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Low Thermal Mass Liquid Chromatography

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A novel technique, low thermal mass liquid chromatography (LTMLC), is introduced in this study. The use of an LTM assembly that utilizes the principle of resistive wire heating and a temperature sensor to accurately deliver unprecedented heating (up to 1800 °C/min) or cooling (100∼200 °C/min) rates is reported. With the use of packed microcolumns (<0.5 mm i.d.), essentially instantaneous heat transfer from the assembly to the mobile phase was obtained. A systematic investigation was conducted to study the performance of the LTMLC technique. Both isocratic and gradient mobile phase conditions were used. For temperature control, isothermal, temperature-increasing, and temperature-decreasing gradients were applied. Three model mixtures, two of which containing neutral and acidic analytes and the other containing neutral, acidic, and basic analytes, were used to study the effect of temperature on elution time, resolution, column efficiency, and selectivity. It was found that the LTMLC experimental setup delivered reliable temperature control, as evidenced by linear van't Hoff plots for neutral and acidic compounds. The effect of temperature on the elution of basic analytes yielded nonlinear van't Hoff plots, explaining the dramatic selectivity changes observed for bases with changes in column temperature. Column efficiency generally increased with the increase in column temperature in the range of 25∼75 °C and decreased in the range of 75∼150 °C at a fixed column flow rate (3 μL/min), when extra column band broadening was taken into account. The increase in efficiency upon the increase in column temperature in the low temperature range was mainly due to the decreased mass transfer term resulting from increased analyte diffusivity. However, under even higher temperatures, the longitudinal diffusion dominated band broadening, explaining the decrease in column efficiency upon a further increase in column temperature. Resolution and selectivity decreased at elevated temperature for neutral and acidic compounds. For mixtures that contain bases, improved resolution was obtained by simultaneously tuning temperature and solvent programming. In addition to heating ability, LTMLC also demonstrated reliable cooling capability, allowing performance of oscillated or cycled temperature programming for fine-tuning the separation of critical band pairs for the first time. Finally, ultrafast reproducible LTMLC was also demonstrated, showing the potential of utilization of this technology for fast and ultrafast separations.

Among the current trends of development in chromatographic analysis, increased speed and high throughput continue to generate interest. The need for faster analyses using liquid chromatography (LC) is evident for drug discovery, for use as the second dimension for comprehensive multidimensional LC and for improvement of analytical productivity in routine industrial laboratories. Current interests have centered in fast LC separations (up to 5 min analysis) and ultrafast LC separations (up to 1 min analysis) which raise new challenges for separation scientists.[1−3] Because of the inherent lower diffusion coefficients of analytes in liquids when compared to gases or supercritical fluids, the interstitial linear flow velocity used in conventional LC is typically lower (in the range of ∼0.1∼1.5 cm/s), when compared to gas chromatography (GC) (2∼10 cm/s).[4] More importantly, the practical limitation of column back pressure prevents the use of faster linear flow velocity in liquid chromatography.

Under typical operating conditions, the van Deemter equation [$H = A + B/u + Cu$, where the plate height H is the sum of the A term (flow multipath band broadening), the product of the C term (the resistance to mass transfer) and mobile phase linear velocity u, and the longitudinal term B/u, which is negligible under the condition of fast linear velocity], suggests that a decrease in the C term must be sought before an increase in the linear velocity, u, in order to keep plate height, H, lower. Currently, several approaches have been explored to decrease the C term in order to attain fast separations. Examples are the use of sub-2 μm particles[5,6] (C is inversely proportional to the square of the particle diameter), monolithic columns[7] (inherent smaller C term

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(1) Nguyen, D. T.-T.; Guillarme, D.; Rudaz, S.; Veuthey, J.-L. *Chimia* **2007**, *61*, 186–189.

(2) Stoll, D. R.; Li, X.; Wang, X.; Carr, P. W.; Porter, S. E. G.; Rutan, S. C. *J. Chromatogr., A* **2007**, *1168*, 3–43.

(3) Colon, I.; Gonzalez, J. R.; Richoll, S. M. *Am. Lab.* **2006**, *38*, 20–26.

(4) Guiochon, G. *Anal. Chem.* **1980**, *52*, 2002–2008.

(5) Dong, M. W. *LCGC North Am.* **2007**, (Suppl.), 89–98.

(6) Mazzeo, J. R.; Neue, U. D.; Kele, M.; Plumb, R. S. *Anal. Chem.* **2005**, *77*, 460A–467A.

(7) Siouffi, A.-M. *J. Chromatogr., A* **2006**, *1126*, 86–94.

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due to the continuous-rod property of the column), shell particles[8,9] (also a reduced C term due to the partially porous features of the particles), and the use of high temperature[10−13] (increased analyte diffusion coefficients resulting in smaller C values).

Of the approaches mentioned above for achieving fast separations, the use of elevated temperature is of particular interest due to the increase in the analyte diffusion coefficient and lowered mobile phase viscosity resulting in lower column back pressure. Thus, it is practically attractive to use elevated temperature to attain fast separations. A systematic theoretical study on the effect of increased temperature for the separation of high-molecular weight analytes showed that an order of magnitude decrease in analysis time could be achieved at 150 °C when compared to 25 °C, if column pressure, length, particle diameter, and retention factor are fixed.[14] In addition, the column plate number increased 1.5∼2 times over that at room temperature. Because the retention process is an equilibrium process, raising temperature will change the process equilibrium constant, depending on the process enthalpy change. For the retention process in reversed-phase LC, raising the temperature decreases the retention of an analyte because the enthalpy change is typically negative (retention is generally an exothermic process). This effect reduces the analysis time further, provided that other separation variables are fixed. If the benefits of the decrease in analysis time and increase in column efficiency are at the expense of resolution, temperature would still not be considered a good variable to achieve fast analysis. Fortunately, a series of studies have shown that temperature is a very powerful variable for tuning selectivity in reversed-phase LC for a large variety of samples, thus yielding better resolution.[15−21]

Temperature has long been neglected as an optimization variable in LC until recently.[15] The main obstacles for its use as a variable in LC are the availability of thermally stable stationary phases and instrumentation capable of temperature control for both isothermal and temperature gradient runs. With the increasing interest in the utilization of temperature in LC, temperature stable particles (some can be used up to 200 °C) have become commercially available. The maximum operating temperatures, bonding phase chemistry, selectivity as well as the column providers was compiled in two recent review articles.[10,11] A commercial instrument dedicated to high temperature LC (HTLC) was also recently introduced.[24] Still, further research is needed to provide thermally stable particles with similar selectivities as conventional C-18 silica particles for wider acceptance of HTLC.

