Elevated-temperature ultrahigh-pressure liquid chromatography using very small polybutadiene-coated nonporous zirconia particles

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Abstract

Capillary columns packed with small diameter particles typically lead to low permeability and long separation times in high-performance liquid chromatography. Ultrahigh pressures (>10 000 p.s.i.; 1 p.s.i. ≡6894.76 Pa) can be used to overcome the limitations that small particles impose. Ultrahigh-pressure liquid chromatography (UHPLC) has demonstrated great potential for high-speed and high-efficiency separations. Decreasing the viscosity of the mobile phase by elevating the temperature could additionally reduce the pressure drop and facilitate the use of longer columns or smaller particles to achieve even higher total plate numbers. For this reason, we investigated the use of elevated temperatures in UHPLC. Water-resistant, flexible heater tape covered with insulation was used to provide the desired heat to the column. Polybutadiene-coated 1 μm nonporous zirconia particles were used because of their chemical stability at elevated temperature. A column efficiency as high as 420 000 plates m^{-1} was obtained. The effects of temperature and pressure on the separation of parabens were investigated. Separation of five herbicides was completed in 60 s using 26 000 p.s.i. and 90 °C.
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1. Introduction

The overall goal of analytical chromatography is to achieve sufficient resolution of analytes of interest within the shortest possible time. Theoretically, fast and high-performance separations can be achieved using long columns packed with small particles [1].

However, the use of very small particles requires significant pressures because the pressure drop across a packed column is inversely proportional to the square of the particle size as given by the following equation [2].

$$\Delta P = \phi \eta L u / d_{\mathrm{p}}^{2} \tag{1}$$

where Δp is the pressure drop, ϕ is the flow resistance factor, L is the column length, η is the

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mobile phase viscosity, u is the linear velocity, and d_p is the particle diameter. Therefore, for columns packed with particles smaller than 2 μm in diameter, much higher pressures are required to operate at optimum flow-rates compared to columns containing large particles [3]. Lower flow-rates result in degraded column efficiency because of increased band broadening by solute longitudinal diffusion. However, the applicability of high flow-rates for fast separations using small particle packed columns is limited by the pressure that the pump system can supply and the back pressure that the other components of the chromatographic system (injector, column and valves) can withstand. Most conventional pumping systems have upper pressure limits of ~400 bar, which would limit a column packed with 1.5 μm particles to ~3.3 cm in length. Although fast separations have been demonstrated using such columns, the loss in efficiency due to such a short column is significant, making them unsuitable for separation of complex samples [4].

In 1997, MacNair et al. first reported the use of ultrahigh pressures in liquid chromatography [5]. Mobile phase pressures as high as 5000 bar (72 000 p.s.i.) were supplied to a 46-cm column packed with 1.0 μm particles to generate more than 200 000 theoretical plates in 6 min [6]. Subsequent studies have further demonstrated the high efficiency and high speed potential of ultrahigh-pressure liquid chromatography (UHPLC) [7–10]. Using pressures near 40 000 p.s.i., separations of combinatorial chemistry samples, pharmaceutical compounds and chiral compounds have been completed in less than 2 min, yielding more than 200 000 theoretical plates.

In LC, elevated column temperature can also be used to lessen the high back pressure caused by small particles [11,12]. Increasing the temperature of the mobile phase decreases its viscosity, allowing the use of flow-rates that otherwise could not be achieved [13–15]. Moreover, elevated temperature facilities high-efficiency separations [16]. Generally, for fast separations, columns are operated at much higher velocities than the optimum velocity in order to reduce the separation time. The resulting efficiency of the column at these high velocities is determined by the mass transfer characteristics of the column packing, typically leading to a loss in efficiency. However, elevated temperatures increase the diffusivities of the analytes, improving mass transfer and efficiency [17,18]. Thus, elevated temperature in UHPLC should assist in improving both speed and efficiency.

