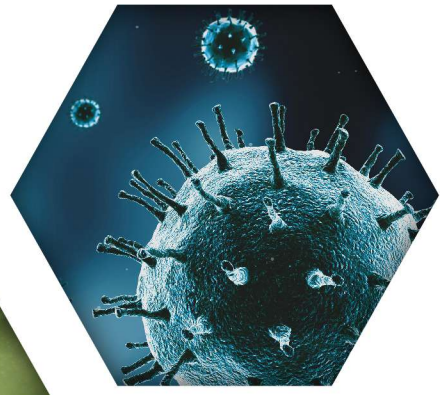

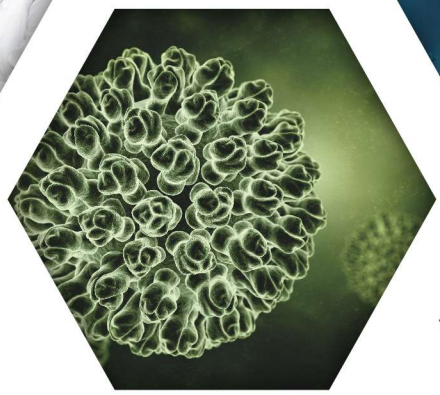

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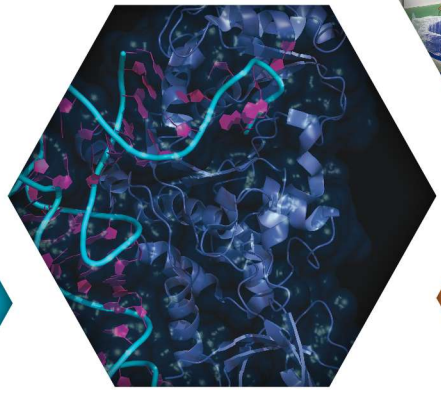
Novel Resin Functionalities Maximize Process Value For Biomolecule Purifications



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Novel Resin Functionalities Maximize Process Value for Biomolecule Purifications — A Preview

With large scale production, biologics such as monoclonal antibodies (mAbs), recombinant proteins, viruses, and more recently, biosimilars and biobetters have become powerful therapeutics and have been shown to be effective in treating various human diseases. Biopharmaceutical companies are continuing to evolve to meet the supply needs of the industry and ever increasing demands from national healthcare systems. The bioproduction industry is actively exploring and implementing several techniques such as disposable manufacturing, continuous processing, process analytical technology, and quality by design to address these challenges. As the structures of novel biotherapeutics become more diverse, new purification schemes are emerging to effectively resolve these biomolecules. Purification scientists are implementing new selectivity and high capacity chromatography techniques to maximize overall process efficiency.

This book highlights several examples where creative use of chromatography resins and their unique functionalities has emerged as powerful tools and have produced tangible benefits such as higher process yield, reduced buffer consumption and processes with fewer unit operations. Several cases spanning a range of biomolecules and viruses are discussed with proven approaches to accelerate method development, thus allowing scientists to fine-tune selectivity and optimize downstream purification platforms.

CONTENTS

"Our commitment extends beyond the lab to more practical matters such as faster product delivery time and being as physically close to our customers as possible to serve them better."

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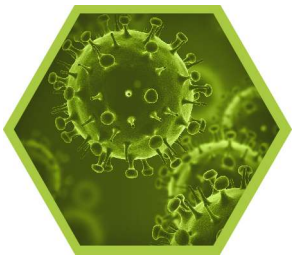

Development of a Two-Column **Manufacturing Process for Adenovirus**

Large-scale downstream processing of viruses for clinical applications poses challenges different from those for many other biotherapeutics. Adenovirus vectors are effective tools for the transfer of genetic material into mammalian cells. They offer several advantages, including the capacity to accommodate up to 37 kb of foreign genetic material, very high infection efficiency, ability to infect a wide variety of both dividing and nondividing cell types, lack of integration into the host chromosome, and availability of production systems capable of generating high virus titers. These and other qualities have led to adenoviruses being the most used gene transfer vectors in experimental therapies, accounting for 25% of all gene therapy trials. As of 2014, they had been used in almost 500 clinical trials.

Simple Method Development

CAPTURE

Table 1. Capture with Nuvia™ cPrime™ Resin. *Column: 5 ml, 0.8 x 10 cm*

Step	Buffer	CV	Flow Rate
Equilibration	25 mM histidine, pH 6.0 (Buffer A)	10	120 cm/hr
Sample Loading	Culture supernatant diluted 1:3 with Buffer A	48	120 cm/hr
Wash	Buffer A	5	120 cm/hr
Elution	75 mM Tris, 525 mM NaCl, pH 8.5	3	120 cm/hr

POLISHING

Table 2. Polishing with Nuvia Q Resin. *Column: 5 ml, 0.8 x 10 cm*

Step	Buffer	CV	Flow Rate
Equilibration	75 mM, pH 8.0 (Buffer A)	10	120 cm/hr
Sample Loading	Nuvia cPrime eluate diluted 1:1 with Buffer A	2	120 cm/hr
Wash 1	75 mM, 250 mM NaCl, pH 8.0	2	120 cm/hr
Wash 2	15 mM Tris, 440 mM NaCl, pH 8.5	3	120 cm/hr
Elution	75 mM Tris, 1 M NaCl, pH 7.5	5	120 cm/hr

Large-scale downstream processing of viruses for clinical applications poses challenges that arise, in part, from the viruses' large size and complexity. In the case of adenovirus, one intact virus particle (vp) contains more than 2,700 protein subunits, with a mass of approximately 165 MDa, and a diameter of approximately 0.1 µm. The complexity of the particle gives rise to thousands of charge variants, making it difficult to establish well-defined binding and elution conditions on charged separation resin. In addition, adenoviruses tend to be acid labile, which further increases the complexity of process development.

A two-column efficient purification process was developed for a recombinant adenovirus after screening five chromatography resins (Table 1 and Table 2). The final process yields an active, concentrated virus product with purity, HCP levels, and DNA contamination comparable to clinical-grade products. The process is readily scalable and is sufficiently simple, rapid, and efficient to be used for the production of clinical-grade viral vectors.

PROCESS DEVELOPMENT

Mass Capture

Initial screening showed that of the five resins selected three resins had potential

for use in a mass capture process — Nuvia cPrime (hydrophobic cation exchange), UNOsphere™ Q (anion exchange), and Nuvia Q (anion exchange) — since the virus was not detected in either the flow-through or wash fractions (Table 3). Nuvia cPrime was selected for capturing the adenovirus as it offered the best clearance of feedstream contaminants. Eluate from this hydrophobic cation exchange resin could be loaded onto the subsequent column following a simple pH adjustment. Fouling of anion exchange chromatography resins in process manufacturing (Close et al. 2013, Drevin et al. 1989) and difficulties in their regeneration (Ng and McLaughlin 2007) have been reported due to the binding of excessive amount of impurities and ineffective cleaning. Therefore, the Q resins are more suitable for polishing purification. Between the two strong anion exchange resins, Nuvia Q was chosen for its higher binding capacity for target virus even in the presence of high NaCl concentrations.

A nuclease digestion was introduced prior to column loading to aid in nucleic acid clearance. Initial mass capture experiments were focused on reducing feedstream volumes and recovering virus. The chromatograms in Figure 1 illustrate the early progression of these experiments and indicate when significant transgene expression was detected.

Anion Exchange Chromatography

Of the two anion exchange resins, UNOsphere Q and Nuvia Q, Nuvia Q was selected because it could adsorb virus at higher NaCl concentrations (Figure 2). It was therefore easier to work with downstream of the Nuvia cPrime capture step, where the eluate had an NaCl concentration of approximately 500 mM going on to the anion exchange column.

The anion exchange experiments with

Table 3. Results from the initial resin screening and their implications.

Column Type	Virus in Flowthrough /Wash	Virus in Eluate	Notes /Implications
UNOsphere S (CEX)	+++	++	Poorly suited for virus purification in both bind-and-elute and flow-through modalities
Nuvia S (CEX)	+++	++	Poorly suited for virus purification in both bind-and-elute and flow-through modalities
Nuvia cPrime (MM)	–	++++	Partial elution in 125 mM NaCl, pH 6.5; hence, dilution of crude harvest required prior to column loading
UNOsphere Q (AEX)	–	++++	Could be considered for direct mass capture
Nuvia Q (AEX)	–	++++	Could be considered for direct mass capture

CEX, cation exchange; MM, mixed mode; AEX, anion exchange

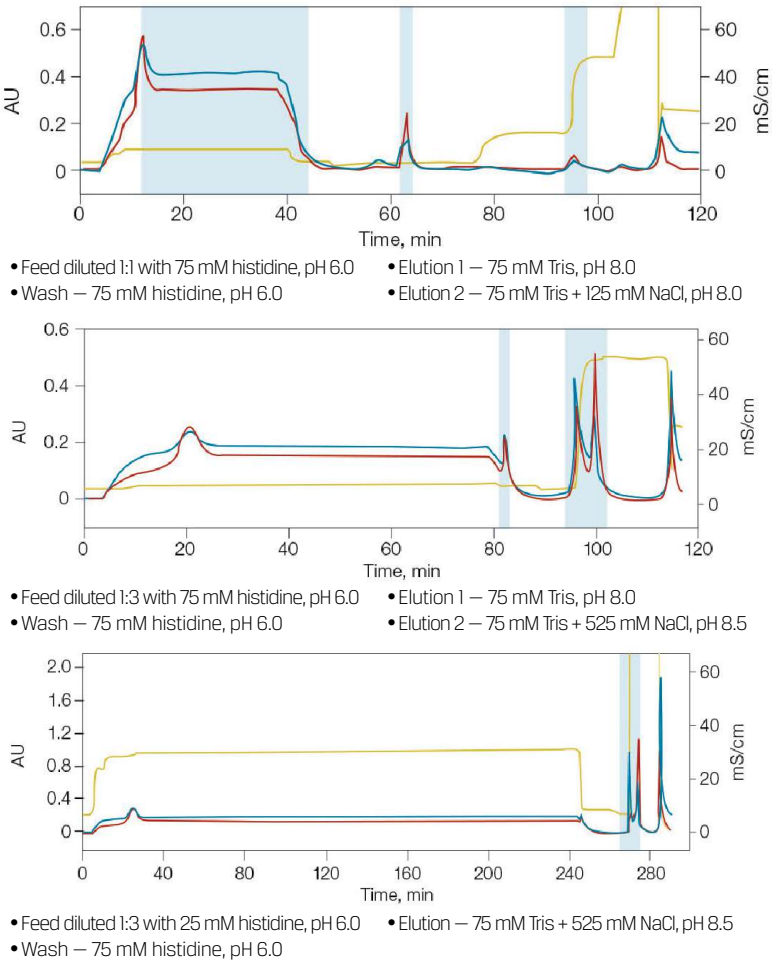

Fig. 1. Iterative development of Nuvia cPrime capture. OD 260 (—); OD 280 (—); conductivity (—). Blue shading indicates detection of significant transgene expression. AU, absorbance units. Conductivity, mS/cm.

