Study of the influence of the aspect ratio on efficiency, flow resistance and retention factors of packed capillary columns in pressure- and electrically-driven liquid chromatography

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Abstract

The influence of the aspect ratio, ρ (ρ = column diameter/particle diameter), on column parameters such as efficiency, retention factors and flow resistance was studied in both high-performance liquid chromatography and capillary electrochromatography with packed capillary columns. In order to compare the true efficiencies of different columns, a procedure to account for external band broadening was applied. High efficiencies (reduced plate height $h \approx 2$) were obtained with capillary columns with internal diameters of 150-, 100-, and 75-μm, packed with 10-μm particles. In contrast to previous reports in the literature, no significant improvements in efficiency or flow resistance were observed when the aspect ratio of such columns was decreased. Our observations suggest that the wall effect in these types of columns is not significant. When the aspect ratio was decreased by increasing the particle size, a decrease in reduced plate height was observed. However, the results of flow resistance measurements showed that the latter effect should be attributed to differences in packing and particle batch quality rather than to differences in the aspect ratio.
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1. Introduction

There is a strong interest in a further miniaturization of high-performance liquid chromatography (HPLC) [1] and the development of capillary electrochromatography (CEC) [2]. In many applications, such as in high-throughput screening of candidate drugs and in proteomics, fast, efficient separation methods are required to analyze very small amounts of sample [3–5]. Also, low volumetric flow rates are advantageous for coupling the separation system to mass-spectrometry using an electrospray interface [6].

The main strategy to improve the efficiency of packed columns is by decreasing the size of the stationary phase particles (d_p). Several authors have shown the possibility to use sub-micrometer sized particles in HPLC and especially CEC [7–9]. It has been suggested that an additional improve-ment in efficiency can be obtained by optimizing the column diameter (d_c) in relation to the particle diameter [10]. An increase in efficiency has been claimed for columns with a low aspect ratio ($\rho = d_c/d_p$). However, this possible effect of the aspect ratio is still not widely documented and not fully understood.

In an early study, Knox and Parcher investigated the effect of the aspect ratio on dispersion in pressure-driven liquid chromatography with columns packed with spherical glass beads [10]. They reported that columns with an aspect ratio between 6 and 8 show a higher efficiency than columns with a higher aspect ratio. The increase in efficiency was attributed to the existence of a dominant wall-effect on the packing structure. In high-aspect ratio columns two different packing regions are expected to be present, one close to the wall and one in the core region of the column. When the packing densities or structures in these two regions are different, differences in mobile-phase flow velocity over the cross-section of the column will exist, which leads to additional peak broadening. When the aspect ratio is decreased,

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the wall effect will influence the packing structure over the entire cross-section and a better flow homogeneity can be obtained. At the same time, a decrease in flow resistance will be obtained. Therefore, longer columns can be used to obtain higher separation efficiencies.

Several authors investigated columns with different aspect ratios, obtained by varying either the column diameter or the particle diameter [11–16]. Karlsson and Novotny observed an increase in efficiency when decreasing ρ through decreasing d_c [11]. Three different column diameters were tested (from 44 to 265 μm) with aspect ratios from 9 to 53. With a decreasing aspect ratio, significant decreases were observed in the eddy-diffusion term (A) and in the mass-transfer term (C) of the van Deemter equation. Yet, the authors concluded that this could not be attributed to a wall-effect, since the column porosity and the flow resistance did not change significantly. According to Kennedy et al., decreasing ρ by changing either d_c or d_p enhanced the performance of columns with porous and pellicular stationary phases [9,10]. In decreasing ρ from 10 to 4, a steady reduction was found for the A- and C-term contributions to peak broadening [12]. No effect of ρ on the chromatographic performance was observed when perfusion (macro-porous) particles were used [13]. The aspect-ratio effect was observed in pressure-driven systems only; in CEC such an effect was not found [14]. A possible explanation is that in electrically-driven systems the local flow velocity is less dependent on the packing porosity and structure than in pressure-driven systems. An effect of a variation in d_c (and therefore in ρ) on the A-term in the pressure-driven mode was also observed by Hsieh and Jorgenson [15]. However, they found an increase in the C-term when the column diameter was decreased. Other authors found that when irregular particles were used, no effect of ρ on the efficiency was observed [16,17].

