Agilent Zorbax Bio Series GF-250

Datasheet

General Description

Zorbax Bio Series GF-250 is a surface-stabilized, hydrophilic gel-filtration column useful for the size separation of biological macromolecules. Proteins are the macromolecules most often used on the GF-250. This column has been specifically designed to provide superior efficiency, pH stability, and operational lifetime when using typical aqueous buffer solutions (pH 3.0-8.5) as the mobile phase.

Size-exclusion chromatography separates compounds on the basis of effective size of the sample molecule, relative to effective pore diameter of the column packing material. Molecular size of the sample molecule is related to its molecular weight. For molecules of a given conformational state (i.e., spherical, coiled, rodlike), retention time is inversely proportional to the logarithm of molecular weight. Very large molecules are totally excluded from the pores of the packing. They have the shortest path through the chromatographic bed, and thus elute first (total exclusion volume). The smallest molecules, which can totally permeate the porous packing, elute last (total permeation volume). Intermediate size molecules permeate only a portion of the available pore volume of the packing, depending on their molecular size, and elute between the total exclusion and total- permeation volumes. For more detailed treatments of size-exclusion chromatography, see References 1-6. Recent reviews of biopolymer size-exclusion chromatography are found in References 7-11.

GF-250 columns are recommended for the size separation of water-soluble macromolecules having molecular weights from 400,000 to 4,000 Daltons (D). The most linear separation range is between 250,000-10,000 D, assuming spherical macromolecules (e.g., globular proteins).

Product Description

GF-250 packing material is based on a wide-pore, small particle-diameter silica support. This base support has been rendered hydrophilic by covalently bonding an organosilane reagent to produce a homogeneous, monolayer surface coverage. Use of appropriate silica particles, combined with a patented zirconium-stabilization process[12] and optimized silane bonding chemistry, gives the packing material superior performance characteristics. This packing is suitable for size-exclusion chromatography of proteins, enzymes, peptides, carbohydrates, nucleic acids, and a variety of other water-soluble macromolecules. The zirconium-stabilized particles are highly resistant to base-catalyzed hydrolysis[13,14]. This proprietary surface-stabilized silica eliminates the requirement for an extended polymeric coating to protect the silica from hydrolytic attack when using higher pH mobile phases. Monolayer surface coating does not reduce the effective pore diameter of porous particles, as is the case with polymeric coating methods. Zorbax silicas are inherently superior in their physical strength because the method of synthesis is based on agglutination of silica sol particles. This high particle strength permits use of high linear velocities (high flow rates) without danger of damage to the particles or disturbing the packed bed.

The use of small-diameter spherical particles in Zorbax GF-250 columns results in high column efficiency (decreased peak volume). Physically stable particles permit highly efficient column packing techniques to be used. GF-250 columns are supplied in a column configuration which represents an appropriate compromise between sample load and cost.

Column Characteristics

Column Packing

• Particle	-Spherical Silica
• Surface Modification	-Zirconium-stabilized
• Bonded Phase	-Hydrophilic molecular mono layer (diol-type)
• Pore Diameter	-150 Å
• Surface Area	-140 m^2/gram
• Particle Diameter	-Nominal 4-4.5 μm

Column Configuration

• Diameter	-4.6 mm, 9.4 mm or 21.2 mm ID
• Length	-250 mm

Column Performance

Column Quality Control

Each column is thoroughly tested prior to shipping. GF-250 packing materials undergo quality analysis with rigorous specifications determined by separating protein mixtures. (See Figure 1 which shows a chromatogram of a typical protein test mixture used to evaluate GF-250 columns.) When calibrating your column, use a freshly prepared protein mixture of commercially available lyophilized proteins. Measure column efficiency using a small-molecule permeation peak (e.g., sodium azide). Prepare samples by dissolving protein and/or sodium azide in mobile phase collected from detector waste effluent when no peaks are eluting. Using this liquid eliminates refractive index disturbances in the chromatogram.

