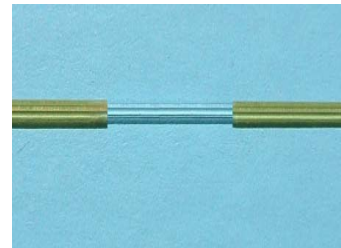
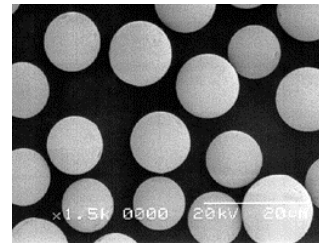
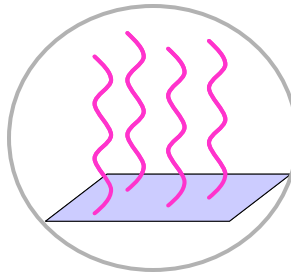


Separation Science & Surface Technology

Better Surface Chemistry for Better Separation



Sepax Technologies, Inc.

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Toll Free: 1-877-Sepax-US
www.sepax-tech.com

Sepax Nanofilm SEC Phases

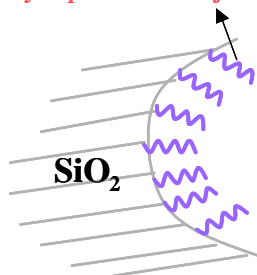
The Best Quality Products

General Description

Utilizing proprietary surface technologies, Sepax *Nanofilm* SEC phases are made of the uniform, hydrophilic, neutral, and polymeric thin films chemically bonded on the high purity and enhanced mechanical stability silica. Sepax *Nanofilm* SEC phases have been innovatively and specially designed to ensure highest resolution and maximum recovery for the separation of biological molecules, such as proteins, nucleic acids, peptides and others. These polymer coatings are a few of nanometer thick. The Sepax proprietary surface technologies allow the chemistry of thin film formation completely controlled that results in high column-to-column reproducibility. The nature of the chemical bonding and densely packed polymer thin film benefit *Nanofilm* SEC phases with exceptional stability. The uniform polymer coating enables high efficiency separation. The narrow dispersed, spherical silica particles of the *Nanofilm* packings for SEC-150, SEC-250, SEC-500, and SEC-1000 have nominal pore sizes at 150 Å, 250 Å, 500 Å, and 1,000 Å, respectively. Their pore volume is *ca.* 0.9 mL/g. *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 the separations of biological molecules in aqueous buffer solutions.

Surface Structure

Hydrophilic nm thin films



Characteristics

- High efficiency and resolution
- Extremely stable in high salt solution
- Great reproducibility

- Protein separation over very low salt concentration
- High protein recovery and maintaining biological activity
- Wide pH application range 2 - 9

High Efficiency Separation

For biological separations, nonspecific interactions with the packing materials are the major contribution for low separation efficiency, such as peak tailing, as well as low recovery. The unique surface chemistry of *Nanofilm* SEC packings 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 allows *Nanofilm* SEC columns to achieve high selectivity and high efficiency separations. For example, uracil, as a test compound could reach as high efficiency as 90,000 plates per meter, as shown in Table 1.

Table 1. Column efficiencies (measured using uracil in 0.1 M phosphate buffer, pH 7.0)

Sepax <i>Nanofilm</i> SEC-150	>90,000 plates/meter
Sepax <i>Nanofilm</i> SEC-250	>90,000 plates/meter
Sepax <i>Nanofilm</i> SEC-500	>85,000 plates/meter
Sepax <i>Nanofilm</i> SEC-1000	>85,000 plates/meter

Separation of Sepax Standard Proteins

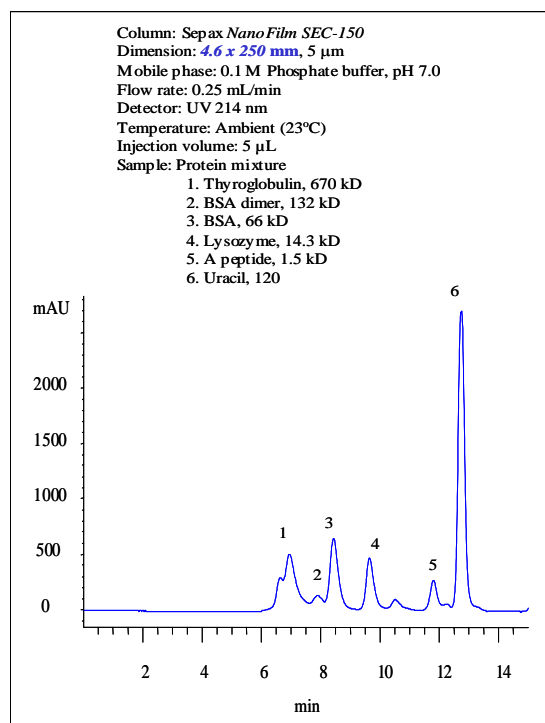


Figure 1. Separation of 4 proteins and a peptide by using a 4.6x250 mm *Nanofilm* SEC-150 column.