When conventional-size columns are used in HTLC, thermal mismatch (thermal gradient along the radius of the column caused by cooler incoming mobile phase and hotter column wall) can result in significant band broadening and skewed or split peaks.[22,23] To alleviate or eliminate thermal mismatch, preheating of the mobile phase is required. Traditional methods involve the housing of small i.d. tubing between the injector and the column in the column oven compartment.[24] Recently, an active mobile phase preheating module which can be operated independently from the column oven became commercially available.[10,25] Special instrumentation is required to perform HTLC or temperature programming LC for conventional-size columns. In contrast, less constraints are encountered when using microcolumns (capillary columns with i.d. <0.5 mm) because the thermal mismatch is greatly alleviated or even negligible.[13] This observation simplifies the instrumentation for temperature programming in microcolumn LC because the requirement is reduced to the control of the column temperature alone. A GC oven is well suited for this purpose, due to the excellent reproducibility in temperature control. For conventional-size columns, the maximum heating rate is 30 °C/min while the maximum cooling rate is 42 °C/min (Selerity Technology Web site, http://www.selerity.com). For capillary columns using a GC oven, the maximum heating rate is ∼100 °C/min and for cooling, 25 °C/min.[26] In order to fully explore the effect of temperature programming on LC separations, an increased rate of heating or cooling is desirable. Recently, a novel design of a GC heating module (termed low thermal mass GC or LTMGC) was introduced, which is based on the principle of resistive wire heating.[27,28] Because of the low thermal mass of the module (composed essentially of the heating wire, resistive temperature detector, capillary column, insulation fiber, and thin aluminum wrapping), an unprecedented heating rate of 1800 °C/min and cooling rate of >400 °C/min were achieved for an open tubular capillary GC column (100 μm i.d. × 2 m long).[26] Another advantage of LTMGC is the lower power consumption, which is approximately 1% of that of a conventional GC oven. More importantly, LTMGC demonstrated good repeatability, with retention time precision (RSD) of 0.04% and area counts (RSD) of <2%.[26]

With the successful implementation of LTMGC, it seemed appropriate to investigate the extension of the concept to LC. Low thermal mass liquid chromatography (LTMLC) refers to the combination of the LTM heating module and the use of packed capillary columns with i.d. smaller than 0.5 mm, both of which have low thermal masses. In addition, in order to reduce the mass to facilitate heating or cooling, capillary columns with protective sheaths (metal or polymer) were not considered. This additional

(8) Kirkland, J.; Truszkowski, F. A.; Ricker, R. D. *J. Chromatogr., A* **2002**, *965*, 25–34.
(9) Wang, X.; Barber, W. E.; Carr, P. W. *J. Chromatogr., A* **2006**, *1107*, 139–151.
(10) McNeff, C.; Yan, B.; Stoll, D. R.; Henry, R. A. *J. Sep. Sci.* **2007**, *30*, 1672–1685.
(11) Vanhoenacker, G.; Sandra, P. *J. Sep. Sci.* **2006**, *29*, 1822–1835.
(12) Greibrokk, T.; Andersen, T. *J. Chromatogr., A* **2003**, *1000*, 743–755.
(13) Greibrokk, T.; Andersen, T. *J. Sep. Sci.* **2001**, *24*, 899–909.
(14) Antia, F. D.; Horváth, C. *J. Chromatogr., A* **1988**, *435*, 1–15.
(15) Dolan, J. W. *J. Chromatogr., A* **2002**, *965*, 195–205.
(16) Dolan, J. W.; Lommen, D. C.; Snyder, L. R. *J. Chromatogr.* **1990**, *535*, 55–74.
(17) Zhu, P. L.; Snyder, L. R.; Dolan, J. W.; Djordjevic, N. M.; Hill, D. W.; Sander, L. C.; Waeghe, T. J. *J. Chromatogr., A* **1996**, *756*, 21–40.
(18) Zhu, P. L.; Dolan, J. W.; Snyder, L. R. *J. Chromatogr., A* **1996**, *756*, 41–50.
(19) Castells, C. B.; Gagliardi, L. G.; Ràfols, C.; Rosés, M.; Bosch, E. *J. Chromatogr., A* **2004**, *1042*, 23–35.
(20) Gagliardi, L. G.; Castells, C. B.; Ràfols, C.; Rosés, M.; Bosch, E. *J. Chromatogr., A* **2005**, *1077*, 159–169.
(21) Pous-Torres, S.; Torres-Lapasió, J. R.; Baeza-Baeza, J. J.; García-Álvarez-Coque, M. C. *J. Chromatogr., A* **2007**, *1163*, 49–62.
(22) Wolcott, R. G.; Dolan, J. W.; Snyder, L. R.; Bakalyar, S. R.; Arnold, M. A.; Nichols, J. A. *J. Chromatogr., A* **2000**, *869*, 211–230.
(23) Thompson, J. D.; Brown, J. S.; Carr, P. W. *Anal. Chem.* **2001**, *73*, 3340–3347.
(24) Jones, B. A. *J. Liq. Chromatogr. Relat. Technol.* **2004**, *27*, 1331–1352.
(25) Holm, A.; Molander, P.; Lundanes, E.; Greibrokk, T. *J. Sep. Sci.* **2003**, *26*, 1147–1153.
(26) Luong, J.; Gras, R.; Mustacich, R.; Cortes, H. J. *J. Chromatogr. Sci.* **2006**, *44*, 253–261.
(27) Mustacich, R.; Richards, J.; Everson, J. U.S. Patent 6,530,260, 2003.
(28) Mustacich, R.; Everson, J. U.S. Patent 6,682,699, 2004.

