Anal. Chem. **2002,** *74,* 4150−4159

High-Speed Liquid Chromatography by Simultaneous Optimization of Temperature and Eluent Composition

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Clearly, a major trend in liquid chromatography is to increase its speed to make it faster. Improving throughput for routine analysis of pharmaceutical release samples and stability assays are all key motivations for improving separation speed. Recent work has focused on the theoretical benefits of temperature on speed in liquid chromatography. We have shown that the 5−10-fold decrease in eluent viscosity that comes from a temperature increase of 175 °C over ambient, and the concomitant increase in analyte diffusitivity, act to dramatically decrease the time needed to generate a theoretical plate. Lower viscosities at elevated temperatures decrease the pressure drop across the column and allow the use of higher linear velocities as the pump pressure limit is approached. Simultaneously, faster analyte diffusion at higher column temperature improves efficiency at high eluent velocity conditions compared to the efficiency at lower temperatures at the same velocity. We find that higher temperature plays a central role in improving speed. In this work, we show that when the percent organic modifier in the eluent and column temperature are adjusted to keep retention factors fixed, highly efficient, subminute separations can be routinely achieved when a hot column is used at the maximum system back pressure. We find that the best way to facilitate such an optimization, assuming constant selectivity, is to use a very retentive column so that one can work at both high temperature and high volume fraction of organic modifier to achieve the lowest possible eluent viscosity. We have also analyzed the effect that key extracolumn contributions have on column selection and system design.

From its inception, liquid chromatography has been slow compared to other separation techniques.[1−4] Efforts aimed at improving the speed of LC have been a primary driving force for improvements in particle technology,[5,6] specialized instrumentation,[6−8] and selectivity optimization.[9−11]

Over three decades have passed since Knox and Saleem published their highly influential paper on speed in LC.[4] They showed that a fast analysis (<20 s for $k' = 5$) could be achieved for a given plate number (5000) at the maximum pump pressure (350 bar), when column length (<1 cm) and particle diameter (<1 μm) are adjusted to give a minimum in the reduced plate height curve (h_o) at the maximum reduced velocity (ν_o) allowed by the maximum pump pressure. All their predicted improvements have yet to be fully achieved due to the lags in particle, column, and instrument advances.

The idea that a short column (<5 cm) packed with small particles (<5 μm) ought to dramatically improve the speed of LC has been the subject of many commercial advertisements. However, all things being equal, these treatments minimize or ignore the fact that major reduction in column length (<5 cm) is a problematic solution to improving speed with *conventional* (that is, off-the-shelf) equipment. This is primarily due to the dominance of the detector time constant[12] and extracolumn tubing broadening over the column broadening when very short, high plate count columns are used. In the absence of major improvements in commercially available equipment, the full speed of LC will not be available to most chromatographers.

Assuming that sufficiently efficient columns can be obtained, the ultimate limitation to speed in LC is set by the maximum pump pressure.[1,4,13] This limit can be significantly mitigated by operating the system at higher temperatures. The 5−10-fold decrease in eluent viscosity [14,15] that comes from a temperature increase of 175 °C over ambient, and the concomitant increase in analyte diffusitivity,[16] act to dramatically reduce the pressure limitations on speed and increase efficiency at high column linear velocity, respectively.[7,13,17] As a consequence of the lower pressure drop, a higher eluent linear velocity can be used. This may improve the

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10.1021/ac0112622 CCC: $22.00
Published on Web 07/16/2002

speed if the column has adequate efficiency. The increased efficiency gained at higher column temperatures from enhanced analyte diffusivity is especially advantageous under high linear velocity conditions where interphase mass-transfer broadening is thought to dominate.[7,13]

As a consequence of the enhanced column dynamics, dramatic improvements in speed can occur as a function of temperature when the resolution of a critical pair is important. By extending the work of Knox and Saleem to include resolution, the shortest analysis time (t_R) at a given plate number (N) and pump pressure *scales with the fourth power of the resolution (R)*[4,18,19] according to the following equation:

$$t_R = 256\varphi h^2 R^4 \frac{(1+k')^5}{(k')^4}\left(\frac{\alpha}{\alpha-1}\right)^4\left(\frac{\eta}{\Delta P}\right) \qquad (1)$$

where φ is related to the external column porosity (see eq 3), h is the reduced plate height, k' is the retention factor, α is the selectivity, η is the viscosity, and ΔP is the system back pressure. At a given resolution, the selectivity contribution to eq 1 dominates, and it is obvious that large gains in speed can be obtained by small improvements in the selectivity. Furthermore, selectivity is a function of temperature and temperature can be used as a variable to tune selectivity.[9,11,18,20−24]

Atamna and Grushka addressed resolution optimization as a function of temperature in their series of papers on "isochronal" analysis.[25,26] The approach adopted in this paper does not consider resolution or selectivity but focuses solely on the fast elution of an individual analyte. It is evident that full practical optimization must encompass consideration of the effect of temperature on selectivity.

The use of temperature as an operating variable in LC has been applied to the theory of speed in LC. Based on approach similar to that of Knox and Saleem, Antia and Horvath[13] predicted that a large decrease in analysis time is feasible if the column could be operated at elevated temperatures (100−200 °C). They also identified the major problems associated with high-temperature analysis such as the importance of chromatographic system design and the problem of on-column analyte instability. In a recent publication,[27] we addressed the critical issues relevant to analyte stability on a fast chromatographic time scale. To get an accurate estimate of the improvement as a function of temperature, Chen and Horvath[14] determined the viscosity of acetonitrile/water mixtures up to temperatures of 120 °C. Here we extend Antia and Horvath's theoretical treatment to include the relationship between retention, percent organic modifier, and temperature.

One of the key problems in elucidating the effect that temperature has on performance is the evaluation of the relative contribution of the analyte's retention and the eluent's transport properties. When neither contribution is restrained as the eluent temperature is raised, the decrease in the retention of a typical analyte dominates the decrease in eluent viscosity.[17] Consequently, as a system (i.e., column and instrument) is optimized for speed, and the eluent temperature is raised, analyte retention becomes too low ($k' < 1$) to be of practical interest since the resolution must fall off at low k'. In this work, we constrain the analyte's retention factor by adjusting the volume fraction of organic composition to hold k' constant as the temperature is raised and the system is optimized. This approach is similar to but distinct from the simultaneous optimization of temperature and organic modifier as described by Atamna and Grushka.[25] In the first part of this paper, we ignore instrumentation issues and focus solely on the role that temperature plays in selection of the eluent volume fraction. Here we address the question of whether a strong eluent at low temperature or a weak eluent at high temperature should be used to speed up LC. The second part of the paper applies well-known equations for extracolumn band broadening to determine which column lengths and particles should be used to optimize high-temperature LC. We also make recommendations for column diameter, detector time constant, and tubing length based on balancing heat transfer, extracolumn broadening, and flow rate.

