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# Comparative study on the column performance of microparticulate 5- $\mu\text{m}$ C<sub>18</sub>-bonded and monolithic C<sub>18</sub>-bonded reversed-phase columns in high-performance liquid chromatography<sup>1</sup>

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## Abstract

In this paper we report on the results of a comparative study on the performance of Purospher RP 18e, 5  $\mu\text{m}$ , columns and prototypes of monolithic columns named SilicaROD from Merck, Darmstadt, Germany. The studies were performed on HPLC equipment with minimum extra column contribution. The plate height linear velocity dependency of the Purospher RP 18e column showed a minimum of  $H$  of about 10–15  $\mu\text{m}$  at a linear velocity of 1 mm/s. The  $H$  versus  $u$  curves of the monolithic columns followed the same course. Yet, the curves remained flat up to a linear velocity of about 7 mm/s, where the Purospher RP 18e column could not be operated anymore due to the extremely high back-pressure. In conclusion the monolithic HPLC columns showed a column performance equivalent to a column with about 5  $\mu\text{m}$  porous particles and were operated at flow-rates up to 9 ml/min on a conventional HPLC equipment without notable loss in column efficiency. The column back-pressure drop at the monolithic columns was three- to five-times lower than that on the 5- $\mu\text{m}$  particulate column. © 1999 Elsevier Science B.V. All rights reserved.

**Keywords:** Stationary phases, LC; Monolithic columns; Toluene; Alkylbenzenes

## 1. Introduction

Although the column technology in high-performance liquid chromatography (HPLC) has now reached a high standard and a high reproducibility, thorough attempts are undertaken to search for a novel column design based on monolithic supports

rather than on microparticulate packings [1–8]. The advancements achieved in the sol-gel chemistry of silica [9] provide the material basis to design composite supports of silica and polymers with a coherent binary phase system [10]. Such composites have been converted into porous bodies after calcination i.e., removal of the polymeric constituent. The porous silica bodies being shaped as rods exhibit a bimodal and highly connected pore system composed of flow-through pores of 1–2  $\mu\text{m}$  in width and mesopores with adjustable average pore diameter [8,10].

Japanese researchers not only developed such

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silica rods but also tested their column performance, retention and selectivity in reversed-phase chromatography [7]. Merck, Darmstadt, Germany will commercialize this product under the name SilicaROD at the beginning of 1999. The aim of this study was to compare the column performance of prototypes of such SilicaROD RP 18 columns with a microparticulate 5- $\mu\text{m}$  Purospher RP 18e column of the same company.

## 2. Experimental

### 2.1. Materials

Seven prototype monolithic columns kindly supplied by Merck, were used with the following properties: diameter of through-pores of about 1.5–2  $\mu\text{m}$ ; diameter of diffusional pores of about 12 nm; specific pore volume of 1.0 ml/g; specific surface area of 300–350  $\text{m}^2/\text{g}$  and total porosity of  $\sim 81\%$ .

The columns originate from different batches in the exploratory phase of the manufacturing process. Thus, data on the reproducibility of these columns are not appropriate to assess.

The microparticulate column for comparison was a Purospher RP 18e column 5  $\mu\text{m}$  of dimension 125  $\times$  4 mm.

The solvents employed were of HPLC quality (Merck). The solvents were acetonitrile, 99.9%, HPLC grade and deionised water.

The analytes were: (1) uracil, (2) toluene, (3) ethylbenzene, (4) *n*-propylbenzene, (5) *n*-butylbenzene, (6) *n*-pentylbenzene.

The following chromatographic equipment was used: a HPLC pump 64 (Knauer, Berlin, Germany), a capillary UV detector 433 (Kontron Instruments, Munich, Germany), an injection valve (Rheodyne 7125); the size of the rod column was 100  $\times$  4.6 mm.