Over the past decade, Carr and co-workers have produced a series of thermally stable stationary phases, which allow very high temperatures to be used in LC. Chemically and thermally stable zirconia particles coated with polybutadiene (PBD) [19–23], polystyrene (PS) [24,25], and carbon (C) [26] have allowed separations at temperatures exceeding 200 °C. Studies on efficiency, selectivity, and thermodynamic properties of PBD–ZrO$_2$, PS–ZrO$_2$, and C–ZrO$_2$ stationary phases have shown the advantages of high-temperature LC. Recently, a liquid chromatographic system [27] that enables high-temperature ultrafast liquid chromatography using conventional diameter LC columns was designed through careful consideration of heat transfer, band broadening and pressure drop. Fast separations (seconds) were achieved. For example, at 150 °C and a flow-rate of 15 ml min^{-1} with a 5-cm × 4.6-mm I.D. column packed with 3 μm polystyrene coated porous zirconia particles, five long-chain alkylphenones were completely resolved in under 30 s. This is a 50-fold reduction in time compared to the same separation at ambient temperature.

In this study, elevated-temperature UHPLC using packed capillary columns was studied. Nonporous PBD coated 1 μm zirconia particles were used to demonstrate high-speed and high-efficiency separations.

2. Experimental

2.1. Materials

Nonporous 1.0 μm zirconia particles were prepared as described in a previous paper [28]. Acetonitrile, isopropanol, chloroform, and water were HPLC grade and were obtained from Fisher Scientific (Fair Lawn, NJ, USA). Reagent grade cyclohexanol was purchased from Mallinckrodt (Phillipsburg, NJ, USA).

Fused-silica capillary tubing was purchased from Polymicro Technologies (Phoenix, AZ, USA). All buffers and solvents were filtered through Durapore

membrane filters (0.22 μm pores) (Milipore, Bedford, MA, USA) before use. Similarly, samples were filtered through polytetrafluoroethylene (PTFE) syringe filters (0.2 μm pores, Chromacol, Trumbull, CT, USA). Zeflor filter membranes with pore size of 3.0 μm were supplied by Gelman Science (Ann Arbor, MI, USA) and used to filter the particle slurry before packing.

2.2. Column preparation

The high-pressure packing system used to prepare the columns for this study was described previously [7]. A Model DSF-150-C1 air-driven pneumatic amplifier pump (Haskel, Burbank, CA, USA) was used for packing the capillary columns. Instead of carbon dioxide, isopropanol was used to push the slurry into the column. The packing materials used for all fabricated columns were spherical 1.0 μm nonporous zirconia particles that were chemically modified with PBD. Zirconia particles were slurried in chloroform–cyclohexanol (50:50, v/v). Slurries were sonicated for more than 1 h before being transferred to the packing reservoir. A syringe pump was used to fill the solvent reservoir of the pump with isopropanol, which was subsequently driven by a compressed nitrogen cylinder. The initial packing pressure was 450 p.s.i. The pressure was raised periodically to keep a nearly constant packing rate until the column was completely filled. The final pressure was 20 000 p.s.i. The column was left to depressurized overnight. It was not possible to sinter the zirconia particles to make frits. Therefore, 1.5 μm bare silica particles were introduced into the capillary for several centimeters both before and after packing with zirconia particles. Internal frits were made by sintering the silica particle beds at a distance of 3 mm from the zirconia particle bed with water flowing through the column under a pressure of 15 000 p.s.i. The non-sintered silica particles were flushed away and a detection window was made after the outlet frit.

2.3. Elevated temperature UHPLC system

The UHPLC system was previously described in detail [7]. Briefly, a double-head air-driven liquid pump (Model DSHF-32, Haskel) with a piston area

ratio (air drive area to liquid piston area) of 346 was used to generate the necessary liquid pressures. The maximum air supply pressure was 150 p.s.i. (10 atm), resulting in a pump pressure limit of 52 000 p.s.i. (3600 atm). The internal volume of the pump was 4.5 ml. A cylinder containing compressed nitrogen was used to drive the pump. The outlet of this pump was connected to a home-built injection system. A static-split injection technique was employed for sample introduction [5,7]. A Model UV3000 scanning detector from Thermo Separations (Sunol, CA, USA) was used to monitor UV absorbance on-column. Data were acquired with ChromQuest 2.5.1 (ThermoQuest, Sunol, CA, USA).

