NMCHT Ceramic Hydroxyapatite

Mixed-mode Chromatography Media

Product Instruction

File code: NM-A-DF-0135

Version: A0

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NanoMicro

NMCHT

Mixed-mode chromatography media

Ceramic hydroxyapatite (CHT) is a hydroxyapatite-based composite material with important application value as a chromatographic medium. It has a spherical appearance and macropores. Unlike traditional chromatographic media, CHT is a mixed-mode resin with both metal affinity and cation ion exchange capabilities offers a unique selectivity that can provide unique and effective purification solutions. CHT chromatography media can be used for the purification of various types of biomacromolecules, including: antibodies, virus particles, vaccines, recombinant proteins and nucleic acid, etc.

We produce hydroxyapatite with high-quality raw material through a proprietary process with stable microstructure (shown in Figure 1), enabling precise control over particle size, pore size, and porosity. The media has the following characteristics:

- Large pore size allows large molecules to be adsorbed and eluted in a short time, leading to a high purification efficiency and yield.
- Good mechanical strength enables a more uniform flow of fluids within the bed layer and reduces pressure drop at a higher column bed.
- Higher manufacturing productivity meets the purification needs of different biological products.

NMCHT Type I has a higher binding capacity and is suitable for small protein purification. NMCHT Type II has a larger pore size and is more suitable for the purification of macromolecules such as viruses, IgM, VLP particles, plasmids, etc.

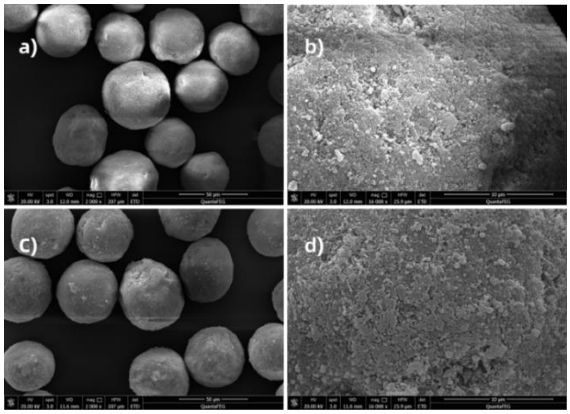

Figure 1. SEM images of NMCHT Type I, 2000×(a), 16000×(b); SEM images of NMCHT Type II, 2000×(c), 16000×(d)

Table 1. Technical parameters for CHT media

Product name	NMCHT Type I	NMCHT Type II
Functional group	Ca^{2+}, PO_4^{3-}, -OH	
Particle size	40±4 μm	
Tap-settled density	0.72 g/ml	
DBC (IgG)	~35 mg/ml	~15 mg/ml
Recommended flow rate	50-1000 cm/h	
Sanitization	1-2 M NaOH	
pH stability	6.5-14, at least 1year in 1 M NaOH	
Regeneration	0.4 M sodium phosphate buffer, pH 7-7.5; 1 M trisodium phosphate, pH 11-12; If a higher phosphate concentration is required, please use 0.4-1 M potassium phosphate buffer	
Autoclavability	121 °C, 20 min, phosphate buffer, pH 7	
Storage*	0.1 M NaOH, room temperature	

* Please store the product under sealed, room temperature (2-30°C), and dry conditions in original packaging. Here are the recommended storage conditions once the package has been opened.

Column packing

CHT media allows low back pressure at high flow rates thanks to its good mechanical strength which is suitable for large-scale purification processes. It attributes to its unique pore structure and optimized back pressure-flow rate curve.

NMCHT Type II undergoes a significant increase in pore structure after high-temperature calcination. When compared to NMCHT Type I, NMCHT Type II exhibits lower backpressure at the same flow rate, enabling a higher throughput during purification, thereby streamlining the process flow and reducing production costs.

