Bio-Beads® S-X Beads
Gel Permeation Chromatography
Instruction Manual

BIO-RAD

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Introduction

Bio-Beads S-X beads are a series of porous crosslinked polystyrene polymers used for gel permeation separations of lipophilic polymers and low molecular weight, hydrophobic materials in the presence of organic solvents. These non-aqueous spherical beads are used in much the same way aqueous gels are used, except that they are swollen with organic solvents during the separation.

Technical Description

Bio-Beads S-X beads are neutral, porous styrene divinylbenzene copolymer beads. The beads in the Bio-Beads S-X series have exclusion limits from 400 to 14,000 daltons. This range makes them particularly suitable for the fractionation and separation of low molecular weight organic polymers and other hydrophobic substances. The amount of divinylbenzene crosslinkage determines the pore size, and hence the molecular weight exclusion limit of a particular gel in this series. The beads are available with crosslinkages from 1-12%. Pore dimensions and exclusion limits are also influenced by the eluant employed; maximal expansion

of the matrix is achieved with relatively nonpolar, aromatic solvents. The beads are typically used with benzene, toluene, xylene, carbon tetrachloride, and mixtures of solvents.

Mechanism

Gel filtration, also called gel permeation, is the mode of separation which occurs with Bio-Beads S-X beads. Large compounds, greater than the molecular exclusion limit, pass through the column unhindered, whereas small compounds, within the molecular weight operating range, will be retained in the column. The small compounds permeate the pores of the Bio-Beads S-X beads, and thus they take longer to pass through the column. This mechanism requires an eluant which is mobile, and, therefore, Bio-Beads S-X beads must always be used in a column mode.

Instructions for Use

Bio-Beads S-X beads are supplied dry, and must be swollen prior to packing into a chromatographic column. The general instructions are:

1. Swell the beads.
2. Assemble the column.
3. Pour the beads into the column.
4. Add the sample and proceed with the separation.

The instructions below describe swelling the beads and packing the column in details.

Swelling the Beads

Before use, swell the Bio-Beads S-X beads in an organic solvent, such as:

Aromatics	Methylene chloride
Benzene	Orthodichlorobenzene
Carbon tetrachloride	Perchloroethylene
Dimethylformamide	Tetrahydrofuran
Ketones	Trichlorobenzene

These organic solvents allow maximal swelling of the beads. If polar solvents, such as water or methanol, are used,

the Bio-Beads S-X beads will not swell, and the pore size will be minimal. The chosen solvent should be the one used for the separation, and the same as the solvent in which the sample is dissolved.

The solvents should be of highest quality available, and preferably redistilled if non-volatile matter is present in them. Some solvents, e.g. tetrahydrofuran, develop peroxides on standing in contact with air. Solvents should generally be degassed prior to use and protected from atmospheric contamination, to prevent later outgassing during the chromatographic run.

Bio-Beads S-X1 beads will swell considerably, and should be placed in at least six times the resin weight of solvent (w/w). The higher crosslinked resins will not swell as much, so they will not require as much elution solvent. The swelling should always be done in the presence of excess solvent to prevent the resin from drawing up all the solvent, and possibly not swelling fully. The higher crosslinked resins will require more time to become fully swollen.

Bio-Beads S-X12 and S-X8 beads may require swelling overnight, whereas the lower crosslinked resins will be fully swollen in a few hours. Complete swelling is necessary to prevent swelling after packing, which could break the col-

umn. If the amount of swelling is unknown, it can be checked by swelling a known weight of beads and measuring the volume.

After the beads are fully swollen, they are packed into a chromatographic column and washed with the solvent in which they were swollen. Normally, the sample is dissolved and the elution is performed with this same solvent, to prevent swelling or shrinking of the resin during the run. If the beads swell during the run, a glass column may break.

When the beads are used for the first time, low molecular weight polystyrene trapped inside the pores of the beads will tend to cause slow column equilibration. Although swelling the beads in one of the solvents listed above will remove some of the low molecular weight polystyrene, several column volumes of the running buffer may be necessary to reach baseline equilibration.

Packing the Column

Metal columns are often used in gel permeation chromatography, though glass columns offer the important advantages of visibility of packing and therefore are better suited.

Put together a clean column assembly. Place a small amount of elution solvent in the column to prevent bubble formation at the base of the poured column packing. Prepare a solvent reservoir, which should be placed at an elevation higher than the top of the column. The solvent reservoir may be connected to the column by tubing. Any type of connection must be air-tight and clean.