Efficiency

GF-250 packing materials are small-particle spherical silicas which produce a highly efficient size-exclusion column. High efficiency results in better resolution. Therefore, one GF-250 column may provide the needed resolution where two "High-Performance Gel-Filtration" columns were previously required. The columns typically exhibit over 18,000 theoretical plates per column for a small-molecule sample (e.g., sodium azide), when tested at a flow rate of 2mL/min. Actual performance of your column is provided on the COLUMN PERFORMANCE REPORT accompanying the column.

Stability

The stability of GF-250 columns was demonstrated by continuously pumping 0.2 M $(NH_4)_2SO_4$, 0.05 M Tris, 0.005% sodium azide, pH 8.25 ± 0.05, for three weeks, while evaluating protein separation performance of the column in protein separation. As a result of the zirconia stabilization, the GF-250 may be used for short periods at pH 8.5. The lifetime of the column will be reduced; however, the effect is highly dependent upon the sample molecule. The chromatography of many proteins is unchanged, even upon several washes of the column with 0.1 N sodium hydroxide. In contrast, very basic samples are sensitive to any change of the packing surface. The columns are stable to a pH as low as 3.

Speed

At moderate flow rates (1.0 mL/min), a GF-250 column can effect separations in less than 15 minutes. This compares with several hours to several days required for conventional organic-gel-based gel-filtration media. In general, lower flow rates will favor higher resolution for high molecular weight materials. The optimum flow rate for separations will be determined by molecular size of the compound of interest. It is well known that the rate of decrease in performance with increasing flow rates is less for smaller particle-diameter silicas. This effect has been confirmed experimentally with this small-particle-diameter GF-250 packing.[15] The strong physical stability of Zorbax GF-250 columns permits use at flow-rates up to 3 mL/min, which will frequently yield adequate resolution for many samples in a very short time (<5 min).

Recovery

Protein recoveries from GF-250 columns are typically 85-100% of the total material applied to the column. This recovery level is dependent on the sample molecule and the mobile phase in use. Biological activity of macromolecules can be fully retained, once mobile-phase compatibility with the biological substance has been established.

Safety Considerations

The following is a list of important points to keep in mind for safe operation with LC components:

- All points of connection in liquid chromatographic systems are potential sources of leaks. Users of this equipment should be aware of the potential hazards from such leaks due to the toxicity or flammability of the chosen mobile phase.
- Because of its small particle size, dry Zorbax packing is respirable. Columns should only be opened in a well ventilated area.
- These columns have not been approved for use in processing products for human use.
- Sodium azide is toxic and potentially a powerful and sensitive explosive. In the presence of azide, moisture, and carbon dioxide, heavy metals (e.g., copper) are capable of forming a number of basic azides which are explosive. In the presence of acids, azide can produce hydrazoic acid which is also explosive.
- Material Safety Data Sheets will be provided upon request.

Operational Guidelines

Installation

- Remove the protective screw plugs from the column.
- Attach the column inlet to the appropriate port on the injector system. There is an arrow on the column to indicate direction of flow of the mobile phase.
- All components should be coupled as closely as possible. Excess dead volume, mixing volumes, and lengths of tubing should be avoided. The use of low-dead-volume connectors is advised.
- DO NOT overtighten fittings since this can damage the fittings and the 1/16" tubing.
- The column is shipped in aqueous phosphate buffer containing sodium azide. Flush the column with at least 10 column volumes (120 mL) of new buffer before use.
- Check the mobile phase for sample solubility and biocompatibility prior to use. All solvents should also be filtered and degassed prior to use.
- When the column has been completely purged with new mobile phase, connect it to the detector.
- Recommended flow rates are from 0.5-3.0 mL/min, typically, 1.0 mL/min. Resolution is flow dependent,[2,15] therefore, the effect of flow rate on resolution should be examined before finalizing method conditions.
- Zorbax GF-250 columns may be operated at temperatures up to 40°C and at any lower temperature which does not induce the formation of solids in the mobile phase. The system back pressure will increase markedly near the freezing point of the mobile phase, as a result of increasing viscosity.
- Rapid changes in column temperature are best avoided, particularly when mobile phase is not being pumped through the column.

