CHT™ Ceramic Hydroxyapatite

Instruction Manual

Please read these instructions prior to using CHT ceramic hydroxyapatite. If you have any questions or comments regarding these instructions, please contact your local Bio-Rad Laboratories sales representative.

BIO-RAD

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Section 1
Introduction

CHT™ ceramic hydroxyapatite is a leading purification medium of biomolecules in today's demanding downstream process industry. Its mixed-mode support offers unique selectivities and often separates biomolecules that appear homogeneous using other chromatographic methods. The diverse binding capabilities of CHT for host cell proteins, leached protein A, antibody dimmers and aggregates, nucleic acids, and viruses allow its use at any stage from initial capture to final polishing.

The robust properties of CHT ceramic hydroxyapatite improve efficiency, yield, and financial value through:

- Excellent capture at elevated flow rates enabling processing at all scales
- Large capacity for higher-titer upstream feedstocks
- Exceptional selectivity allowing for a two-step chromatographic process

This manual is a guide for the use of CHT as a media support in your purification process. The manual is organized into four main topics:

- Product Description
- Chromatography
- Regeneration, Sanitization, and Storage
- Column Packing Protocols
- Case Studies

Throughout this manual, we have incorporated recommendations ranging from method scouting and optimization to column packing techniques that represent feedback from process chromatographers globally. Should you have further questions, contact either your local Bio-Rad process chromatography sales representative or the Bio-Rad chromatography technical support department for further assistance at 1-510-741-6563.

Section 2
Product Description

2.1 What is CHT™ Ceramic Hydroxyapatite?

Hydroxyapatite ($Ca_5(PO_4)_3OH)_2$ is a form of calcium phosphate used in the chromatographic separation of biomolecules. Sets of five calcium doublets (C-sites) and pairs of –OH containing phosphate triplets (P-sites) are arranged in a repeating geometric pattern. Repeating hexagonal structures can be seen in electron micrographs of the material. 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. Applications of hydroxyapatite chromatography include the purification of different subclasses of monoclonal and polyclonal antibodies, antibodies that differ in light chain composition, antibody fragments, isozymes, supercoiled DNA from linear duplexes, and single-stranded from double-stranded DNA.

CHT ceramic hydroxyapatite is a spherical, macroporous form of hydroxyapatite. It has been sintered at high temperatures to modify it from a crystalline to a ceramic form. The ceramic material overcomes many of the limitations of traditional crystalline hydroxyapatite that prevent its use in industrial-scale applications. The ceramic material retains the unique separation properties of crystalline hydroxyapatite, but can be used reproducibly for many cycles at high flow rates and in large columns. Unlike most other chromatography adsorbents, CHT is both the ligand and the support matrix. Separation protocols originally developed on crystalline hydroxyapatite can often be transferred directly to the ceramic material with only minor modifications. Two types of CHT ceramic hydroxyapatite, Type I and Type II, are available in three particle sizes, 20, 40, and 80 µm. Both types have elution characteristics similar to crystalline hydroxyapatite, but also have some important differences. 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.

2.2 Specifications

	Type I	Type II
Functional groups	Ca^{2+}, PO_4, OH	Ca^{2+}, PO_4, OH
Observed dynamic binding capacity lysozyme (Lys)	≥ 25 mg Lys/g CHT	≥ 12.5 mg Lys/g CHT
Nominal pore diameter	600–800 Å	800–1,000 Å
Maximum backpressure	100 bar (1,500 psi)	100 bar (1,500 psi)
Nominal mean particle size	20 ± 2, 40 ± 4, and 80 ± 8 µm	20 ± 2, 40 ± 4, and 80 ± 8 µm
Bulk density	0.63 g/ml	0.63 g/ml

2.3 Characteristics

	Type I	Type II
Observed dynamic binding capacity IgG	25–60 mg IgG/ml CHT*	15–25 mg IgG/ml CHT**
Typical linear flow rate range	50–1,000 cm/hr	
pH stability* **	6.5–14 pH	
Base stability	at least 21 months in 1 N NaOH	
Regeneration	500 mM sodium phosphate, pH 7	
	1,000 mM trisodium phosphate, pH 11–12	
Autoclavability (bulk)	121°C, 20 min in phosphate buffer, pH 7	
Sanitization	1–2 N NaOH	
Recommended column storage	0.1 M NaOH	
Shelf life (dry, unused material)	85 months stored dry, sealed, and at room temperature	

* 40 µm particles, 300 cm/hr, 5 mM sodium phosphate, pH 6.5

** 40 µm particles, 300 cm/hr, 5 mM sodium phosphate, pH 6.5

*** For pH 5.5–6.0, see Section 3.1.1 Buffers and Table 1

Purity

In the preparation of ceramic hydroxyapatite, use of high-purity raw materials results in low levels of contaminants as determined by ICP mass spectrometry for metal analysis and ion chromatography for anions.

Impurity	Levels
Chloride	≤ 0.005%
Sulfate	≤ 0.01%
Carbonate	≤ 0.01%
Lead	≤ 0.001%
Cadmium	≤ 0.0001%
Barium	≤ 0.001%
Arsenic	≤ 0.001%

2.4 General Handling and Packing

CHT ceramic hydroxyapatite is a rigid support and can operate under high flow rates and pressures. However, excessive physical force beyond typical operating conditions can result in bead damage. In order to optimize the chromatographic properties of CHT, avoid excessive stirring or agitation that may lead to mechanical damage and bead fracture.

See General Handling and Powder Preparation, Section 5.1, for more details.

Section 3
Chromatography

3.1 CHT™ Ceramic Hydroxyapatite Mechanism

Hydroxyapatite contains two types of binding sites, positively charged calcium and negatively charged phosphate groups. These sites are distributed regularly throughout the crystal structure of the matrix. Solute species dominantly interact through cation exchange via the phosphate groups and/or metal affinity via the calcium atoms.

Cation exchange occurs when protein amino groups interact ionically with the negatively charged phosphates. The amino groups are similarly repelled by the calcium sites. Binding depends upon the combined effects of these interactions. These ion exchange interactions can be disrupted by adding neutral salts such as sodium chloride or buffering species such as phosphate to the mobile phase. Cation exchange interactions also weaken with increasing pH. Hence, the addition of salt or phosphate, or an increase in pH, can be used to weaken the interaction. Studies with model proteins have demonstrated that anion exchange, which might be expected from interactions of negatively charged surface residues with calcium, does not make a significant contribution.

Calcium affinity occurs via interactions with carboxyl clusters and/or phosphoryl groups on proteins or other molecules (e.g., nucleic acids); these groups are simultaneously repelled by the negative charge of the CHT phosphate groups. The affinity interaction is between 15 and 60 times stronger than ionic interactions alone and, like classical metal-affinity interactions, is not affected by increasing ionic strength using typical elution ions (e.g., chloride). Species binding through calcium affinity may adsorb more strongly as the ionic strength increases due to ionic shielding of the charge repulsion from the CHT phosphate sites. Metal affinity interactions can be dissociated by phosphate in the mobile phase.

Most large proteins bind by a combination of mechanisms (Figure 1):

Dominantly acidic proteins, such as albumin, bind chiefly by metal affinity interactions. Sodium chloride at 1.0 M reduces retention time by approximately 10% in the presence of phosphate gradients, indicating a minor contribution by cation exchange. To elute acidic proteins, phosphate buffers are required.

Dominantly basic proteins, such as IgG, bind chiefly by cation exchange interactions. Sodium chloride reduces retention time in the presence of phosphate gradients, indicating a minor contribution by metal-affinity. Basic proteins may be selectively eluted with either phosphate or salts.

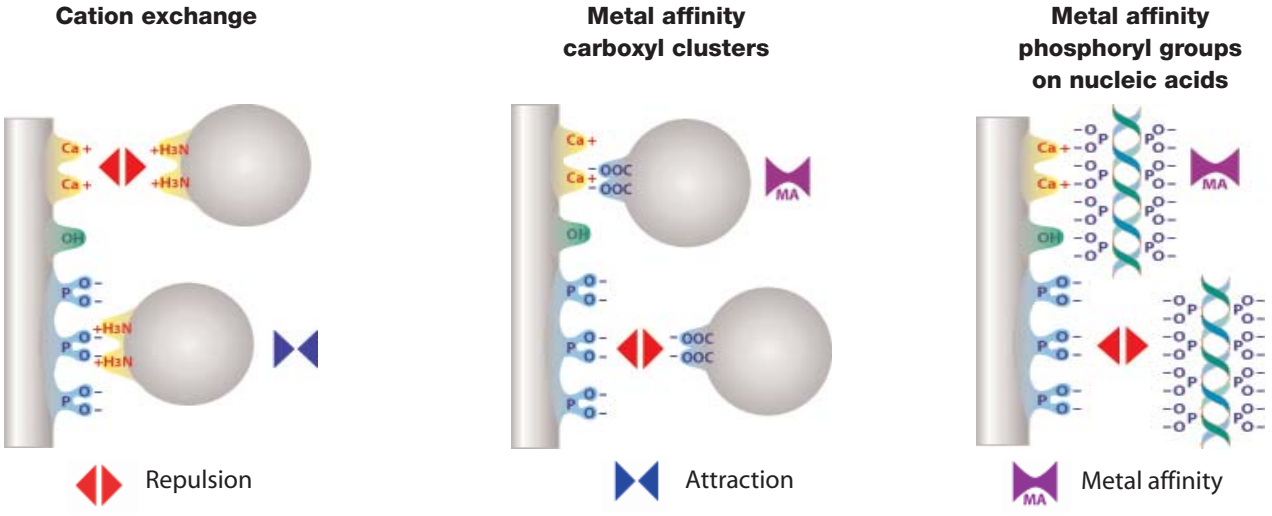


Fig. 1. Schematic Representation of CHT binding mechanisms.

3.1.1 Buffers

A key advantage to CHT is its compatibility with a wide range of salts and buffers.

While phosphate buffers are the most widely used with CHT, buffer systems composed of, for example, MES, HEPES, Tris, imidazole, or acetate can support at least 50 life cycles of use. Table 1 highlights a variety of buffer conditions that optimize CHT sustainability. Prolonged exposure to pH 6.5 reduces the cycle life of CHT. This is attributed to the breakdown of the CHT matrix.

In order to promote the stability of CHT, we recommend that low concentrations of either phosphate or calcium be included in buffers. Calcium may improve binding of weakly acidic proteins.

Table 1. CHT Stability in Various Buffers.*

		CHT Suitability	
pH	**Buffer**	**10 cycles**	**50 cycles**
5.0	Acetate + 5 mM PO_4	—	n/a
5.5	Acetate + 5 mM PO_4	-	—
6.0	Acetate + 5 mM PO_4	+	-
6.0	Succinate + 5 mM PO_4	-	—
6.5	Succinate + 5 mM PO_4	+/-	+/-
6.5	Acetate + 5 mM PO_4	+	n/a
6.5	Phosphate (5 mM)	+	n/a
6.5	Acetate + 5 mM PO_4	+	+
6.5	MES + 5 mM PO_4	+	+
6.5	Imidazole + 5 mM PO_4	+	+/-
6.5	Glycine + 5 mM PO_4	+	+
6.5	Arginine + 5 mM PO_4	+	+
6.5	Tris + 5 mM PO_4	+	n/a
7.0	Phosphate (5 mM)	+	+
7.0	MES + 5 mM PO_4	+	n/a
7.0	Acetate + 5 mM PO_4	+	n/a
7.0	Imidazole + 5 mM PO_4	+	+
7.0	Glycine + 5 mM PO_4	+	n/a
7.0	Arginine + 5 mM PO_4	+	n/a
7.0	HEPES + 5 mM PO_4	+	n/a
7.0	Tris + 5 mM PO_4	+	n/a
7.5	Phosphate (5 mM)	+	n/a
7.5	MES + 5 mM PO_4	+	n/a
7.5	Imidazole + 5 mM PO_4	+	+
7.5	Acetate + 5 mM PO_4	+	n/a
7.5	HEPES + 2 mM PO_4	+	n/a
7.5	HEPES + 5 mM PO_4	+	n/a
7.5	Tris + 2 mM PO_4	+	n/a
7.5	Tris + 5 mM PO_4	+	+
8.5	Tris + 5 mM PO_4	+	n/a

+	No statically significant mass loss observed
+/-	Slight (0–1%) loss
-	Small (1–2%) loss
—	Significant (>2%) loss
n/a	Not applicable

* All experiments performed in small scale columns. Each cycle used approximately 35 column volumes of buffer to simulate an equilibration and long gradient, as well as five column volumes of 1 N NaOH to simulate regeneration.

3.1.2 Elution

During the course of operation any step change in the buffer conditions (increase or decrease in salt, buffering species, or other components) can lead to a transient change in the pH of the mobile phase. This phenomenon can be attributed to the interaction of the mobile phase ions with the phosphate surface groups of CHT. The extent of this pH shift, generally less than 0.5 units, depends on the degree to which components are increased or decreased. The addition of nonphosphate buffer can be used to stabilize pH shift.

3.1.3 Trace Metal Contamination

CHT will also bind to trace metals, such as iron, that may be present in buffer solutions. The metal contaminants may originate from production media, buffers and salts, process water, and/or corroded stainless steel. The degree of trace-metal deposition will manifest itself as a visible discoloration at the top of the column over time. If this becomes an issue, two potential solutions are to either pretreat buffers by incubating with a small amount of CHT prior to filtration, or to install a CHT guard column for use during buffer and/or load application.

If bulk buffer pretreatment with CHT incubation is used, take care to avoid damage to pumps and tubing due to abrasion by CHT particles. Vessel cleaning can be accomplished by an acid wash, which will completely solubilize CHT.

3.1.4 Phosphate

Generally, 5 mM phosphate should be included in all buffer solutions. When operating at pH >7.0, lower amounts of phosphate down to 2 mM may suffice. Phosphate concentrations above 5 mM in these buffers will not improve stability and may decrease protein binding. As illustrated in Figure 2, CHT binding capacity decreases in 50 mM MES with increasing phosphate concentration.

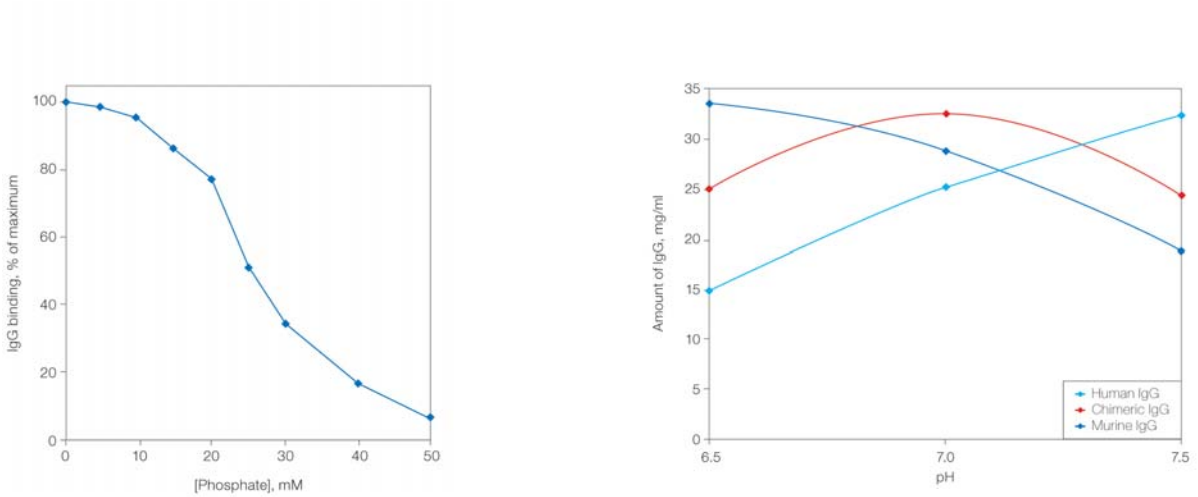


Fig. 2. IgG binding capacity of CHT vs. phosphate concentration and pH.

Hydrated phosphate salts should be used in all buffer preparations. Nonhydrated phosphates should not be used because the manufacturing process for these salts leads to pyrophosphate formation. Pyrophosphates inhibit the binding of some macromolecules and reduce CHT selectivity. Avoid back-titrating buffer pH as this increases conductivity and may reduce target protein binding on CHT.

3.1.5 Calcium

Calcium chloride can be used as a CHT stabilizing agent at a general concentration of 3 mM. More or less calcium chloride may be used dependent on phosphate concentration. Note that calcium deposits onto the matrix and precipitates with other solution species, especially phosphate.

3.1.6 Chemical Compatibility/Load Preparation

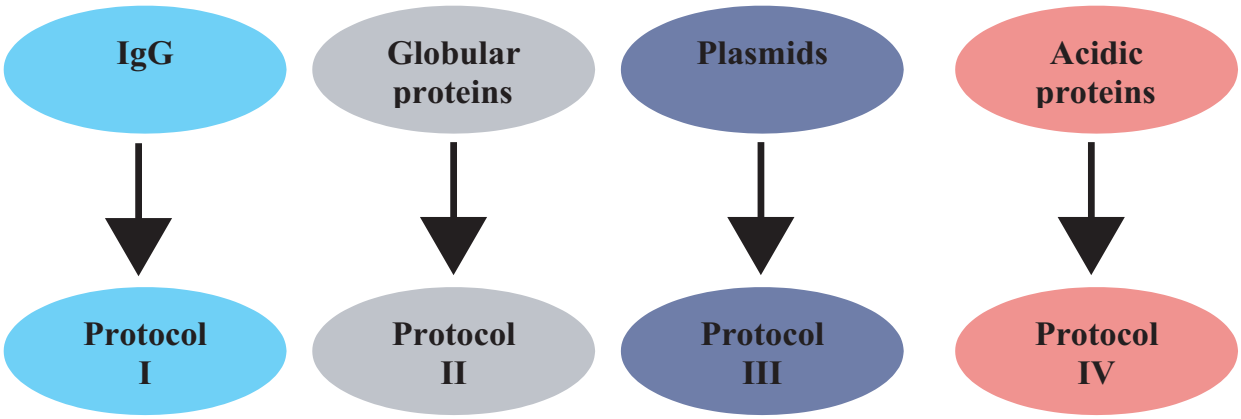
Loads should be free of agents such as citrate or EDTA that could degrade CHT via chelation. CHT is chemically compatible with the following solutions at pH 6.5–14 in the presence of calcium or phosphate.

