Versatile Capillary Column Temperature Control Using a Thermoelectric Array Based Platform

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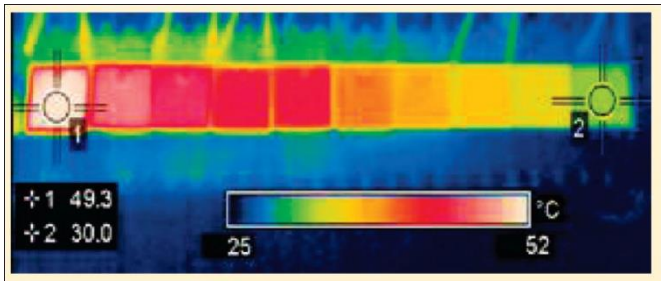
ABSTRACT



A new direct contact platform for capillary column precise temperature control based upon the use of individually controlled sequentially aligned Peltier thermoelectric units is presented. The platform provides rapid temperature control for capillary and microbore liquid chromatography columns and allows simultaneous temporal and spatial temperature programming. The operating temperature range of the platform was between 15 and 200 ^{0}C for each of 10 aligned Peltier units, with a ramp rate of approximately 400 ^{0}C/min. The system was evaluated for a number of nonstandard capillary based applications, such as the direct application of temperature gradients with both linear and nonlinear profiles, including both static column temperature gradients and temporal temperature gradients, and the formation of in-capillary monolithic stationary phases with gradient polymerization through precise temperature control.

Introduction

Temperature is an important, yet often neglected, separation parameter in liquid chromatography. Precise control of column temperature can, and has been used to manipulate run times, affect peak efficiency and resolution, increase analyte signal-to-noise ratios, and even reduce mobile phase solvent consumption.1-7 Chen and Horvath8 showed that isobaric temperature programming can be a promising alternative to gradient elution in cases where the required range of the elution strength is not too wide, for instance, for the separation of closely related macromolecules. Varying temperature has also been shown to both reduce overall retention and, in cases, improve selectivity for smaller molecules in both reversed-phase9 and in ion exchange chromatography10,11 and greatly affect the retention and resolution of biomolecules.12,13

Separations carried out at high temperatures also provide the opportunity to apply higher flow rates without the usual increased pressure penalty, due to the decreased viscosity of the mobile phase. The combination of the above-mentioned decrease in retention (particularly in reversed-phase mode) and the ability to apply higher flow rates mean that high-temperature

separations can achieve tremendous reductions in analysis times.' In addition, separation temperatures between 80 and 250 °C have been shown to facilitate the successful use of pure water as the mobile phase for reversed-phase separations.[15] High-temperature HPLC could be defined as extending from 60 to 374 °C,[16] since many of the commonly used solvents in reversed-phase separations would otherwise boil at approximately 65 °C, and 374 °C is the highest critical temperature observed for water. There are many techniques that go beyond these limits, such as those that rely on the use of super critically heated water as the mobile phase,[17-19] or those at the other end of the temperature scale, including 0,some approaches which utilize column cooling below 0 °C. The task of precise temperature control throughout the column in HPLC is not trivial, and achieving temperature precision while applying rapid gradients generates more difficulty. In addition to "programmed" or intentional applied thermal profiles, there may also be unintentional heating within the column from frictional forces or other causes of nonuniform temperature profiles, which may even cause band broadening and loss of efficiency.[22'23] In some cases, longitudinal temperature gradients within the column originating from frictional forces may increase to the point that the column outlet temperature can be over 10 °C higher than the column inlet.[24]

To overcome the problem of temperature differentials at the column inlet, Wolcott et al.[22] stated that when elevated temperatures are utilized for the separation, the temperature of the incoming mobile phase should be within ±6 °C of the oven/column temperature to minimize any band broadening resulting from radial temperature gradients.

At the same time, to efficiently counteract the effects of frictional heating, a column should be cooled to lower temperatures along its length, ensuring that heat sinks directly outward from the column to the cooling surface and not to cooler areas of the column itself. In order to achieve this type of segmented heating control, a direct contact type system is required, one which is capable of applying a different temperature to each segment.