Water-resistant, flexible heater tape (Watlow Heatcon, Seattle WA, USA) covered with insulation was used to provide the desired heat. Due to differences in their thermal conductivities and masses, tubing between the pump and the injector, the laboratory-designed ultrahigh-pressure injector, and the column were heated separately. Heating the injector block was challenging due to its large volume and mass. Each side of the injector block was covered with heat tape which heated the whole injector block homogenously. In addition, the original small diameter connection tubing was changed to a larger one (9/16 in O.D.×3/16 in I.D., 1 in≡2.54 cm), which preheated a larger amount of mobile phase. Otherwise, flushing excess sample away during split injection could cause significant loss of preheated mobile phase, and cold mobile phase might enter the separation column and cause a temperature difference which would lead to band broadening and/or peak splitting [29]. Each heater cover was connected to a temperature controller (Omega, Stamford, CT, USA), which thermostated the whole system to ±0.2 °C at temperatures up to 150 °C. A linear restrictor (1 m×30 μm I.D.) was attached to the end of the column to prevent bubble formation, especially at temperatures higher than the boiling points of water and acetonitrile.

3. Results and discussion

Fig. 1 shows the relationship between column inlet pressure and mobile phase linear velocity at different temperatures for 1 μm PBD-coated particles. The
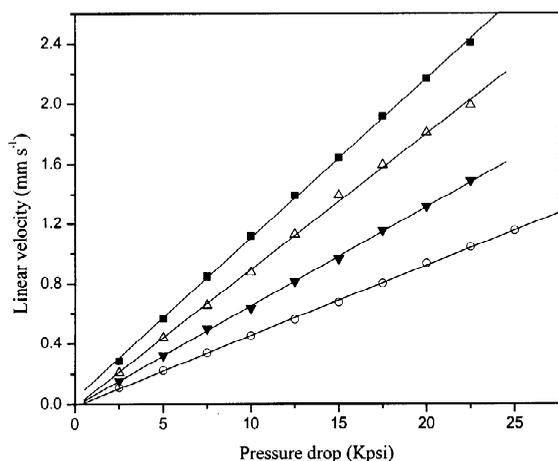
Fig. 1. Effect of temperature on pressure drop. Conditions: 13 cm ×50 μm I.D. fused-silica capillary column packed with 1.0 μm PBD-coated nonporous zirconia particles; water (40 mM NaH$_2$PO$_4$, pH 7.0)–acetonitrile (65:35, v/v); 215-nm UV detection; uracil as marker; parabens as test solutes. (○) 22 °C; (▼) 40 °C; (▲) 60 °C; (■) 80 °C.

average linear velocity at 80 °C is approximately 2.6 times higher than that at room temperature when the same inlet pressure is used. As expected, elevated column temperature decreases the pressure drop by decreasing the mobile phase viscosity as predicted in Eq. (1). Therefore, faster separation should be possible using elevated temperature. It should be noted that linear velocity and not pressure itself is the actual parameter that affects chromatographic performance. For convenience, however, since the outlet of the column is always at atmospheric pressure and the pressure is easier to measure than the linear velocity, pressure is used in this study to indicate a relative increase or decrease in velocity.