THEORETICAL SECTION

The equations that relate analysis time to efficiency as a function of column format (i.e., column length and particle diameter) given below are well known and are similar to the equations given by Knox [4] and Guiochon.[1] The focus of this work is to understand the role of temperature in the equations based upon the work of Antia and Horvath.[13] The primary difference between our work and previous work is the introduction of equations used to restrain the calculation to hold the retention factor constant. We will also discuss how the chromatographic system was modeled to elucidate its effect on column efficiency.

The Fastest Analysis with a Given Column Format. The fastest elution with a given column occurs at the maximum pressure deliverable by the pump. In the following derivation, we fix the maximum pump pressure and calculate the linear velocity. This linear velocity is then used to calculate the plate height. We will also fix the column length and particle diameter as is usually done in LC. This means that efficiency and retention time are dependent variables.

The back pressure of a packed column can be estimated by combining Darcy's equation for the velocity of the mobile phase in a laminar flow system and the Kozeny−Carman equation for the specific column permeability.[28] Darcy's law is given by

$$\Delta P = B^\circ L \eta u \qquad (2)$$

where the pressure drop ΔP (dyn/cm^2) is proportional to the column linear velocity (u, cm/s), the length of the column (L, cm), and the eluent viscosity (η, dyn·cm/s). The column velocity is related to the superficial linear velocity by ϵ_e as per Horvath and Lin.[29] The specific column permeability (B°, cm^{-2})

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can be estimated from the Kozeny−Carman equation for porous particles:[30]

$$B^\circ = \frac{\varphi}{{d_p}^2} = \frac{180}{{d_p}^2}\frac{(1-\epsilon_e)^2}{{\epsilon_e}^3} \quad (3)$$

where ϵ_e (typically 0.38) is the external packing porosity and d_p (cm) is the particle diameter. The column's specific permeability is proportional to the hydraulic radius. Depending primarily on the structure of the packed bed, the hydraulic radius generally correlates well with the inverse square of the particle diameter.[30]

The Peclet number (reduced velocity) describes the eluent's linear velocity (u, cm/s) scaled to the particle diameter (d_p, cm), relative to the diffusion coefficient (D_m, cm^2/s) of the analyte in the eluent and is given by

$$\nu = ud_p/D_m \quad (4)$$

At the maximum pump pressure, the maximum Peclet number can be calculated by combining eqs 2−4 to give

$$\nu_{max} = dp^3\Delta P_{max}/\eta\varphi D_m L \quad (5)$$

The reduced plate height at ν_{max} can be calculated from the Knox equation,[31] which is given by

$$h = B/\nu + A\nu^{1/3} + C\nu \quad (6)$$

where h is the reduced plate height, ν is the Peclet number, A is the term used to measure hydrodynamic dispersion in the moving zone, B is the term associated with axial diffusion, and C is the resistance to interphase mass-transfer term.[32] The C term is given by

$$C = k''/30\gamma_{sm}(1+k'')^2 \quad (7)$$

where k'' is the retention factor in the *moving zone* and is estimated from the retention factor in the mobile phase (k') and other considerations related to particle porosity and pore space accessibility.[32] The parameter γ_{sm} is proportional to the ratio of the analyte diffusion coefficient in the stationary phase to the analyte diffusion coefficient in the eluent and is estimated to be 0.6.[32] The C term depends on temperature only via k', since we have held γ_{sm} constant with temperature. For our purposes, the B term was estimated to be 1.5 and was not made temperature dependent because its contribution to the plate height at high linear velocity is unimportant to the analysis. The A term is assumed to be temperature independent. Experiments conducted by Yan et al.[7] suggest that the A term obtained by fitting the vanDeemter equation is independent of temperature.

Under isocratic conditions, the analyte retention time (t_R, s) is given by

$$t_R = (L/u)(1+k') \quad (8)$$

and is dependent on the column length (L, cm), the chromatographic linear velocity (u, cm/s), and the retention factor. The number of plates (N) depends directly on column length (L, cm) and inversely on the reduced plate height (h) and particle diameter (d_p, cm) as is given by

$$N = L/hd_p \quad (9)$$

The time to generate one unit of efficiency (t_R/N, s) can be calculated by solving eq 9 for the column length and eq 4 for linear velocity and substituting them into eq 8 to give

$$\frac{t_R}{N} = \frac{(1+k'){d_p}^2}{D_m}\frac{h}{\nu} \quad (10)$$

By substituting eq 5 into eq 10, the performance (t_R/N) at the maximum possible Peclet number can be calculated and is given by

$$\frac{t_R}{N} = \frac{(1+k')\eta hL\varphi}{d_p\Delta P_{max}} \quad (11)$$

The plate number at maximum pressure and Peclet number for a given column length and particle size can be calculated by substituting eq 5 into eq 9:

$$N = {d_p}^2\Delta P_{max}/\varphi\eta D_m h\nu_{max} \quad (12)$$

Temperature-Dependent Variables. These equations can be cast as functions of temperature and eluent composition using empirical correlations for viscosity, analyte diffusion coefficient, and retention factor. The viscosity of aqueous acetonitrile mixtures as a function of temperature and composition can be estimated from the correlation developed by Chen and Horvath:[14]

$$\eta(\phi,T) = \exp\left[\phi\left(-3.476+\frac{726}{T}\right) + (1-\phi)\left(-5.414+\frac{1566}{T}\right) + \phi\left(-1.762+\frac{929}{T}\right)\right] \quad (13)$$

where η is the viscosity (in cP), ϕ is the volume fraction of acetonitrile in the eluent, and T is the absolute temperature. This correlation has been verified for the entire range of mixtures up to 120 °C.[6] The diffusion coefficient for small molecules can be estimated using the Wilke−Chang correlation:[16]

$$D_A = 7.4\times10^{-8}(\sqrt{\Psi_B MW_B}T/\eta {V_A}^{0.6}) \quad (14)$$

where A and B denote the solute and the eluent, respectively, D_A (cm^2/s) is the diffusion coefficient of the analyte in the eluent, Ψ_B is the solvent association factor, MW_B is the calculated

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molecular weight (g/mol) of the eluent, T is the absolute temperature, η is the eluent viscosity (in cP), and V_A is the molar volume (mL/mol) of the analyte at its normal boiling point and can be calculated by a group contribution approach.