2 M NaOH*	1% SDS and other surfactants
6 M Guanidine-HCl	4 M NaCl
8 M Urea	1 M Potassium phosphate
100% Acetonitrile	0.5 M Sodium phosphate
100% Ethanol/methanol	

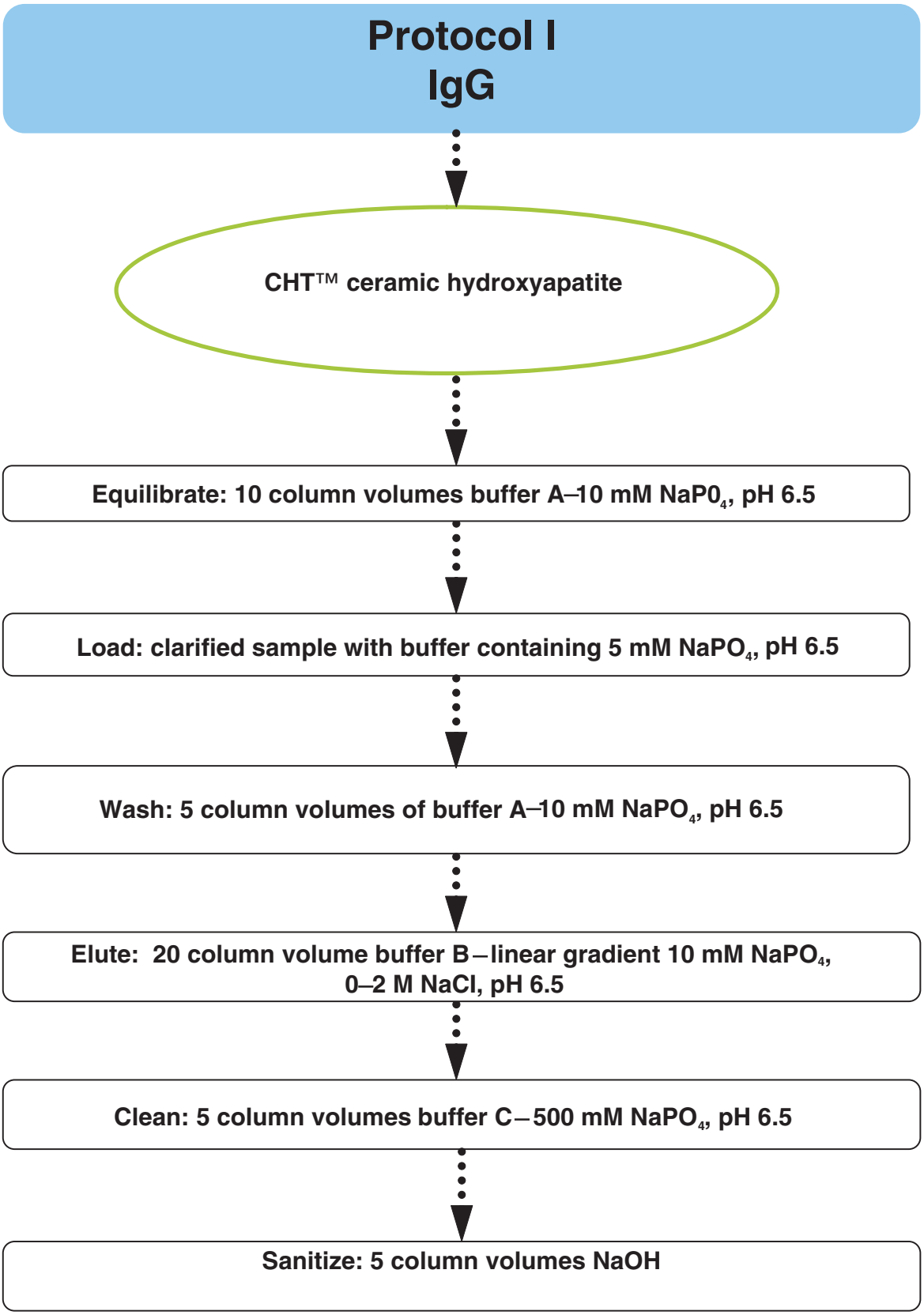
* No Ca or PO_4 required

3.2 Method Development

Optimizing the bioprocess requires management of multiple variables ranging from matrix interaction, elution characteristics, scale-up from bench, regulation and drug safety requirements, and process robustness and economics. The following four protocols have been developed as general starting guidelines for the purification of most proteins and nucleic acids and may help to reduce time spent in methodology development.**



** Optimal experimental conditions vary on a case-by-case basis depending on desired results. These protocols serve only as a screening tool for biomolecule purification.


Protocol I
IgG
CHT™ ceramic hydroxyapatite
Equilibrate: 10 column volumes buffer A–10 mM NaP0$_4$, pH 6.5
Load: clarified sample with buffer containing 5 mM NaPO$_4$, pH 6.5
Wash: 5 column volumes of buffer A–10 mM NaPO$_4$, pH 6.5
Elute: 20 column volume buffer B – linear gradient 10 mM NaPO$_4$, 0–2 M NaCl, pH 6.5
Clean: 5 column volumes buffer C – 500 mM NaPO$_4$, pH 6.5
Sanitize: 5 column volumes NaOH

3.2.1 Protocol I: IgG Monoclonal Antibodies

Flow rate: 300 cm/hr

Buffer A: 10 mM $NaPO_4$, pH 6.5

Buffer B: 10 mM $NaPO_4$, 2 M NaCl, pH 6.5

Buffer C: 500 mM $NaPO_4$, pH 6.5

Equilibrate the column with approximately 10 column volumes of buffer A. Prepare the sample, adjusting the pH and conductivity to those of buffer A. Load the sample in 5 mM $NaPO_4$; wash the column with approximately 5 column volumes of buffer A; and elute with 20 column volumes of buffer B linear gradient. The target protein will usually elute within the NaCl gradient. Buffer at higher pH decreases binding and retention time; conversely, lower pH increases binding capacity and sample retention times. If elution does not occur, increase the phosphate concentration. Slope and amplitude can be adjusted based on initial results. Flow rate may also be converted to a step format or run in flow-through mode. A typical chromatograph using NaCl for the elution of a protein A purified mouse IgG chimera is shown in Figure 3. After elution clean the column with 5 column volumes of buffer C followed by a sanitation step using 5 column volumes of 1 M NaOH.

Protocol Optimization

1. Start with 10 mM $NaPO_4$ in buffer A. Titrate buffer B down to 5 mM $NaPO_4$ or up to 15 mM $NaPO_4$ depending on desired results. Rare IgGs may require 40 mM $NaPO_4$.
2. Select the lowest phosphate concentration that supports NaCl elution, but do not use concentrations lower than 5 mM.

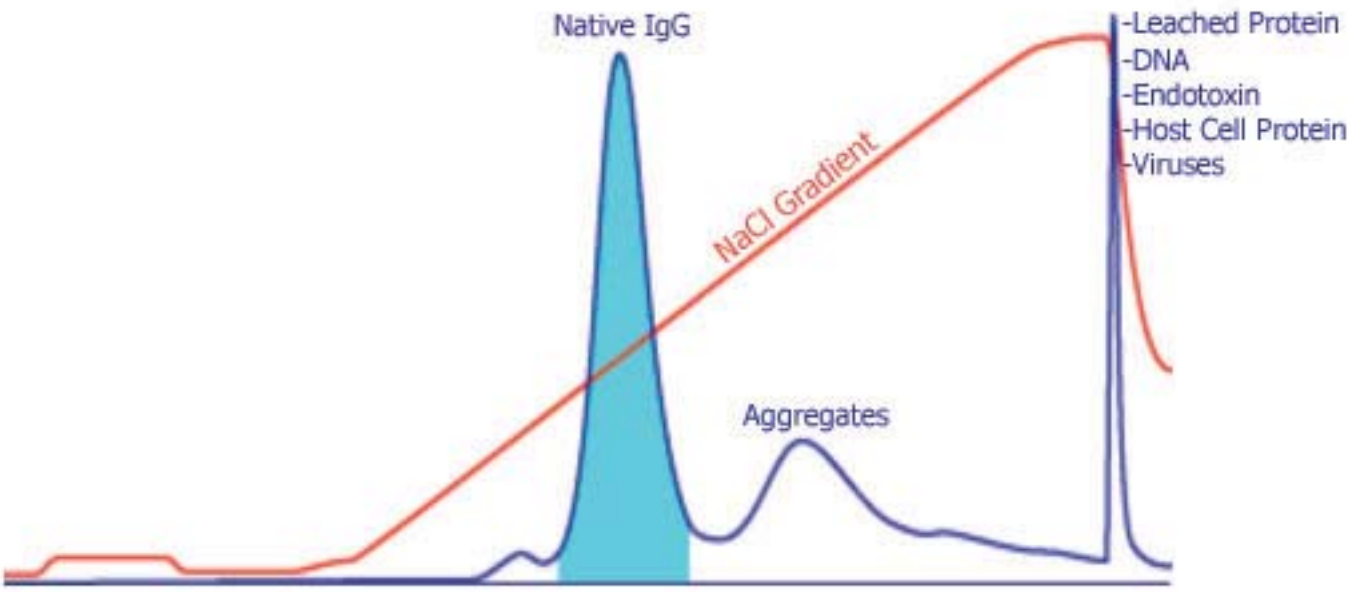
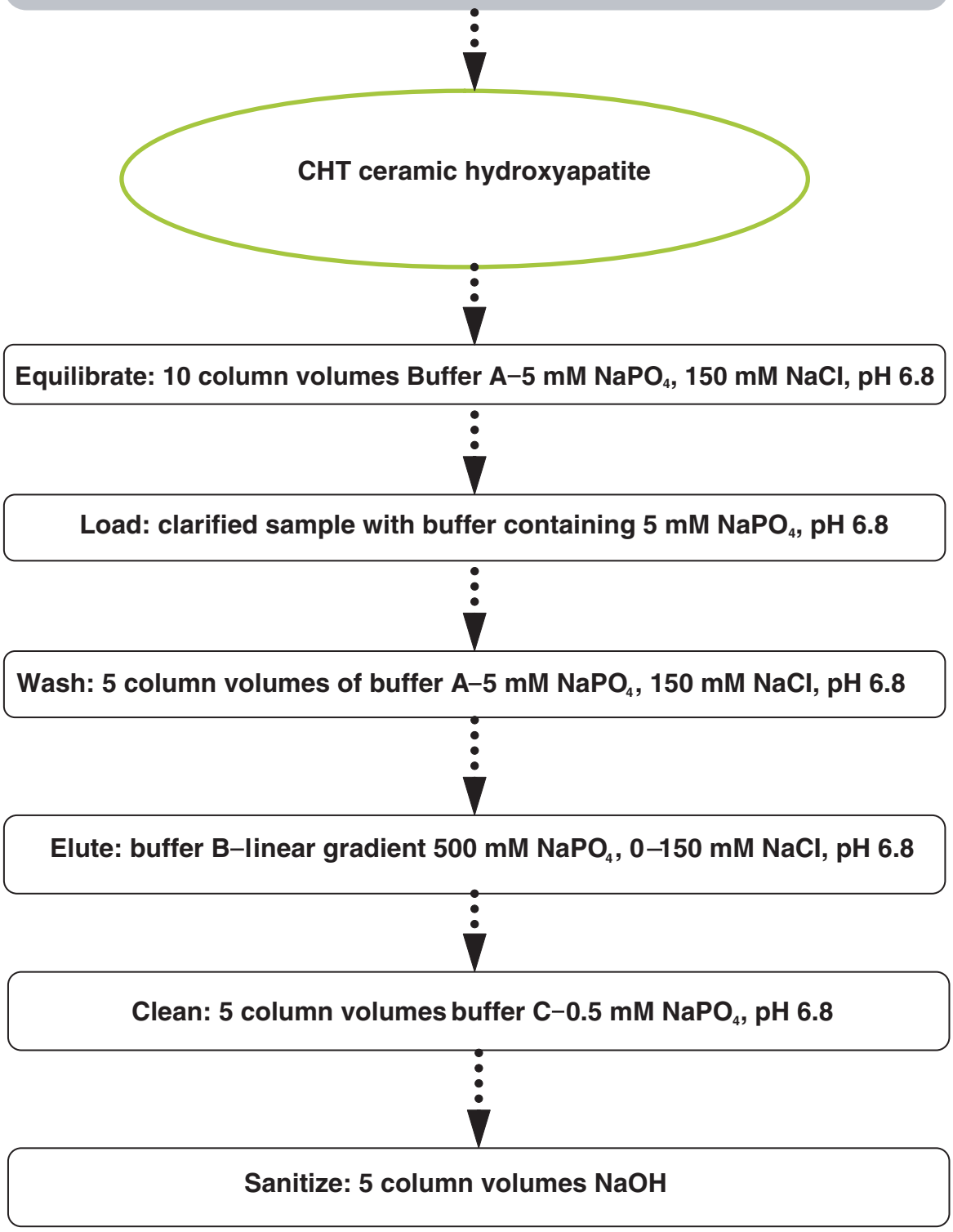


Fig. 3. Elution of monoclonal antibody in a 40 column volume linear gradient to 1 M NaCl.

Protocol II
Globular Proteins



3.2.2 Protocol II: Globular Proteins

Flow rate: 300 cm/hr

Buffer A: 5 mM $NaPO_4$, 150 mM NaCl, pH 6.8

Buffer B: 500 mM $NaPO_4$, 150 mM NaCl, pH 6.8

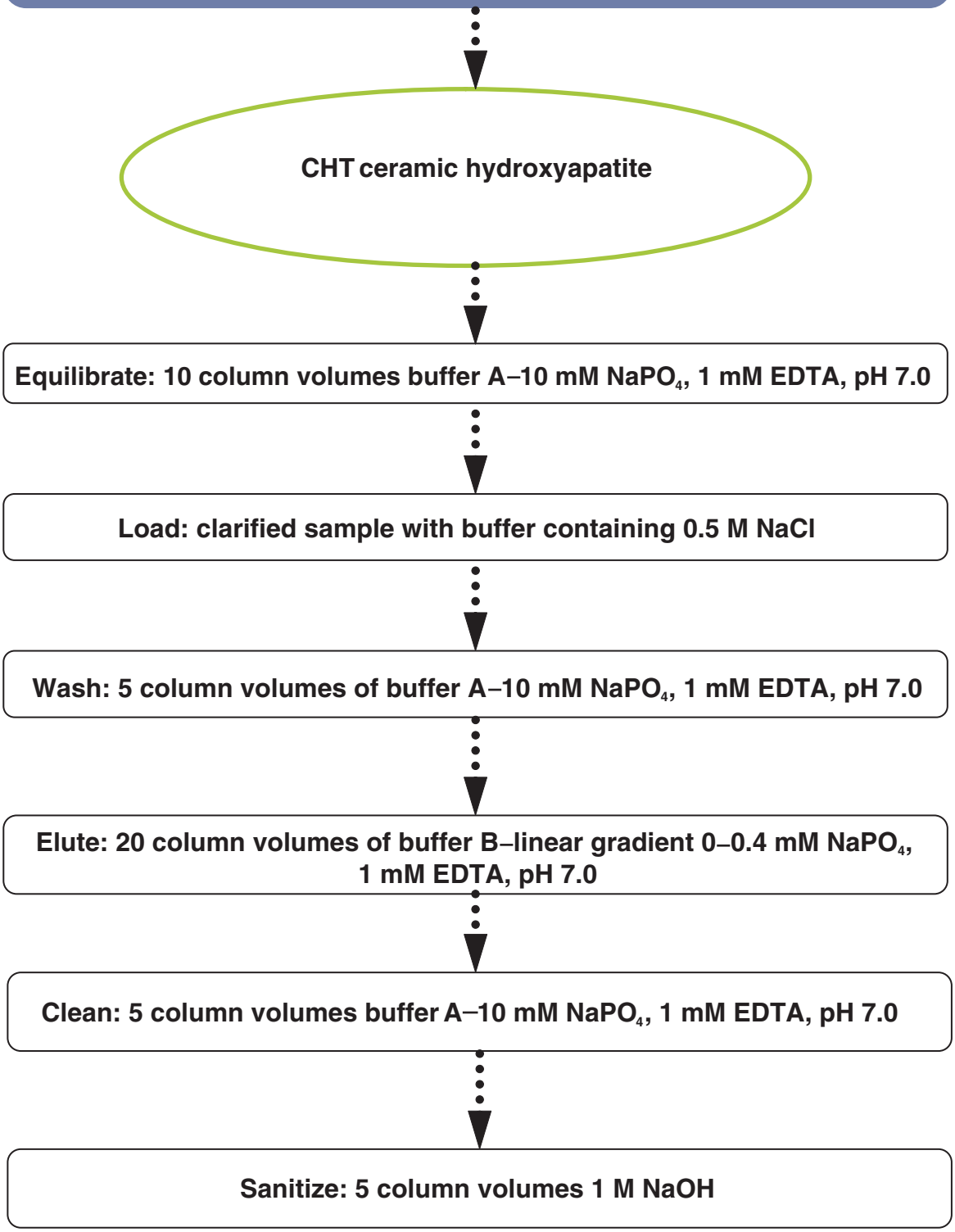
Buffer C: 500 mM $NaPO_4$, pH 6.8

Equilibrate the column with approximately 10 column volumes of buffer A. Prepare the sample, adjusting the pH and conductivity to those of buffer A. Load the sample in 5 mM $NaPO_4$, pH 6.8; wash the column with approximately 5 column volumes of buffer A; and elute with approximately 20 column volumes of buffer B linear gradient. Slope and amplitude can be adjusted based on initial results. After elution clean the column with approximately 5 column volumes of buffer C followed by a sanitation step using approximately 5 column volumes of 1 M NaOH.

