Waters
Lab Highlights

An Internal Communicatio
of Applications and Technique

LAH 0296 2/86
AN/LS/MD/PR/EZ

NO. 165 NEW COLUMNS FOR HYDROPHOBIC INTERACTION CHROMATOGRAPHY OF PROTEINS

S. A. COHEN, N. Z. THIMOT, T. L. TARVIN and B. A. BIDLINGMEYER
Waters Chromatography Division of Millipore Corp.,
34 Maple St. , Milford, MA 01757

Along with the increasing popularity of HPLC for protein separations has come a search for columns and conditions that yield high recoveries of both mass and biological activity. Gel filtration chromatography on modified silicas was the first mode of HPLC applied to proteins, but low resolution on these columns limits their utility to crude separations, or cleanup of relatively simple mixtures. More recently high performance ion-exchange columns based on hydrophilic organic polymers have successfully been employed for biopolymer separations. Favorable characteristics include excellent recoveries and good resolution and peak capacity.

There have been numerous attempts to use reverse phase chromatography on alkyl or aryl bonded silica as a complementary technique to ion-exchange chromatography. Best results to date have been achieved on silica supports with mean pore diameters greater than 250Å. However, even though these packings provide very good resolution and often yield good recovery of protein mass, the harsh conditions (low pH, organic solvent concentration greater than 20 percent) required for protein desorption too often result in loss of biological activity.

These observations indicate that commercially available reverse phase columns are too hydrophobic to allow routine recovery of active proteins. Accordingly, we have taken an alternate approach in developing suitable packings for mild hydrophobic interaction chromatography (HIC). The packings are polar bonded phases which exhibit weak hydrophobicity. Retention of proteins is accomplished via loading in high salt buffers (e. g. 1-2 M ammonium sulfate in 0.1 M phosphate) and eluting with a gradient decreasing in salt and/or organic modifier such as ethylene glycol. The most successful of these packings has a modified diol-type ligand on a silica support. Proteins are poorly retained in low salt mobile phase on these columns, but are well retained with high salt loading.

Effects of bonding chemistry and silica characteristics will be examined for a variety of chromatographic properties. A series of small molecule probes has been studied to determine variations in packing hydrophobicity and ionic interactions. Closely related proteins have also been used to determine variations in these properties due to changes in packing synthesis.

These columns exhibit low hydrophobicity and consequently show poor retention of hydrophilic proteins such as cytochrome C, but more hydrophobic proteins show good retention properties yet can be eluted more easily under mild conditions than from reverse phase packings. High recoveries of mass and protein activity are characteristic of the diol column. High resolution separations of serum, snake venoms and closely related proteins differing in only a few amino acids will also be shown.

HIC is particularly well suited to protein purifications involving multiple chromatographic steps by providing a high-resolution orthogonal separation mode to ion-exchange chromatography. Purification of yeast enzymes will be shown demonstrating the utility of HIC in a multi-step purification scheme.