Another way to evaluate the properties of a packed bed is to measure the flow resistance, as is normally done to evaluate chromatographic columns [18]. In low-aspect-ratio columns, the wall effect should create a more-open bed structure, leading to a lower flow resistance, compared to columns with a high aspect ratio. This trend was confirmed for gas-chromatography columns [19]. Kennedy and Jorgenson found a slight downward trend in flow resistance when the column diameter was decreased for HPLC columns [12]. However, the scatter in their experimental data was large. Other researchers found opposite results to the expected trend in flow resistance. Novotny et al. observed a decrease in the total porosity when the aspect ratio was decreased [11,20]. The packing density was also studied using other methods. In early experiments, columns of 30.0-mm i.d. packed with 2.3-mm glass beads revealed regions of ideal packing and regions showing defects. These defects were found mostly near the wall of the column [21]. With static magnetic-resonance imaging (MRI), Lightfoot et al. observed opposite results [22]. Packing non-uniformity and void formation were found to be smallest near the wall, while the core region was more-loosely packed. Shalliker

et al. observed two different packing regions using optical on-column visualization [23]. The first region, containing voids, was found adjacent to the wall. A second region gave rise to variation in sample migration, with retention increasing from the wall to the center of the column.

In the present study, the influence of the aspect ratio on efficiency, flow resistance, and retention parameters of slurry packed columns with i.d. 75–150 μm was investigated. Aspect ratios from 7.5 to 30 were obtained by varying the column and particle diameters. The efficiencies of columns with different aspect ratios were compared in pressure-driven as well as in electrically-driven liquid chromatography.

2. Experimental

2.1. Chemicals, solutions and materials

Acetonitrile (ACN) was obtained from Rathburn (Walkerburn, UK), and tetrahydrofuran (THF) from Acros (Geel, Belgium). Tris(hydroxymethyl)aminoethane (Tris) and hydrochloric acid (37%) were purchased from Merck (Darmstadt, Germany). Thiourea, naphthalene, fluorene, pyrene, anthracene, and benzanthracene were purchased from various suppliers.

A mixture of ACN–water (80:20, v/v) was used as mobile phase in HPLC. A solution of the mobile phase containing thiourea and polyaromatic hydrocarbons, with concentrations between 5 and 10 mg/L for each component, was used for injection. In CEC experiments a mobile-phase composition of ACN–aqueous Tris buffer (pH 7.5) (80:20, v/v) was used, with a total ionic strength of 1 mM.

Fused-silica capillaries (375-μm O.D., 75-, 100-, and 150-μm i.d.) were purchased from Polymicro Technologies (Phoenix, AZ, USA). The stationary phases, Hypersil ODS 5 and 10 μm, both with an average pore diameter of 120 Å, were obtained from Agilent Technologies (Waldbronn, Germany).

2.2. Instrumentation

In the pressure-driven mode, the eluent was degassed using a micro degasser (Agilent). An Agilent 1100 series capillary pump coupled to a home-made splitter provided the flow. The spit ratio was set at 1:120. A wide capillary was used for the connection between the pump and the splitter, so that the pressure readout of the instrument could be used as a measure of the inlet pressure of the column. The column was directly coupled to a 10-nL injector equipped with a pneumatic actuator and high speed switching accessory (Valco, Schenkon, Switzerland). On-column UV detection was performed using the diode array detector of an HP [3D]CE instrument (Agilent). Therefore, a mass spectrometry cartridge was used in a way that the electrospray outlet was used as column inlet (coupled to the injector) and an UV detection window could be used as normal in CE.