The first example for high efficiency separation is shown in Figure 1. Four proteins (thyroglobulin, BSA dimer, BSA, lysozyme) and a peptide are well separated with 30,000 plates per meter for lysozyme. Even an impurity from lysozyme was very well separated. This was only seen separated with high efficiency capillary electrophoresis. (Reference: Sepax Capillary Electrophoresis Catalog). For standard test of size exclusion columns, manufacturers normally use Biorad standard proteins (thyroglobulin, IgG, ovalbumin, and myoglobin). The reason is that those proteins have pI less than 7.5, which are not positively charged at pH 7.0 separation condition. The highly positively charged proteins, such as lysozyme (pI=11.0), cytochrome C (pI=10.6), aprotin (pI=11.0) are very difficult to elute due to the strong electrostatic interactions with the surface of the packing materials manufactured by other vendors. However, this is no longer a problem for Sepax *Nanofilm* SEC columns. Sepax sets its own standard of test proteins including lysozyme, a very sensitive protein for testing the quality of bioseparations.

Separation of BSA dimer from BSA protein

The second example for high efficiency *Nanofilm* SEC columns is the separation of BSA dimer from BSA, as shown in Figure 2. A *Nanofilm* SEC-250 column, 4.6x300 mm, achieved baseline separation of BSA dimer from BSA at 150 mM phosphate, pH 7.0.

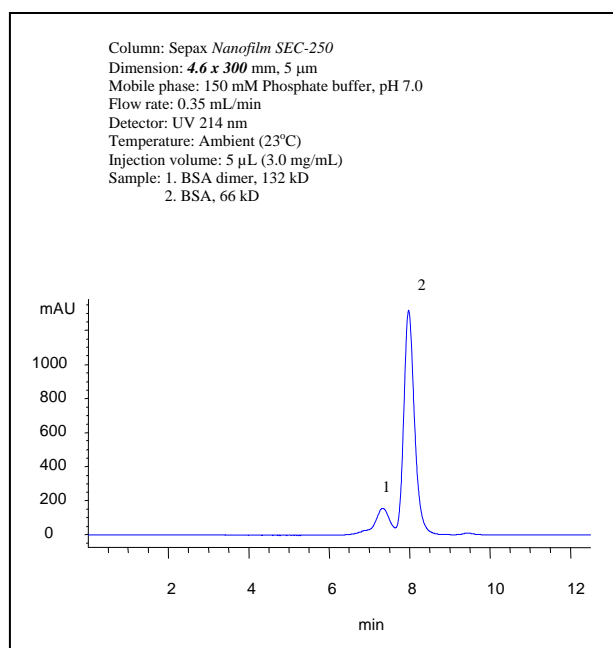


Figure 2. Separation of BSA dimer from BSA by using a 4.6x300 mm *Nanofilm* SEC-250 column.

Separation of the aggregates from thyroglobulin

Thyroglobulin is a huge protein (MW 670,000). The commercial sample of thyroglobulin contains the aggregate impurity, possibly dimer or tetramer. Sepax *Nanofilm* SEC-500 provides a baseline separation of the aggregates from thyroglobulin, as shown in Figure 3.

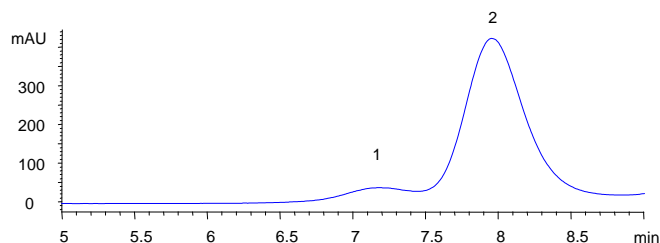


Figure 3. Separation of the aggregates from thyroglobulin by a *Nanofilm* SEC-500 column (5 μ m, 4.6x300 mm). The separation conditions: mobile phase, 150 mM Phosphate buffer, pH 7.0; flow rate, 0.35 mL/min; detection: UV 214 nm; temperature, ambient (23 °C); and injection volume, 5 μ L. The protein sample: (1) the aggregates; and (2) Thyroglobulin (1.0 mg/mL), 670 kD.

