

EcoSpheres



Introduction

The EcoSpheres product line from Varian, Inc. is specifically designed for the clean up of environmental samples by GPC (gel permeation chromatography). EcoSpheres are microporous crosslinked polystyrene-divinyl benzene materials that are used to perform size exclusion (gel permeation) separations of low molecular weight, hydrophobic materials from higher molecular weight fats and lipids in the presence of organic solvents.

EcoSpheres are prepared by swelling in an appropriate solvent and gravity packing into a glass column. This user guide describes the preparation of a glass column of EcoSpheres ready for use.

PRODUCT DESCRIPTION

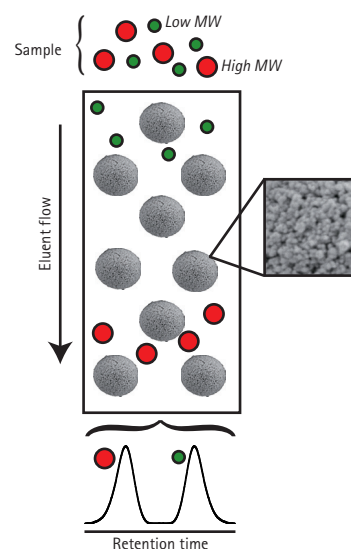
EcoSpheres are spherical beads of a microporous, chemically neutral copolymer of styrene and divinylbenzene of a controlled particle size.

The beads have an effective separation range that is dependent on the solvent used to swell them but is typically of a few thousand g/mol, making them ideal for separation or fractionation of low molecular weight organic species and other hydrophobic materials from high molecular weight sample components.

EcoSpheres beads must be swollen in an appropriate solvent prior to use to generate the pore volume and size. The maximum expansion of the resin and therefore largest pore volume and size may be achieved by swelling the beads in non-polar, aromatic solvents such as toluene or xylene, although they may also be used with other organic solvents such as ethyl acetate, dichloromethane or solvent mixtures.

THE SIZE EXCLUSION (GEL PERMEATION) SEPARATION MECHANISM

EcoSpheres separate materials via a chromatography method known as size exclusion or gel permeation chromatography. This separation mechanism partitions a solute between a mobile flowing liquid, the eluent, and stationary solvent trapped within pores of a packed bed. In this case the swollen EcoSphere beads compose the packed bed. The mechanism is shown schematically in the following figure.



Features of the separation mechanism:

- Mobile phase is a flowing solvent (the eluent)
- Stationary phase is the stagnant eluent trapped in the pores
- Large molecules (above exclusion limit) pass through the column unhindered as they are too big to enter the pores
- Small molecules (within the separation range) are retained in the column by entry into the pores, permeating within the pore structure of the EcoSpheres and as a result taking longer to pass through the column
- Separation is therefore achieved on the basis of size – largest first, smallest last
- The separated fractions may then be collected for further analysis

NOTICE: This document contains references to Varian. Please note that Varian, Inc. is now part of Agilent Technologies. For more information, go to www.agilent.com/chem.

INSTRUCTIONS FOR USE

EcoSpheres material can be supplied as a dry, free-flowing powder. The general preparation and use of the material involves four steps:

1. Swell the beads in a suitable solvent
2. Assemble the column
3. Pack the beads into the column
4. Add the column to a system and perform a separation

Step four concerns the operation of the system you are using for your separations, and is outside the remit of this user guide. The following pages describe steps 1, 2 and 3 in more detail.

SWELLING ECOSPHERES

Prior to use EcoSpheres beads must be swollen in organic solvents to generate the pore volume that will be used to perform the separation. A wide range of organic solvents may be used from aromatics such as toluene and orthodichlorobenzene, chlorinated solvents such as dichloromethane and trichlorobenzene to polar organics such as tetrahydrofuran, ketones, ethyl acetate and dimethylformamide. Highly polar solvents such as methanol or water should not be used with EcoSpheres material as no swelling will occur. EcoSpheres can be considered chemically neutral, non-polar and hydrophobic in nature.

Most organic solvents will give a usable degree of swell for the EcoSpheres material. Only in the case of some solvents where the EcoSpheres are less dense than the solvent (such as dichloromethane) and will float on the liquid surface should another swelling solvent be employed. In this case EcoSpheres material should be swollen and packed (see below) in tetrahydrofuran before transfer into the solvent of choice. If in doubt as to the suitability of a solvent, perform an initial swelling test on a small sample of EcoSpheres prior to working with the rest of the material.