The following parameters were calculated:

(1) Retention coefficient  $k$  using uracil as dead time marker

$$k = \frac{t_{\text{R}} - t_{\text{M}}}{t_{\text{M}}} \quad (1)$$

where  $t_{\text{R}}$  is the retention time and  $t_{\text{M}}$  the column dead time

(2) the plate number  $N$

$$N = 5.54 \cdot \left( \frac{t_{\text{R}i}}{w_{0.5}} \right) \quad (2)$$

where  $w_{0.5}$  is the peak width at half peak height and  $t_{\text{R}i}$  the retention time of substance  $i$   
(3) the plate height  $H$

$$H = \frac{L}{N} \quad (3)$$

where  $L$  is the length of the column  
(4) the reduced plate height  $h$

$$h = \frac{H}{d_{\text{p}}} \quad (4)$$

where  $d_{\text{p}}$  is the particle diameter  
(5) the reduced linear velocity  $\nu$

$$\nu = \frac{ud_{\text{p}}}{D_{\text{m}}} \quad (5)$$

where  $u$  is the linear velocity and  $D_{\text{m}}$  is the diffusion coefficient of the solute in the mobile phase

(6) the total porosity of the column  $\epsilon_{\text{T}}$

$$\epsilon_{\text{T}} = \frac{F}{ur_{\text{c}}^2\pi} = \frac{Ft_{\text{M}}}{Lr_{\text{c}}^2\pi} \quad (6)$$

where  $F$  is the flow-rate and  $r_{\text{c}}$  is the radius of the column

(7) the column permeability  $K_{\text{F}}$

$$K_{\text{F}} = \frac{F\eta L}{\Delta p r_{\text{c}}^2 \pi} \quad (7)$$

where  $\eta$  is the viscosity of the mobile phase and  $\Delta p$  is the column pressure

$$K_{\text{F}} = \frac{u\eta L}{\Delta p} \quad (8)$$

and

$$F = u\epsilon_{\text{f}} \quad (9)$$

where  $\epsilon_{\text{f}}$  is the free cross-section of the column

### 3. Results and discussion

#### 3.1. Comparison of the hydrodynamic properties of monolithic versus microparticulate columns

The hydrodynamic properties of a HPLC column are commonly described by the column pressure drop  $\Delta p$ , the chromatographic permeability  $K$  and the column resistance factor  $\Phi$  [11]. Usually, for well-packed columns the dimensionless column resistance factor varies between 500 and 1000.

As reported in the literature, monolithic HPLC columns possess a higher porosity than microparticulate columns [12]. The total column porosities of the SilicaRODs and the Purospher RP 18e column were determined by the Eqs. (8) and (9) to  $0.780 \pm 0.01$  (rod 178),  $0.736 \pm 0.08$  (rod 216),  $0.723 \pm 0.01$  (rod 225) and  $0.452 \pm 0.02$  for the Purospher RP 18e column.

It is evident that the microparticulate reversed-phase column exhibits a significantly lower total

column porosity than the monolithic columns, which is reflected by the  $K_F$  and  $\Delta p$  values. Fig. 1 shows the dependencies of the column permeability  $K_F$  calculated according to Eq. (8) on the flow-rate  $F$  for the two types of columns.

$K_F$  is by a factor of four higher on the monolithic columns as compared to the microparticulate column. For practical use the plot of the column back-pressure against the flow-rate is more informative (Fig. 2). The mean standard deviation of the curve  $\Delta p$  vs.  $F$  for the three rods calculates to  $\pm 3\%$ . The correlation coefficient is 0.9991.

Inspection of the plots of Fig. 2 shows, (i) that at a flow-rate of 1 ml/min  $\Delta p$  is about five-times smaller on the rods than on the Purospher RP 18e column and (ii) the Purospher RP 18e column reaches a maximum back-pressure of about 350 bar at 3 ml/min whereas the rod columns can be operated at 6 ml/min with a back-pressure of 150 bar. The lower pressure drop of monolithic columns is caused by the higher column porosity or more precisely by the higher macroporosity.