The CEC experiments were performed on an HP [3D]CE instrument (Agilent). A nitrogen pressure of 10 bar was applied on both ends of the column during the separation. During all experiments, the column was thermostatted at 25 °C. Samples were injected electrokinetically at 5 kV for 3 s. On-column detection at 254 nm was performed using a diode-array detector (HP [3D]CE, Agilent). Eluent for CEC was degassed by ultrasonication, prior to use.

Particle size distributions were measured using a Coulter Multisizer II (Luton, UK).

2.3. Column preparation

Column packing was performed by modifying a previously described high-pressure slurry method [24]. Capillaries with a length of 50 cm were attached to a slurry reservoir. A temporary frit, a screen with 2-μm pores, was fitted onto the column. A slurry containing 0.05 g/mL of the packing material was prepared in THF and homogenized for 10 min by ultrasonication. The slurry reservoir was filled using a Rheodyne switching valve. A maximum pressure of 650 bar was applied to the column for 2 h, using ACN–water (20:80, v/v) as a packing liquid. During this time, ultrasonication was applied to the slurry reservoir and column. An outlet frit was prepared by hydrothermal treatment, using a heating element and the temporary frit was removed. After packing, the columns were coiled with an approximate coiling radius of 3 cm and flushed with ACN for 4 days. Afterwards, the column was flushed with ACN–water (20:80, v/v) and an inlet frit was prepared by hydrothermal treatment of the stationary phase. Finally, a detection window was made next to the outlet frit. Columns of about 30-cm effective length were used in both pressure and electrically-driven mode.

Prior to the CEC experiments, the column was flushed with eluent (20% Tris buffer in ACN). A pre-conditioning step was performed by applying a stepwise increase in voltage up to 30 kV over the column, until a stable current was observed.

3. Results and discussion

Due to the small peak volumes obtained when using capillary columns, it is mandatory to minimize external band broadening in order to preserve high separation efficiencies. Therefore, packed capillary columns, complete with an on-column-detection window, were directly connected to a 10-nL high-speed air-actuated injector. In order to compare the true efficiencies of different columns, a correction for external band broadening may be necessary.

The external-band-broadening contribution, $\sigma_{v,\text{ext}}^2$ was estimated directly from the obtained chromatograms by separating a standard mixture on a packed column and applying a method published previously [25]. To a first approximation, it may be expected that for chemically similar compounds the same separation efficiency will be obtained. When this is the case, the observed (total) peak variance in volume units for compound i can be written as:

$$\sigma_{v,\text{tot}}^2 = \frac{V_{\text{r},i}^2}{N} + \sigma_{v,\text{ext}}^2$$

where $V_{\text{r},i}$ is the retention volume for compound i and N is the plate number. A plot of the measured peak variances against the squared V_{r} values for the separated compounds is expected to yield a straight line, with a slope equal to $1/N$ and the extra-column dispersion, $\sigma_{v,\text{ext}}^2$, as the intercept.

In practice, linear relationships between $\sigma_{v,\text{tot}}^2$ and $V_{\text{r},i}^2$ were observed for the polyaromatic hydrocarbons, with an average correlation coefficient (r^2) of 0.999. Apparently, the variation between the test compounds with respect to their diffusion coefficients is small enough to justify the assumption that their plate numbers have equal values.