Retention Restraints on the Calculation. We developed a retention correlation for *n*-butylbenzene on a bonded-phase silica column as a function of temperature (30−80 °C) and fraction of organic modifier (30−60% acetonitrile, ACN) from retention data of Mao.[33] The log of retention was regressed against temperature and composition to produce

$$\log k' = -1.1 - 0.4\phi + \frac{1490}{T} - \frac{1262\phi}{T} \qquad (15)$$

where k' is the retention factor, ϕ is the volume fraction of organic in the eluent, and T is the temperature (in °C). We extrapolated this correlation ($R^2 = 0.994$; SE = 0.04) to the extremes in temperature and fraction of organic studied in this work. The standard change in enthalpy of transfer for *n*-butylbenzene was calculated to be −4.9 kcal/mol at 30% ACN, and the solvent strength parameter (S) was calculated to be 4.35 at 38 °C. The standard change in enthalpy is slightly lower than that given by Alvarez-Zapeda[34] and Ranathunga.[35] However, it satisfies our requirements for a fairly typical low molecular weight analyte.

Equation 15 was used to determine the volume fraction of organic modifier required to maintain k' at a specified value over a range of specified system temperatures. This volume fraction (ϕ) was then used to calculate the viscosity of the eluent (eq 13), which, in turn, was used to calculate the diffusion coefficient (eq 14) for *n*-butylbenzene. Then the maximum Peclet number (eq 5) and finally the reduced plate height (eq 6) were calculated. Once these parameters were determined, t_R/N and N were calculated via eqs 11 and 12. From these values, t_R could be determined. The maximum pump pressure, the particle diameter, the permeability, and the column length were held constant for the calculation of one set of t_R/N and N values as a function of temperature. Then different combinations of L, and d_p were used to calculate further values of t_R/N and N.

Calculation of the System Efficiency. Band broadening occurs not only in the column but also in the injector, the detector, and the connecting tubing. To elucidate the effect that system efficiency has on the column efficiency, we used well-known equations[12,36] for the extracolumn contribution to column broadening. The total peak variance (σ^2_{Total}) is the sum of all band-broadening possibilities:

$$\sigma^2_{Total} = \sigma^2_c + \sigma^2_{inj} + \sigma^2_d + \sigma^2_\tau + \sum\sigma^2_{other} \qquad (16)$$

where σ^2_c, σ^2_{inj}, σ^2_d, and σ^2_τ are the variance contributions of the column, injector, detector flow cell volume, and detector time constant, respectively. The term $\sum\sigma^2_{other}$ is the sum of all other broadening mechanisms. Injector and detector broadening is, to a first approximation, independent of the flow rate.[37] Band broadening in the detector depends on the detector volume and the time constant. The dispersion caused by the injector depends on the volume injected and the type of injector. Hydrodyamic dispersion in the tubing depends on the flow rate, the tubing length, the tubing inner diameter, and the temperature of the eluent both radially and axially. When the flow rate is very high, the peak variance contribution of both the injector and detector *volumes* becomes negligible and the variances due to the tubing and the detector time constant become dominant. Throughout the entire study, we assume that the eluent flowing through the injector and the detector flow cell is at ambient. This means that precolumn tubing is required to heat the eluent to column temperature and postcolumn tubing is required to cool the eluent prior to the detector. Typical specifications for injector rotor materials give an upper limit of 75 °C at 400 bar. Due to noise and flow cell longevity, a typical maximum flow cell temperature is ∼60 °C; thus, the eluent must be cooled if the column is hot. If better materials were available, lengths of heating and cooling tubing would no longer be required and concomitant improvements in system efficiency would follow.

A previously published heat transfer correlation was used to estimate the length of tubing needed to heat the eluent to within 1 °C of the column temperature and, thus, its contribution to the overall broadening and pressure.[7,17] We have presented in detail the effect that preheating and cooling tubing has on the column broadening in a prior publication.[7] Under high-speed, high-temperature conditions, the detector time constant broadening ($\sigma^2_{\tau,v}$ in volume units) dominates the extracolumn broadening and is given by

$$\sigma^2_{\tau,v} = (F\cdot\tau)^2 \qquad (17)$$

where τ is the detector time constant (s) and F is the flow rate (in mL/s).[38] As the temperature is raised, the fractional contribution of the time constant to the total second moment increases due to the improvement in the time variance of the Gaussian chromatographic peak and the increased flow rate allowed by lower eluent viscosity.

EXPERIMENTAL SECTION

Instrument. All viscosity measurements were taken with a Hewlett-Packard 1090 chromatographic instrument controlled by Chemstation software (Hewlett-Packard S.A., Wilmington, DE). The instrument was equipped with a ternary pump, a helium sparger, an autosampler, a thermostated-column compartment, and a diode-array UV detector. The column compartment and the detector were bypassed for the viscosity measurements. The temperature control of the analytical column and the preheating of the eluent were achieved with a stirred silicone oil bath controlled to ±1 °C. The preheater was a 0.010 in. i.d. × 1 m long 316 SS tube and was fully immersed in the oil bath. An in-line filter with a 0.45-μm frit was inserted upstream of the analytical column to prevent tubing or frit blockage.

Reagents. The eluent contained ChromAR HPLC grade ACN from Mallinckrodt Chemical Co. (Paris, KY). HPLC water was

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obtained from a Barnsted Nanopure deionizing system (Dubuque, IA) with an "organic-free" cartridge and a 0.2-μm filter. The water was subsequently boiled to remove carbon dioxide. All solvents were filtered through a 0.45-μm filter (Lida Manufacturing Corp., Kenosha, WI) before use.

Analytical Columns. Carbon-coated zirconia (C-ZrO_2) particles (3 μm) used in the viscosity measurements were provided by ZirChrom (ZirChrom Separations, Inc., Anoka, MN). They were packed into a 4.6 × 50 mm column (Isolation Technologies, Inc., Hopedale, MA) using a downward slurry method that was developed in-house.