The temperature of the column can be controlled in various ways: heating blocks, water jackets and baths, as well as the common circulating air ovens. Heaters based upon water jackets and baths have been found to be the most efficient, due to their superior heat capacity. However, such column heaters generally exhibit a rather limited temperature range (though water can be replaced with other liquids with greater heat capacity for temperatures greater than 100 °C) and a prohibitively slow rate of heating and cooling, in applications where any form of temperature gradient is required. In the case of heating blocks, performance strongly depends on the degree of contact with the column, and in commercial examples where this close contact is maintained, these type of heaters are generally efficient in heat transfer[25] and also exhibit reasonable heating rates of approximately 20-30 °C min-1.

Circulating air ovens have a heating capacity that depends on the heating rate of the air and speed at which this heated air can be circulated around the column and are mainly suitable for isothermal operation (very limited in their heating and cooling rates, typically <10 °C min-1). In addition, very few commercially available column ovens are capable of 06-3° heating beyond 80 while fewer still are capable of cooling below 10 °C[29]

Capillary and microscale HPLC lends itself very well to rapid heating and high-temperature operation, as the column mass is small and the columns have thin walls (predominantly manufactured in fused silica housing), thus possessing low thermal mass and high thermal conductivity. However, currently specifically designed column heaters for both capillary and micro-scale HPLC are few, and those available are very limited in heating rate and range. Moreover, none can provide the generation of longitudinal gradients and most have only limited temperature data acquisition capability.[26-29] Column heating/ cooling platforms based upon Peltier thermoelectric (TEC) units could combine the advantages of direct contact ovens (fast thermal transfer rates) and of circulating air ovens (broad elevated temperature range). Indeed, rapid direct contact heating or cooling can be applied through an array of TEC units, which would consist of many distinct thermally isolated zones, making it possible to generate both temporal and spatial temperature gradients. This approach introduces a number of novel features previously unavailable, including the ability to spatially apply heated or cooled zones for potential on-column thermally controlled trap-and-release applications or to apply instant or dynamic temperature gradients to the column. In addition, such a heating/cooling platform could also find use as a tool in various hyphenated techniques that demand minimal extra column band broadening and require either high or low temperatures. Such precise and rapid localized control of temperature using single TEC units has previously been reported in microfluidic platforms, mainly for control of fluid flow[31-34] but also for PCR amplification of DNA,[35] and the thermally actuated trap and release from thermo-responsive polymer gels.[36]

With the above advantages in mind, a capillary column heating/cooling platform based upon a Peltier TEC unit array has been designed. The direct contact segmented heater is capable of a broad heating and cooling range and exhibits a very rapid response, with heating and cooling rates considerably better than 50 °C min^{-1}. In addition, the module was designed with full independent TEC unit control and temperature feed-back and developed to provide excellent thermal stability at all temperatures. The following technical note reports upon the unique performance and novel applications of the new platform.

EXPERIMENTAL SECTION

Work was undertaken to construct an initial prototype comprising of ten 12 mm x 12 mm single stage TEC units (TEC Microsystems GmbH, Berlin, Germany) arranged in a longitudinal array, using a simple forced air cooled heat exchanger to dissipate heat during cooling operations (see Figure 1). During operation below ambient temperature, air is passed from the heat exchanger through a series of fins attached to the bottom surface of each TEC unit (the side which would be heating up), and so by dissipating this heat it allows the working surface of the unit to cool. Each TEC unit was wired independently so that both heating and cooling can take place simultaneously and independently along the array. For the prototype device, the capillary column was attached to the array of TEC units using commercially available thermal paste, ensuring good thermal conductivity between the TEC unit surfaces and the column. The small thermal mass of the capillary columns immobilized using the thermal paste meant any radial thermal gradients, which could possibly result in

larger diameter columns, would be negligible in this instance. Since the thermal paste is not an adhesive, it also allows for the easy removal of the column.

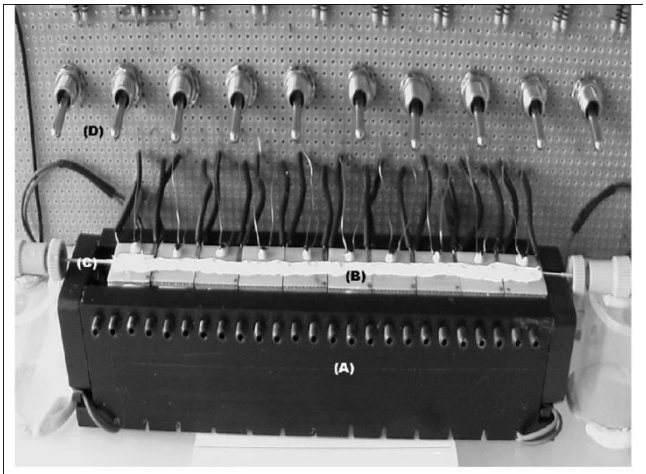


Figure 1. Capillary column heater with capillary column attached, showing (A) heat exchanger, (B) TEC units, (C) capillary column, and (D) control board.

