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Mesoporous polybutadiene-modified zirconia for high-temperature packed capillary liquid chromatography: column preparation and temperature programming stability

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Abstract

In the present study, three different methods for packing of 3 μm PBD–ZrO_2 particles in 0.5 mm i.d. glass-lined stainless steel columns have been examined. The two first methods were based on a traditional downstream high-pressure technique using tetrachloromethane (Method I) or aqueous Triton X-100 (Method II) as slurry solvents, while Method III was an upstream high-pressure flocculating method with stirring, using isopropanol both as the slurry and packing solvent. Method I was found to be superior in terms of efficiency, producing 0.5 mm i.d. × 10 cm columns with almost 90,000 plates m^{-1} for toluene (R.S.D. = 8.7%, $n = 3$), using a slurry concentration of 600 mg ml^{-1}, ACN–water (50:50 (v/v)) as the packing solvent and a packing pressure of 650 bars. For Method I, the slurry concentration, column i.d., column length and initial packing pressure were found to have a significant effect on column efficiency. Finally, the long-term temperature stability of the prepared columns was investigated. In isothermal mode, using ACN–20 mM phosphate buffer, pH 7 (50:50 (v/v)) as the mobile phase, the columns were found to be stable for at least 3,000 void volumes at 100 °C. At this temperature, the solute efficiencies changed about 5–18% and the retention factors changed about 6–8%. In temperature programming mode (not exceeding 100 °C), on the other hand, a rapid decrease in both column efficiency and retention factors was observed. However, when the columns were packed as initially described, ramped up and down from 50 to 100 °C for 48 h and refilled, fairly stable columns with acceptable efficiencies were obtained. Although not fully regaining their initial efficiency after refilling, the solute efficiencies changed about 19–28% (32–37%) and the retention factors changed about 4–5% (13–17%) after running 3,000 (25,000) void volumes or 500 (3,900) temperature programs.

Keywords: Column packing; Temperature programming; Stationary phases, LC; Packed capillary; Polybutadiene; Zirconia; Zirconium oxide

1. Introduction

The advantages of performing liquid chromatography (LC) at elevated temperatures are unambiguous [1–3]. The lower viscosity of most solvents at elevated temperatures allows the use of smaller stationary phase

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particles, longer columns or higher volumetric effluent flow rates without exceeding the pressure limit of conventional HPLC pumps [1]. At the same time, the increased solute diffusivity speeds up the mass transfer between the phases and reduce the significance of the C-term in the van Deemter equation, which normally is dominating at high effluent flow rates. Hence, the speed of analysis can be dramatically increased without significant loss in column efficiency [4–9]. Higher column temperatures might also give different selectivity and improved efficiency, particularly for large molecules, and are also reported to reduce secondary interactions in reversed-phase LC [10]. High column temperatures also offer the possibility of separating compounds with low solubility in any solvent at room temperature [11].

Furthermore, the ongoing trend of miniaturization of column i.d. in LC, which mainly is catalyzed by the rapid developments within LC–ESI–MS, also creates new opportunities for selectivity and retention control based on temperature programming [12]. The low heat capacity of narrow LC columns (i.d. <0.5 mm) enables fast thermal equilibration, and since transfer of solutes from the mobile to stationary phases usually is an exothermic process in most chromatographic systems, temperature programming can be used to optimize resolution. For example, temperature-programmed LC has been applied for the separation of oligonucleotides [13], transfer-RNA [14], fatty acids in fish oils [15], retinyl esters [16], polyglycerol fatty acid esters [17], ceramides [18], X-ray contrast agents [19], polyethylene glycols [20], polystyrenes [21–23], technical waxes [24], polymer additives [25–27] and different polymers [28]. Temperature-programmed LC is also believed to have a great potential for detection of sequence variations in human genes, since mutated DNA usually responds different to temperature than the wild-type [29].

Although the arguments for performing LC at elevated temperatures weigh heavily, the potential of temperature as a variable is only scarcely exploited by chromatographers. Mainly, this is due to the well-known fact that the thermal stability of most silica-based materials columns in aqueous environment is limited, particularly in acidic or basic buffered effluents (often $<60\,^{\circ}$C). Since the vast majority of LC separations are performed under such conditions nowadays, i.e. in reversed-phase mode, the natural acceptance of temperature as valuable tool to optimize chromatographic parameters has been retarded. However, the gradual implementation of more thermally (and chemically) stable supports than the silica-based ones is expected to attract more attention to high-temperature LC, because such phases allow the column temperature to be varied over a wider range than before. Among the alternative high-performance stationary phases introduced over the years, which mainly are based on transition metal oxides or synthetic polymers, surface modified zirconium oxide (ZrO_2) shows interesting chromatographic properties. Uniform, spherical, porous, micro-particulate ZrO_2 with high mechanical strengths, sufficiently high surface areas and reproducible pore geometries have already been prepared for more than one decade [30,31], and is today commercially available with several types of surface modifications. The deposition and cross-linking of polybutadiene (PBD) on the ZrO_2 surface has proven to provide a reversed-phase material with remarkable chemical and thermal stability, e.g. no sign of degradation was observed after exposure to 1 M sodium hydroxide at 100 °C for 3.25 h [31]. However, for chromatography purposes, the surface chemistry of ZrO_2 is extraordinary; it possesses both acidic and basic, as well as oxidizing and reducing, chemical properties [32]. Some of these surface sites are still available after surface modification, and particularly are Lewis bases, e.g. organophosphates and carboxylic acids, strongly adsorbed. Even the thickest layers of PBD allowed the adsorption of about 2.3 μmol m^{-2} of phosphate [33]. Hence, mobile phases containing 10–50 μM of a strong Lewis base, e.g. fluoride or phosphate, are commonly used to reduce peak tailing, increase column efficiency and improve recoveries of strongly adsorbed compounds [31]. It is also worthy noting that with the application of phosphate buffers on PBD–ZrO_2, positively charged compounds can undergo mixed-mode retention mechanisms that often provide different selectivity than what obtained on conventional C18-bonded silica-based materials. For uncharged non-polar solutes, on the other hand, the selectivity is often comparable, only with slightly less retention.

