uno

chromatography
BIOCHROMATOGRAPHY COLUMNS

USING THE UNO S1 COLUMN IN THIS SEPARATION SCHEME ALLOWED:

- **PURIFICATION OF CRUDE INSULIN UP TO THREE TIMES FASTER THAN A MONODISPERSED BEADED COLUMN**
- **FAST SEPARATION WITHOUT SACRIFICING RESOLUTION OR PURITY DUE TO THE UNO COLUMN'S UNIQUE CONTINUOUS BED**

Purification of Insulin on UNO™ S1 Column

Insulin is a peptide hormone which stimulates anabolic reactions for carbohydrates, proteins, and fats resulting in lowered blood glucose. It is used therapeutically for treatment of diabetes mellitus and was the first recombinant protein on the market. Although insulin is well characterized and understood, it is often difficult to purify because of the harsh pH and denaturing conditions needed to solubilize and disaggregate the protein. A comparison of the UNO S1 (Bio-Rad catalog # 720-0021) and a popular monodispersed cation exchange column showed that it was advantageous to use the UNO S1 column for small scale insulin separation or analysis.

Experimental

The insulin used in this study was an acid precipitate in suspension. The insulin was solubilized in acidic buffer with urea. The insulin sample was applied separately to an UNO S1 and a monodispersed cation exchange column and eluted by linear gradients. Fractions were collected and neutralized with ammonium hydroxide to pH 7.0. Due to the viscosity of the buffers, both columns were run at half the maximum recommended flow rates. The separation performed on the UNO S1 was run at 3 ml/min (Figure 1). The separation performed on the monodispersed cation exchanger was performed at 1 ml/min (Figure 2). Fractions 43–50 and fractions 30–34 were pooled from the separation performed on the UNO S1 and the monodispersed column respectively and analyzed by reversed phase HPLC. Analysis performed on Hi-Pore® RP-304 reversed phase column (Bio-Rad catalog # 125-0551). The products from both columns were 98% pure. Figure 3 shows the HPLC chromatogram of the pooled fractions from the UNO S1 column after subtraction of the background UV from the buffer.

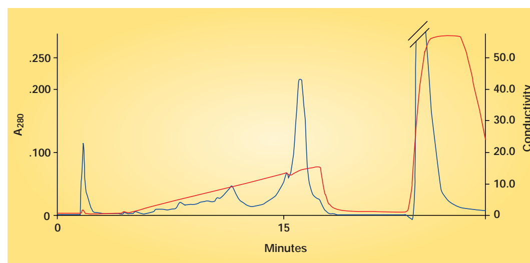

Fig. 1. Chromatogram of insulin purification on UNO S1 column. Buffer A: 0.25 M HOAc, 7.0 M urea. Buffer B: A +1.0 M NaCl. Gradient: 0–30% B over 40 column volumes. Flow rate: 3 ml/min. Sample size: 0.25 ml.

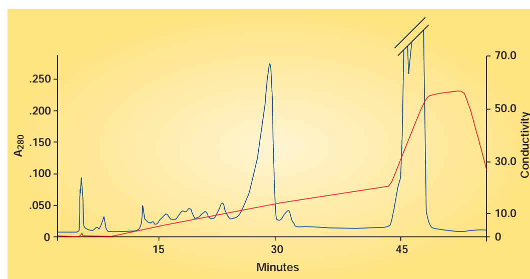

Fig. 2. Chromatogram of insulin purification on monodispersed cation exchange column. Conditions were as in Figure 1 except flow rate was 1 ml/min.

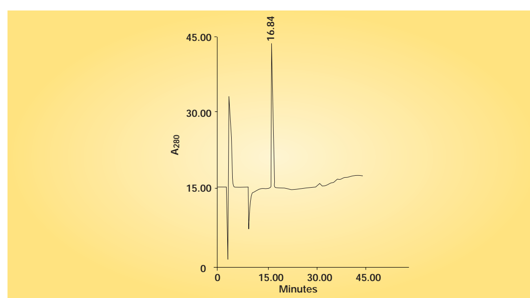

Fig. 3. Chromatogram of HPLC analysis on fractions 43–50 from UNO S1. Buffer A: 0.01% TFA. Buffer B; 95% Acetonitrile 5% buffer A. Flow rate: 1 ml/min.

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