Guide to Lentiviral Vector Purification with CHT™ Ceramic Hydroxyapatite

Introduction

Cell and gene therapy (CGT) represents a groundbreaking approach in modern healthcare, offering promising treatment options for previously incurable diseases. Through targets at the genetic or cellular level, these therapies offer exceptional prospects for many kinds of conditions. Consequently, growing interest has led it to become one of the fastest growing fields in modern healthcare. Recent FDA approvals for Lyfgenia (lentiviral vector) and Casgevy (CRISPR/Cas9) for sickle cell disease[1] are perfect examples of how gene therapy provides treatment options for diseases which previously had limited treatment options.

Lentiviruses are enveloped viruses of the Retroviradae family that are coming to the forefront of these new treatment options as lentiviral vectors (LVVs). These are used as vehicles for efficient delivery of genes into a wide variety of cells, enabling the expression of transgene(s). Consequently, LVVs have broad usages, from functional genomics to recombinant protein production and clinical gene therapy[2,3].

Despite their versatility, challenges persist in the production of LVVs, such as low functional viral titers and high impurity levels. As their usage extends from ex vivo transduction of cells to in vivo administration, regulatory requirements on impurity removal are expected to increase. This article aims to guide scientists and pharmaceutical companies on the use of Ceramic Hydroxyapatite to overcome LVV purification challenges.

Ceramic Hydroxyapatite

Ceramic Hydroxyapatite as chromatography media is mostly a spherical macroporous form of hydroxyapatite known for its physical and chemical robustness. Functioning as both the ligand and support matrix, it is a multimodal resin that features metal affinity interactions with calcium sites (C-sites) and cation exchange interactions with phosphate sites (P-sites). These sites allow for unique and precise separation of an extremely broad range of therapeutic modalities, including viruses[4].

LVVs have an isoelectric point (pI) of between 6.0 to 6.5, and therefore are negatively charged at neutral pH. Anion exchange chromatography (AEX) is a commonly employed strategy for LVV purifications, where both weak and strong AEX resins have been used for down-stream purifications. However, elution of LVVs from AEX resins require the use of high sodium chloride concentrations (up to 1M), which has been shown to be extremely detrimental to its stability. Infective titer of LVVs drops significantly when incubated in different buffer systems with 1M sodium chloride, with phosphate buffer at pH 7.0 showing the least decrease[3].

CHT™ Type II* Characteristics

Table 1. CHT™ Type II* Characteristics**

Functional groups	Ca^{2+}, PO_4^{3-}, OH^-
Particle sizes	20, 40, and 80 µm (nominal)
Recommended linear flow rate	50 –1,000 cm/hr
Operating pH range	6.5 – 14
Chemical compatibility (>24 hr)	1 N NaOH, 6 M urea, 8 M guanidine-HCl, ethanol, methanol, 100% acetonitrile
Sanitization	1–2 N NaOH
Autoclavability (121ºC, 20 min)	Yes
Packing density (g/ml packed bed)	0.63 g/ml Type II
Dynamic binding capacity	>12.5 mg lysozyme/g
Nominal pore diameter	800–1,000 Å
Maximum operating pressure	100 bar (1,500 psi)

**CHT™ Type II is manufactured by HOYA Technosurgical Corporation and distributed globally by Bio-Rad Laboratories, Inc. Hercules, CA USA.*