Carr and co-workers [8,33], Li and Carr [34,35], Thompson and Carr [36], and others [37,38] have convincingly demonstrated the high-temperature stability of conventional-sized PBD–ZrO_2 columns, but this is

not a guarantee itself for obtaining temperature-stable packed capillary LC columns, unfortunately. Preparation of highly efficient densely packed porous PBD–ZrO_2 capillary columns is challenging, and thus the application of high-temperatures, and particularly the repeated thermal expansions of the column during temperature programming, might have a compressing effect on the packed bed. Accordingly, the aim of the present study was to investigate the potential of PBD–ZrO_2 as a stable stationary phase for high-temperature and temperature-programmed packed capillary LC. The work was divided in two parts: (1) to examine three different packing methods for preparation of efficient and densely packed PBD–ZrO_2 capillary columns, and (2) to evaluate the long-term stability of these columns when subjected to high-temperatures or numerous temperature programs.

2. Experimental

2.1. Chemicals and materials

Benzyl alcohol, toluene, potassium dihydrogen phosphate (all from Merck, Darmstadt, Germany), fluorene (Supelco, Bellefonte, PA, USA), amitriptyline, Triton X-100 (both from Sigma–Aldrich Chemie, Steinheim, Germany) and benzene (Prolabo, Fontenay S/Bois, France) were all of PA-quality, while isopropanol, hexane (both from Lab-Scan, Dublin, Ireland), ACN (Rathburn Chemicals, Walkerburn, Scotland) and tetrachloromethane (CCl_4) from VWR (Briare Le Canal, France) were of HPLC-grade quality. Water was deionized and glass-distilled in-house. Totally porous spherical 3 μm PBD–ZrO_2 particles with an average pore diameter of 300 Å and surface area of 30 $m^2\,g^{-1}$ were obtained from ZirChrom Separations (Anoka, MN, USA).

2.2. Chromatographic instrumentation

A Merck-Hitachi LaChrom L-7100 pump (Merck, Darmstadt, Germany) was used to deliver a constant flow rate throughout the study. Manual injections were performed with a Valco ChemInert model C4 injection valve (Valco Instruments, Houston, TX, USA) equipped with a 50 nl internal loop. An HP 5900 GC oven (Agilent, Palo Alto, CA, USA) was used to control the temperature of the column, while detection was accomplished using a Spectra 200 UV-Vis Detector (Spectra-Physics, San Jose, CA, USA) equipped with an on-column optical cell built in-house. The detector was operated at 215 nm, while the response time was set to 0.2 s. The column inlet was connected to the injector with a 50 μm i.d. × 20 cm fused silica capillary, while the column outlet was connected directly to the UV-detector with a 100 μm i.d. × 15 cm fused silica capillary. A 20 μm i.d. × 10 cm capillary was mounted after the UV-detector to prevent the mobile from boiling at higher temperatures. TotalChrom 6.2 Workstation software (Perkin-Elmer Instruments, Wellesley, MA, USA) was used for data sampling.

2.3. Packing equipment and column preparation

The packing equipment consisted of an Isco 100DM syringe pump (Isco, Lincoln, NE, USA) providing a maximum pressure of 10,000 psi (690 bars), a Manual Autoclave valve (Autoclave Engineers, Erie, PA, USA) and two different slurry reservoirs. Three different packing methods, i.e. Methods I–III, were applied (Table 1). In Methods I and II, a 2 mm i.d. × 5 cm long stainless steel slurry reservoir was used, while

Table 1
Column packing conditions

	Slurry concentration (mg ml^{-1})	Slurry solvent	Packing solvent
Method I[a]	200–1000	Tetrachloromethane	ACN–water (50:50 (v/v))
Method II[a]	200–1000	10% Triton X-100[b] in water	ACN–water (50:50 (v/v))
Method III[c]	20–100	Isopropanol	Isopropanol

[a] Downstream mode.
[b] Alkylated polyethylene glycols.
[c] Upstream mode.

a 4.6 mm i.d. × 20 cm long reservoir was applied in Method III. In the latter method, the reservoir was submerged into a sonicating bath during the packing. The column hardware was constructed by mounting a Valco ZU1XC internal union (VICI, Schenkon, Switzerland) in each end of a 0.5 mm i.d. × 10 cm long 1/16 in. o.d. glass-lined stainless steel capillary (GLT) from SGE Europe (Kiln Farm, Milton Keynes, UK). A Valco stainless steel screen (pore size = 2 μm, ϕ = 1/16 in., thickness = 75 μm) was placed inside each union to immobilize the packing material. Unless stated otherwise, the appropriate amount of PBD–ZrO_2 was suspended in slurry liquid by sonication for 10 min, the final pressure was held for 15 min before closing the valve, and the columns were allowed to decompress for approximately 30 min before use.

