DIONEX

Application Note 88

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Medium-Pressure Gel Filtration Chromatography Using the Dionex DX 500 HPLC System

INTRODUCTION

During the final stages of protein purification, a protein is often characterized by high-pressure and/or medium-pressure liquid chromatography. Whenever high- and medium-pressure applications must be performed on the same system, HPLC systems are usually preferred. HPLC systems are able to operate at both high and medium pressures and they often have more advanced automation capabilities, but some HPLCs can exhibit irreproducible flow at pressures below 3.4 MPa (500 psi). The DX 500 HPLC system, designed for operation at pressures as low as 1.4 MPa (200 psi) and as high as 34 MPa (5000 psi), was used with both medium-pressure and high-pressure gel filtration columns for the separation of five proteins based on their sizes.

EQUIPMENT

Dionex DX 500 HPLC system consisting of:
- GP40 Gradient Pump
- AD20 UV/Visible Absorbance Detector
- LC20 Chromatography Enclosure

Dionex PeakNet Chromatography Workstation

Pharmacia Superose® 12 HR 10/30 Column:
- 10 x 300 mm
- 12% cross-linked agarose
- Separation Range: 1000–300,000 daltons
- Exclusion Limit: approx. 2,000,000 daltons

Zorbax® SE-250 Column:
- 9.4 x 250 mm
- Silica-based
- Separation Range: 4000–400,000 daltons

REAGENTS AND STANDARDS

All reagents and proteins were obtained from (Sigma USA) and used without further purification. The molecular weights (daltons) for the reagents and proteins are listed below.

Bovine Thyroglobulin	660,000
Yeast Alcohol Dehydrogenase	141,000
Bovine Serum Albumin (BSA)	66,000
Bovine Zrythocyte Carbonic Anhydrase	30,000
Turkey Egg White Lysozyme	14,000

CONDITIONS

Columns and Back Pressures:	Pharmacia LKB Superose 12 HR 10/30 Column: 1.2–1.4 MPa (180–200 psi) Zorbax SE-250 Column: 2.7 MPa (390 psi)
Injection Volume:	100 µL
Flow Rate:	0.5 mL/min
Detector:	UV, 280 nm
Buffers:	200 mM Sodium phosphate, pH 7.0

PREPARATION OF SAMPLES AND REAGENTS

Buffer

To make up a 1M stock buffer, 142 g of sodium phosphate (Na_2HPO_4) was dissolved in 1 L of deionized water (Type I reagent grade) and the pH was adjusted to 7.0. The stock buffer was then diluted with deionized water to the different final concentrations.

Protein Standards

For each protein standard, 1 mg of the protein was dissolved into 1 mL of 200 mM sodium phosphate buffer (pH 7.0) to make up a protein stock. Different concentrations of protein standards were then obtained by mixing aliquots of the protein stocks into a 1.5-mL sample tube. Final sample volume was then adjusted to 500 µL by adding the 200 mM sodium phosphate buffer.

RESULTS

Resolution

Figure 1a shows the separations of five proteins on a Superose 12 column using a DX 500 HPLC system. Thyroglobulin, carbonic anhydrase, and lysozyme are baseline resolved, while alcohol dehydrogenase and BSA are 50% resolved. When the Superose 12 column is run by a competitive medium-pressure liquid chromatography system, as shown in Figure 1b, identical resolution is obtained. Clearly, the DX 500 system provides equivalent flow rates when the back pressure is maintained at 1.4 MPa (200 psi).

When the Zorbax SE-250 column is run at the same flow rate on the DX 500 system (see Figure 2), thyroglobulin, carbonic anhydrase, and lysozyme are completely resolved. In addition, alcohol dehydrogenase and BSA are approximately 90% resolved. The Zorbax SE-250 column, operated at 2.7 MPa (390 psi), clearly provides higher resolution and better peak efficiencies than those of the Superose 12 column. Total separation time is also 9 minutes faster than the separation using the DX 500/Superose combination.

Molecular Weight Calibration

Molecular weight calibration curves provide analysts with optimal size separation ranges for gel filtration columns. As shown in Figure 3, the Superose 12 column shows a separation range for proteins with molecular weights from 10 kDal to 600 kDal. The two calibration curves are essentially identical, indicating that the isocratic flow produced by the GP40 gradient pump matches the flow produced by the pump from the competitive medium-pressure LC system, even at back pressures as low as 1.4 MPa (200 psi). The slight offset of the DX 500/Superose 12 calibration curve is due to differences in dead volume created by the different lengths of tubing used.

