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CNT Ion-Exchange Phases

Column Information

The *CNT* ion-exchange columns are specially designed for high resolution and high efficiency chiral separation of DNA wrapped carbon nanotubes. The packing support is composed of a rigid, spherical, highly cross-linked poly(styrene divinylbenzene) (PS/DVB) bead. The porous PS/DVB resin has particle size of 5 and 10 μm , and pore size of 500 \AA . The PS/DVB resin surface is grafted with a highly hydrophilic, neutral polymer thin layer with the thickness in the range of nanometer. The hydrophobic PS/DVB resin surface is totally covered by such a hydrophilic coating that eliminates non-specific bindings with biological analytes, leading to high efficiency and high recovery separations for carbon nanotubes. On the top of the hydrophilic layer, an ion-exchange functional group is attached via chemical bonding. A proprietary chemistry was developed to synthesize a densely packed and uniform ion-exchange layer. The *CNT* ion-exchange phases are composed of SAX and WAX. The *CNT* SAX column is a strong anion exchanger with quaternary ammonium functional groups chemically bonded to the top of the hydrophilic coating covalently bonded on the surface of the porous PS/DVB resins. The *CNT* WAX column is a weak anion exchanger with tertiary amine functional groups chemically bonded to the top of the hydrophilic coating covalently bonded on the surface of the porous PS/DVB resins.

Column Stability and Performance

The *CNT* ion-exchange columns are based on PS/DVB resin and all the surface coatings are chemically bonded onto PS/DVB support, which allows exceptional high stability. They are compatible with most aqueous buffers, such as ammonium acetate, phosphate, tris and so on. When ovalbumin was used as the standard protein sample for test and the 20 mM Tris buffer at pH 8.0 was used as the mobile phase to run the *CNT* SAX and WAX columns, 1,000 injections or 3 months of usage has negligible deterioration for the columns.

The *CNT* SAX and WAX resins are based on porous PS/DVB resins that are coated with a proprietary hydrophilic layer and functionalized with a uniform ion-exchange layer. The pore size is 500 \AA . These phases have three unique features. First, the nanometer thick hydrophilic layer completely eliminates the non-specific interactions with biological analytes. Secondly, Sepax's proprietary technology synthesizes a uniform and densely packed

layer of ion-exchange functional groups. Thirdly, porous beads provide high surface area, leading to high capacity separation. Such uniquely designed *CNT* SAX-POR and WAX-POR phases offer high resolution and high capacity chiral separation for the carbon nanotubes. The *CNT* SAX-POR and WAX-POR phases are well suited for semi-preparative and preparative separation and purification of the carbon nanotubes.

The column dimensions of *CNT* SAX and WAX are 0.75, 2.1, 3.0, 4.6, 7.8, 10, and 21.2 mm I.D., and 2, 3, 5, 10, 15, 25, and 30 cm length. Sepax also offers custom-made columns.

Technical Specifications

<i>CNT</i> IEC Phases	SAX-POR5 and SAX-POR10 WAX-POR5 and WAX-POR10
Packing	Highly cross-linked PS/DVB resin support grafted with a densely packed, nanometer thick hydrophilic coating which is chemically bonded with an uniform ion-exchange layer
Particle size	5 and 10 μm
Pore structure	Porous, 500 \AA
Dynamic Binding Capacity (BSA used as the standard)	~35 mg/mL for <i>CNT</i> SAX-POR5 ~30 mg/mL for <i>CNT</i> WAX-POR5
pH stability	2-12
Operating temperature limit	80 $^{\circ}\text{C}$
Operating pressure limit	4,500 psi
Mobile phase compatibility	Compatible with aqueous solution, a mixture of water and acetonitrile, acetone, or methanol. Typical buffers: phosphate, tris, and acetate
Flow rate	Typical 0.25-1.0 mL/min for a 4.6 mm I.D. column

Safety Precaution

The *CNT* ion-exchange columns are normally operated under moderate pressure. Loose connections will cause leaking of organic solvents and injected samples, all of which should be considered as the hazards. In the case of leaking, proper gloves should be worn for handling the leaked columns. When open the columns, proper protections should be used to avoid inhalation of the small polymer particles.

