Sepax Technologies, Inc.

Delaware Technology Park
5 Innovation Way, Newark, DE 19711, USA
Tel: (302) 366-1101; Fax: (302) 366-1151
www.sepax-tech.com

Proteomix Ion-Exchange Phases

Column Information

Proteomix ion-exchange columns are specially designed for high resolution, high efficiency and high recovery separations of proteins, oligonucleotides, and peptides. The packing support is composed of a rigid, spherical, highly cross-linked poly(styrene divinylbenzene) (PS/DVB) beads with a choice of particle sizes from 1.7, 3, 5 and 10 µm. 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. On the top of the hydrophilic layer, a densely packed and uniform ion-exchange layer is attached via proprietary chemistry developed at Sepax, leading to high efficiency and high recovery separations for biological molecules.

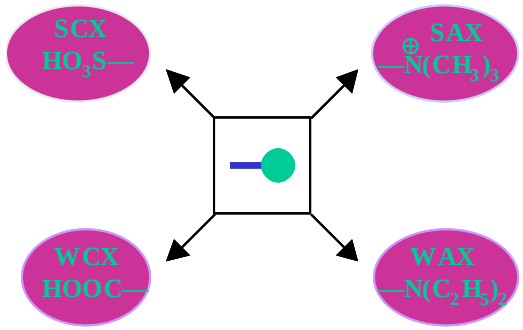

Figure 1. Chemical compositions of *Proteomix* SCX, WCX, SAX, and WAX phases.

As shown in Figure 1, *Proteomix* ion-exchange phases are composed of SCX, WCX, SAX, and WAX. *Proteomix* SCX column is a strong cation exchanger with sulfonate functional groups chemically bonded to the top of the hydrophilic coating. *Proteomix* WCX column is a weak cation exchanger with carboxylate functional groups. *Proteomix* SAX column is a strong anion exchanger with quaternary ammonium functional groups. *Proteomix* WAX column is a weak anion exchanger with tertiary amine functional groups chemically bonded to the hydrophilic coating.

Column Stability and Performance

Proteomix 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 20 mM sodium phosphate buffer at pH 6.0 was used as the mobile phase to run *the Proteomix* SCX and WCX columns, or 20 mM Tris buffer at pH 8.0 was used as the mobile phase to run *the Proteomix* SAX and WAX columns, 1,000 injections or 3 months of usage has negligible deterioration for the columns.

Figure 2 is a typical test chromatogram for separation of three proteins: ribonuclease A, cytochrome C, and lysozyme on a 4.6 x 50 mm, *Proteomix* SCX-NP3 column (3 µm). The efficiency of lysozyme reaches 100,000 plates with a 5 cm long column. Such a high efficiency separation is unprecedented.

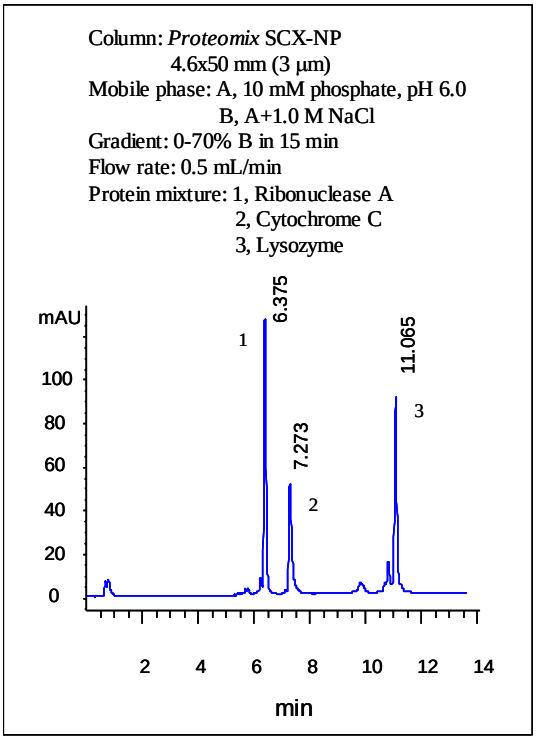

Figure 2. Separation of a protein mixture by a *Proteomix* SCX-NP3 column.