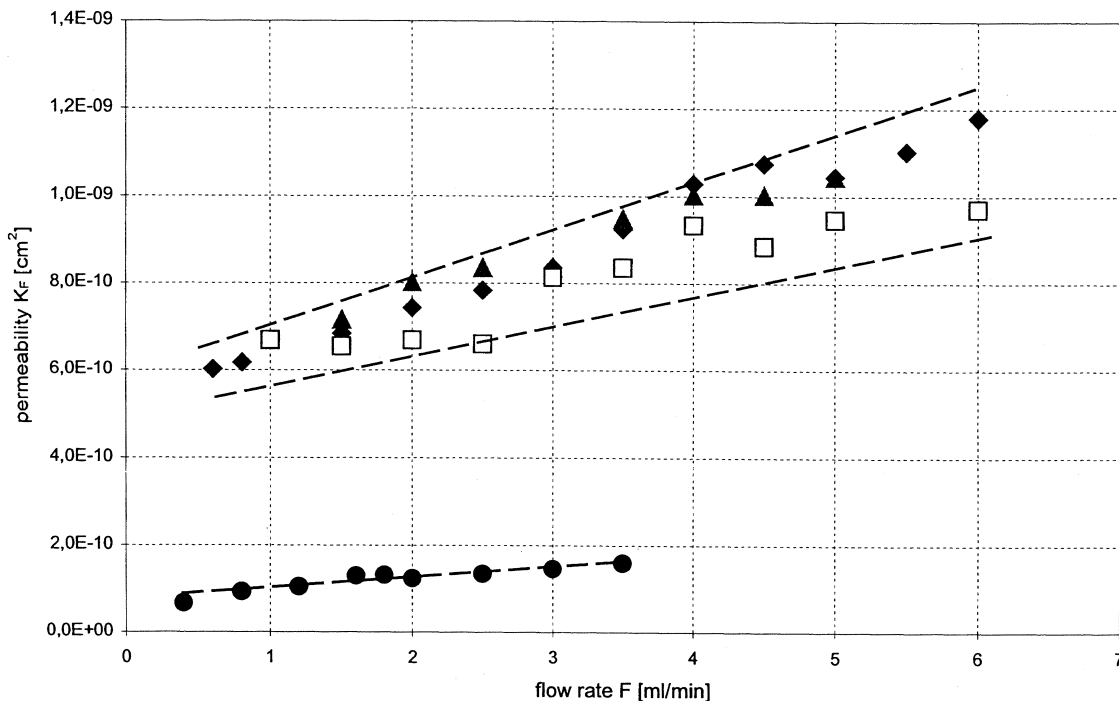


Fig. 1. Permeability  $K_F$  as a function of the flow-rate  $F$  for three SilicaROD columns and the Purospher RP 18e column. ( $\blacklozenge$ ) Rod 178, ( $\blacktriangle$ ) rod 216, ( $\square$ ) rod 225, ( $\bullet$ ) Purospher RP 18e column.