Temperature control of the device was achieved by monitoring the temperature of each individual TEC unit. A thermistor (EPCOS AG, Munich, Germany) mounted on the surface of each unit measured the surface temperature and the data was fed to a data acquisition circuit which collected data from each segment of the module. Each value was fed into a temperature management program which automatically controlled the temperature of each unit through closed loop control. Closed loop control (in this case, proportional—integral—derivative (PID) control) constantly compared the desired set point with the process value, changing the output to the system (the TEC units) to reach that set point.

Each module had two dedicated PID controllers, one PID loop handling heating operations, while the other controlled cooling. This setup was essential in this case as the thermal response of each TEC unit was very different depending on whether the unit was in heating or cooling mode. By using this approach, the user could very effectively "tune" the control loops for heating and cooling, and so high ramp rates and fast thermal response were possible while obtaining a high precision with minimal overshoot. For the chromatographic studies, a Dionex Ultimate 3000 nano-HPLC system (Dionex, Sunnyvale, CA) was used, incorporating an FLM3100 column compartment which was used only for the performance comparison with the TEC array module. For data acquisition, Chromeleon 6.8 software (Dionex, Sunnyvale, CA) was utilized. Chromatography was performed with a flow rate of 1 RL min-1, and detection was by UV at 254 nm using a 3 nL flow cell.

A SputterCoater S150B (BOC Edwards, Sussex, U.K.) was utilized for coating capillary monolithic stationary phase samples with a 60 nm gold layer prior to scanning electron microscopy (SEM) analysis, which was performed on a S-3400N instrument (Hitachi,

Maidenhead, U.K.). Optical microscopy evaluation of microfluidic chip samples was performed on a Meiji Techno EMZ-8TR stereomicroscope (Meiji Techno UK Ltd., Somerset, U.K.). Thermal imaging was performed using a Thermovision A20 infrared camera (FLIR Systems, West Malling, U.K.). Fused silica capillaries were initially pretreated through activation of the surface silanol groups of the inner walls by sequential flushing with 1 M NaOH, deionized water, 0.1 M HC1, deionized water, and acetone. The pretreated capillary was silanized using a 50 wt % solution of trimethoxysilylpropyl methacrylate in toluene at 60 °C for 24 h. Chromatographic separations were performed on a lauryl methacrylate (LMA)—ethylene dimetha-crylate (EDMA) monolithic column. The LMA-EDMA column was prepared according to the procedure described by Eeltink et al.37 The monomer mixture consisted of 24% wt LMA, 16% wt EDMA, 45.5% wt 1-propanol, 14.5% wt 1-4-butanediol, and 0.4% wt of dimethoxy-2-phenyacetophenone (with respect to monomers).

The initiator (DAP) was weighed out into the mixture vessel, and the porogen mixture (1-propanol and 1-4- butanediol) was added, followed by the monomers. The mixture was vortexed and deoxygenated under a flow of nitrogen for 10 min. A desired length of 100 ,um i.d. silanized capillary was filled with the monomer mixture and exposed to 2 J cm-2 of UV radiation. The resultant monolithic column was washed with Me0H to remove residual porogen and unreacted monomers. Gradient polymerization was performed on a butyl methacry-late monomer mixture. The monomer mixture consisted of 24% wt BuMA, 16% wt EDMA, 60% wt 1-decanol, and 1% AIBN. The monomer mixture was prepared as per the procedure described by Nesterenko et al.38 As per this procedure, AIBN was first dissolved in the porogen, then monomers were added to the mixture, which was then vortexed, deoxygenated under the flow of nitrogen for 10 min, and centrifuged. A length of silanized capillary was filled with the monomer mixture and exposed to a profiled heating program on the TEC array module. Lauryl methacrylate (LMA), ethylene dimethacrylate (EDMA), butyl methacrylate (BuMA), 1-propanol, 1-4-butanediol, styrene,