Proteomix SCX-NP, WCX-NP, SAX-NP, and WAX-NP resins are based on a non-porous PS/DVB particle and offer three features. First, the nanometer thick hydrophilic layer eliminates the non-specific interactions with biological analytes. Secondly, non-porous beads minimize biological analytes' lateral diffusion and suppress their diffusion into the chromatographic bed. Thirdly, Sepax's proprietary technology is used to synthesize a uniform and densely packed layer of ion-exchange functional groups. Such uniquely designed IEX phases offer the highest resolution and efficiency separations for proteins, oligonucleotides, carbohydrates, and peptides.

Figure 3 is a typical test chromatogram for a 5 µm, *Proteomix* SAX-NP5 column for separation of a mixture of ovalbumin and

BSA. The high resolution and high selectivity of *Proteomix* SAX-NP5 phase separates well the impurities contained in the ovalbumin mixture, as well as the BSA dimer from BSA.

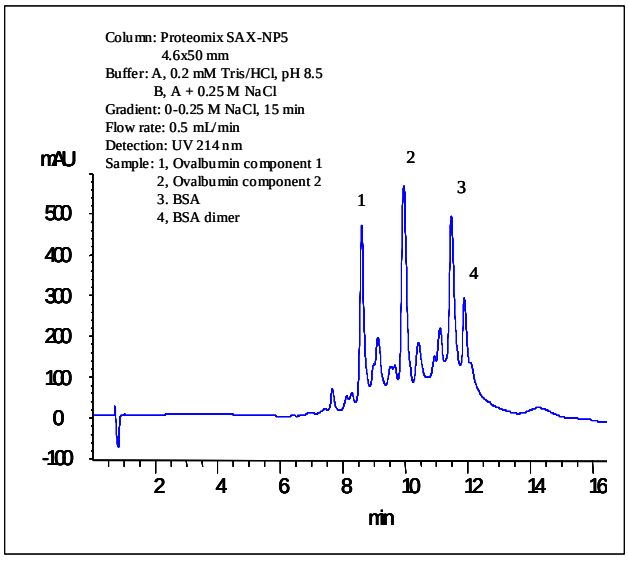

Figure 3. Elution profile of a mixture of ovalbumin and BSA by a Proteomix SAX-NP5 column.

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

Safety Precaution

Proteomix ion-exchange columns are normally operated under high pressure. Loose connections will cause leaking of buffers 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 opening the columns, proper protections should be used to avoid inhalation of the small polymer particles.

Column Installation and Operation

When a column is shipped or not in use, it is always capped at both ends. When installing the column to the system, first remove the end caps. Make the flow direction as marked on the column. Unless a user has a 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 finger tighten the nut.

(c) While continuing to press the tube firmly into the endfitting, use a 1/4" wrench to further tighten.

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

Technical Specifications

Proteomix Phases	SCX-NP1.7, 3, 5, 10 WCX-NP1.7, 3, 5, 10 SAX-NP1.7, 3, 5, 10 WAX-NP1.7, 3, 5, 10
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	1.7, 3, 5, and 10 µm
Pore structure	Non-porous
Dynamic Binding Capacity	~53, 38, and 20 mg/mL for *Proteomix* SCX-NP3, 5, and 10 resins ~19, 15, and 10 mg/mL for *Proteomix* WCX-NP3, 5, and 10 resins ~35, 28, and 17 mg/mL for *Proteomix* SAX-NP3, 5, and 10 resins ~26, 18, and 12 mg/mL for *Proteomix* WAX-NP3, 5, and 10 resins
pH stability	2-12
Operating temperature limit	80 °C
Operating pressure limit	8,000 psi for 3, 5 and 10 µm 10,000 psi for 1.7 µm
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 0.1-1.0 mL/min for a 4.6 mm I.D. 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. It is also strongly recommended to use a pre-column filter (0.5µm frit) or a guard column to protect the column. 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 use an inline degasser or degas the mobile phase prior to use. A simple way for degassing is to sonicate it for 5 minutes under water pumped vacuum.

Proteomix SAX and WAX columns are compatible with nonionic and zwitterionic detergents. ***The Proteomix SAX and WAX columns are incompatible with anionic detergents.*** The Proteomix SCX and WCX columns are compatible with nonionic and zwitterionic detergents. ***The Proteomix SCX and WCX columns are incompatible with cationic detergents.***

Column Care

Shipping solvent New *Proteomix* SAX and WAX columns are shipped in 20 mM Tris at pH 8.0. New *Proteomix* SCX and WCX columns are shipped in 20 mM phosphate buffer at pH 6.0.