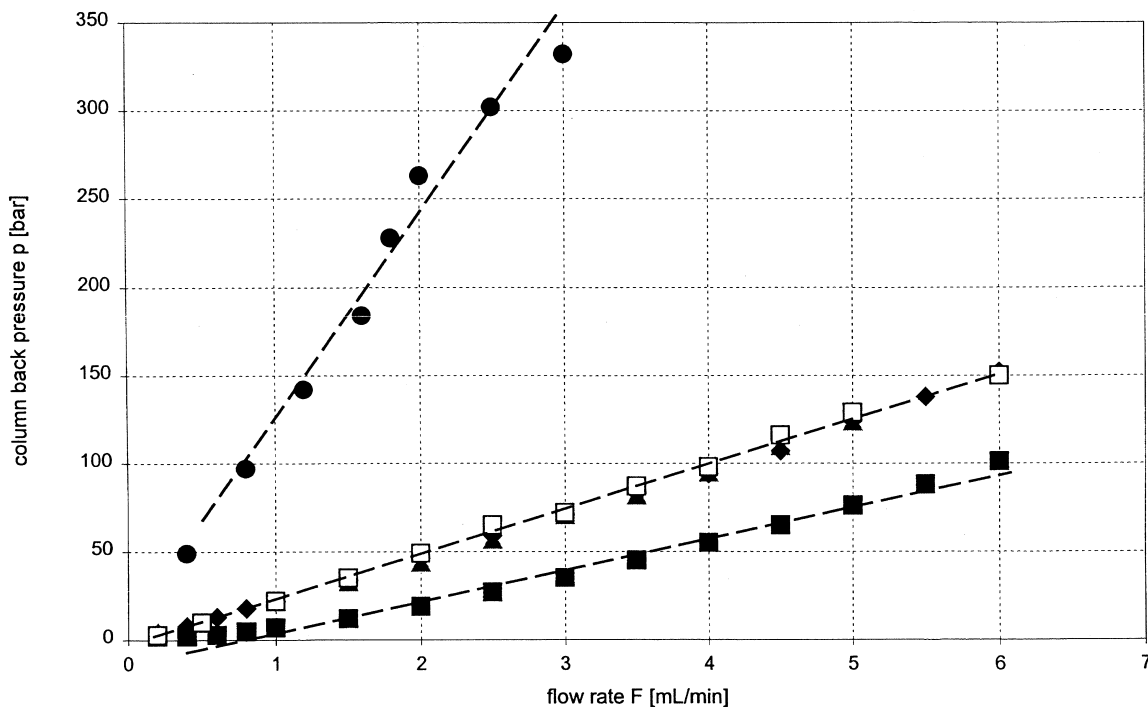


Fig. 2. Column back pressure  $\Delta p$  as a function of the flow-rate  $F$  on three monolithic and a microparticulate column. (■) Pressure drop due to equipment (without a column), (◆) rod 178, (▲) rod 216, (□) rod 225, (●) Purospher RP 18e column.

### 3.2. Plate height versus linear flow velocity characteristics of monolithic and microparticulate columns

Chromatograms of homologous  $n$ -alkylbenzenes as test compounds in acetonitrile–water (58:42, v/v) resulted in a slightly better peak symmetry on the Purospher RP 18e column than on all monolithic columns tested. Fig. 4 shows a chromatogram of a homologue series of  $n$ -alkylbenzenes on rod 216.  $n$ -Alkylbenzenes were also applied to analyse the dependency of the plate height  $H$  on the retention coefficient  $k$  at various linear velocities (Fig. 3).

It can be concluded from the data (Fig. 4) that the plate height values are nearly independent on the retention coefficient in the range between  $2 < k < 10$ .

Furthermore, the minimum plate height was about 13–15  $\mu\text{m}$  at an optimum linear velocity of about 3 mm/s on rod 216. The minimum plate height at the Purospher RP 18e column was assessed to about 10  $\mu\text{m}$  at an optimum linear velocity of about 1 mm/s (not shown).

The analysis of the  $H$  versus  $u$  curve for Purospher RP 18e using an iteration function for fitting gave the following values for  $A$ ,  $B$  and  $C$  in the van Deemter equation:  $A = 0.5 \pm 0.1$ ,  $B = 1.0 \pm 0.2$  and  $C = 0.8 \pm 1$ .

Stout et al. [13] reported the following values on Zorbax columns  $A = 1$ ,  $B = 2$ ,  $C = 0.02\text{--}0.05$  taken from  $h$  vs.  $\nu$  curves. The experimental  $H$  vs.  $u$  curves of the SilicaROD columns were fitted with an apparent average particle diameter of 5 and 7  $\mu\text{m}$ , respectively into  $h$  vs.  $\nu$  dependencies. The values were  $A = 1.1 \pm 0.2$  ( $1.45 \pm 0.2$ ),  $B = 0.75 \pm 0.2$  ( $1.2 \pm 0.2$ ),  $C = 0.05 \pm 0.02$  ( $0.05 \pm 0.02$ ) for an average particle diameter for a rod column of 5- and 7- $\mu\text{m}$  particle size assumed, respectively. Thus the performance of the monolithic columns studied corresponded to the performance of a 5- $\mu\text{m}$  reversed-phase column.