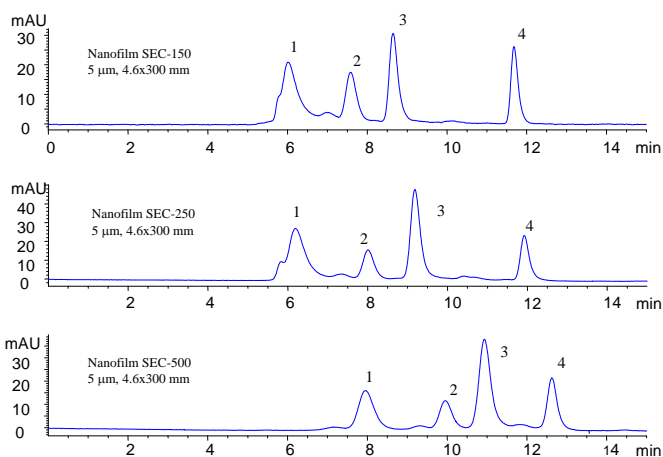


Figure 4. Comparison of the separation patterns by *Nanofilm* SEC-150, *Nanofilm* SEC-250 and *Nanofilm* SEC-500. The separation conditions: mobile phase, 150 mM Phosphate buffer, pH 7.0; flow rate, 0.25 mL/min; detection: UV 280 nm; temperature, ambient (23 °C); and injection volume, 5 μ L. The protein mixture: (1) Thyroglobulin (1.0 mg/mL), 670 kD; (2) BSA (1.0 mg/mL), 66 kD; (3) Ribonuclease A (1.0 mg/mL), 13.7 kD, and (4) Uracil (2.5 μ g/mL), 120

The Impact of Pore Structure on Separation

The pore size of the SEC packings plays a critical role on protein separations. The selection of a *Nanofilm* SEC phase for a particular molecular weight range protein sample is based on the exclusion limit and linear molecular weight range of the *Nanofilm* packings that are shown below.

<i>Nanofilm</i> Packings	Protein MW exclusion limit
SEC-150	750,000
SEC-250	1,250,000
SEC-500	2,500,000
SEC-1000	5,000,000

To demonstrate the impact of the *Nanofilm* SEC packings with different pore size on the separation pattern, Figure 4 shows the separation of three proteins and a small molecule with the MW range from 120 to 670,000.

High Protein Recovery and Maintaining Activity

The *Nanofilm* SEC phases are composed of a hydrophilic and neutral polymer thin film with certain thickness that is chemically bonded on the silica surface. Proteins and other biological molecules have negligible nonspecific interactions with such stationary phases. The protein adsorption to the silica is suppressed, leading to high recovery and maintaining the activity after separations. Table 2 shows the recovery results of BSA and lysozyme, the representatives for acidic and basic proteins, separately.

Table 2. Recovery of proteins from the *Nanofilm* SEC columns (measured with a 4.6x250 mm column in 0.15 M phosphate buffer, pH 7.0)

Column	Protein	Recovery %
<i>Nanofilm</i> SEC-150	BSA	>95%
	Lysozyme	>95%
<i>Nanofilm</i> SEC-250	BSA	>95%
	Lysozyme	>95%
<i>Nanofilm</i> SEC-500	BSA	>95%
	Lysozyme	>95%
<i>Nanofilm</i> SEC-1000	BSA	>95%
	Lysozyme	>95%

High Stability of Sepax *Nanofilm* SEC Phases

The *Nanofilm* SEC columns uses full coverage bonded silica packing. The polymeric thin film is densely packed on the silica surface which greatly hinders the diffusion of the attacking molecules to the silica-polymer bonding, thus enabling exceptional high stability. The *Nanofilm* SEC phases are compatible with most aqueous buffers, such as ammonium acetate, phosphate, trizma and so on. When 150 mM phosphate buffer at pH 7.0 was used as the mobile phase to run the *Nanofilm* SEC columns, 1,000 injections

or 3 months of usage made negligible deterioration for its performance. After 3 months of running in 150 mM phosphate buffer at pH 7.0, the average retention time change was within 5%.

Figure 5 shows the separation profiles of a *Nanofilm* SEC-150 at the day 1, 5, and 10 with 10 hours/day running at the flow rate of 0.35 mL/min in 150 mM and 100 mM phosphate buffer, respectively. The separations are almost identical.