Viscosity Measurements. We measured the viscosity of aqueous acetonitrile mixtures as a function of temperature by monitoring the pressure drop across the column. This was done with two pressure gauges (Alltech Associates, Inc. Deerfield, IL) connected to the column by Rheodyne "T"s (Alltech Associates, Inc.). The T's were connected to the inlet and outlet ends of the column by 5 cm × 0.005 in. i.d. 316 SS tubing. The flow rate was checked for accuracy both gravimetrically and volumetrically. The eluents were prepared in the range of 10−90% w/w, and density tables were utilized to convert them to volume fraction. Hand mixing was done in order to avoid errors associated with binary on-line mixing. To prevent the eluent from boiling, a back pressure regulator adjusted to ∼25 bar was connected to the outlet T piece via a counterflow heat exchanger.

The specific column permeability ($B°$, cm^{-2}) was determined by measuring the pressure across the column at different temperatures (100−200 °C) and different flow rates (0.5−2.5 mL/min) using pure water and pure methanol. Superficial linear velocity was regressed against the system pressure drop. The slopes were corrected for the pressure drop across the 10 cm length of 0.005-in.-i.d. connection tubing using Poiseuille's equation[39] for the pressure drop across a length of tubing for an incompressible eluent in laminar flow. Established viscosity correlations for pure water and methanol,[40] which were validated for temperatures exceeding 200 °C, were then used to determine the specific column permeability from the slope.

The viscosities of the aqueous acetonitrile eluents were determined by measuring the pressure across the column at different temperatures (100−200 °C) and different flow rates (0.5−2.5 mL/min). The average specific column permeability was then used with Darcy's law (eq 2) to calculate the viscosity from the slope of the superficial linear velocity versus the system pressure drop. The slope was also corrected for the 10 cm of connection tubing. We assumed that the permeability did not change for aqueous acetonitrile mixtures and used the average specific permeability to calculate the viscosity. After the measurements were taken, water was perfused through the column to verify that the permeability of the column had not changed over time.

DISCUSSION

Aqueous Acetonitrile Eluent Viscosity as a Function of Temperature. The validity of the Chen−Horvath viscosity correlation for temperatures exceeding 120 °C is important for the accuracy of our theoretical predictions presented in the following figures and tables. First, the specific permeability for the column as a function of eluent and temperature had to be determined. The results are shown in Table 1. The table shows that the permeability of the column is largely independent of the eluent that was perfused through the column and independent of the column temperature except for methanol at high temperature. Once the experimental viscosity had been calculated, the viscosity as a function of temperature and composition was compared to the correlation developed by Chen and Horvath. Figure 1A shows the difference between the calculated eluent viscosity and the measured eluent viscosity. A clear trend is observed for the errors: under high aqueous conditions and high temperature, the correlation underestimates the viscosity. Conversely, at low aqueous conditions and at lower temperatures, the viscosity is overestimated. The plot shows that the correlation is good to within ∼±20% from 100 to 200 °C, which is appropriate for most transport property estimations of interest to us.

Table 1. Specific Permeability[a] of a 4.6 × 50 mm Column Packed with 3-μm C-ZrO_2 Particles in Methanol and Water at Various Temperatures

	viscosity (cP)		pecific permeability $\times 10^{10}$ (Darcy)	
T (°C)	water[b]	methanol[b]	water	methanol
100	0.290	0.247	1.492	1.425
120	0.241	0.202	1.406	1.450
140	0.206	0.160	1.584	1.538
160	0.179	0.123	1.482	1.740
180	0.159	0.090	1.465	1.958
200	0.142	0.063	1.597	2.557

[a] Calculated using eq 2. [b] Calculated using correlations in ref 40.

A possible source of experimental error is the effect of pressure on the viscosity. It is known that, with the exception of water at low temperatures (0−10 °C), all liquids will show a roughly exponential increase in viscosity with increasing pressure.[40] Darcy's law predicts that linear velocity should be directly proportional to the pressure drop across a packed bed. If the range in column pressure was large enough, we might expect to see curvature in the plot of system pressure versus linear velocity due to the effect of pressure on viscosity. However, inspection of the plots indicates no curvature, which suggests that the effect is negligible over the observed pressure range (500−1000 psi).

Modified Viscosity Correlation. For those applications that require a more accurate correlation, we modified the correlation using the method outlined by Chen and Horvath to give the following correlation:

$$\eta(\phi,T) = \exp\left[(1-\phi)\left(-4.367+\frac{1150}{T}\right)+(1-\phi)\left(-3.93+\frac{858}{T}\right)+\phi(1-\phi)\left(0.16+\frac{137}{T}\right)\right] \quad (18)$$

where T is the absolute temperature, η is the viscosity (in cP), and ϕ is the volume fraction of acetonitrile of the eluent. The

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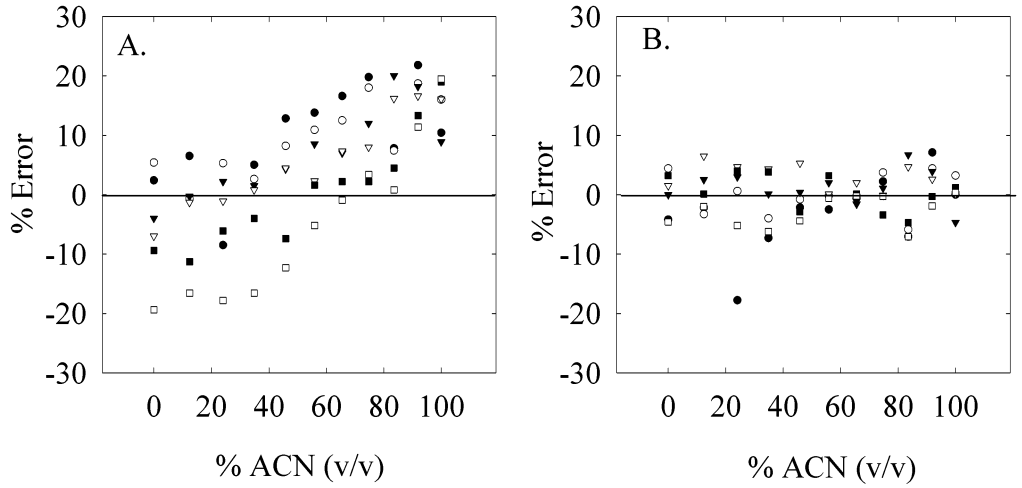

