Developing and Scaling Up A Purification Procedure With Two New Ion Exchange Supports

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Introduction

A new 10 µm Macro-Prep® support has been developed to complement the 50 µm Macro-Prep chromatography support. The new 10 µm support is based on the same macro-porous methacrylate copolymer in the 50 µm material, albeit with a higher degree of cross linking. The material is available in pre-packed Bio-Scale columns in different dimensions ranging from 2 to 20 ml. The 50 µm Macro-Prep material is available in packages ranging from 100 ml to 10 liter.

The new 10 µm support provides a tool for development of separation methods on an analytical and semi-preparative scale. Methods developed using the Bio-Scale columns can then easily be transferred to production scale, without modifications necessary when going from one type of bead to another.

This poster demonstrates scale up from analytical HPLC (2 ml and 5 ml columns, 10 µm beads) to a 60 ml preparative column utilizing 50 µm beads.

Separation of Rattlesnake Venom

Snake venoms are a rich source of pharmacologically-active substances including proteins and peptides.[1] Figures 1 A to D show the scaling up of a separation of venom from the Diamondback rattlesnake *(Crotalus atrox*).

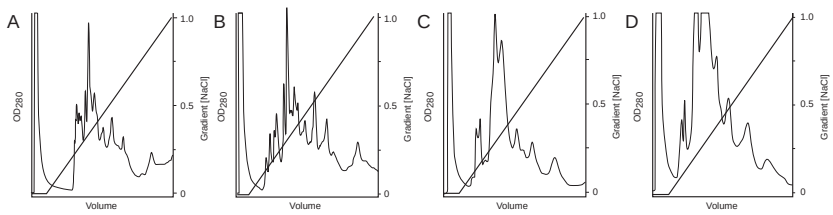
The study was started at 2 mg per ml of support in an analytical 2 ml Bio-Scale S2 column packed with 10 µm material (Figure 1 A), and then step wise transferred to larger columns with increasing sample loads (Figures 1 B and C). The last column, packed with 57 ml of 50 µm Macro-Prep high S support, was used to separate 1 gram of sample (Figure 1 D).

Separation of Yeast Enzyme Extract

Genetically engineered yeast (*Saccharomyces cerevisiae*) is emerging as an important expression system for recombinant proteins.[2] The advantages of yeast over *E. coli* expression systems include the post-translational modifications of proteins. Figure 2 illustrates how a crude extract of enzymes from yeast is separated using Macro-Prep supports.

The study was started at 2 mg per ml of support in an analytical 2 ml Bio-Scale Q2 column (Figure 2 A), and then step wise transferred to larger columns with increasing sample loads (Figure 2 B and C). The last column, packed with 60 ml of the 50 µm Macro-Prep high Q support, was used to separate 1 gram of sample (Figure 2 D).

Fig. 1. Separation of venom from the Diamondback rattlesnake.



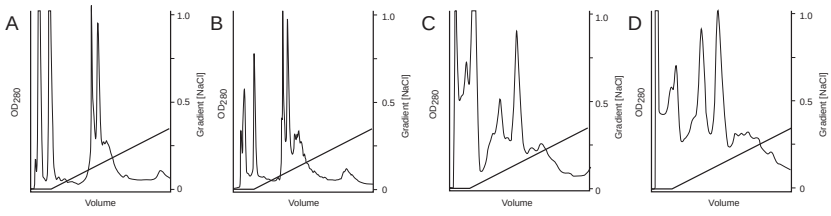
Sample: 4 mg
Column: Bio-Scale S2 column (2 ml of 10 µm Macro-Prep high S support, I.D. 0.7 cm)
Flow Rate: 78 cm/h (0.5 ml/min)
Gradient time: 90 minutes
Gradient volume: 45 ml
Buffer A: 20 mM sodium acetate, pH 4.8
Buffer B: A + 1.0 M NaCl
Gradient:
0% B in 2.5 column volumes
0-100% B in 22.5 column volumes

Sample: 20 mg
Column: Bio-Scale S5 column (5 ml of 10 µm Macro-Prep high S support, I.D. 1.0 cm)
Flow Rate: 78 cm/h (1.02 ml/min)
Gradient time: 112.5 minutes
Gradient volume: 112.5 ml
Buffer A: 20 mM sodium acetate, pH 4.8
Buffer B: A + 1.0 M NaCl
Gradient:
0% B in 2.5 column volumes
0-100% B in 22.5 column volumes