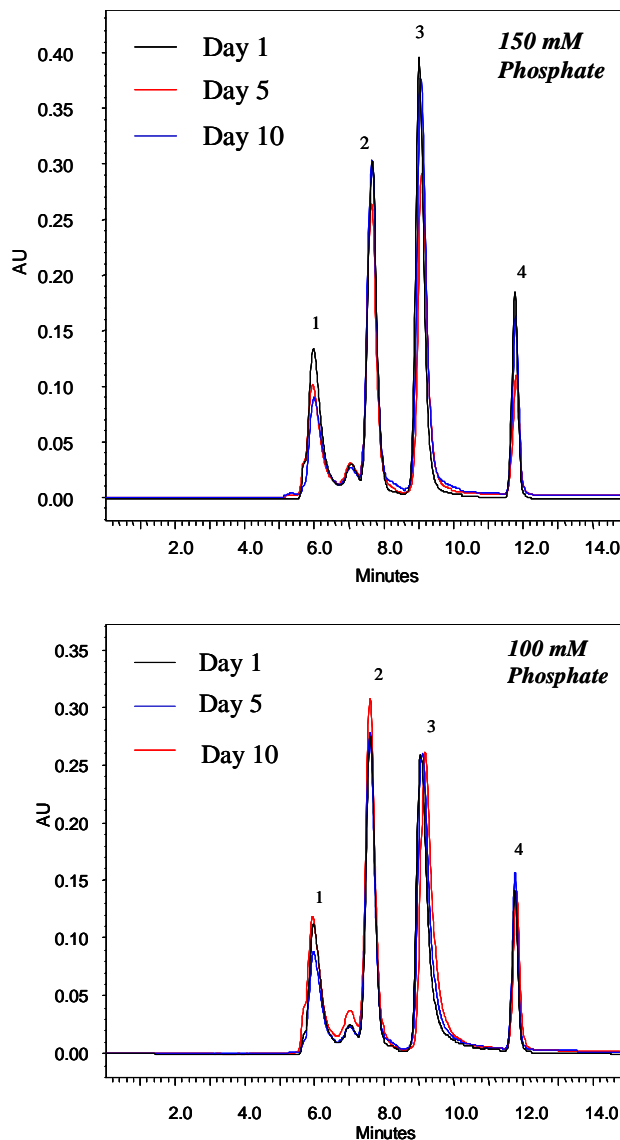


Figure 5. Column: *Nanofilm* SEC-150 (5mm, 4.6x300mm)
 Mobile phase: 150 mM and 100 mM PBS, pH 7.0
 Flow rate: 0.35 mL/min
 Running for 10 h/day
 Temperature: Ambient
 Sample: 1. Thyroglobulin (1.0 mg/mL)
 2. BSA (1.0 mg/mL)
 3. Lysozyme (1.0 mg/mL)
 4. Uracil (50 µg/mL)

Figure 6 is the stability test for retention time of proteins and a small molecule over 500 column volume running in two weeks. It shows negligible retention time change for each of those three proteins as well as uracil within 500 column volume testing.

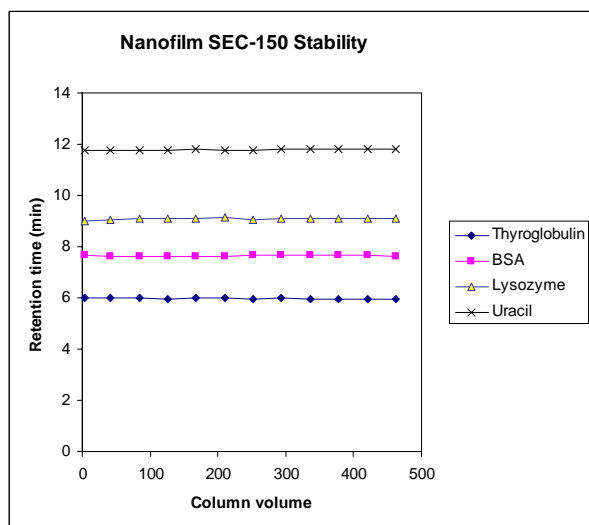


Figure 6. Column: *Nanofilm* SEC-150 (5mm, 4.6x300mm)
 Mobile phase: 150 mM and 100 mM PBS, pH 7.0
 Flow rate: 0.35 mL/min
 Running for 10 h/day
 Temperature: Ambient
 Sample: 1. Thyroglobulin (1.0 mg/mL)
 2. BSA (1.0 mg/mL)
 3. Lysozyme (1.0 mg/mL)
 4. Uracil (50 µg/mL)

The *Nanofilm* SEC phases can tolerate very high concentration of salts, such as 1.0 M. The *Nanofilm* SEC columns are very stable in both organic solvents, such as methanol, ethanol, THF, DMF, DMSO, and so on, and the mixture of water and organic solvents.

The *Nanofilm* SEC columns have very good stability over a wide range of pH from 2 to 8.5. In addition, they can even tolerate very higher pH, such as pH 8.5-10 for temporary using.

The tolerance of temperature for the *Nanofilm* SEC phases is also excellent. The working temperature can go as high as 80 °C.

Reproducibility of the *Nanofilm* SEC columns

The controlled surface chemistry for synthesizing the *Nanofilm* SEC phases makes the surface coating very reproducible, leading to very consistent columns. The separation variation from batch to batch is within 5% for the retention time. Figure 7 is the separation of the Sepax standard protein mixture by the *Nanofilm*

SEC-250 columns from two different lots. The largest variation is the retention time for lysozyme, which is *ca.* 2%.