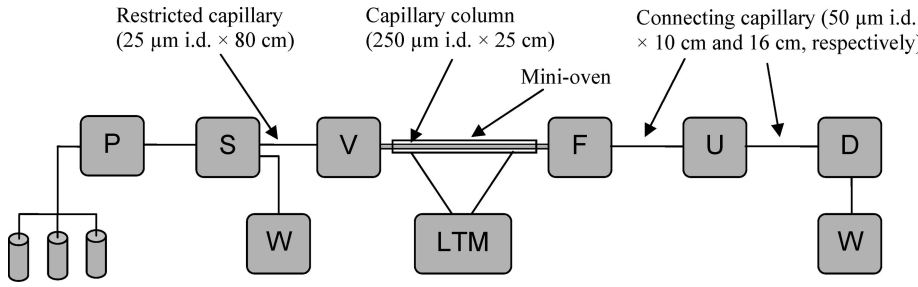

Figure 1. Schematic diagram of LTMLC experimental setup: P, pump; S, splitter; W, waste; V, injection valve; LTM, low thermal mass controller; F, inline filter, also served as column outlet retaining frit; U, union; and D, detector.

requirement excludes the use of most commercially available capillary columns.[29] In this study, a systematic evaluation of the effect of temperature on efficiency, resolution, speed of analysis, and selectivity under LTMLC conditions is presented. Both isocratic and gradient elution with the combined use of isothermal, temperature-increasing, and temperature-decreasing gradients were used. Neutral, acidic, and basic compounds were used as model analytes to explore the temperature effects under LTMLC conditions.

EXPERIMENTAL SECTION

Chemicals and Reagents. Three model mixtures were used to conduct the LTMLC experiments. Mixture I (neutral and acidic) contained uracil, benzoic acid, 2,4-dichlorophenoxy acetic acid, 4-phenylphenol, ethylbenzoate, benzophenone, naphthalene, and 4-hexylbenzoic acid. Mixture II (neutral, acidic, and basic) was composed of uracil, diphenhydramine hydrochloride, nortriptyline hydrochloride, benzoic acid, dimethyl phthalate, sulfinpyrazone, terfenadine, and 4-phenylphenol, respectively. Mixture III was the same as mixture I except that 2,4-dichlorophenoxy acetic acid and ethylbenzoate were not included. All of the chemicals were purchased from Sigma-Aldrich (Milwaukee, WI). HPLC grade acetonitrile was obtained from J. T. Baker (Phillipsburg, NJ). Water was purified by running deionized water through an EASYpure UV Ultrapure Water System (Barnstead, Dubuque, IA). Spectrophotometric grade trifluoroacetic acid (TFA, Sigma) was used as a mobile phase additive. Phosphoric acid (85 wt %, Sigma) and potassium dihydrogen phosphate (Fisher Scientific, Fair Lawn, NJ) were used to prepare buffered mobile phases.

Capillary Column Packing. A Mini Microfilter (M537, Upchurch, Oak Harbor, WA) was used as the retaining frit for the column packing and column end-fitting. One end of the Microfilter was connected to a fused silica capillary (250 μm i.d., 360 μm o.d., 0.8 m long, Polymicro Technology, Phoenix, AZ) that was also connected to a packing reservoir, while the other end of the filter was connected to a restricted capillary (50 μm i.d., 360 μm o.d., 1 m long) to control the packing speed. The slurry in the packing reservoir was composed of 10 w/v % of packing material in acetone/hexane (1:2 v/v ratio). The packing materials used were Pinnacle II C-18 (5 μm, 110 Å pore-size, Restek, Bellefonte, PA) and Zorbax SB-C18 (5 μm, 80 Å). The Zorbax particles were obtained by unpacking an analytical-size Zorbax column (Agilent, Santa Clara CA). The upper temperature limits for the Pinnacle II C-18 and Zorbax SB-C18 are 80 and 90 °C, respectively, based on the vendors' product specification. However, for demonstration purposes, up to 150 °C was used for a short period of time (e.g., 10 min). No significant decrease in retention factor or column efficiency was observed in the experiments. However, long-term stability studies of the stationary phases were not conducted. An ISCO model 100 DM syringe pump (Isco Inc., Lincoln, NE) was used to pack the capillary column. The packing solvent was the same as the slurry solvent. The packing was accomplished with a pressure gradient that started at 1000 psi (held for 1 min) and ramped to 5000 psi in 20 min. After packing, the capillary was depressurized from 5000 psi to atmospheric pressure in 60 min.

Instrumentation. The experimental setup for LTMLC experiments is shown in Figure 1. A conventional LC pump (Hitachi L6200, Tokyo, Japan) was used to deliver mobile phase at various flow rates. An Acurate flow splitter with a split ratio of ~1:100 (LC Packings, San Francisco, CA) was used to deliver flow rates in the microliters per minute range. The column flow rate was measured by weighing the mobile phase delivered in a given period of time. The flow rate was calculated using the mobile phase density. Samples were introduced using an electronically actuated internal sample injector with a rotor volume of 20 nL (VICI, Houston, TX). The capillary column was threaded through the LTM assembly before connection to the injection valve. No inlet retaining frit was used in order to decrease extra column band broadening. The capillary column outlet retaining frit was made up of an inline filter (M537, Upchurch). The capillary column was connected to a UV detector (Spectroflow 757, ABI Analytical, Foster City, CA) through a zero dead-volume union (P720, Upchurch Scientific). The capillary flow cell used in the detector was 45 nL, 10 mm path length, 201LCP91 (LC Packings). A capillary column temperature control module LTM A68 (RVM Scientific, Santa Barbara, CA) was used for temperature control. It was composed of an aluminum tube (0.5 mm i.d., 0.55 mm o.d., 20 cm long), surrounded by a nickel resistive wire, resistive temperature detector, an insulating fiber, and controlled by the LTM A68 controller. The upper temperature limit of the LTM A68 controller was 400 °C. Temperature camping as fast as 1800 °C/min or −1800 °C/min can be achieved by the controller. However, the actual heating or cooling rate was limited by the LTM assembly (i.e., depending on column i.d. and length). In the current experimental setup, the maximum heating and cooling rates were approximately 1800 or 100~200 °C/min, respectively. The LTM assembly is also equipped with a fan to expedite cooling, but this capability was not used in the current work as cooling rates faster than 100−200 °C were deemed unnecessary for the

(29) Prüss, A.; Kempter, C.; Gysler, J.; Jira, T. *J. Chromatogr., A* **2004**, *1030*, 167–176.

