Waters
Lab Highlights
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Increased Temperature May Actually Decrease Column Efficiency in Reversed Phase Liquid Chromatography

As a result of information supplied by our colleagues, J. Chinen, H. Hirasawa and T. Sakai, at Nihon Waters (Japan) who observed decreased column efficiency with a reverse phase Radial-Pak™ column at elevated temperatures and our interest in understanding the parameters which influence column efficiency in LC (1), we chose to investigate the influence of temperature on column efficiency across a wide range of eluent flow rates. This report substantiates our Japanese colleagues' observations and concludes that improvements in column efficiency for reverse phase LC systems are not a guaranteed consequence of increased column temperature. Therefore, when a heater is used in LC the motivation should be improved precision or improved selectivity, not increased efficiency.

Figure 1 presents a typical chromatogram from the study.

Figure 1. Typical chromatogram, showing separation of the test solutes acetone (1) acenaphthene (2) and dibutyl phthalate (3) using a radially compressed Nova-Pak® C_{18} cartridge (batch 1036). The eluent was 50% acetonitrile/50% water. The flow rate was 3.0 mL/min and the temperature was 55° C. Temperature control was provided by TCM temperature control module connected to an RCM®100/column heater. The k' values were greater than 7 for acenaphthene and 12.5 for dibutyl phthalate at all temperatures. A study of peak asymmetry as a function of amount injected was done in order to determine the dilution of the test mixture which should be injected for the efficiency checks.

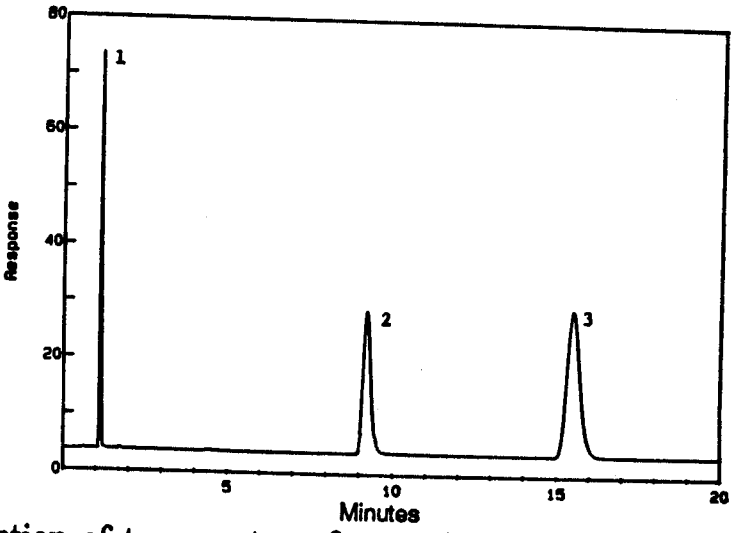

Figure 2 shows efficiency as a function of temperature for steel and RCSS using acenaphthene as the test solute. (The plots for dibutyl phthalate were very similar.) Contrary to expectations, an increase in column temperature leads to higher H values regardless of eluent flow rate. There is an overall upward shift in the van Deemter curves for both column types, reflecting an unanticipated effect of temperature on the A term of the van Deemter equation.

The effect of temperature on the van Deempter curve is more dramatic for the radially compressed cartridge. Across the entire flow rate range, the plate count for the cartridge is 26-30% lower at 65° than at ambient temperature. For the steel column, the largest difference in plate count is 20% at 0.3 to 0.5 mL/min. In this

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comparison it is of interest that the minimum H value for the cartridge is observed to be lower than the of the steel column. This is consistent with a previous report (2) which indicated a substantial reduction of the A term due to radial compression.

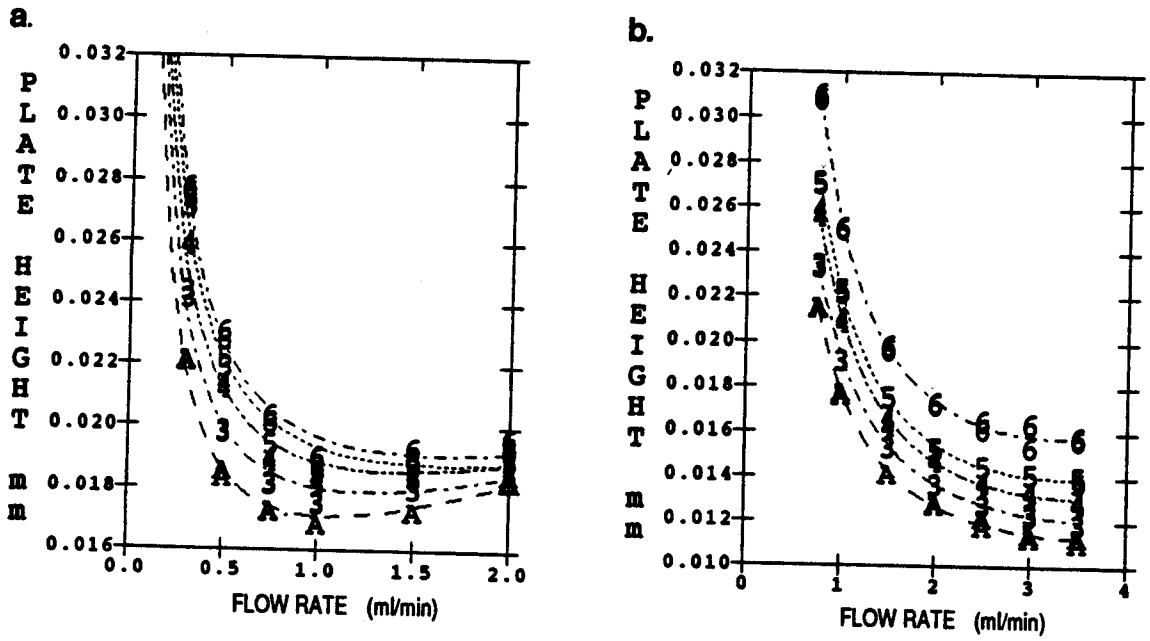

Figure 2. Experimental van Deempter curves for acenaphthene. a) Steel column. b) Radial-Pak cartridge. Curve symbols: A = ambient, 3 = 35° C, 4 = 45° C, 5 = 55° C and 6 = 65° C. Duplicate chromatograms were run at every temperature and flow rate. Pressure and flow rate were monitored throughout the study as a check for stable performance of the pumping system. Pressure fluctuations less than about 50 psi were considered acceptable. Plate counts were calculated by the area/height method.[1] If pressure fluctuated more than ± 25 during a run or if the average pressure was not constant, the data were not accepted.

Some authors have previously reported a negative impact of increased temperature on column efficiency of bonded phases (3-6), although we are not aware of another report which presents the full van Deempter curve across a range of temperatures for modern (4-10 μ) packings. Poppe et al. (3,4) and others (5) attribute the negative impact of temperature on H to radial thermal gradients which may exist for thermostatted columns if the eluent temperature is not precisely equal to the temperature of the outer walls of the column. Axial thermal gradients (7,8) may also occur due to resistive heating in the column. Either effect may explain the trend observed in Figure 2. Indeed, this may be "fact of life" for commercially available column heaters.

The results presented here do not support the "conventional belief" that, efficiency of all types of columns will rise with temperature. Therefore, the decision to use column temperature control in reverse phase systems should not be dictated solely by a requirement for increased efficiency, but by other goals such as the improvement of retention time precision or the control of selectivity.

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