2.4. Column evaluation

The prepared columns were evaluated using a mobile phase consisting of ACN–20 mM phosphate buffer, pH 7.0 (50:50 (v/v)) and a column temperature of 50 °C. The mobile phase was filtered through a 0.45 μm Minisart-RC25 (Sartorius, Göttingen, Germany) and degassed with helium for 10 min prior to use. The column efficiency was measured by calculating the number of theoretical plates obtained when injecting 0.1 mg ml^{-1} toluene (dissolved in mobile phase). The calculations are described in Section 2.6. The dead volume of the system was calculated to approximately 1.7 μl.

2.5. Evaluation of temperature stability

The same mobile phase as described in Section 2.4 was used for evaluation of the high-temperature stability of the columns. For evaluation of the isothermal temperature stability, the column was continuously operated at high-temperatures (100–150 °C), only interrupted by three subsequent injections of sample solution at 50 °C isothermally each 24 h. For evaluation of the temperature programming stability, the columns were continuously subjected to a temperature program starting at 50 °C going up to the temperature limit found during the isothermal investigations. One temperature program cycle, including cooling and re-equilibration, took 11 min, thus allowing approximately 130 cycles per 24 h. The sample solution consisted of benzyl alcohol (t_0), toluene, fluorene and amitriptyline, and the concentrations were 0.1, 0.9, 0.4 and 0.2 mg ml^{-1}, respectively. The column efficiencies and retention factors of these solutes were calculated as described in Section 2.6.

2.6. Calculations

The number of theoretical plates N, the retention factor k and total column porosity ε_T were calculated as described below:

$$N = 5.54\left(\frac{t_r}{w_{0.5}}\right)^2$$

where t_r is the retention time and $w_{0.5}$ the peak width at 50% peak height.

$$k = \frac{t_r - t_0}{t_0}$$

where t_0 is the retention time of a non-retained compound.

$$\varepsilon_T = \frac{Ft_0 - V_{ex}}{\pi\,(d/2)^2\,L}$$

where F is the flow rate, V_{ex} is the extra-column dead volume, d is the column i.d., and L is the column length.

2.7. ICP–AES

The ICP–AES analyses of the effluent samples were performed with a Vista AX CCD Simultaneous ICP–AES (Varian, Victoria, Australia). The effluent samples were collected in polyethylene vials and diluted 10× with pure water to reduce the ACN content. The *c*LOD of zirconium was estimated to 0.05 μg ml^{-1}.

3. Results and discussion

3.1. Column preparation

3.1.1. General considerations

Preparation of highly efficient packed capillary LC columns ($h < 3$) is generally more challenging than preparing conventional sized columns [39]. Mainly, it

impose more stringent requirements to the homogeneity and pureness of the slurry, since the disparaging effect on column efficiency caused by irregularities in the packing structure, e.g. small voids due to the presence of fines or particle agglomerates, is more or less inversely proportional to the column i.d. Moreover, the narrow i.d. of packed capillary LC columns generally requires more stable slurries, since rapid sedimentation tend to cause a "traffic jam" at the inlet and cause slow and inhomogeneous packing. The latter is probably a key issue when packing capillary LC columns with ZrO_2-based materials, since the apparent density of ZrO_2 is approximately $6\,g\,ml^{-1}$, that is, 3–4× higher than that of silica [33]. Consequently, the sedimentation of these materials is rapid in practically all solvents, even when suspended in the halogenated ones. For example, the time required to settle $100\,mg\,ml^{-1}$ suspensions of 3 μm PBD–ZrO_2 in acetone and CCl_4 (2 cm high) was estimated to 12 and 50 s ($n = 2$), respectively.

Nevertheless, a commonly applied principle to obtain a homogenously packed bed of reversed-phase particles is to use a non-flocculating slurry based on a medium to non-polar organic solvent, followed by intra-column compression of the bed with a flocculating polar packing solvent. For packing of reversed-phase silica-based particles in 320 μm i.d. fused silica capillaries, Vissers et al. showed that it is preferable to have a flocculating packing solvent and that this was the major factor in determining the quality of the packed capillary [40,41]. Furthermore, Xiang et al. used chloroform and isopropanol as slurry and packing solvents, respectively, for packing of 1 μm non-porous PBD–ZrO_2 particles in <100 μm i.d. capillaries [42,43]. A simple method for estimation of the non-flocculating properties of different potential slurry solvents is to simply measure the sediment height H_f after complete sedimentation, since the highest sediments are expected to occur in the most non-flocculating solvents. Consequently, the H_f of PBD–ZrO_2 particles in a series of polar to non-polar solvents were investigated. As shown in Fig. 1, ACN–water (50:50 (v/v)) gave the lowest sediment and was, therefore, chosen as the packing solvent, while CCl_4, and particularly aqueous Triton X-100 (surfactant), provided significantly higher sediments than the other solvents. Accordingly, it was chosen to use a traditional downstream highly concentrated slurry method using CCl_4 (Method I) and aqueous Triton X-100 [44] (Method II) as slurry solvents. In addition, an upstream flocculating slurry method with stirring was investigated, since this principle have been commonly applied for large particles that sedimentate rapidly (Method III). However, in this method, a relatively large packing reservoir was required to obtain proper stirring, and thus, the same