Figure 1. Percent error as a function of percent organic modifier by volume and temperature for (A) the Chen−Horvath and (B) the modified Chen−Horvath viscosity correlations. Eluent temperatures are given by the following symbols: ○, 100 °C; ●, 120 °C; ▽, 140 °C; ▼, 160 °C; □, 180 °C; ■, 200 °C.

method combines the Grunberg and Nissan equation[42] to determine the interaction between temperature and eluent composition and the Andrade equation[40] to determine the dependence of viscosity of the pure eluents on temperature. Figure 1B shows that the modified correlation gives more accurate approximations of viscosity within the temperature range of 100−200 °C and is good to within ±7%. We excluded the discrepant data seen in the figure via an outlier test at the 95% confidence level.

Determination of Optimal Eluent Composition and Temperature. Obviously, the fastest elution for a given column and chromatographic system without regard to k' occurs at a fixed temperature when the percent organic composition is 100%. Likewise, for a given composition, the fastest elution will occur at the highest operating temperature allowed. Both of these situations are hardly practical because neither consider the interrelationship between T, ϕ, and k'. Conventional wisdom typically ignores this paradigm and opts for simple, inaccurate concepts such as the fastest analysis occurs at the highest percent organic. We consider here the fastest elution of a peak at a specified k'.

Since the time to generate a unit of efficiency (t_R/N) is directly proportional to the viscosity (η) (eq 11), any decrease in eluent viscosity will improve the performance. When temperature and pressure are fixed, faster elution of a peak could be accomplished by *increasing* the percent organic composition (i.e., decreasing the viscosity) until a lower limit of k' was reached. Table 2 shows that for a k' of 5 at 25 °C, a maximum of 69% ACN can be used. The viscosity of such an eluent is 0.55 cP. In light of the relationship of t_R/N and viscosity in eq 11, it is obvious that an eluent-based speed optimization is inferior to the speed optimization approach that we are about to describe. The eluent-based optimization approach to increasing speed represents the best of conventional wisdom on the subject of speed in LC.

In contrast to an eluent-based speed optimization of fixed temperature, Table 2 shows that even faster elution can be accomplished at constant retention factor as the eluent temperature is raised (column 1) and the volume fraction organic is simultaneously *decreased* (column 2) to compensate for the effect of increased temperature on analyte retention. Column 5 represents the contribution that decreased eluent viscosity makes to speeding up elution. The table clearly shows that an increase in eluent temperature will decrease the elution time by decreasing the viscosity, as the retention factor is fixed by weakening the eluent.

Table 2. Effect of Temperature and Retention Factor on the Percent Acetonitrile and Corresponding Eluent Viscosity[a]

k'[a]	% ACN (v/v)	T (°C)	η[b] (cP)	$\eta\,(T)/\eta\,(25\ °C)$
1	84.4	25	0.44	1.00
1	76.9	100	0.26	0.60
1	72.1	150	0.19	0.43
1	67.5	200	0.14	0.32
5	69.4	25	0.55	1.00
5	58.6	100	0.29	0.53
5	51.7	150	0.20	0.36
5	44.9	200	0.14	0.25
10	63.0	25	0.60	1.00
10	50.7	100	0.30	0.51
10	42.8	150	0.20	0.33
10	35.2	200	0.14	0.23

[a] The table gives the value of ϕ_{ACN} and T required to obtain the indicated k' for *n*-butylbenzene on a Zorbax ODS phase calculated from eq 15. [b] Eluent viscosity calculated from eq 13 at the indicated ϕ_{ACN} and T.

Further examination of the data in Table 2 suggests that to make LC as fast as possible, the most retentive column should be used. A very retentive column compensates for reduced retention at high temperature or high organic modifier. The greater is the column retention at some initial temperature and composition, the smaller is the viscosity at any given temperature/eluent composition. As a consequence, elution would be faster.

What Column Should Be Used? In 1974, Guiochon and coworkers published a paper entitled "Optimization of Column Design and Operating Parameters in High-Speed Liquid Chromatography". We thought it appropriate to revisit their approach using temperature as the operating variable of interest. Figure 2A shows the effect that temperature and column format have on

(42) Grunberg, L.; Nissan, A. H. *Nature* **1930**, *125*, 309.