CONCLUSIONS

The DX 500 HPLC system delivers performance equivalent to a competitive medium-pressure liquid chromatography system when a medium-pressure column such as the Pharmacia Superose 12 is used. The Zorbax SE-250 column, however, provides faster separations than the Superose 12 column for the five proteins tested.

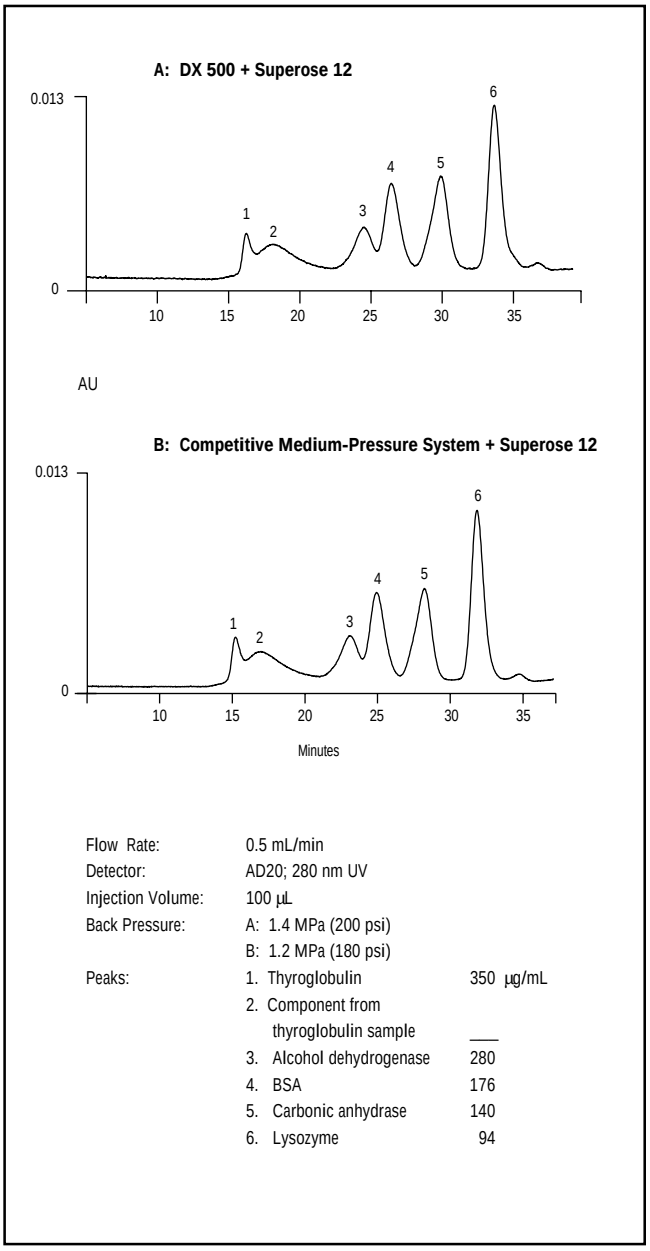

Figure 1 *Separation of five proteins using a DX 500 HPLC system and a competitive medium-pressure system with a Superose 12 column.*