The Bio-Beads S-X beads should be placed in an approximately 50% slurry of beads and elution solvent. A good practice at this point is to degas this mixture by sonicating it under vacuum. Safety precautions should be observed. Double check that the stopcock below the column is closed. Swirl the slurry to get a homogeneous mixture, then slowly and consistently pour the slurry into the column. It is best to pour a long narrow column in sections, in order to obtain a uniform and reproducible column. A glass rod may be used to facilitate pouring the mixture down the side of the column. This will also help eliminate the formation of bubbles. When it is clear that a few centimeters of packed bed have settled, open the stopcock below the column and allow the mobile phase to begin flowing. Begin with a slow flow rate and gradually increase to 10-15% above the final operating flow rate, if possible. The purpose of this elevated flow rate is to pack the column under more pressure than

will be used during the separation. (This is not necessary for Bio-Beads S-X1 beads.) Maintain at least a few centimeters of liquid above the resin bed.

Never allow the packed beads to become dry, because this will cause air pockets and channeling within the bed, resulting in poor efficiency and low resolution. If a small column does go dry, add extra solvent, cap both ends, invert several times until the resin is fully slurried, allow the resin bed to settle, and begin flow as normal. An alternative technique, for larger columns, is to backwash the resin. This will resuspend the beads and allow repacking. This technique should only be used if the packed bed fills less than half the column. Simply connect a piece of tubing to the column outlet, and, at a very slow flow rate, flow the elution solvent into the column. This will resuspend the packing material. Care must be taken that enough space is available for the resuspended material, as well as the additional solvent. When the material is fully suspended, and the air pockets have risen above the packing material, stop the flow. Allow the material to settle.

Note: Bio-Beads S-X beads will float in high density solvents, e.g. chloroform. First swell the media in tetrahydrofuran and pack the column as previously described. Insert an adjustable flow adaptor into the column to constrain the beads in a packed

position. Then, displace the tetrahydrofuran with 3 bed volumes of chloroform.

Flow Rate

Bio-Beads S-X beads have the capacity for different flow rates, depending on the crosslinkage. The 1% and 2% crosslinked resins (Bio-Beads S-X1 beads) are very soft when fully swollen and should only be used in gravity flow procedures. Bio-Beads S-X3 beads can withstand 5 ml/min with a backpressure of 300 psi. Bio-Beads S-X8 and S-X12 beads can withstand up to 5,000 psi backpressure.

Collecting Fractions

Separated compounds are often collected for further analysis. Fractions can be constantly collected, for example in 2 ml increments, or they can be collected specifically when the compound of interest is eluting. In order to know when the compounds are eluting from the column, pass the eluant through a detector. Ultraviolet (UV) detectors are commonly used, though a preparative cell may be necessary so that the flow is not restricted too much. Use the information from the UV chro-

matogram to establish the time periods to use when collecting a specific fraction.

Regeneration

Regeneration of Bio-Beads S-X beads may be necessary if compounds have become trapped within the pores of the resin, for example if a series of eluants has been used. Bio-Beads S-X beads are hydrophobic, and can also absorb compounds. To wash the resin, swell it to its maximum with a solvent such as methylene chloride, toluene, or tetrahydrofuran.

After long periods of storage, small loose polystyrene which had been trapped inside the beads will leach out. This polystryrene (2-10 chain length) has a very high extinction coefficient, so even trace amounts will have a high UV absorption. To eliminate this problem, wash with several bed volumes of solvent.

Chemical Resistance

Bio-Beads S-X beads are highly chemically resistant, though explosive mixtures may form in the presence of strong oxidizing reagents such as chromic acid, nitric acid, and hydrogen peroxide. The beads may be autoclaved.