divinylbenzene (DVB), 3-methoxysilylpropyl methacrylate, 1-decanol and UV-initiator dimethoxy-2-phenyacetophenone (DAP) were all purchased from Sigma-Aldrich (Gillingham, U.K.). All solvents which were used for the preparation of HPLC mobile phases and for the synthesis and washing of prepared monoliths, namely, tetrahydrafuran (THF), acetonitrile (ACN), and methanol (MEOH), were purchased from Lab Scan (Gliwice, Poland). The thermal initiator, 1,1'-azobisiziobutyro-nitrile (AIBN), was obtained from DuPont (Le Grand Sacconex, Switzerland). Standard solutions of ethylbenzene, propylben-zene, butylbenzene, and pentylbenzene (purchased from Sigma Aldrich, Gillingham, U.K.) and toluene (purchased from Lab Scan, Gliwice, Poland) were prepared in a 50:50 ACN/H20 mixture, at a concentration of 0.05 mg/mL for each analyte. Deionized water purified by a Milli-Q system (Millipore, Bedford) was utilized throughout the experiments. Teflon-coated (15 ,um thickness) fused silica capillary, 100 ,um i.d., 0.375 mm o.d. was purchased from Composite Metal Services Ltd. (Charlestown, U.K.).

RESULTS AND DISCUSSION

The working temperature range of the prototype TEC array module was restricted to 15-200 °C. It was found that the system response was extremely fast with heating and cooling rates of up to and beyond 360 °C min-1. To place this in context, a direct comparison of the module and a leading commercial air bath column oven was made, with the latter exhibiting a max-imum heating rate of just 9 °C min-1. With comparison to the commercial column oven, the response of the TEC array heater was approximately 20 times faster.

Figure 2 shows this comparison graphically, comparing the rate of climb (ROC, degrees Celsius minute-1) against response time (seconds) for (a) the TEC array module and (b) a leading commercially available air bath oven. The performance of the system was also tested by measuring the individual response of each of the units against time and by also monitoring their thermal stability over time.

The system response and stability were investigated while continuously cycling between two set point temperatures of 16 and 40 °C (see Figure 3, inset shows temperature stability at 40 °C over approximately a 15 min period). It was found that the deviation from steady state temperature was in the region of ±0.2 °C over 1 h period and ±0.5 °C over 24 h.

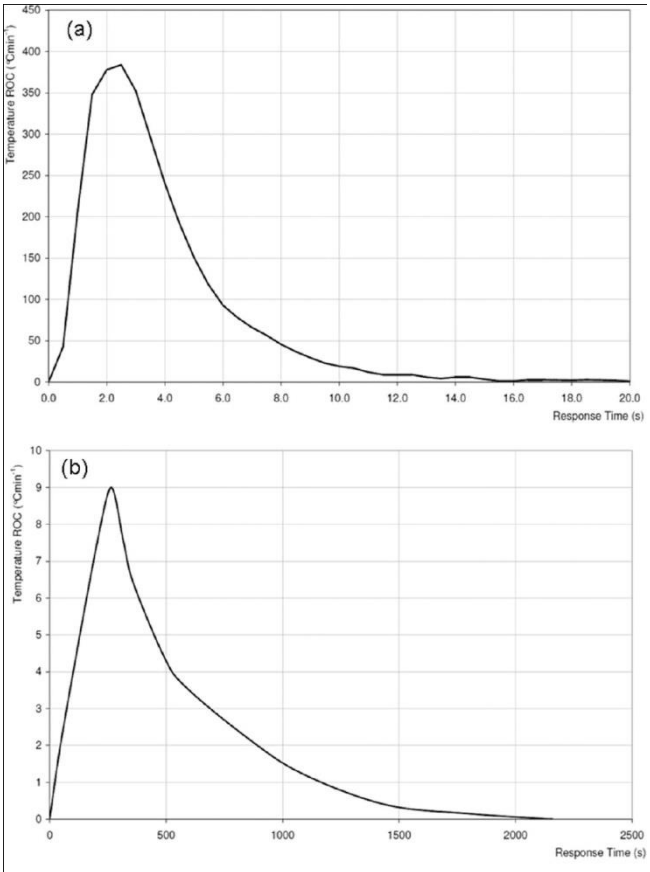


Figure 2. Comparison of heating rates between the (a) TEC column oven and (b) a leading commercially available air bath oven.