It was found experimentally that the connection between the chromatographic column and the injector was a critical factor for generating external band broadening and peak tailing. A void between the injector and the chromatographic column behaves as an ideal mixer. Due to the exponential time constant of this mixing process tailing may be observed, depending on the volume of the void, the flow rate and the flow profile. The same 150-μm i.d. column packed with 10-μm particles was connected twice to the injector device. In the first experiment, the plot of $\sigma_{v,\text{tot}}^2$ versus $V_{\text{r},i}^2$ showed a significant intercept, corresponding to an external peak broadening volume standard deviation, $\sigma_{v,\text{ext}}$, of approximately 80 nL, as shown in Fig. 1. After reconnecting the column, no external-band-broadening contribution was found. In further experiments, this approach was used for every newly connected column. When the external peak broadening was significant, the column was reconnected.
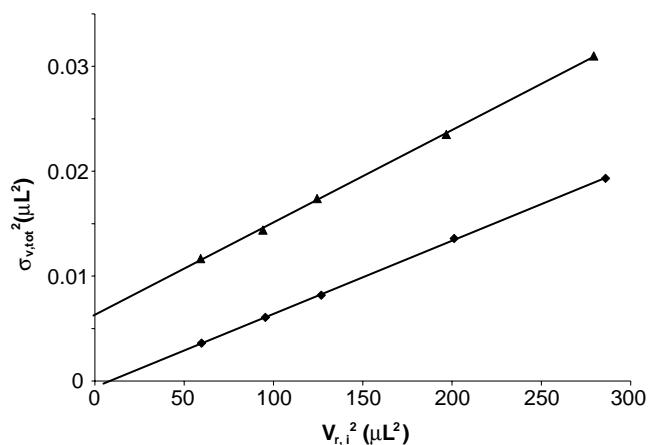

Fig. 1. Dependency of total peak variance on squared retention volume for a series of polyaromatic hydrocarbons, separated on a 150-μm i.d. column packed with 10-μm particles. The two data series were obtained with the same column, before (▲) and after (◆) re-installation in the equipment.
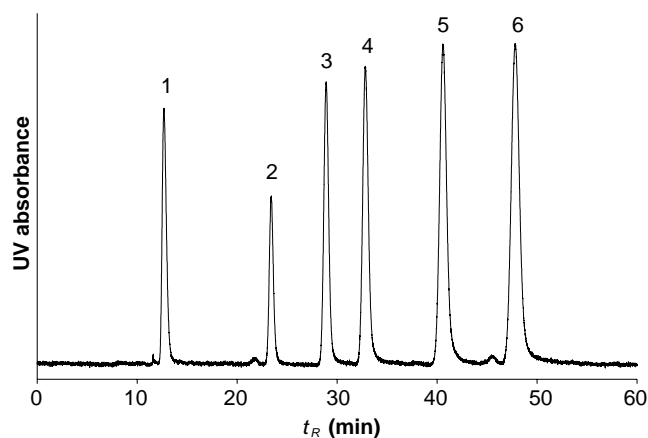
Fig. 2. Test chromatogram of a HPLC separation of a mixture of polyaromatic hydrocarbons using a 75-μm i.d. capillary packed with 10-μm particles. The eluent composition was ACN–water (80:20, v/v). The peaks represent (1) thiourea, (2) naphthalene, (3) fluorene, (4) anthracene, (5) pyrene, and (6) benzanthracene, respectively.

In general, in the HPLC mode, for columns with i.d. ≥75 μm, the external band broadening volume standard deviation could be kept below 3 nL, as was verified with the approach described above, and tailing could be prevented. When using CEC, no external-band-broadening contribution was observed.

In Fig. 2, we show a typical chromatogram obtained in the pressure-driven mode, using a 75-μm i.d. column packed with 10-μm particles, at a flow rate close to its optimum value. The chromatogram shows a good efficiency for retained solutes ($1 < k < 3$) with plate numbers around 16 000 on a 30-cm column (plate height, $H \approx 20$ μm; reduced plate height, $h \approx 2$).

The influence of the aspect ratio on the column efficiency was investigated by changing the column diameter and keeping the particle size constant. For columns with small diameters, a higher efficiency may be expected due to a combination of the wall effect with enhanced radial diffusion. Columns with 75-, 100-, and 150-μm i.d. were slurry-packed with 10-μm particles ($7.5 < \rho < 15.0$) and their averaged data, obtained in the pressure-driven mode, are given in Table 1. All columns exhibited a good efficiency with minimum reduced plate heights (h_{min}) between 1.6 and 2.7 and with optimum reduced velocities (ν_{opt}) between 2.6 and 5.6. However, no increase in efficiency was observed