Sample: 105 mg
Column: 20 ml of 50 µm Macro-Prep high S support, I.D. 1.5 cm
Flow Rate: 78 cm/h (2.3 ml/min)
Gradient time: 195.6 min
Gradient volume: 450 ml
Buffer A: 20 mM sodium acetate, pH 4.8
Buffer B: A + 1.0 M NaCl
Gradient:
0% B in 2.5 column volumes
0-100% B in 22.5 column volumes

Sample: 1 gram
Column: 57 ml of 50 µm Macro-Prep high S support, I.D. 2.5 cm.
Flow Rate: 25 ml/min (306 cm/h)
Gradient time: 217 min
Gradient volume: 1,358 min
Buffer A: 20 mM sodium acetate, pH 4.8
Buffer B: A + 1.0 M NaCl
Gradient:
0% B in 2.5 column volumes
0-100% B in 22.5 column volumes

Fig. 2. Separation of yeast extract.



Sample: 4 mg
Column: Bio-Scale Q2 column (2 ml of 10 µm Macro-Prep high Q support, I.D. 0.7 cm)
Flow Rate: 78 cm/h (0.5 ml/min)
Gradient time: 90 min
Gradient volume: 45 ml
Buffer A: 50 mM Tris-HCl, pH 8.3
Buffer B: A + 1.0 M NaCl
Gradient:
0% B in 2.5 column volumes
0-100% B in 22.5 column volumes

Sample: 20 mg
Column: Bio-Scale Q5 column (5 ml of 10 µm Macro-Prep high Q support, I.D. 1.0 cm)
Flow Rate: 78 cm/h (1.02 ml/min)
Gradient time: 112.5 min
Gradient volume: 112.5 ml
Buffer A: 50 mM Tris-HCl, pH 8.3
Buffer B: A + 1.0 M NaCl
Gradient:
0% B in 2.5 column volumes
0-100% B in 22.5 column volumes

Sample: 105 mg
Column: 20 ml of 50 µm Macro-Prep high Q support
Flow Rate: 78 cm/h (2.3 ml/min)
Gradient time: 195.6 min
Gradient volume: 450 ml
Buffer A: 50 mM Tris-HCl, pH 8.3
Buffer B: A + 1.0 M NaCl
Gradient:
0% B in 2.5 column volumes
0-100% B in 22.5 column volumes

Sample: 1 gram
Column: 60 ml of 50 µm Macro-Prep high Q support
Flow Rate: 78 cm/h (6.4 ml/min)
Gradient time: 217 min
Gradient volume: 1,358 ml
Buffer A: 50 mM Tris-HCl, pH 8.3
Buffer B: A + 1.0 M NaCl
Gradient:
0% B in 2.5 column volumes
0-100% B in 22.5 column volumes

Materials and Methods

Both samples were obtained from Sigma Chemical Company (St. Louis, MO). The snake venom (catalog number V-7000) was dissolved in 20 mM sodium acetate, pH 4.8, and filtered prior to application. The yeast extract (catalog number Y-3000) was dissolved in 50 mM Tris-HCl, pH 8.3, and filtered prior to application.

All chemicals were of analytical grade. All buffers were filtered and degassed prior to use.

All separations were performed using a Bio-Rad HPLC system. The $O.D._{280}$ detection was done using a Bio-Dimension® UV/Vis monitor with the standard analytical cell.

Discussion

Developing methods using the high resolution Bio-Scale columns allows rapid development and optimization, using minimal amounts of buffer and sample. Once the method is developed, it is easily transferred to the larger 50 µm Macro-Prep bead.

Through utilizing the same methacrylate copolymer in both the 10 and the 50 µm material, the problems associated with transferring a method from a small synthetic bead to a larger bead which is based on natural polymers, can be avoided.

We believe that the new Bio-Scale columns will provide a valuable tool for process development and analytical chromatographic work.

References

1. Faure, G., *et al., European Journal of Biochemistry,* **214** (2), 491-496 (1993).

2. Manivasakam, P., and Shiestl, R., H., *Nucleic Acids Research,* **21,** 18, 4414-4415 (1993).