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Antibodix Ion-Exchange Phases

Column Information

Antibodix ion-exchange columns are specially designed for high resolution, high efficiency and high recovery separation of antibody proteins. The packing support is composed of a rigid, spherical, highly cross-linked non-porous poly(styrene divinylbenzene) (PS/DVB) bead. The non-porous particles have particle size selection of 1.7, 3, 5 and 10 µm. The PS/DVB particle surface is grafted with a highly hydrophilic, neutral polymer layer with 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 antibody proteins, leading to high efficiency and high recovery separations. On the top of the hydrophilic layer, a weak cation exchanger with carboxylate functional groups is chemically bonded to the top of the hydrophilic coating. A proprietary chemistry was developed to synthesize a densely packed and uniform ion-exchange layer.

Column Stability and Performance

Antibodix ion-exchange columns are based on PS/DVB resin and all the surface coatings are chemically bonded onto PS/DVB support, which allows high stability.

Safety Precaution

Antibodix 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 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 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.

Technical Specifications

Phases	Weak cation exchanger
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
pH stability	2-12
Operating temperature limit	80 °C
Operating pressure limit	8,000 psi for3, 5, 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

(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 further tighten.

(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. It is also strongly recommended to use a pre-column filter (0.5µm frit) or a guard column to protect the column. Antibodix 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 degassor or degas the mobile phase prior to use. A simple way for degassing is to sonicate it for 5 minutes under water pumped vacuum.

Antibodix columns are compatible with nonionic and zwitterionic detergents. ***Antibodix columns are incompatible with cationic detergents.***

Column Care

Shipping Solvent New Antibodix 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 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 10 µm, 4.6x250 mm column. If the mobile phase or pH is quite different from the stock buffer in the column, it is recommended that the column be 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 Antibodix ion-exchange columns can operate at pressure up to 10,000 psi for 1.7 µm and 8,000 for 3, 5 and 10 µm particles, respectively, the normal operation is usually under 8,000 psi for 1.7 µm particles and 5,000 psi for 3, 5 and 10 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 plugged. 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 Antibodix columns in 20 mM phosphate buffer at pH 6.0. 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 50 mM phosphate buffer in 1.0 M NaCl at pH 10.

(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. For general cleaning, 50 mM phosphate buffer in 1.0 M NaCl at pH 10 is recommended.

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 into 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.