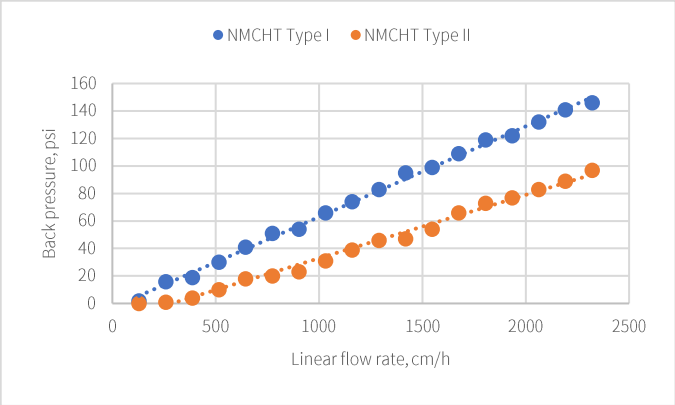

Figure 2. Pressure-flow rate curve of NMCHT Type I (blue) and NMCHT Type II (orange). I. D.: 7.7 mm, height: 100 mm

Unlike traditional media, CHT media settles quickly and cannot be compressed, so it is necessary to develop a new column packing method.

Notice:
A magnetic stirring rod is not recommended and do not compress the column bed to avoid any wear and tear that may produce small particles that may affect the chromatography effect.

Choose the column packing buffer:

Please use a buffer with an ionic strength ＞ 150 mM and a pH ＞ 6.8. The following three buffers are recommended:

- 20 mM sodium phosphate+150 mM NaCl, pH 7.2-8.0
- 200-400 mM potassium phosphate or sodium phosphate, pH 6.8-10
- 0.15-1 M NaOH

Laboratory scale:

Calculate the weight of the media needed:

$$Weight\ of\ CHT\ media = Column\ volume \times bulk\ density$$

Taking a 20 mL packed column as an example, the required amount of CHT media is:

$$20\ mL \times 0.72\ g/mL \approx 14.4\ g\ CHT\ media$$

Prepare the slurry and pack the column:

a) Suspend the CHT media with a plastic stirring rod in a 3.5 times volume of packing buffer to prepare the slurry;
b) Leave the slurry stand still for 5 min to remove the air enclosed in the media particles;
c) Resuspend the CHT media again with a plastic stirring rod to prepare a slurry for later use;
d) Connect the column extension tube to the chromatography column;
e) Fill the column with 1-2 cm of packing buffer to wet the bottom frit to ensure that no air is trapped in the bottom adapter;
f) Transfer the CHT slurry into the column;
g) Open the outlet and pack the media through gravity flow until the column bed is stable;
h) Close the outlet and remove the extension tube. Install the column adapter until it can come into contact with the surface of the column bed;
i) Connect the chromatography system and open the outlet, equilibrate the column with 3 CV of the packing buffer at a flow rate of 250 cm/h;
j) Adjust the adapter until it can come into contact with the surface of the column bed.

Notice:
Avoid introducing any air into the column. If any air enters, please follow the following steps:
Please equilibrate the column with 3 CV of equilibrium buffer, 3 CV of 0.5-1 M NaOH, 3 CV of 0.4-0.5 M PB buffer (pH 6.5~7.2) and then 3 CV of equilibrium buffer in sequence.
An equilibrium buffer should be a buffer with low ionic strength, such as 2-20 mM PB. If 2-10 mM PB buffer is needed, Good’ s buffer should be added.

Manufacturable-scale

The packing methods for CHT media vary based on the type of column and the equipment being used. Please refer to the relevant manuals for columns, media transfer equipment, and packing equipment before packing.

Calculate the weight of the media needed:

Whether it is an open or closed column, the maximum column height cannot exceed 50% of the distance between the surfaces of the media retention plate. Calculate the column volume and the required weight of CHT media using the following formula:

Weight of CHT media =Column volume × bulk density

Weigh the required CHT media according to the column volume, and weigh 720 g of dry powder and 1.84 L of packing buffer per liter of column volume to prepare 50% (V/V) slurry.

Prepare the slurry and pack the column (open column):

a) Closed outlet and pour the packing buffer into the column before adding CHT dry powder;
b) Use a plastic stirrer to stir the mixture to form a slurry, and then stir in the opposite direction to minimize the movement of the slurry;
c) Install the top adapter into the column and allow the slurry to settle for 5 minutes. Lower the adapter slowly to remove the top air once the supernatant appears and clean the collector and inlet lines on the adapter with packing buffer;
d) Run the system with 2 CV of packing buffer at a flow rate of 200-300 cm/h. Lower the adapter once the column bed stops sinking and leave a gap of 1-5 mm between the media retention plate and the top of the column; do not lower the adapter into the column bed, as this can cause irreversible damage to the CHT particles.