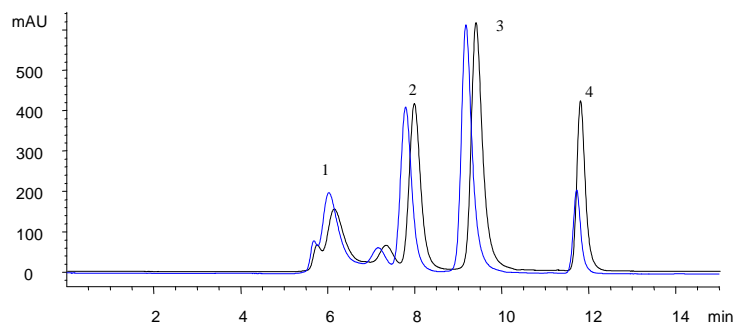


Figure 7. Comparison of the separations by two different batches of *Nanofilm* SEC-250 (5 µm), 4.6x300 mm columns. The separation conditions: mobile phase, 150 mM Phosphate buffer, pH 7.0; flow rate, 0.35 mL/min; detection: UV 214 nm; temperature, ambient (23 °C); and injection volume, 5 µL. The protein mixture: (1) Thyroglobulin (1.0 mg/mL), (2) BSA (1.0 mg/mL), (3) Lysozyme (1.0 mg/mL), and (4) Uracil (2.5 µg/mL).

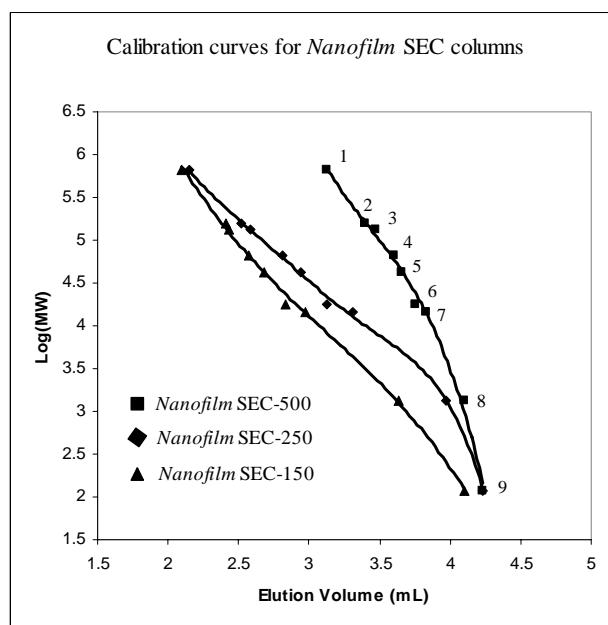


Figure 8. Protein MW calibration with *Nanofilm* SEC-150, *Nanofilm* SEC-250, and *Nanofilm* SEC-500 phases.

Columns: 4.6x300 mm, 5 µm
 Mobile phase: 150 mM Phosphate buffer, pH 7.0
 Flow rate: 0.25 mL/min
 Detector: UV 214 nm
 Injection volume: 5 µL
 Sample: 1. Thyroglobulin, 670 kD
 2. IgG, 150 kD
 3. BSA dimer, 132 kD
 4. BSA, 66 kD
 5. Ovalbumin, 44 kD
 6. Myoglobin, 17.6 kD
 7. Lysozyme, 14.3 kD
 8. B12, 1.35 kD
 9. Uracil, 120

MW Calibration for Protein Separation

For size exclusion chromatography, individual pore size packings determine the range of molecular weight for separation, while the pore volume controls the separation capacity. Sepax's four pore size packings cover whole range of biological molecules. The protein calibration curves for *Nanofilm* SEC-150, *Nanofilm* SEC-250, and *Nanofilm* SEC-500 are shown in Figure 8.

Another widely used method to characterize the SEC phases is the calibration curve between protein MW and the distribution coefficient (K_d). K_d value is defined by the equation below:

$$K_d = (V_e - V_o) / (V_T - V_o)$$

Where V_e , V_T , and V_o are the sample elution volume, the column total liquid volume, and the column void volume, respectively. The linear region between the protein MW and K_d is frequently used as the reference for the selection of the SEC phases. As plotted in Figure 9, the *Nanofilm* SEC-150 phase has an excellent linear relationship between protein MW and K_d in a wide range of MW.

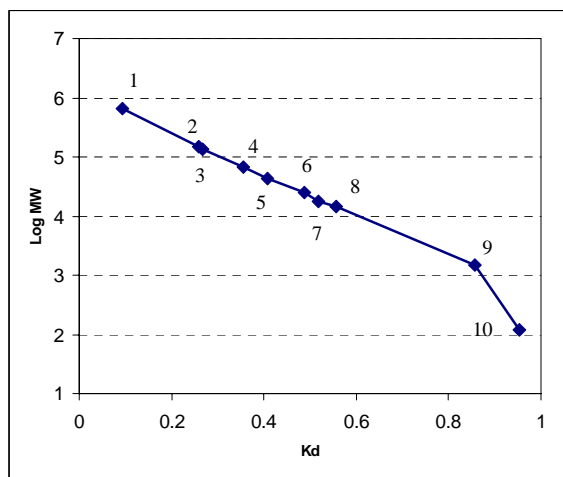


Figure 9. Protein MW calibration curve for *Nanofilm* SEC-150

Column: *Nanofilm* SEC-150, 4.6x150 mm, 5 μ m

Mobile phase: 100 mM Phosphate buffer, pH 7.0

Flow rate: 0.25 mL/min

Detector: UV 214 nm

Temperature: Ambient (23 °C)

