

## Ready-to-Use, Sterile, Endotoxin-Free Adsorbent Cartridges for Purifying Biological Preparations

*Sterile, endotoxin-free Porozorb cartridges effectively remove detergents and other nonpolar, hydrophobic materials from biological or food preparations. They are available in sizes appropriate for analytical to process scale purification schemes. Amberlite XAD-4 and Amberlite XAD-16 resins, the specially cleaned adsorbents in Porozorb cartridges, are polymeric, hydrophobic materials that are chemically inert and stable at autoclave temperatures. They are highly effective in adsorbing relatively low molecular weight, water soluble organic compounds from aqueous systems.*

### Key Words

- Porozorb cartridges
- hydrophobic solutes
- surfactants
- Triton X-100

### Convenience

Analysts processing protein or other biological preparations, sterile pharmaceuticals, foods, or beverages must separate wanted products from unwanted process components. Porozorb™ cartridges are sterile, endotoxin-free, clean, ready-to-use adsorbent columns that effectively remove surfactants (e.g., Triton® X-100, Tween®, SDS, CHAPS<sup>▲</sup>, cholate, etc.) or other nonpolar, hydrophobic materials from such preparations. Porozorb cartridges are produced following current Good Manufacturing Practices (cGMP). They are available in four sizes, 250mL to 1500mL, making them useful in analytical scale to process scale purification schemes. Porozorb cartridges ensure consistent bed weight, void volume, and adsorption capacity (Table 1), as well as sterility and absence of endotoxins. Porozorb cartridges are currently available packed with either Amberlite® XAD®-4 or XAD-16 resins, although any of Supelco's large selection of resin and media can be custom prepared in the Porozorb cartridge format. Table 2 presents the characteristics of Porozorb cartridges.

### Proven Technology

Amberlite XAD-4 and Amberlite XAD-16 resins, the adsorbents in Porozorb cartridges, are polymeric, hydrophobic materials that adsorb hydrophobic solutes from polar solvents. They are particularly effective in adsorbing relatively low molecular weight, water soluble organic compounds from aqueous systems. The resins' adsorptive properties are derived from their macroreticular structure, pore size range, and large surface area, and the aromatic nature of the surface. Each bead is an agglomeration of many much smaller microspheres. Adsorbate molecules in the aqueous phase are adsorbed to the microsphere surface through interaction

between the resin surface and hydrophobic regions of the molecules (Figure A). Amberlite XAD-4 and Amberlite XAD-16 resins are chemically inert and stable at autoclave temperatures. If desired, Porozorb cartridges can be regenerated with cleaning agents (e.g., most weak acids and bases, etc.), or autoclaved at 121°C.

### Applications

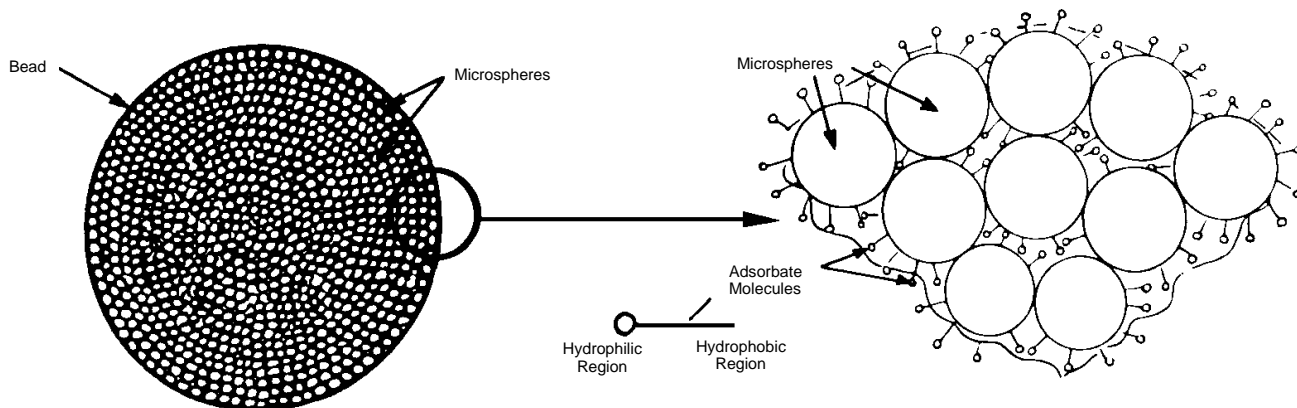
- Vaccine production
- Organics from aqueous food streams
- Removal of surfactants
- Color-producing compounds from industrial effluents
- Recovery, isolation, and purification of water-soluble steroids, amino acids, and polypeptides
- Bitter components from fruit juices
- Decaffeination
- Recovery of tubomycin, antibiotics
- Separation of sodium cilastatin from broths
- Vitamin processing
- Removal and recovery of phenol, chlorinated organics, and pesticides



