Macro-Prep® DEAE Support for Chromatography of Biomolecules

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Introduction

Anion exchange chromatography using DEAE supports is a commonly used chromatographic separation technique for protein purification on all scales. The Macro-Prep DEAE derivative is a weak anion exchange support developed using a macroporous methacrylate polymer (Figure 1). We demonstrate here the exceptional flow properties of the Macro-Prep DEAE support and its ability to maintain its resolving power at flow rates in excess of 1,500 cm/hr. These flow properties, combined with the material's high dynamic binding capacity, make Macro-Prep DEAE support suitable for processing large sample volumes in a short time. Macro-Prep DEAE support is stable in a wide variety of organic solvents, which makes it possible to thoroughly sanitize and regenerate the material. The combination of elution characteristics and chemical and physical stability makes the Macro-Prep DEAE support a valuable tool for purification and production of biomolecules.

Physical Characteristics

The Macro-Prep DEAE support is a weak anion exchange support that is generated by derivatizing a glycidyl methacrylate matrix with diethylamine under carefully controlled conditions; the unreacted epoxide groups are converted to diols to further reduce nonspecific interaction (Figure 2). Its working pH range is roughly 3–8.5 (Figure 3).

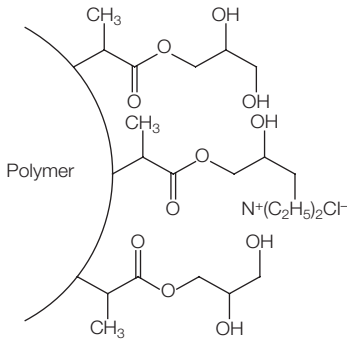

Fig. 1. The Macro-Prep epoxide bead is derivatized with diethylamine to form the Macro-Prep DEAE weak anion exchange support.

The particle size distribution of the Macro-Prep DEAE support is narrow, with a nominal particle size of 50 µm. There are neither fines, which generate unnecessary backpressure, nor oversized particles, which can decrease resolution (Figure 4). Though a 50 µm bead is often perceived to pose potential pressure problems, this is not the case with the Macro-Prep support (Figure 5).

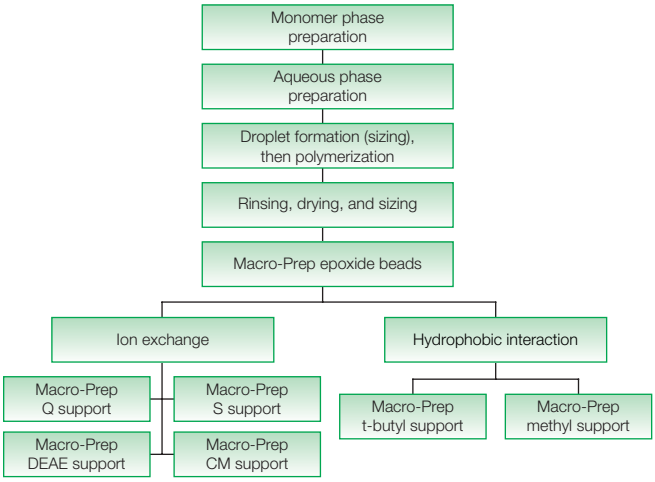

Fig. 2. The epoxide-activated Macro-Prep base bead is prepared from monomer and crosslinked, rinsed, sized, and derivatized with different ligands.

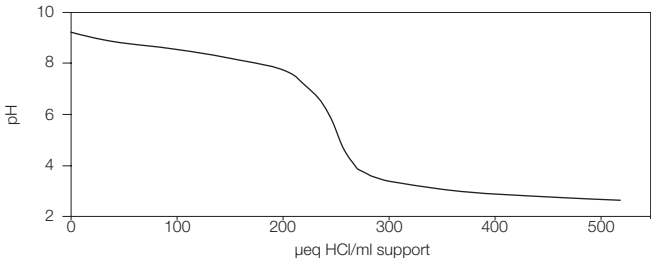

Fig. 3. Titration curve of the Macro-Prep DEAE support.