Table 1. Comparison of Column Efficiency for Model Mixture I under Isocratic and Isothermal Conditions

	25 °C	50 °C	75 °C	100 °C	125 °C	150 °C
	Column Efficiency (Plates) with the Same Column Flow Rate (3 μL/min)					
uracil	12 300	13 200	14 900	12 500	11 500	9 100
benzoic acid	9 300	10 000	11 400	9 800	8 100	9 200
2,4-D	7 800	9 000	8 900	8 500	7 000	6 100
4-phenylphenol	10 000	9 800	9 100	9 000	7 700	6 700
ethylbenzoate	11 300	11 500	10 900	9 700	7 400	6 300
benzophenone	11 300	11 700	10 900	10 000	7 900	6 300
naphthalene	9 700	8 800	7 500	6 600	4 600	4 000
4-hexylbenzoic acid	8 600	8 300	7 200	5 300	3 800	3 500
	Column Efficiency (Plates) with Column Flow Rate Uncontrolled					
flow rate (μL/min)	3.00	3.68	4.26	4.82	5.34	5.78
uracil	12 300	11 700	11 100	9 200	8 900	4 900
benzoic acid	9 300	10 900	11 400	9 800	7 100	6 200
2,4-D	7 800	7 500	8 000	6 700	5 500	4 400
4-phenylphenol	10 000	8 900	7 700	6 700	4 700	4 600
ethylbenzoate	11 300	11 200	10 100	8 200	5 700	5 000
benzophenone	11 400	10 800	8 700	6 800	5 300	3 900
naphthalene	9 700	8 900	7 300	5 500	4 000	3 200
4-hexylbenzoic acid	8 600	7 800	5 600	4 900	3 000	2 600

[a] Experimental conditions: column, 250 μm × 25 cm, Restek Pinacle II C-18, 5 μm particle size; mobile phase, 60% acetonitrile containing 0.1% trifluoroacetic acid; injection volume, 20 nL; UV detection wavelength, 220 nm; analytes, model mixture I consisting of uracil, benzoic acid, 2,4-dichlorophenoxy acetic acid, 4-phenylphenol, ethylbenzoate, benzopheneone, naphthalene, and 4-hexylbenzoic acid, respectively; column temperature, various temperatures were applied. The pump master flow rate was set at 0.4 mL/min, which resulted in column flow rates of 3.00, 3.68, 4.26, 4.82, 5.34, and 5.78 μL/min at column temperature of 25, 50, 75, 100, 125, and 150 °C, respectively. If the same column flow rate (e.g., 3 μL/min) was used, the pump master flow was adjusted to set at 0.400, 0.326, 0.282, 0.249, 0.225, and 0.208 mL/min at column temperatures of 25, 50, 75, 100, 125, and 150 °C, respectively.

current work. Compared to LTMGC, the LTMLC module is somewhat different. While in GC the capillary is wound together with resistive heating wires, temperature resistive sensor, and insulating fiber to form a torus, the capillary column in LC was threaded through the aluminum tubing. Temperature control was obtained by precisely controlling the aluminum tubing temperature. The LTMLC design was more flexible and convenient in that different capillary columns can be used with the same LTM assembly design as long as the o.d. of the capillary column is smaller than the i.d. of the aluminum tubing.

Data were acquired using the Atlas v8.2 Chromatography data system (Thermo Electron, Waltham, MA). Chromatograms were redrawn using Origin (OriginLab, Northampton, MA).

Extracolumn Volume and Gradient Dwell Volume Measurement. Because two sections of connecting capillaries and a union (see Figure 1) are used in the setup, the extracolumn volume and extracolumn band broadening were measured according to procedures reported elsewhere with slight modification.[30] A short piece of capillary (25 μm × 9 cm) was used to replace the capillary column, and naphthalene was injected at various column flow rates and detected at 220 nm to estimate extracolumn volume and band broadening. The extra column volume was determined to be 1.1 μL, independent of the column flow rates used, as expected. Peak widths (4σ) were measured to be 0.42, 0.58, 0.69, and 0.80 μL at column flow rates of 2.6, 5.5, 8.3, and 11.1 μL/min, respectively.

The gradient dwell volume was determined by replacing the microcolumn with a smaller i.d capillary (25 μm i.d., 50 cm long) and running a linear 10 min AB gradient, where mobile phase A was water and B was water containing 0.2% acetone. The pump flow rate was set at 0.4 mL/min, which resulted in a column flow rate of 6.7 μL/min. On the basis of the gradient output profile at a detection wavelength of 265 nm, the gradient dwell time was calculated to be 2.2 min. Under the current experimental setup, the gradient dwell volume between the gradient mixer and the splitter was calculated to be 0.76 mL and the dwell volume between the splitter and injector was 2 μL.

Retention Factor and Column Efficiency. Because of the extracolumn volume and extracolumn band broadening of the system, the retention factor and column efficiency were corrected for extracolumn contributions using the following equations.

$$t_M = t_{M,meas} - t_{ex}$$
$$t_R = t_{R,meas} - t_{ex}$$
$$t_{ex} = V_{ex}/F$$
$$k = (t_R - t_M)/t_M$$
$$N = 16[t_R^2/(w_{meas}^2 - w_{ex}^2)]$$

where t_M and $t_{M,meas}$ are the corrected and measured retention times of uracil, respectively; t_R and $t_{R,meas}$ are the corrected and measured retention times of analytes, respectively; t_{ex} is the time the analyte spent outside of column, calculated from the ratio of extracolumn volume V_{ex} (1.1 μL in this case), and column flow rate F. k is the retention factor; N is the corrected column efficiency; w_{meas} is the measured peak width of analytes at the baseline; and w_{ex} is the extracolumn bandwidth. As described above, w_{ex} is column flow rate dependent. For various column flow rates, the w_{ex} value was linearly extrapolated from the measured values.