Nuvia Q were focused on attaining high product purity (Figure 3).

As shown in Figure 3, increasing the pre-elution wash NaCl concentration from 400 to 440 mM prevented a suspected HCP contaminant from eluting with the product. Therefore, it would be possible to further improve the efficiency of this chromatography step by directly equilibrating the Nuvia Q Column with 75 mM Tris + 440 mM NaCl, pH 8.0.

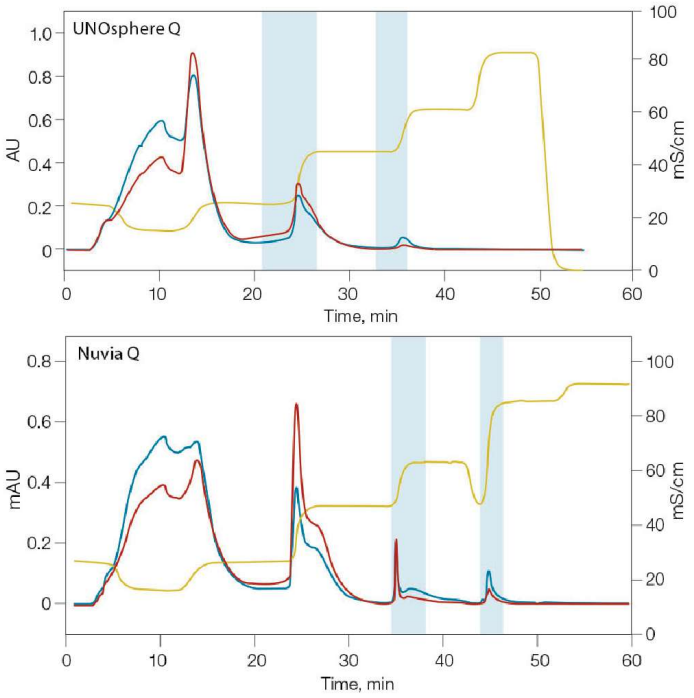

Fig. 2. Behavior of the crude harvest on the UNOsphere Q and Nuvia Q Columns. OD 260 (—); OD 280 (—); conductivity (—). Blue shading indicates detection of significant transgene expression. AU, absorbance units. Conductivity, mS/cm.

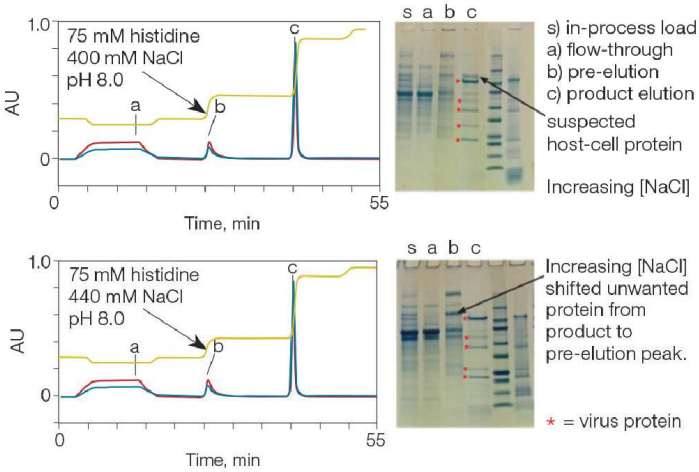

Fig. 3. Chromatograms and gels from AEX process development experiments. OD 260 (—); OD 280 (—); conductivity (—). * Proteins presumed to be virus capsid components. AU, absorbance units.

FINAL PROCESS

Mixed-Mode Chromatography

Initial capture was accomplished using Nuvia cPrime Mixed-Mode Resin (Figure 4). This portion of the process achieved a ten-fold reduction in processing volume and a significant reduction in feedstream contaminants (Figure 6, lanes 2–4).

Anion Exchange Chromatography

Final virus purification was accomplished using Nuvia Q Resin (Figure 5). This portion of the process achieved an additional two-fold reduction of product volume along with a significant improvement in product purity (Figure 6, lanes 4–7). Following this operation, nonvirus proteins were no longer evident by SDS-PAGE (Figure 6, lane 7).

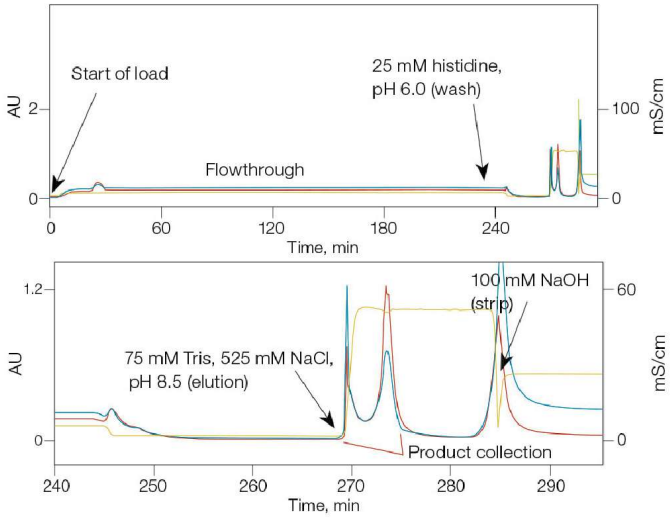

Fig. 4. Representative mixed-mode chromatogram. OD 260 (—); OD 280 (—); conductivity (—). AU, absorbance units. Conductivity, mS/cm.

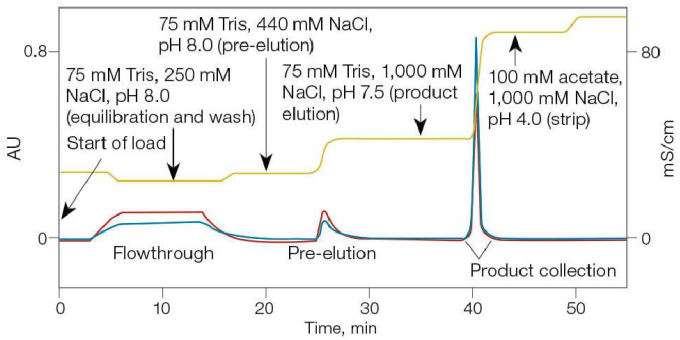

Fig. 5. AEX chromatogram. OD 260 (—); OD 280 (—); conductivity (—). AU, absorbance units. Conductivity, mS/cm.

Analysis of In-Process and Final Product

SDS-PAGE was used for analysis to visualize the progressive reduction of contaminating proteins at each step of the purification process (Figure 6). Cell culture media components such as albumin and transferrin are clearly evident in the virus culture harvest, that is, the sample loaded onto the Nuvia cPrime Column (Figure 6, lane 2). These proteins are effectively separated from the viral protein components, as they are highly visible in the flow-through fraction from this column, under the current chromatography conditions (Figure 6, lane 3). Significantly fewer contaminants are seen in the sample loaded onto the Nuvia Q Column (Figure 6, lane 4). During the anion exchange process, the virus bound to the resin while contaminants either flowed through the column (Figure 7, lane 5) or were pre-eluted (Figure 6, lane 6). The five most prominent viral proteins — hexon, penton, core (V), hexon (VI), and core (VII) — are readily visible in the final purified product (Figure 6, lane 7).

Virus concentration and DNA levels were evaluated at select points along the downstream process (Table 4). The data demonstrates an overall recovery of virus particles of approximately 54% ng/10^{13}, with DNA levels below detection and HCP at 2 ng/10^{10} particles. These values are well within current guidelines for clinical and perhaps commercial use.

Table 4: Viral particle recovery and impurity clearance.

Sample	Total virus (x10^{11} particles)	Impurity Levels (ng/10^{13} particles)	
		DNA	HCP
Bulk harvest	30.6	3,144	ND
Nuclease-treated harvest	31.6	30	3,022
Nuvia cPrime eluate	18.4	ND	58
Nuvia Q eluate	16.4	<0.02	2

ND, not determined.

SUMMARY

The final process yields an active, concentrated virus product with purity, HCP, and DNA levels comparable to clinical-grade products. While the purification methods presented here were developed using the Ad5-E1+GFP model virus, they are expected to be applicable to recombinant adenoviruses in general, and to constructs derived from serotype 5 viruses in particular. The process is readily scalable and uses procedures and reagents compatible with cGMPs. Also, it is sufficiently simple, rapid, and efficient to be used for the production of clinical-grade virus-based gene therapy products and vaccines.

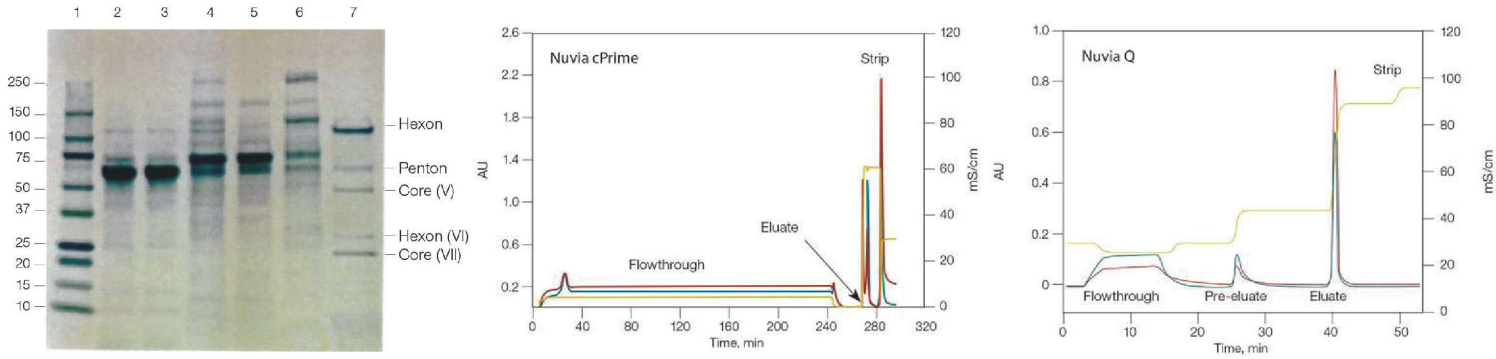

Fig. 6. SDS-PAGE of intermediates and the final product. Lane 1, MW marker; lane 2, Nuvia cPrime load; lane 3, Nuvia cPrime flowthrough; lane 4, Nuvia cPrime elution/Nuvia Q load; lane 5, Nuvia Q flowthrough; lane 6, Nuvia Q pre-elution; lane 7, Nuvia Q product. OD 260 (—); OD 280 (—); conductivity (—).