Table 1
Influence of the aspect ratio on efficiency and flow velocity in HPLC

d_c (μm)	d_p (μm)	ρ	$n^{[a]}$	$h_{min}^{[b]}$	$\nu_{opt}^{[b]}$
150	10	15.0	8	2.2 ± 0.5	5.0 ± 0.9
100	10	10.0	8	1.8 ± 0.2	4.1 ± 0.5
75	10	7.5	6	2.3 ± 0.4	3.3 ± 0.7

All columns were packed with particles from the same batch of Hypersil-ODS.
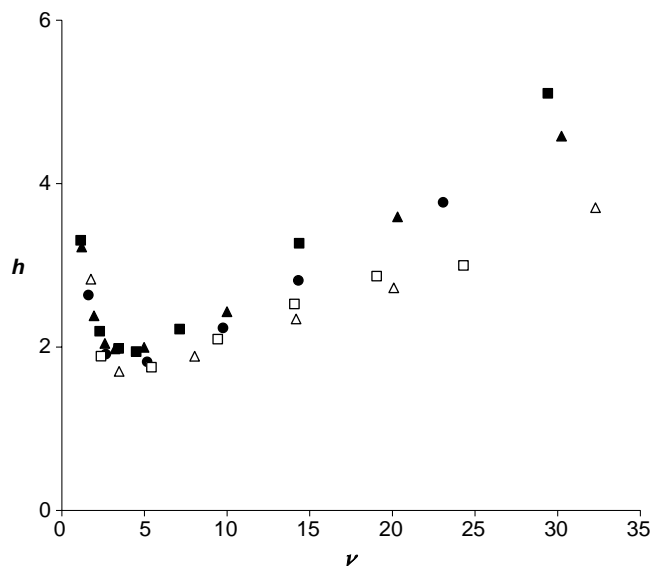[a] Number of columns studied.
[b] Mean ± standard deviation.

Fig. 3. Reduced H vs. ν curves for 75-μm (▲), 100-μm (●) and 150-μm (◆) i.d. columns packed with 10-μm particles. Pressure-driven mode (solid symbols); electrically-driven mode (75 and 150 μm i.d. columns only; open symbols).

with decreasing aspect ratio (i.e. decreasing the column diameter). Random variations in efficiency due to differences in column-packing quality were found to be more significant than a possible systematic wall effect or systematically enhanced radial diffusion.

Fig. 3 shows the comparison between typical h versus ν curves of columns with different aspect-ratios, measured in the pressure and electrically-driven modes. A higher efficiency was obtained in CEC than in HPLC. Since the columns were very well packed ($h_{min} \approx 2$), the differences between the eddy-diffusion and mass-transfer contributions for HPLC and CEC were not as pronounced as reported by other authors [13]. In the electrically-driven mode, the flat flow profile of the electro-osmotic flow is probably not influenced by possible inhomogeneities in the packed bed. No systematic trend in the efficiency was observed in CEC when the aspect ratio was reduced from 15.0 to 7.5.