## 4. Conclusions

The SilicaROD columns tested on HPLC equip-

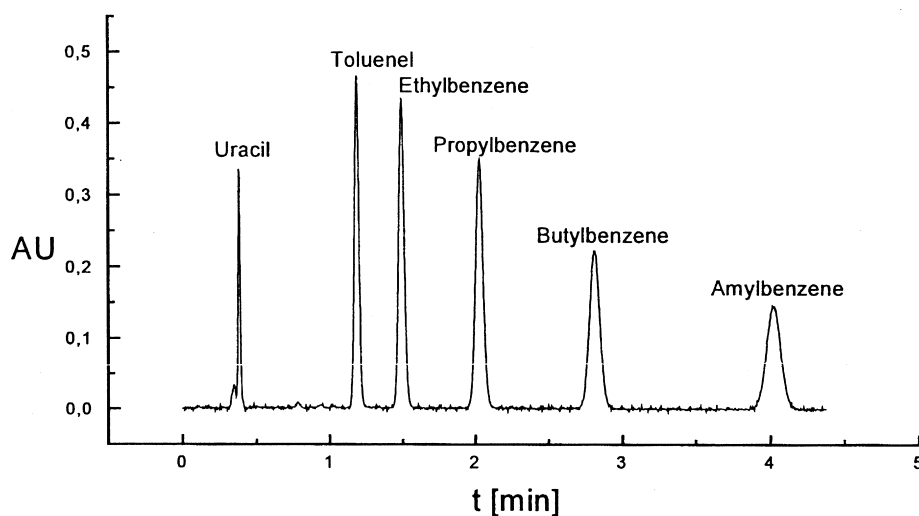


Fig. 3. Chromatogram of homologues *n*-alkylbenzenes on SilicaROD 216. Conditions: mobile phase: acetonitrile–water (73:27, v/v); column temperature: 25°C; flow-rate: 2 ml/min; detection: UV 254 nm; injection: 2  $\mu$ l.

ment with minimum dead volume showed a similar column performance as the Purospher RP 18e column. Due to the larger total porosities the SilicaROD columns can be operated up to flow-rates of about 7

ml/min, whereas the maximum flow-rate of the Purospher RP 18e column was about 3 ml/min. Even at these high flow-rate the excellent performance of the SilicaROD columns is maintained. One

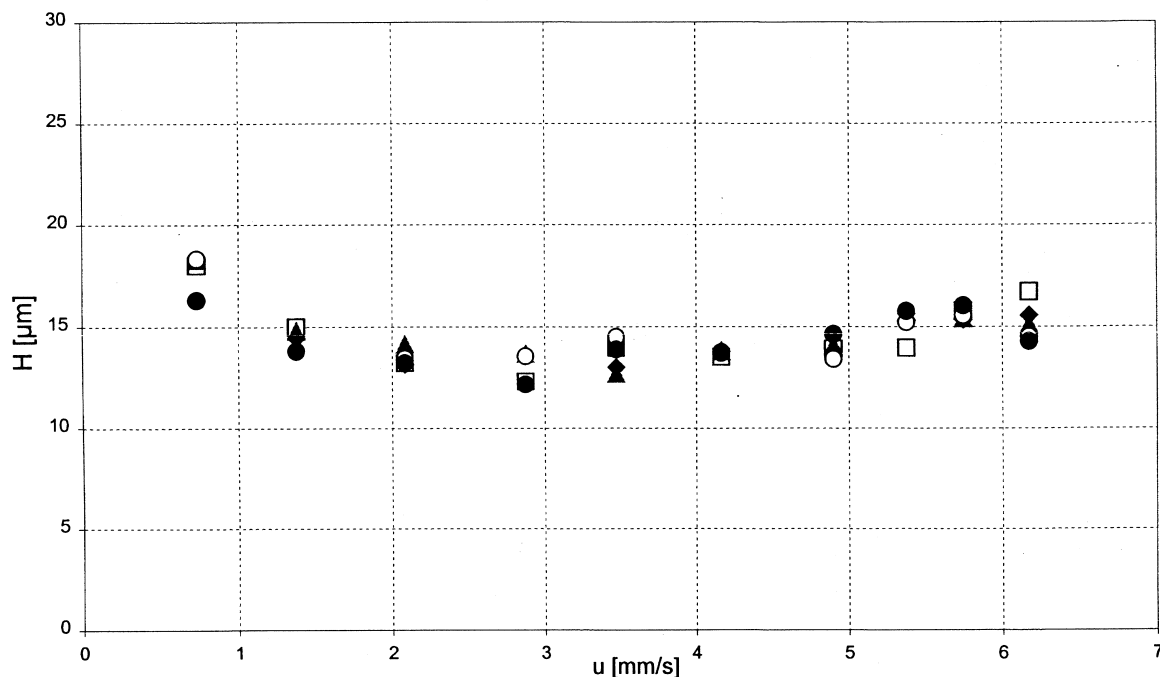


Fig. 4.  $H$  vs.  $u$  curve for *n*-alkylbenzenes on Rod 216 (for conditions see Section 2). (♦) Toluene,  $k=1.55$ ; (□) ethylbenzene,  $k=2.32$ ; (▲) propylbenzene,  $k=3.67$ ; (○) butylbenzene,  $k=5.74$ ; (●) pentylbenzene,  $k=9.22$ .

of the most advantages features of the monolithic columns is the drastically reduced column pressure drop as compared for microparticulate column.

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