In the current design, units in the cooling mode and subsequently "switched off" automatically return to heating mode (default state), thus residual current in the circuit causes an instant slight heating effect, the result of which can be seen at 3200 s within Figure 3. The column heating/cooling effectiveness of the TEC array module was evaluated through investigating the rate of column back-pressure change with alteration of temperature. For this study, a thermal step gradient program was applied and the column backpressure change was recorded. These results were again compared to those obtained for a commercial air bath oven, for which the same temperature program was used and the backpressure change was studied on the same column (LMA-EDMA polymer monolithic column, 150 mm x 100 μm i.d.). For both experiments, the flow rate was set to 4 μL min^{-1} (pumping 50% acetonitrile) and the starting temperature was 25 °C. The temperature was ramped up to 60 °C in a single step, and once the column temperature reached 60 °C, the tempera-ture was set to return directly to 25 °C.

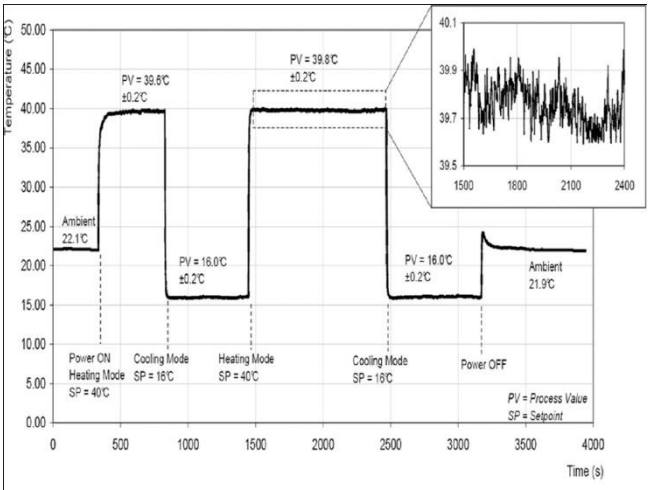


Figure 3. TEC unit response during 1 h cycling between 16 and 40 °C.

Figure 4a shows the temperature and pressure response of the column for the air bath oven, which had a maximum heating rate of 9 °C min^{-1}, taking 28 min to reach set point temperature. This compared with the TEC array module which took less than 30 s (see Figure 4b). The completion of the full heating/cooling cycle took approximately 60 min for the air bath oven, compared to less than 4 min for the TEC array module. This demonstration highlights the fact that current commercial air bath type ovens can support only very shallow gradients, outside of which the programmed temperature profile does not match that experienced by the column. Column back pressure profiles, as shown in Figure 4, are a convenient way of graphically visualizing the rate and degree of actual column temperature change (which is directly related to mobile phase viscosity and therefore column back-pressure), in response to changes in the programmed column temperature.

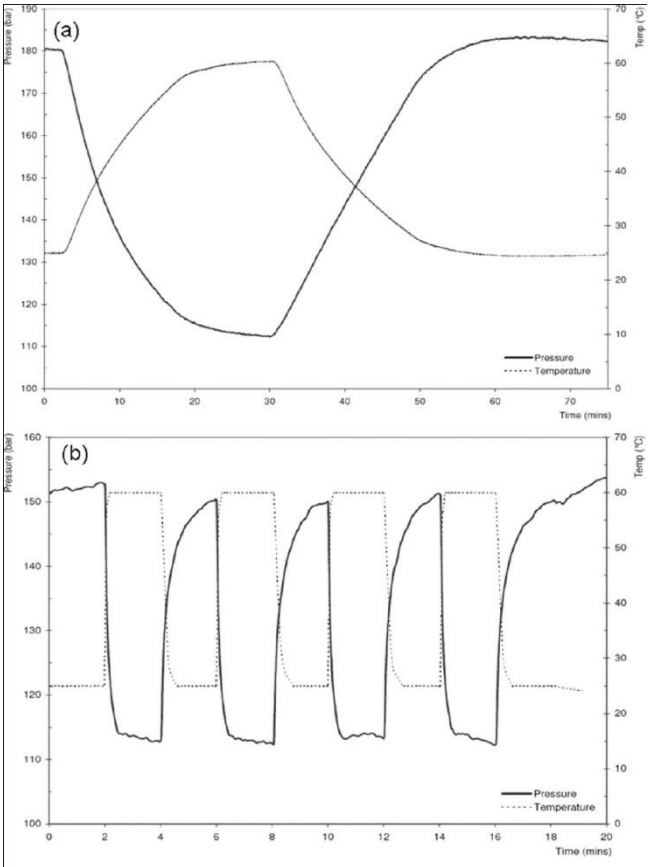


Figure 4. Comparison of column temperature and pressure under maximum heating rates for (a) a leading commercially available air bath oven and (b) the TEC column oven.