Protocol Optimization

1. Start with 150 mM NaCl in buffer A. Titrate buffer B down to 50 mM NaCl or up to 100 mM NaCl depending on desired results.
2. If 500 mM $NaPO_4$ is not sufficient for protein elution (this is rare), try 500 mM KPO_4.

Protocol III
Plasmids



3.2.3 Protocol III: Plasmids

Flow rate: 300 cm/hr

Buffer A: 10 mM $NaPO_4$, 1 mM EDTA, pH 7.0

Buffer B: 400 mM $NaPO_4$, 1 mM EDTA, pH 7.0

Equilibrate the column with approximately 10 column volumes of buffer A. Prepare the sample, adjusting the pH and conductivity to those of buffer A. Load the sample in 0.5 M NaCl pH 7.0; wash the column with approximately 5 column volumes of buffer A; and elute with approximately 20 column volumes of buffer B linear gradient. Slope and amplitude can be adjusted based on initial results. After elution clean the column with approximately 5 column volumes of buffer A followed by a sanitation step using approximately 5 column volumes of 1 M NaOH.

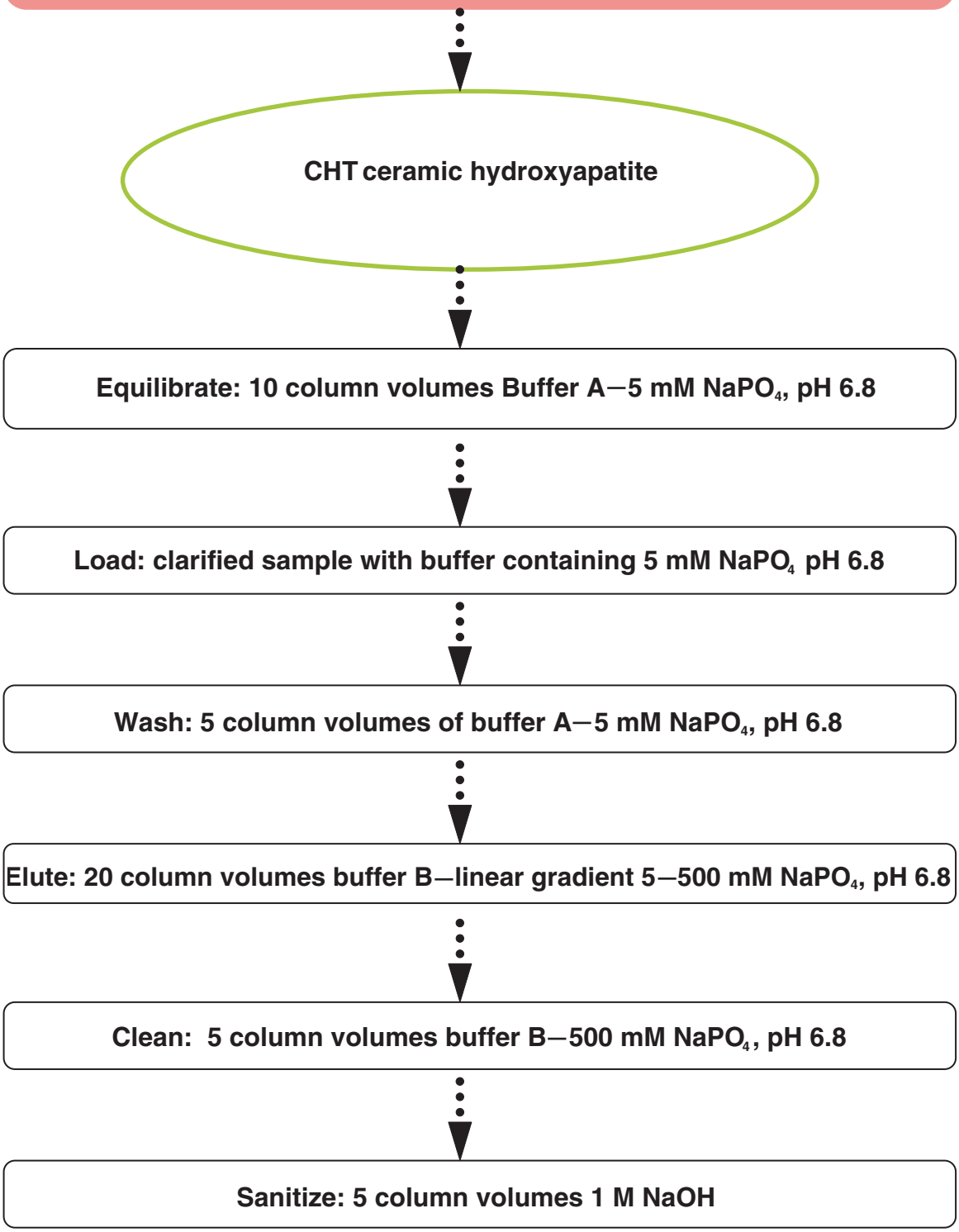
Protocol Optimization

1. Alkaline cell lysate containing plasmid DNA should be acidified by a mineral acid in the presence of inorganic salt. The use of mineral acid and inorganic salts avoids the use of acetate ion, which degrades CHT ceramic hydroxyapatite.

For detailed methods and protocol optimization refer to Tech Note 2731: Plasmid Purification Using CHT Ceramic Hydroxyapatite Support.

Acetate-Free Purification of Plasmid DNA On Hydroxyapatite. Patent No.: US 6,406,892 B1, EU 02771845.1-2404-US0215705.

Protocol IV
Acidic Proteins



3.2.4 Protocol IV: Acidic Proteins

Flow rate: 300 cm/hr

Buffer A: 5 mM $NaPO_4$, pH 6.8

Buffer B: 500 mM $NaPO_4$, pH 6.8

Equilibrate the column with approximately 10 column volumes of buffer A. Prepare the sample, adjusting the pH and conductivity to those of buffer A. Load the sample in 5 mM $NaPO_4$, pH 6.8; wash the column with approximately 5 column volumes of buffer A; and elute with approximately 20 column volumes of buffer B linear gradient. After elution clean the column with approximately 5 column volumes of buffer B followed by a sanitation step using approximately 5 column volumes of 1 M NaOH.

Protocol Optimization

1. If binding capacity is not sufficient, replace phosphate with MES and add 1–10 mM calcium chloride to load and buffer solutions.
2. To increase the binding efficiency of acidic proteins, calcium chloride may be added to the mobile phase. Due to the low solubility of calcium phosphate, however, extra care is required. Do not exceed the following concentrations of calcium chloride in phosphate buffers: 0.3 mM calcium chloride for 10 mM phosphate, 0.01 mM calcium chloride for 300 mM phosphate, and 0.0075 mM calcium chloride for 400 mM phosphate. At higher concentrations of calcium chloride, calcium phosphate will precipitate. Calcium phosphate is extremely difficult to dissolve and will appear white and cloudy in the supernatant. If higher levels of calcium are desired then a compatible buffering system must be used. However, high calcium levels can lead to additional calcium deposition onto CHT; the effects of this have not been fully elucidated.

3.2.5 Scouting Tips

The target protein will usually elute within the phosphate gradient. Slope and amplitude can be adjusted based on initial results. Flow rate may also be converted to a step format or run in flow-through mode. We studied protein retention using the phosphate elution procedure. Fourteen purified proteins were loaded and eluted with a linear gradient of sodium phosphate at pH 6, 7, 8, and 9. In general, retention time of proteins increase with increasing pI.

Optimizing Tips for Protocols I-IV

1. Select the optimum buffering agent (Table 1) making sure to add phosphate to stabilize the CHT matrix.
2. The ionic strength in samples containing a high concentration of salt should be reduced to be equivalent to the starting buffer. Dilution, diafiltration, or buffer exchange using Bio-Gel P-6DG gel may also be used.
3. As with any chromatographic step, buffer solutions and samples should be filtered through a 0.20–0.45 µm filter before use.
4. If 500 mM sodium phosphate is not sufficient for protein elution (this is rare), try 500 mM potassium phosphate.
5. If the elution peak is not sharp enough, try 40 CV linear gradient elution.
6. Where appropriate, convert linear gradient elution to step elution. Use the information from the gradient to devise an intermediate wash step if desired for increased purity.
7. Determine the pH that gives the highest binding capacity at a phosphate concentration of 5 mM.

Section 4
Regeneration, Sanitization, and Storage

4.1 Regeneration

CHT™ ceramic hydroxyapatite columns should be regenerated at the completion of each run with 3–5 column volumes of 500 mM potassium or sodium phosphate buffer at neutral pH, or 400 mM trisodium phosphate, pH 11–12. The column can also be stripped with other cleaning solutions (1–2 M KCl or NaCl, 6 M urea, or 8 M guanidine-HCl) containing 5 mM phosphate at neutral pH.

4.2 Sanitization

The column can be sanitized in up to 2 N NaOH and stored in 0.1–1.0 N NaOH if desired. For sanitization, a contact time in sodium hydroxide of at least 1 hour is recommended.

Carbonate reacts with small amounts of calcium ions released from hydroxyapatite to form a precipitate. The reaction could result in a crust of calcium carbonate at the top of the column or as an opaque white layer if the eluate is collected during the cleaning cycle. This carbonate reaction can occur in a CIP/SIP cycle with base since NaOH has a high affinity for carbonate. To minimize this reaction a 25 mM phosphate rinse is recommended whenever NaOH is used in the cleaning cycle.

4.3 Storage

Unused CHT ceramic hydroxyapatite should be stored in the original container at room temperature. Once wetted, CHT may be stored at room temperature in 0.1 M NaOH. Higher concentrations of NaOH may be used if desired. Used CHT, after being regenerated and sanitized, can be stored in solution up to 1.0 N NaOH in room temperature and away from direct light.

Section 5
Column Packing Protocols

This section offers guidelines for packing process scale columns. Topics include handling and column packing of CHT™ ceramic hydroxyapatite into a limited number of commercially available process columns, considerations for packing an open column or a closed system, and whether a media transfer device is being used for packing. Please read over the protocols carefully and follow the protocol for packing your specific column. Should you have further questions, contact either your local process chromatography sales representative or the chromatography technical support department for further assistance (1-510-741-6563). Not following the protocols may lead to poor chromatographic performance such as shortened column lifetimes or damaged ceramic hydroxyapatite particles.

5.1 General Handling and Powder Preparation

CHT ceramic hydroxyapatite is supplied as a dry powder. A dust mask, gloves, and laboratory coat are advisable while transferring the powder. The 5 kg containers of CHT have a plastic seal covering the container and screw closure. The seal ensures that the container has not been opened after it was filled. The screw closure is a secondary closure that secures a powder seal onto the container's opening.

Clean the container surface if it has accumulated dust. Wipe it with a clean damp cloth and dry it with a clean dry cloth. Remove the plastic seal. Reclean the container surface by wiping with a clean damp cloth and dry it with a clean dry cloth.

Invert the container several times to loosen the CHT into a dry free-flowing powder. Repeat this step just prior to dispensing the powder.

5.2 Guidelines for Packing Low-Pressure Process Columns

Several methods exist for packing columns with CHT that depend on the type of column and equipment used. Always read the relevant column instruction and associated media transfer skid or media packing skid manuals carefully. Where appropriate, make the recommended changes according to the guidelines.

CHT ceramic hydroxyapatite is rigid and exhibits high flow rates at low pressure relative to its average particle size; refer to Figure 4 for 40 µm CHT and Figure 5 for 80 µm CHT. Bead damage through excessive physical force is possible. Fine particles generated in this manner may clog the column and increase backpressure. The following packing methods are used for packing CHT ceramic hydroxyapatite:

- Gas-assisted axial compression packing of open columns with motorized adjustable inlet adaptors
- Gas-assisted flow packing of open columns with adjustable adaptors at less than 700 cm/hr flow rate
- Gas-assisted flow packing of open columns with adjustable inlet adaptors capable of 700 cm/hr
- Axial compression packing of closed columns with motorized adjustable inlet adaptors (media transfer stations)
- Pressure packing of closed columns (media packing stations)

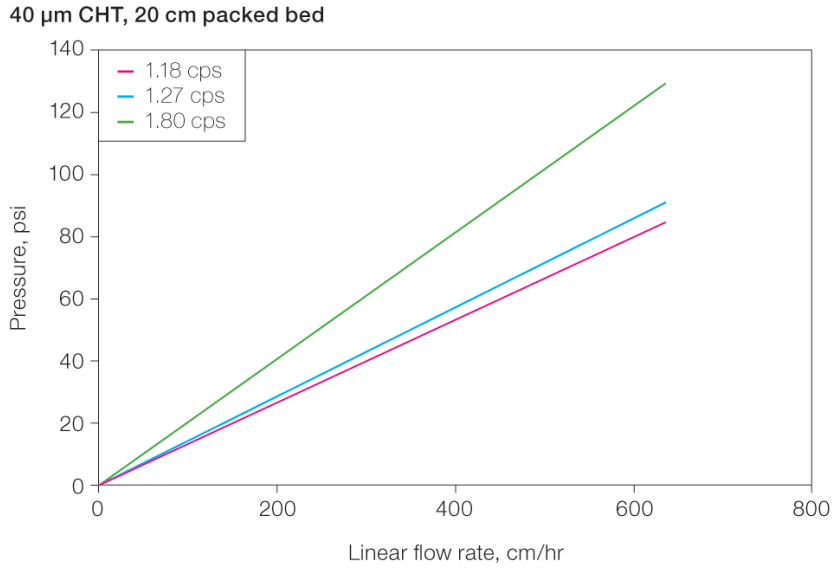


Fig. 4. Estimated pressure for 40 µm CHT packed to 20.0 cm bed height vs. 1.0 M NaOH (1.80 cps), 0.5 M sodium phosphate buffer, pH 6.8 (1.27 cps), and phosphate buffered saline (1.18 cps).

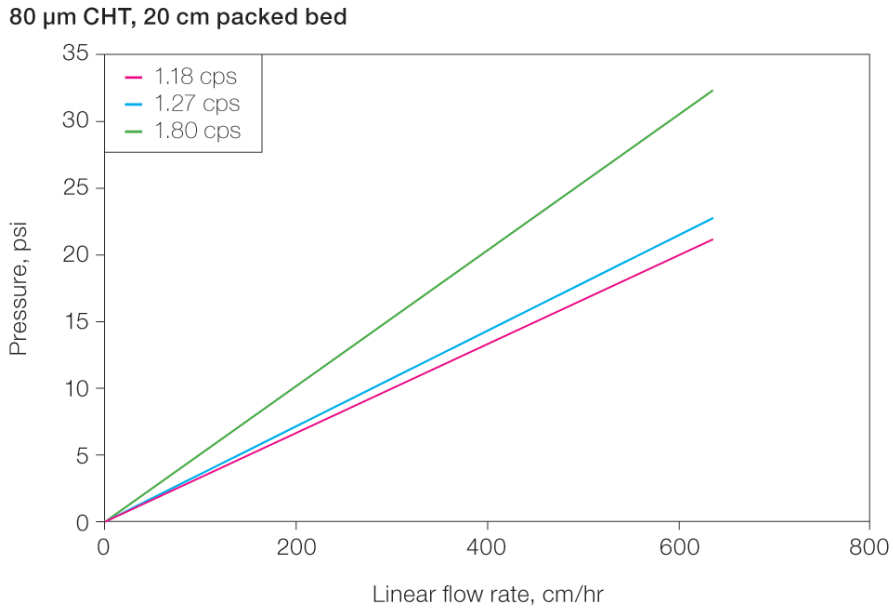


Fig. 5. Estimated pressure for 80 µm CHT packed to 20.0 cm bed height vs. 1.0 M NaOH (1.80 cps), 0.5 M sodium phosphate buffer, pH 6.8 (1.27 cps), and phosphate buffered saline (1.18 cps).

Well-packed columns, in which the beds are homogeneous and continuous from top to bottom, exhibit the best chromatographic separations. It is therefore very important to pack your columns according to these guidelines.

Each packing method covers packing, repacking, and unpacking using sequential steps to aid the technician. The following sections discuss recommended packing solutions, packed column qualification, and column conditioning for the purification application.

Best Practices

Open-column methods: Whenever possible, mix the slurry iof powder and buffer in the column. Mixing with a one-piece polypropylene paddle or with gas sparging minimizes mechanical damage. Slurries up to 47% v/v (29.6% w/v) in composition can be mixed with either method.