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<sup>▲</sup>3-(3-cholamidopropyl)dimethylammonio-1-propanesulfonate

**Figure A. Structure of Macroreticular Adsorbent**



85-274, 275

**Table 1. Porozorb Cartridges Ensure Consistent Behavior**

| Porozorb Cartridge      | Bed Vol. (mL) | Void Vol. (mL) | Bed Weight (g) | ID (cm) | Length (cm) | Calculated Saturation Capacity |                  |                    |
|-------------------------|---------------|----------------|----------------|---------|-------------|--------------------------------|------------------|--------------------|
|                         |               |                |                |         |             | g Triton X-100/ Cartridge      | g SDS/ Cartridge | g CHAPS/ Cartridge |
| <b>Amberlite XAD-4</b>  |               |                |                |         |             |                                |                  |                    |
| 254                     | 250           | 70 ± 10        | 100 ± 5        | 8       | 6.55        | 44.0                           | 125              | 78                 |
| 504                     | 500           | 145 ± 10       | 173 ± 5        | 8       | 13.10       | 76.1                           | 216              | 135                |
| 1004                    | 1000          | 296 ± 10       | 370 ± 5        | 8       | 26.20       | 168.8                          | 462              | 288                |
| 1504                    | 1500          | 380 ± 10       | 510 ± 5        | 8       | 39.30       | 224.4                          | 638              | 398                |
| <b>Amberlite XAD-16</b> |               |                |                |         |             |                                |                  |                    |
| 266                     | 250           | 85 ± 10        | 65 ± 5         | 8       | 6.55        | 46.2                           | na               | na                 |
| 516                     | 500           | 150 ± 10       | 138 ± 8        | 8       | 13.10       | 98.0                           | na               | na                 |
| 1016                    | 1000          | 270 ± 10       | 390 ± 5        | 8       | 26.20       | 276.9                          | na               | na                 |
| 1516                    | 1500          | 406 ± 10       | 688 ± 5        | 8       | 39.30       | 376.3                          | na               | na                 |

na = not available

Porozorb cartridges are highly effective for removing surfactants such as Triton X-100 from vaccine preparations. The data summarized in Table 2 show that there is a balance between the breakthrough volume for a detergent and the time required to efficiently clear it from a preparation. This table presents data for Triton X-100, but similar results would be obtained with any surfactant. If you need both a high flow rate and rapid clearing, you can link two or more Porozorb cartridges together in series. Additionally, the resins' mean pore size affects selectivity. Amberlite XAD-4 resin has a mean pore size of 40Å, while Amberlite XAD-16 resin has 100Å pores. As a result, the linear Triton X-100 molecules rapidly migrate into the adsorbing surface of Amberlite XAD-16 resin. However, the larger pores also may allow some biomolecules of interest to adsorb on Amberlite XAD-16 resin.

A Porozorb cartridge's selectivity for a solute, and the extent of adsorption, increases as the hydrophobicity of the solute increases. Organic acids, for example, are generally more strongly adsorbed than are their salts. You can use Porozorb cartridges with high flow rates or large volumes, as in recirculation/regeneration procedures. They can be used to back pressures of 30psi (2.1kg/cm<sup>2</sup>).

**Table 2. Characteristics of Porozorb Cartridges\***

|                       |   |
|-----------------------|---|
| Resin Composition:    | styrene/divinylbenzene, cleaned   |
| Particle Shape/Size:  | spherical, 500µm  |
| Mean Pore Diameter:   | 40Å for Amberlite XAD-4<br>100Å for Amberlite XAD-16  |
| Mean Surface Area:    | 725m <sup>2</sup> /g for Amberlite XAD-4<br>800m <sup>2</sup> /g for Amberlite XAD-16       |
| Cartridge Dimensions: | 6.55 x 8cm (250mL)<br>13.10 x 8cm (500mL)<br>26.20 x 8cm (1000mL)<br>39.30cm x 8cm (1500mL) |
| Nipple Connections:   | 3/16" ID, 1/4" OD   |
| Sanitary Fittings:    | 1"  |
| Shell:                | clear polycarbonate   |
| Retaining Screens:    | stainless steel, 50x250 mesh,<br>highest quality  |
| Gaskets:              | Melrath medical grade   |
| Maximum Pressure:     | 30psi (2.1kg/cm <sup>2</sup> )  |
| Sterility:            | shipped sterile and endotoxin-free  |

\* We can make other Porozorb cartridges on request.

