ISOCRATIC SEPARATION OF PROTEINS USING A THERMAL GRADIENT

INTRODUCTION

HPLC analysis at elevated temperatures reduces analysis time and improves resolution. Reduced viscosities at elevated temperatures result in significantly lower backpressure, allowing for higher flow rates and shorter analysis times. It also permits the use of smaller particle size packings to increase efficiency while operating at lower backpressure. Flatter van Deemter curves mean that operating at higher flow rates does not sacrifice efficiency. Solvent properties also change as the temperature is increased. Hydrogen bonding interactions in water are reduced at higher temperatures, making water less polar so that it behaves more like an organic solvent as the temperature is elevated. This means that most solvent gradients can be replaced with temperature gradients so that samples requiring complex solvent gradients can be separated isocratically using a simple temperature program.

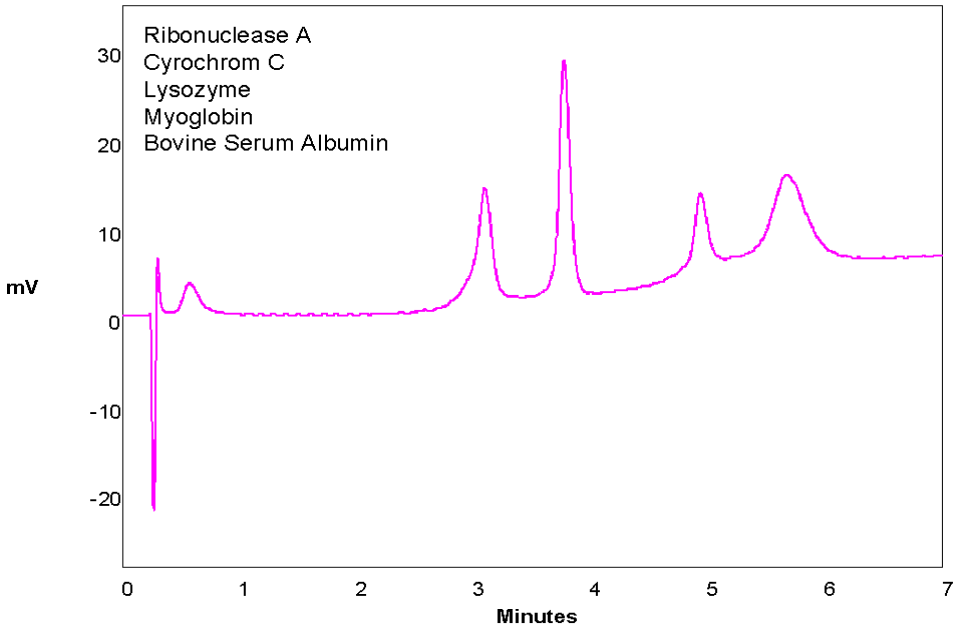
EXPERIMENTAL CONDITIONS

Conditions are summarized in Table 1. A Milton Roy CM4000 pump, Alltech vacuum degasser, Thermo Separations UV2000 variable wavelength detector and Alcott autosampler were used in conjunction with a Selerity Technologies Series 8000 programmable HTLC oven.

TABLE 1 :
HTLC CONDITIONS FOR THE ISOCRATIC SEPARATION OF PROTEINS

COLUMN:	HAMILTON PRP-3® 100 x 2.1 MM, 3 µM
MOBILE PHASE:	25:75 ACETONITRILE:WATER WITH 0.1 %TFA
FLOW:	1.0 ML/MIN
DETECTION:	UV @ 215 NM
INJECTION:	5 µL
TEMPERATURE:	THERMAL GRADIENT FROM 40° TO 150°C AT 30°/MIN. HOLD FOR FIVE MINUTES.

FIGURE 1 : *Separation of proteins using a thermal gradient from 40°C to 150°C.*



RESULTS

Figure 1 shows the separation of protein standards on a Hamilton PRP-3® column using an isocratic mobile phase and a temperature gradient. Baseline resolution of five standards is achieved in about six minutes. Reduced viscosity of the mobile phase at higher temperature allows a flow rate of 1.0 mL/min – a high flow rate for a 2.1 mm column with a 3mm particle size! The backpressure at 50°C was about 3100 psi. A backpressure regulator installed on the system after the detector contributes 250 psi to that total.