Media transfer stations: Media transfer stations can be used to transfer dense slurries from a mixing tank to the column through large orifices. When transferring the CHT slurry to the column the concentration should be less than 47% v/v (29.6% w/v). Lower concentrations ensure more efficient packing of columns; however, a further increase in efficiency is negligible below 15% v/v (9.5% w/v). Damage to the chromatography medium due to excessive physical force is possible. Excessive mixing or use of impellers other than low-shear hydrofoil

impellers may fracture the particles. The fine particles resulting from excessive mixing or use of improper impellers may increase the column's backpressure. Peristaltic and diaphragm pumps fracture the particles and are generally the major cause of particle damage. A single-pass operation of the slurry with these pumps minimizes damage. Never recycle CHT with these pumps or any other pumps as the shear forces and particle-to-particle collisions in piping, bends in piping, and other connections accelerate damage. Whenever possible, avoid pumps for media transfer. Pressurized slurry vessels are preferred.

Media packing stations: Media packing stations can be used to pack thin slurries from a mixing tank into the column through a packing nozzle containing multiple small-diameter orifices. When packing such slurries of CHT, the slurry concentration should be less than 25% v/v (15.8% w/v). Lower concentrations ensure more efficient packing; however, a further increase in efficiency is negligible below 15% v/v (9.5% w/v). Damage to the chromatography medium due to excessive physical force is possible. Excessive mixing or using impellers other than low-shear hydrofoil impellers may fracture the particles. The fine particles resulting from excessive mixing or use of improper impellers may increase the column's backpressure. Peristaltic and diaphragm pumps fracture the particles and are generally the major cause of particle damage. Thin slurries minimize the shear forces and particle-to-particle collisions in piping, bends in piping, other connections, and the packing nozzle. Thin slurries are defined as less than 26% v/v or 16.0% w/v. A single-pass operation of the slurry through these pumps, piping, valves, and nozzle minimizes damage. Never recycle CHT with these pumps or any other pumps as the shear forces and particle-to-particle collisions in piping, bends in piping, and other connections accelerate damage. Whenever possible, avoid pumps for media transfer. Pressurized slurry vessels are preferred.

5.2.1 Recommended Column Packing Solutions

Refer to your buffer preparation instructions to make your choice of packing solution that is at least 150 mM in ionic strength and pH 6.8 or greater. The following packing solutions have been used to successfully pack efficient columns of 40 µm and 80 µm CHT.

- 20 mM phosphate buffer, 150 mM sodium chloride, pH 7.2 to 7.4 (phosphate buffered saline)
- Sodium or potassium phosphate buffer, 200 mM, pH 6.8–10
- Sodium or potassium phosphate buffer, 400 mM, pH 6.8–10
- 0.15 N to 1 N NaOH

Best Practices: Avoid using packing solutions that are less than 150 mM in ionic strength for the first contact of the powder with liquid. Lower concentrations may cause turbid supernatants in freshly prepared slurries of CHT. The turbidity results from the first contact of the powder with the solution but is minimized or eliminated when using ionic strength solutions greater than 150 mM and pH of 6.8 or greater.

5.3 Open-Column Methods

Determine the dry weight of CHT and volume of packing solutions for open columns such as the following:

- Bio-Rad EasyPack™
- Bio-Rad OCS
- BPG columns (GE Healthcare)
- Moduline 2 columns (Millipore)

Tables 5–9 in Section 7 "Appendices" cover tube lengths of 500, 600, 700, and 900 mm, where the maximum fill heights are 400, 500, 600, and 800 mm, respectively. The 100 mm difference between tube height and fill height is due to the minimum length required by the inlet adaptor. The tables also list the appropriate dry weight of CHT and volume of packing solution necessary to prepare the slurry in the column to obtain a packed bed for the specified diameter and height. Table 9 provides similar information for contained columns. For example, a BPG 450/500, which is a 45 cm ID column with a packed bed height of 15 cm, requires 15.03 kg of CHT and 48.6 L of packing solution to make a 37.5% v/v slurry. The 500 and 600 mm tube length columns can be packed to no more than 20 cm bed heights. A 25 cm bed height can be packed into the 700 mm tube length column and the 900 mm tube length column can be packed up to a 30 cm bed height.

5.3.1 Gas-Assisted Axial Compression Packing of Open Columns With Motorized Adjustable Inlet Adaptors

Gas-assisted slurry packing methods use air, argon, helium, or nitrogen gas to uniformly agitate the mixture of CHT and packing buffer within the column. The method applies to 29.6% w/v slurries. This is approximately equal to 47% v/v slurries. The height of the column limits the length of the packed bed as a function of practical filling volume. Most motorized systems have a gauge indicating the position of the inlet adaptor surface from the column's bottom surface. Consult your column vendor's manual for guidance. The following instructions contain comments relative to the guideline.

Packing

1. Level the column; otherwise, the distance between packed bed surface and inlet adaptor surface will not be uniform across the column's diameter.
2. Connect the gas line to the column outlet. While air may be used with some column hardware, the other gases prevent bubbles from being trapped by the bed supports and media. Air should be avoided with columns that contain porous polyethylene or polypropylene bed supports or unsupported nylon or stainless-steel screens.

3. Read the amount of packing solution and CHT from the appropriate appendix. Invert the containers of CHT repeatedly to loosen the powder. This will enable it to pour easily into the column.
    a. Dispense the packing solution into the column.
    b. Turn on the gas line and adjust the metering valve so that the packing solution is slightly turbulent. Record the metering position or rate of flow.
    c. Insert the paddle and stir the solution.
    d. Dispense the CHT into the column. Remove the paddle.
4. Continue mixing with gas until the swirling action created by the paddle ceases.
5. With the inlet valve open, insert the flow adaptor into the column, lower it to 2 cm above the liquid level, and secure its position.
6. Seal the adaptor's sealing device marginally (O-ring, inflatable bladder, or compression seal). Marginal sealing is the sealing force recommended for the column where liquid will not bypass the seal while the adaptor is lowered.
7. Turn off the gas flow and close the column outlet valve. Disconnect the gas line from the outlet valve.
8. Allow the CHT to settle for 1 min. Turn on the motor drive and lower the inlet adaptor 4–6 cm to release the air trapped between the bed support and the packing solution.
9. Close the inlet adaptor valve and open the outlet valve. Read the advisory statement and instructions before proceeding to the next step.

Best Practices: The following steps pack the bed under flow conditions using the inlet adaptor to drive the packing solution. The height of the packed bed may be 5% higher than the target value but is generally equal to the target in 40 cm ID columns and larger. IF SUDDEN RESISTANCE IS NOTED AS THE ADAPTOR APPROACHES THE TARGET HEIGHT, STOP THE DESCENT IMMEDIATELY. CLOSE THE OUTLET VALVE. OPEN THE INLET VALVE, THEN PUMP PACKING BUFFER INTO THE INLET VALVE AT 50 CM/HR WHILE RAISING THE ADAPTOR AT 50 CM/HR. STOP WHEN THE ADAPTOR HAS TRAVELED 0.5 CM.

10. Turn on the motor drive so that the inlet adaptor descends at 300 cm/hr.
11. Continue the descent until the adaptor is 1.0 cm per 10 cm of packed bed above the target height. A gap of about 0.5–1.0 cm is acceptable between the packed bed and the inlet adaptor bed support for a 10 cm packed bed with the adaptor set to 11 cm. A gap of 0.5–2 cm is acceptable between the packed bed and the inlet adaptor bed support for a 20 cm packed bed with the adaptor set to 22 cm. The gap iensures that the surface of the packed bed is undisturbed by contact with the uneven geometry characteristic of most inlet adaptors.
12. Close the outlet.
13. Connect the column to the chromatography station.
14. Condition with 5 column volumes of packing buffer at 125–200% of the selected operating flow rate.
15. Continue with packed column qualification.

Repacking

1. Estimate the bed height for the repacked column based on results from Comments 1 or 2 in topic 5.8.1 "Comments on Column Qualification for Columns With Adjustable Inlet Adaptors".
2. If the column has moved since it was packed, level the column. If not level, the distance between packed bed surface and inlet adaptor surface will not be uniform across the column's diameter.
3. Sanitize the top plate of the inlet adaptor and the wall of the column using your sanitization protocol. Rinse the surfaces with water-for-injection and remove the excess liquid by aspirating under sanitary conditions.
4. Deflate the adaptor's sealing device to its marginal setting (O-ring, inflatable bladder, or compression seal). Marginal sealing is the sealing force recommended for the column where liquid will not bypass the seal while the adaptor is raised or lowered.
5. Raise the inlet adaptor to the same set point used when it was inserted and marginally sealed.
6. Connect the gas line to the column outlet using a valve that can switch between the gas connection and the buffer connection. While air may be used with some column hardware, the other gases prevent bubbles from being trapped by the bed supports and medium. Air should be avoided with columns that contain porous polyethylene or polypropylene bed supports or unsupported nylon or stainless-steel screens.

7. Pump test buffer into the column by upflow at 150 cm/hr to raise the bed with sufficient buffer to equal 80% of the original slurry volume. Stop the upflow and switch the valve to the gas connection.
8. Turn on the gas in upflow to the same metering position or rate of flow used when packing the column.
    a. Agitate for 10 min.
    b. Stop the gas upflow and switch the valve to the buffer connection.
    c. Pump test buffer into the column by upflow at 150 cm/hr to fill the column with sufficient buffer to equal 100% of the original slurry volume.
    d. Stop the upflow.
9. Allow the CHT to settle for 1 min. Turn on the motor drive and lower the inlet adaptor 4–6 cm to release air trapped between the bed support and the packing solution.
10. Close the inlet adaptor valve and open the outlet valve. Read the advisory statement and instructions before proceeding to the next step.

Best Practices: The following steps pack the bed under flow conditions using the inlet adaptor to drive the packing solution. The height of the packed bed may be 5% higher than the target value but is generally equal to the target in 40 cm ID columns and larger. IF SUDDEN RESISTANCE IS NOTED AS THE ADAPTOR APPROACHES THE TARGET HEIGHT, STOP THE DESCENT IMMEDIATELY. CLOSE THE OUTLET VALVE. OPEN THE INLET VALVE, THEN PUMP PACKING BUFFER INTO THE INLET VALVE AT 50 CM/HR WHILE RAISING THE ADAPTOR AT 50 CM/HR. STOP WHEN THE ADAPTOR HAS TRAVELED 0.5 CM.

11. Turn on the motor drive so that the inlet adaptor descends at 300 cm/hr.
12. Continue the descent until the adaptor is 1.0 cm above the new target height. A gap of about 0.5–1.0 cm is acceptable between the packed bed and the inlet adaptor bed. The gap ensures that the surface of the packed bed is undisturbed by contact with the uneven geometry characteristic of most inlet adaptors.
13. Close the outlet.
14. Connect the column to the chromatography station.
15. Condition with 5 column volumes of packing buffer at 125–200% of the selected operating flow rate.
16. Continue with column qualification.

Unpacking

1. Deflate the adaptor's sealing device.
2. Remove the adaptor.
3. Connect the gas line to the column outlet using a valve that can switch between the gas connection and the buffer connection.
4. Pump test buffer into the column by upflow at 150 cm/hr. Raise the bed with sufficient buffer to equal 50% of the original slurry volume.
    a. Insert a paddle into the column and agitate the medium.
    b. Stop the upflow and switch the valve to the gas connection.
5. Turn on the gas in upflow to the same metering position or rate of flow used when packing the column.
    a. Agitate for 10 min using the paddle to disperse the medium.
    b. Stop the gas upflow and switch the valve to the buffer connection.
6. Pump the slurry into waste containers.
    a. Allow the CHT to settle. Aspirate the excess water.
    b. Dispose of the medium per the Material Safety Data Sheet (MSDS) and in accordance with your facility's guidelines.

5.3.2 Gas-Assisted Flow Packing of Open Columns With Adjustable Adaptors at Less Than 700 cm/hr Flow Rate

Gas-assisted slurry packing methods use air, argon, helium, or nitrogen gas to uniformly agitate the mixture of CHT and packing buffer within the column. The method applies to up to 29.6% w/v slurries. This is approximately equal to 47% v/v. The height of the column limits the length of the packed bed as a function of practical filling volume. Mark a reference point on the central screw of the column adaptor so that you can determine the distance between the surface of the inlet adaptor and the column's bottom surface. Consult your column vendor's manual for guidance. The following instructions contain comments relative to the guideline.

Packing

1 Level the column; otherwise, the distance between packed bed surface and inlet adaptor surface will not be uniform across the column's diameter.

2 Connect the gas line to the column outlet. While air may be used with some column hardware, the other gases prevent bubbles from being trapped by the bed supports and medium. Air should be avoided with columns that contain porous polyethylene or polypropylene bed supports or unsupported nylon or stainless-steel screens.

3. Read the amount of packing solution and CHT from the appropriate appendix table. Invert the containers of CHT repeatedly to loosen the powder. This will enable it to pour easily into the column.

   a. Dispense the packing solution into the column.

   b. Turn on the gas line and adjust the metering valve so that the packing solution is slightly turbulent.

   c. Insert the paddle and stir the solution.

   d. Dispense the CHT into the column. Remove the paddle.

4. Continue mixing with gas until the swirling action created by the paddle ceases.

5. With the inlet valve open, insert the flow adaptor into the column, lower it to 2 cm above the liquid level, and secure its position.

6. Seal the adaptor's sealing device marginally (O-ring, inflatable bladder, or compression seal). Marginal sealing is the sealing force recommended for the column where liquid will not bypass the seal while the adaptor is lowered.

7. Turn off the gas flow and close the column outlet valve. Disconnect the gas line from the outlet valve.

8. Allow the CHT to settle for 1 min. Lower the inlet adaptor 4–6 cm to release the air trapped between the inlet adaptor bed support and the packing solution.

9. Close the inlet adaptor valve and open the outlet valve.

10. Fully seal the adaptor's sealing device.

11. Open the outlet valve, then flow-pack the column at 300 cm/hr using packing solution equivalent to 3 times the slurry volume.

12. Stop the flow. Close the outlet valve.

13. Read the advisory statement and instructions before proceeding to the next step.

Best Practices: The following step releases excess packing solution through the inlet adaptor. The height of the packed bed may be 5% higher than the target value but is generally equal to the target in 40 cm ID columns and larger. IF SUDDEN RESISTANCE IS NOTED AS THE ADAPTOR APPROACHES THE TARGET HEIGHT, STOP THE DESCENT IMMEDIATELY. PUMP PACKING BUFFER INTO THE INLET VALVE AT 50 CM/HR WHILE RAISING THE ADAPTOR AT 50 CM/HR. STOP WHEN THE ADAPTOR HAS TRAVELED 0.5 CM.

14. Lower the inlet adaptor, allowing the packing solution to drain through the inlet port.

15. Continue the descent until the adaptor is 1.0 cm per 10 cm of packed bed above the target height. A gap of about 0.5–1.0 cm is acceptable between the packed bed and the inlet adaptor bed support for a 10 cm packed bed with the adaptor set to 11 cm. A gap of 0.5–2 cm is acceptable between the packed bed and the inlet adaptor bed support for a 20 cm packed bed with the adaptor set to 22 cm. The gap ensures that the surface of the packed bed is undisturbed by contact with the uneven geometry characteristic of most inlet adaptors.

16. Close the inlet valve.

17. Connect the column to the chromatography station.

18. Condition with 5 column volumes of packing buffer at 125–200% of the selected operating flow rate.

19. Continue with packed column qualification.

Repacking

1. Estimate the bed height for the repacked column based on results from Comments 1 or 2 in topic 5.8.1 "Comments on Column Qualification for Columns With Adjustable Inlet Adaptors".

2. If the column has moved since it was packed, level the column. If not level, the distance between packed bed surface and inlet adaptor surface will not be uniform across the column's diameter.

3. Sanitize the top plate of the inlet adaptor and the wall of the column using your sanitization protocol. Rinse the surfaces with water-for-injection and remove the excess liquid by aspirating under sanitary conditions.

4. Deflate the adaptor's sealing device to its marginal setting (O-ring, inflatable bladder, or compression seal). Marginal sealing is the sealing force recommended for the column where liquid will not bypass the seal while the adaptor is raised or lowered.
5. Raise the inlet adaptor to the same set point used when it was inserted and marginally sealed.
6. Connect the gas line to the column outlet using a valve that can switch between the gas connection and the buffer connection. While air may be used with some column hardware, the other gases prevent bubbles from being trapped by the bed supports and medium. Air should be avoided with columns that contain porous polyethylene or polypropylene bed supports or unsupported nylon or stainless-steel screens.
7. Pump test buffer into the column by upflow at 150 cm/hr to raise the bed with sufficient buffer to equal 80% of the original slurry volume. Stop the upflow and switch the valve to the gas connection.
8. Turn on the gas in upflow to the same metering position or rate of flow used when packing the column.
    a. Agitate for 10 min.
    b. Stop the gas upflow and switch the valve to the buffer connection.
    c. Pump test buffer into the column by upflow at 150 cm/hr to fill the column with sufficient buffer to equal 100% of the original slurry volume.
    d. Stop the upflow.
9. Allow the CHT to settle for 1 min. Turn on the motor drive and lower the inlet adaptor 4–6 cm to release the air trapped between the bed support and the packing solution.
10. Close the inlet adaptor valve and open the outlet valve. Read the advisory statement and instructions before proceeding to the next step.

Best Practices: The following steps pack the bed under flow conditions using the inlet adaptor to drive the packing solution. The height of the packed bed may be 5% higher than the target value but is generally equal to the target in 40 cm ID columns and larger. IF SUDDEN RESISTANCE IS NOTED AS THE ADAPTOR APPROACHES THE TARGET HEIGHT, STOP THE DESCENT IMMEDIATELY. CLOSE THE OUTLET VALVE. OPEN THE INLET VALVE, THEN PUMP PACKING BUFFER INTO THE INLET VALVE AT 50 CM/HR WHILE RAISING THE ADAPTOR AT 50 CM/HR. STOP WHEN THE ADAPTOR HAS TRAVELED 0.5 CM.