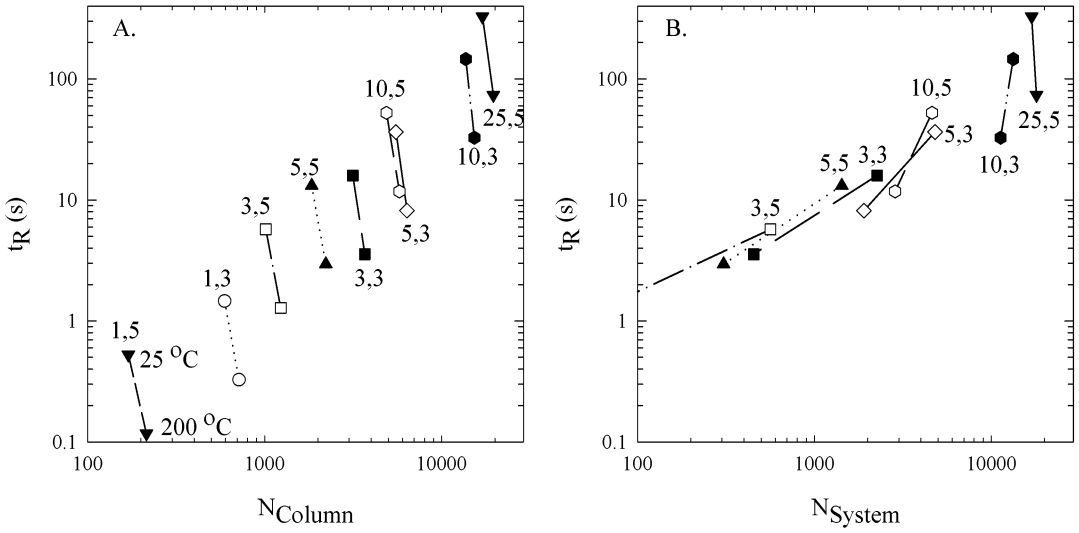

Figure 2. Calculated relationship between retention time, efficiency, and operating temperature using hypothetical columns and systems. Retention time (t_R) at a k' of 5 is plotted vs column efficiency (A) and system efficiency (B). Column efficiency (350 bar; $\epsilon_e = 0.38$, A = 1; B = 1.5; C ($f(k',T)$)), denoted by N_{Column}, was calculated for different combinations of column length (cm) and particle diameter (μm) which are respectively represented by the numbers separated by commas. The top and bottom points in both (A) and (B) are calculated for column temperatures of 25 and 200 °C, respectively. The volume fraction of organic modifier was adjusted at each temperature to hold retention constant ($k' = 5$). A hypothetical system was assumed to calculate system efficiency (N_{System}) for each column format at 25 and 200 °C. The column i.d. is 2.1 mm; the detector time constant is 0.15 s; the injection volume is 0.2 μL; the detector volume is 0.8 μL; the tubing i.d. is 0.005-in. i.d. and 0.0625-in. o.d.; the tubing length is 10 cm plus the length needed to heat the eluent from 25 °C to within 1 °C of the column temperature.

retention time and number of plates generated by the column. The eluent velocities for all traces in the plot were adjusted to hold pressure constant at 350 bar. The T,ϕ pairs from Table 2 for a retention factor of 5 were used. First, Figure 2A clearly shows that, for any column/particle configuration, an increase in column temperature decreases analysis time and increases the number of plates. Second, Figure 2A shows that a decrease in the column length does not always give an improvement in analysis time. For example, a 5,5 column (denoting a column of length 5 cm, packed with particles of 5 μm in diameter) would be faster than a 3,3 column but would be less efficient. These data are in agreement with Knox's analysis[42] that shows that once a separation has been achieved, a faster separation can be done by using larger particles with a longer column. Similarly, a 10,3 column operated at high temperature would be equally as fast as a 5,3 column operated at ambient temperature.

For a separation requiring a minimum of 2000 plates, 10,5, 5,3, and 3,3 column formats would be acceptable. However, the traces in Figure 2A must be viewed in the light of Figure 2B, which shows the limitations to the column format that are imposed by a low dead volume chromatographic system. The shorter columns are no longer able to generate any significant number of plates, and the efficiency benefits that are gained as a function of temperature are lost when extracolumn processes are taken into account. Shorter columns should only be used at ambient temperature or when low plate counts are acceptable. Certainly, a shorter column (1−3 cm) packed with conventional particles (3−5 μm) will reduce the column residence time. But the loss of plates by extracolumn broadening makes such column formats untenable for fast, high-resolution LC because commercial systems are made for much slower chromatography. Obviously, the longer the column, the better the system efficiency. This suggests that one ought to be able to achieve immediate and dramatic improvements in the speed and plate counts in preparative separations as the temperature is raised with no or very minor changes to existing systems. Since selectivity often limits the loading capacity in preparative LC, the use of high temperatures may very well improve the productivity. This analysis does not consider the effect of selectivity on speed. It may be that a short column with very low efficiency could be very fast if there is adequate selectivity between the critical pairs.

The origin of the plate loss in Figure 2B comes about as a function of temperature as two things happen simultaneously. First, an enhanced analyte diffusion coefficient and eluent viscosity dramatically improves the efficiency generated per unit time (N/t_R). This will decrease the time-based variance of the chromatographic peak. As a result, contribution of the detector time constant and tubing broadening to the overall broadening increases dramatically. Second, as the column temperature is raised and the linear velocity is adjusted to keep the pressure constant, the length of tubing needed to heat the eluent increases. It is primarily these two contributions, with the former overwhelmingly dominating over the latter, that act together to bring about the intolerable loss of efficiency.

Other interesting conclusions emerge from Figure 2B. Column formats 10,5 and 5,3 at 25 °C and formats 10,3 and 25,5 at 200 °C all have about the same elution time. However, the 10,3 and 25,5 formats have 3−5 times the efficiency. The plots show that very fast separations can be obtained using the 10,5 and 5,3 formats at lower temperatures, and efficiency can be traded for higher speed as the column temperature is raised. We conclude that the 10,3 and 25,5 are the more desirable formats with the 10,3 being an excellent compromise between plate count and time and are highly desirable for very efficient subminute separations *at elevated temperature*. In the following discussion, we will focus on these

(43) Knox, J. H. *J. Chem. Soc.* **1961**, 433−441.

