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 methacrylamide 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 ranging from 2 ml to 20 ml. The 10 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 scale with an eye towards scaled up production. 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 2 ml to 10 liter.

Separation of Rattlesnake Venom
Snake venoms are a rich source of pharmacologically-active substances including proteins and peptides. 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 Q2 column packed with 57 ml of 50 µm Macro-Prep high S support, I.D. 0.7 cm (Figure 1 A), and then steps were transferred to larger columns. Figure 1 B shows a separation on a 10 ml Bio-Scale Q5 column (10 µm Macro-Prep high S support, I.D. 1.0 cm) with 200 ml of support. 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 (S. cerevisiae) containing a recombinant expression system for recombinant proteins will provide a valuable tool for process development and analytical chromatographic work.

The advantages of yeast over E. coli expression system for recombinant proteins is emerging as an important application. 2

Materials and Methods
Both samples were obtained from Sigma Chemical Company (St. Louis, MO). The crude 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 20 mM sodium acetate, pH 4.8, and filtered prior to application.

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

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Discussion
Developing methods using the high resolution Bio-Scale columns is essential for 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 methacrylamide-co-polymer to build the 10 and 50 µm materials, 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

Fig 1. Separation of venom from the Diamondback rattlesnake.

Fig 2. Separation of yeast extract.