11. Turn on the motor drive so that the inlet adaptor descends at 300 cm/hr.
12. Continue the descent until the adaptor is 1.0 cm above the new target height. A gap of about 0.5–1.0 cm is acceptable between the packed bed and the inlet adaptor bed. The gap ensures that the surface of the packed bed is undisturbed by contact with the uneven geometry characteristic of most inlet adaptors.
13. Close the outlet.
14. Connect the column to the chromatography station.
15. Condition with 5 column volumes of packing buffer at 125–200% of the selected operating flow rate.
16. Continue with column qualification.

Unpacking

1. Deflate the adaptor's sealing device.
2. Remove the adaptor.
3. Connect the gas line to the column outlet using a valve that can switch between the gas connection and the buffer connection.
4. Pump test buffer into the column by upflow at 150 cm/hr. Raise the bed with sufficient buffer to equal 50% of the original slurry volume.
    a. Insert a paddle into the column and agitate the medium.
    b. Stop the upflow and switch the valve to the gas connection.
5. Turn on the gas in upflow to the same metering position or rate of flow used when packing the column.
    a. Agitate for 10 min using the paddle to disperse the medium.
    b. Stop the gas upflow and switch the valve to the buffer connection.
6. Pump the slurry into waste containers.
    a. Allow the CHT to settle. Aspirate the excess water.
    b. Dispose of the medium per the MSDS and in accordance with your facility's guidelines.

5.3.3 Gas-Assisted Flow Packing of Open Columns With Adjustable Inlet Adaptors Capable of 700 cm/hr

Packing

Gas-assisted slurry packing methods use air, argon, helium, or nitrogen gas to uniformly agitate the mixture of CHT and packing buffer within the column. The method applies to up to 29.6% w/v slurries. This is approximately equal to 47% v/v. The height of the column limits the length of the packed bed as a function of practical filling volume. Mark a reference point on the central screw of the column adaptor so that you can determine the distance between the surface of the inlet adaptor and the column's bottom surface. Consult your column vendor's manual for guidance. The following instructions contain comments relative to the guideline.

1. Level the column; otherwise, the distance between packed bed surface and inlet adaptor surface will not be uniform across the column's diameter.
2. Connect the gas line to the column outlet. While air may be used with some column hardware, the other gases prevent bubbles from being trapped by the bed supports and media. Air should be avoided with columns that contain porous polyethylene or polypropylene bed supports or unsupported nylon or stainless-steel screens.
3. Obtain the amount of packing solution and CHT from the appropriate table. Invert the containers of CHT repeatedly to loosen the powder. This will enable it to pour easily when dispensing it into the column.
    a. Dispense the packing solution into the column.
    b. Turn on the gas line and adjust the metering valve so that the packing solution is slightly turbulent.
    c. Insert the paddle and stir the solution.
    d. Dispense the CHT into the column. Remove the paddle.
4. Continue mixing with gas until the swirling action created by the paddle ceases.
5. With the inlet valve open, insert the flow adaptor into the column, lower it to 2 cm above the liquid level, and secure its position.
6. Seal the adaptor's sealing device marginally (O-ring, inflatable bladder, or compression seal). Marginal sealing is the sealing force recommended for the column where liquid will not bypass the seal while the adaptor is lowered.
7. Turn off the gas flow and close the column outlet valve. Disconnect the gas line from the outlet valve.
8. Allow the CHT to settle for 1 min. Lower the inlet adaptor 4–6 cm to release the air trapped between the inlet adaptor bed support and the packing solution.
9. Close the inlet adaptor valve and open the outlet valve.
10. Fully seal the adaptor's sealing device.
11. Open the outlet valve, then flow pack the column at 700–1,000 cm/hr (for 80 µm CHT) or 300–500 cm/hr (for 40 µm CHT) using packing solution equivalent to 3 times the slurry volume. Do not exceed the pressure limit for the column.
12. Stop the flow. Close the outlet valve.
13. Read the advisory statement and instructions before proceeding to the next step.

Best Practices: The following step releases excess packing solution through the inlet adaptor. The height of the packed bed may be 5% higher than the target value but is generally equal to the target in 40 cm ID columns and larger. IF SUDDEN RESISTANCE IS NOTED AS THE ADAPTOR APPROACHES THE TARGET HEIGHT, STOP THE DESCENT IMMEDIATELY. PUMP PACKING BUFFER INTO THE INLET VALVE AT 50 CM/HR WHILE RAISING THE ADAPTOR AT 50 CM/HR. STOP WHEN THE ADAPTOR HAS TRAVELED 0.5 CM.

14. Lower the inlet adaptor and allow the packing solution to drain through the inlet port.
15. Continue the descent until the adaptor is 1.0 cm per 10 cm of packed bed above the target height. A gap of about 0.5–1.0 cm is acceptable between the packed bed and the inlet adaptor bed support for a 10 cm packed bed with the adaptor set to 11 cm. A gap of 0.5–2 cm is acceptable between the packed bed and the inlet adaptor bed support for a 20 cm packed bed with the adaptor set to 22 cm. The gap ensures that the surface of the packed bed is undisturbed by contact with the uneven geometry characteristic of most inlet adaptors.
16. Close the inlet valve.
17. Connect the column to the chromatography station.
18. Condition with 5 column volumes of packing buffer at 125–200% of the selected operating flow rate.
19. Continue with packed column qualification.

Repacking

1. Estimate the bed height for the repacked column based on results from Comments 1 or 2 in topic 5.8.1 “Comments on Column Qualification for Columns With Adjustable Inlet Adaptors”.
2. If the column has moved since it was packed, level the column. If not level, the distance between packed bed surface and inlet adaptor surface will not be uniform across the column’s diameter.
3. Sanitize the top plate of the inlet adaptor and the wall of the column using your sanitization protocol. Rinse the surfaces with water-for-injection and remove the excess liquid by aspirating under sanitary conditions.
4. Deflate the adaptor’s sealing device to its marginal setting (O-ring, inflatable bladder, or compression seal). Marginal sealing is the sealing force recommended for the column where liquid will not bypass the seal while the adaptor is raised or lowered.
5. Raise the inlet adaptor to the same set point used when it was inserted and marginally sealed.
6. Connect the gas line to the column outlet using a valve that can switch between the gas connection and the buffer connection. While air may be used with some column hardware, the other gases prevent bubbles from being trapped by the bed supports and media. Air should be avoided with columns that contain porous polyethylene or polypropylene bed supports or unsupported nylon or stainless-steel screens.
7. Pump test buffer into the column by upflow at 150 cm/hr to raise the bed with sufficient buffer to equal 80% of the original slurry volume. Stop the upflow and switch the valve to the gas connection.
8. Turn on the gas in upflow to the same metering position or rate of flow used when packing the column.
    a. Agitate for 10 min.
    b. Stop the gas upflow and switch the valve to the buffer connection.
    c. Pump test buffer into the column by upflow at 150 cm/hr to fill the column with sufficient buffer to equal 100% of the original slurry volume.
    d. Stop the upflow.
9. Allow the CHT to settle for 1 min. Lower the inlet adaptor 4–6 cm to release air trapped between the inlet adaptor bed support and the packing solution.
10. Fully seal the adaptor’s sealing device.
11. Open the outlet valve, then flow pack the column at 700–1,000 cm/hr (for 80 µm CHT) or 300–500 cm/hr (for 40 µm CHT) using packing solution equivalent to 3 times the slurry volume. Do not exceed the pressure limit for the column.
12. Stop the flow. Close the outlet valve.
13. Read the advisory statement and instructions before proceeding to the next step.

Best Practices: The following step releases excess packing solution through the inlet adaptor. The height of the packed bed may be 5% higher than the target value but is generally equal to the target in 40 cm ID columns and larger. IF SUDDEN RESISTANCE IS NOTED AS THE ADAPTOR APPROACHES THE TARGET HEIGHT, STOP THE DESCENT IMMEDIATELY. PUMP PACKING BUFFER INTO THE INLET VALVE AT 50 CM/HR WHILE RAISING THE ADAPTOR AT 50 CM/HR. STOP WHEN THE ADAPTOR HAS TRAVELED 0.5 CM.

14. Lower the inlet adaptor and allow the packing solution to drain through the inlet port.
15. Continue the descent until the adaptor is 1.0 cm above the new target height. A gap of about 0.5–1.0 cm is acceptable between the packed bed and the inlet adaptor bed. The gap ensures that the surface of the packed bed is undisturbed by contact with the uneven geometry characteristic of most inlet adaptors.
16. Close the inlet valve.
17. Connect the column to the chromatography station.
18. Condition with 5 column volumes of packing buffer at 125–200% of the selected operating flow rate.
19. Continue with column qualification.

Unpacking

1. Deflate the adaptor’s sealing device.
2. Remove the adaptor.
3. Connect the gas line to the column outlet using a valve that can switch between the gas connection and the buffer connection.
4. Pump test buffer into the column by upflow at 150 cm/hr. Raise the bed with sufficient buffer to equal 50% of the original slurry volume.

a. Insert a paddle into the column and agitate the medium.

b. Stop the upflow and switch the valve to the gas connection.

5. Turn on the gas in upflow to the same metering position or rate of flow used when packing the column.

   a. Agitate for 10 min using the paddle to disperse the medium.

   b. Stop the gas upflow and switch the valve to the buffer connection.

6. Pump the slurry into waste containers.

   a. Allow the CHT to settle. Aspirate the excess water.

   b. Dispose of the medium per the MSDS and in accordance with your facility's guidelines.

5.4 Media Transfer Station Methods

Determine the dry weight of CHT and volume of packing solutions for media transfer methods into columns such as the following:

- Bio-Rad GelTec™ columns
- BioProcess LPLC columns (GE Healthcare) use the bottom slurry port to fill the column

Best Practices: The media transfer method is used for systems designed to inject particle slurries into specially designed columns. CHT particles are mechanically agitated to maintain slurry consistency, then packed into the column using a slurry transfer unit. Carefully planned packing strategies are especially necessary for CHT slurries. High solids concentration, prolonged agitation, repeated agitation, and use of the cycling option on slurry transfer units will cause irreversible damage to CHT particles.

Tables 5–8 in Section 7 "Appendices" cover tube lengths of 500, 600, 700, and 900 mm where the maximum fill heights are 400, 500, 600, and 800 mm, respectively. The 100 mm difference between tube height and fill height is due to the minimum length required by the inlet adaptor. The tables also list the appropriate dry weight of CHT and volume of packing solution necessary to prepare the slurry in the column to obtain a packed bed for the specified diameter and height. For example, the Bio-Rad GelTec™ 450/600 or BioProcess LPLC 45/700 are 45 cm ID columns with a packed bed height of 20 cm. The Bio-Rad Geltec 450/600 requires 20.04 kg of CHT and 59.5 of packing solution to make a 40.0% v/v and 25.2 w/v slurry. While the LPLC 45/700 with identical amount and volume of CHT and packing solution makes a 33.3% f/f and 21.0% w/v slurry. The 500 and 600 mm tube length columns can be packed to no more than 20 cm bed heights. A 25 cm bed height can be packed into the 700 mm tube length column and the 900 mm tube length column can be packed up to a 30 cm bed height.

5.4.1 Axial Compression Packing of Closed Columns With Motorized Adjustable Inlet Adaptors

Packing

1. Level the column; otherwise, the distance between packed bed surface and inlet adaptor surface will not be uniform across the column's diameter.
2. Leave the inlet adaptor fully extended on the GelTec 450/600 or just below the upper slurry transfer port on the LPLC 45/700.
3. Seal the adaptor's sealing device marginally (O-ring, inflatable bladder, or compression seal). Marginal sealing is the sealing force recommended for the column where liquid will not bypass the seal while the adaptor is lowered.
4. Read the amount of packing solution and CHT from Tables 5–8 as explained above. Invert the containers of CHT repeatedly to loosen the powder. This will enable it to pour easily when dispensing into the column.
   a. Dispense the packing solution into the slurry transfer vessel.
   b. Agitate the solution using a low shear hydrofoil impeller, or stir with a paddle. Do not mix or agitate the slurry with the transfer pumps.
   c. Dispense the CHT to the slurry transfer vessel and mix for 5 min following the last addition.
2. Transfer the CHT slurry to the column through the bottom port at a fast rate to facilitate adequate mixing as it enters the column.
3. Close the filling port.
4. Allow the CHT to settle for 1 min. Lower the inlet adaptor 4–6 cm to release air trapped between the bed support and the packing solution.
5. Close the inlet adaptor valve and open the outlet valve. Read the advisory statement and instructions before proceeding to the next step.

Best Practices: The following two steps pack the bed under flow conditions using the inlet adaptor to drive the packing solution. The height of the packed bed may be 5% higher than the target value but is generally equal to the target in 40 cm ID columns and larger. IF SUDDEN RESISTANCE IS NOTED AS THE ADAPTOR APPROACHES THE TARGET HEIGHT, STOP THE DESCENT IMMEDIATELY. CLOSE THE OUTLET VALVE. OPEN THE INLET VALVE, THEN PUMP PACKING BUFFER INTO THE INLET VALVE AT 50 CM/HR WHILE RAISING THE ADAPTOR AT 50 CM/HR. STOP WHEN THE ADAPTOR HAS TRAVELED 0.5 CM.

6. Turn on the motor drive so that the inlet adaptor descends at 300 cm/hr on the Bio-Rad GelTec and up to 700 cm/hr (80 µm CHT) and 500 cm/hr (40 µm CHT) on the LPLC. Do not exceed the maximum recommend pressure for the seal.
7. Continue the descent until the adaptor is 1.0 cm per 10 cm of packed bed above the target height. A gap of about 0.5–1.0 cm is acceptable between the packed bed and the inlet adaptor bed support for a 10 cm packed bed with the adaptor set to 11 cm. A gap of 0.5–2 cm is acceptable between the packed bed and the inlet adaptor bed support for a 20 cm packed bed with the adaptor set to 22 cm. The gap ensures that the surface of the packed bed is undisturbed by contact with the uneven geometry characteristic of most inlet adaptors.
8. Close the outlet.
9. Connect the column to the chromatography station.
10. Condition with 5 column volumes of packing buffer at 125–200% of the selected operating flow rate.
11. Continue with packed column qualification.

Repacking

1. Estimate the bed height for the repacked column based on results from Comments 1 or 2 in topic 5.8.1 “Comments on Column Qualification for Columns With Adjustable Inlet Adaptors”.
2. If the column has moved since it was packed, level the column. If not level, the distance between packed bed surface and inlet adaptor surface will not be uniform across the column’s diameter.
3. Sanitize the top plate of the inlet adaptor and the wall of the column using your sanitization protocol. Rinse the surfaces with water-for-injection and remove the excess liquid by aspirating under sanitary conditions.
4. Deflate the adaptor’s sealing device to its marginal setting (O-ring, inflatable bladder, or compression seal). Marginal sealing is the sealing force recommended for the column where liquid will not bypass the seal while the adaptor is raised or lowered.
5. Raise the inlet adaptor to the same set point used when it was inserted and marginally sealed.
6. Connect the gas line to the column outlet using a valve that can switch between the gas connection and the buffer connection. While air may be used with some column hardware, the other gases prevent bubbles from being trapped by the bed supports and media. Air should be avoided with columns that contain porous polyethylene or polypropylene bed supports or unsupported nylon or stainless-steel screens.
7. Pump test buffer into the column by upflow at 150 cm/hr to raise the bed with sufficient buffer to equal 80% of the original slurry volume. Stop the upflow and switch the valve to the gas connection.
8. Turn on the gas in upflow to the same metering position or rate of flow used when packing the column.
    a. Agitate for 10 min.
    b. Stop the gas upflow and switch the valve to the buffer connection.
    c. Pump test buffer into the column by upflow at 150 cm/hr to fill the column with sufficient buffer to equal 100% of the original slurry volume.
    d. Stop the upflow.
9. Allow the CHT to settle for 1 min. Lower the inlet adaptor 4–6 cm to release air trapped between the bed support and the packing solution.
10. Close the inlet adaptor valve and open the outlet valve. Read the advisory statement and instructions before proceeding to the next step.

Best Practices: The following two steps pack the bed under flow conditions using the inlet adaptor to drive the packing solution. The height of the packed bed may be 5% higher than the target value but is generally equal to the target in 40 cm ID columns and larger. IF SUDDEN RESISTANCE IS NOTED AS THE ADAPTOR APPROACHES THE TARGET HEIGHT, STOP THE DESCENT IMMEDIATELY. CLOSE THE OUTLET VALVE. OPEN THE INLET VALVE THEN PUMP-PACKING BUFFER INTO THE INLET VALVE AT 50 CM/HR WHILE RAISING THE ADAPTOR AT 50 CM/HR. STOP WHEN THE ADAPTOR HAS TRAVELED 0.5 CM.

11. Turn on the motor drive so that the inlet adaptor descends at 300 cm/hr on the Bio-Rad GelTec™ or up to 700 cm/hr (80 µm CHT) and 500 cm/hr (40 µm CHT) on the LPLC. Do not exceed the maximum recommend pressure for the seal.

12. Continue the descent until the adaptor is 1.0 cm per 10 cm of packed bed above the target height. A gap of about 0.5–1.0 cm is acceptable between the packed bed and the inlet adaptor bed support for a 10 cm packed bed with the adaptor set to 11 cm. A gap of 0.5–2 cm is acceptable between the packed bed and the inlet adaptor bed support for a 20 cm packed bed with the adaptor set to 22 cm. The gap ensures that the surface of the packed bed is undisturbed by contact with the uneven geometry characteristic of most inlet adaptors.
13. Close the outlet.
14. Connect the column to the chromatography station.
15. Condition with 5 column volumes of packing buffer at 125–200% of the selected operating flow rate.
16. Continue with packed column qualification.