Injection volume: 5 μ L

Sample: 1. Thyroglobulin, 670 kD

2. IgG, 150 kD

3. BSA dimer, 132 kD

4. BSA, 66 kD

5. Ovalbumin, 44 kD

6. Alpha-chymotrypsin, 25 kD

7. Myoglobin, 17.6 kD

8. Lysozyme, 14.3 kD

9. A peptide, 1.5 kD

10. Uracil, 120

Proteins Separations at very low salt concentration

In separation of positively charged proteins, a high concentration of salt is needed to shield the strong electrostatic interaction between the silica packing and the proteins. For example, cytochrome C, a highly positively charged protein ($pI=9.6$) is very difficult to be eluted at a low salt concentration, such as 50 mM phosphate buffer at pH 7.0. The need for high concentration salt to elute proteins greatly limits the applications. Sepax designed a special *Nanofilm* SEC phase that could well elute positively charged proteins. As shown in Figure 10, a specially designed *Nanofilm* SEC-500 eluted cytochrome C at 50 mM, phosphate buffer at pH 7.0, which could not be done with any other silica based SEC phases. Such specially designed *Nanofilm* SEC phases can expand size exclusion chromatography to a variety of new applications, such as separation of salt sensitive biological molecules, protein binding studies, and LC/MS detections.

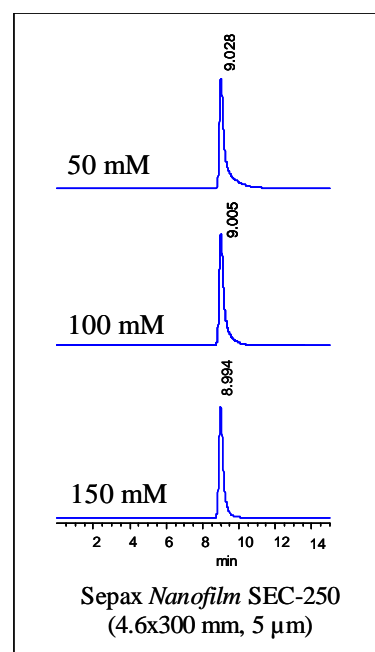


Figure 10. Cytochrome C elution profile with phosphate buffer at pH 7.0.

Column: *Nanofilm* SEC-250 (5 μ m), 4.6x300 mm

Flow rate: 0.35 mL/min

Detection: UV 214 nm

Temperature: ambient (23 °C)

Injection volume: 5 μ L

Sample: 1.0 mg/mL.

Nanofilm SEC Applications

Separation of proteins and peptides

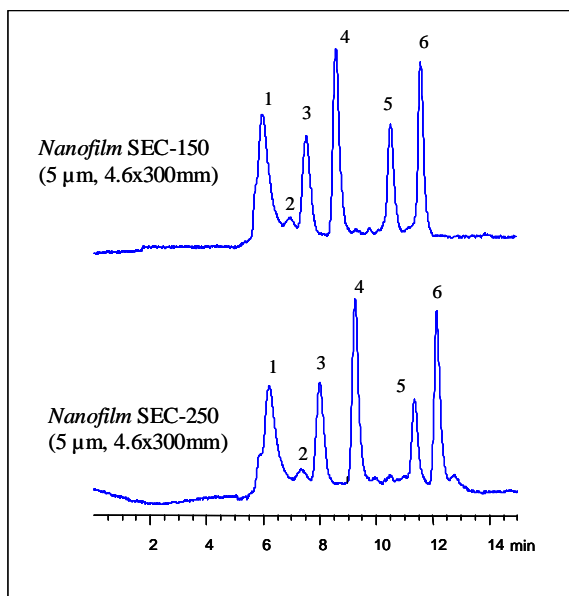


Figure 11. Separation of a mixture of proteins, a peptide and uracil by a Nanofilm SEC-150 and a Nanofilm SEC-250 column

Mobile phase: 0.15 M Phosphate buffer, pH 7.0

Flow rate: 0.35 mL/min

Detector: UV 280 nm

Injection volume: 5 μ L

Sample: 1. Thyroglobulin (1.0 mg/mL), 670 kD

2. BSA dimer, 132 kD

3. BSA (1.0 mg/mL), 66 kD

4. Ribonuclease A (1.0 mg/mL), 13.7 kD

5. A peptide (0.1 mg/mL), 1.5 kD

6. Uracil (0.02 mg/mL), 120

Applications

- Protein, nucleic acid and peptide separations
- Natural and synthetic polymer separations
- Protein binding studies
- Analytical, semi-preparative, and preparative separations
- Proteomics
- Special Applications for LC/MS