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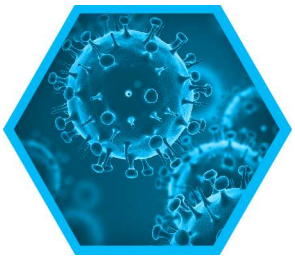


Purification of Mammalian Virus **in a Single Step**

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, Moritz 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 case study, ceramic hydroxyapatite media is used for purification of a wide variety of mammalian viruses (Table 1). Viral activity was determined using the assays shown in Table 2. Chromatography using ceramic hydroxyapatite media is simple, easily scalable, and results in a concentrated preparation of highly active virus.

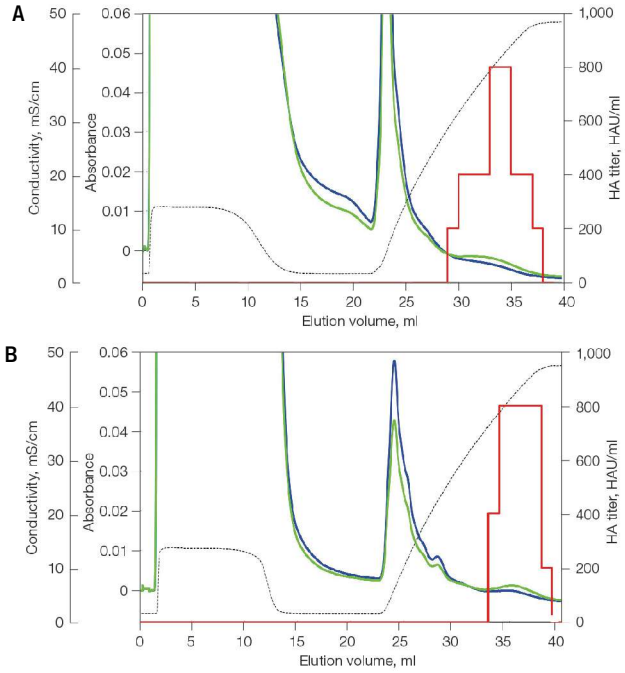


Fig. 1. Chromatograms of the separation of dengue virus type 2 by CHT Type II Media. A, flow rate at 1.0 ml/min.; **B.** flow rate at 0.1 ml/min. UV absorbance at 260 nm (–); UV absorbance at 280 nm (–); conductivity (–); viral activity in HA test (–). Method development: Table 4 and 5.

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

Table 1. Wide variety of viruses that can be purified using CHT.

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

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

the gradient, separated from the bulk of A280-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™ Ceramic Hydroxyapatite Type II Media is similar to their adsorption to cells (Saito et al. 2013).

Other serotypes of dengue virus were 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 3. Types 2 and 4 were eluted at roughly the same sodium phosphate concentration.

Table 3. Elution points of dengue serotypes in a sodium phosphate

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

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.

HA activity was 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 were 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).

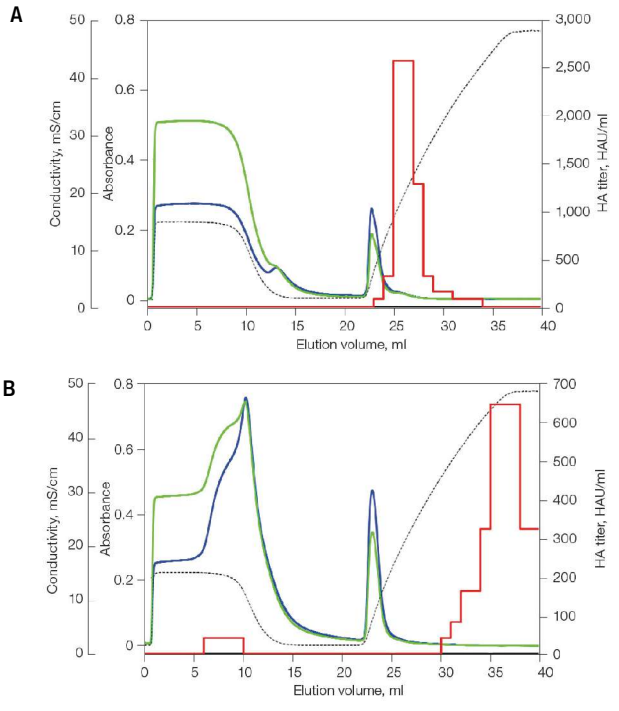


Fig. 2. Chromatograms of influenza virus. A. A/Beijing/262/95; **B.** A/Panama/2007/99. UV absorbance at 260 nm (–); UV absorbance at 280 nm (–); conductivity (--); viral activity in HA test (–). Method development: Table 4.

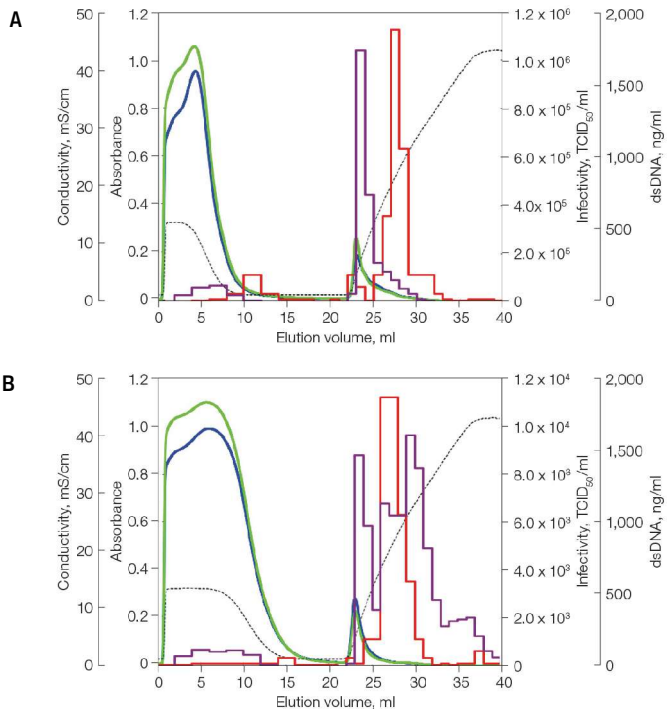
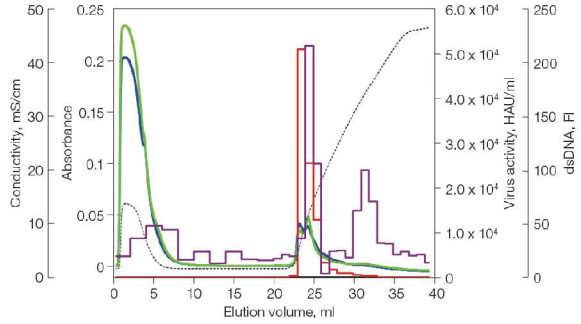


Fig. 3. Chromatograpms of two strains of MHV. A, MHV-NuU; **B,** MHV-S. UV absorbance at 260 nm (–); UV absorbance at 280 nm (–); conductivity (--); viral infectivity in $TCID_{50}$ (–); dsDNA (–). Culture fluid contained 10% fetal bovine serum (FBS). Method development: Table 4.

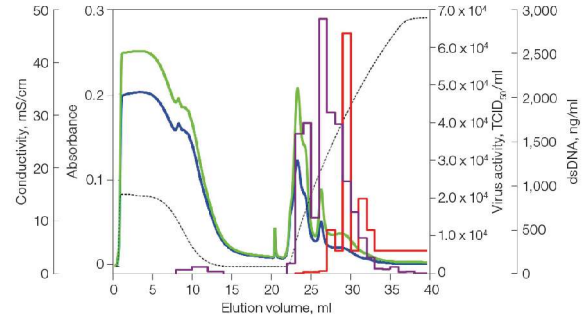
Nonenveloped Viral Particles

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 were all adsorbed to CHT Type II Media (Figure 4), although they showed different elution times.

A. Adenovirus



B. Feline calicivirus



C. Poliovirus

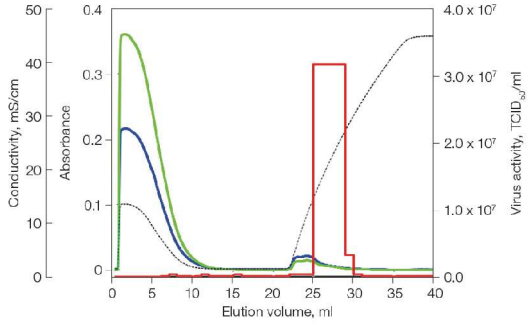


Fig. 4. Chromatograms of the separation of cell lysate (A) or culture fluid (B, C) containing nonenveloped viral particles by CHT Ceramic Hydroxyapatite Type II Media. A, AdV type 27; **B,** FCV A391; **C,** PV Sabin type 2. UV absorbance at 260 nm (–); UV absorbance at 280 nm (–); conductivity (--);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

METHOD DEVELOPMENT

Table 4. Purification with CHT Ceramic Hydroxyapatite Media, Type II, 40 µm. This method applies to dengue virus type 2 (Figure 1A and 7B), influenza virus (Figure 2), mouse hepatitis virus (Figure 3), adenovirus (Figure 4A), feline calicivirus (Figure 4B), poliovirus (Figure 4C and 8A). *Column: 0.6 ml, 0.46 x 3.5 cm*

Step	Buffer	CV	Flow Rate
Pre-Wash	600 mM sodium phosphate, pH 7.2 (Buffer B)	8	350 cm/hr
Equilibration	10 mM sodium phosphate, pH 7.2 (Buffer A)	16	350 cm/hr
Sample Loading	Virus preparation in Buffer A	16	350 cm/hr
Wash	Buffer A	16	350 cm/hr
Elution 1	Gradient of Buffer A to Buffer B	24	350 cm/hr
Elution 2	Buffer B	8	350 cm/hr

Table 5. Purification with CHT Media Type II, 40 µm. This method applies to dengue virus type 2 (Figure 1B). *Column: 0.6 ml, 0.46 x 3.5 cm*

Step	Buffer	CV	Flow Rate
Pre-wash	600 mM sodium phosphate, pH 7.2 (Buffer B)	8	35 cm/hr
Equilibration	10 mM sodium phosphate, pH 7.2 (Buffer A)	16	35 cm/hr
Sample Loading	Virus preparation in Buffer A	16	35 cm/hr
Wash	Buffer A	16	35 cm/hr
Elution 1	Gradient of Buffer A to Buffer B	24	35 cm/hr
Elution 2	Buffer B	8	35 cm/hr

Table 6. Purification with CHT Media Type II, 40 µm. This method applies to Japanese encephalitis virus (Figure 5). *Column: 0.6 ml, 0.46 x 3.5 cm*

Step	Buffer	CV	Flow Rate
Pre-wash	400 mM sodium phosphate, pH 7.2 (Buffer B)	5	160 cm/hr
Equilibration	10 mM sodium phosphate, pH 7.2 (Buffer A)	16	160 cm/hr
Sample Loading	Virus preparation in Buffer A	16	160 cm/hr
Wash	Buffer A	16	160 cm/hr
Elution 1	Gradient of Buffer A to Buffer B	24	160 cm/hr
Elution 2	Buffer B	8	160 cm/hr

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 was eluted at approximately 350 mM sodium phosphate (note that the gradient used in these two cases was 10–400 mM and the column size is 6.8 x 20 mm). Again, there is good separation between protein contamination and the virus.

