Waters
Lab Highlights
An Internal Communication of Applications and Techniques
LAH 0081 12/82
Doc#M1022
AN/LS,/MD,RS/PR/LP

REVERSED-PHASE PROTEIN SEPARATIONS

The use of reversed-phase HPLC for protein separations has expanded rapidly in the past several years. A common misconception often stated in the literature is that successful separations require the use of the so-called wide-pore (> 300Å) silica based packings. This is not true for protein separations on the μBONDAPAK™ family.

In fact it is possible to use both μBONDAPAK™ Phenyl and μBONDAPAK™ C_{18} columns for the chromatography of proteins up to molecular weight 50,000. Henderson and co-workers[1] have demonstrated the utility of these columns (Figure 1) using a gradient system of 0.05% TFA and acetonitrile.

The μBONDAPAK™ C_{18} column has been used by several groups, especially those of T. H. J. Huisman[2] and W. A. Schroeder[3] for the analysis of hemoglobin chains (MW=16,000) from human blood samples (Figure 2). This difficult separation, in which the hemoglobin subunits can vary by as little as one amino acid, usually employs a mobile phase of acidic phosphate and a mixture of methanol and acetonitrile as the B solvent.

Radial-PAK™ C_{18} (10μm) has also been used successfully in the resolution of C-apolipoproteins (MW=9,000) and A-I apolipoprotein polymorphs (MW=26,000) from human very low density lipoproteins[4].

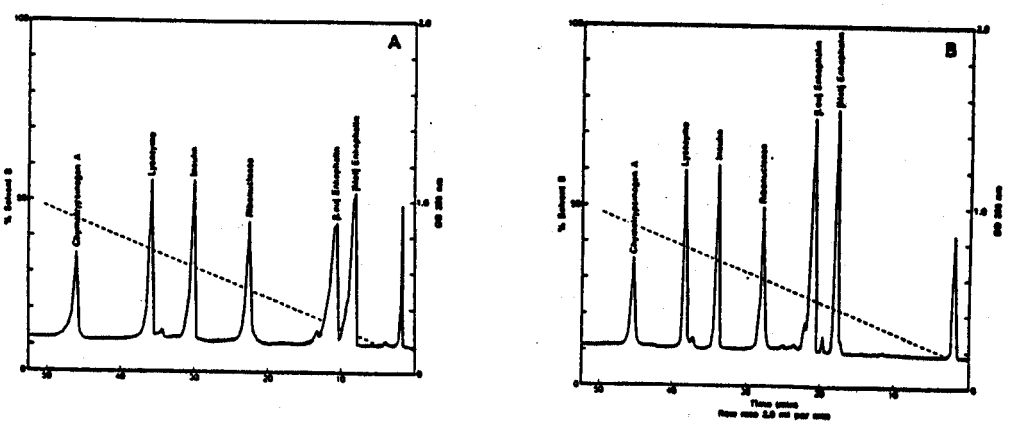

Figure 1. Separation of a mixture of peptides and proteins containing 50 μg of each of the components shown in the diagram on μBONDAPAK™ phenylalkyl support (panel A) and μBONDAPAK™ C_{18} (panel B): Solvent A:0.05% TFA in water. Solvent B: 0.05% TFA in acetonitrile; Gradient: 10% Solvent B to 60% Solvent B over 1.0 hr at a flow rate of 2.0 ml per min.

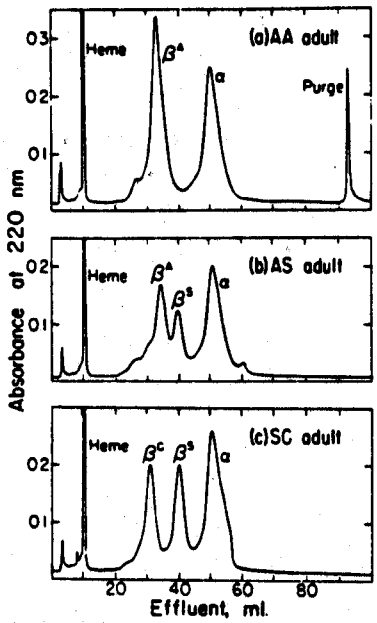

Figure 2. Separation of chains of hemoglobins from adults with AA, AS, and AC hemoglobins on a Waters μBONDAPAK™ C_{18} column. Solvent A was 80:5:15:0.1 (v/v/v/v) 0.15M $NaClO_4$-methanol-acetonitrile-H_3PO_4 and Solvent B was 20:5:75:0.1 ratio of the same components. The gradient ran from 66% to 70% B in 80 min at a flow rate of 1.5 ml per minute.

Steve Cohen

1. L. E. Henderson et al. in Chemical Synthesis and Sequencing of Peptides and Proteins, Liu, Schecter, Heinrikson and Condliffe eds, Elsevier North Holland, Inc. (1981) p 251.
2. T. H. J. Huisman et al., Blood, 57, 75 (1981).
3. J. B. Shelton, J. R. Shelton and W. A. Schroeder, J. Liq. Chromatogr. 4, 1381 (1981).
4. W. S. Hancock and J. T. Sparrow, J. Chromatogr., 206, 71 (1981).