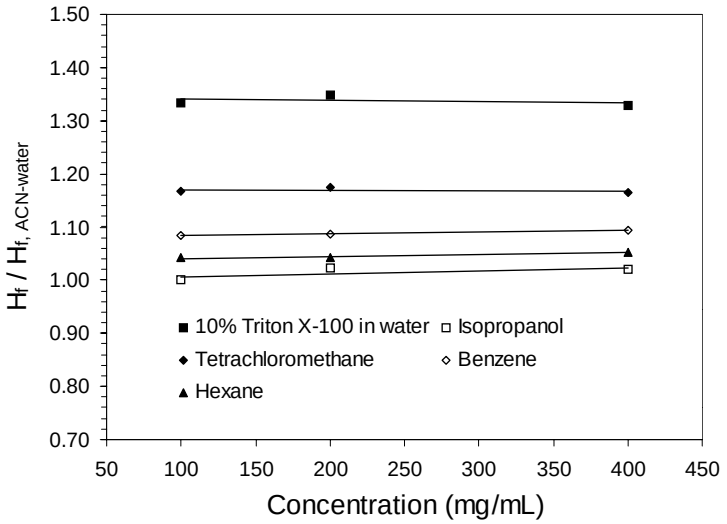

Fig. 1. Solvent effects on PBD–ZrO_2 particle flocculation measured by normalized sediment height. Appropriate amounts of 3 μm PBD–ZrO_2 particles were placed into 4 × 100 mm glass tubes, suspended in 1 ml solvent via 10 min sonication, and allowed to settle 1 h ($n = 2$).

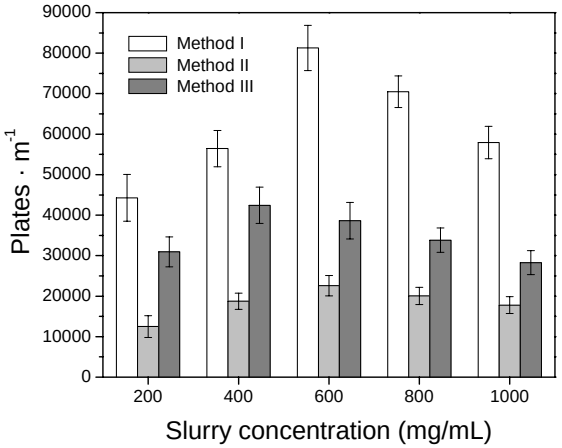

Fig. 2. Influence of slurry concentration on column efficiency (Methods I–III). The packing solvent was ACN–water (50:50 (v/v)) in Methods I and II, while isopropanol was used in Method III. Packing pressure: 650 bars. Note that the slurry concentrations in Method III are multiplied by a factor of 10 to fit the diagram. The error bars represent the standard deviation of the measurements ($n = 3$).

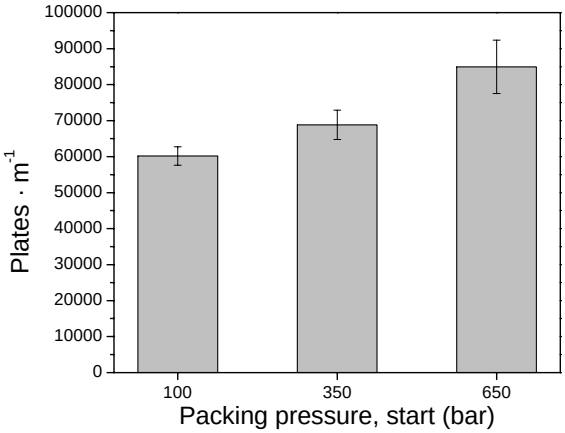

Fig. 3. Influence of initial packing pressure on column efficiency (Method I). The packing pressure was immediately ramped with 200 bars min^{-1} up to the packing end pressure at 650 bars. Slurry: 600 mg ml^{-1} of 3 μm PBD–ZrO_2 in CCl_4. Packing solvent: ACN–water (50:50 (v/v)). The error bars represent the standard deviation of the measurements ($n = 3$).

solvent was used both as slurry and packing solvent to prevent any intra-reservoir mixing. Moreover, isopropanol was preferred instead of ACN–water (50:50 (v/v)), since the lower viscosity of the former was expected to give an overall rapid packing process, particularly during the final part [45].

3.1.2. Influence of slurry concentration on column efficiency

Initially, trial-and-error methodology was applied to find the optimum slurry concentration for each packing method. The slurry concentration was varied while all other conditions were held constant. As shown in Fig. 2, the highest column efficiencies for Methods I and II were obtained with slurry concentrations in the range of 200–1000 mg ml^{-1}, while the corresponding concentration range for Method III was approximately 20–100 mg ml^{-1}. Fig. 2 also shows that the best column efficiency was obtained with Method I using a slurry concentration of 600 mg ml^{-1}, which produced columns with almost 90,000 plates m^{-1} (R.S.D. = 8.7%, $n = 3$). Method II was also performed with pure water instead of ACN–water (50:50 (v/v)) as the packing solvent, but no significant difference in column efficiency was observed. Based on the results shown in Fig. 2, the rest of the experiments were performed with columns prepared using Method I.