RESULTS AND DISCUSSION

LTMLC of Model Mixture I: Isocratic and Isothermal Conditions. With the use of a 250 μm i.d × 25 cm column packed with the Restek Pincale II C-18 particles, an isocratic run of the

(30) Majors, R. E. *LCGC Asia Pacific* **2004**, *7*, 1124–1133.

model mixture I under various column temperature conditions controlled by the LTM assembly were conducted (refer to Table 1 for experimental conditions). Visual examination of the chromatograms obtained revealed that temperature of 125 °C could be used for the current experimental setup. When 150 °C was applied however, the baseline became unstable. Note that no preheating of the incoming mobile phase, cooling of outlet mobile phase before the detector, or pressure restrictor after the detector were used in the instrumentation. Because of the low mass of the capillary column as well as low flow rates used, mobile phase preheating was not required.

The empirical expression for heat transfer inside a tubing is as follows.[22]

$$\log\left(1-\frac{T_2-T_1}{T_3-T_1}\right)=-B\left(\frac{L}{F}\right)$$

where T_1, T_2, and T_3 are the column inlet, outlet, and oven temperatures, respectively, in Kelvin; B is a constant (for a circulated oven, B is approximately 0.025 cm^2/min); L is column length in centimeters; and F is the column flow rate in milliliters per minute. Under typical column flow rate of 3∼6 μL/min, if we assume $T_1 = 25$ °C, $T_3 = 150$ °C, and $T_2 = 145$ °C, a simple calculation reveals that the column length L required to preheat the mobile phase from 25 to 145 °C in a circulated air oven of 150 °C is 0.17−0.34 cm. A similar calculation under an extreme case of 300 °C (T_1 = 25 °C, T_3 = 295 °C, and T_2 = 300 °C) resulted in the length of 0.21−0.42 cm. These calculations indicate that no obvious thermal lag is expected when performing capillary LC experiments.

Under isocratic and isothermal conditions, the analyte retention should follow the van't Hoff equation, which has the following expression.

$$\ln k=-\frac{\Delta H}{R}\frac{1}{T}+\frac{\Delta S}{R}+\ln\varphi$$

where k is the retention factor; ΔH and ΔS are the enthalpy and entropy of the retention process, respectively; T is the absolute temperature; R is the universal gas constant; and ϕ is the phase ratio (the stationary to mobile phase volume ratio). The van't Hoff plot obtained under LTMLC conditions yielded straight lines for all the analytes with correlation coefficients greater than 0.998. From the slopes of these lines, the ΔH was estimated to be −6∼11 kJ/mol, which is in agreement with the literature values for small molecules.[12] This indicated the efficient temperature control of the LTM module. It should be noted that the linear plots were obtained only when extracolumn volume was taken into consideration for the calculation of retention factors. Column flow rates increased with the increase in column temperature due to the passive splitter used. However, because the retention factor is independent of the column flow rate, it was unnecessary to maintain the same column flow rate for the purpose of calculating retention factors.

The resolution for the critical band pair (ethylbenzoate and benzophenone) decreased from 3.3 to 1.0 when temperature increased from 25 to 125 °C. For other band pairs, resolution also decreased with increasing temperature. This behavior was typically observed for neutral or acidic analytes, as repeatedly reported in the literature. For basic analytes, however, the effect of temperature on selectivity was dramatic (see next section).

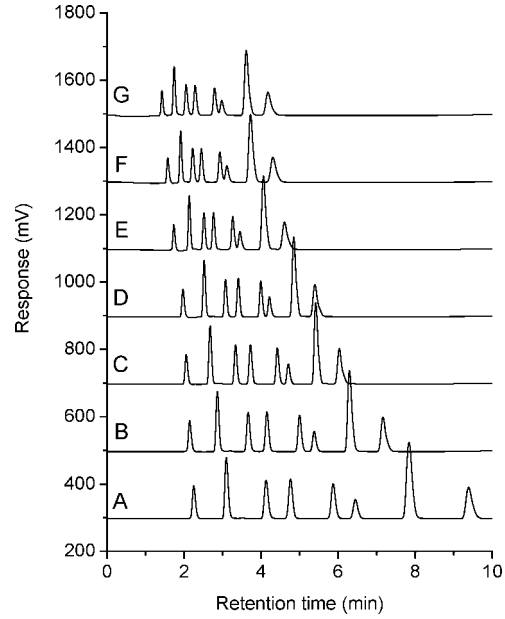

Figure 2. LTMLC of model mixture I under isocratic and thermal gradient conditions. Conditions: the same as shown in Table 1 where the column flow rate was not controlled and the pump master flow rate was set at 0.4 mL/min except that thermal gradients of 6 (A), 12 (B), 18 (C), 24 (D), 50 (E), 100 (F), and 1800 (G) °C/min from 25 to 125 °C were applied. At the end of the thermal gradient, the temperature was held at 125 °C until all analytes were eluted. Note that the column flow rate was changed during chromatographic runs due to the passive splitter used.