**Table 3. Effect of Flow Rate and Bed Volume on Triton X-100 Removal by Porozorb Cartridges<sup>▼</sup>**

| Bed Volume (mL)         | Flow Rate 3.6L/hr.      |                                     | Flow Rate 10.3L/hr.     |                                      | Flow Rate 22.8L/hr.     |                                      |
|-------------------------|-------------------------|-------------------------------------|-------------------------|--------------------------------------|-------------------------|--------------------------------------|
|                         | Breakthrough Volume (L) | Recirculation Time(hr) <sup>■</sup> | Breakthrough Volume (L) | Recirculation Time (hr) <sup>■</sup> | Breakthrough Volume (L) | Recirculation Time (hr) <sup>■</sup> |
| <b>Amberlite XAD-4</b>  |                         |                                     |                         |                                      |                         |                                      |
| 250                     | 3±1                     | 11 ± 0.5                            | <1                      | 6 ± 0.5                              | —                       | 3 ± 0.5                              |
| 500                     | >25                     | 11 ± 0.5                            | 6.5±1                   | 3.5 ± 0.5                            | <1                      | 2 ± 0.5                              |
| 1000                    | —                       | 9 ± 0.5                             | 50±1                    | 3.5 ± 0.5                            | 1±1                     | 2 ± 0.5                              |
| 1500                    | —                       | 9 ± 0.5                             | —                       | 3.5 ± 0.5                            | >40                     | 2 ± 0.5                              |
| <b>Amberlite XAD-16</b> |                         |                                     |                         |                                      |                         |                                      |
| 250                     | 9±1                     | 8 ± 0.5                             | 2±1                     | 4 ± 0.5                              | 1±1                     | 1.5 ± 0.5                            |
| 500                     | —                       | 8 ± 0.5                             | 43±1                    | 3.5 ± 0.5                            | 17.5±1                  | 1.5 ± 0.5                            |
| 1000                    | —                       | 8 ± 0.5                             | —                       | 3.5 ± 0.5                            | —                       | 1.5 ± 0.5                            |
| 1500                    | —                       | 8 ± 0.5                             | —                       | 3.5 ± 0.5                            | —                       | 1.5 ± 0.5                            |

<sup>▼</sup> Solution of 0.1% Triton X-100 in water.

<sup>■</sup> Time required to reduce detergent in 10 liters of 0.1% Triton X-100 solution to below detection level. Detergent detected by monitoring the absorbance at 275nm, with the lowest detectable limit ( $0.05 = A_{275}$ ) indicating a positive result.

### Tested and Certified

The adsorbent material is cleaned without the use of organics and tested for residues (FCR 21: 173.65) and organics. Each cartridge is packaged separately, and is accompanied by a Certificate of Analysis for the sterility and endotoxicity tests. Sterility testing follows USP XXIII guidelines. Endotoxicity is tested using a validated LAL kit, with any detectable level of endotoxin (0.03EU/mL) indicating a positive test.

#### Trademarks

Amberlite, XAD — Rohm and Haas Company  
 Porozorb — Supelco, Inc.  
 Triton — Union Carbide Corp.  
 Tween — ICI Americas, Inc.

### Ordering Information:

#### Porozorb Cartridges

With nipple connections. Inquire about cartridges with sanitary endfittings.

| Description                   | Cat. No. |
|-------------------------------|----------|
| <b>Amberlite XAD-4 Resin</b>  |          |
| Porozorb 254 (250mL)          | 57500    |
| Porozorb 504 (500mL)          | 57501    |
| Porozorb 1004 (1000mL)        | 57502    |
| Porozorb 1504 (1500mL)        | 57503    |
| <b>Amberlite XAD-16 Resin</b> |          |
| Porozorb 266 (250mL)          | 57504    |
| Porozorb 516 (500mL)          | 57505    |
| Porozorb 1016 (1000mL)        | 57506    |
| Porozorb 1516 (1500mL)        | 57507    |

**NOTE:** Porozorb cartridges are not for clinical or diagnostic use. Sterility and endotoxin-free certification are dependent on and limited to results from a single testing procedure. Liability is limited to replacement of the product. Determination of appropriate cartridge purity for a particular application, and techniques used to achieve this purity, are the purchaser's responsibility. Equilibrate the cartridge with matrix buffer prior to use. Porozorb cartridges contain patented materials.

For more information, or current prices, contact your nearest Supelco subsidiary listed below. To obtain further contact information, visit our website ([www.sigma-aldrich.com](http://www.sigma-aldrich.com)), see the Supelco catalog, or contact Supelco, Bellefonte, PA 16823-0048 USA.

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