3.1.3. Influence of initial packing pressure on column efficiency

Fig. 3 shows the column efficiencies obtained with different initial packing pressures. After the valve was opened, the packing pressure was immediately ramped with 200 bars min^{-1} to the final pressure (i.e. 650 bars), which was the highest pressure obtainable with the present packing equipment. The best column efficiencies were obtained with an initial packing pressure of 650 bars. However, the results indicate that even better column performance is achievable with initial packing start pressures above 650 bars, provided that the stationary phase particles tolerate this violent treatment. It is also worthy noting that the R.S.D.s of the obtained column efficiencies were 4.2, 5.9 and 8.7% ($n = 3$) at 100, 350 and 650 bars, respectively, which probably indicates that a high packing start pressure causes a more random and less reproducible packing process.

3.1.4. Influence of column i.d. and length on column efficiency

To evaluate the influence of column i.d. on the column efficiency, columns with 0.3, 0.5 and 1.0 mm i.d.

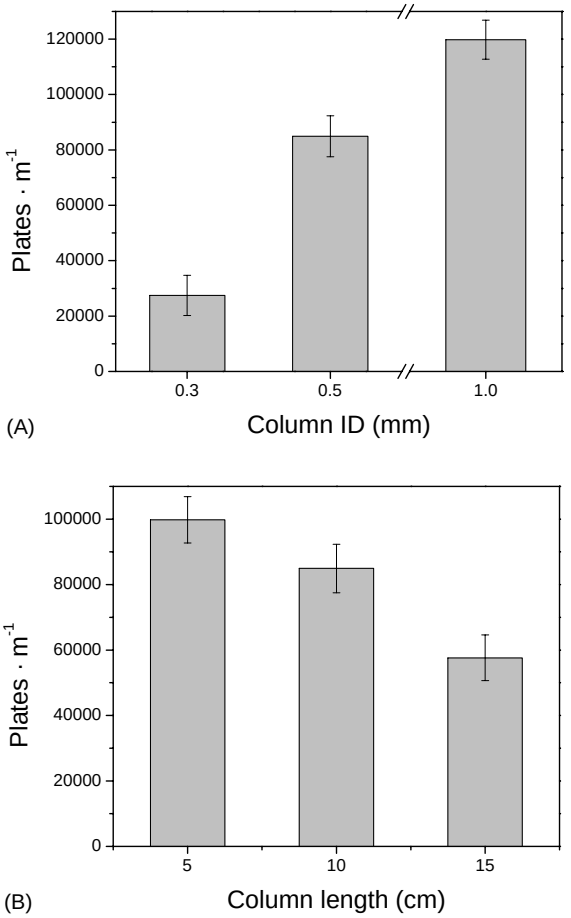

Fig. 4. Influence of: (A) i.d. and (B) column length on column efficiency (Method I). Slurry: 600 mg ml^{-1} of 3 μm PBD–ZrO_2 in CCl_4. Packing solvent: ACN–water (50:50 (v/v)). Packing pressure: 650 bars. The error bars represent the standard deviation of the measurements ($n = 3$).

were prepared. As shown in Fig. 4(A), the column efficiency drops when the column i.d. is reduced. It should be noted, however, that the 0.3 mm i.d. columns were prepared in fused silica capillaries instead of GLT, which made it impossible to use a high initial packing pressure without bursting the column. It is, therefore, reasonable to expect that somewhat higher efficiencies can be obtained by using 0.3 mm i.d. GLT, instead. Furthermore, the column length was found to have a significant influence on column efficiency. Fig. 4(B) shows the efficiencies obtained when packing 0.5 mm i.d. columns of 5, 10 and 15 cm lengths. Not surprisingly, the column efficiency decreased with increased

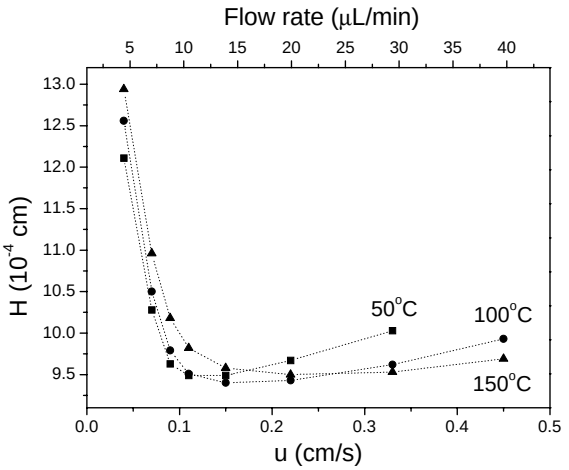

Fig. 5. Van Deemter curves for different temperatures. Column: 3 μm PBD–ZrO_2, 0.5 mm i.d. × 10 cm. Mobile phase: ACN–20 mM phosphate buffer, pH 7.0 (50:50 (v/v)).

column length, and highly efficient columns longer than 10 cm were difficult to obtain. Therefore, it was chosen to use 0.5 mm i.d. × 10 cm columns for the temperature stability studies, since this length was a good compromise between resolving power and column efficiency.

3.2. Operation at high-temperatures and temperature programming

3.2.1. Column efficiency as function of flow rate and temperature

The influence of flow rate and temperature on column efficiency was examined by plotting van Deemter curves in the flow range of 4–40 μl min^{-1} at three different temperatures (i.e. 50, 100 and 150 °C). As shown in Fig. 5, a slight gain in efficiency was observed when the column temperature was increased from 50 to 100 °C, while no gain was observed when going from 50 to 150 °C. This is most likely explained by the fact that the longitudinal diffusion (the B-term) becomes dominating above 100 °C, since the probe solute (i.e. toluene) is a small neutral molecule with a relatively high diffusion coefficient. Furthermore, the van Deemter curves became flatter at the higher temperatures, allowing the flow rate to be markedly increased without significant loss in efficiency. For example, at 150 °C the flow rate could be increased by at least a factor of 4 with only a small increase in plate height.