The column efficiency under LTMLC conditions was also examined, and results are listed in Table 1. When the same column flow rate (3 μL/min) was used, the column efficiency increased in the range of 25∼75 °C and decreased upon further increase in column temperature in the range of 75∼150 °C. It is believed that the dominant band broadening was from the mass transfer resistance in the low temperature range while longitudinal diffusion became predominant at higher temperatures, as has been reported previously.[11,31−33]

LTMLC of Model Mixture I: Isocratic and Thermal Gradient Conditions. The utility of the LTMLC lies in the capability to achieve fast temperature programs. Figure 2 represents an isocratic separation under temperature increasing programs at various programming rates, from 6 to 1800 °C/min. Compared to the isothermal separations, peaks are sharper and the decrease in resolution was less drastic. The resolution between the critical band pair (ethylbenzoate and benzophenone) was 1.2 even under the fastest temperature programming rate. The decreases in retention time of uracil, which reflects the column flow rate changes, indicated that essentially instantaneous heat transfer from the LTM assembly to the capillary column was achieved. In addition to heating, the LTMLC system was also able to provide fast cooling programs. Figure 3 represents the overlay of the LC separations under temperature decreasing program conditions.

(31) Andersen, T.; Nguyen, Q. T.; Trones, R.; Greibrokk, T. *J. Chromatogr., A* **2003**, *1018*, 7–18.
(32) Sheng, G.; Shen, Y.; Lee, M. L. *J. Microcolumn Sep.* **1997**, *9*, 63–72.
(33) Yang, Y. *Anal. Chim. Acta* **2006**, *558*, 7–10.

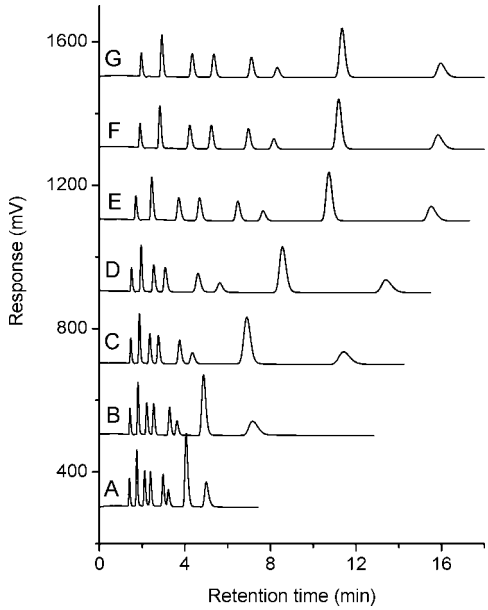

Figure 3. LTMLC of model mixture I under isocratic and temperature decreasing gradient conditions: Experimental conditions were the same as in Figure 2 except that a temperature decreasing gradient of 6 (A), 12 (B), 18 (C), 24 (D), 50 (E), 100 (F), and 200 (G) °C/min from 125 to 25 °C was applied. At the end of the gradient, the temperature was held at 25 °C until all analytes were eluted from the column.

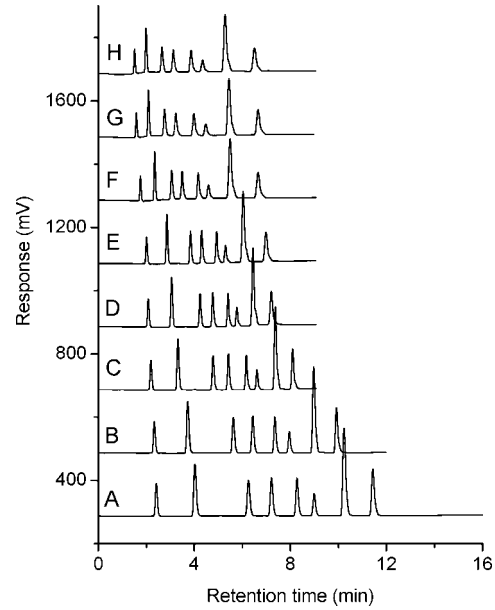

Figure 4. LTMLC of model mixture I under solvent gradient and temperature increasing gradient conditions. Mobile phase A was water containing 0.1% TFA and B, acetonitrile containing 0.1% TFA; linear gradient from 50 to 95% B in 15 min; temperature increasing gradients of 3 (A), 6 (B), 12 (C), 18 (D), 24 (E), 50 (F), 100 (G), and 200 (H) °C/min from 25 to 125 °C, respectively. All other conditions were the same as in Figure 2.

The LTMLC oven could sense the difference between the 100 and 200 °C/min cooling rates by monitoring the retention times of uracil. Note that no cryogen was used. The cooling of the LTM assembly and thus the capillary column was attained by the heat dissipation to the laboratory environment. The resolution for the critical band pair, as expected, increased from 1.3 to 3.3 when the temperature decreasing program rates of 6 and 200 °C/min from 125 to 25 °C were applied.

LTMLC of Model Mixture I: Simultaneous Solvent and Thermal Gradient Conditions. The same sets of temperature programming conditions (isothermal, temperature increasing, and temperature decreasing) were applied to model mixture I under linear solvent gradient conditions. A similar effect of temperature on resolution was obtained. Figure 4 represents an example of LTMLC experiments under simultaneous solvent and temperature increasing program conditions. When both solvent and temperature gradients were used, the solvent gradient had a greater effect on analyte retention than the temperature program.

LTMLC of Model Mixture II. The decrease in resolution for neutral and acidic compounds is undesirable when elevated temperature or temperature increasing programs are applied. For basic analytes, however, temperature might not only shorten analysis time but also increase the resolution. Figure 5 represents LTMLC of model mixture II under isocratic and isothermal conditions. A small change in temperature (from 25 to 50 °C) dramatically improved resolution for some band pairs. The van't Hoff plot for model mixture II is presented in Figure 6. For acidic and neutral analytes (benzoic acid, dimethylphthalate, and 4-phenylphenol), straight lines were obtained as expected. For all basic analytes (diphenhydramine, nortriptyline, sulfinpyrazone, and terfenadine), nonlinear van't Hoff plots were obtained, indicating that the enthalpy change ΔH is temperature dependent or that a mixed-mode retention mechanism existed. The nonlinear van't Hoff plots for bases were believed to be due to the change of buffer acid dissociation constant pK_a or the change of analyte dissociation constant pK_a or both upon the change of column temperature.[19,20,34–36]

Examination of the van't Hoff plots for band pair benzoic acid and nortriptyline revealed that they crossed at temperatures 25 and 100 °C. In the temperature range between these two temperatures, the retention of nortriptyline was slightly greater than that of benzoic acid suggesting that an optimum temperature could be obtained to maximize the resolution if this band pair was of interest. A similar strategy could be applied to optimize the separations between sulfinpyrazone, 4-phenylphenol, and terfenadine. This example demonstrated that temperature is a powerful variable for selectivity tuning of basic analytes.