Prepare the slurry and pack the column (closed column):

a) Prepare the slurry according to the SOP and remove the bubbles from the column before packing;
b) Open the inlet valve and move the piston upwards to extract the material at a pumping speed of 200-300cm/h. Determine the amount of slurry to be extracted based on the concentration of the slurry, and calculate the piston movement distance. Close the inlet valve and move the piston downwards to press the column at a speed of 200-300cm/h.
c) When the piston is about to press onto the column bed, reduce the pressing speed, and stop pressing the column when the upper sieve plate is 5 mm away from the column bed. Do not lower the adapter into the column bed, causing irreversible damage to CHT particles. If using a stainless steel column, maintain axial flow until the adapter reaches 2 cm above the target height, and then reduce the flow rate to 10 cm/h until the adapter signal sensor contacts the top of the packed bed.

Qualify the column

Evaluation of the column efficiency:

Perform column efficiency testing on the packed column according to the chromatography condition in Table 2.

Table 2. Column efficiency testing condition

Sample	20 mM Sodium phosphate, 1 M NaCl, pH 7.2-7.4
Loading volume	1.0% CV
Elution buffer	20 mM Sodium phosphate, 0.15 M NaCl, pH 7.2-7.4
Linear flow rate*	60-100 cm/h
Detector	Conductivity

Calculate the column efficiency:

Calculate the theoretical plate height (HETP), theoretical plate number (N) and asymmetry factor (As) according to the UV or conductivity curve with the following equation:

$$HETP = L/N$$

$$N = 5.54 \times \frac{{V_R}^2}{{W_h}^2}$$

Where,

V_R is retained volume;

W_h is peak width at half-height;

L is the column height;

N is the number of theoretical plates.

The units of V_R and W_h should be the same.

$$A_s = \frac{b}{a}$$

Where,

a is the first half peak width at 10% peak height;

b is the second half peak width at 10% peak height.

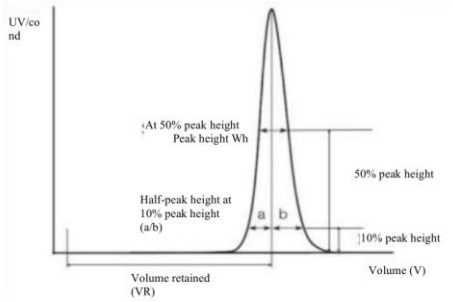

Figure 3. Column efficiency test

Result evaluation

It is judged to be qualified if the value of HETP is less than three times the average particle diameter of the resin beads and the asymmetry factor is between 0.8 and 1.8. It is necessary to analyze the reasons and repack the column, if the HETP and A_s is unsatisfactory.

Chromatography method

Purification principle of CHT media

The surface of CHT media particles contains two types of binding sites, the C (calcium) site and the P (phosphate) site. The C site has metal affinity and can form coordination bonds with phosphate groups on biological molecules, making it suitable for adsorbing DNA, RNA, or removing endotoxin and lipid-coated virus particles. Additionally, the C site can also form coordination bonds with multiple carboxyl groups on aspartic acid and glutamic acid residues, making it suitable for purifying protein substances such as antibodies or recombinant proteins with effective removal of HCP and aggregates. The P site has cation exchange ability and binds alkaline proteins through the P site to effectively remove impurities from the target molecule.

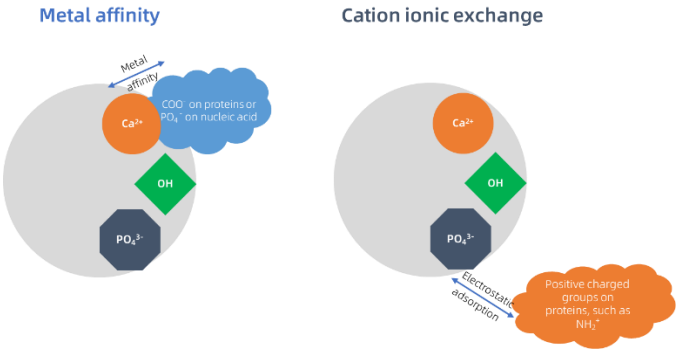

Figure 4. Schematic diagram of CHT media purification principle

Antibody purification

CHT media is often used for the capture and polishing of antibodies which can efficiently separate antibody aggerates and fragments and remove protein A, DNA, endotoxin and viruses.