In order to demonstrate the practical application of the module for capillary HPLC, a simple mixture of alkylbenzenes (toluene, ethylbenzene, propylbenzene, butylbenzene, and pentylbenzene) was separated on a reversed-phase LMA-EDMA monolithic capillary column (150 mm x 100μm i.d.) attached to the TEC unit array. The separation was performed under both isothermal and isofluentic conditions and with various combinations of single and dual temporal gradients of temperature and flow rate. The performed separations are presented in Figure 5, with all other parameters, such as analyte concentration, injection volume, and mobile phase composition, kept constant. The peak parameters (area, height, resolution, width at half height, and asymmetry) were calculated for all the separations shown under each of the applied conditions (see Table 1 in the Supporting Information).

Initially,the separation was performed under ambient temperature at a set flow rate of 1 ,μL min^{-1}.As shown in Figure 5a, under these conditions a complete resolution of all five analytes was achieved in approximately 20 min. Using the TEC array module set to a constant 85 °C, while maintaining the flow rate at 1 μL min^{-1}, resulted in a faster overall separation, complete in just 12 min (see Figure 5c).

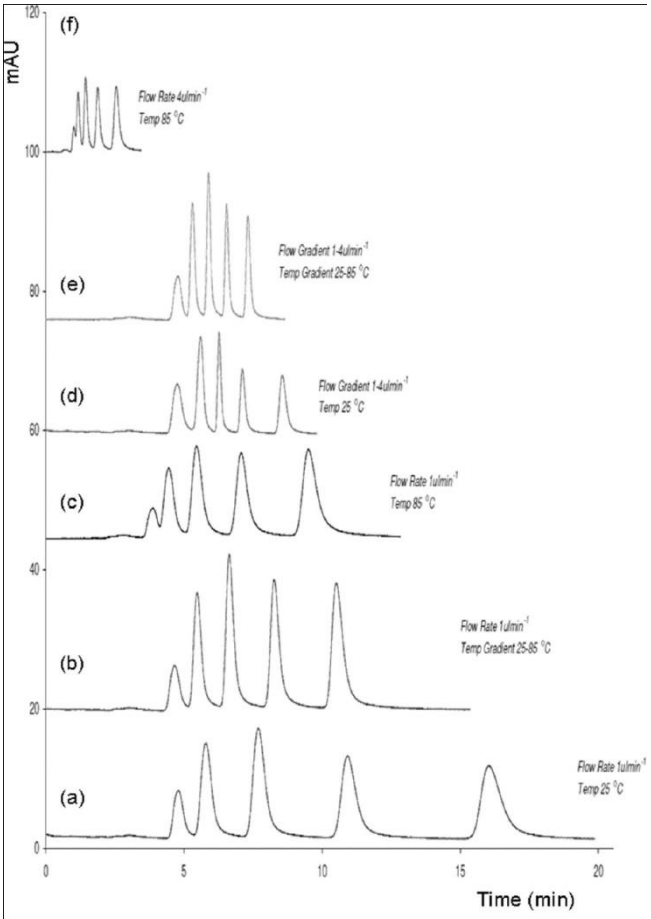


Figure 5. Separation of five alkylbenzenes (toluene, ethylbenzene, propylbenzene, butylbenzene, and pentylbenzene) at varying flow rate and temperature: (a) temperature 25 °C, flow rate 1 μL min^{-1}, (b) temperature gradient 25−85 °C from 3.5 to 6.5 min, flow rate 1 μL min^{-1}, (c) temperature 85 °C, flow rate 1 μL min^{-1}, (d) temperature 25 °C, flow gradient 1−4 μL min^{-1} from 5.3 to 6.3 min, (e) temperature gradient 25−85 °C from 3.5 to 6.5 min, flow gradient 1−4 μL min^{-1} from 4.3 to 8.0 min, (f) temperature 85 °C, flow rate 4 μL min^{-1}; LMA-EDMA monolithic column, 150 mm × 100 μm i.d.; mobile phase 50:50 ACN/H_2O. Injection volume = 100 nL. UV detection at 254 nm.