The van Deemter equation can be used to evaluate column efficiency at different temperatures:

$$H = A + \frac{B}{u} + C\mu \qquad (2)$$

where

$$A = 2\lambda d_p \qquad (3)$$

$$B = 2\gamma D_m \qquad (4)$$

$$C = \frac{1 + 6k + 11k^2}{24(1 + k)^2} \cdot \frac{d_p^2}{D_m} + \frac{8}{\pi^2} \cdot \frac{k}{(1 + k)^2} \cdot \frac{d_f^2}{D_s} \qquad (5)$$

and where λ is the packing constant, d_p is the mean particle diameter of the packing, γ is the tortuosity constant due to the packing, k is the retention factor, d_f is the mean stationary phase film thickness, D_m is the diffusion coefficient of the solute in the mobile phase and D_s is the diffusion coefficient of the solute in the stationary phase.

Theoretically, the effect of temperature on the A term is uncertain. The B term, representing longitudinal diffusion, increases with increasing temperature, but only becomes significant at low linear velocities. The C term, which represents adsorption/ desorption kinetics and mass transfer between phases, decreases with increasing temperature. Therefore, efficiencies at linear velocities greater than the optimum are better at higher than room temperature.

The dependence of plate height (H) on interstitial linear velocity (μ_e) at different temperatures was plotted as shown in Fig. 2. The actual data, represented by markers, were fitted using the van Deemter equation. Efficiencies as high as 420 000 plates m^{-1} were obtained at the optimum linear velocities at different temperatures. As expected, efficiencies at linear velocities >1.0 mm s^{-1} were better at elevated temperatures than at room temperature. Temperature effects on the A, B, and C terms were investigated and the results are listed in Table 1. It can be seen that the B term increases with increasing
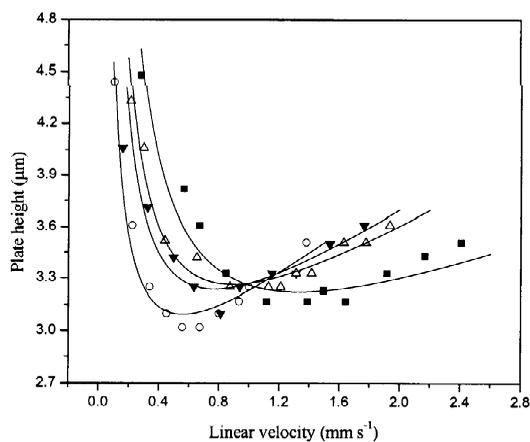

Fig. 2. Van Deemter curves for different temperatures. Conditions as in Fig. 1. Lines represent fitted data. (○) 22 °C; (▼) 40 °C; (▲) 60 °C; (■) 80 °C.

Table 1
Effect of temperature on the *A*, *B*, and *C* terms in the van Deemter equation

T (°C)	A (μm)	B (10^3 μm s^{-1})	C (10^3 s^{-1})
22	2.32	0.22	0.70
40	2.30	0.36	0.61
60	2.28	0.44	0.55
80	2.27	0.63	0.36

temperature, which is mainly due to an increase in D_m at elevated temperature. Compared to room temperature, the *C* term at 40, 60 and 80 °C decreases as expected and is much smaller at 80 °C than at room temperature. As described in Eq. (5), both changes in *k* and D_m at elevated temperatures could have an effect on the *C* term. Generally, an increase in the diffusion coefficient of the solute in the mobile phase is the main reason for the decrease in the *C* term, which helps to produce faster and higher-efficiency separations.

Lower efficiencies were obtained in this study than we typically obtain in capillary LC, especially when using 1 μm silica particles in UHPLC. The reasons for this are: (a) coated zirconia particles are known to provide lower efficiencies than bonded silica particles, (b) efficient packing of zirconia particles is challenging due to their tendency to agglomerate, and (c) higher than optimum linear velocities were used.

The effect of temperature on retention factor, k, can be described using the Van't Hoff equation,

$$\ln k = -\frac{\Delta H}{RT} + \frac{\Delta S^\circ}{R} + \ln \phi \qquad (6)$$

where ΔH and ΔS° are the enthalpy and entropy of transfer from the stationary phase to the mobile phase, respectively, and ϕ is the phase ratio of the column. The dependence of retention factor on temperature for four parabens over the temperature range of 22–80 °C was observed. The analytes eluted with good peak shapes at each temperature tested. Linear relationships for ln *k* vs. 1/*T* were obtained with correlation coefficients greater than 0.98 for each compound. The enthalpy values are consistent with typical enthalpy interactions in reversed-phase systems [16].