***Referenced from Bio-Rad Bulletin 5667*

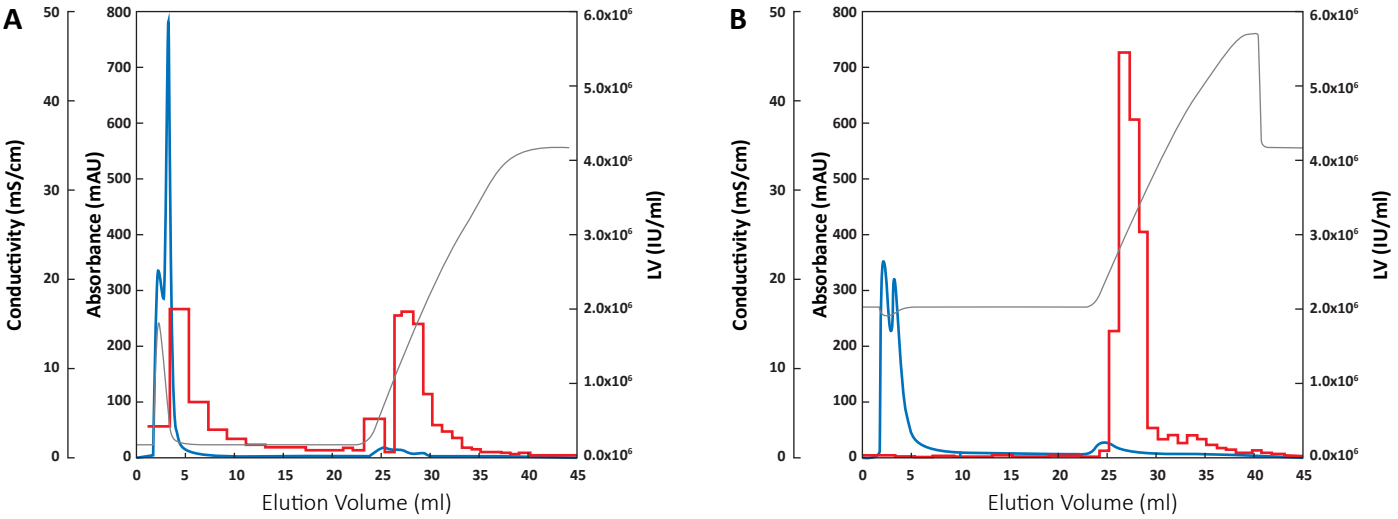

Figure 1. Adsorption of LVVs without **(A)** and with **(B)** the addition of sodium chloride in the equilibration buffer (Buffer A) and elution buffer (Buffer B). The sample (1 mL) was loaded into the column without any adjustment and washed with the respective equilibration buffer (Buffer A) before a gradient elution (Buffer B) was carried out. Infective LVV titer (IU/mL) was measured through detection of MpGFP by flow cytometry **(red)**. Without sodium chloride addition **(A)**, 48.3% of the total infective LVVs were found in the load and wash fractions and 51.7% were found in elution fractions. With sodium chloride addition **(B)**, 0.2% of the total infective LVVs were found in the load and wash fractions and 99.8% were found in the elution fractions. UV absorbance measured at 280 nm **(blue)** and conductivity **(black)** were also monitored.

Sodium Chloride for Improved Adsorption

Column:	4.6mm x 35mm
Resin:	CHT™ Type II* 40 µm
Sample:	MpGFP HEK293T supernatant containing 10% FBS, 3.9 x 10^7 IU/mL
Buffer A:	• 10 mM Sodium Phosphate, pH 7.2 • 10 mM Sodium Phosphate, 150 mM Sodium Chloride, pH 7.2
Buffer B:	• 400 mM Sodium Phosphate, pH 7.2 • 400 mM Sodium Phosphate, 150 mM Sodium Chloride, pH 7.2
Flowrate:	360 cm/hr
System:	ÄKTA™ Avant Chromatography System

Sodium chloride addition is required for improved LVV adsorption to Ceramic Hydroxyapatite. To demonstrate this, purifications were carried out with and without the presence of sodium chloride (Figure 1). The infective titer of the LVVs was measured through detection of transduced Green Fluorescent Protein (Gene of Interest) by flow cytometry. When no sodium chloride is added, only 51.7% of the total infective LVVs were detected in the fractions obtained from the elution gradient. When sodium chloride was added to the equilibration and elution buffers, this increased to 99.8%. Therefore, it is recommended that sodium chloride is added, at a concentration that is suitable both for LVV stability and purification performance.