However, simply increasing the temperature also resulted in decreased resolution, leaving toluene and ethylbenzene peaks unresolved. Neither was any significant beneficial increase in peak height or area observed, with peak shape (peak width and asymmetry) for both separations approximately the same. Combining the application of high temperature (85 °C) and increased flow rate (4 μL min) significantly shortened the analysis time to just 4 min. In this case, although peak width and asymmetry improved, resolution be-tween early eluting peaks deteriorated further, leaving toluene and ethylbenzene unresolved, with both peak area and height also significantly reduced (see Figure 5f).

In order to speed up the separation while maintaining peak resolution, a series of gradient conditions were investigated. Single gradients of temperature and flow rate were applied separately and were then compared to a dual temperature/flow gradient. In the first instance, a single temperature gradient from 25 to 85 °C was applied from 3.5 to 6.5 min while

maintaining a constant flow rate of 1 μL min-1. The start of the temperature gradient was delayed to ensure complete separation of the first two peaks. During the application of the gradient, the column backpressure change was recorded, confirming that the shape of the programmed gradient was identical and simultaneous to that generated by the TEC array module. With comparison of chromatograms obtained using the temperature gradient, shown as Figure 5b, to the isothermal separations at 25 °C (Figure 5a) and 85 °C (Figure 5c), a reduction in peak width was observed, causing an increase in peak height while simultaneously main-taining asymmetry values and resolution between peaks. It can also be seen that overall separation time decreased by 40% compared with the separation at ambient temperature (Figure 5a).

In the second instance, a flow gradient from 1 ,uL min-1 to 4 yL min-1 was applied from 5.3 to 6.3 min, while maintaining a constant temperature of 25 °C, shown as Figure 5d. In this case, the start of the flow gradient was delayed to ensure complete separation of the first two peaks. With comparison of the peak shapes and overall chromatogram to those achieved under isofluentic conditions at 25 °C (Figure 5a) and 85 °C (Figure 5c), an improvement was observed in peak resolution (compared with Figure 5c), with peaks for toluene and ethyl-benzene being fully resolved while width and asymmetry values noticeably improved. In addition, the application of a flow gradient reduced the time separation by 48% compared to the separation at 25 °C (Figure 5a). Finally, in order to achieve the complete resolution of all peaks, yet maintaining a fast run time, a rapid dual gradient of both flow and temperature was applied, shown here as Figure 5e. In this case, the applied temperature gradient was run from 25 to 85 °C over 3.5 to 6.5 min, while a simultaneous flow gradient was applied from 1,uL min-1 to 4 μL min-1 over 5.3 to 6.3 min. With comparison of this separation with the individual gradient runs, namely, the temperature gradient (Figure 5b) and flow rate gradient (Figure 5d), it can be seen that the overall separation time has been further reduced to just under 8 min, equaling a 66% reduction compared to the isofluentic separation at 25 °C (Figure 5a).

Furthermore, the applied dual gradient resulted in the complete resolution of all sample components, with both peak width and asymmetry improved compared to each of the previous runs and peak heights generally unaffected.

The above chromatographic experiments utilized in-house fabricated polymer monolithic columns. The reproducible production of such monoliths with ideal pore structure for selected applications remains a challenge for separation scientists. It is well-known that in order to obtain fine control of monolith porosity during thermal polymerization, the precise control of the reaction temperature is crucial. The polymerization process itself is a complex series of reactions, each effected to some degree by the exact system temperature. The most important of these is the initiation rate, which is highly dependent on temperature, since the half-life of initiators decreases with increases in temperature. As a result, the rate of the formation of free radicals and, subsequently, the speed of the chain growth and formation of globules and the overall polymerization rate are each higher at elevated temperatures. As the formation of new polymerization centers is faster than the growth of globules, the supply of monomers runs low fast and the number of globules is large, but their size stays small,

leading to smaller voids between globules. Essentially, the entire pore size distribution is shifted smaller with an increase in temperature, although most obviously seen for the larger flow-through pores.