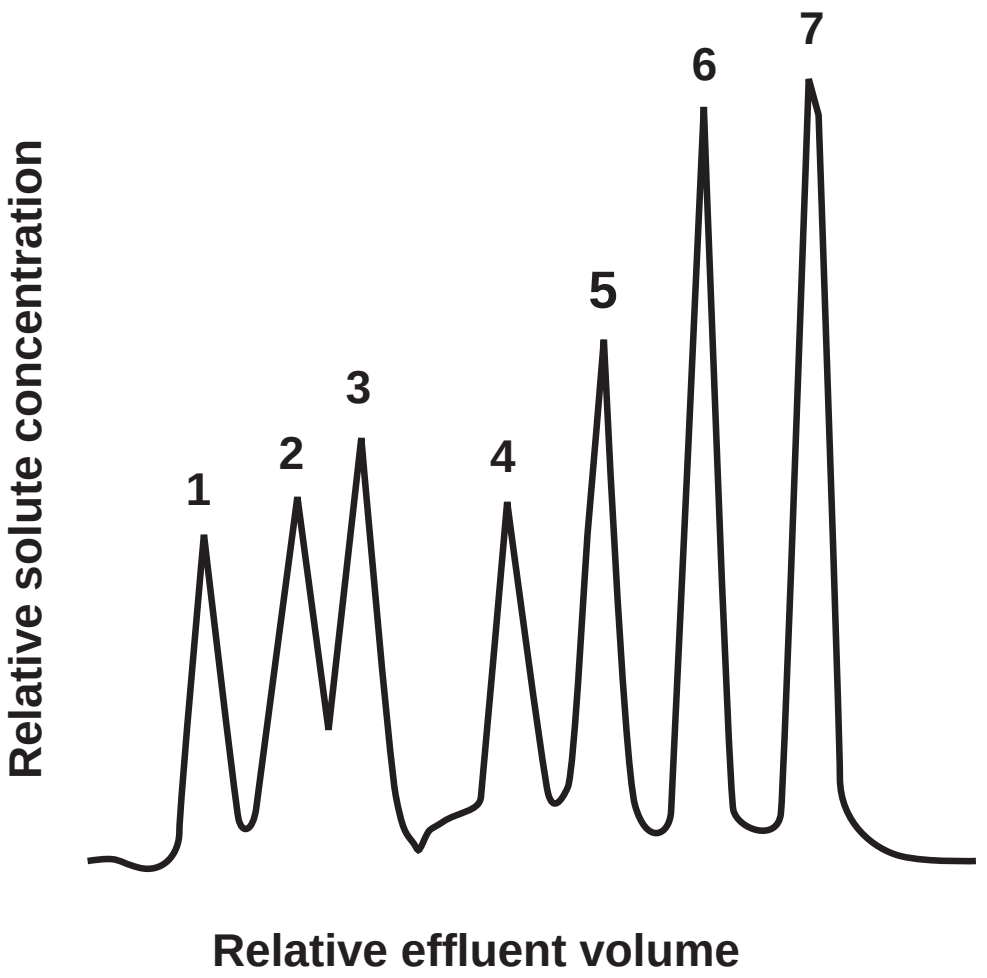
Applications

Harmon reviewed the many different types of gels that are available to the chromatographer, and reported that the relatively soft gels, such as the lower Bio-Beads (particularly S-X1) products swell appreciably in many solvents.[1] Remarkable separations can therefore be achieved at low flow rates.

Many different types of compounds have been separated on Bio-Beads S-X beads. The beads have been used for analysis and quantification of pesticides[2-5] and rodenticides.[6,7] Bio-Beads S-X beads are the basis of the official EPA procedure for the measurement of organic priority pollutants in sludge.[8] The beads are useful for separation of polycyclic aromatic compounds,[9-11] assessment of tissue reaction to biomaterial,[12, 13] and fractionation of halogenated environmental contaminants.[14-17] Other applications include separation of tall oil components,[18] lipids,[19, 20] alkalines,[21] fatty acids,[22] a variety of hydrocarbons,[23] and polystyrenes.[24] These beads can also be used to determine polymer molecular weights and molecular weight distributions.[24-28] They have been used for the separation of low molecular weight trimethylsilylated silicic acid,[29] and for the isolation of low molecular weight polar organics in fatty tissues for subsequent GLC-MA analysis.[30] They have also been used for the

analysis of fish lipid extracts,[31] and for fractionation of food grade poly (vinyl chloride) resin.[32, 33]

A variety of lipophilic polymer substances has been successfully purified on beds of Bio-Beads S-X beads (Figure



1).

Fig. 1. Separation of triglycerides and hydrocarbons on Bio-Beads S-X1 and S-X2 beads in benzene (two beds in series). 1 = tristearin; 2 = trimyristein; 3 = trilaurin; 4 = tri-caprylin; 5 = tricaproin; 6 = hexadecane; 7 = undecane.[21]

Bio-Beads S-X2 and S-X8 beads have been used in multi-column system for separating various components of tall oil, wood resin, and gum resin by gel permeation chromatography.[18] Figure 2 shows the fractionation of tall oil with Bio-Beads S-X beads. All of the injected acid sample was fully recovered, and no delay in elution time was noticed.