Changes in selectivity with temperature can be caused by either enthalpy or entropy changes. The

latter is usually brought about by shape related changes in the stationary phase. In this study, no major changes in selectivity were observed as the temperature was varied, presumably because the sample studied was a homologous series.

Fig. 3 shows separations of four parabens at different temperatures. All separations were conducted at constant pressure. With an increase in temperature, the linear velocities (flow-rates) were increased due to a decrease in mobile phase viscosity (dead time decreased from 3.68 to 1.62 min); at the same time, solute retention also decreased due to an increase in speed of adsorption/desorption (retention factor decreased from 0.54 to 0.25). Therefore, there was an approximate 3-fold reduction in the analysis time (from 6 min at 25 °C to about 2 min at 80 °C). Even though there was a significant decrease in analysis time, the overall quality of the separation is still quite acceptable.
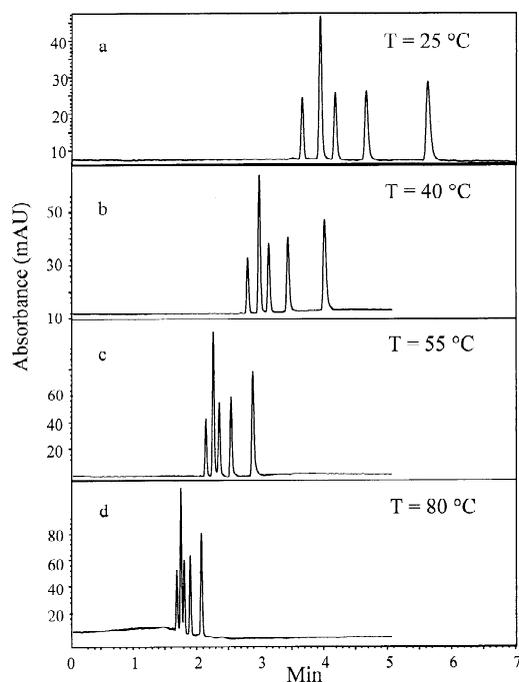

Fig. 3. Effect of temperature on the separation of parabens. Conditions: (a) 25 °C; 15 000 p.s.i. inlet pressure; 15 cm×50 μm I.D. fused-silica capillary column packed with 1.0 μm PBD coated nonporous zirconia particles; water (40 m*M* NaH$_2$PO$_4$, pH 7.0)–acetonitrile (60:40, *v*/*v*); other conditions as in Fig. 1; (b) 40 °C; (c) 55 °C; (d) 80 °C; other conditions as in (a).
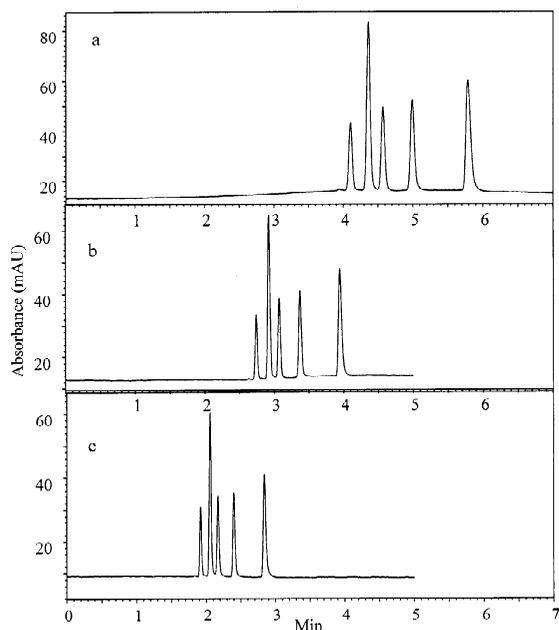
Fig. 4. Effect of pressure on the separation of parabens. Conditions: (a) 40 °C; 10 000 p.s.i. inlet pressure; other conditions as in Fig. 3; (b) 15 000 p.s.i.; and (c) 20 000 p.s.i.; other conditions as in (a).