Mobile Phase Selection

The choice of mobile phase is critical for separating biopolymers. Separation efficiency, preservation of biological activity and/or conformation, and column longevity are all important for successful solution of separation problems. Typical buffered solutions used for classical gel filtration of proteins are compatible with the GF-250 column. These include concentrated solutions of protein denaturants (e.g., guanidinium-HCl or urea), mild chemical oxidizing and reducing agents (e.g., DTT or mercaptoethanol), soluble metal ions or complexes, a variety of metal-ion chelating agents, various detergent additives, and aqueous eluents containing a wide range of miscible organic solvent additives. Using certain detergents, such as sodium dodecylsulfate (SDS), may require dedication of the column to specific applications.

All silica-based, and most organic-polymer-based size-exclusion columns, possess a slight negative charge. With silica-based packing materials, this characteristic is mainly due to unreacted silanol groups on the silica surface. For molecules that possess a highly positive overall surface charge (i.e., certain very basic proteins or amino acid homopolymers), non-ideal size-exclusion effects can occur as a result of ion exchange or coulombic interactions of solutes with the packings. GF-250 column packing is synthesized in a manner that reduces the silanol-group interaction potential of its surface. However, certain mobile phase considerations are important. Examples of routinely-used buffered mobile phases are 0.2 M sodium phosphate (pH 7.0), or phosphate buffered saline (0.05 M sodium phosphate, (pH 6.8), 0.13 M sodium chloride, and 0.02 M potassium chloride). Many organic buffers have also been found to be useful, including Bis-Tris, Bicine, Tris, HEPES, and MOPS. Although many macromolecules elute from the GF-250 column as expected when using these mobile phase conditions, sample/packing interactions may still be observed with some molecules. In most cases these interactions may be effectively overcome by increasing the ionic strength of the mobile phase (e.g., 0.3 to 0.6 M sodium phosphate). If it is desirable to work with minimal buffer solution (e.g., 10-50 mM phosphate), 0.2 to 1.0 M sodium chloride, sodium acetate, sodium sulfate, or potassium chloride work well at pH 7.0 to reduce these interactions. As ionic interactions are reduced by increasing the ionic strength of the mobile phase, the possibility of hydrophobic interactions increases. In this respect, however, GF-250 Bio-Series columns have a large operational range of ionic strength (i.e., well-behaved size-exclusion separations up to 600 mM sodium phosphate or 2 M sodium chloride). Hydrophobic effects can be reduced by the addition of methanol (e.g., 5%) to the mobile phase.

To date, no chemical incompatibilities of the GF-250 packing are known to exist within the recommended pH limits. When choosing mobile phase buffers, one should consider both the ability to detect important components relative to the mobile phase background (i.e., spectral properties) and minimization of column deterioration. The zirconium-stabilized silica surface of GF-250 packing is significantly more tolerant of higher pH operation than conventional silica-based column packings. (See "**Stability**" section.)

Buffers with pH values less than 4 should be avoided because of possible alterations in samples of interest. Such alterations may include protein unfolding, a change in aggregation state, precipitation, etc. Such changes to the sample may adversely affect the desired separation.

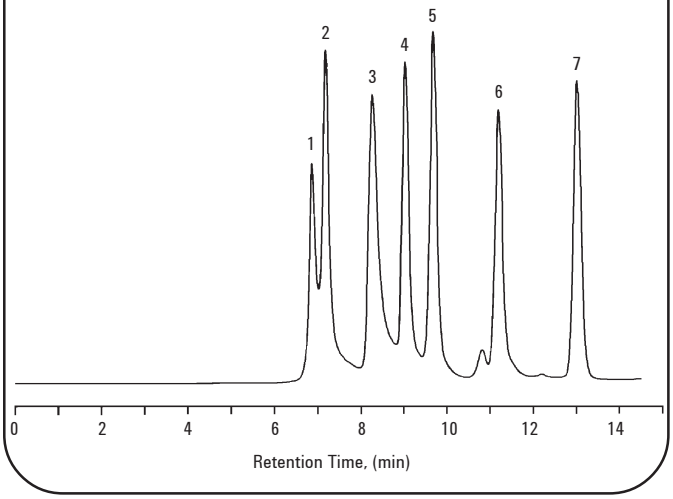
The use of particulate-free samples is recommended to reduce obstruction of flow by plugging of the column frit or packed-bed. Freshly filtered mobile phases are also recommended. An antimicrobial agent such as 0.005% sodium azide may be added to the mobile phase to extend the time between successive preparations.