In order to evaluate the packing density of the different columns, retention factors ($k = KV_s/V_m$) and flow-resistance parameters (ϕ) were determined. In Fig. 4 the observed optimum efficiency of 75- and 150-μm i.d. columns, packed with 10-μm particles, is plotted as a function of the averaged retention factor of fluorene, and measured at different mobile phase velocities. Every data point in the figure represents a column. Significantly lower retention factors were observed on the 75-μm columns than on the 150-μm columns. This indicates that a lower ratio between the stationary-phase and mobile-phase volumes, V_s/V_m, is obtained with narrower columns. The latter, therefore, have a lower packing density and a more-open packing structure. One k value of a 150-μm column was considered as an outlier, and was therefore not included in the trendline. For the 100-μm i.d. columns, retention factors were obtained in between those of the
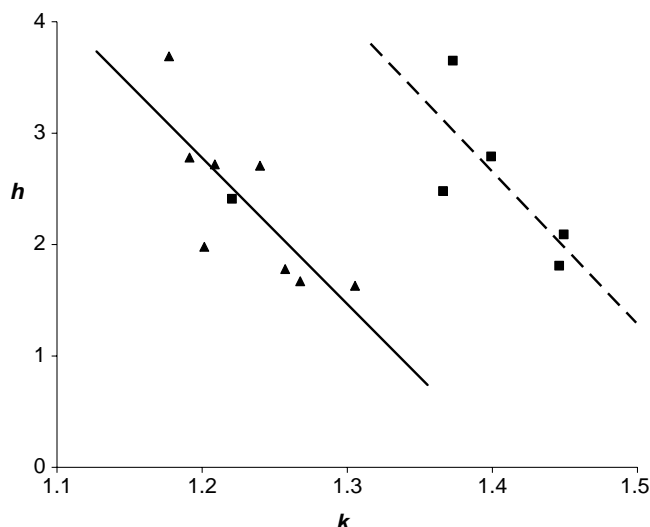
Fig. 4. Relationship between the observed optimum reduced plate height and the observed retention factor of fluorene for 75-μm (▲) and 150-μm (■) i.d. columns packed with 10-μm particles.

75- and 150-μm i.d. columns (data not shown). At a given aspect ratio, higher efficiencies were observed for columns that were more densely packed (exhibiting higher k values).

The concept of the flow-resistance parameter (ϕ) is based on the idea that a chromatographic bed can be represented as a bundle of capillaries [26]. It is defined as:

$$\phi = \frac{\Delta P d_{\mathrm{p}}^2}{uL\eta}$$

where ΔP is the pressure drop across the column, u the mobile phase velocity, L the column length, and h the viscosity of the eluent. Thiourea was used as an unretained marker for the determination of the mobile phase velocity. It should be noted that we used the average mobile phase velocity in the calculation of the flow resistance and not the interstitial velocity. For the viscosity of the mobile phase, the value of 0.52 cP was used [27]. The flow resistance depends on the total porosity, ε_{t}, (which is the sum of interstitial, ε_{m}, and intra-particle, ε_{p}, porosities) of the column, the particle-size distribution, particle shape, and packing geometry. With a decreasing aspect ratio, an increase in interstitial porosity, ε_{m}, can be expected, due to the stronger influence of the wall on the packing structure. For 75-, 100-, and 150-μm i.d. columns, the experimental flow-resistance parameters were calculated to 930 ± 140, 920 ± 90, and 960 ± 130, respectively. In contrast to the results obtained when comparing the retention factors, no decrease in flow resistance was observed when the aspect ratio was changed by decreasing the column diameter. This indicates that there is no noticeable wall effect, which would lead to a more-open packing structure in this region.

Another way to change the aspect ratio is to pack columns of a given diameter with particles of different size. The effects of differences in particle diameters can be accounted
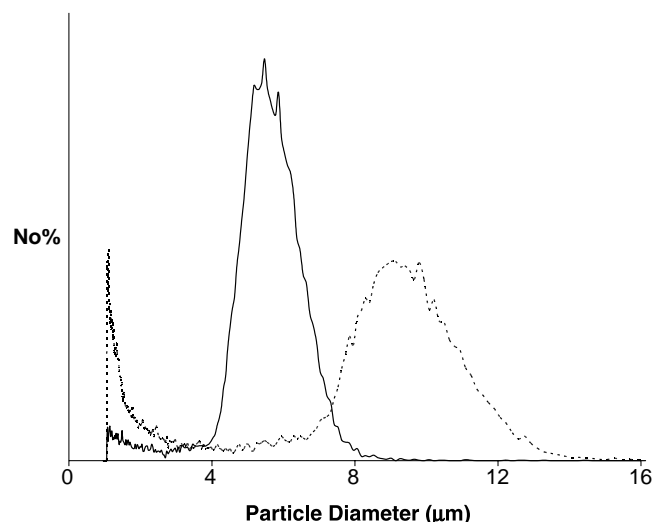
for by using reduced parameters, such as the reduced plate height ($h = H/d_{\mathrm{p}}$).

