Sepax Technologies, Inc.

Delaware Technology Park
5-100 Innovation Way, Newark, DE 19711, USA
Phone: (302) 366-1101; Fax: (302) 366-1151
Toll Free: 1-877-SEPAX-US; www.sepax-tech.com

Nanofilm SEC Column Manual

Column Information

Utilizing highest purity and enhanced mechanical stability silica, pure bonding reagents, as well as a proprietary surface technology, the Nanofilm SEC phases have been innovatively and specially designed to ensure highest resolution and maximum recovery for biological molecule separations, such as proteins, nucleotides, peptides and others. The Nanofilm SEC phases are a few nanometer-thick organic thin films covalently bonded to the silica. The thin film is neutral, hydrophilic, and uniform. Its proprietary surface technology allows the chemistry of thin film formation to be completely controlled, resulting in highly reliable column-to-column reproducibility. The nature of the chemical bonding and densely packed polymeric thin film benefit the Nanofilm SEC phases with exceptional stability. The uniform, spherical particles of the Nanofilm SEC-150, 250, 500 and 1000 packings have a nominal pore size at 150 Å, 250 Å, 450 Å, and 950 Å with the surface area of 100, 86, 70, and 25 m^2/g, respectively. Nanofilm SEC columns are packed with a proprietary slurry technique to achieve uniform and stable packing bed density for maximum column efficiency. Typical applications for Nanofilm SEC columns are separations of biological molecules in aqueous buffer solutions.

Column Stability and Performance

Nanofilm SEC columns use full coverage bonded silica packing, which allows exceptional high stability. They are compatible with most aqueous buffers, such as ammonium acetate, phosphate, tris etc. When 150 mM phosphate buffer at pH 7.2 is used as the mobile phase to run Nanofilm SEC columns, 300 injections or 1 month of usage has negligible deterioration for the Nanofilm SEC columns.

The unique surface chemistry of Nanofilm SEC packing generates uniform polymer chains covalently bonded on the silica. Such a uniform stationary phase is neutral and hydrophilic, which has negligible nonspecific interactions with biological molecules, especially proteins. This special coating technology allows Nanofilm SEC columns to achieve separations with high selectivity, efficiency, and recovery. A typical quality control chromatogram is shown in Figure 1 for a 4.6x250mm Nanofilm SEC column.

Column Characteristics

Silica: *Spherical, high purity (<10 ppm metals)*
Particle size: *5 μm*
Pore size: *150 Å, MW range 200 ~ $5.0x10^5$*
250 Å, MW range 1,500 ~ $7.0x10^5$
450 Å, MW range $1.5x10^4$ ~ $2.5x10^6$
950 Å, MW range $5.0x10^4$ ~ $5.0x10^6$

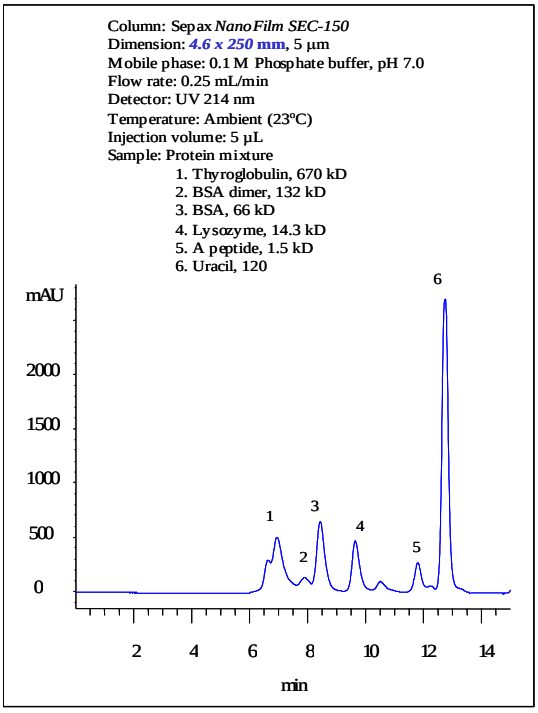

Figure 1. Separation of 4 proteins and a peptide using a 4.6x250 mm Nanofilm SEC-150 column.