Effect of Hydroxyapatite Type on Separation

Hydroxyapatite, $Ca_{10}(PO_4)_6(OH)_2$, has been widely used in the chromatographic separation of biomolecules. In its crystal lattice, sets of five calcium doublets (C sites) and pairs of –OH containing phosphate triplets (P sites) are arranged in a repeating geometric pattern. Space-filling models and repeat structure from Raman spectroscopy have also been constructed. Hydroxyapatite has unique separation properties and unparalleled selectivity and resolution. It often separates proteins shown to be homogeneous by electrophoretic and other chromatographic techniques.

METHOD DEVELOPMENT

Table 7. Purification with CHT Media Type I, 40 µm. This method applies to dengue virus type 2 (Figure 7A). *Column: 0.6 ml, 0.46 x 3.5 cm*

Step	Buffer	CV	Flow Rate
Pre-wash	600 mM sodium phosphate, pH 7.2 (Buffer B)	6	350 cm/hr
Equilibration	10 mM sodium phosphate, pH 7.2 (Buffer A)	16	350 cm/hr
Sample Loading	Virus preparation in Buffer A	16	350 cm/hr
Wash	Buffer A	16	350 cm/hr
Elution 1	Gradient of Buffer A to Buffer B	24	350 cm/hr
Elution 2	Buffer B	8	350 cm/hr

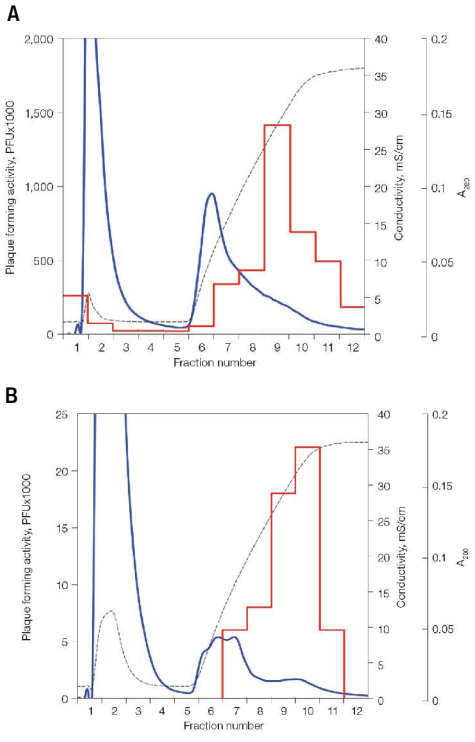


Fig. 5. JEV chromatography at pH 7.0. A, mouse brain homogenate infected with JEV JaGAr01; **B,** cell culture fluid of JEV Beijing. UV absorbance at 280 nm (–); conductivity (--); infectious activity in plaque assay (–). Method development: Table 6.
Note: *Figure 5A is modified from Kurosawa et al. 2012a.*

CHT Ceramic Hydroxyapatite is a spherical, macroporous form of hydroxyapatite. It is manufactured by sintering at high temperatures to be converted from a nanocrystalline to ceramic form. Unlike most other chromatography adsorbents, CHT is both the ligand and the support matrix. As shown in Figure 6, hydroxyapatite contains two types of binding sites; positively charged calcium and negatively charged phosphate groups. These sites are distributed throughout the crystal structure of the matrix.

Two types of CHT Ceramic Hydroxyapatite, Type I and Type II, are available for process separations. CHT Type I has a higher protein binding capacity and better capacity for acidic proteins. CHT Type II has a lower protein binding capacity but has better resolution of nucleic acids and certain proteins. The Type II material also has a very low affinity for albumin and is especially suitable for the purification of many species and classes of immunoglobulins.

$Ca_1(PO_4)_6(OH)_{1.5}(F)_{0.5}$ – MPC™ Ceramic Hydroxyfluoroapatite – like CHT, is a macroporous ceramic hydroxyapatite with 25% of the hydroxyl groups substituted with fluoride. The incorporation of fluoride creates a more chemically stable form of the matrix.

CFT™ Ceramic Fluoroapatite – $Ca_{10}(PO_4)_6F_2$ – is a rigid, spherical macroporous, apatite-based media and is a composite of fluoroapatite and

METHOD DEVELOPMENT

Table 8. Purification with CFT™ Ceramic Fluoroapatite Media, Type II, 40 µm. This method applies to dengue virus type 2 (Figure 7C) and poliovirus (Figure 8B). *Column: 0.6 ml, 0.46 x 3.5 cm*

Step	Buffer	CV	Flow Rate
Pre-wash	600 mM sodium phosphate, pH 7.2 (Buffer B)	6	350 cm/hr
Equilibration	10 mM sodium phosphate, pH 7.2 (Buffer A)	16	350 cm/hr
Sample Loading	Virus preparation in Buffer A	16	350 cm/hr
Wash	Buffer A	16	350 cm/hr
Elution 1	Gradient of Buffer A to Buffer B	24	350 cm/hr
Elution 2	Buffer B	8	350 cm/hr

Table 9. Purification with MPC™ Ceramic Hydroxyfluoroapatite Media, 40 µm. This method applies to dengue virus type 2 (Figure 7D).
Column: 0.6 ml, 0.46 x 3.5 cm

Step	Buffer	CV	Flow Rate
Pre-wash	600 mM sodium phosphate, pH 7.2 (Buffer B)	8	350 cm/hr
Equilibration	10 mM sodium phosphate, pH 7.2 (Buffer A)	16	350 cm/hr
Sample Loading	Virus preparation in Buffer A	16	350 cm/hr
Wash	Buffer A	16	350 cm/hr
Elution 1	Gradient of Buffer A to Buffer B	24	350 cm/hr
Elution 2	Buffer B	8	350 cm/hr

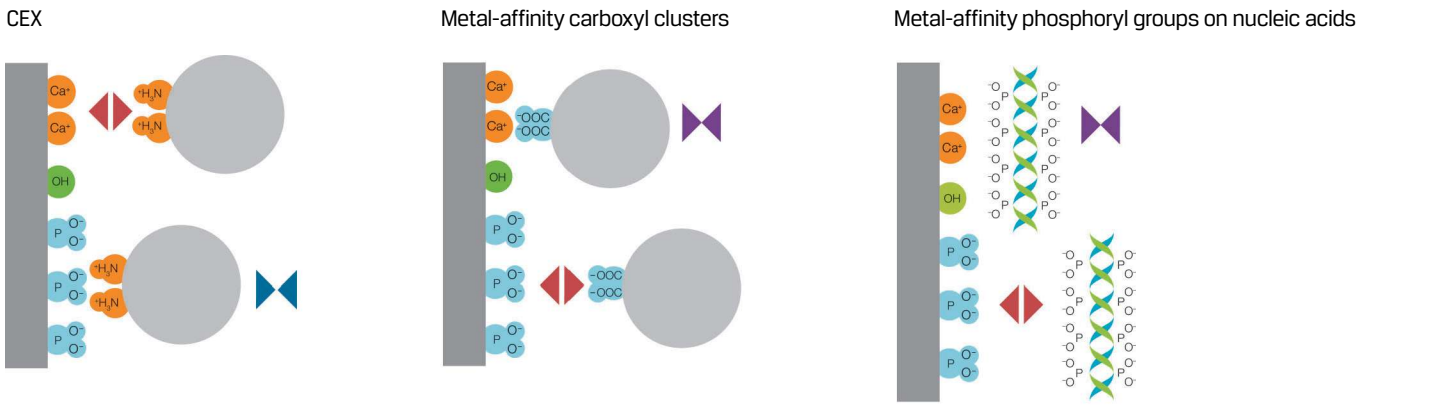


Fig. 6. Schematic representation of Ceramic Hydroxyapatite binding mechanism. Biomolecule (•); metal affinity (▶◀); electrostatic repulsion (◀▶); electrostatic attraction (▶◀).