Separation of horse serum at low salt concentration

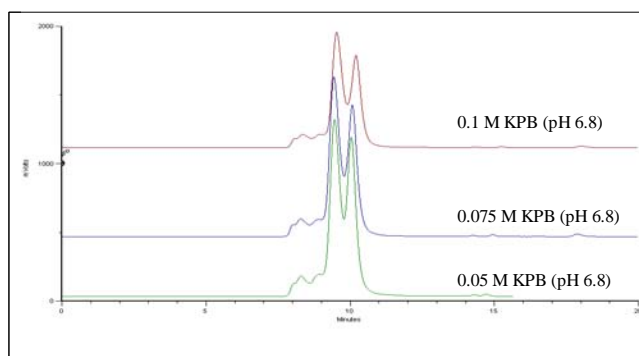


Figure 12. Separation of horse serum at various buffers with low salt concentrations

Column: Nanofilm SEC-150 (5 μ , 4.6x300mm)

Mobile phase: phosphate buffer at pH 6.8

Flow rate: 0.25 mL/min

Injection volume: 1.0 μ L

Detection: 280 nm

(Courtesy of Miyako Kawakatsu, M&S Instruments Inc.)



Nanofilm SEC Technical Specifications

Phase	Nanofilm SEC-150	Nanofilm SEC-250	Nanofilm SEC-500	Nanofilm SEC-1000
Material	Nanometer, hydrophilic film bonded silica	Nanometer, hydrophilic film bonded silica	Nanometer, hydrophilic film bonded silica	Nanometer, hydrophilic film bonded silica
Particle size	5, 10 μm	5, 10 μm	5, 10 μm	5, 10 μm
Pore size (\AA)	~ 150	~ 250	~ 500	~ 1,000
Protein MW range (native)	200 - 750,000	1,500 - 1,250,000	15,000 - 2,500,000	50,000 - 5,000,000
pH stability	2 - 8.5 (pH 9.0 can be tolerated temporarily.)	2 - 8.5 (pH 9.0 can be tolerated temporarily.)	2 - 8.5 (pH 9.0 can be tolerated temporarily.)	2 - 8.5 (pH 9.0 can be tolerated temporarily.)
Backpressure (psi for a 4.6x300 mm)	~ 700	~ 700	~650	~650
Maximum backpressure (psi)	~ 3,500	~ 3,500	~ 3,000	~ 3,000
Salt concentration range	20 mM - 2.0 M	20 mM - 2.0 M	20 mM - 2.0 M	20 mM - 2.0 M
Maximum temperature ($^{\circ}\text{C}$)	~ 80	~ 80	~ 80	~ 80

Ordering Information

Nanofilm SEC-150 (5 μm , 150 \AA)

Length x ID (mm x mm)	P/N	Price
350x21.2	201150-21230	\$3150.00
250x21.2	201150-21225	\$2790.00
150x21.2	201150-21215	\$2150.00
100x21.2	201150-21210	\$1550.00
Guard	201150-21207	\$895.00
75x21.2		
300x10	201150-10030	\$1950.00
250x10	201150-10025	\$1750.00
150x10	201150-10015	\$1350.00
100x10	201150-10010	\$1150.00
Guard	201150-10007	\$695.00
75x10		
300x7.8	201150-7830	\$1095.00
250x7.8	201150-7825	\$950.00
150x7.8	201150-7815	\$785.00
Guard	201150-7805	\$375.00
50x7.8		
300x4.6	201150-4630	\$950.00
250x4.6	201150-4625	\$850.00
150x4.6	201150-4615	\$735.00
Guard	201150-4605	\$365.00
50x4.6		

Nanofilm SEC-250 (5 μm , 250 \AA)

Length x ID (mm x mm)	P/N	Price
300x21.2	201250-21230	\$3150.00
250x21.2	201250-21225	\$2790.00
150x21.2	201250-21215	\$2150.00
100x21.2	201250-21210	\$1550.00
Guard	201250-21207	\$895.00
75x21.2		
300x10	201250-10030	\$1950.00
250x10	201250-10025	\$1750.00
150x10	201250-10015	\$1350.00
100x10	201250-10010	\$1150.00
Guard	201250-10007	\$695.00
75x10		
300x7.8	201250-7830	\$1095.00
250x7.8	201250-7825	\$950.00
150x7.8	201250-7815	\$785.00
Guard	201250-7805	\$375.00
50x7.8		
300x4.6	201250-4630	\$950.00
250x4.6	201250-4625	\$850.00
150x4.6	201250-4615	\$735.00
Guard	201250-4605	\$365.00
50x4.6		

Ordering Information

Nanofilm SEC-500 (5 μm , 450 Å)