Sanitize:	1.0 M NaOH, 5CV
Wash:	deionized water, 0.5 CV
Equilibrate:	400 mM PB, pH 6.5, 3 CV
	10 mM PB+4-8 ppm Ca^{2+}, pH 6.5, 10 CV（Increase the concentration of Ca^{2+} to improve the chemical stability of CHT)
Load:	Prepare sample with 10 mM PB+4-8 ppm Ca^{2+}, pH 6.5 (Loading amount should not exceed 80% of the DBC)
Wash:	10 mM PB+4-8 ppm Ca^{2+}, pH 6.5, 5 CV
Elute:	10 mM PB+15 ppm Ca^{2+}, 0-2 M NaCl（linear）, pH 6.5, 20 CV (if there is no elution peak, please increase the phosphate concentration)
Clean:	400 mM PB, pH 7.0-7.5, 5 CV
	Deionized water, 0.5 CV
Sanitize:	1.0 M NaOH, 5CV
Equilibrate:	400 mM PB, pH 6.5, 3 CV
	10 mM PB+4-8 ppm Ca^{2+}, pH 6.5, 10 CV

The CHT media can also be used for the purification of antibody fragments, Fab, VHH, FC fusion proteins, IgM, and IgA. Method screening can be carried out by referring to the IgG purification guidelines for sample loading, elution, and elution conditions.

DNA and RNA purification

Phosphate groups on the DNA and RNA can interact with the citrate ion on the CHT media, allowing for the purification of DNA and RNA. This property can be advantageously applied in lieu of traditional anion exchange resins, as the retention time of nucleic acid increases with the increase in base number, resulting in better resolution between nucleic acids of different lengths.

An example of a chromatography method using CHT media for plasmid purification can be found below:

Sanitize:	1.0 M NaOH, 5 CV
Wash:	Deionized water, 0.5 CV
Equilibrate:	400 mM PB, pH 7.0, 3 CV
	10 mM PB+1 mM EDTA, pH 7.0, 5 CV (Note: Adding EDTA will shorten the life of the media)
Load:	Loading amount should not exceed 80% of the DBC (The sample solution does not contain non acetate alkaline lysis substances)
Wash:	10 mM PB+1 mM EDTA, pH 7.0, 5 CV
Elute:	0-400 mM PB (linear)+1 mM EDTA, pH 7.0, 10 CV
Clean:	400 mM PB, pH 7.0-7.5, 5 CV
	Deionized water, 0.5 CV
Sanitize:	1.0 M NaOH, 5 CV
Equilibrate:	400 mM PB, pH 7.0, 3 CV
	10 mM PB+1 mM EDTA, pH 7.0, 5 CV

Virus purification

The CHT media can form coordination bonds with phosphate groups in lipid encapsulated viruses, which have a strong affinity and require elution with high concentrations of phosphate.

Sanitize:	1.0 M NaOH, 5 CV
Wash:	600 mM PB, pH 7.2, 5 CV
Equilibrate:	10 mM PB, pH 7.2, 10 CV
Load:	Prepare sample with 10 mM PB, pH 7.2, 10 CV (Loading amount should not exceed 80% of the DBC)
Wash:	10 mM PB+1 mM EDTA, pH 7.0, 5 CV
Elute:	10 -600mM PB (gradient), 15 CV（If sodium phosphate precipitation occurs, the concentration can be appropriately reduced or use potassium phosphate solution instead)
Clean:	600 mM PB, pH 7.2, 5 CV
	800 mM potassium phosphate, pH 7-7.5, 3 CV
Sanitize:	1.0 M NaOH, 5 CV
Equilibrate:	10 mM PB, pH 7.2, 10 CV

Acid protein purification

Acidic proteins can bind to the C site on the CHT media through electrostatic interactions, and some phosphorylated protein phosphate groups can also form coordination bonds with the C site, often having a significant impact on protein elution. Therefore, it is necessary to try using phosphate and non-phosphate gradient elution methods for screening during pre-experiments. The following is an example of a chromatography method using CHT media for acidic protein purification.