Unpacking

1. Deflate the adaptor's sealing device.
2. Remove the adaptor.
3. Connect the gas line to the column outlet using a valve that can switch between the gas connection and the buffer connection.
4. Pump test buffer into the column by upflow at 150 cm/hr. Raise the bed with sufficient buffer to equal 50% of the original slurry volume.
    a. Insert a paddle into the column and agitate the medium.
    b. Stop the upflow and switch the valve to the gas connection.
5. Turn on the gas upflow to the same metering position or rate of flow used when packing the column.
    a. Agitate for 10 min using the paddle to disperse the medium.
    b. Stop the gas upflow and switch the valve to the buffer connection.
6. Pump the slurry into waste containers through the column's unpacking port.
    a. Allow the CHT to settle. Aspirate the excess water.
    b. Dispose of the medium per the MSDS and in accordance with your facility's guidelines.

5.5 Media Packing Station Methods

Pressure packing contained operating systems, closed columns from 40 cm–240 cm ID (12 to 900 L)

Estimating quantities of CHT and column packing solutions for closed columns

- Chromaflow column (GE Healthcare)
- Resolute column (Pall)
- IsoPak column (Millipore)
- Eastern River

Best Practices: The media transfer method is used for systems designed to inject particle slurries into specially designed columns. CHT particles are mechanically agitated to maintain slurry consistency, then packed into the column using a slurry transfer unit. Carefully planned packing strategies are especially necessary for CHT slurries. High solids concentration, prolonged agitation, repeated agitation, and use of the cycling option on slurry packing units will cause irreversible damage to CHT particles.

Appendix table 9 (Section 7) lists packed bed lengths from 10–20 cm for contained operating system columns that are 40 cm to 240 cm ID. The typical tube length is 50 cm and the inlet adaptor can be fixed so that the packed bed can be between 5 cm and 40 cm in height.

Packing

1. Level the column, otherwise, the distance between packed bed surface and inlet adaptor surface will not be uniform across the column's diameter.
2. Set the inlet adaptor to the desired packed bed height.
3. Seal the column's adaptor per the instructions for the specific column.
4. Prime the slurry transfer skid, fill the column completely with packing solution, clear any air from the column, and transfer lines according to the manufacturer's instructions.

5. Determine the amount of packing solution and CHT according to Appendix table 6 (Section 7). Invert the containers of CHT repeatedly to loosen the powder. This will enable it to pour easily when dispensing into the column.

    a. Dispense approximately the necessary amount (as determined from appendix tables) of packing solution into the slurry transfer vessel.

    b. Agitate the solution using a low-shear hydrofoil impeller or stir with a paddle.

Best Practices: Use low-shear hydrofoil impellers for slurry volumes larger than 200 L. It is advisable to use multiple impellers on the shaft spaced approximately 1 m apart for deep slurries. Do not mix or agitate the slurry with the transfer pumps.

    c. Dispense the CHT to the slurry transfer vessel and mix for 5 min following the last addition.

6. Pressure-pack the CHT slurry into the column through the top-filling nozzle at the fastest rate permitted by the pressure limit of the column and media packing station.

    a. Continue until the media packing station stalls. Refer to the manufacturer's instructions.

    b. Close the column outlet valve.

7. Close the packing nozzle, then clear the packing lines. Refer to the manufacturer's instructions.

Best Practices: Manufacturers of closed columns recommend reverse flow of the column after packing it to remove entrapped air from the flow distributor of the column inlet. For CHT packed columns, we advise reverse flows into closed columns only at less than 37 cm/hr. Reverse flow at higher rates may create channels in the packed bed.

8. Connect the column to the chromatography station.

9. Condition with 5 column volumes of packing buffer at 125–200% of the selected operating flow rate.

10. Continue with packed column qualification.

Unpacking to Prepare for Repacking

Best Practices: The media packing station is used to inject particle slurries into specially designed columns and to remove the packed media from these columns. Do not use the instructions from the manufacturer for unpacking CHT from these columns without using the additional instructions in this guideline. Carefully planned unpacking and repacking strategies are especially necessary for CHT. High solids concentration, prolonged agitation, repeated agitation, and use of the cycling option on slurry packing units will cause irreversible damage to CHT particles. The media packing skid should be used only to loosen the packed CHT and direct the flow to the slurry or collection tank. Do not cycle the collected slurry back to the column. Never allow any slurry to settle in the packing station, the packing lines, and especially in the nozzles. If this happens, it may be necessary to disassemble any components clogged with settled medium.

1. Plan on using about 10 times the packed column volume of unpacking solution. For example, you will need a 1,000 L collection capacity for a 100 L packed column.

2. Set both nozzles to "Unpack".

3. Using the packing pump, pump the unpacking solution (test buffer or packing solution) into the column and collect the evacuating slurry in the collection vessel.

    a. It is best to use the slurry tank to collect the evacuated medium; however, the capacity is often too small.

        i) If this is the case, unpack the column in increments equal to the capacity of the slurry tank.

        ii) At each increment, stop the pump, close the nozzles, and clear the evacuation lines of CHT.

        iii) Allow the CHT to settle in the slurry tank. The settling rate is approximately 180 cm/hr for the smallest particles in 40 µm CHT. The settling rate for the largest particles in 80 µm CHT is 1,000 cm/hr.

        iv) Aspirate the supernatant from the tank.

    b. Repeat all of Step 3 until CHT particles are no longer seen in the evacuated unpacking solution. The final aspiration of the collected CHT removes particulate developed during the unpacking process.

4. Rinse the column with water using the packing station.

Repacking

1. Level the column; otherwise, the distance between packed bed surface and inlet adaptor surface will not be uniform across the column's diameter.

2. Fill the column completely with packing solution and clear any air from the column and transfer lines according to the manufacturer's instructions.

3. Prepare the slurry.
    a. Dispense enough packing solution to the slurry transfer vessel through the outlet port of the slurry tank so that the total is approximately equal to the amount used in the initial packing (determined from Table 5).
        i) Dispense packing solution through the slurry tank outlet port to clear the port of settled CHT and to facilitate mixing.
    b. Agitate the solution using a low-shear hydrofoil impeller or stir with a paddle.

Best Practices: Use low-shear hydrofoil impellers for slurry volumes larger than 200 L. It is advisable to use multiple impellers on the shaft spaced approximately 1 m apart for deep slurries. Do not mix or agitate the slurry with the transfer pumps.

    c. Dispense CHT equal to about 5% of the initial packing volume to the slurry transfer vessel and mix for 5 min. The additional amount ensures there is enough CHT to pack the column.
4. Pressure-pack the CHT slurry into the column through the top-filling nozzle at the fastest rate permitted by the pressure limit of the column and media packing station.
    a. Continue until the media packing station stalls. Refer to the manufacturer's instructions.
    b. Close the column outlet valve.
5. Close the packing nozzle then clear the packing lines. Refer to the manufacturer's instructions.

Best Practices: Manufacturers of closed columns recommend reverse flow of the column after packing to remove entrapped air from the flow distributor of the column inlet. For CHT packed columns, we advise conducting reverse flows only at less than 37 cm/hr. Reverse flows at higher rates may create channels in the packed bed.

6. Connect the column to the chromatography station.
7. Condition with 5 column volumes of packing buffer at 125–200% of the selected operating flow rate.
8. Continue with packed column qualification.

5.6 Unpacking for Disposal

1. Follow the instructions in the "Unpacking to Prepare for Repacking" section above, but direct the effluent into 30 waste containers.
2. Aspirate excess liquid from the waste containers.
3. Dispose of the medium per the MSDS and in accordance with your facility's guidelines.

5.7 Packed Column Qualification

Column efficiency should be tested directly after packing, at regular intervals afterward, and when separation performance is seen to deteriorate. Tests should check the quality of the packing and monitor it during the working life of the column.

The best method for determining the efficiency of a packed column is in terms of the height equivalent to a theoretical plate (HETP), and the asymmetry factor (As). These values are easily obtained by applying a test probe such as 2% acetone/test buffer solution or 1.75 M NaCl/test buffer solution to the column. The preferred test buffer is 0.15 M NaCl in 20 mM phosphate buffer, pH 7.2–7.4 (PBS).

The calculated plate number (n), will vary depending on the test conditions and should therefore be used as a reference value only. It is also important to maintain constant conditions and equipment when comparing results. Changes in the test probe concentration, test buffer, sample volume, flow rate, liquid pathway, temperature, etc., will affect the results.

For optimal results, the test probe volume should be at least 2.5% and up to 5% of the column volume. The flow rate should range between 75 and 90 cm/hr but should be the same from test to test. If an acceptance limit is defined in relation to column performance, the column plate number can be used as part of the acceptance criteria for column use. Avoid sample dilution by applying it as close to the column inlet as possible without interrupting the flow rate.

To begin the qualification, follow these steps:

1. Equilibrate the column with 5 column volumes of test buffer using the chromatography station at 150 cm/hr.
2. Record the pressure.
3. Reduce the flow rate to that listed in "Conditions", below, and conduct the remaining tests.

Conditions

- Sample volume: 2.5% of the bed volume
- Sample concentration: 2.0% v/v acetone in test buffer or 1.75 M NaCl in test buffer
- Flow rate: 75 cm/hr
- UV: 280 nm, 1 cm, 0.1 AU
- Conductivity: 100 mS

Calculate HETP and the reduced plate height, H, from the UV or conductivity curve (Figure 6) as follows:

HETP = L/N where

L = bed height (cm)

$N = 5.54(V_e/W_{1/2})^2$

V_e = peak elution distance

$W_{1/2}$ = peak width at half peak height. V_e and $W_{1/2}$ are measured in the same units

H = HETP/d

d = mean particle diameter of the medium in cm

Typical HETP values for 40 µm CHT range from 0.016–0.021 cm, and the range for 80 µm is 0.032–0.041 cm. Reduced plate height is often used to compare column performance to that expected for the mean particle size of the chromatography medium. As a guideline for rigid, incompressible chromatography media, a H value of less than 5.5 is normally acceptable.

Calculate the peak asymmetry factor, A_f, as follows:

A_f = b/a where

a = 1st half peak width at 10% of peak height

b = 2nd half peak width at 10% of peak height

The asymmetry factor should be as close as possible to 1.0 (values from 0.8–2.3 are usually acceptable). A change in the shape of the peak is usually the first indication of bed deterioration due to use.

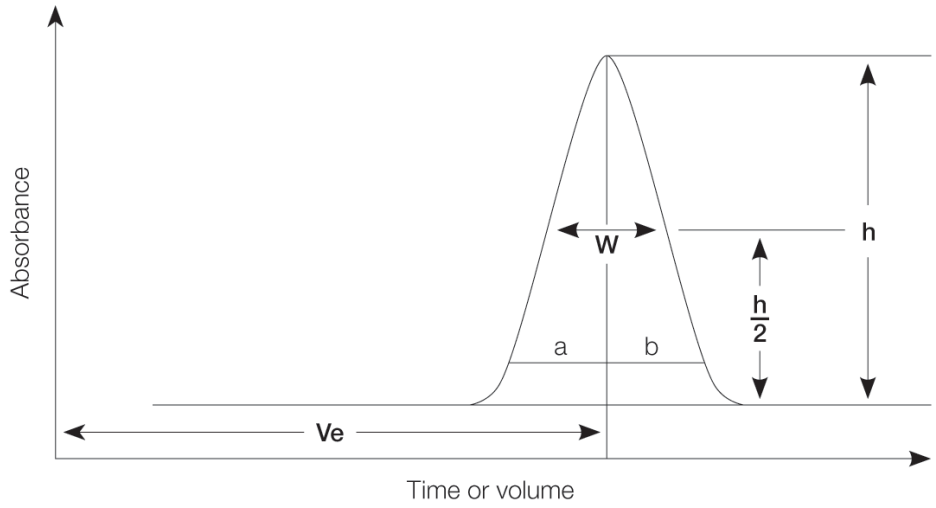


Fig. 6. UV or conductance trace for test.

5.8 Comments on Column Packing

The following comments are provided to further assist your column packing.

5.8.1 Comments on Column Qualification for Columns With Adjustable Inlet Adaptors

Comment 1: If the column test failed the initial qualification tests because the A_f is greater than 2.3 but the HETP and reduced plate height are acceptable, the inlet adaptor descended too far and is compressed firmly against the bed support but loosely at the wall of the column. This may occur to packed beds with adjustable inlet adaptors if the advisory statement regarding the inlet adjustment to the target bed height was ignored. The deficiency may be eliminated by following the instructions in the advisory statement.

Comment 2: If the column failed the initial qualification test because the HETP and reduced plate height were too large but the A_f was acceptable, the inlet adaptor did not descend enough. This may occur when the column is not precision bored. The deficiency may be eliminated by lowering the adaptor 0.5 cm and then retesting the column. Continue lowering the adaptor in 0.5 cm increments and retesting between each increment until HETP, h, and A_f are acceptable.

Comment 3: If the results of the column qualification tests are unsatisfactory and are not correctable using the advice in Comments 1 or 2, the column can be repacked according to the directions in the appropriate section in this guide. The cause of the deficiency may be channeling, heterogeneous bed formation, or gas entrapped in the packed bed. Proceed to the instructions in the "Column Repacking" section.

5.8.2 Comments on Column Qualification for Contained Operating System Pressure-Packed Closed Columns

Comment 4: If the results of the column qualification tests are unsatisfactory, repack the column according to the directions in the appropriate section of this guide. The cause of the deficiency may be channeling, heterogenous bed formation, or gas entrapped in the packed bed. Proceed to the instructions in the "Column Repacking" section.

5.8.3 Comments on Column Qualification for Columns Used in Purification Campaigns

Comment 5: If the column was used for purification cycles and has developed symptoms of deterioration such as higher operating pressure, larger HETP, reduced plate height, and/or poor A_f, we recommend discarding the chromatography medium. Follow the directions for unpacking the column for media disposal supplied by the column manufacturer. Alternatively, drain the excess buffer from the column including that between and in the particles. Remove the column inlet. Scoop the medium from the column, discarding it according to your facility's guidelines and per the recommendation in the product Material Safety Data Sheet.

5.8.4 Conditioning the Column for the Purification Application

If the column was tested with the recommended test buffer proceed directly to the column conditioning protocol established for your purification process.

When using another test buffer at a pH greater than 8, or when packing with buffers above pH 8, adjust the pH of a packed CHT column by equilibrating it with 3 column volumes of 200 mM sodium phosphate at pH 6.5 to 6.8. Proceed to the column conditioning protocol established for your purification process.

Section 6
Case Studies

Examples of protocols for packing CHT™ ceramic hydroxyapatite into different types of columns used in the industry have been provided to help guide you. Read each protocol thoroughly.

Variance in specifications and construction of the columns should be taken into consideration when packing your own column.

6.1 Packing Results — Custom GE Healthcare Chromaflow 900/200–400

Objective

To pack approximately 222 L CHT ceramic hydroxyapatite, Type II, 40 µm, to a height of 35 cm in a 90 cm ID Chromaflow column as a 45% v/v (28% w/v) slurry in 200 mM disodium phosphate buffer, pH 9–10 (DSPB) by pressurizing a 700 L tank containing 510 L of the CHT slurry.

Slurry preparation

Dispense 400 L of DSPB to the slurry tank (316 SS 60° taper, hydrofoil impeller at end of shaft, flat blade impeller at midpoint of shaft). Agitate the buffer while de-aerating with nitrogen for 10 min. Prime the line to the column with 10 L of buffer.

Mix 145 kg of CHT into the remaining 390 L of DSPB while mixing (approximately 3% more slurry than is required to fill the column). Mix for an additional 10 min following the addition of the 29th CHT container while closing and sealing the column hatch.

Column packing

Pressurize the tank to 45 psi with nitrogen gas and hold for 1 min. Open the tank valve to fill the column while maintaining 45 psi tank pressure until the packing nozzle on the column stalls. Immediately close the packing nozzle.

Post-packing cleanup

Close the tank valve and release the pressure on the tank. Disconnect the line from the slurry tank at the column filling connection and direct it to a collection container. Open the tank valve, then pressurize the tank if necessary to empty it of excess slurry. Turn off the nitrogen line if used in the prior step. Rinse the interior with WFI with the tank spraying system, collecting the contents in the collection container.

Connect the GE Healthcare Chromaflow Pack 50 packing station to the column filling line. Flush and clean the filling line according to the instructions in the Chromaflow column and packing station instruction manuals.

Column evaluation

Connect the column to the process chromatography workstation. Apply phosphate buffered saline (PBS) test solution to the column at 25 cm/hr in an upward direction to purge air from the column's inlet distributor for 5 min. Apply PBS buffer in the downward direction at 170 cm/hr until the pH and conductivity of the effluent equals that of the PBS (the pressure will be about 38 psi).

Program the workstation for 350 L of test buffer at 100 cm/hr following the sample injection of 5.55 L of 2% acetone in PBS. Obtain and summarize the results.