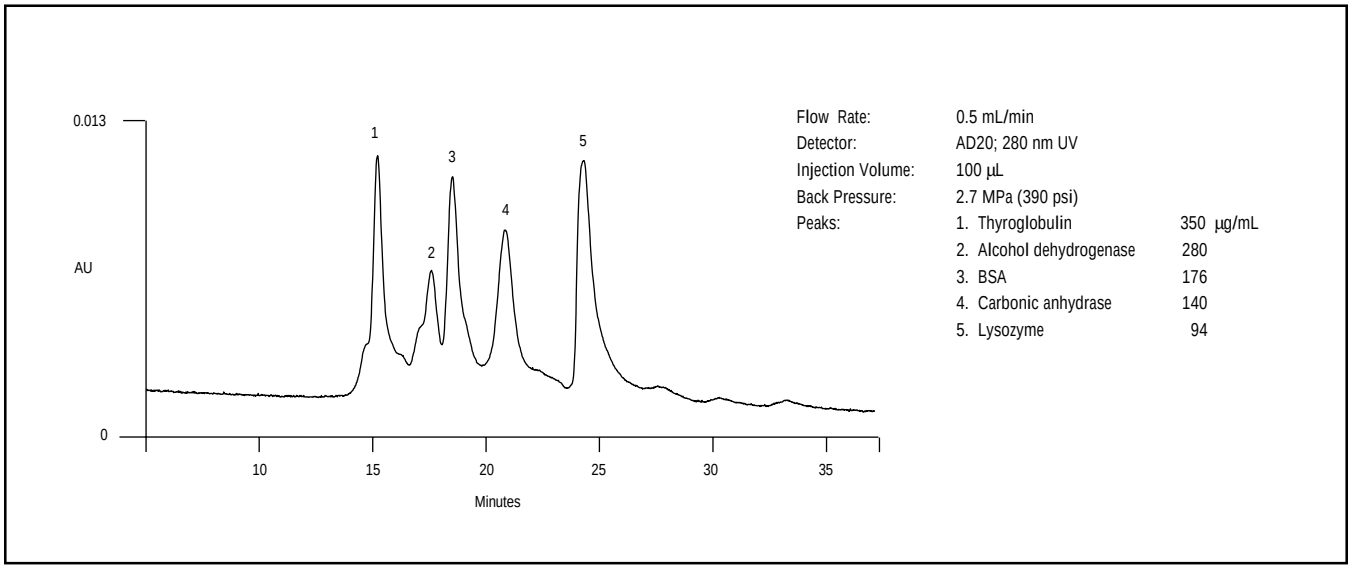

Figure 2 *Separation of five proteins using a DX 500 system and a Zorbax SE-250 column.*

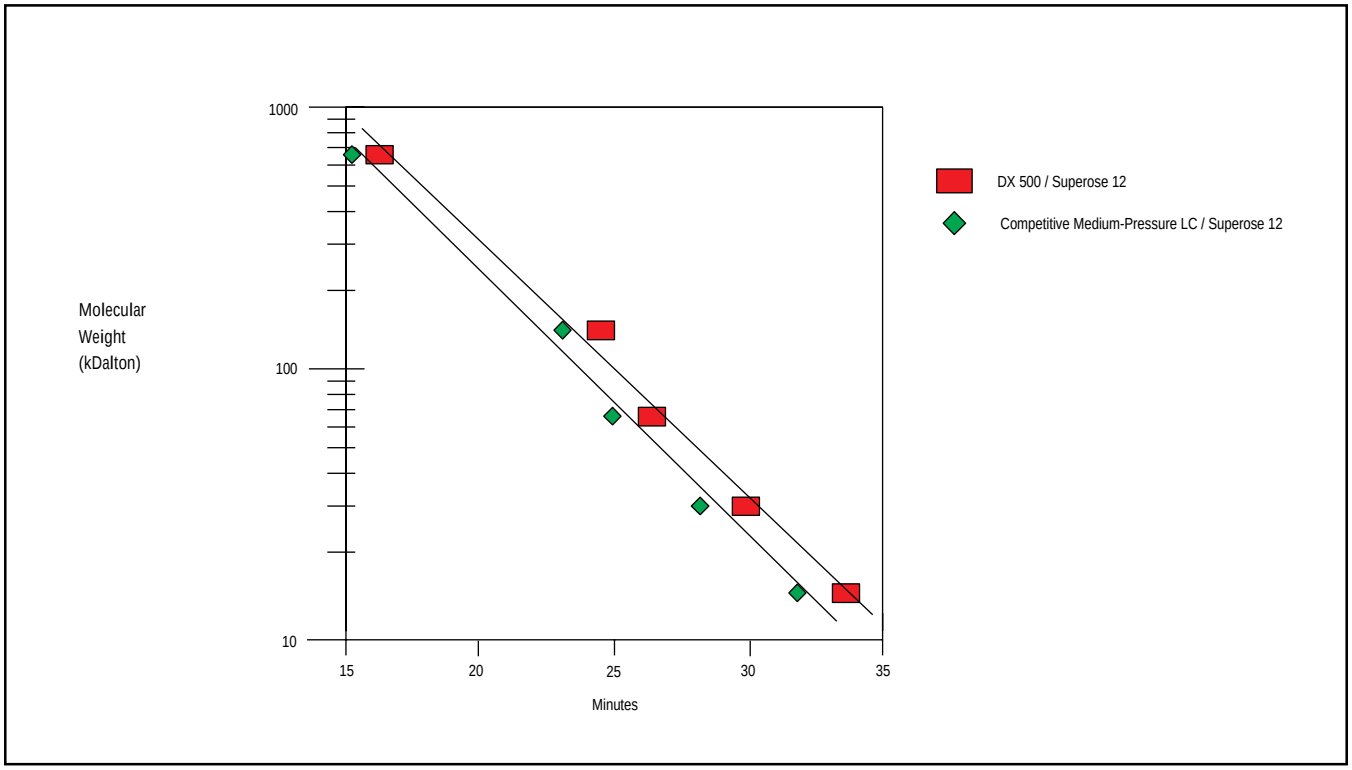

Figure 3 *Calibration curves for a Superose 12 column.*

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