Waters
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Higher Purification on Waters PROTEIN-PAK™ DEAE 5 PW Ion Exchange Column When Loading the Sample in a Specified Salt Concentration

A paper by Alexander Vardanis [1] describes a fortuitous result when a peak fraction from a previous ion exchange separation was directly injected on a Waters PROTEIN-PAK DEAE 5PW ion exchange column. An increase in resolution from the separation resulted when the eluting salt was left in the re-injected sample as compared to the resolution obtained when the sample was de-salted.

The author states that the results are not always predictable. To investigate this effect we conducted two experiments on a protein sample being purified. Increases in resolution were monitored by determining purification factors from the separations. The purification factor is the ratio of the enzymic activity of the fraction versus the enzymic activity of the starting material. Increases in enzyme purifications are accomplished by further removing other proteins from the fraction containing the enzyme being purified.

A Waters PROTEIN-PAK DEAE 5PW ion-exchange column was used to purify glucose-6-phosphate dehydrogenase from a 1 mg sample of crude yeast enzyme concentrate. Chromatographic conditions were as follows: flow rate 1 mL/min., 20 mM Tris buffer pH=8.0, 0.0 to 0.5 M NaCl linear gradient in 20 minutes, and detection at 280 nm.

In the first experiment we varied the salt concentration between 0.0 M and 0.5 M NaCl in a 100 µL sample of yeast enzyme concentrate. The highest purification factor was achieved at 0.38 M NaCl which represented a 50% increase in purification over the sample containing no salt.

We varied the volume from 33 µL to 200 µL in the second experiment while maintaining a salt concentration of 0.25 M NaCl. The results of this experiment showed the purification factor changing little with volumes up to 100 µL, but then decreasing significantly with volumes greater than 100 µL.

TAKE HOME MESSAGE

When using Waters PROTEIN-PAK ion exchange columns, it may be worthwhile to experiment with dilution of the eluting salt concentration or direct loading of fractions from a preceding ion exchange or hydrophobic interaction separation. The benefits may be two-fold; higher purifications, and the time-consuming step of desalting samples can be eliminated.

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Reference

1) A. Vardanis, *Journal of Chromatography* 350 (1985) 299-303

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