Fig. 5. Fast separation of herbicides. Conditions: (a) 22 °C; 10 000 p.s.i. inlet pressure; 13 cm×50 μm I.D. fused-silica capillary column packed with 1.0 μm PBD coated nonporous zirconia particles; water (40 mM NaH$_2$PO$_4$, pH 7.0)–acetonitrile (55:45, v/v); 215-nm UV detection; uracil as marker. (b) 90 °C; 26 000 p.s.i. inlet pressure; other conditions as in (a).

Fig. 4 shows separations of four parabens at different pressures. When the pressure increased from 10 000 to 20 000 p.s.i., the analysis time was reduced from 5.8 to 2.8 min, or approximately 2-fold. However, resolution values for peaks 2 and 3 were 2.18 and 2.08 at 10 000 and 20 000 p.s.i., respectively, indicating no significant loss in resolution. This may be due to two reasons. First, the van Deemter curve for the small particle packed column tends to be relatively flat, which means there is little loss in efficiency with an increase in pressure [5,7,10]. Second, retention factors of solutes increase with an increase in pressure [5,10].

High-speed separation of herbicides using elevated-temperature UHPLC is demonstrated in Fig. 5. At room temperature, the separation was completed in 8 min as shown in Fig. 5(a). Using a pressure of 26 000 p.s.i. and a temperature of 90 °C, the separation was achieved in 60 s with all peaks baseline separated, except peaks 4 and 5 (Fig. 5(b)). In addition, an efficiency of 200 000 plates m^{-1} was obtained for this separation. Comparatively, it would be necessary to increase the pressure to approximate-
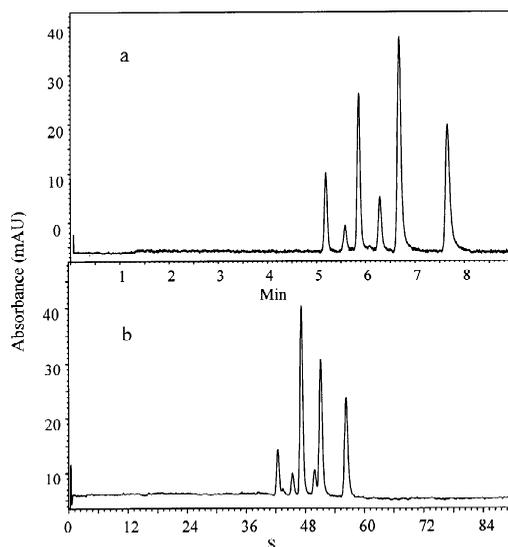
ly 80 000 p.s.i. to achieve a 60-s separation of herbicides at room temperature.

Operating at higher than optimum linear velocities produces very fast separations, but results in some efficiency loss. The separation in Fig. 5(b) was conducted at a linear velocity of approximately 3.0 mm s^{-1}, while the optimum linear velocity at 80 °C was approximately 1.2 mm s^{-1}. At a linear velocity 2.5 times higher than the optimum, 26 000 plates for a 13-cm long column is still quite good. The inherently high efficiencies of columns containing small particles allow us to sacrifice some efficiency for speed.

4. Conclusions

In this study, a combination of elevated temperature with ultrahigh pressure was used to decrease the tremendous pressure drops that small diameter packing materials create in HPLC. Elevated temperature reduces the mobile phase viscosity and increases the speed of sorption/desorption, leading to fast separation without significant loss of resolution. This new

technique was used with PBD-coated nonporous zirconia particles because of their noted high temperature stability. A good separation of herbicide standards was completed in 60 s.

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