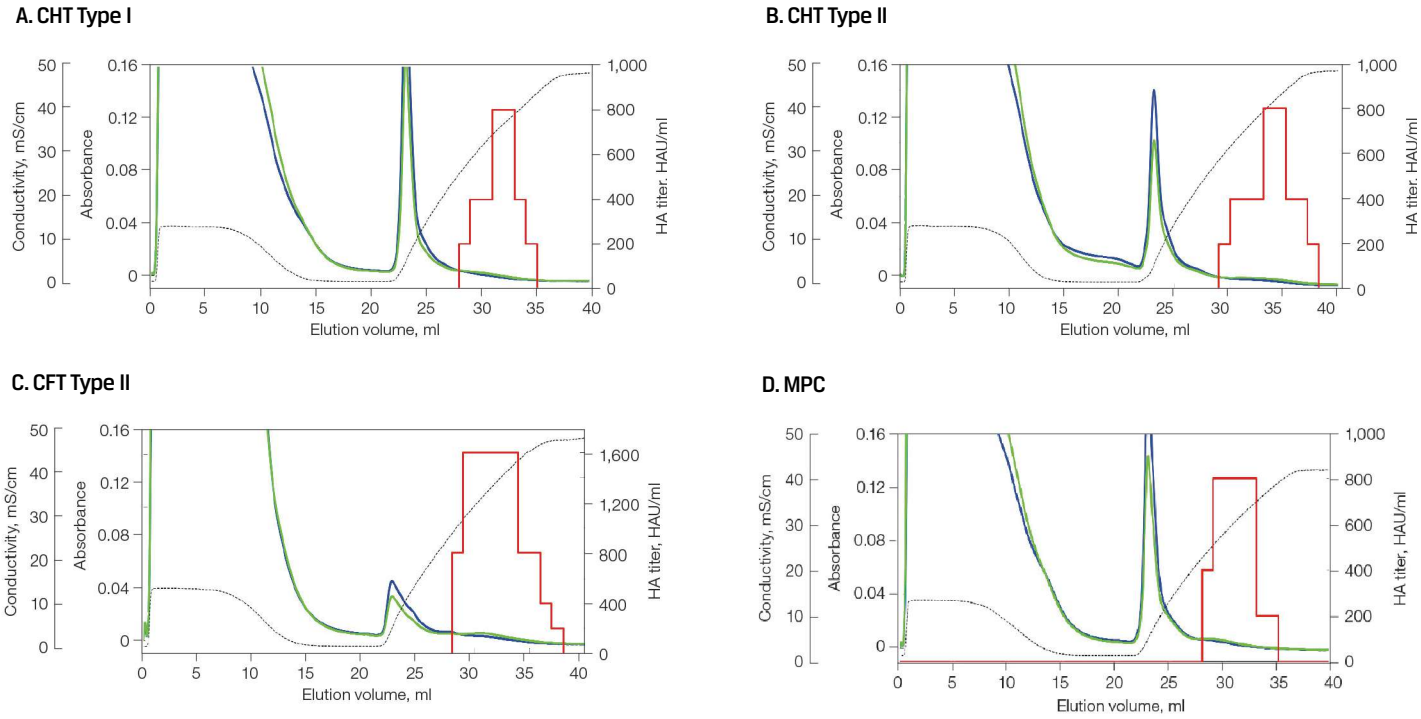


Fig. 7. Chromatograms of dengue virus type 2 . A, CHT Type I Media; **B,** CHT Type II Media; **C,** CFT Type II Media; **D**, MPC Media. UV absorbance at 260 nm (—);UV absorbance at 280 nm (—); conductivity (--); viral activity in HA test (—). Method development: Table 4,7,8, and 9.

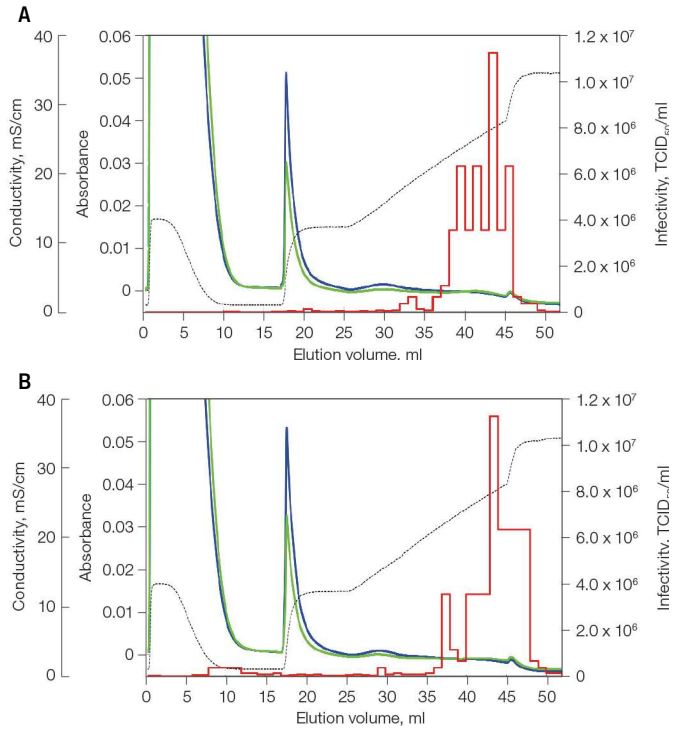


Fig. 8. Chromatograms of culture fluid of poliovirus at pH 6.4.
A, CHT Type II Media; **B,** CFT Type II Media. UV absorbance at 260 nm (—); UV absorbance at 280 nm (—); conductivity of elution buffer (--); infectious activity in $TCID_{50}$ (—). Method development: Table 4 and 8.
Note: *The gradient in these two cases is 150–450 mM at pH 6.4 for 20 ml.*

hydroxyapatite prepared by chemically converting hydroxyapatite nanocrystals to fluoroapatite with a fluorine reagent. CFT is a multimodal chromatographic media that interacts with biomolecules through cation exchange via phosphate groups, through metal affinity via calcium atoms, or both.

CFT Ceramic Fluoroapatite possesses separation characteristics similar to CHT Ceramic Hydroxyapatite. CFT purifications can be performed at lower pH. In addition, CFT can be used under stringent chromatography conditions to separate acidic proteins. The unique selectivity of CFT Ceramic Fluoroapatite is attributed to its multiple interactions with biomolecules. Amino groups are attracted to phosphate (P) sites but repelled by calcium (C) sites; the situation is reversed for carboxyl groups (Figure 6).

Figure 7 shows the separation of dengue virus type 2 from cell culture contaminants on four apatites: CHT Type I, CHT Type II, CFT Type II, and MPC 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 8 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.

SUMMARY

Ceramic hydroxyapatite chromatography provided high purity, recovery, and viral activity for seven mammalian viruses of varying size and different families. We have shown that slowing the flow rate and decreasing the gradient slope may allow 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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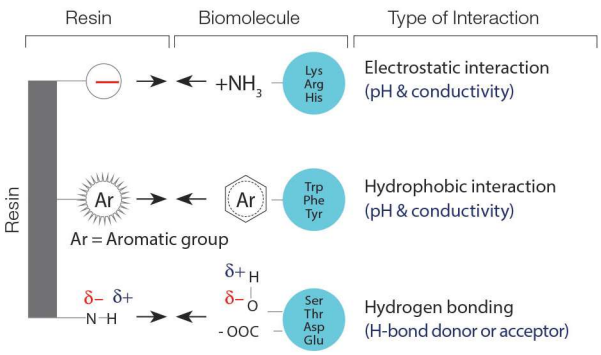
An Efficient Purification Platform **for Recombinant Proteins**

Mixed-mode chromatography has become an important purification tool for downstream process developers, who are always seeking highly selective yet robust methods for the purification of recombinant proteins. Mixed-mode chromatography matrices are designed to present multiple interaction modes to resolve target proteins and impurities, entities that are themselves complex multimodal molecules. Under specific purification conditions, one or more such interaction modes may be involved in the binding or repulsion between the target protein and the chromatography media. Therefore, the behavior of a protein during purification by mixed-mode chromatography is often not predictable on the basis of its pI or amino acid sequence.

Here, we describe the use of a design of experiment (DOE) approach in the initial screening of chromatographic conditions for the purification of a diverse set of protein targets on Nuvia™ cPrime™ Hydrophobic Cation Exchange Media. The ligand on this media has three major functionalities: A weak carboxylic acid end group, an aromatic hydrophobic ring, and an amide bond serving as a potential hydrogen bond donor/acceptor (Figure 1). The combined effect of these structural elements provides unique selectivity and good conductivity tolerance, allowing protein purifications to be conducted effectively under gentle conditions. Our studies show that, with a limited amount of protein sample and chromatography media, a simple DOE setup can be used to determine the effects of buffer pH and conductivity on selectivity, recovery, and robustness of protein purification on Nuvia cPrime. Working conditions established by such scale-down studies can be used for the purification of a target protein on a preparative scale.

Fig. 1. Interactive permutations of the ligand and biomolecule.



METHOD DEVELOPMENT

Bovine serum albumin, bovine carbonic anhydrase, and conalbumin were purchased from Sigma-Aldrich. Lactoferrin was obtained from Glanbia Nutritionals, Inc. Monoclonal antibody, mAbX, was overproduced in a Chinese hamster ovary (CHO) cell culture and previously purified by column chromatographic methods. Protein fractions were analyzed by SDS-PAGE using Criterion™ Tris-HCl 4–20% Linear Gradient Gels (Bio-Rad) stained with Bio-Safe™ Coomassie Stain (Bio-Rad), and quantified on a GS-800™ Calibrated Densitometer (Bio-Rad). The clearance of *E. coli* host cell proteins (HCPs) and double-stranded DNA (dsDNA) were determined by *E. coli* HCP ELISA kit F410 (Cygnus Technologies) and Quant-iT dsDNA High-Sensitivity Assay Kit (Invitrogen), respectively. Protein concentration was determined by UV absorption at 280 nm, using the respective coefficients at 1 mg/ml.

Design of experiments setup

JMP software (www.jmp.com) was used in the design of experiment studies to identify optimal binding and elution conditions on Nuvia cPrime for a set of five proteins. These proteins vary in their molecular

Table 1. List of proteins used in the study.

Test Protein	pI	Molecular Mass
Bovine serum albumin	4.7	67 kD
Bovine carbonic anhydrase	5.9	29 kD
Conalbumin	6.9	78 kD
Lactoferrin	9.2	78 kD
mAbX	9.5	150 kD

mass and pI (Table 1). The effects of two parameters, buffer pH, and conductivity, were evaluated by a two-level fractional factorial experimental design, with three center points and a total of 11 experiments (Table 2). They were executed in spin column format. The static binding capacity and recovery of target proteins were plotted against the binding or elution buffer pH and sodium chloride concentration. A standard least squares model was employed to obtain the response surfaces and to predict the optimal conditions for maximum target protein binding capacity and recovery.

Table 2. Design of experiment setup.

Experiment	Binding pH	Binding [NaCl], mM	Elution pH	Elution [NaCl], mM
1	4.0	400	8.0	10
2	8.0	10	8.0	10
3	6.0	205	6.0	505
4	4.0	400	4.0	1,000
5	8.0	400	8.0	1,000
6	8.0	10	4.0	1,000
7	6.0	205	6.0	505
8	4.0	10	8.0	1,000
9	4.0	10	4.0	10
10	6.0	205	6.0	505
11	8.0	400	4.0	10

Note: Experiments 3, 7, and 10 are the center points.

Developing Methods for Protein Purification on Nuvia cPrime Media Using DOE

Mini Bio-Spin® Columns (Bio-Rad), each containing 100 µl of pre-equilibrated Nuvia cPrime, were used in the tests. Nuvia cPrime Resin was mixed with 500 µl of test protein solution (6 mg/ml in respective binding buffer) at room temperature with constant agitation for 3 minutes. The unbound protein was collected at the end of the incubation by spinning at 1,000 x g for 1 minute. Loading was repeated once. Each spin column was washed once with 5 CV of binding buffer. All unbound proteins collected during column loading and wash were combined. To each spin column, 500 µl of respective elution buffer were added and mixed with loaded resin at room temperature for 3 minutes with agitation. The eluted protein was collected by spinning at 1,000 x g for 1 minute. This was repeated once and eluates from both runs were combined. The protein concentrations in these samples were quantified for the determination of static binding capacity and recovery of each test protein under specific conditions.