Separation of Impurities

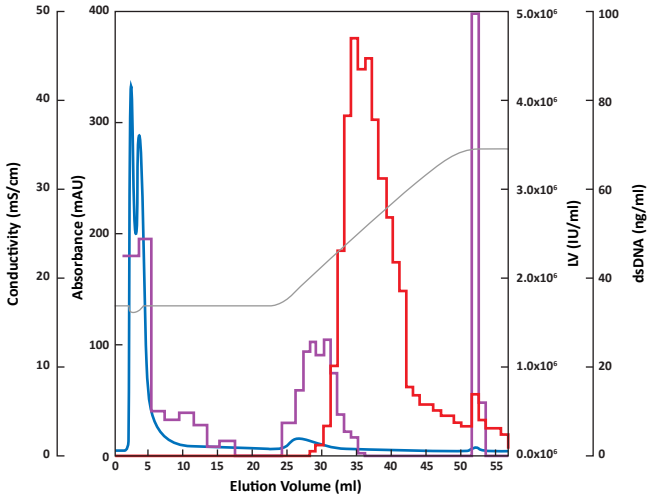

Figure 2. Separation of infective LVVs detected by flow cytometry for GFP **(red)**, from protein impurities at the start of the phosphate gradient detected by UV280 **(blue)** and dsDNA impurities at the end of the gradient detected by picogreen **(purple)**. Conductivity was monitored **(black)**.

Therefore, an appropriate wash and elution condition can be easily derived to achieve good impurity clearance from infective LVVs. To achieve effective impurity clearance, the following workflow is recommended as an initial purification strategy (Figure 3):

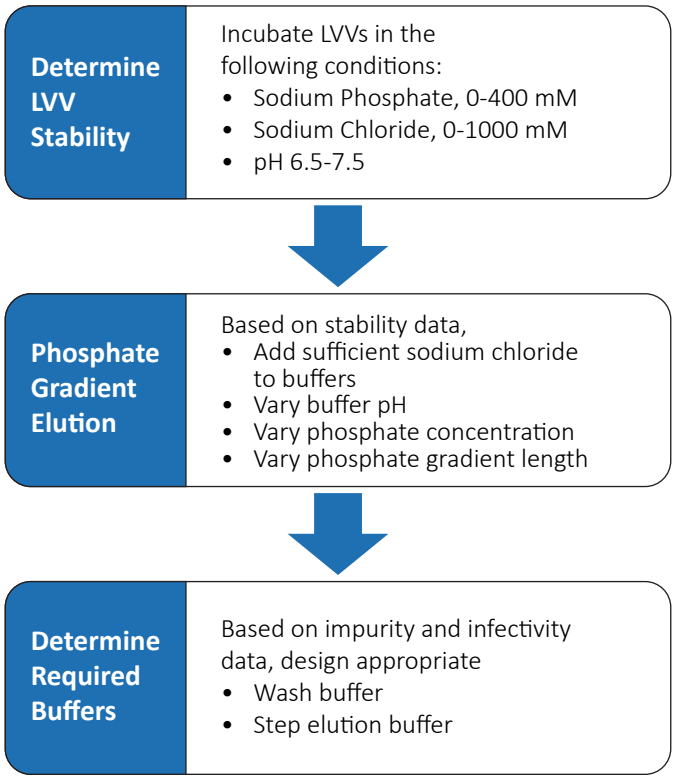

Figure 3. Recommended workflow for initial purification of LVVs with Ceramic Hydroxyapatite

Table 2. Recommended buffers to be used for phosphate gradient elution experiments

Step Name	Buffer Composition	Details
Equilibration	10 mM Phosphate, 150 mM NaCl	Until pH and Conductivity is stable
Sample Loading	Clarified, serum removal not required	10^7 IU / mL of of resin*
Wash	10 mM Sodium Phosphate, 150 mM NaCl	Ensure minimal LV is detected in wash
Elution	300 mM Sodium Phosphate, 150 mM NaCl	Start with 20CV, adjust gradient as necessary

*Recommended resin loading amount for initial experiments. Actual sample loading amount will depend on lentiviral characteristics and loading condition. Serum removal will increase binding capacity.

The results presented indicate that Ceramic Hydroxyapatite can achieve excellent binding to LVVs, while maintaining effective protein and DNA separation from the main infective LVV peak. Additionally, it can be eluted using phosphate with minimal sodium chloride (~ 150 mM) addition. Compared to typical AEX resins which require much higher sodium chloride concentrations[3], Ceramic Hydroxyapatite elution conditions for LVVs are significantly milder and can be expected to improve its stability.

Ceramic Hydroxyapatite, specifically CHT™ Type II* can address the challenges of LVV production and purification, with similar results obtained across the same family of resins.

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