Sanitize:	1.0 M NaOH, 5 CV
Wash:	Deionized water, 0.5 CV
Equilibrate:	400 mM PB, pH 6.7, 3 CV
	5 mM PB+12-20 ppm Ca^{2+}+ 50-100 mM NaCl, pH 6.7, 10 CV（Increase the concentration of Ca^{2+} to improve the chemical stability of CHT）
Load:	Prepare sample with 5 mM PB+12-20 ppm Ca^{2+}+ 50-100 mM NaCl, pH 6.7 (Loading amount should not exceed 80% of the DBC)
Wash:	5 mM PB+12-20 ppm Ca^{2+}+ 50-100 mM NaCl, pH 6.7, 5 CV
Elute:	Linear gradient 5 mM PB+12-20 ppm Ca^{2+}+ 50-100 mM NaCl, pH 6.7 to 120 mM PB+ 50-100 mM NaCl, pH 6.7, 20 CV
Clean:	400 mM PB, pH 6.7, 5 CV
	Deionized, 0.5 CV
Sanitize:	1.0 M NaOH, 5 CV
Equilibrate:	400 mM PB, pH 6.7, 3 CV
	5 mM PB+12-20 ppm Ca^{2+}+ 50-100 mM NaCl, pH 6.7, 10 CV

Cleaning and sanitization

After using the media for a certain period of time, the column efficiency may decrease and the separation effect may worsen. The following procedure can be used for cleaning and regenerating the media.

a) Wash the column with 2 CV of equilibrium buffer;

b) Wash with 3-5 CV of 400 mM phosphate (if higher concentrations of phosphate are needed to remove tightly bound impurities, potassium phosphate can be used instead of sodium phosphate taking into account the solubility.; 1-2 M KCl+5 mM NaCl, 8 M urea, or 6 M guanidine hydrochloride at pH 6.5-7.5 is also recommended;

c) Wash with 1-2 CV of less than 20 mM phosphate;

d) Wash with 1-2 CV of 1.0 M NaOH solution;

e) Wash with 4 CV of equilibrium buffer.

Sterilization

The CHT media can be treated with phosphate buffer (pH 7.0) at 121 °C for 20 min or 1 M NaOH for 1 hour to achieve sterilization and pyrogen removal.

Long-term storage

Unsealed dry powder media can remain stable at room temperature for extended periods when stored in a dry and sealed environment.
If you intend to store sealed media for an extended duration, we recommend using a 0.1 M NaOH solution and keeping it securely stored in a cool, dry place within a temperature range of 2-30 °C.
It is advisable to avoid preservation media that contain azide compounds or chlorhexidine in antibacterial agents to prevent bacterial growth.

Trouble shooting

If you meet any issues when using the CHT media NMCHT Type I or NMCHT Type II, please refer to the table below for resolution or contact us

Q 1	The CHT media exhibits discoloration.
	The combination of media and other non-ferrous metal ions can accumulate at the top of the column, leading to discoloration. The pollutants mainly come from water, cell culture, or may have been left over during the last purification. CHT media can be used to remove metal ions that cannot be removed by other resins, including various heavy metals such as chromium, lead, cadmium, and aluminum ions.
Q 2	The elution peak contains calcium ions.
	The elution using CHT media often contains about 1 mM of calcium ions, which is equivalent to the concentration of calcium ions in human body, and no adverse reactions have been reported.
Q 3	Can chelating agents such as EDTA and EGTA be used?
	Chelating agents such as EDTA and EGTA, as well as citrate salts, including other compounds that can dissolve or chelate metal ions with aspartic acid and glutamic acid, can affect the stability of CHT media. Please try to avoid using relevant additives to affect the service life of the media.

Order information

Product name	Package	Cat. No.
NMCHT Type I	10 g	80000-040001-4010
	100 g	80000-040001-4100
	1 KG	80000-040001-3001
	5 KG	80000-040001-3005
NMCHT Type II	10 g	80000-040002-4010
	100 g	80000-040002-4100
	1 KG	80000-040002-3001
	5 KG	80000-040002-3005

We also offer 7.7 mm × 22 mm, 16 mm × 25 mm, 7.7 mm × 100 mm prepacked columns. More requirements about products or customized needs, please contact us.

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