The van Deemter curves also revealed that somewhat better efficiencies could have been presented by testing the packed columns at other conditions than the ones applied, i.e. using a flow rate of 15 μl min^{-1} at 100 °C.

3.2.2. Column stability at high isothermal temperatures

The long-term high-temperature stability of the prepared PBD–ZrO_2 columns was investigated by operating the LC system at high-temperatures continuously. Initially, the column temperature was set to 150 °C, since the manufacturer claims that conventional sized PBD–ZrO_2 columns are stable at this temperature. However, as illustrated in Fig. 6, a relatively rapid decrease, not only in column efficiency, but also in retention factors, was observed at this temperature. Furthermore, when the column was opened after approximately 3,200 void volumes or 40 h operation to examine the packed bed, an 8 mm long void at the inlet

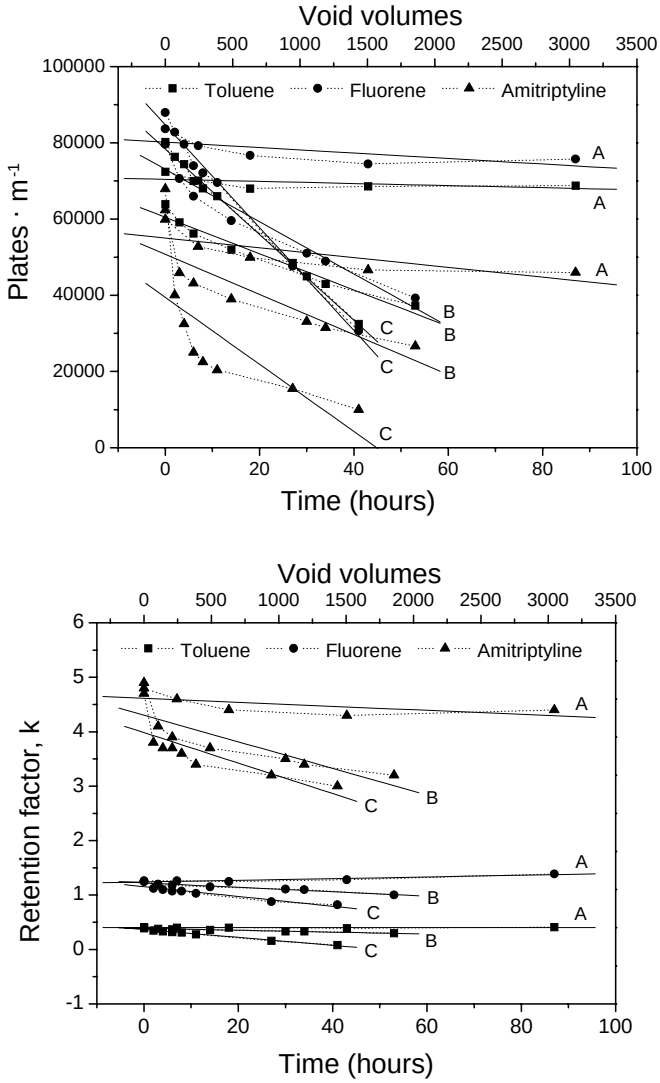

Fig. 6. The long-term high-temperature (A, 100 °C; B, 125 °C; C, 150 °C) stability of a 0.5 mm i.d. × 10 cm column packed with 3 μm PBD–ZrO_2 particles ($n = 2$). Mobile phase: ACN–20 mM phosphate buffer, pH 7.0 (50:50 (v/v)). Flow rate: 10 μl ml^{-1}.

(8% of total column length), was discovered. To investigate if the void was caused by dissolution of the ZrO_2 support, the column effluent, which was collected during the experiments, was analyzed by ICP–AES. In agreement with the analogous work performed by Carr and co-workers [31], the effluent showed no measurable level (cLOD $< 0.05\,\mu g\,ml^{-1}$) of zirconium. Furthermore, scanning electron microscopy (SEM) was used to compare the exposed particles at the column inlet with unused particles. No apparent difference was observed, neither in diameter nor exterior surface (Fig. 7). However, the SEM pictures suggest that the particles are not too monodisperse [46], which is critical to obtaining a well-packed bed. The initial total column porosity of the temperature-exposed column was calculated to $\varepsilon_T = (16.9–1.7)\mu l/19.6\,\mu l = 0.78$, which indicates a relatively loose packing structure. As a comparison, the ε_T of a well-packed 0.32 mm i.d. × 15 cm capillary filled with 3 μm 300 Å silica-based particles was found to be 0.61 [47]. Thus, since the dead volume was practically unchanged during the experiment, a possible explanation for the column instability is a temperature-induced compression (rearrangement) of the packed bed. This is also supported by the shape of the retention factor versus time curves in Fig. 6, since stationary phase degradation

