and PV in $TCID_{50}$) (—); dsDNA (—). Cell culture fluid contained 10% FBS. FI, fluorescence intensity.

Japanese Encephalitis Virus

Japanese encephalitis virus (JEV) chromatography is shown in Figure 5 (Kurosawa et al. 2009, 2012a). Irrespective of the source or strain, the virus elutes at approximately 350 mM sodium phosphate (note that the gradient in these two cases is 10–400 mM and the column size is 6.8 x 20 mm). Again, there is good separation between protein contamination and the virus.

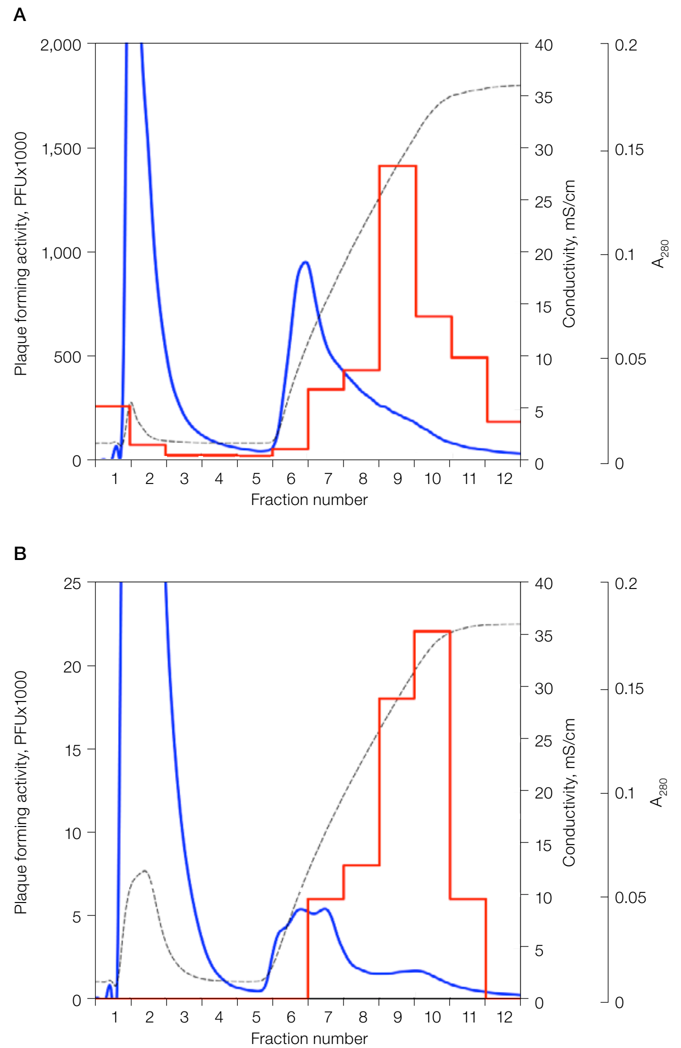

Fig. 5. JEV chromatography at pH 7.0. A, mouse brain homogenate infected with JEV JaGAr01; **B**, cell culture fluid of JEV Beijing. UV absorbance at 280 nm (—); conductivity (**--**); infectious activity in plaque assay (—).

Note: Figure 5A is modified from Kurosawa et al. 2012a.

Effect of Hydroxyapatite Type on Separation

Figure 6 shows the separation of dengue virus type 2 from cell culture contaminants on four apatites: CHT Type I, CHT Type II, CFT™ Ceramic Fluorapatite Type II, and MPC™ Ceramic Hydroxyfluoroapatite Media. Yields were 80% or higher for each media type except for MPC, where the yield was 50%. Although binding and elution was achieved on all four media, the separation of virus from impurities was best on CHT Type II Media. Figure 7 shows a similar study using CHT Type II and CFT Type II Media for the purification of poliovirus, with recoveries of 88% and 102%, respectively. These results illustrate the importance of choosing the appropriate media for the separation in question.

