Waters
Lab Highlights
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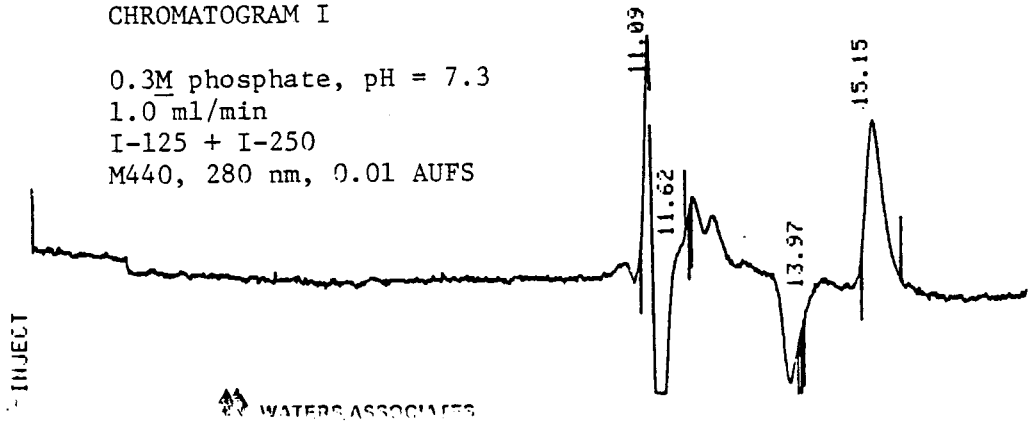
SOLVENT CONSIDERATIONS IN PROTEIN SEPARATIONS

The most frequently used and preferred mode of operation for the Series I Protein Columns is size separation; however, the mobile phase may have profound effects on the separation and the extent of interactions between the protein and the stationary phase. In an attempt to duplicate an earlier separation of nuclear matrix protein on a column set comprised of an I-125 and an I-250 column, Chromatogram I was obtained with a mobile phase of 0.3M phosphate buffer, pH=7.3. The earliest eluting material was barely included in the size separation range of the column set (K_d=1.00 for guanosine, RT=12.1 min, the totally included marker) and material elutes as late as K_d=1.55, RT=15.15 min, definitely a retained peak. Additionally, the back pressure increased, indicating that a portion of the sample did not elute from the column.

When the mobile phase was changed to 0.1M phosphate, pH=6.8, Chromatogram II was obtained. The detector response was greater, no increase in back pressure was observed, and the majority of the peak area was within the size separation range of the column bank. Only a single peak (RT=13.96 min) eluted in a retention mode. If the isoelectric point, and the behavior of the protein in various salt concentrations were known, further refinements in the mobile phase should permit all material to elute in the size mode.

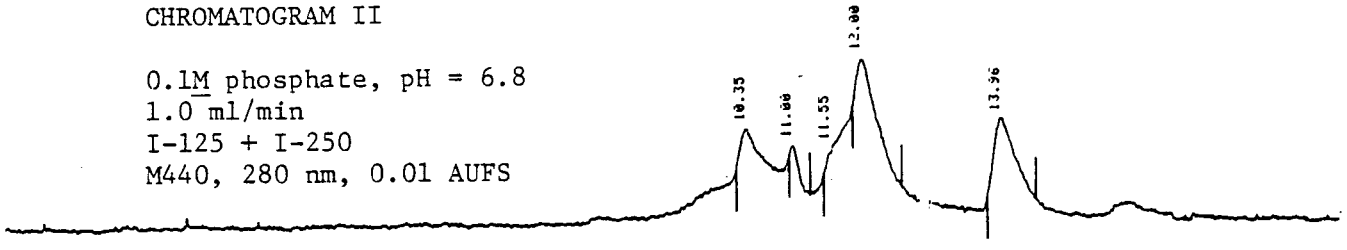
CHROMATOGRAM I

0.3M phosphate, pH = 7.3
1.0 ml/min
I-125 + I-250
M440, 280 nm, 0.01 AUFS



CHROMATOGRAM II

0.1M phosphate, pH = 6.8
1.0 ml/min
I-125 + I-250
M440, 280 nm, 0.01 AUFS



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