Particle-size distributions were determined using a Coulter-counter method. In Fig. 5, the number fractions of particles are plotted against the particle diameter. The batch of 10-μm particles appeared to have a broader particle-size distribution than the 5-μm particles and also contains more small particles. For samples of the 5- and 10-μm particles, average volume particle diameters of 6 and 10 μm were measured, respectively. These average values were used in our calculations.

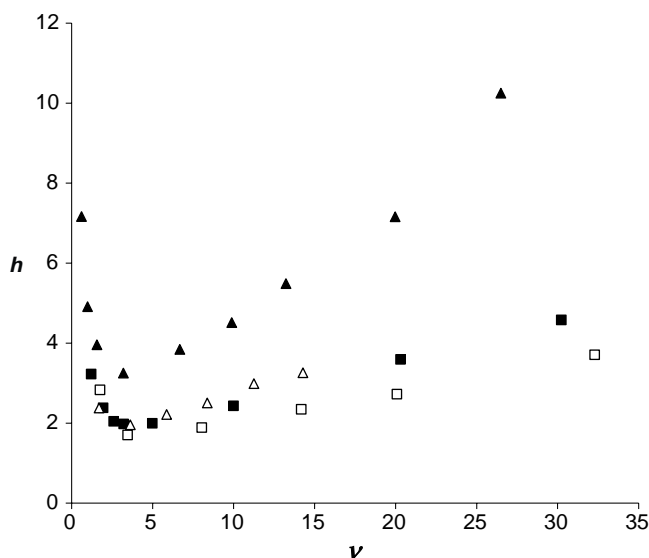Fig. 6 shows h versus v curves of 75-μm i.d. columns packed with the nominal 5- and 10-μm particles, obtained

Fig. 5. Particle size number distributions of the 5-μm (—) and 10-μm (---) Hypersil-ODS particles used in this study.

Fig. 6. Reduced H vs. v curves for 75-μm i.d. columns with different aspect ratios. Columns packed with 5-μm (▲) and 10-μm (■) particles measured in the pressure-driven mode (solid symbols) and in the electrically-driven mode (open symbols).

in the pressure and electrically-driven modes. In the pressure-driven mode a large increase in the efficiency was observed when the particle diameter was increased (aspect ratio decreased). In the electrically-driven mode no such effect could be detected. However, the value of the flow-resistance parameter for both particle diameters was virtually identical, namely 1040 and 1020 for columns with aspect ratios of 15.0 and 7.5, respectively. It should be noted, however, that the presence of fines in the batches of stationary phases and the difference in particle size distributions will have a strong influence on the flow resistance. We also assume that packing columns with smaller particles is more difficult. The effect of the aspect ratio on column efficiency and flow resistance as observed by various authors can possibly also be explained with such considerations. Therefore, investigations into wall effects by using different particle sizes should be performed with great care.

4. Conclusions

The external band broadening procedure used in this study can be recommended to test the ability of miniaturized HPLC systems to function properly. With proper connections, no correction for external band broadening was needed. The effects of the aspect ratio on various column parameters was studied, either by changing the column diameter while keeping the particle size constant, or by changing the particle size, while keeping the column diameter constant. In the former case, at least six columns of each aspect ratio (15.0, 10.0, or 7.5) were studied. All columns exhibited high efficiencies ($h \approx 2$), but no systematic effect on the efficiency or the flow resistance was observed. Changing the aspect ratio by decreasing the particle diameter did result in a systematic effect on the observed efficiency. However, the flow-resistance parameters of the different columns were comparable. Differences in packing quality were found to be far more important than a possible aspect-ratio effect.

This work constitutes the most comprehensive experimental study to date into the possible beneficial effects of working with low-aspect-ratio columns in HPLC. No convincing evidence was found to support the existence of such an aspect-ratio effect.

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