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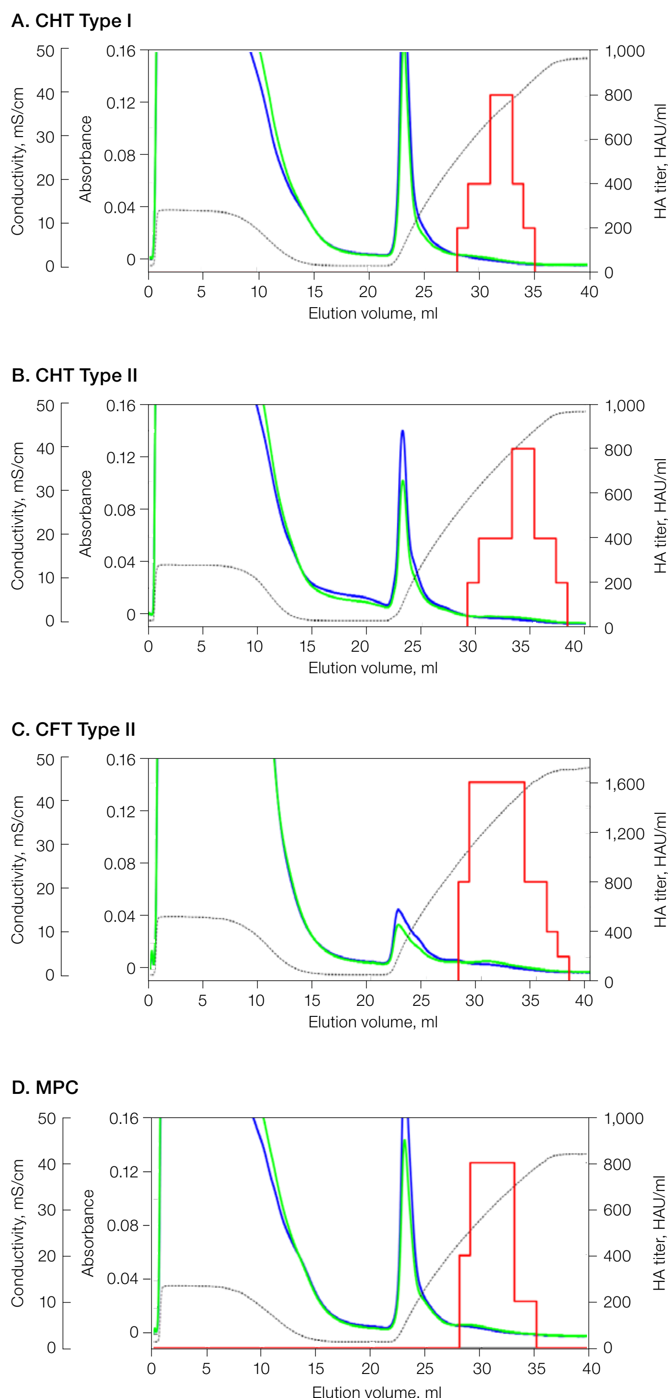

Fig. 6. Chromatograms of dengue virus type 2. A, CHT Type I Media; **B**, CHT Type II Media; **C**, CFT Type II Media; **D**, MPC Media. UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity (--); viral activity in HA test (—).

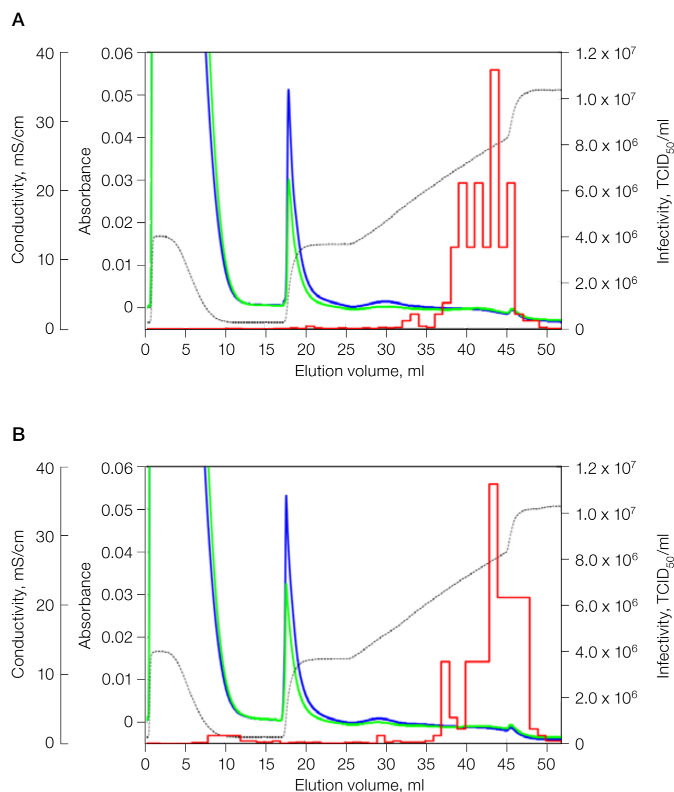

Fig. 7. Chromatograms of culture fluid of polio virus at pH 6.4. A, CHT Type II Media; **B**, CFT Type II Media. UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity of elution buffer (--); infectious activity in $TCID_{50}$ (—). Note: the gradient in these two cases is 150–450 mM at pH 6.4 for 20 ml.

Conclusion

Using ceramic hydroxyapatite media provided high purity, recovery, and viral activity for seven mammalian viruses of varying size and belonging to different families. We have shown that, in at least one case, slowing the flow rate and decreasing the gradient slope allowed better purification of viral particles on CHT Type II Media, signifying the importance of determining the best settings for such factors when using apatite media. Testing different apatites is significant for determining which media type will work best for a specific virus. A larger pore size, as provided by the CHT Type II Media, allowed better separation of the dengue virus from contaminants, compared to other apatite media.

Of equal significance, the use of ceramic hydroxyapatite media is simple and provides reproducible results, allowing an alternative to the conventional methods of viral purification.

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