Column Installation and Operation

When column is shipped or not in use, it is always capped at both ends. When install the column to the system, first remove the end caps. Make the flow direction as marked on the column. Unless a user has special purpose to reverse the flow direction, for example, removal of the inlet pluggage, follow the flow direction as labeled. Column connections are an integral part of the chromatographic process. If ferrules are over tightened, not set properly, or are not specific for the fitting, leakage can occur. Set the ferrules for column installation to the HPLC system as follows:

(a) Place the male nut and ferrule, in order, onto a 1/16" o.d. piece of tubing. Be certain that the wider end of the ferrule is against the nut.

(b) Press tubing firmly into the column end fitting. Slide the nut and ferrule forward, engage the threads, and fingertighten the nut.

(c) While continuing to press the tube firmly into the endfitting, use a 1/4" wrench to tighten the nut 90 degrees past fingertightness.

(d) Repeat this coupling procedure for the other end of the column.

Samples and Mobile Phases

To avoid clogging the column, all samples and solvents including buffers should be filtered through 0.45 µm or 0.2 µm filters before use. The Proteomix ion-exchange columns are compatible with aqueous mobile phase or a mixture of organic and water, such as methanol or acetonitrile and water. Typical eluents contain sodium, potassium salts of phosphate, chloride, acetate, or Tris. Always degas the mobile phase. A simple way for degassing is to sonicate it for 5 minutes under water pumped vacuum.

The *CNT SAX* and *WAX* columns are compatible with nonionic and zwitterionic detergents. ***The CNT SAX and WAX columns are incompatible with anionic detergents.***

Column Care

Shipping Solvent New *CNT SAX* and *WAX* columns are shipped in 20 mM Tris at pH 8.0/0.1% NaN₃. During stocking and shipping, the packing could be dried out. It is recommended that 10-20 column volumes of the starting run buffer be purged to activate the column. Flush the column with your mobile phase with gradual increasing the flow rate from 0.1 mL/min to your operation condition, until the baseline is stable. If the column backpressure and baseline fluctuate, this might be due to the air bubbles trapped inside the column. Flush the column with higher flow rate for 2-5 minutes, for example 1.0 mL/min for a 4.6x250 mm column.

PH The optimum performance and operation for longest lifetime are at pH 2 - 12.

Pressure Even though the *CNT* ion-exchange columns can operate at pressure up to 4,500 psi, the normal operation is usually under 3,000 psi. Continuous use at high pressure may eventually damage the column. Since the pressure is generated by the flow rate. The maximum flow rate is limited by the backpressure. It is expected that the backpressure might gradually increase with its service. A sudden increase in backpressure suggests that the column inlet frit might be plugged. In this case it is recommended that the column be flushed with reverse flow in an appropriate solvent.

Temperature The maximum operating temperature is 80°C. The optimum temperature operation for longest lifetime is 10 - 50°C. Continuous use of the column at higher temperature (>80°C) can damage the column, especially under extremely pH (>12 or <2.0).

Flow Rate Range Normal operation is 0.1-1.0 mL/min for 4.6 mm I.D. columns, respectively.

Storage When not in use for extended time, store the *CNT SAX* and *WAX* columns in 20 mM Tris at pH 8.0/0.1% NaN₃. Each column is shipped with two removable end plugs. To prevent the drying of the column bed, seal both ends of the column with the end plugs provided.

CNT SAX-POR5 Column Product

Length x ID (mm x mm)	Particle Size	Pore size	P/N
250x21.2	5 µm	500 Å	417505-21225
250x10	5 µm	500 Å	417505-10025
150x7.8	5 µm	500 Å	417505-7815
100x7.8	5 µm	500 Å	417505-7810
75x7.8	5 µm	500 Å	417505-7807
250x4.6	5 µm	500 Å	417505-4625
150x4.6	5 µm	500 Å	417505-4615
30x4.6 (Guard)	5 µm	500 Å	417505-4603

CNT WAX-POR5 Column Product

Length x ID (mm x mm)	Particle Size	Pore size	P/N
250x21.2	5 µm	500 Å	418505-21225
250x10	5 µm	500 Å	418505-10025
150x7.8	5 µm	500 Å	418505-7815
100x7.8	5 µm	500 Å	418505-7810
75x7.8	5 µm	500 Å	418505-7807
250x4.6	5 µm	500 Å	418505-4625
150x4.6	5 µm	500 Å	418505-4615
30x4.6 (Guard)	5 µm	500 Å	418505-4603