Length x ID (mm x mm)	P/N	Price
350x21.2	201500-21230	\$3650.00
250x21.2	201500-21225	\$3450.00
150x21.2	201500-21215	\$2450.00
100x21.2	201500-21210	\$1750.00
Guard	201500-21207	\$895.00
75x21.2		
300x10	201500-10030	\$2150.00
250x10	201500-10025	\$1850.00
150x10	201500-10015	\$1350.00
100x10	201500-10010	\$1150.00
Guard	201500-10007	\$695.00
75x10		
300x7.8	201500-7830	\$1150.00
250x7.8	201500-7825	\$1050.00
150x7.8	201500-7815	\$875.00
Guard	201500-7805	\$375.00
50x7.8		
300x4.6	201500-4630	\$950.00
250x4.6	201500-4625	\$850.00
150x4.6	201500-4615	\$735.00
Guard	201500-4605	\$365.00
50x4.6		

Nanofilm SEC-1000 (5 μm , 950 Å)

Length x ID (mm x mm)	P/N	Price
300x21.2	201950-21230	\$3650.00
250x21.2	201950-21225	\$3450.00
150x21.2	201950-21215	\$2450.00
100x21.2	201950-21210	\$1750.00
Guard	201950-21207	\$895.00
75x21.2		
300x10	201950-10030	\$2150.00
250x10	201950-10025	\$1850.00
150x10	201950-10015	\$1350.00
100x10	201950-10010	\$1150.00
Guard	201950-10007	\$695.00
75x10		
300x7.8	201950-7830	\$1150.00
250x7.8	201950-7825	\$1050.00
150x7.8	201950-7815	\$875.00
Guard	201950-7805	\$375.00
50x7.8		
300x4.6	201950-4630	\$950.00
250x4.6	201950-4625	\$850.00
150x4.6	201950-4615	\$735.00
Guard	201950-4605	\$365.00
50x4.6		

* Sepax packs columns with the I.D. from 75 μm to 2 inches and the length from 2 cm to 60 cm. For the products not listed here, please contact Sepax Sales Department.

10 μm Nanofilm SEC Analytical and Preparative Columns

Inquire price

Length x ID (mm)	Nanofilm SEC-150	Nanofilm SEC-250	Nanofilm SEC-500	Nanofilm SEC-1000
300x4.6	203150-4630	203250-4630	203500-4630	203950-4630
50x4.6 (Guard)	203150-4605	203250-4605	203500-4605	203950-4605
300x7.8	203150-7830	203250-7830	203500-7830	203950-7830
50x7.8 (Guard)	203150-7805	203250-7805	203500-7805	203950-7805
300x10	203150-1030	203250-1030	203500-1030	203950-1030
75x10 (Guard)	203150-10007	203250-10007	203500-10007	203950-10007
300x21.2	203150-21230	203250-21230	203500-21230	203950-21230
75x21.2 (Guard)	203150-21207	203250-21207	203500-21207	203950-21207

Ordering Information

How to Order

It's fast and easy to order from the Sepax on-line store at

www.sepax-tech.com

Or, contact Sepax sales department

By Phone: (302) 366-1101

By Fax: (302) 366-1151

By e-mail

Lchen@sepax-tech.com

By mail

1 Innovation Way, Suite 303

Delaware Technology Park

Newark, Delaware 19711

USA

Pricing

Prices in this catalog are effective May 1, 2006 and are subject to change without notice.

Discounts

Sepax Technologies offers best discounts determined by the volume of the purchase. Please contact the Sepax Sales Department for your maximum discount.

Opening a Sepax account

Call the Sepax Sales Department and supply your business information, and billing and shipping address to set up a Sepax account. Open account terms are subject to credit approval.

Payment Terms

Terms of payment are net 30 days. Mastercard®, Visa®, and American Express® are accepted. There is no minimum order.

Shipping

If items are damaged in transit, simply follow these instructions:

- If shipment is visibly damaged on arrival, do not accept it until the delivery person has endorsed it with a statement for the extent of damage.
- Notify us immediately of the damaged shipment in order for us to make the appropriate adjustment and/or provide you with return instructions.

Returns

Returns are accepted only with prior authorization. Call Sepax Technical Support to describe the problem that happened. Please provide us with the sales order number, product number, and quantity damaged. Sepax Technical Support will give you instructions for returns. All claims must be made within 15 business days after receipt of product. A 10% charge will be made on cancelled orders or customer order errors.

Warranty

Sepax Technologies warrants its products to be free from manufacturing defects for 90 days after the shipment. Sepax will accept for return or replacement any product which fails to meet the stated specifications. This warranty shall not apply to any defect, failure or damage caused by improper use or improper or inadequate maintenance and care. This warranty is exclusive and no other warranty, whether written or oral is expressed or implied. Sepax specifically disclaims the implied warranties of merchantability and fitness for a particular purpose. Under no circumstance shall Sepax be liable for direct, indirect or consequential damages arising from the use of its products. The maximum liability that Sepax will assume should be no more than the invoice price of the product.