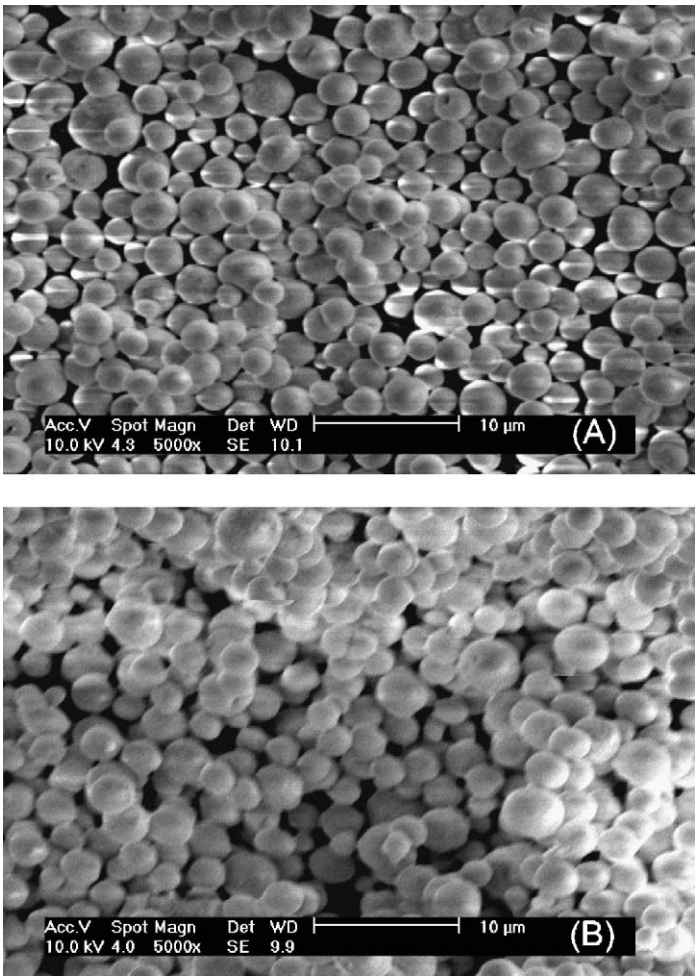

Fig. 7. SEM pictures of: (A) unused and (B) high-temperature exposed 3 μm PBD–ZrO_2 particles. Picture (B) was made by tapping out a small amount of exposed particles from the column inlet.

usually gives a linear decrease in retention factors that do not reach an asymptotic limit. However, the initial reduction in retention is worthy noting, if assuming that no PBD-coating or ZrO_2 support were lost from the column. A possible explanation can be based on the fact that the individual solute molecules are exposed to a minor portion of the total amount of stationary phase since they travel through the column in narrow, distinct channels. Thus, the surface area available for each solute molecule within the compressed bed is probably smaller than what available within the initially longer and more loosely packed column.

Nevertheless, to find the maximum temperature limit of the column, the stability testing was repeated with 25 °C decrements in temperature until stable conditions were obtained (i.e. <20% change in solute efficiency and 10% change in retention factors after passing 3,000 void volumes of mobile phase through the column). As shown in Fig. 6, the column was found to be stable for at least 3,000 void volumes or 90 h operation at 100 °C. At this temperature, the solute efficiencies changed about 5–18% and the retention factors changed about 6–8%. As a comparison, Li and Carr concluded that a 4.6 mm i.d. PBD–ZrO_2 column was stable for at least 7,000 void volumes at 100 °C [35].

3.2.3. Column stability in temperature programming mode

Since the 0.5 mm i.d. PBD–ZrO_2 columns were defined stable at 100 °C in isothermal mode, similar freshly packed columns were subjected to numerous temperature programs ending at this temperature. Unfortunately, a rapid decrease in both efficiency and retention factors was observed. When the column was opened after approximately 40 h operation, a 5 mm long void at the inlet was discovered. The fact that temperature programming had a more pronounced effect on the bed stability than high isothermal temperatures strengthens the theory of compression of the packed bed, since temperature programming imposes more physical stress on the column due to the repeated thermal expansions. Moreover, dissolution of the stationary phase should be more pronounced at high isothermal temperatures than in temperature programming mode.

To improve the temperature programming stability and at the same time maintain high column efficiency, several alternative temperature-related packing methods were tested, e.g. packing at high isothermal temperatures or during temperature programming. Unfortunately, all these methods produced columns with less than 10,000 plates m^{-1}, e.g. due to the