Capture of Lysozyme from *E. coli* Lysate

The same DOE approach was used in the screening of chromatographic conditions for capturing lysozyme (pI 9.3) from an *E. coli* lysate. Approximately 500 µl of preconditioned *E. coli* lysate containing 3 mg of lysozyme, were loaded onto each spin column with 100 µl pre-equilibrated Nuvia cPrime Resin media. The yield and purity of eluted lysozyme from each set of binding and elution condition combinations were chosen as the responses to parameter variations. Eluates were analyzed by reducing SDS-PAGE. The band density of lysozyme in each eluate was used as the indicator for its yield under specific conditions; the abundance of lysozyme among all proteins in each eluate represented the purity of a particular lysozyme preparation. The A280/A260 ratio was used to evaluate the efficiency of host cell nucleic acid clearance. A higher A280/A260 ratio indicates a relatively lower nucleic acid contamination level. The design space for optimal yield and purity of lysozyme was identified from the response surfaces generated by the JMP software.

For scale-up preparation of lysozyme from *E. coli* lysate, a 1 ml column of Nuvia cPrime Resin media was used. This column was equilibrated with 20 mM sodium acetate and 150 mM NaCl (pH 4.0). *E. coli* lysate containing 3 mg/ml lysozyme was adjusted to pH 4.0 and applied onto the column at a linear velocity of 250 cm/hr. The dynamic binding capacity of lysozyme at 10% breakthrough was determined by inline UV absorption at 280 nm. Bound lysozyme was eluted using 10 CV of 20 mM sodium phosphate and 1 M sodium chloride (pH 7.5). Recovery of lysozyme was calculated by comparing the amount of lysozyme eluted from the column and the total amount of lysozyme loaded. The reported value was the average of two runs. This procedure was repeated for determining the 10% breakthrough dynamic binding capacity of lysozyme in the same buffer at pH 4.5. The purity of lysozyme and

dsDNA content in eluates were also quantified.

RESULTS AND DISCUSSION

Increasingly diverse therapeutic protein candidates are entering drug development pipelines. The pI and molecular mass of these recombinant proteins can be predicted from their encoding DNA sequences. However, information on other aspects of their physiochemical/conformational properties is often incomplete, posing a challenge to downstream purification process developers who are tasked to elucidate a purification strategy. We have explored the possibility of using DOE, a fractional factorial design consisting of 11 experiments, for initial chromatographic conditions screening. A set of five diverse proteins with differing pI value and molecular mass have been included in this study (Table 1). The binding capacity and recovery of these proteins on Nuvia cPrime, in response to changes in buffer pH and sodium chloride concentration, were assessed statistically. The predicted optimal binding and elution conditions are summarized in Table 3. From the data, we can find areas of optimal performance where target protein binding and recovery are maximized. Our results also suggest that these proteins employ dramatically different modes of interaction with Nuvia cPrime under the conditions explored.

Table 3. Optimal binding and elution conditions of various proteins on Nuvia cPrime.

Test Protein	pI	Optimal Conditions Predicted by DOE
Bovine serum albumin	4.7	Binding: 10 mM NaCl, pH 4.0 Elution: 1,000 mM NaCl, pH 8.0
Bovine carbonic anhydrase	5.9	Binding: 10 mM NaCl, pH 4.6 Elution: 1,000 mM NaCl, pH 8.0
Conalbumin	6.9	Binding: 10 mM NaCl, pH 4.0 Elution: 505 mM NaCl, pH 6.0
Lactoferrin	9.2	Binding: 205 mM NaCl, pH 4.0 Elution: 1,000 mM NaCl, pH 8.0
mAbX	9.5	Binding: 300 mM NaCl, pH 4.6 Elution: 800 mM NaCl, pH 8.0

Maximum binding of bovine serum albumin and bovine carbonic anhydrase are reached at the lowest tested pH and sodium chloride concentration, while high pH and high sodium chloride concentrations are required for their complete recovery from Nuvia cPrime. Therefore, it can be concluded that the main interaction between these test proteins and Nuvia cPrime is electrostatic in nature. In other words, the cation exchange character of Nuvia cPrime is more dominant in the purification of these bovine proteins.

The best binding condition for conalbumin is the same as that for bovine serum albumin and bovine carbonic anhydrase, indicating that charge-charge interaction is predominant in initial binding as well. Interestingly however, maximum recovery of this protein is achieved at mildly acidic pH (~6.0) in the presence of modest salt concentration (~600 mM). This would indicate that the use of higher salt concentrations may promote hydrophobic interactions between conalbumin and Nuvia cPrime, thus hampering its elution. It is also possible that an increase in buffer pH causes conalbumin to adopt an alternative conformation, which exposes more hydrophobic regions for a stronger association with the chromatography media.

Lactoferrin and mAbX are two basic proteins with a pI of around 9. They are expected to be positively charged in the entire test pH range and interact strongly with Nuvia cPrime via electrostatic interaction. Interestingly, optimal binding capacity for these proteins was observed in the presence of substantial concentration of sodium chloride, which suggests the binding of these proteins is enhanced by the hydrophobic interactions. Both high pH and high salt concentration are needed for the proteins' complete elution. Therefore, charge-charge interaction is the main driving force for the association of these proteins with Nuvia cPrime.

The data suggest that a simple DOE screening study is sufficient for predicting the chromatographic behavior of these test proteins on Nuvia cPrime, despite the fact that they are very different in size, charge state, and hydrophobicity.

In a real-world protein separation scenario, more exhaustive DOE can be performed to fine-tune the separation condition. An alternative approach is to create buffer gradients on a traditional packed column to understand the impact of buffer pH, conductivity, and additives on column chromatography performance, following the initial DOE screening.

The same DOE strategy was applied to process

development for the purification of lysozyme from an *E. coli* lysate. At pH 4.0–5.0, lysozyme can be efficiently captured from *E. coli* lysate in the presence of up to 100 mM of sodium chloride. Under this condition, nucleic acids as well as acidic proteins from the expression host cells are expected to flow through the column. While loading lysate at pH near 8.0 may also increase the selectivity for lysozyme over other protein impurities, higher binding buffer pH does compromise the yield and nucleic acid impurity removal according to the model. The elution of lysozyme from Nuvia cPrime is robust in the entire pH range tested. However, higher sodium chloride concentration in elution buffer is required for maximum recovery of this protein and optimal nucleic acid clearance. The response contours from modeling also define the design space for the purification of target protein (Figure 2). It is worth mentioning that lysozyme is a basic protein, with a pI value very close to that of lactoferrin and mAbX. However, hydrophobic interaction does not seem to play any important role in its interaction with Nuvia cPrime. In other words, the pI of an unknown protein alone is not sufficient for predicting its chromatographic behavior on Nuvia cPrime. This is consistent with observations made on other multimodal chromatography media (Cramer 2013).

A scale-up preparation of lysozyme was prepared on the basis of conditions predicted from the above DOE screening. Feed stream loading was performed at pH 4.0 or 4.5. Such buffer pH variation has no impact on dynamic binding capacity for lysozyme or the clearance of impurities from host cells (Table 4 and

Table 4. Capture of lysozyme from an *E.coli* lysate

Binding Buffer	10% DBC, mg/ml	Recovery, %	Purity, %	dsDNA, ppm
20 mM sodium acetate, 150 mM NaCl (pH 4.0)	59	94	91	ND*
20 mM sodium acetate, 150 mM NaCl (pH 4.5)	67	100	92	22 ppm

*ND, not determined.

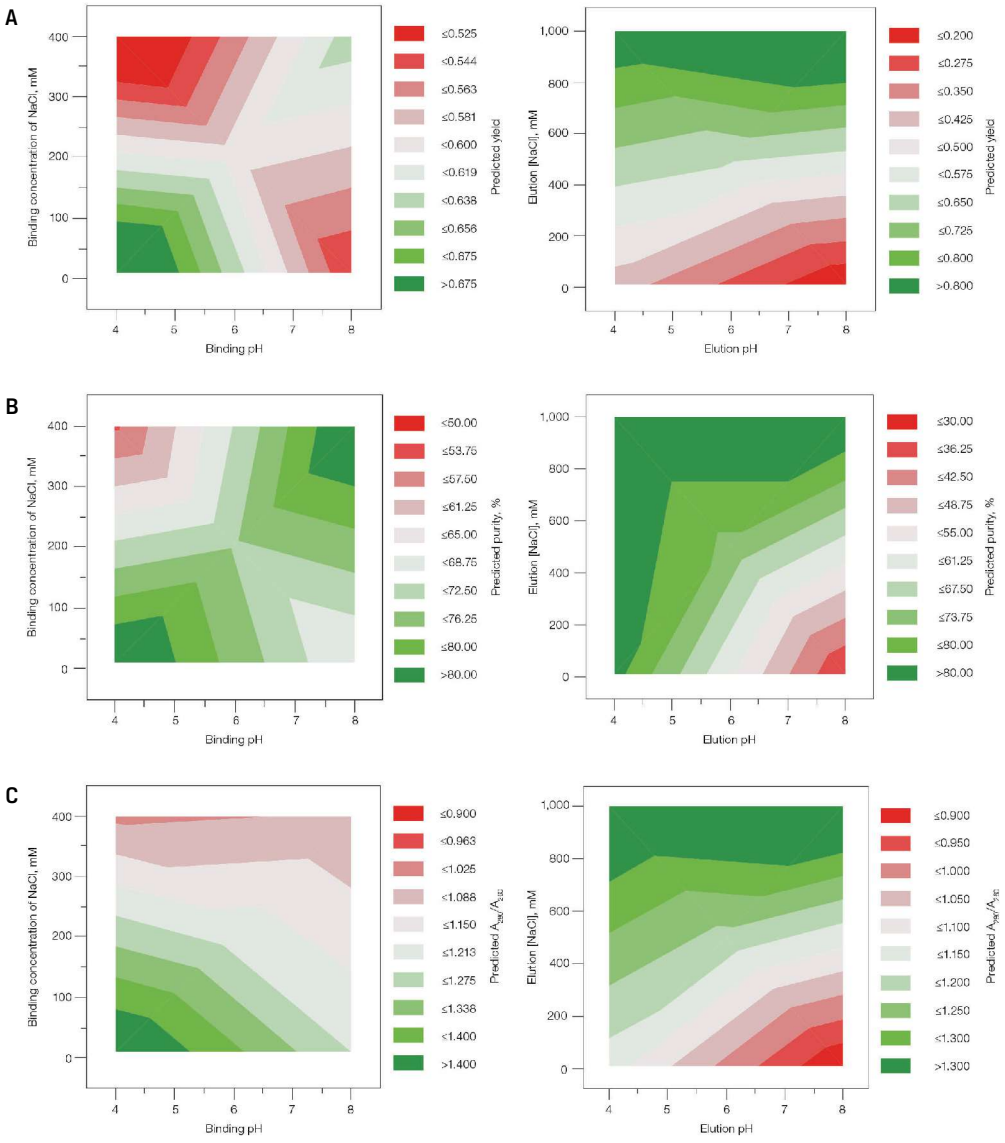


Fig. 2. Capturing lysozyme from *E.coli* lysate using Nuvia cPrime Column. A, effect of buffer pH and sodium chloride concentration on the yield of lysozyme; **B,** effect of buffer pH and sodium chloride concentration on the purity of lysozyme; **C,** effect of buffer pH and sodium chloride concentration on the removal of host cell nucleic acids, as indicated by A_{280}/A_{200}.