Temperature can also have a greater effect on the selectivity change under solvent gradient programming. Figure 7 represents the separation of model mixture II under solvent gradient and isothermal conditions. For band pairs 7 and 8, the elution order was reversed and resolution improved upon the increase of temperature from 25 to 50 °C. Even better resolution for this band pair was obtained at higher temperature of 75 or 100 °C. For band pairs 6 and 7, they were well separated at 50 °C but coeluted at 100 °C. For band pairs 2 and 3, the increase in temperature decreased their resolution. Overall, numerous changes of selectivity were observed when the simultaneous combination of solvent gradient and temperature were applied.

Figure 8 represents the optimization of the separation of model mixture II under solvent isocratic conditions. The bottom panel

(34) Mao, Y.; Carr, P. W. *Anal. Chem.* **2001**, *73*, 4478–4485.
(35) Buckenmaier, S. M. C.; McCalley, D. V.; Euerby, M. R. *J. Chromatogr., A* **2004**, *1060*, 117–126.
(36) Dolan, J. W. *LCGC North Am.* **2007**, *25*, 448–454.

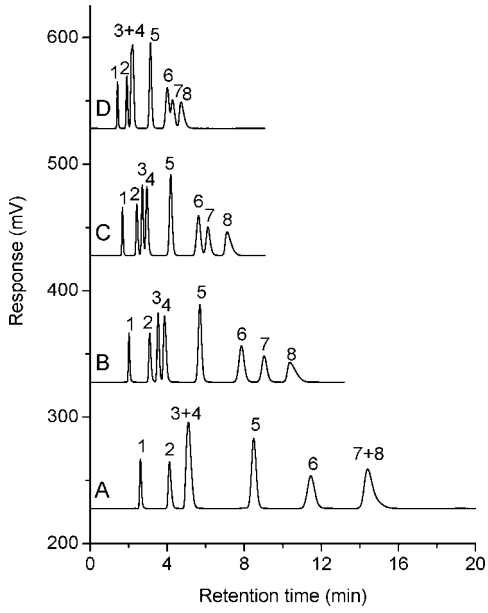

Figure 5. LTMLC of model mixture II under isocratic and isothermal conditions. Chromatographic conditions: column, 250 μm $\times$ 25 cm, Zorbax SB C-18, 5 μm particle size; mobile phase, 35% B isocratically where A was 30% of acetonitrile/70% of 40 mM potassium phosphate, pH 2.30, and B, 75% of acetonitrile/25% of 40 mM potassium phosphate, pH 2.30; injection volume, 20 nL; UV detection wavelength, 220 nm; analytes, model mixture II consisting of uracil (1), diphenhydramine (2), benzoic acid (3), nortriptyline (4), dimethylphthalate (5), sulfinpyrazone (6), 4-phenylphenol (7), and terfenadine (8); column temperature, 25 (A), 50 (B), 75 (C), 100 (D) °C, respectively; pump master flow rate of 0.4 mL/min resulting in column flow rates of 2.51 (A), 3.25 (B), 3.91 (C), and 4.62 (D) μL/min, respectively.

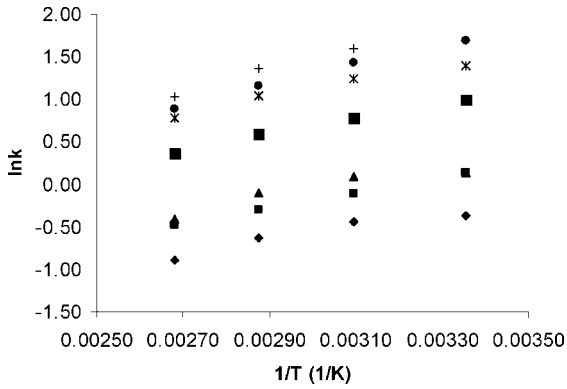

Figure 6. Van't Hoff plots for model mixture II under the conditions shown in Figure 5. From bottom to top, diphenhydramine, benzoic acid, nortriptyline, dimethylphthalate, sulfinpyrazone, 4-phenylphenol, and terfenadine.

shows the separation under isothermal conditions (panel A, 25 °C) where coelution was observed. With the use of a segmented temperature gradient (panel B), baseline separation of all analytes of interest was achieved in a shorter time. Alternatively, an oscillated thermal gradient (panel C) can be applied to optimize the separation. Careful examination on the separations between panel B and C indicates that the first peak was narrower, the resolution between analytes 3 and 4 was better (1.81 vs 1.62), and the run time was shorter in panel C than panel B, although the peak widths were broader for analytes 2,3 and 4. This example demonstrates that the device, capable of providing fast cooling

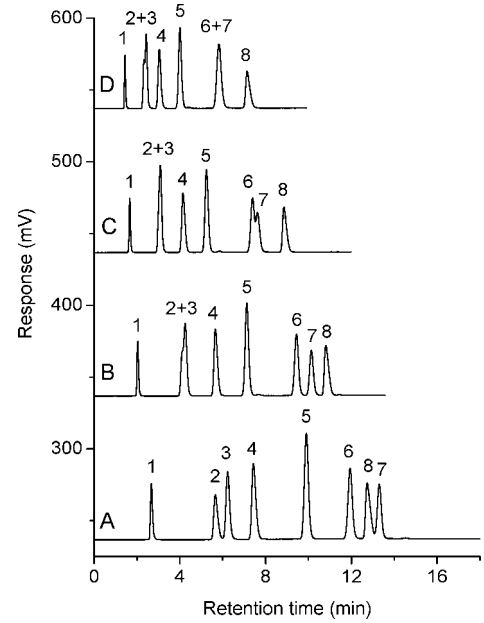

Figure 7. LTMLC of model mixture II under solvent gradient and isothermal conditions. Chromatographic conditions were the same as those in Figure 5 except that a linear 15 min of AB gradient was applied.

