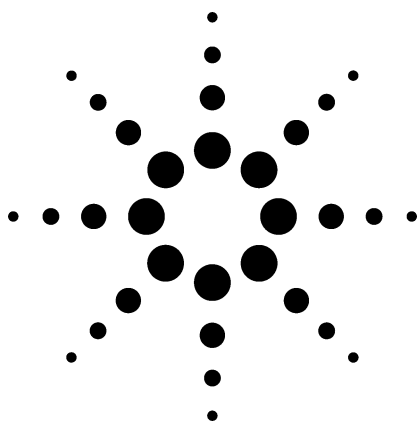


Effect of Temperature on Ultrafast Separation of Macromolecules

Application



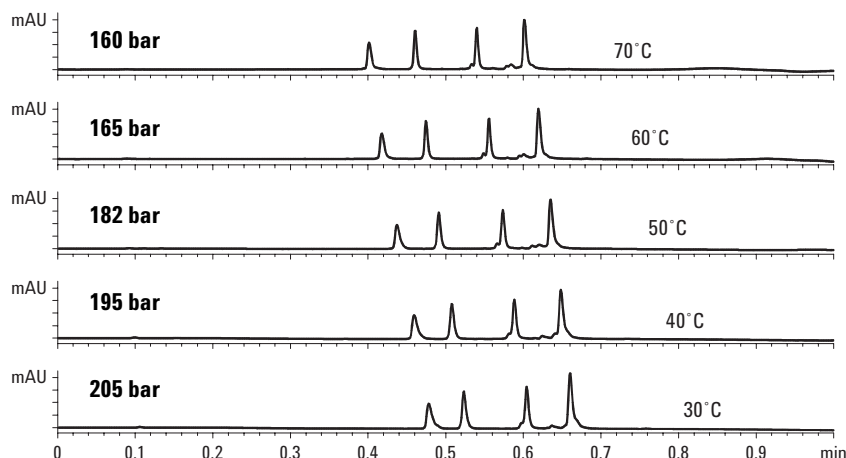
Technical

Robert D. Ricker and Cliff Woodward*

In the modern protein laboratory, sensitivity, speed, and resolution are sometimes competing goals. Frequently the analyst is working under conditions of limited sample size, a large number of samples, and a need for resolution of contaminants in the shortest possible time. Under these circumstances, Poroshell 300SB shines, delivering ultrasharp peaks with ultrashort run times and less required sample (20% that of a 4.6-mm id column). As may be seen in the chromatograms below, increased temperature enables shorter run times, increased resolution by sharpened peaks, and reduced mobile-phase viscosity for maximal throughput as a result of higher flow rates.

Poroshell 300SB-C18

2.1 × 75 mm, 5 µm; part no: 660750-902



Conditions:

Agilent 1100 HPLC; WPS with delay-volume reduction

Thermostatted column compartment

Mobile phase:

A = 95% H₂O, 5% ACN with 0.1% TFA, pH 2.1

B = 5% H₂O, 95% ACN with 0.07% TFA

Flow = 2 mL/min

Piston stroke: 20 µL

UV: 215 nm

Temperature: 70°C

Compound analyzed (1 µL/injection, approximate 250 µg/mL):

1. Neurotensin
2. RNase A
3. Lysozyme
4. Myoglobin

Highlights

- Poroshell 300SB-C18 uses proven StableBond technology to maintain stability at elevated temperatures and low pH.
- Because of the thin superficially porous layer, Poroshell 300SB-C18 allows rapid equilibration with large molecules having slower diffusion into pores.
- Use of higher temperatures reduces system back pressure and mobile-phase viscosity enabling higher flow rates to be used.
- Poroshell 300SB-C18 allows the use of higher linear velocities, with no loss in resolution. This translates into shorter run times.
- Poroshell columns were designed with a 2.1-mm id so that high linear velocities could be achieved at typical flow rates; 2 mL/min on this column compares to nearly 10 mL/min on a conventional 4.6-mm id column.

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