Figure 3). The recovery of lysozyme was close to quantitative. These results are in good agreement with the predictions from the DOE screening study.

SUMMARY

This case study demonstrates a simple yet effective approach to obtain the optimum binding and elution conditions for a diverse set of proteins on Nuvia cPrime Hydrophobic Cation Exchange Media. A total of 11 experiments from a two-parameter, two-level fractional factorial DOE approach was sufficient to assess the effects of buffer pH and conductivity on the chromatographic behavior of a particular protein.

Using the purification of lysozyme from *E. coli* lysate as an example, this has demonstrated that a design space for maximum target protein purity and yield can be identified from response surfaces generated in the DOE screening. Following the operational conditions suggested by DOE, the scale-up purification of lysozyme from crude expression harvest was achieved with remarkable purity.

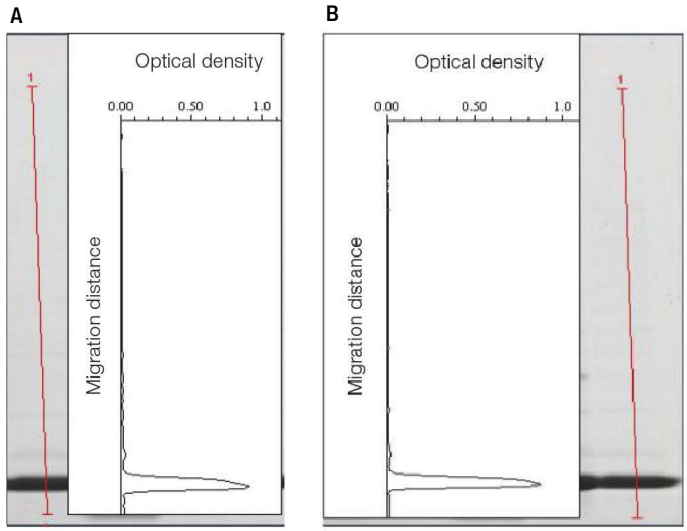


Fig. 3. Electrophoretograms of purified lysozyme. A, *E. coli* lysate was loaded onto the Nuvia cPrime column at pH 4.0 and eluted with 20 mM sodium phosphate and 1 M sodium chloride (pH 7.5); **B**, *E. coli* lysate was loaded onto the Nuvia cPrime Column at pH 4.5 and eluted with 20 mM sodium phosphate and 1 M sodium chloride (pH 7.5). The reducing SDS-PAGE gel was scanned with a GS-800 Densitometer. The purity of lysozyme in each eluate was illustrated by its optical density trace.

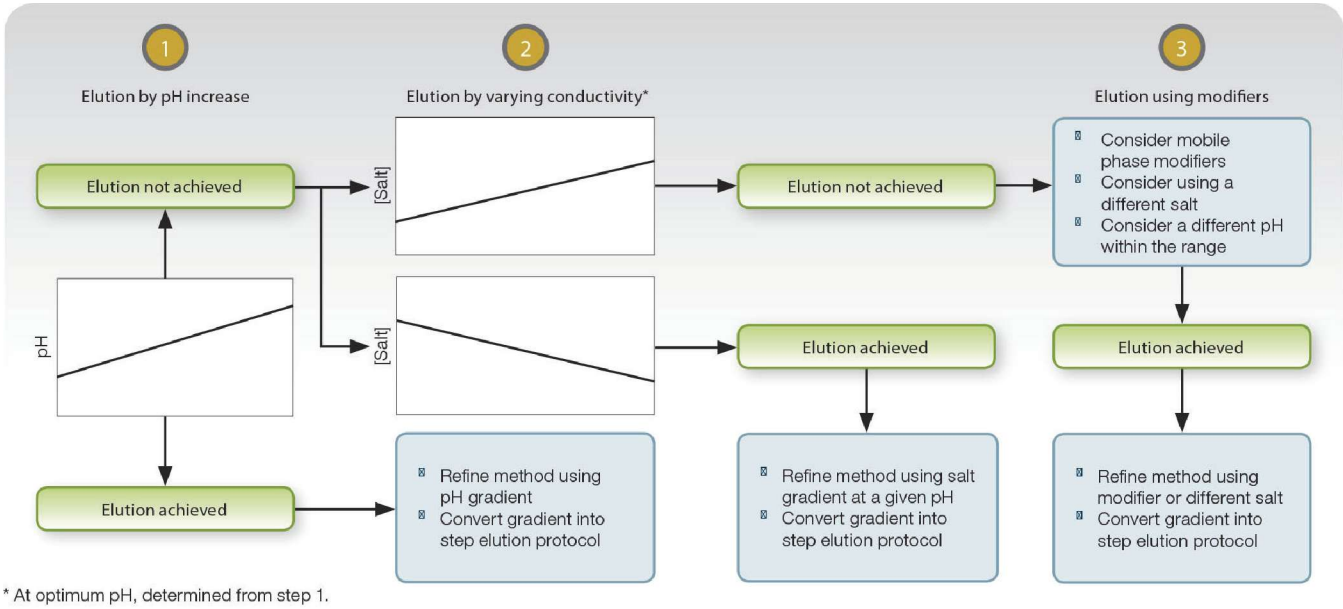


Fig. 4. Recommended approach to method development using Nuvia cPrime.

The method development for protein purification on Nuvia cPrime Hydrophobic Cation Exchange Media is straightforward. The modes of interaction, as well as the extent of these interactions between feed stream components and Nuvia cPrime can be elucidated early in the process, thus allowing users to quickly and effectively optimize their method for best selectivity and robustness (Figure 4). •

Reference

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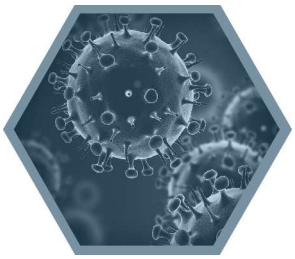

Rapid Single–Step Purification **of Proteins from Whey**

Whey proteins play a vital role in the formulation of food supplements because of numerous desirable nutritional and functional properties. This case study depicts the establishment of a process workflow for whey purification using Nuvia™ S and Nuvia Q Ion Exchange Resins.

The major whey proteins are α-lactalbumin (ALA), β-lactoglobulin (BLG), bovine serum albumin (BSA), and bovine immunoglobulins (Hahn et al. 1998) (Table 1). Minor proteins such as lactoperoxidase, lactoferrin, and proteose-peptone account for the other protein components in whey.

The objective of this study was to develop a separation process to fractionate usable proteins from whey (Figure 1). First, total whey protein isolate (WPI) was purified because of its high-end functional advantages, as evidenced by a demand from nutrition and health segments. Second, ALA and BLG were purified because of their value in infant formula and confections, respectively.

Table 1. Protein composition of bovine whey.

PROTEIN	AVERAGE CONCENTRATION, g/L	PROPORTION IN WHEY, %	MOLECULAR WEIGHT	ISOELECTRIC POINT
ALA	1.5	22	14,200	4.7 - 5.1
BLG	3–4	51	18,400	5.2
BSA	0.3–0.6	6.6	65,000	4.9
IgG, IgA, IgM	0.6–0.9	11	150,000–900,000	5.8–7.3
Lactoperoxidase	0.06	<1	78,000	9.6
Lactoferrin	0.05	<1	78,000	8.0
Proteose-peptone	0.5	7	4,000–20,000	–

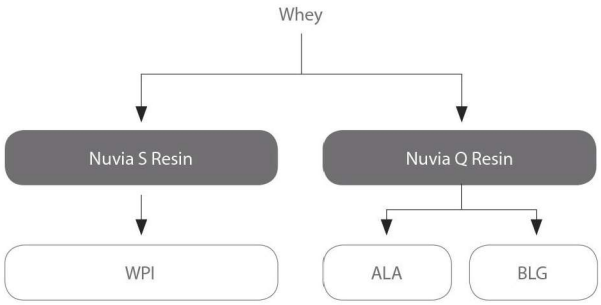

Fig. 1. Protein Purification Workflow for Whey using Nuvia S and Nuvia Q Resins.

METHOD DEVELOPMENT

Workflow for protein purification from Whey with Nuvia™ S and Nuvia Q Resins.

Nuvia S Cation Exchange Resin and Nuvia Q Anion Exchange Resin are designed to meet the pressure-flow requirements of demanding purification processes. With optimized bead size distribution and mechanical properties, Nuvia Resins consistently deliver very high binding capacity at fast linear velocities while maintaining low column pressure. The wide operating window allows users to operate at increasingly higher flow rates without compromising binding capacity.

RESULTS

Preparation of Whey Protein Isolate

As defined by the Whey Protein Institute (wheyoflife.org), WPI is the most pure and concentrated form of whey protein with few or no lipids or lactose. Since the majority of the proteins have isoelectric points above 4.7, loading of whey at pH 4.0 onto Nuvia S Resin resulted in the capture of these proteins (Etzel 2004, Hahn et al. 1998). Lipids, lactose, and unbound proteins remain in the flowthrough and the wash. As shown in Figure 2, a single peak was collected upon elution with pH 8.0 elution buffer. Capacity of the Nuvia S Column, based on the measurement of the protein content in the eluate pool, was 60± 2 g/L (60 CV/hr, n = 4). As shown in Figure 3, proteins in WPI and in whey are similar. Interestingly, densitometry of the major species, including ALA, BLG, and BSA, in whey and WPI showed nearly identical distributions.