Safety Precaution

The 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 hazards. In the case of leaking, proper gloves should be worn for handling the leaking columns. While opening the columns, proper protections should be used to avoid inhalation of the small silica particles.

Column Installation and Operation

The column should always be capped at both ends when it is not in use. When installing the column to the system, first remove the end caps. Unless a user has special purpose to reverse the flow

direction, for example, removal of the inlet blockage, follow the flow direction as marked on the column. 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" outer diameter 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 finger-tighten the nut.

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

Samples and Mobile Phases

To avoid clogging the column, all samples and solvents should be filtered through 0.45 µm or 0.2 µm filters before use. The Nanofilm SEC columns are compatible with an aqueous mobile phase or a mixture of organic solvent and water, such as methanol or acetonitrile and water. 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 columns are shipped in 150 mM sodium phosphate buffer, pH 7.0. During stocking and shipping, the silica packing may become dried out. It is recommended that 10-20 column volumes of 50 mM sodium phosphate buffer at pH 7.0 be purged to activate the column. Flush the column with your mobile phase while gradually increasing the flow rate from 0.1 mL/min to your operating condition, until the baseline is stable. If the column backpressure and baseline fluctuate, this might be due to 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 4.6x300 mm column.

pH For optimum performance and operation during the longest lifetime keep pH between 2 and 8.5.

Pressure Even though the columns can operate at a pressure up to 3,500 psi, the normal operating pressure is usually under 2,000 psi. Continuous use at a 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 operating temperature for the longest lifetime is 10 - 30°C. Continuous use of the column at a higher temperature (>80°C) can damage the column, especially under high pH (>8).

Flow rate Range Normal operating flow rate 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 the column is not in use for an extended time, store the column in a mobile phase of 150 mM sodium phosphate buffer at pH 7.0. 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.

Cleaning From time to time, some samples could get adsorbed onto the inlet frit or the packing material. When the adsorption accumulates to a certain level, it is usually indicated by an increase in backpressure and a broader peak. When this occurs, it is time to clean your column. The general procedure for column cleaning is as follows:

1. Disconnect the column from the detector.
2. Clean your column in the reverse flow direction.
3. Run the column at less than 50% of the maximum recommended flow rate. Monitor the backpressure. If you see the pressure is much higher than the normal operating conditions, you need to lower the flow rate or change the washing buffer as the cleaning solutions may be of different viscosities.
4. Typically, 10-15 column volumes of cleaning solution are sufficient. Rinse well with 3-5 column volume of distilled, deionized water between each solution.

Cleaning solutions Low pH salt solutions help remove basic proteins. Organics are useful when removing hydrophobic proteins. Chaotropic agents help remove strongly adsorbed materials (e.g., via hydrogen bonding). Only use chaotropic agents when neutral salts or organics have not improved resolution. Two cleaning solutions are recommended for general cleaning:

1. Concentrated neutral salt (e.g., 0.5 M Na_2SO_4) at low pH (e.g., pH 3.0)
2. Water soluble organic (MeOH, ACN, EtOH, 10 %-20 %) in aqueous buffer (e.g., 50 mM phosphate, pH 7.0)

Column Protection

In addition to filtering the sample and the mobile phase, the best way to protect the column is to install a guard column or a pre-column filter in front of it. In most cases, a pre-column filter helps remove the residual particulates that are in the sample or the mobile phase, or leached from the HPLC system, such as pump and injector seals. However, a guard column is highly recommended because it is more effective in trapping highly adsorptive sample components and residual particulates in the sample, the mobile phase or from the HPLC system.

Nanofilm *SEC Products*

Length x ID (mm x mm)	Particle size	Pore size	P/N
	Nanofilm SEC -150		
300x7.8	5 µm	150Å	201150-7830
300x4.6	5 µm	150Å	201150-4630
	Nanofilm SEC-250		
300x7.8	5 µm	250Å	201250-7830
300x4.6	5 µm	250Å	201250-4630
	Nanofilm SEC-500		
300x7.8	5 µm	450Å	201500-7830
300x4.6	5 µm	450Å	201500-4630