Results

Slurry preparation:	45 min CHT addition time for 29 x 5 kg
	13 min additional mixing time including 3 min pressurization
Column packing duration:	16 min
Average packing flow rate:	30.8 L/min
Flow rate range start to finish:	75.0–21.6 L/min

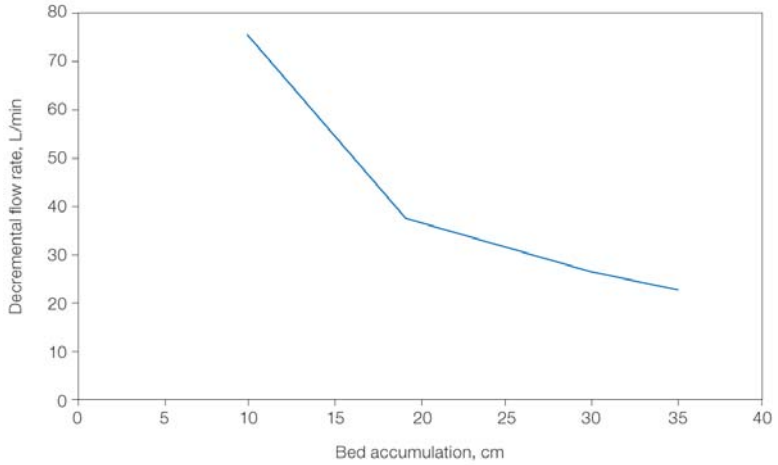


Fig. 7. Decremental flow rate chart.

Peak retention:	193.53 L
Peak retention %CV:	87.17
Plates for column:	2827.5
Plates/meter:	8079
HETP:	0.01238 cm
Reduced plate height:	3.10
Asymmetry A_f:	1.14
Peak width volume:	26.75 L
Injection volume:	5.55 L
Dilution factor:	4.82

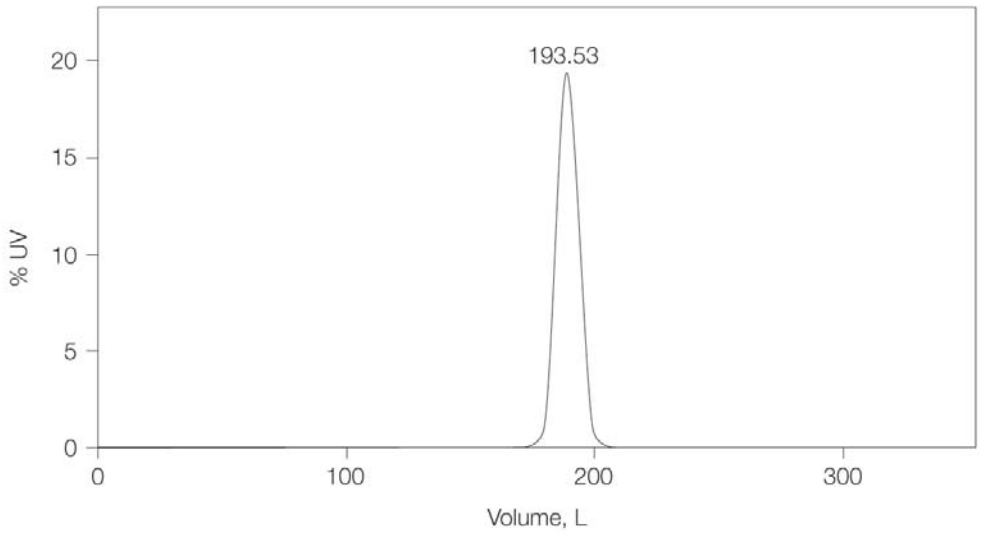


Fig. 8. Acetone test chromatogram.

6.2 Packing Results — Prototype Millipore IsoPak IPP350/500

Objectives

To demonstrate that the IsoPak packing system is able (1) to pack two media types, CHT ceramic hydroxyapatite,Type I 40 µm and 80 µm without damage (2) to give good chromatographic results, and (3) to determine how many times the media can be repacked before extensive damage occurs.

Slurry preparation

CHT Type I, 40 µm, was obtained as slurries from single flow-packed experiments conducted to establish baselines for the medium and relevant packed column volume. The slurry was transferred from the column to a 45° taper polypropylene slurry tank after agitating by upflow with 200 mM disodium phosphate buffer, (DSPB) pH 8–9 to a 25% v/v consistency. It was diluted to 11–14% v/v with DSPB in the tank. The slurry was mixed with a polypropylene paddle to homogeneity just prior to packing the column with the IsoPak packing station.

Column preparation

The clean, dry IPP350 packing station was adjusted to a volume of about 5% less than the available amount of CHT. The line to the slurry tank was back-flushed with air to clear the exit port of any settled CHT. The packing line was connected to the bottom-filling valve on the IPP350 packing station. The inlet adaptor was set to effluent mode. The bottom-filling valve was opened.

Column packing

The packing station was set to 30 psi and the filling line engaged. The slurry filled the column from the bottom, expelling air and excess DSPB through the inlet frit. The bed accumulated against the frit and increased in length from top to bottom during the filling. The system stalled after completely filling the column. The bottom-filling valve was closed when the system stalled.

Post-packing cleanup

The bottom-filling valve and lines were cleaned following the steps in the column and packing station instruction manuals.

Column evaluation

The column was connected to the process chromatography workstation. We applied 75 mM sodium phosphate/100 mM sodium chloride, pH 7.4 test solution (PBNaCl) to the column at 200 cm/hr for 2.6 column volumes.

The column was tested at 200 cm/hr with 500 mL of 2% acetone in PBNaCl, loaded in a 10 sec pulse.

6.3 Table 3. Summary for Packing CHT Type I, 40 µm in Isopak IPP350

Packing number	2	3	4	5	6	7
Slurry concentration, %v/v	13	14	15	8	14	14
Average exhaust flow rate, L/min	10.9	10.9	11.1	11.1	9.75	10.4
Packing time to stall	8.25	7.50	6.30	13.0	8.00	8.00
Test flow rate	200	200	200	200	200	200
Peak retention time	4.02	3.90	3.88	3.90	3.90	3.92
HETP, cm	0.0109	0.0134	0.0157	0.0132	0.0147	0.0151
Asymmetry at 10% peak height	1.064	1.047	0.990	0.909	0.879	0.866
Sample loading time, seconds	10	10	10	10	10	10
Peak width at 10% peak height min	0.526	0.544	0.603	0.544	0.571	0.587
Dilution factor, D_1	3.16	3.26	3.62	3.26	3.43	3.52
Column inlet pressure, bar	1.2	1.18	1.21	1.27	1.25	1.3
Bed height, cm	13	13	13	13	13	13

6.4 Table 4. Summary for CHT Type I, 80 µm in Isopak IPP350

Packing number	1	2	3	4	5	6	7
Slurry concentration, %v/v	25	25	16	25	11	21	16
Average exhaust flow rate, L/min	27.7	24.6	23.1	23.5	21.8	21.3	20.0
Packing time to stall	1.70	1.95	3.60	2.20	6.25	3.00	4.50
Test flow rate	200	200	200	200	200	200	200
Peak retention time	4.02	4.02	3.98	3.97	3.83	3.93	3.85
HETP, cm	0.026	0.030	0.029	0.030	0.030	0.032	0.039
Asymmetry at 10% peak height	1.265	1.090	1.133	1.137	1.429	1.347	1.545
Sample loading time, seconds	10	10	10	10	10	10	10
Peak width at 10% peak height min	0.805	0.819	0.804	0.812	0.888	0.895	1.008
Dilution factor, D_1	4.83	4.91	4.82	4.87	5.33	5.37	6.05
Column inlet pressure, bar	0.66	0.70	0.73	0.69	0.76	0.74	0.75
Bed height, cm	13	13	13	13	13	13	13

Conclusion

CHT Type I (both 40 and 80 µm) was shown to efficiently pack into the Millipore IsoPak IPP350 column using a slurry packing station. IsoPak provided additional convenience, containment, and greatly reduced packing and unpacking time. Moreover, the reproducibility of the IsoPak pump-packed results is excellent.

CHT friable in the extreme. After 40 passes through the packing station, the backpressures increased by 15–18%.

Section 7
Appendices

Tables 5–8 cover tube lengths that are 500, 600, 700, and 900 mm where the maximum fill heights are 400, 500, 600, and 800 mm respectively. The 100 mm difference between tube height and fill height is due to the minimum length required by the inlet adaptor. Tables 5–8 also list the appropriate dry weight of CHT and volume of packing solution necessary to prepare the slurry in the column to obtain a packed bed for the specified diameter and height. Table 9 lists packed bed lengths from 10–20 cm for contained operating system columns that are 40 cm to 240 cm inner diameter. The typical tube is 50 cm in length and the inlet adaptor can be fixed so that the packed bed can be between 5 cm and 40 cm in height.

For example, a BPG 450/500, a 45 cm ID column with a packed bed height of 15 cm requires 15.03 kg of CHT and 48.6 L of packing solution to make a 37.5% volume slurry. The 500 and 600 mm tube length columns can be packed to no more than 20 cm height beds. A 25 cm bed height can be packed into the 700 mm tube length tube column and the 900 mm tube length column can be packed up to a 30 cm bed height.

7.1 Table 5: CHT to Buffer for Packing 50 cm High Open Columns

50 cm tube open columns and media transfer methods, weight of CHT, and volume of buffer to prepare recommended slurry volume for packing designated column ID to designated height.

Column ID (cm)	Bed Height (cm)	Packed Volume (L)	Maximum Fill Height (cm)	Maximum Slurry Volume (L)	CHT (kg)	Volume Buffer (L)	Slurry (%v/v)	Slurry (%w/v)
20	10	3.1	40	12.6	1.98	10.6	25.0	15.8
20	15	4.7	40	12.6	2.97	9.6	37.5	23.6
20	20	6.3	40	12.6	3.96	8.6	50.0	31.5
30	10	7.1	40	28.3	4.45	23.8	25.0	15.8
30	15	10.6	40	28.3	6.68	21.6	37.5	23.6
30	20	14.1	40	28.3	8.91	19.4	50.0	31.5
40	10	12.6	40	50.3	7.92	42.3	25.0	15.8
40	15	18.8	40	50.3	11.88	38.4	37.5	23.6
40	20	25.1	40	50.3	15.83	34.4	50.0	31.5
45	10	15.9	40	63.6	10.02	53.6	25.0	15.8
45	15	23.9	40	63.6	15.03	48.6	37.5	23.6
45	20	31.8	40	63.6	20.04	43.6	50.0	31.5
50	10	19.6	40	78.5	12.37	66.2	25.0	15.8
50	15	29.5	40	78.5	18.56	60.0	37.5	23.6
50	20	39.3	40	78.5	24.74	53.8	50.0	31.5
60	10	28.3	40	113.1	17.81	95.3	25.0	15.8
60	15	42.4	40	113.1	26.72	86.4	37.5	23.6
60	20	56.5	40	113.1	35.63	77.5	50.0	31.5
70	10	38.5	40	153.9	24.25	129.7	25.0	15.8
70	15	57.7	40	153.9	36.37	117.6	37.5	23.6
70	20	77.0	40	153.9	48.49	105.4	50.0	31.5
80	10	50.3	40	201.1	31.67	169.4	25.0	15.8
80	15	75.4	40	201.1	47.50	153.6	37.5	23.6
80	20	100.5	40	201.1	63.33	137.7	50.0	31.5
90	10	63.6	40	254.5	40.08	214.4	25.0	15.8
90	15	95.4	40	254.5	60.12	194.4	37.5	23.6
90	20	127.2	40	254.5	80.16	174.3	50.0	31.5
100	10	78.5	40	314.2	49.48	264.7	25.0	15.8
100	15	117.8	40	314.2	74.22	239.9	37.5	23.6
100	20	157.1	40	314.2	98.96	215.2	50.0	31.5
120	10	113.1	40	452.4	71.25	381.1	25.0	15.8
120	15	169.6	40	452.4	106.88	345.5	37.5	23.6
120	20	226.2	40	452.4	142.50	309.9	50.0	31.5
140	10	153.9	40	615.8	96.98	518.8	25.0	15.8
140	15	230.9	40	615.8	145.47	470.3	37.5	23.6
140	20	307.9	40	615.8	196.96	421.8	50.0	31.5
200	10	3147.2	40	1,256.6	197.92	1,058.7	25.0	15.8
200	15	471.2	40	1,256.6	296.88	959.8	37.5	23.6
200	20	628.3	40	1,256.6	395.84	860.8	50.0	31.5
220	10	380.1	40	1,520.5	239.48	1,281.0	25.0	15.8
220	15	570.2	40	1,520.5	359.23	1,161.3	37.5	23.6
220	20	760.3	40	1,520.5	478.97	1,041.6	50.0	31.5
240	10	452.4	40	1,809.6	285.01	1,524.6	25.0	15.8
240	15	678.6	40	1,809.6	427.51	1,382.0	37.5	23.6
240	20	904.8	40	1,809.6	570.01	1,239.5	50.0	31.5

7.2 Table 6: CHT to Buffer for Packing 60 cm High Open Columns

60 cm tube open columns and media transfer methods, weight of CHT and volume of buffer to prepare recommended slurry volume for packing designated column ID to designated height.

Column ID (cm)	Bed Height (cm)	Packed Volume (L)	Maximum Fill Height (cm)	Maximum Slurry Volume (L)	CHT (kg)	Volume Buffer (L)	Slurry (%v/v)	Slurry (%w/v)
20	10	3.1	50	15.7	1.98	13.7	20.0	12.6
20	15	4.7	50	15.7	2.97	12.7	30.0	18.9
20	20	6.3	50	15.7	3.96	11.7	40.0	25.2
30	10	7.1	50	35.3	4.45	30.9	20.0	12.6
30	15	10.6	50	35.3	6.68	28.7	30.0	18.9
30	20	14.1	50	35.3	8.91	26.4	40.0	25.2
40	10	12.6	50	62.8	7.92	54.9	20.0	12.6
40	15	18.8	50	62.8	11.88	51.0	30.0	18.9
40	20	25.1	50	62.8	15.83	47.0	40.0	25.2
45	10	15.9	50	79.5	10.02	69.5	20.0	12.6
45	15	23.9	50	79.5	15.03	64.5	30.0	18.9
45	20	31.8	50	79.5	20.04	59.5	40.0	25.2
50	10	19.6	50	98.2	12.37	85.8	20.0	12.6
50	15	29.5	50	98.2	18.56	79.6	30.0	18.9
50	20	39.3	50	98.2	24.74	73.4	40.0	25.2
60	10	28.3	50	141.4	17.81	123.6	20.0	12.6
60	15	42.4	50	141.4	26.72	114.7	30.0	18.9
60	20	56.5	50	141.4	35.63	105.7	40.0	25.2
70	10	38.5	50	192.4	24.25	168.2	20.0	12.6
70	15	57.7	50	192.4	36.37	156.1	30.0	18.9
70	20	77.0	50	192.4	48.49	143.9	40.0	25.2
80	10	50.3	50	251.3	31.67	219.7	20.0	12.6
80	15	75.4	50	251.3	47.50	203.8	30.0	18.9
80	20	100.5	50	251.3	63.33	188.0	40.0	25.2
90	10	63.6	50	318.1	40.08	278.0	20.0	12.6
90	15	95.4	50	318.1	60.12	258.0	30.0	18.9
90	20	127.2	50	318.1	80.16	237.9	40.0	25.2
100	10	78.5	50	392.7	49.48	343.2	20.0	12.6
100	15	117.8	50	392.7	74.22	318.5	30.0	18.9
100	20	157.1	50	392.7	98.96	293.7	40.0	25.2
120	10	113.1	50	565.5	71.25	494.2	20.0	12.6
120	15	169.6	50	565.5	106.88	458.6	30.0	18.9
120	20	226.2	50	565.5	142.50	423.0	40.0	25.2
140	10	153.9	50	769.7	96.98	672.7	20.0	12.6
140	15	230.9	50	769.7	145.47	624.2	30.0	18.9
140	20	307.9	50	769.7	193.96	575.7	40.0	25.2
200	10	314.2	50	1,570.8	197.92	1,372.9	20.0	12.6
200	15	471.2	50	1,570.8	296.88	1,273.9	30.0	18.9
200	20	628.3	50	1,570.8	395.84	1,175.0	40.0	25.2
220	10	380.1	50	1,900.7	239.48	1,661.2	20.0	12.6
220	15	570.2	50	1,900.7	359.23	1,541.4	30.0	18.9
220	20	760.3	50	1,900.7	478.97	1,421.7	40.0	25.2
240	10	452.4	50	2,261.9	285.01	1,976.9	20.0	12.6
240	15	678.6	50	2,261.9	427.51	1,834.4	30.0	18.9
240	20	904.8	50	2,261.9	570.01	1,691.9	40.0	25.2

7.3 Table 7: CHT to Buffer for Packing 70 cm High Open Columns

70 cm tube open columns and media transfer methods, weight of CHT, and volume of buffer to prepare recommended slurry volume for packing designated column ID to designated height.