EcoSpheres material will swell by a considerable amount and therefore should be placed in at least 10 times (w/w) the packing material weight of solvent. The swelling should always be performed in an excess of solvent to allow the EcoSpheres material to swell to the maximum extent. The solvents used for swelling, sample preparation and separation should be the highest quality available. Also, solvents should be thoroughly degassed prior to use.

To ensure complete swell EcoSpheres material should be left in excess solvent for a period of around 6 hours. Complete swelling is required to prevent the EcoSpheres material from further swelling during and after the packing procedure, which can be potentially hazardous to the performance of the column. After swelling is complete the EcoSpheres material may be packed into a suitable chromatographic column and washed with the solvent in which they were swollen (this is outlined below).

When swelling and using EcoSpheres for the first time, it is possible that low molecular weight fractions of polystyrene trapped

within the dry beads may wash from the column resulting in slow detector equilibration. Several column volumes of eluent may be required to remove the leachables and reach full detector baseline equilibration.

PACKING IN A COLUMN

Preparative scale 25 mm internal diameter glass or stainless steel GPC columns are best suited to the clean up of environmental samples. Typically a single 300–400 mm column length is employed with at least one adjustable end fitting. The column is packed with EcoSpheres material following the general procedure outlined below.

1. At least 30 g of the EcoSpheres is swelled in 300 mL of the swelling solvent for a minimum of 6 hours.
2. A 300–400 x 25 mm adjustable column is washed in the solvent used for swelling the EcoSpheres material, along with the frit/seal/end fitting assemblies.
3. One end fitting is attached to the column, including the end plug, and the column is supported vertically in a clamp and stand with the plugged end at the bottom.
4. Roughly 5 mL of the swelling solvent is added to the column.
5. The end plug is removed to allow the swelling solvent to slowly drip through the frit and seal at the bottom of the column.
6. EcoSpheres in the swelling solvent are stirred to form a uniform slurry and then poured slowly into the column in 10–20 mL increments, allowing the solvent to drip from the column until the surface of the EcoSpheres material is just visible. A glass rod may also be used to enable the pouring of the slurry.
7. This is to be repeated until the full packed bed is achieved.
8. The top, adjustable, end fitting is attached and a flow of 5 mL/min of the swelling solvent is pumped through the column from bottom to top for ten minutes.
9. The void formed in the bottom of the column is then removed by adjusting the position of the adjustable end fitting, the column is then ready for use.
10. The flow through the column should be from bottom to top to limit the possibility of the column drying through siphoning.
11. During operation of the column any air pockets that form above or below the column bed can be removed by adjusting the adjustable end fittings.

Both metal and glass columns may be utilized with EcoSpheres material. Glass columns offer the user the advantage of visibility of packing.

Note that the column must not be allowed to run dry. If the column does become dry, then the material should be removed from the column, re-swelled and the column re-packed as above.

When using EcoSpheres material with high-density solvents such as dichloromethane the beads may float. If this occurs, first swell the beads in an alternative suitable solvent e.g. tetrahydrofuran and pack the column as previously described. After packing, switch solvent by flushing the column with at least 3 column volumes of desired separation solvent.

PRESSURE AND FLOW RATE

EcoSpheres material can withstand flow rates up to 10 mL/min and back pressures of up to 1 MPa.

The recommended flow rate for EcoSpheres material is between 5 and 10 mL/min.

REGENERATION OF PACKING MATERIAL

EcoSpheres material may require regeneration in the following situations:

1. Compounds have become trapped within the pores of the resin.
2. Absorption of hydrophobic materials onto the surface of the packing material.

Regeneration of the EcoSpheres may be achieved via swelling the beads to the maximum size using solvents such as toluene or tetrahydrofuran, re-packing the column in the swelling solvent and washing the packed bed with at least three column volumes of solvent.

CHEMICAL COMPATIBILITY

The EcoSpheres material is highly chemically resistant. However, exposure to strong oxidizing reagents (e.g. chromic/nitric acid or hydrogen peroxide) should be avoided. The beads may also be autoclaved.

WARRANTY

EcoSpheres beads are covered by a warranty for 60 days following delivery. Varian, Inc., cannot accept liability for deterioration or loss of column performance as a result of improper handling or use.

EcoSpheres

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