The GF-250 columns are compatible with most organic solvents. When using mixtures containing organic solvents and aqueous solutions, it is necessary to ensure that all of the mobile-phase components remain soluble. In many cases, salts that are highly soluble in aqueous media are only sparingly soluble in organic solvent mixtures. The final mobile phase should always be checked for insoluble materials before use. Because certain precipitates form very slowly, filtration of the mobile phase does not guarantee the absence of particulates. In cases where formation of precipitates is possible, an in-line filter or guard column may be used to safeguard the analytical column.

FIGURE 1
Zorbax GF-250 QA CHROMATOGRAM

OPERATING CONDITIONS
Column: Zorbax GF-250, 9.4 mm ID x 25 cm (4-4.5 µm)
Mobile Phase: 130 mM NaCl/20 mM Na_2HPO_4, pH 7.0
Flow: 1.0 mL/min
Temperature: Ambient (23°C)
Detector: UV (210 nm)
Peak Identity:

1. Mouse IgM	900,000 D
2. Bovine Thyroglobulin	670,000 D
3. Sweet Potato B-Amylase	200,000 D
4. Bovine Serum Albumin	67,000 D
5. Chicken Albumin	45,000 D
6. Bovine RNAse	13,700 D
7. Azide	65 D



Sample Introduction

- Samples should be free of any particulates. Filter if necessary.
- Samples should be injected using a continuous-flow injection device. Standard loop injectors on most HPLC systems are suitable devices.
- When using a single GF-250 column (9.4 mm x 250 mm), injection volumes of 1-100 µL may be used without any significant alterations of resolution. Injection volumes of 300 µL or greater may be used; however, a major decrease in resolution may be observed.
- Concentrated proteins may be injected directly onto the column as long as major differences in viscosity between the sample and the mobile phase are avoided.
- When injecting reaction mixtures (e.g., enzyme digests), always check for compatibility of the sample and mobile phase, and solubility of the mixture prior to use.

Applications

Typical uses of the Zorbax GF-250 column include:

- Purification of Large Biomolecules
- Monitoring Specific Proteins in a Mixture
- Evaluating Reaction Mixtures
- Screening Commercial Products
- Binding Studies
- Quantitation Studies
- Buffer Exchange
- Protein Identification Assay for Other Purification Steps
- Determination of Molecular Weight or Molecular Volume

Column Care

To protect the column and increase its life, we recommend the use of a guard column inserted between the injector and the column to protect against particulates in the sample. The Reliance cartridge guard column is recommended. This requires a fittings kit.

Halide salts, such as sodium chloride, are corrosive to steel. If it is necessary to use such salts, thoroughly flush the HPLC system after use.

If the column becomes plugged, try to clear the blockage by backflushing the column. If this procedure is not successful, replace the column inlet frit using the following procedure:

- Remove the column from the HPLC system and carefully open the inlet fitting, using care not to disturb the packed bed.
- Inspect the frit and top of the packed bed for discoloration. If there is a void in the packed bed, do not continue to use the column.
- Replace the inlet frit and reassemble.

If the packing is significantly discolored, back-flush the column (after reassembly) with 30% isopropanol; 30% isopropanol with 1mM EDTA (pH 4.5); or 50% acetonitrile, containing 0.05% TFA. This last cleaning procedure is quite stringent and may restore column performance. If not, you can assume that the column has been compromised beyond repair.

Storage Recommendations

- When the column is in frequent use, it is not necessary to flush out the mobile phase daily; although, care should be taken to avoid potential bacterial growth. If the column will not be used for several days, it is advised that the mobile phase be flushed and replaced with one containing an antimicrobial agent (e.g., sodium azide).
- Do not store the column in a mobile phase which contains halides.
- Storage in 100% methanol or ethanol is not necessary or recommended. A recommended long-term storage liquid is 0.1 M sodium phosphate, pH 7.0, containing an antimicrobial agent (0.005% sodium azide).
- Remove the column from the HPLC instrument and seal the ends tightly with the protective screw fittings used during shipping.
- Columns can be stored at room temperature.

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