



Sepax CNT SEC Column Manual

Column Information

Utilizing proprietary surface technologies, the Sepax CNT SEC phases are made of specially coated porous silica materials. The silica support has high purity and enhanced mechanical stability. The Sepax CNT SEC phases have been innovatively and specially designed to ensure highest resolution and maximum recovery for the separation of nanotubes, such as carbon nanotubes, and nanorods. The Sepax proprietary surface technology allows extremely high column-to-column reproducibility and provides exceptional phase stability. The uniform, spherical particles of the Sepax CNT SEC phases have a nominal pore size at 300 Å, 500 Å, 1,000 Å, and 2,000 Å with the pore volume at *ca.* 1.0 mL/g. The Sepax CNT SEC phases are packed with a proprietary slurry technique to achieve uniform and stable packing bed density for maximum column efficiency. Typical applications for the Sepax CNT SEC columns are the separations of nanotubes in aqueous buffer solutions as well as normal organic solvents, such as acetonitrile, methanol and THF.

Column Stability and Performance

The specially designed CNT SEC columns allow exceptional high stability. They are compatible with most aqueous buffers, such as ammonium acetate, phosphate, tris and so on. They are extremely stable in both low and high concentration salts. The highly stable stationary phases enable usage at high temperature up to 80 °C in aqueous solution.

The unique surface chemistry of the CNT SEC packing provides very high column reproducibility from batch to batch. The variation of retention time for the same sample from any different batch is less than 5%.

The CNT SEC stationary phases are hydrophilic, which has negligible nonspecific interactions with carbon nanotubes, resulting in very high recovery for the separation of carbon nanotubes. For example, the recovery for DNA wrapped carbon nanotubes is higher than 95%.

A typical test chromatogram for DNA wrapped carbon nanotube separation is shown in Figure 1 for a series of 4.6x250mm CNT SEC columns with the pore size at 300 Å, 1,000 Å, and 2,000 Å. A random fraction was chosen to measure the CNT length uniformity, as shown in Figure 2. The fraction # 25 generates a uniform length carbon nanotubes of 200 nm.

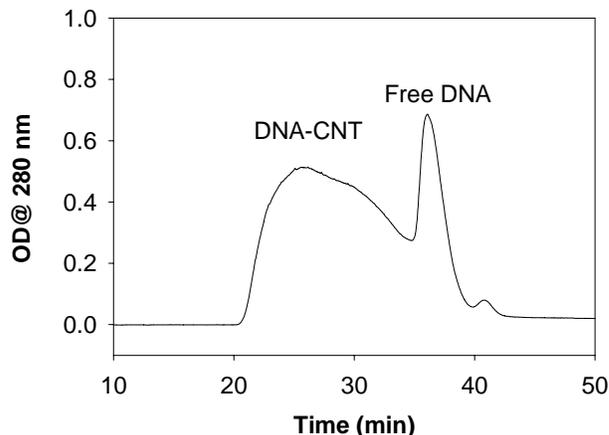


Figure 1. Separation of DNA wrapped carbon nanotubes by a combination of Sepax CNT SEC-300, CNT SEC-1000, and CNT SEC-2000 columns (4.6x250 mm, 5µm). Mobile phase: 40 mM Tris + 0.5 mM EDTA + 0.2 M NaCl, pH 7.0. Flow rate: 0.25 mL/min.

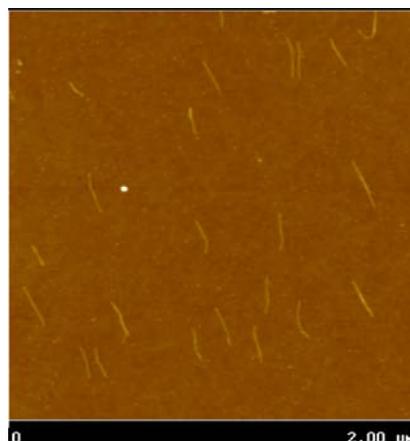


Figure 2. AFM image of fraction 25. A statistical analysis of tube length distribution from 50 randomly chosen tubes results in 192±19 nm.

Safety Precaution

The CNT SEC columns are normally operated under moderate pressure. Loose connections will cause leaking of mobile phase 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 silica particles.

Column Characteristics

Silica: *Spherical, high purity (<10 ppm metals)*

Particle size: *5 and 10 μm*

Pore size: *300, 500, 1,000, and 2,000 Å*

Separation of carbon nanotubes and nanorods

CNT SEC	Pore size (Å)	Particle size (μm)	CNT Dimension Range
300	300	5, 10	Length 1-100 nm Diameter 1-5 nm
500	500	5, 10	Length 25-250 nm Diameter 1-10 nm
1000	1,000	5, 10	Length 100-500 nm Diameter 1-25 nm
2000	2,000	5, 10	Length 300-1000 nm Diameter 1-50 nm

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 CNT SEC columns are compatible with aqueous mobile phase, organic solvent, or a mixture of organic and water, such as methanol or acetonitrile and water. ***The CNT SEC columns are incompatible with cationic species, such as cationic detergents, polymers and proteins.*** Always degas the mobile phase. A simple way for degassing is to sonicate it for 5 minutes under water pumped vacuum.

Column Care

Shipping Solvent New CNT SEC columns are shipped in 0.05% NaN₃ in 0.1 M phosphate buffer, pH 7.0. During stocking and shipping, the silica packing could be dried out. It is

recommended that 10-20 column volumes of 0.1 M phosphate buffer at pH 7.0 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 0.5 mL/min for a 4.6x250 mm column.

pH The optimum performance and operation for longest lifetime are at pH 2 - 8.5.

Pressure Even though the CNT SEC columns can be operated at pressure up to 3,500 psi, the normal operation is usually under 2,000 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 recommend that the column be flushed with reverse flow in an appropriate solvent.

Temperature The maximum operating temperature is 120°C. The optimum temperature operation for longest lifetime is 10 - 60°C. Continuous use of the column at higher temperature (>100°C) can damage the column, especially under high pH (>8).

Flow rate Range Normal operation is 0.1 - 0.4 and 0.1 - 1.25 mL/min for 4.6 mm and 7.8 mm I.D. columns, respectively.

Storage When not in use for extended time, store the column in a mobile phase containing 0.05% NaN₃ or 20% ethanol. 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.

Sepax CNT SEC Products

CNT SEC	ID x Length (mm x mm)	Particle size	Pore size	P/N
300	4.6x250	5 μm	300Å	205300-4625
	7.8x250	5 μm	300 Å	205300-7825
	21.2x250	5 μm	300Å	205300-21225
500	4.6x250	5 μm	500Å	205500-4625
	7.8x250	5 μm	500 Å	205500-7825
	21.2x250	5 μm	500Å	205500-21225
1000	4.6x250	5 μm	1000Å	211000-4625
	7.8x250	5 μm	1000 Å	211000-7825
	21.2x250	5 μm	1000Å	211000-21225
2000	4.6x250	5 μm	2000Å	212000-4625
	7.8x250	5 μm	2000 Å	212000-7825
	21.2x250	5 μm	2000Å	212000-21225