First-time use During stocking and shipping, the packing could be dried out. It is recommended that 10-20 column volume of the running buffer be purged to activate the column. Flush the column with your mobile phase with gradual increasing of 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 4.6 x 250 mm column. If the mobile phase or pH is quite different from the stock buffer in the column, it is recommended that the column is washed first with the new mobile phases for 10 column volume.

A typical IEX analytical method usually involves two mobile phases. One is binding buffer with low or zero amount of chaotropic salt, the other is eluting buffer with high concentration of chaotropic salt. Two examples are shown in Figure 2 and 3. Another popular IEX analytical method uses the mobile phases of two different pH. To make the column ready, wash the column with elution buffer till the baseline is stabilized. This typical wash takes 5-10 column volumes. Then equilibrate the column with binding buffer (typically 10-20 column volumes) till a stable baseline is reached, followed by injections of analytical sample.

PH The recommended pH range is from pH 2 to 12. However, it is preferred that the column be used between pH 3 and pH 11 to achieve optimum performance and operation for long lifetime. Extensive usage at very low or high pH will shorten the column lifetime.

Pressure Even though the non-porous *Proteomix* ion-exchange columns can operate at pressure up to 10,000 for 1.7 and 8000 psi for 3, 5 and 10 μm particles, the normal operation is usually under 3,500 psi (5,000 psi for 1.7 μm particles). 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 back pressure. 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 clogged. In this case it is recommended that the column be flushed with reverse flow in an appropriate solvent. It is recommended to wait until the pressure drops to zero to safely disconnect the column from the testing apparatus at the end of the test. Important Note: All PEEK columns cannot be operated beyond 8000 psi.

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.

Storage When not in use for extended time, store the *Proteomix* SAX and WAX columns in 20 mM Tris at pH 8.0/0.1% NaN_3, and the *Proteomix* SCX and WCX columns in 20 mM phosphate buffer at pH 6.0/0.1% NaN_3. Flush the column with the storage buffer for at least 15 column volumes. And then seal both ends with the removable end plugs provided with the column, to prevent the drying of the column bed.

Column clean-up (1) If a pre-column filter or a guard column is used before the separation column, clean the pre-column filter or the guard column first by flushing the filter or the guard column in reverse flow direction using washing solutions for 15-30 min, or replace the filter or the guard column if washing does not improve the column performance. The washing solutions are 150 mM potassium nitrate in 75% acetonitrile at pH 2 (adjusted by HCl) for Proteomix anion-exchange columns and 50 mM phosphate buffer in 1.0 M NaCl at pH 10 for Proteomix cation-exchange columns.

(2) From time to time, some samples could get absorbed onto the inlet frit or the packing material. When the adsorption accumulates to a certain level, it is usually indicated by that the backpressure is increased and the peak becomes broader. When this occurs, it is time to clean your column. The general guidelines for column cleaning are the followings:

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 back pressure. 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. Some general guidelines are recommended for choosing cleaning solutions here. A low pH salt solution will help to remove basic proteins. A high pH salt solution will help to remove acidic proteins. Organics will help to remove hydrophobic proteins. Two cleaning solutions are recommended for general cleaning: 150 mM potassium nitrate in 75% acetonitrile at pH 2 (adjusted pH by HCl) for Proteomix anion-exchange columns and 50 mM phosphate buffer in 1.0 M NaCl at pH 10 for Proteomix cation-exchange columns.

Column Protection

To extend the column life, it is necessary to filter both sample and mobile phases daily with 0.2 μm filters, especially for sub 2 μm IEX columns. To further block the residual particulates in the sample or the mobile phase entering columns: one of the following two additional measures are recommended:

The 1st recommendation is to install a guard column, 2.0 x 10 mm or 4.0 x 10 mm. It will more effectively trap highly adsorptive sample components and residual particulates in the sample, the mobile phase or from the HPLC system. After the use of guard column for certain period of time, it is recommended to back flush the guard column daily with high salt (1 M NaCl) for 10 column volumes.

The 2nd recommendation is to install a precolumn filter with a cut off ≤0.3 μm. It is required to change and replace the filter, once the back pressure is built up or the column performance is decreased. It is recommended to back flush the column with high salt (1 M NaCl) for 10 column volumes, each time the precolumn filter is cleaned or replaced.