

Waters

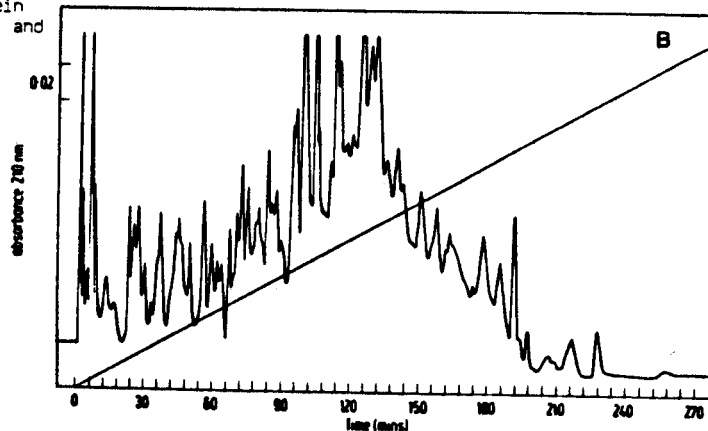
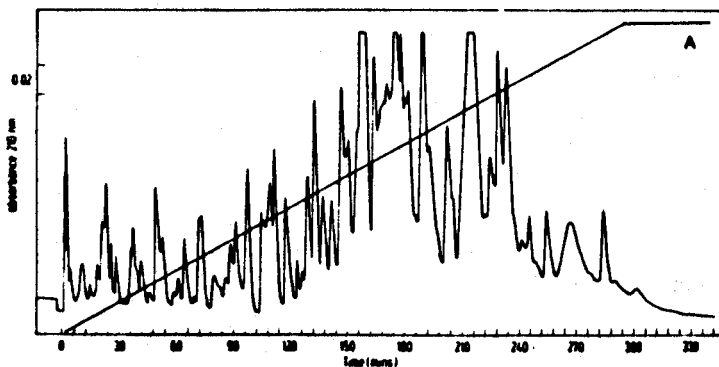
Lab Highlights

RCSS MAKES PEPTIDE SEPARATIONS EASIER

In a previous Lab Highlight (LAH 0090), the popularity of volatile mobile phases for reverse phase peptide separations was discussed. Mostly these eluents have consisted of low concentrations (0.05 - 0.1%) of perfluorinated acids such as trifluoroacetic and heptafluorobutyric, with acetonitrile as the organic modifier. A convenient alternative ionic modifier, according to Professor W. S. Hancock of the Massey University, Palmerston North, New Zealand (1) and Professor M. T. W. Hearn of St. Vincent's School of Medical Research, Melbourne, Australia, (2) is ammonium bicarbonate. Significantly, ammonium bicarbonate buffers are sufficiently volatile to allow salt-free peptide recovery via lyophilization of buffer-acetonitrile mixtures. A potential drawback, though, is the high pH of these buffers, about 7.9, and consequent decrease in column life. Fortunately, this limitation can be overcome through the use of Radial-PAK™ cartridges, which, as we show in an accompanying Lab Highlight (LAH 0183), exhibit at least fourfold longer lifetimes than comparable steel columns in high pH mobile phases. In fact, Hancock and co-workers have used cartridges for more than six months in this alkaline eluent (1).

For example, using a Radial-PAK™ RESOLVE™ C₁₈ cartridge Hearn was able to show (Figure 1) excellent resolution of an extremely complex tryptic digest of thyroglobulin 19S iodoprotein (MW 500,000). They also reported good selectivity and excellent recovery (> 85%) with the ammonium bicarbonate-containing mobile phase.

Column: Radial PAK™ RESOLVE™ C₁₈, 10 micron
Mobile Phase: Eluent A: 100 mM ammonium bicarbonate
Eluent B: 50% CH₃CN, 50% water -
100 mM ammonium bicarbonate
Flow Rate: 1.5 ml/minute
Gradient: 0-100% B in 5 hours
Detection: M450 variable UV detector, set at 210 nm
Sample: 18h tryptic digest of
(A) human thyroglobulin 19S iodoprotein and
(B) guinea pig thyroglobulin 19S iodoprotein
Chromatographic System: 2 X M6000A pumps, M660 gradient controller, and
a U6K injector.



The use of Radial-PAK™ cartridges gave several other advantages for peptide separations. Hearn and co-workers reported, "No significant loss of resolution was noted with the Radial-PAK™ A/C₁₈ (Sic) support, packed into 10 X 0.8 cm cartridges, for sample loadings between one and two orders of magnitude greater than those typical for standard (25 X 0.4 cm) stainless steel HPLC columns." Hancock has also noted that larger than expected capacities (25 mg!) are routinely possible with the Radial-PAK™ C₁₈ cartridges. Column re-equilibration was also much shorter because the Radial-PAK™ cartridge could withstand much higher flow rates than conventional columns.

Cartridges are also available with NOVA-PAK™ C₁₈ packing material (for small peptides) or μBONDAPAK™ C₁₈ packing material (the most popular column for all peptides including cyanogen bromide fragments). With both low and high pH volatile buffers accessible to RCSS users, and three different column chemistries for peptides, even the most complex mixture can be resolved.

1. D. R. Knighton, D. R. K. Harding, J. R. Napier and W. S. Hancock, J. Chromatogr., 249, 193-198 (1982).
2. M. T. W. Hearn, B. Grego and C. A. Bishop, J. Liq. Chromatog., 4 (10), 1725-1744 (1981).