SINGLE STEP USING CATION EXCHANGE

Table 2. Cation Exchange Chromatography Using Nuvia S Resin
Column: 1 ml, 0.7 x 2.7 cm

Step	Buffer	CV	Flow Rate
Equilibration	0.04 M sodium lactate, pH 4.0 (Buffer A)	3	150 cm/hr
Sample Loading	Whey concentrate diluted 1:10 in Buffer A, 1.2 mM filtered	20	150 cm/hr
Wash 1	Buffer A	10	150 cm/hr
Elution	0.04 M sodium phosphate, pH 8.0	20	150 cm/hr
Wash 2	1 M NaCl	3	150 cm/hr
Wash 3	1 M NaCl	3	150 cm/hr
Equilibration	0.02 M sodium lactate, pH 4.0	3	150 cm/hr
Re-Equilibration	Buffer A	5	150 cm/hr

SINGLE STEP USING ANION EXCHANGE

Table 3. Anion Exchange Chromatography Using Nuvia Q Resin
Column: 1 ml, 0.7 x 2.7 cm

Step	Buffer	CV	Flow Rate
Equilibration	0.02 M sodium phosphate, pH 6.0 (Buffer A)	3	150 cm/hr
Sample Loading	Whey concentrate diluted 1:10 in Buffer A, 1.2 mM filtered	5	150 cm/hr
Wash	Buffer A	4	150 cm/hr
Elution 1	Gradient of Buffer A to Buffer A + 0.01 M NaCl	10	150 cm/hr
Elution 2	Buffer A + 0.1 M NaCl	5	150 cm/hr
Elution 3	Gradient of Buffer A + 0.01 M NaCl to Buffer A + 0.3 M NaCl	10	150 cm/hr
Elution 4	Buffer A + 0.3 M NaCl	5	150 cm/hr
Elution 5	Gradient of Buffer A + 0.3 M NaCl to Buffer A + 0.9 M NaCl	10	150 cm/hr
Elution 6	Buffer A + 0.9 M NaCl	10	150 cm/hr

Anion Exchange Resin Selection and Optimization

ALA and BLG account for about 75% of the proteins in whey. These two model proteins were therefore selected for further experimentation. In an initial screen, Nuvia Q Resin was compared to two resins with high binding capacity (resin A and resin B). The

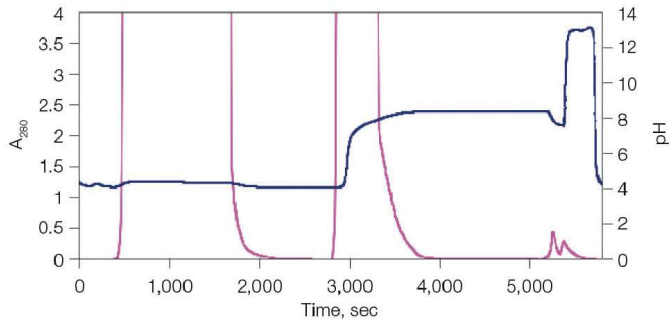

Fig. 2. CEX chromatography of 80 mg of crude whey on a Nuvia S Column. Column: 0.7 x 2.7 cm; 2 ml fractions were collected; flow rate: 60 CV/hr; Buffer A: 0.04 M Na lactate, pH 4.0; Buffer B: 0.04 M Na phosphate, pH 8.0. A_{280} (–); pH (–).

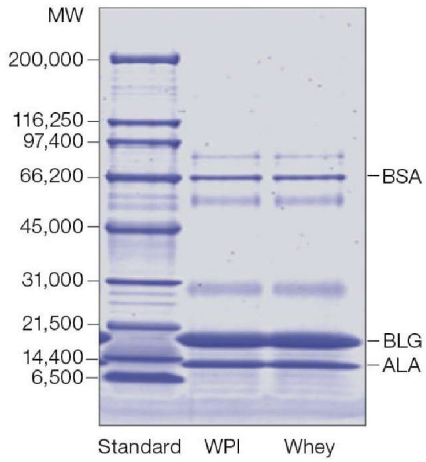

Fig. 3. SDS-PAGE analysis of WPI and whey. Whey protein and WPI (10 mg) along with Bio-Rad SDS-PAGE standards were run on a 4-20% Criterion™ TGX™ Precast Gel. Proteins were stained using BioSafe™ Coomassie Stain.

resins were tested using a small mixture of ALA and BLG (10% CV) that was eluted using a shallow NaCl gradient (0–0.3 M). This permits rapid and uniform evaluation of process parameters, but further optimization will be needed for the selected resin. The resulting chromatograms from this screening experiment were compared (Figure 4). All three resins produced two equally well-resolved peaks. Peaks of resin A eluted sooner, suggesting weaker AEX properties than Nuvia Q Resin and resin B. The bead size of resin A is the smallest of the three resins, which may lead to higher column pressure upon scale-up. Additionally, rigidity, cleanability, capacity, and cost will have to be factored in when choosing the appropriate resin for large-scale purification.

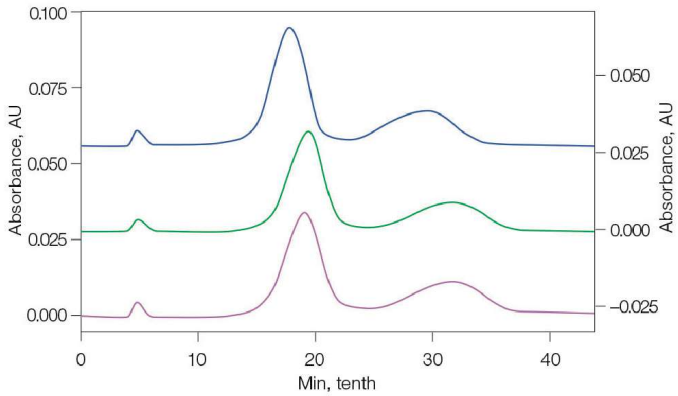

Fig. 4. ALA and BLG purification chromatograms on three anion exchangers. Comparison of Nuvia Q Resin (–), resin A (–), and resin B (–) performance.

Purification of ALA and BLG from Whey

To minimize processing costs, a purification scheme for ALA and BLG from whey ideally should require a single chromatographic step to purify both proteins. Initial studies (Kim et al. 2003, Gerberding and Byers 1998) demonstrated the removal of the major whey proteins (ALA, BSA, and BLG) in a segmented gradient (data not shown). Further work was performed to optimize these conditions. The purification profile of each protein was first identified by injecting the purified protein alone and then a mixture containing the three purified proteins. As expected, separation of the mixture paralleled that of the individual proteins. When whey was used as the feed stock, two major pools were resolved upon adsorption and elution with the segmented gradient (Figure 5).

SDS-PAGE analysis (Figure 6) of the fractions showed two peaks, one containing predominantly ALA (Pool 1) and the other containing predominantly BLG (Pool 2). The corresponding ALA and BLG standards are also shown in the gel scan. Purities of the two whey-derived proteins in each peak were estimated by densitometry to be at least 85%.

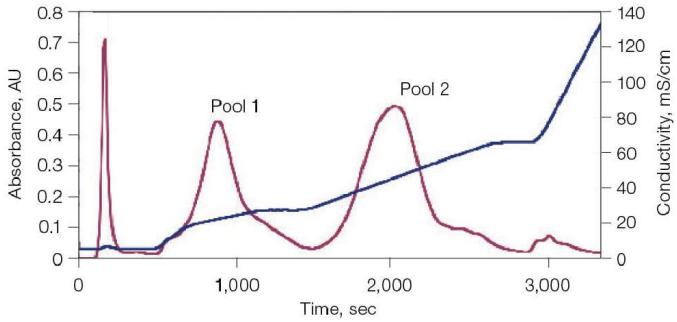

Fig. 5. AEX chromatography of 8 mg of crude whey on a 0.7 x 2.7 cm Nuvia Q Column. Fractions of 2 ml were collected; flow rate: 60 CV/hr; buffer: 0.02 M Na phosphate, pH 6.0; segmented gradient 0-0.1 M NaCl, 0.1-0.3 M NaCl, and 0.3-0.9 M NaCl. A_{280} (–); conductivity (–).

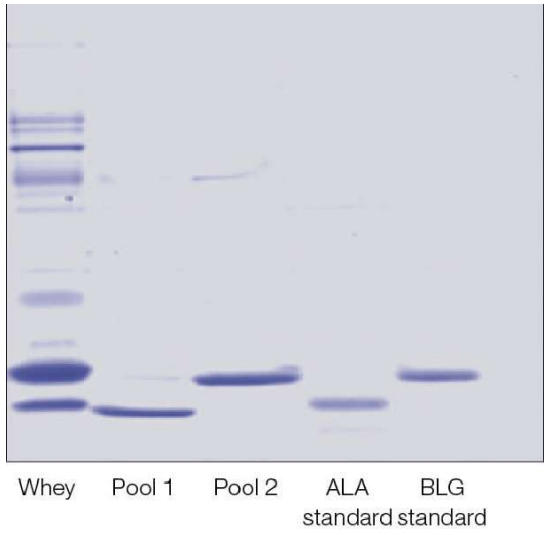

Fig. 6. SDS-PAGE analysis of whey, purified ALA (Pool 1), and purified BLG (Pool 2) against protein standards.

CONCLUSIONS

Nuvia S and Nuvia Q Resins, two novel high-capacity resins for ion exchange chromatography, allow efficient enrichment of proteins from crude mixtures. The feasibility to prepare WPI, purified ALA, and purified BLG has been demonstrated. Because of the high-throughput properties of Nuvia Resins, a rapid single-step purification of proteins from whey is possible. •

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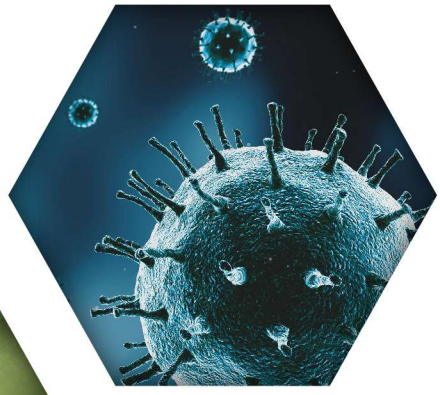

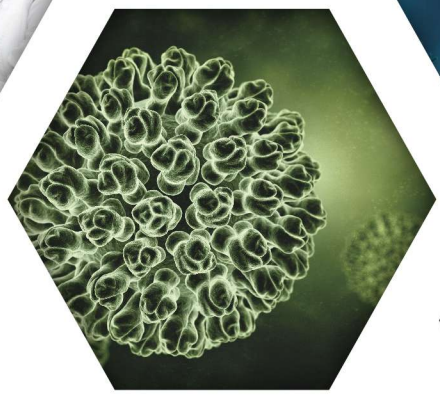

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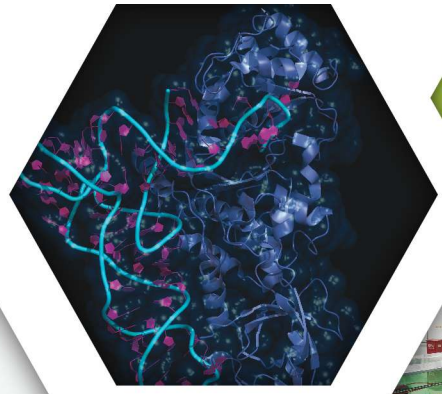

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