Waters
Lab Highlights
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Rechromatography of Proteins Separated by Ion Exchange

Proteins that have been separated by ion exchange are often rechromatographed on the same column for further purification or for analysis of peak purity and mass recovery. A concern with the reinjection of such samples is that the salt concentration in which the peak is collected may affect the elution behavior and yield poor results.

To investigate this, a protein peak was collected for reinjection and its eluting salt concentration determined. The volume for reinjection was held constant at 1 mL while the salt concentration was varied between 10% and 133% of the salt concentration at which the protein was initially eluted.

Figure 1 shows the initial ion exchange separation of 250 µg of a semi-purified preparation of alpha-lactalbumin on a Protein-Pak™ DEAE 5PW ion exchange column. The peak labeled A was collected in 1 mL and its elution concentration determined to be ~85 mM NaCl.

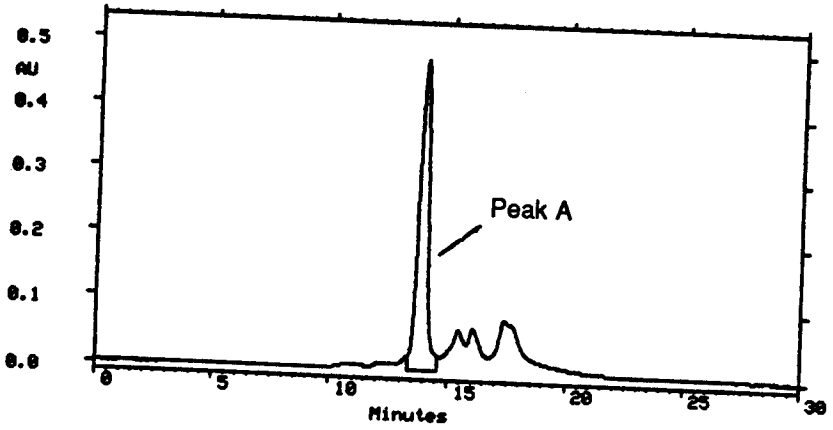

Figure 1. The ion exchange separation of 250µg alpha-lactalbumin on a PROTEIN-PAK DEAE 5PW column. Flow rate 0.8 mL/min of 25mM phosphate buffer pH 7.0. Linear NaCl gradient from 5mM to 210mM in 10 minutes. The peak labeled A was collected between13.30 minutes and 14.55 minutes.

For the reinjection, 100 µl aliquots of the collected fraction were mixed with varying amounts of 0.5 M NaCl and buffer to a total volume of 1 mL. This gave a range of NaCl concentrations for reinjection of the sample. A total of sixteen different dilutions were prepared and analyzed by reinjection onto the Protein-Pak DEAE 5PW column for each sample. The recovery was determined by comparing the area of the peak from the reinjected aliquots with the area of the initial peak.

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Figure 2 shows a 98 ± 6% recovery for reinjected aliquots when the salt concentration in the diluted sample was less than the elution concentration of 85 mM NaCl (dotted vertical line). Dilution in eluents of higher than 85 mM NaCl is clearly inadvisable.

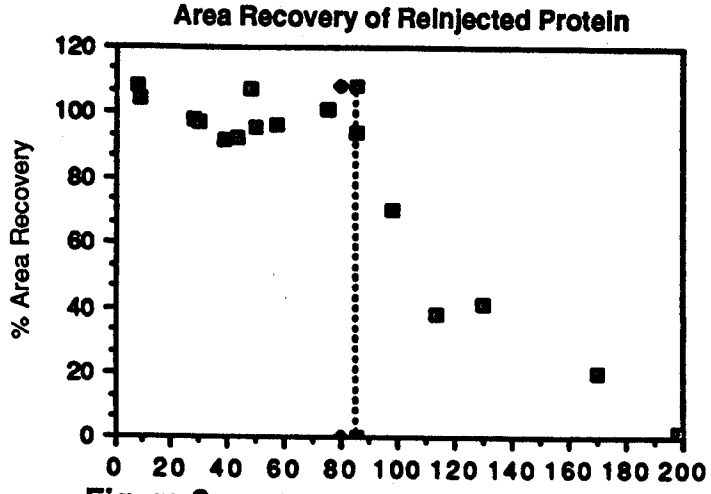

Figure 2.

Actual chromatograms are shown in Figure 3 for dilutions having NaCl concentrations of 8.5 mM, 43 mM, 85 mM, and 113 mM, the peak shape is significantly affected by salt concentration. The peak shape for the 85 mM NaCl reinjection has a large front and a decreased peak height, although the recovery was 94%. The retention time also decreases with increasing salt concentration.

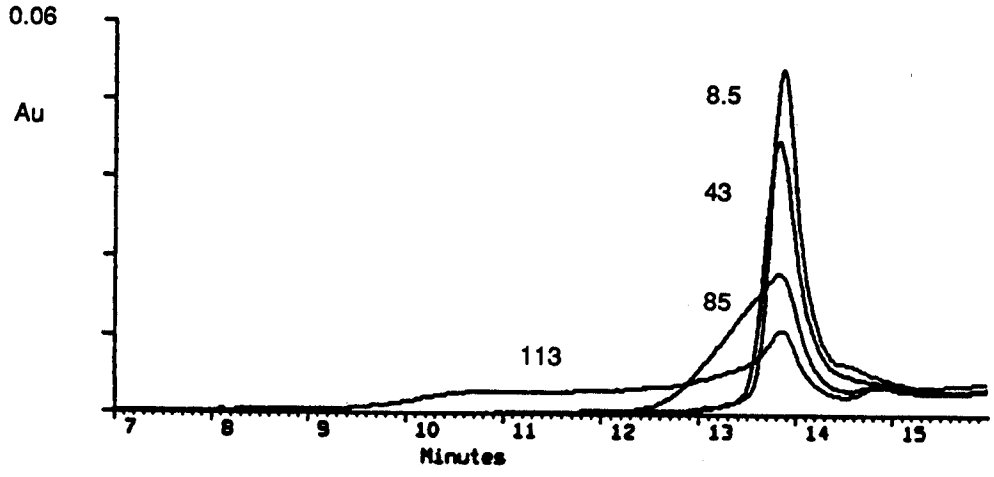

Figure 3. Reinjection of aliquots adjusted to various mM NaCl concentrations. Gradient conditions and column are identical to those in Figure 1.

In summary, for reinjection of samples in ion exchange systems, the peak shape and retention times are affected by the salt concentration of the sample. Nearly quantitative recovery can be achieved if the injected salt concentration is below the elution concentration, but better peak shape and resolution will be obtained if the salt concentration of the collected fraction is diluted prior to reinjection. In this work, a dilution to about 50% of the strength of the eluting solvent was found to be effective.

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