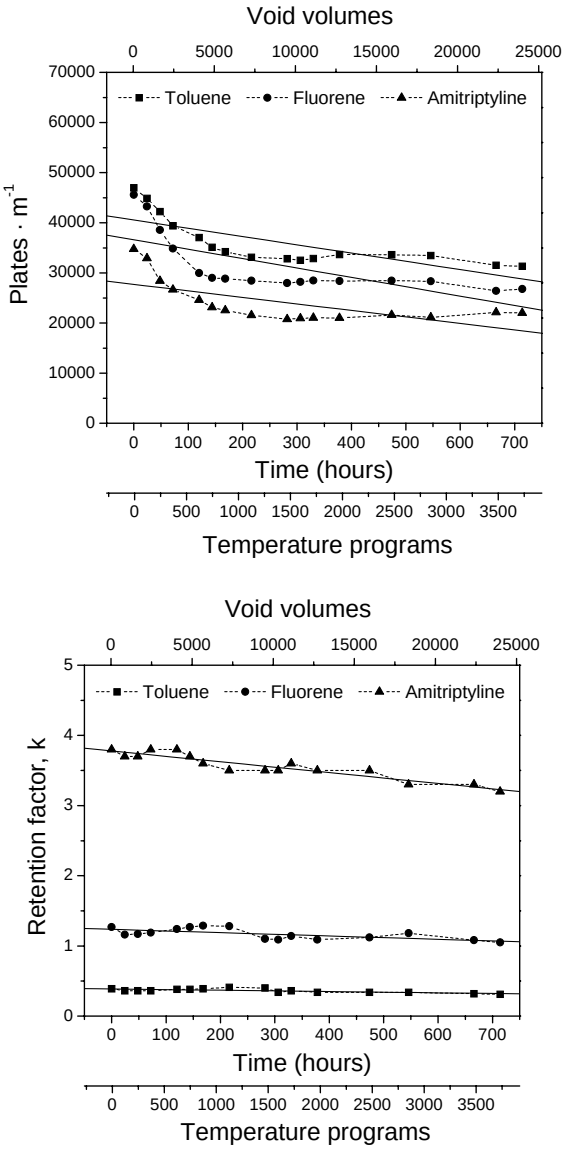

Fig. 8. The long-term temperature programming stability of a 0.5 mm i.d. × 10 cm column packed with 3 μm PBD–ZrO_2 particles ($n = 2$). Mobile phase: ACN–20 mM phosphate buffer, pH 7.0 (50:50 (v/v)). Flow rate: 10 μl min^{-1}. Temperature program: 50 °C (0 min), then 10 °C min^{-1} to 100 °C. Total cycle time: 11 min.

inevitable application of a flow restrictor at the column outlet to prevent boiling that probably caused slow packing processes or formation of channels in the packed bed. However, when columns were packed as initially described, ramped up and down from 50 to 100 °C for 48 h and then refilled, fairly stable columns with acceptable efficiencies were obtained (Fig. 8). Although not fully regaining their initial efficiency after refilling, the solute efficiencies changed about 19–28% and the retention factors changed about 4–5% after running 3,000 void volumes or approximately 500 temperature programs. Furthermore, after passing nearly 25,000 void volumes of mobile phase through the column (about 3,900 temperature programs), the corresponding figures were 32–37 and 13–17%, respectively. As shown in Fig. 8, the column efficiencies reach an asymptotic limit, while the retention factors continue to decrease. When reopening the columns, an approximately 0.5 mm long void at the column inlet was observed. This indicates a continued, but markedly reduced, change in bed stability, which probably explains the continuous change in retention factors. The corresponding chromatograms of the probe solutes after 0 and 3,900 temperature programs are shown in Fig. 9.

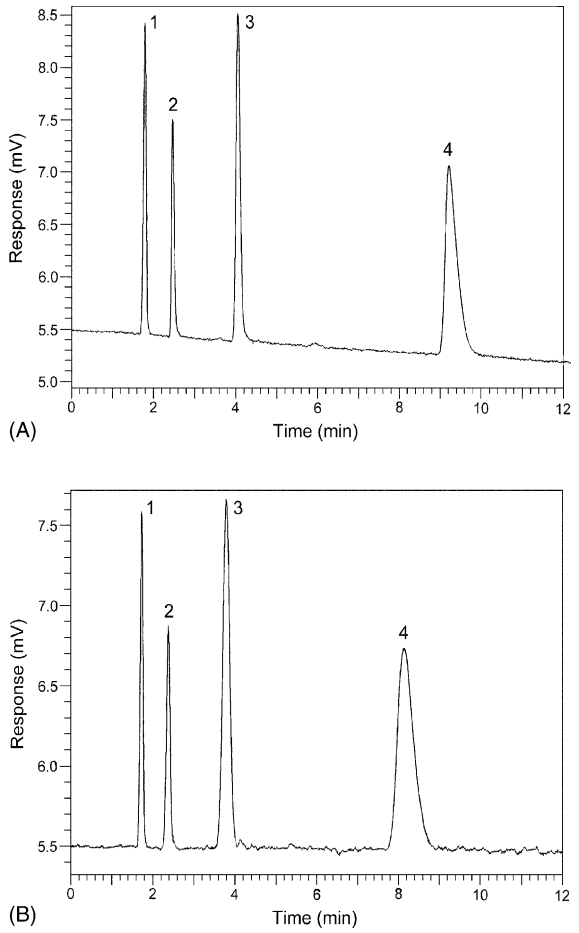

Fig. 9. Isothermal separation of: (1) benzyl alcohol; (2) toluene; (3) fluorene; and (4) amitriptyline: after (A) 0 and (B) 3900 temperature programs. Column: 3 μm PBD–ZrO_2, 0.5 mm i.d. × 10 cm. Mobile phase: ACN–20 mM phosphate buffer, pH 7.0 (50:50 (v/v)). Flow rate: 10 μl min^{-1}. Column temperature: 50 °C.

4. Conclusions

A truly thermally stable packed column, not only temperature stable stationary phase particles, is a prerequisite for performing LC at elevated temperatures. Although mesoporous PBD–ZrO_2 conventional-sized columns has been demonstrated to be highly temperature stable, the reasonably good packing methods (i.e. 90,000 plates m^{-1}) presented in this paper failed to produce capillary columns that were truly stable above 100 °C. However, the development of even better packing methods will most likely extend the applicable temperature range and thus allow chromatographers to fully exploit the high-temperature stability of mesoporous PBD–ZrO_2 in capillary LC.

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