Column ID (cm)	Bed Height (cm)	Packed Volume (L)	Maximum Fill Height (cm)	Maximum Slurry Volume (L)	CHT (kg)	Volume Buffer (L)	Slurry (%v/v)	Slurry (%w/v)
20	10	3.1	60	18.8	1.98	16.9	16.7	10.5
20	15	4.7	60	18.8	2.97	15.9	25.0	15.8
20	20	6.3	60	18.8	3.96	14.9	33.3	21.0
20	25	7.9	60	18.8	4.95	13.9	41.7	26.3
30	10	7.1	60	42.4	4.45	38.0	16.7	10.5
30	15	10.6	60	42.4	6.68	35.7	25.0	15.8
30	20	14.1	60	42.4	8.91	33.5	33.3	21.0
30	25	17.7	60	42.4	11.13	31.3	41.7	26.3
40	10	12.6	60	75.4	7.92	67.5	16.7	10.5
40	15	18.8	60	75.4	11.88	63.5	25.0	15.8
40	20	25.1	60	75.4	15.83	59.6	33.3	21.0
40	25	31.4	60	75.4	19.79	55.6	41.7	26.3
45	10	15.9	60	95.4	10.02	85.4	16.7	10.5
45	15	23.9	60	95.4	15.03	80.4	25.0	15.8
45	20	31.8	60	95.4	20.04	75.4	33.3	21.0
45	25	39.8	60	95.4	25.05	70.4	41.7	26.3
50	10	19.6	60	117.8	12.37	105.4	16.7	10.5
50	15	29.5	60	117.8	18.56	99.3	25.0	15.8
50	20	39.3	60	117.8	24.74	93.1	33.3	21.0
50	25	49.1	60	117.8	30.93	86.9	41.7	26.3
60	10	28.3	60	169.6	17.81	151.8	16.7	10.5
60	15	42.4	60	169.6	26.72	142.9	25.0	15.8
60	20	56.5	60	169.6	35.63	134.0	33.3	21.0
60	25	70.7	60	169.6	44.53	125.1	41.7	26.3
70	10	38.5	60	230.9	24.25	206.7	16.7	10.5
70	15	57.7	60	230.9	36.37	194.5	25.0	15.8
70	20	77.0	60	230.9	48.49	182.4	33.3	21.0
70	25	96.2	60	230.9	60.61	170.3	41.7	26.3
80	10	50.3	60	301.6	31.67	269.9	16.7	10.5
80	15	75.4	60	301.6	47.50	254.1	25.0	15.8
80	20	100.5	60	301.6	63.33	238.3	33.3	21.0
80	25	125.7	60	301.6	79.17	222.4	41.7	26.3
90	10	63.6	60	381.7	40.08	341.6	16.7	10.5
90	15	95.4	60	381.7	60.12	321.6	25.0	15.8
90	20	127.2	60	381.7	80.16	301.5	33.3	21.0
90	25	159.0	60	381.7	100.20	281.5	41.7	26.3
100	10	78.5	60	471.2	49.48	421.8	16.7	10.5
100	15	117.8	60	471.2	74.22	397.0	25.0	15.8
100	20	157.1	60	471.2	98.96	372.3	33.3	21.0
100	25	196.3	60	471.2	123.70	347.5	41.7	26.3
120	10	113.1	60	678.6	71.25	607.3	16.7	10.5
120	15	169.6	60	678.6	106.88	571.7	25.0	15.8
120	20	226.2	60	678.6	142.50	536.1	33.3	21.0
120	25	282.7	60	678.6	178.13	500.5	41.7	26.3
140	10	153.9	60	923.6	96.98	826.6	16.7	10.5
140	15	230.9	60	923.6	145.47	778.2	25.0	15.8
140	20	307.9	60	923.6	193.96	729.7	33.3	21.0
140	25	384.8	60	923.6	242.45	681.2	41.7	26.3
200	10	314.2	60	1,885.0	197.92	1,687.0	16.7	10.5
200	15	471.2	60	1,885.0	296.88	1,588.1	25.0	15.8
200	20	628.3	60	1,885.0	395.84	1,489.1	33.3	21.0
200	25	785.4	60	1,885.0	494.80	1,390.2	41.7	26.3
220	10	380.1	60	2,280.8	239.48	2,041.3	16.7	10.5
220	15	570.2	60	2,280.8	359.23	1,921.6	25.0	15.8
220	20	760.3	60	2,280.8	478.97	1,801.8	33.3	21.0
220	25	950.3	60	2,280.8	598.71	1,682.1	41.7	26.3
240	10	452.4	60	2,714.3	285.01	2,429.3	16.7	10.5
240	15	678.6	60	2,714.3	427.51	2,286.8	25.0	15.8
240	20	904.8	60	2,714.3	570.01	2,144.3	33.3	21.0
240	25	1,131.0	60	2,714.3	712.51	2,001.8	41.7	26.3

7.4 Table 8: CHT to Buffer for Packing 90 cm High Open Columns

90 cm tube open columns and media transfer methods, weight of CHT, and volume of buffer to prepare recommended slurry volume for packing designated column ID to designated height.

Column ID (cm)	Bed Height (cm)	Packed Volume (L)	Maximum Fill Height (cm)	Maximum Slurry Volume (L)	CHT Dry Weight (kg)	Volume of Buffer (L)	Slurry (%v/v)	Slurry (%w/v)
20	10	3.1	80	25.1	1.98	23.2	12.5	7.9
20	15	4.7	80	25.1	2.97	22.2	18.8	11.8
20	20	6.3	80	25.1	3.96	21.2	25.0	15.8
20	25	7.9	80	25.1	4.95	20.2	31.9	19.7
20	30	9.4	80	25.1	5.94	19.2	37.5	23.6
30	10	7.1	80	56.5	4.45	52.1	12.5	7.9
30	15	10.6	80	56.5	6.68	49.9	18.8	11.8
30	20	14.1	80	56.5	8.91	47.6	25.0	15.8
30	25	17.7	80	56.5	11.13	45.4	31.9	19.7
30	30	21.2	80	56.5	13.36	43.2	37.5	23.6
40	10	12.6	80	100.5	7.92	92.6	12.5	7.9
40	15	18.8	80	100.5	11.88	88.7	18.8	11.8
40	20	25.1	80	100.5	15.83	84.7	25.0	15.8
40	25	31.4	80	100.5	19.79	80.7	31.9	19.7
40	30	37.7	80	100.5	23.75	76.8	37.5	23.6
45	10	15.9	80	127.2	10.02	117.2	12.5	7.9
45	15	23.9	80	127.2	15.03	112.2	18.8	11.8
45	20	31.8	80	127.2	20.04	107.2	25.0	15.8
45	25	39.8	80	127.2	25.05	102.2	31.9	19.7
45	30	47.7	80	127.2	30.06	97.2	37.5	23.6
50	10	19.6	80	157.1	12.37	144.7	12.5	7.9
50	15	29.5	80	157.1	18.56	138.5	18.8	11.8
50	20	39.3	80	157.1	24.74	132.3	25.0	15.8
50	25	49.1	80	157.1	30.93	126.2	31.9	19.7
50	30	58.9	80	157.1	37.11	120.0	37.5	23.6
60	10	28.3	80	226.2	17.81	208.4	12.5	7.9
60	15	42.4	80	226.2	26.72	199.5	18.8	11.8
60	20	56.5	80	226.2	35.63	190.6	25.0	15.8
60	25	70.7	80	226.2	44.53	181.7	31.9	19.7
60	30	84.8	80	226.2	53.44	172.8	37.5	23.6
70	10	38.5	80	307.9	24.25	283.6	12.5	7.9
70	15	57.7	80	307.9	36.37	271.5	18.8	11.8
70	20	77.0	80	307.9	48.49	259.4	25.0	15.8
70	25	96.2	80	307.9	60.61	247.3	31.9	19.7
70	30	115.5	80	307.9	72.74	235.1	37.5	23.6
80	10	80.3	80	402.1	31.67	370.5	12.5	7.9
80	15	75.4	80	402.1	47.50	354.6	18.8	11.8
80	20	100.5	80	402.1	63.33	338.8	25.0	15.8
80	25	125.7	80	402.1	79.17	323.0	31.9	19.7
80	30	150.8	80	402.1	95.00	307.1	37.5	23.6
90	10	63.6	80	508.9	40.08	468.9	12.5	7.9
90	15	95.4	80	508.9	60.12	448.8	18.8	11.8
90	20	127.2	80	508.9	80.16	428.8	25.0	15.8
90	25	159.0	80	508.9	100.20	408.7	31.9	19.7
90	30	190.9	80	508.9	120.24	388.7	37.5	23.6
100	10	78.5	80	628.3	49.48	578.8	12.5	7.9
100	15	117.8	80	628.3	74.22	554.1	18.8	11.8
100	20	157.1	80	628.3	98.96	529.4	25.0	15.8
100	25	196.3	80	628.3	123.70	504.6	31.9	19.7
100	30	235.6	80	628.3	148.44	479.9	37.5	23.6
120	10	113.1	80	904.8	71.25	833.5	12.5	7.9
120	15	169.6	80	904.8	106.88	797.9	18.8	11.8
120	20	226.2	80	904.8	142.50	762.3	25.0	15.8
120	25	282.7	80	904.8	178.13	726.7	31.9	19.7
120	30	339.3	80	904.8	213.75	691.0	37.5	23.6
140	10	153.9	80	1,231.5	96.98	1,134.5	12.5	7.9
140	15	230.9	80	1,231.5	145.47	1,086.0	18.8	11.8
140	20	307.9	80	1,231.5	193.96	1,037.5	25.0	15.8
140	25	384.8	80	1,231.5	242.45	989.1	31.9	19.7
140	30	461.8	80	1,231.5	290.94	940.6	37.5	23.6
200	10	314.2	80	2,513.3	197.92	2,315.4	12.5	7.9
200	15	471.2	80	2,513.3	296.88	2,216.4	18.8	11.8
200	20	628.3	80	2,513.3	395.84	2,117.4	25.0	15.8
200	25	785.4	80	2,513.3	494.80	2,018.5	31.9	19.7
200	30	942.5	80	2,513.3	593.76	1,919.5	37.5	23.6
220	10	380.1	80	3,041.1	239.48	2,801.6	12.5	7.9
220	15	570.2	80	3,041.1	359.23	2,681.8	18.8	11.8
220	20	760.3	80	3,041.1	478.97	2,562.1	25.0	15.8
220	25	950.3	80	3,041.1	598.71	2,442.4	31.9	19.7
220	30	1,140.4	80	3,041.1	718.45	2,322.6	37.5	23.6
240	10	452.4	80	3,619.1	285.01	3,334.1	12.5	7.9
240	15	678.6	80	3,619.1	427.51	3,191.6	18.8	11.8

7.5 Table 9: CHT to Buffer Guide for Contained Operating System Columns

Contained operating system columns, weight of CHT, and volume of buffer to prepare recommended slurry volume for packing designated column ID to designated height.

Column ID (cm)	Bed Height (cm)	Packed Volume (L)	Recommended Slurry Volume (L)	CHT Dry Weight (kg)	Volume of Buffer (L)	Slurry (%v/v)	Slurry (%w/v)
40	10	12.6	50.0	7.92	42.1	25.1	15.8
40	15	18.8	75.0	11.88	63.1	25.1	15.8
40	20	25.1	100.0	15.83	74.2	25.1	15.8
40	25	31.4	125.0	19.79	105.2	25.1	15.8
40	30	37.7	150.0	23.75	126.2	25.1	15.8
45	10	15.9	63.0	10.02	53.0	25.2	15.9
45	15	23.9	95.0	15.03	80.0	25.1	15.8
45	20	31.8	126.0	20.04	106.0	25.2	15.9
45	25	39.8	158.0	25.05	133.0	25.2	15.9
45	30	47.7	189.0	30.06	158.9	25.2	15.9
50	10	19.6	78.0	12.37	65.6	25.2	15.9
50	15	29.5	117.0	18.56	98.4	25.2	15.9
50	20	39.3	156.0	24.74	131.3	25.2	15.9
50	25	49.1	195.0	30.93	164.1	25.2	15.9
50	30	58.9	234.0	37.11	196.9	25.2	15.9
60	10	28.3	112.0	17.81	94.2	25.2	15.9
60	15	42.4	168.0	26.72	141.3	25.2	15.9
60	20	56.5	224.0	35.63	188.4	25.2	15.9
60	25	70.7	280.0	44.53	235.5	25.2	15.9
60	30	84.8	336.0	53.44	282.6	25.2	15.9
70	10	38.5	153.0	24.25	128.8	25.2	15.8
70	15	57.7	230.0	36.37	193.6	25.1	15.8
70	20	77.0	306.0	48.49	257.5	25.2	15.8
70	25	96.2	383.0	60.61	322.4	25.1	15.8
70	30	115.5	459.0	72.74	286.3	25.2	15.8
80	10	50.3	200.0	31.67	168.3	25.1	15.8
80	15	75.4	300.0	47.50	252.5	25.1	15.8
80	20	100.5	400.0	63.33	336.7	25.1	15.8
80	25	125.7	500.0	79.17	420.8	25.1	15.8
80	30	150.8	600.0	95.00	505.0	25.1	15.8
90	10	63.6	254.0	40.08	213.9	25.0	15.8
90	15	95.4	381.0	60.12	320.9	25.0	15.8
90	20	127.2	508.0	80.16	427.8	25.0	15.8
90	25	159.0	635.0	100.20	534.8	25.0	15.8
90	30	190.9	762.0	120.24	641.8	25.0	15.8
100	10	78.5	314.0	49.48	264.5	25.0	15.8
100	15	117.8	471.0	74.22	396.8	25.0	15.8
100	20	157.1	628.0	98.96	529.0	25.0	15.8
100	25	196.3	785.0	12.70	661.3	25.0	15.8
100	30	235.6	942.0	148.44	793.6	25.0	15.8
120	10	113.1	450.0	71.25	378.7	25.1	15.8
120	15	169.6	675.0	106.88	568.1	25.1	15.8
120	20	226.2	900.0	142.50	757.5	25.1	15.8
120	25	282.7	1,125.0	148.13	946.9	25.1	15.8
120	30	339.3	1,350.0	213.75	1,136.2	25.1	15.8
140	10	153.9	615.0	96.98	518.0	25.0	15.8
140	15	230.9	922.0	145.47	776.5	25.0	15.8
140	20	307.9	1,230.0	193.96	1,036.0	25.0	15.8
140	25	384.8	1,537.0	242.45	1,294.5	25.0	15.8
140	30	461.8	1,845.0	290.94	1,554.1	25.0	15.8
200	10	314.2	1,250.0	197.92	1,052.1	25.1	15.8
200	15	471.2	1,875.0	296.88	1,578.1	25.1	15.8
200	20	628.3	3,750.0	395.84	2,104.2	25.1	15.8
200	25	785.4	2,500.0	494.80	2,630.2	25.1	15.8
200	30	942.5	3,125.0	593.76	3,156.2	25.1	15.8
220	10	380.1	3,750.0	239.48	1,280.5	25.0	15.8
220	15	570.2	1,520.0	359.23	1,920.8	25.0	15.8
220	20	760.3	2,280.0	478.97	2,561.0	25.0	15.8
220	25	950.3	3,040.0	598.71	3,201.3	25.0	15.8
220	30	1,140.4	4,560.0	718.45	3,841.5	25.0	15.8
240	10	452.4	1,800.0	285.01	1,515.0	25.1	15.8
240	15	678.6	2,700.0	427.51	2,272.5	25.1	15.8
240	20	904.8	3,600.0	571.01	3,030.0	25.1	15.8
240	25	1,131.0	4,500.0	712.51	3,787.5	25.1	15.8
240	30	1,357.2	5,400.0	855.02	4,545.0	25.1	15.8

Section 8
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Section 9
Ordering Information

CHT Ceramic Hydroxyapatite, Type I

Catalog #	Description
158-2000	**CHT Ceramic Hydroxyapatite**, 20 µm, Type I, 10 g
157-0020	**CHT Ceramic Hydroxyapatite** 20 µm, Type I, 100 g
157-0021	**CHT Ceramic Hydroxyapatite**, 20 µm, Type I, 1 kg
157-0025	**CHT Ceramic Hydroxyapatite**, 20 µm, Type I, 5 kg
158-4000	**CHT Ceramic Hydroxyapatite**, 40 µm, Type I, 10 g
157-0040	**CHT Ceramic Hydroxyapatite** 40 µm, Type I, 100 g
157-0041	**CHT Ceramic Hydroxyapatite** 40 µm, Type I, 1 kg
157-0045	**CHT Ceramic Hydroxyapatite**, 40 µm, Type I, 5 kg
158-8000	**CHT Ceramic Hydroxyapatite**, 80 µm, Type I, 10 g
157-0080	**CHT Ceramic Hydroxyapatite**, 80 µm, Type I, 100 g
157-0081	**CHT Ceramic Hydroxyapatite**, 80 µm, Type I, 1 kg
157-0085	**CHT Ceramic Hydroxyapatite**, 80 µm, Type I, 5 kg

CHT Ceramic Hydroxyapatite, Type II

Catalog #	Description
157-2200	**CHT Ceramic Hydroxyapatite**, 20 µm, Type II, 10 g
157-2000	**CHT Ceramic Hydroxyapatite**, 20 µm, Type II, 100 g
157-2100	**CHT Ceramic Hydroxyapatite**, 20 µm, Type II, 1 kg
157-2500	**CHT Ceramic Hydroxyapatite**, 20 µm, Type II, 5 kg
157-4200	**CHT Ceramic Hydroxyapatite** 40 µm, Type II, 10 g
157-4000	**CHT Ceramic Hydroxyapatite**, 40 µm, Type II, 100 g
157-4100	**CHT Ceramic Hydroxyapatite**, 40 µm, Type II, 1 kg
157-4500	**CHT Ceramic Hydroxyapatite**, 40 µm, Type II, 5 kg
157-8200	**CHT Ceramic Hydroxyapatite**, 80 µm, Type II, 10 g
157-8000	**CHT Ceramic Hydroxyapatite**, 80 µm, Type II, 100 g
157-8100	**CHT Ceramic Hydroxyapatite**, 80 µm, Type II, 1 kg
157-8500	**CHT Ceramic Hydroxyapatite**, 80 µm, Type II, 5 kg

Larger volumes and special packaging are available upon request.

CHT Ceramic Hydroxyapatite, Type I Coming Soon!

Catalog #	Description
732-4322	Bio-Scale Mini CHT-I, 40 µm, 1 x 5 ml
732-4324	Bio-Scale Mini CHT-I, 40 µm, 5 x 5 ml

CHT Ceramic Hydroxyapatite, Type II Coming Soon!

Catalog #	Description
732-4332	Bio-Scale Mini CHT-II, 40 µm, 1 x 5 ml
732-4334	Bio-Scale Mini CHT II, 40 µm, 5 x 5 ml

Bio-Rad Laboratories, Inc.

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