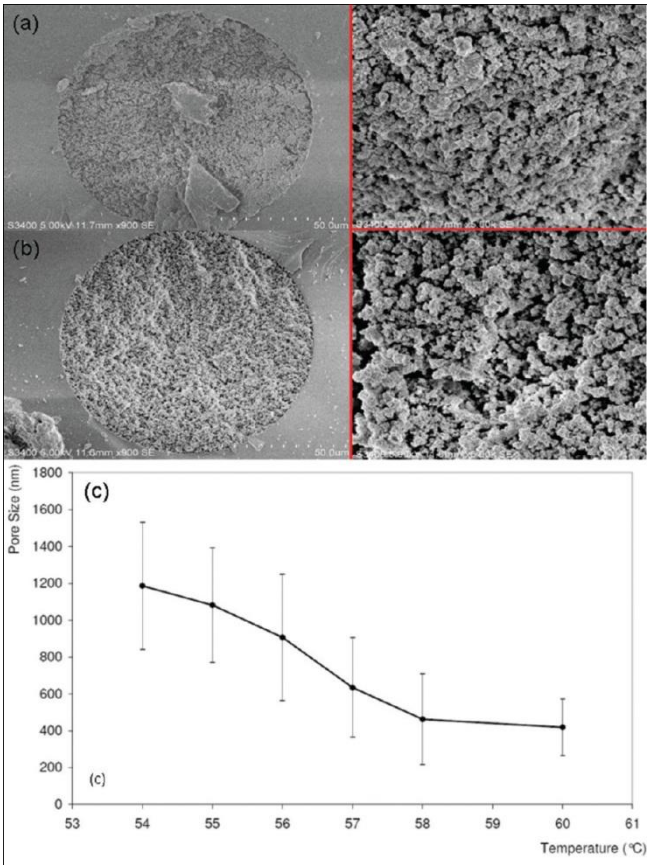


Figure 6. SEM images of BuMA-EDMA monolith formed using a thermal gradient for 16 h at (a) 60 °C and at (b) 54 °C. (c) Plot of average pore sizes against increasing polymerization temperatures for a single capillary housed BuMA-EDMA monolith, polymerized over 16 h on the TEC array column heater.

The ability of the designed module to precisely control temperature of separate zones within a capillary was exploited to optimize and better understand the fabrication of porous polymer monoliths within capillary columns. In this experiment, the TEC array module was used to polymerize a monolithic column with a longitudinal density gradient. To achieve this, a capillary filled with BuMA-EDMA polymerization mixture was attached to the TEC units and a heating profile from 60 to 54 °C programmed over six distinct thermal zones, with 1 °C spatial increments. Polymerization of the complete monolith was performed for 16 h, after which the monolith was washed with Me0H for 1 h, cut into segments, corresponding to each temperature zone, and dried. The porous structure of each of these segments was characterized using SEM. Figure 6 shows SEM images of two selected zones of the monolith polymerized at (a) 60 °C and (b) 54 °C. It can be clearly seen that the size of polymer globules and flow-through pores differ for these sections. From each of the six sets of SEM

images, the average size (n = 30) of the pores were determined and plotted against the exact polymerization temperature. This relationship is presented as Figure 6c. The graph clearly shows an increase in pore size with a decrease in polymerization temperature. The graph also shows how the TEC array can also be utilized to produce polymer monoliths of relatively predictable porous structure resulting in the controlled formation of axial gradients of porosity, something very much more difficult to achieve using alternative air heaters, water baths, or indeed UV polymerization approaches.

CONCLUSIONS

The results presented herein have demonstrated the design and versatile capabilities of a novel direct contact TEC array based column heater/cooler. It was shown that the designed system can provide very rapid heating/cooling with a rate of up to 400 °C/min and that thermal equilibration of the capillary happens at the same rate. The unique platform was demonstrated with chromatographic separations involving the application of dual temperature/flow rate gradients, with beneficial results. Because of the specific features of thermoelectric modules (precise temperature control, fast response), further demonstra-tive applications were possible, such as the fabrication of mono-liths incorporating a gradient of porosity (in capillary format) and the precise positioning of monoliths with well-defined boundaries in chip formats, where in both instances the precise spatial control of temperature during polymerization is essential.

ASSOCIATED CONTENT

Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at http ://pubs.acs.org.

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