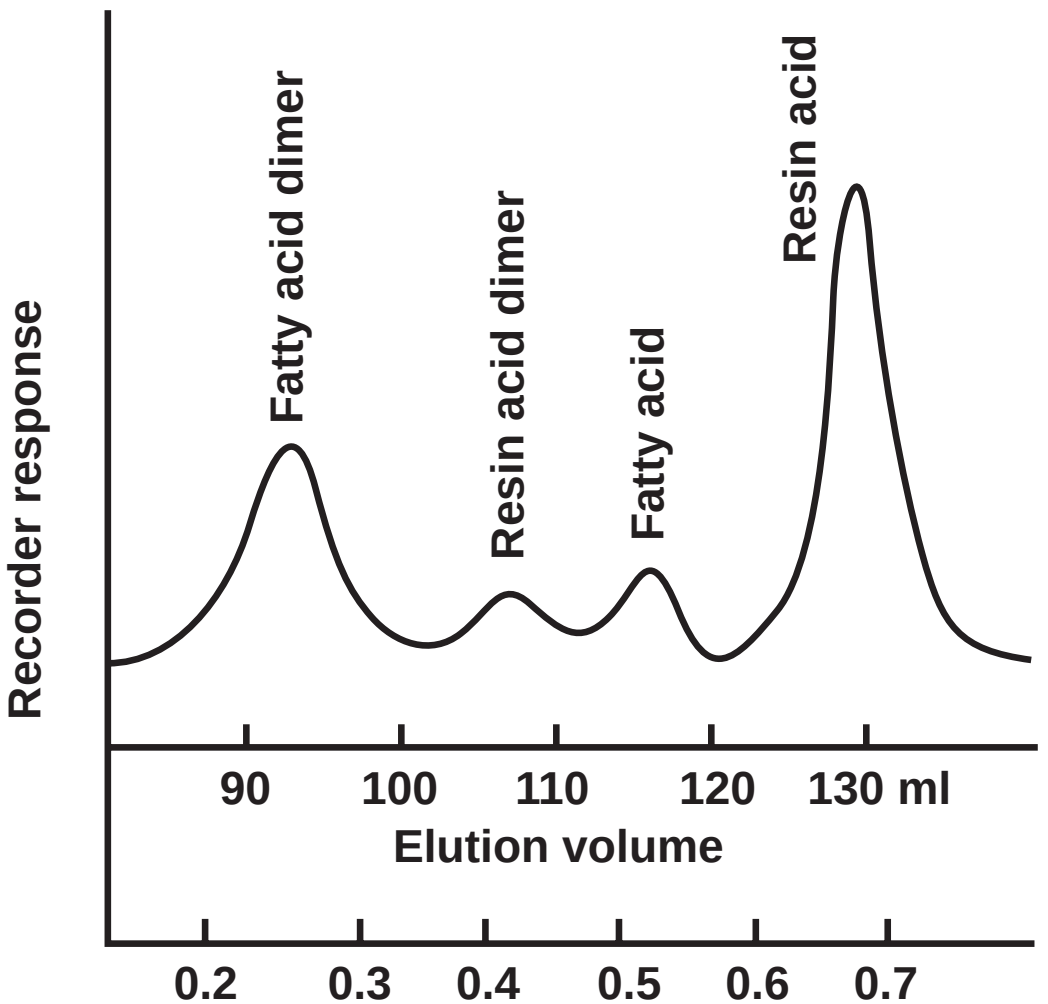


Fig. 2. Gel permeation chromatogram of tall oil.

Ault and Spurgeon used Bio-Beads S-X3 beads for specific separation of chlorinated pesticides form animal fat.[34]

Aitzetmuller has used Bio-Beads S-X beads in benzene to fractionate a 1 g sample of polymeric trioleins. The large pore size of the gel effectively resolved dimeric triglycerides (m.w. ca 1,800) from monomeric triglycerides.[35]

References

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Product Information

Catalog Number	Product Description	Mesh Size	M.W. Exclusion Limit	M.W. Operating Range	Swollen Bed Vol. ml/g Benzene
152-2150	**Bio-Beads S-X1 Beads**, 100 g	200-400	14,000	600-14,000	7.5
152-2151	**Bio-Beads S-X1 Beads**, 1 kg	200-400	14,000	600-14,000	7.5
152-2750	**Bio-Beads S-X3 Beads**, 100 g	200-400	2,000	up to 2,000	4.75
152-3350	**Bio-Beads S-X8 Beads**, 100 g	200-400	1,000	up to 1,000	3.1
152-3650	**Bio-Beads S-X12 Beads**, 100 g	200-400	400	up to 400	2.5

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