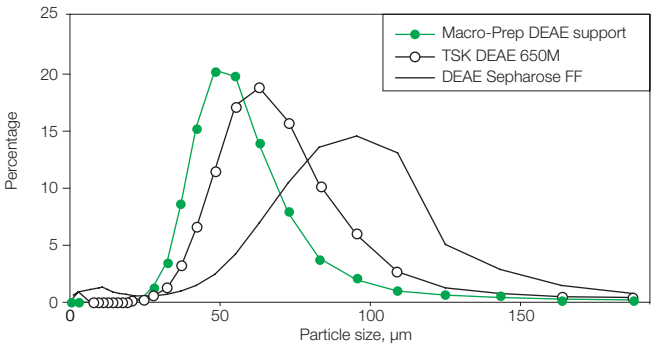

Fig. 4. Particle size distribution of the Macro-Prep DEAE support and two other commercially available DEAE supports.

The porosity of the bead is matched to exact specifications through a precise polymerization process. Regular pores with a narrow size distribution ensure consistent, reliable behavior. The nominal pore size of the bead is 1,000 Å.

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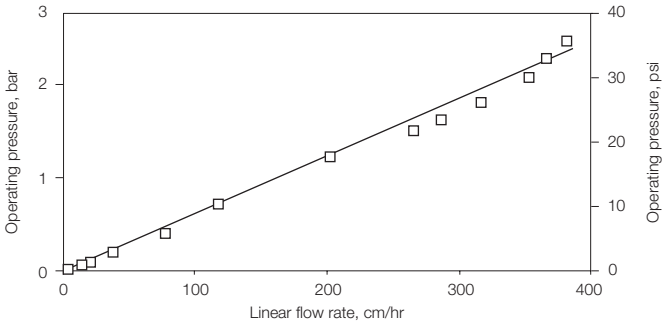

Fig. 5. Operating pressure of a 50 µm Macro-Prep support in a 14 cm ID column.

Support:	Macro-Prep Q support
Column:	Amicon Moduline glass, 14 cm ID, 1/4" plumbing
Bed height:	17.4 cm
Pump:	Cole-Parmer Masterflex pump, size 18 Norprene tubing Easy-Load pumphead

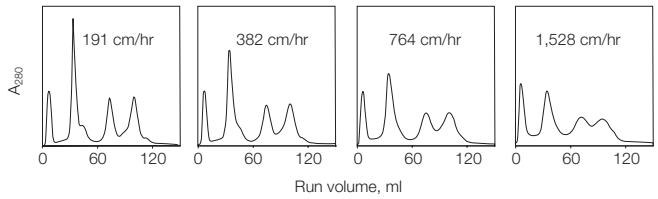

Fig. 6. The effect of flow rate on separation and peak symmetry. A 5 ml sample containing 3.65 mg/ml each myoglobin, conalbumin, BSA, and soybean trypsin inhibitor was loaded onto a column containing 6.56 ml Macro-Prep DEAE support. The sample was loaded in 50 mM Tris-HCl, pH 7.6 (buffer A) and eluted in a gradient of 0–1 M NaCl in buffer A as follows: buffer A for 15 ml, 0–0.35 M NaCl in 85 ml, 0.35–0.65 M NaCl in 15 ml, 0.65–1 M NaCl in 15 ml.

Chromatographic Properties

The Macro-Prep DEAE derivative has high dynamic binding capacity and exhibits consistently high resolution even at high flow rates and high sample loads (Figures 6, 7, and 8). This weak anion exchange support shows higher retention times and better separation properties than other commercially available supports (Figure 9).

Chemical Stability and Sanitization

The Macro-Prep DEAE support is chemically stable to a wide variety of organic solvents, including alcohols, and shrinks and swells very little or not at all with changes of organic solvent.

Information in this tech note was current as of the date of writing (1994) and not necessarily the date this version (Rev C, 2005) was published.