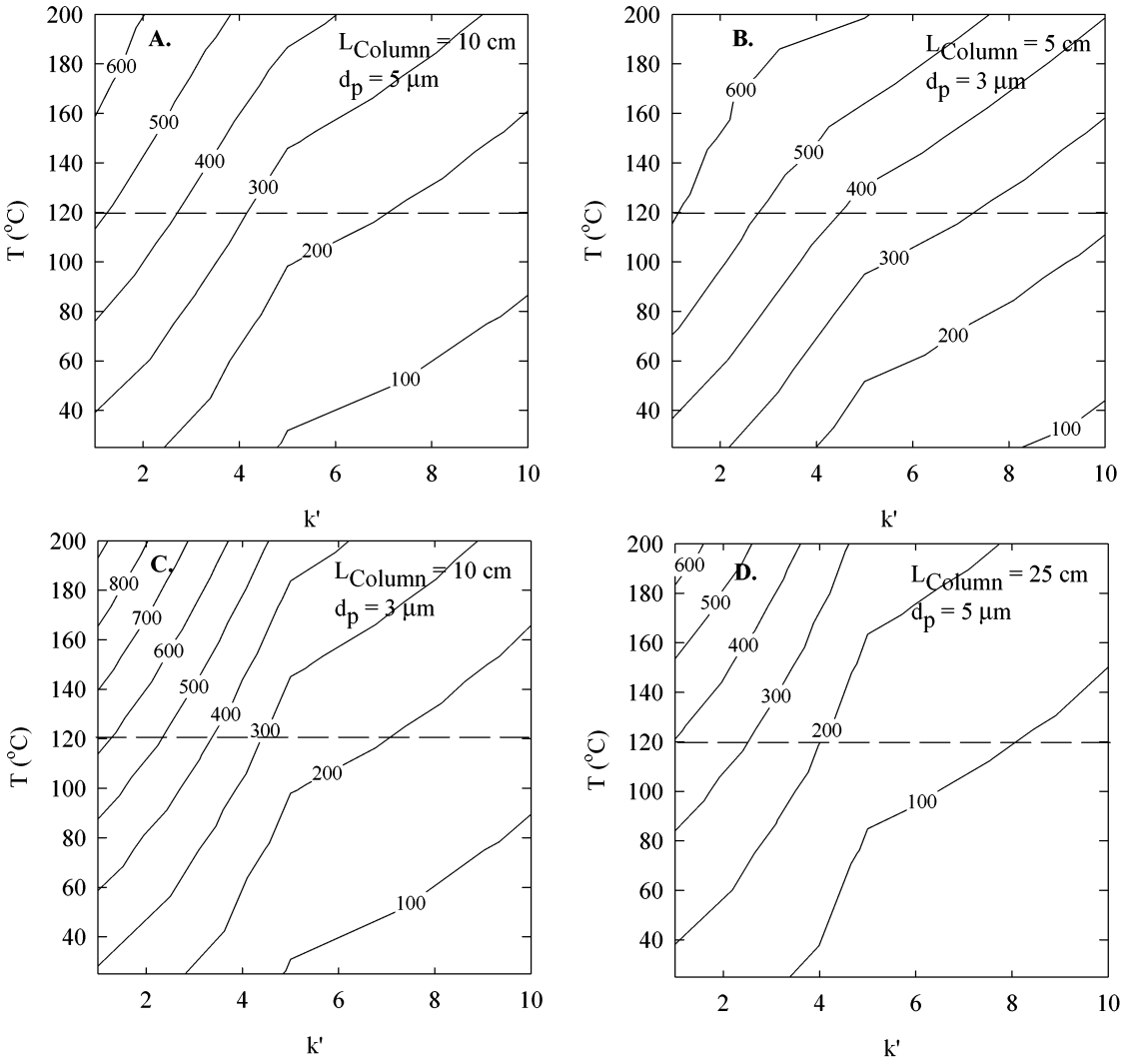

Figure 3. Comparison of the *system* efficiency generated per unit time for the fast columns selected from Figure 2. The contours give the ratio of the system efficiency to the retention time (N_{System}/t_R) (in s^{-1}) and are plotted as a function of temperature and retention factor. The column format is given in each plot. Calculation conditions are given in Figure 2.

four column formats. The plots show that these columns would be suitable for a majority of separations.

Column Efficiency Generated per Unit Time. Figure 3 shows the effect that temperature and retention have on performance for each of the four columns. The contours give the system efficiency generated in 1 s as a function of temperature and retention factor. *The first conclusion is that at any given retention factor, an increase in temperature will decrease the time needed to generate a given efficiency.* Second, column temperatures of approximately 50, 100, 100, and 160 °C are required for columns B, A, C, and D, respectively, to generate 200 plates in 1 s for a species with a $k' = 5$. Clearly, the 5,3 column requires a lower temperature to give the same efficiency rate.

What Column Diameter Should Be Used? When column diameter is selected to use for the fastest analysis, a balance must be struck between the column volume, system volume, rate of heat transfer, and flow rate. It is evident that the column flow rate needed to produce the same column linear velocity scales with the square of the column diameter.[17] The tradeoff incurred for a reduction in column diameter is an increased fractional contribution of extracolumn broadening to the overall broadening. Figure 4 shows the effect that column diameter has on the flow rate (*y*-axis, right) and on the ratio of the system efficiency to the column efficiency (*y*-axis, left).

The flow rates for conventional column diameters are prohibitive due to two factors. First, the flow rate limit of most pumps is 5 mL/min or less; second, the length of heater tubing needed to heat the eluent to the column temperature would dominate column band broadening for shorter columns. The calculation keeps the length of tubing constant at 45 cm, which is sufficient to heat most RPLC eluents in an oil bath at 5 mL/min from 25 to 195 °C. Although the 4.6-mm-i.d. columns show only a small loss in efficiency, the actual loss of plates would be much greater because, as temperature is increased, the length of required preheater tubing greatly exceeds 45 cm so the eluent would not be at column temperature and thermal mismatch broadening would dominate. Based on our prior work,[17] thermal mismatch broadening is a very serious form of broadening and must be avoided. *The plot shows that conventional bore columns (4.6-mm i.d.) are less suitable for fast LC.*

Similarly, the use of 1-mm-i.d. columns is not recommended with conventional LC systems because column efficiency is eroded

