CAPSULES
preliminary notes and applications from Bioanalytical Systems, Inc.

A Reversed Phase Protein Separation on the BAS-200B

Purpose

This capsule shows a separation of a protein mixture on a wide pore reversed phase silica based support. A linear gradient of acetonitrile containing trifluoroacetic acid (TFA), a common mobile phase for peptides and small proteins, was used to separate the components. The proteins ranged in size in molecular weight from 12400 to 66000 Daltons. A BAS-200B liquid chromatograph with ChromGraph® software was used to achieve the separation. A chromatogram was obtained, then the following conditions were altered: gradient slope, gradient time, and organic starting percentage. The chromatographic runs were repeated with the new conditions, which resulted in an optimized separation in shorter time.

Conditions

Instrument: BAS-200B equipped with variable wavelength UV detector and ChromGraph software.
Wavelength: 254 nm
Column: SynChropak RPP (C_{18}), 300Å pore size, 250x4.6 mm, 6 µm particle size.
Gradient: 10%-100%B in 30 min
Flowrate: 1 mL/min
Mobile Phase A: 0.1% TFA in H_2O
Mobile Phase B: 0.05% TFA in 99.5% Acetonitrile/ 0.5% H_2O
Temperature: ambient
Sample loop: 20 µL

Sample:		
	Ribonuclease A	250 µg
	Cytochrome c	20 µg
	Lysozyme	5 µg
	Bovine Serum Albumin	100 µg
	Carbonic Anhydrase	50 µg
	Ovalbumin	100 µg

[Samples were dissolved in H_2O.]

All proteins were obtained from Sigma Chemical Company (St. Louis, MO) under the following product numbers: R-5503, C-2037, L-6876, A-8531, C-3934 and A-7641

Notes

A chromatogram resulting from the above gradient conditions is shown in F1. The gradient time was 30 minutes, the slope of %B per minute was 3, and the starting acetonitrile concentration was 10%. These conditions were favorable for this protein mixture. The sharp, separated peaks suggest good recovery.

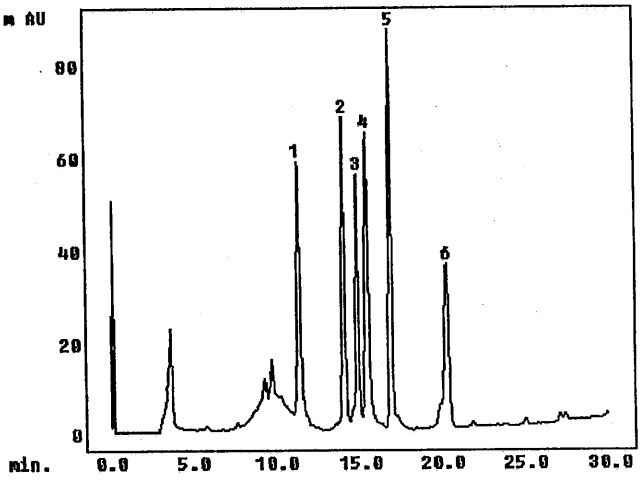

Figure 1. Mobile phase conditions: 10-100%B in 30 minutes. Identified peaks: 1. Ribonuclease A 2. Cytochrome c 3. Lysozyme 4. Bovine Serum Albumin 5. Carbonic Anhydrase 6. Ovalbumin. (Other conditions per text.)

To increase throughput in a laboratory situation it may be necessary to elute the analytes earlier. The BAS-200B chromatographic system was rapidly reprogrammed to a new set of gradient conditions. Data from the new chromatographic run is depicted in F2. In this case the gradient time was 20 minutes instead of 30, and the starting condition was 25% acetonitrile instead of 10%. The slope, therefore, was 3.75%B per minute. The proteins eluted earlier but some of the components were not resolved.

[continued on back]

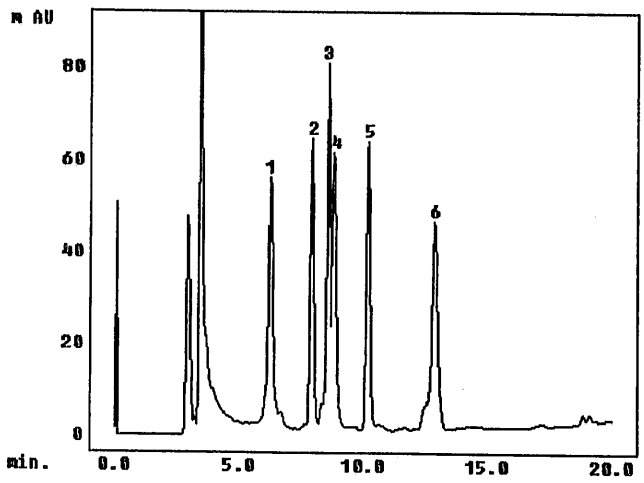

Figure 2. Mobile phase conditions: 25-100%B in 20 minutes. (Other conditions per text.)

To increase the resolution between the coeluted components, it is necessary to make the slope more shallow while maintaining the other parameters. Therefore, the linear gradient was changed to 25%-85%B in 20 minutes, allowing for the same starting conditions but a slope of 3%B per minute. The resulting chromatogram is shown in F3. The peaks are now resolved. The higher organic starting

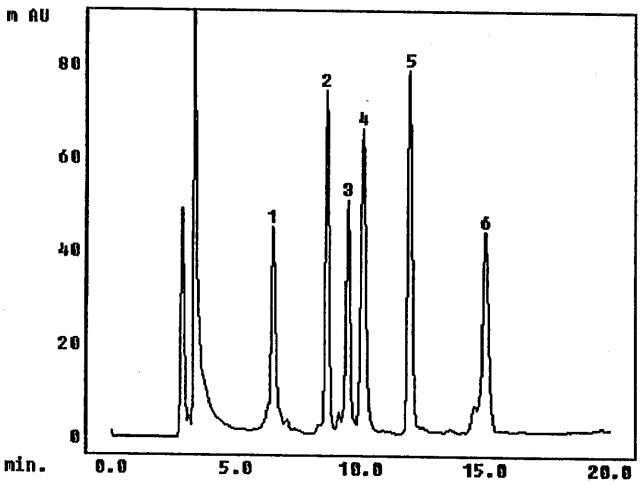

Figure 3. Mobile phase conditions: 25-85%B in 20 minutes. (Other conditions per text.)

percentage along with the shallow slope resulted in peak widths that were not much different from those in the chromatogram of F1.

Care must always be taken not to use too high a concentration of organic solvent in the starting conditions, because (depending on the protein and the type of organic solvent used) peak quality may degrade. Also, when the starting concentration of organic solvent exceeds a critical value, which is different for each protein, the protein may be completely unretained [1].

Reference

1. J. Koyama, J Nomura, Y Shiojima, Y. Ohtsu and I. Horii, J. Chromatogr. 625 (1992) 217-222.

Literature on Reversed Phase Protein and Peptide Separations

1. C.T. Mant and R.S. Hodges (Editors), HPLC of Peptides and Proteins: Separation, Analysis and Conformation, CRC Press, Boca Raton, Fl, (1991).