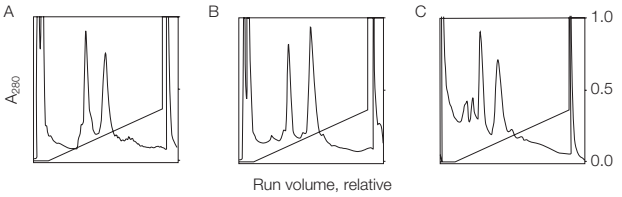

Fig. 7. Scalability of separations on Macro-Prep DEAE support. Separation of a yeast enzyme concentrate (Sigma) under various conditions of scale. A, a 50 mg sample was separated on a 7.4 ml column (10 mm ID) using a flow rate of 78 cm/hr (1 ml/min) in an analytical flow cell; B, a 500 mg sample was separated on a 64 ml column (25 mm ID) using a flow rate of 78 cm/hr (6.4 ml/min) in an analytical flow cell; C, a 1 g sample was separated on a 64 ml column (25 mm ID) using a flow rate of 305 cm/hr (25 ml/min) in a preparative flow cell. For all separations, sample was loaded in 50 mM Tris-HCl, pH 8.3, washed with 1 column volume of the same buffer, and eluted in a linear gradient of 0–0.35 M NaCl in the same buffer for 22.5 column volumes.

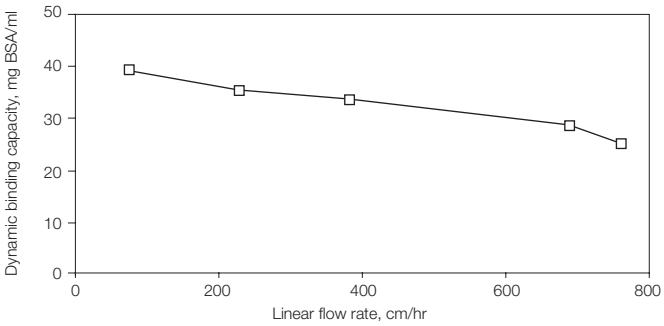

Fig. 8. The effect of flow rate on the dynamic binding capacity of the Macro-Prep DEAE support. A sample containing 3 mg/ml BSA was separated on a 1 x 10 cm (3 ml) Macro-Prep DEAE column. Sample was loaded onto the column in 10 mM Tris-HCl, pH 7.6 (buffer A) and was eluted in 1 M NaCl in buffer A. Sample was applied until the eluting buffer had an A_{280} value 50% of the incoming sample.

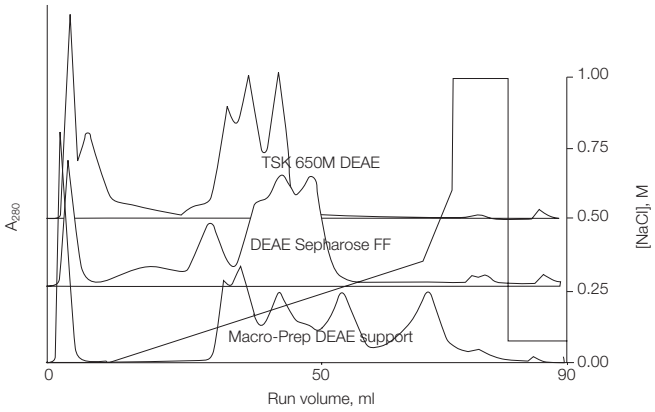

Fig. 9. Sample separation with the Macro-Prep DEAE support and two other commercially available DEAE supports. A 100 µl sample containing 9 mg/ml each of myoglobin, conalbumin, ovalbumin, BSA, and soybean trypsin inhibitor was loaded onto a 0.5 cm ID column containing 2 ml of support. Sample was loaded onto the column in 50 mM Tris-HCl, pH 7.6 (buffer A) and was eluted with 1 M NaCl in buffer A. The flow rate was 153 cm/hr (0.5 ml/min). Scales offset for comparison.

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