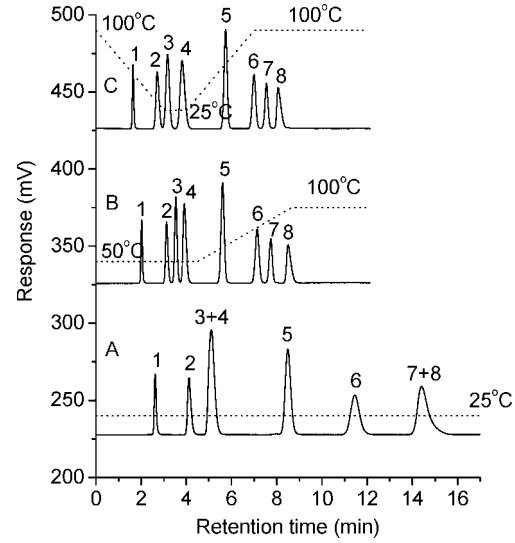

Figure 8. LTMLC of model mixture II under isocratic and different temperature programming conditions. Chromatographic conditions were the same as those in Figure 5 except that different temperature programming was applied. The temperature programming was indicated as a dotted line in the figure where panel A operated isothermally at 25 °C, panel B used a segmented gradient (50 °C isothermal for 4.5 min and 12 °C/min to 100 °C and final hold at 100 °C for 3.5 min), and panel C applied an oscillated gradient (25 °C/min from 100 to 25 °C, followed by a 1 min hold at 25 °C, then ramped at 25 °C/min from 25 to 100 °C, and a final hold at 100 °C for 5 min).

rates (e.g., 25 °C/min in this example), offers another possibility to fine-tune the separation.

Ultrafast LTMLC of Model Mixture III. Under fast temperature program conditions (e.g., 100 °C/min), temperature increased from 25 to 125 °C in about a minute. Some separations under fast temperature increasing program conditions as demonstrated earlier were essentially performed under isothermal

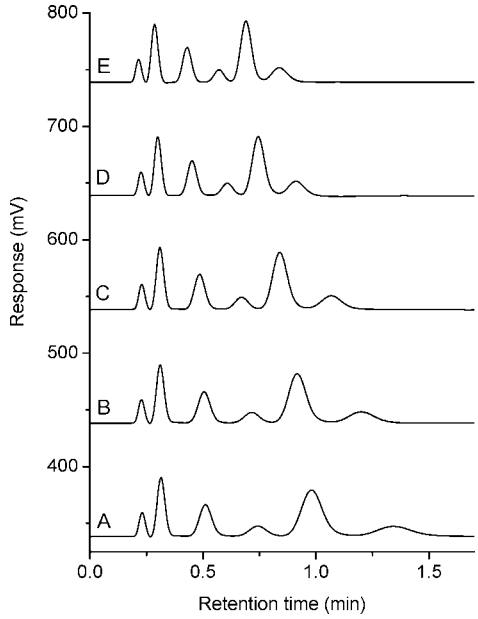

Figure 9. Ultrafast LTMLC of model mixture III. Chromatographic conditions: column, 250 μm $\times$ 8 cm, Zorbax SB C-18, 5 μm particle size; mobile phase, 60% acetonitrile containing 0.1% trifluoroacetic acid; injection volume, 20 nL; UV detection wavelength, 220 nm; analytes, model mixture III consisting of uracil, benzoic acid, 4-phenylphenol, benzopheneone, naphthalene, and 4-hexylbenzoic acid, respectively; column flow rate, 12.3 μL/min under 25 °C; isothermal at 25 °C (A) and thermal gradients of 20 (B), 40 (C), 75 (D), 100 (E) °C/min from 25 to 100 °C were applied.

conditions, since final temperature was reached before analyte elution. In order to demonstrate the further effectiveness of LTMLC, ultrafast capillary LC was performed by using a shorter column and faster column flow rates. As seen in Figure 9, significant changes were observed under different heating rates, again demonstrating the instantaneous heat transfer capabilities of the LTM assembly. With comparison of the separations under isothermal (25 °C) and fast thermal gradient (100 °C/min) conditions, the advantages of temperature gradient were obvious. First, the resolution among the first three analytes was not affected. Second, the peak heights for the last-eluting analytes was increased under fast thermal gradient conditions, resulting in an improvement in detectability. Finally, run time was shortened by 41%. This result demonstrates the potential for utilization of LTMLC as the second dimension in comprehensive multidimensional liquid chromatography, where speed is critical and an isocratic separation is preferred to reduce cycle times. Not only did the LTM module enable fast heating/cooling, but it also provided excellent reproducibility. The RSD (n = 5) under very fast heating rate of 100 °C/min ranged between 0.5−1.3% and 1.3−2.9% for retention time and peak area, respectively.

CONCLUSIONS

The combined use of capillary columns and an LTM assembly controlled by the LTM module allowed unprecedented heating (up to 1800 °C/min) or cooling rates (100∼200 °C/min) reproducibly. With examination of the van't Hoff plots of neutral, acidic, and basic analytes, it was found that the LTMLC instrumentation delivered reliable heating and cooling capabilities.

Both solvent gradients and temperature programs were demonstrated for selectivity tuning of model mixtures that contained basic compounds. This study also demonstrated the feasibility of applying oscillating temperature gradients for selectivity tuning in LC for the first time. In addition, ultrafast LTMLC of neutral and acidic compounds under very fast temperature increasing program conditions was also demonstrated.

The increases in column temperature in the range below 75 °C generally resulted in an increase in column efficiency at constant column flow rate (close to optimum) where mass transfer resistance dominated the band broadening. However, at higher temperatures, greater than 75 °C, a decrease in column efficiency was observed, principally because longitudinal diffusion becomes predominant at higher temperatures.

Future research will be directed toward the utilization of temperature stable particles or polymer monoliths in LTMLC for the application to real-world problems.

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