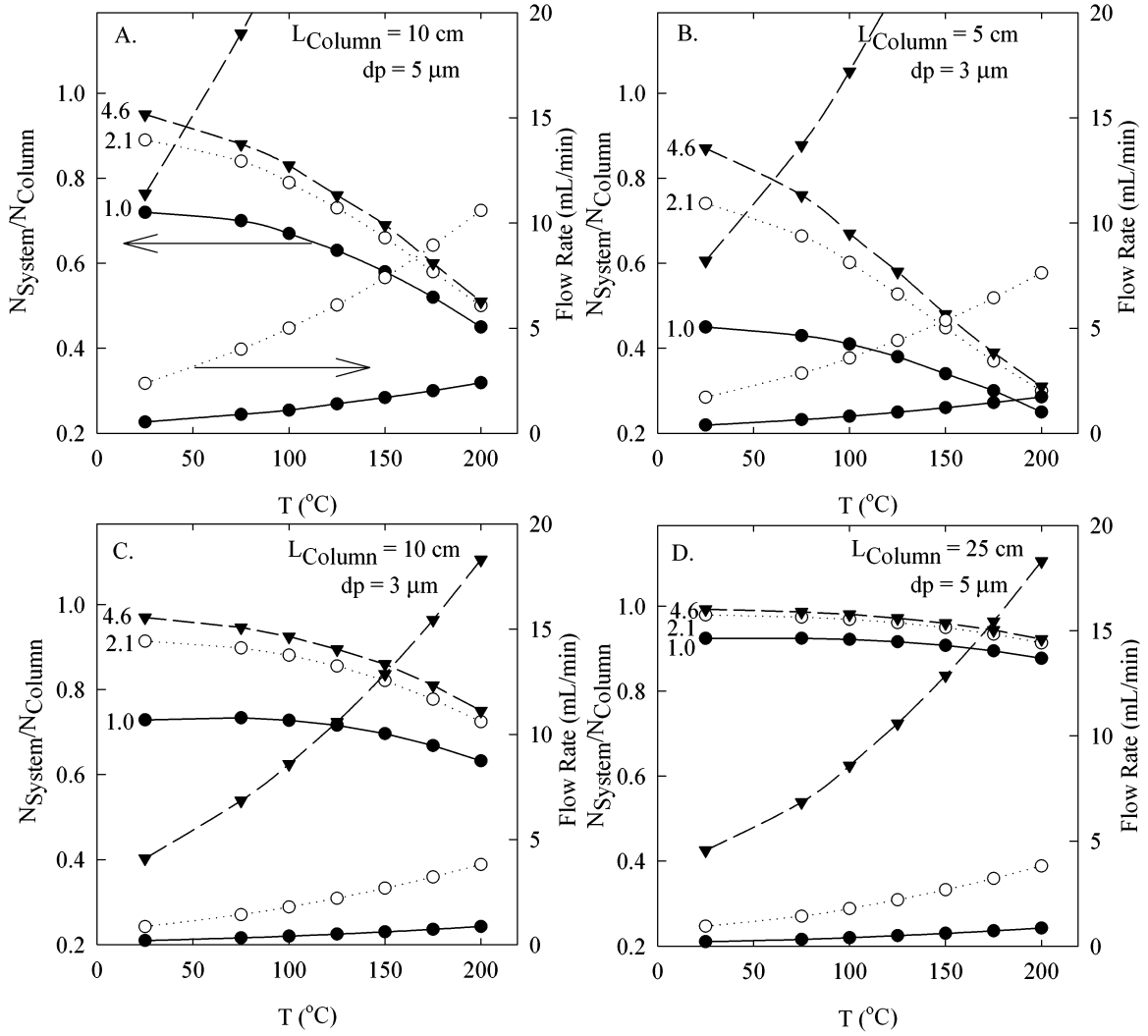

Figure 4. Role of column diameter in determining the efficiency loss due to the extra column (N_{System}/N_{Column}) and the flow rate. Tubing length is held constant at 45 cm. All other conditions are identical to those given in Figure 2. Column formats and column diameters (mm i.d.) for 4.6 (▼), 2.1 (○), and 1.0 (●) are given in the plot.

Table 3. Calculated System Parameters for Fast Columns[a]

	column length (cm), particle diameter (μm)							
	10,3		10,5		5,3		25,5	
system requirements	25 °C	200 °C	25 °C	200 °C	25 °C	200 °C	25 °C	200 °C
max L_{tubing}[b] (cm)	28	40	29	38	9	12	128	174
L_{tubing}[c] (cm)	6.2	4.4	6.1	4.6	20.0	15.0	1.8	1.3
max τ[d] (s)	0.1	0.02	0.06	0.01	0.42	0.009	0.21	0.05
τ[e] (s)	1.5	32	4.0	93.0	9.3	215	0.4	8.1

[a] Calculation conditions described in Figure 2. [b] Maximum length of 0.005-in.-i.d. tubing (cm) for a 5% contribution to column broadening (σ). [c] Percentage contribution of a typical length of system and preheater 0.005-in.-i.d. tubing (45 cm). [d] Maximum time constant (s) for a 5% contribution to column broadening (σ). [e] Percentage contribution of a typical detector time constant (0.15 s) to column broadening.

to the point that such columns are unusable. We recommend the use of 2.1-mm-i.d. columns for use with conventional systems because they strike a balance between the flow rate and the efficiency ratio. Comparisons of the plots for all four columns show the expected conclusion that the longer the column length, the less susceptible the column is to extracolumn band broadening.

What System Should Be Used? Table 3 shows the maximum system requirements for a 5% contribution to column broadening (σ^2) for each column format. The first line gives the maximum length of tubing for a 5% contribution to column broadening for each column at 25 and 200 °C. The maximum allowable tubing length will always increase as the column temperature increases due to the improvement in column efficiency with temperature. The longest amount of connecting tubing can be used with a 25,5 column and the minimum length of tubing should be used with the 5,3 column. Again a useful compromise can be struck by using 10,3 and 10,5 columns. The second row shows the percent contribution to column broadening for 45 cm of 0.005-in.-i.d.

tubing. Since the contribution of the tubing to peak variance is proportional to the fourth power of the tubing diameter, we recommend that tubing inner diameter throughout the system be reduced.

The third row shows the maximum time constant for a 5% contribution to column broadening. Clearly, the detector time constant is the limiting factor in achieving high-temperature, high-speed LC because the shortest time constant available on a typical commercially available LC instrument is ~0.15 s. The fourth row shows the percent contribution to column broadening for a detector time constant of 0.15 s. Obviously, the time constant dominates all other contributions to the extra column and limits the use of short columns at high temperature because such columns generate too much efficiency, in too little time.

CONCLUSIONS

Conventional wisdom by and large leads to the conclusion that, to speed up LC, the shortest column should be used at the highest possible volume fraction of organic modifier. The chief conclusion from the results presented here is that conventional wisdom is at best incomplete and at worst incorrect. We conclude that any complete theory of speed must take into account the relationship between retention, temperature, and volume fraction of organic modifier. We have shown that as temperature is increased, a lower volume fraction of organic modifier, not more is needed to speed up HPLC. A highly retentive column should be used to counteract the loss of retention by temperature. Similarly, isothermal separations should use the most retentive (and most selective) column possible as the percent organic is raised. We showed that column formats 5,3 and 10,5 can be used for fast separations with conventional systems requiring low to moderate efficiency. Columns 10,3 and 25,5 should be used at high temperature for highly efficient, subminute separations. We also showed that the efficiency generation rate improves as temperature is increased for any column. Narrow-bore columns (2.1-mm i.d.) strike the right balance between extracolumn broadening, flow rate, and required heat-transfer tubing. In conclusion, major improvements in detector time constants are needed before short columns (<5 cm) can be used at high temperature.

ACKNOWLEDGMENT

The authors acknowledge the financial support of the National Institutes of Health. We are also thankful to ZirChrom Separations Inc. for the donation of the C-ZrO_2 stationary phase. We acknowledge the helpful comments by Jason Le Prec and Mark Vitha.

Received for review December 14, 2001. Accepted April 18, 2002.

AC0112622