Capillary electrochromatography with 1.8-μm ODS-modified porous silica particles

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Abstract

The feasibility of capillary electrochromatography with 1.8-μm ODS-modified porous Zorbax particles was studied. It appeared not possible to prepare stable frits from this material by in situ sintering; therefore, frits were made from another packing material. Addition of sodium dodecyl sulfate to the mobile phase was not necessary for operation of the columns without bubble formation. The columns could be operated with field strengths up to 125 kV m^{-1}. The effect of the mobile phase composition on electroosmotic mobility and plate heights was investigated. The electroosmotic mobility was almost constant in the pH range from 6 to 9, but declined steeply to half the value in the pH range 3–5. The ionic strength of the mobile phase had only a small effect on the plate heights and electroosmotic flow. Efficiencies up to 310 000 plates m^{-1} (h=1.7) were obtained with 80% (v/v) acetonitrile in the mobile phase. Compared to previous experiments with 1.5-μm non-porous ODS modified particles, capacity factors were 12–15-times higher, while the observed loading capacity of the particles was approximately 50-times larger. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Capillary electrochromatography (CEC) is a separation technique that combines the high selectivity of liquid chromatography (LC) with the high efficiency of capillary electrophoresis (CE) [1,2]. In conventional LC a further reduction of the particle diameter below 3 μm is practically limited, due to the high pressure required to attain an acceptable flow. In CEC this limitation of the particle size is absent, since the flow-rate is independent of the particle size [3]. This advantage of CEC over LC potentially enables us to obtain very high efficiencies by the use of very small particles as packing material.

Until recently, difficulties in packing the capillaries and ''air'' bubble formation during the measurements [4–7] has delayed the development of CEC and the application of very small particles. So far, most of the publications on CEC are dealing with 3 or 5 μm particles [8–13]. However, there are some reports in which the applicability of very small particles is demonstrated. Knox and Grant [4] applied 1.5-μm non-porous silica particles for CEC and observed a reduced plate height of about one for an unretained solute. Yamamoto et al. [14] studied CEC with 1.6-μm non-porous monospher ODS and obtained a reduced plate height of 1.9 for an unretained solute. However, the observed efficiency for retained solutes declined by a factor of five. Smith and Evans [15] used pressurized CEC with 1.8-μm porous

Zorbax SB/C_8 particles and obtained a reduced plate height of 1.6 for retained compounds. Recently we demonstrated CEC with 1.5-μm non-porous ODS modified Chromspher particles and realized reduced plate heights of 1.3 [16]. The observed high efficiency with the non-porous particles can be attributed to a fast mass transfer [17].

The disadvantage of using non-porous particles is the low phase ratio, giving low retention and loading capacity. Therefore we have also studied the use of small diameter porous particles in CEC. To date, the smallest available porous particles have a diameter of 1.8 μm. In this report the feasibility of the application of 1.8-μm ODS-modified Zorbax particles is compared with that of non-porous particles. Attention will be given to the effect of the mobile phase composition on electroosmotic mobility and efficiency. The loading capacity has been determined and compared with non-porous particles.

2. Experimental

2.1. Apparatus

All experiments were performed with a HP [3d]CE system (Hewlett-Packard, Waldbronn, Germany), equipped with a diode array detector operated at 254 nm. Samples were introduced electrokinetically at the anodic side (20 kV, 1 s). The air cooling system was used to keep the temperature at 25°C in all experiments. A HPLC pump was used to flush freshly packed capillaries. The 50 μm I.D.×375 μm O.D. fused-silica capillaries were obtained from Polymicro Technologies (Phoenix, AZ, USA). The total length of the capillary was ≈32 cm of which ≈24 cm was packed with the particles.

2.2. Chemicals and solutions

The 1.8-μm ODS-modified Zorbax porous particles were kindly donated by Professor Dr. J.J. Kirkland (Hewlett-Packard, Newport, DE, USA). Reversed-phase Hypersil-ODS 3 μm was obtained from Shandon (Astmoor, UK). HPLC grade acetonitrile was purchased from J.T. Baker (Deventer, Netherlands). The solutes and other ordinary chemicals were obtained from different commercial

sources. Stock solutions of polycyclic aromatic hydrocarbons (PAHs) were prepared in acetonitrile and those of the steroids in methanol. All solutes were diluted to $5 \cdot 10^{-4}$ mol l^{-1} with mobile phase prior to injection. The pH of the buffer solutions was measured in the aqueous phase prior to mixing with acetonitrile. The ionic strength of the mobile phases was $1.6 \cdot 10^{-3}$ mol l^{-1}, unless stated otherwise. All mobile phases were filtered over a 0.45-μm HVLP Durapore filter (Millipore, Etten Leur, Netherlands) and degassed with a stream of helium gas for about 20 min prior to use.

2.3. Column preparation

In CEC the preparation of the frits appears to be of crucial importance to obtain a stable system. In our previous reports the preparation of capillary columns packed with 5-, 3-, or 1.5-μm particles was extensively described [11,13,16]. In all of these procedures frits were fabricated by local sintering of the packing material with a heating filament. The same technique was initially applied for the preparation of the frits with the 1.8-μm porous particles. However, with these particles it appeared that no mechanically stable frits would be fabricated. The most likely reason for this is the lack of sodium in the Zorbax silica particles. It is known that in the presence of sodium, polysilicates can be formed upon the heating of silica to 550°C [18].

Hypersil silica particles contain a considerable amount of sodium; mechanically stable frits can be fabricated by sintering of these particles. Therefore, first an inlet frit was prepared from 3-μm ODS-Hypersil particles. For this, the capillary was connected to an in-line HPLC filter and to the other side the slurry reservoir (a 200×1 mm I.D. tube). The Hypersil slurry (15 mg ml^{-1}) was pumped into the capillary at 350 bar with a high-pressure membrane pump. After packing of a bed with a length of ≈5 cm, the column was flushed with water at 350 bar for 5 min to remove the organic solvent. While flushing the column at 350 bar, the inlet frit was made by sintering the Hypersil particles for 10 s at 550°C. The in-line filter was disconnected and the remainder of the particles was flushed away with acetone at 60 bar.

Subsequently, the column was slurry packed with

the Zorbax particles (15 mg ml^{-1} in acetone). The pressure was gradually increased to 350 bar to prevent damaging the inlet Hypersil frit. Packing of a bed of 24 cm took less than 10 min. After completion of packing until the desired length, the column was depressurized in $\approx$5 min. Then the column was placed in an ultrasonic bath and flushed with water at 350 bar for 30 min. The outlet frit at the detection side was made by locally sintering of the Zorbax particles at two points next to each other. The sintering process was performed in 10 s at 550°C while flushing with water at 350 bar. The mechanical strength of the outlet frits appeared to be adequate for this side of the packed bed. The detection window was made by burning off the polyimide coating next to the outlet frit while the column was still flushed with water. Depressurizing of the column at this time was done slowly during the night. Finally the column was flushed with buffer solution for 2 h at 60 bar with a HPLC pump.

3. Results and discussion

3.1. Electroosmotic flow

The effect of the mobile phase composition (surfactant concentration, acetonitrile content, pH and ionic strength) on the electroosmotic flow (EOF) was studied. For the measurement of the EOF acetone was used as the t_0 marker. Variation of the field strength between 200 and 1100 V cm^{-1} had no effect on calculated values of the electroosmotic mobility μ_{eo}. As expected with the low electrophoretic currents observed ($\leq$6 μA), Joule heating effects were apparently negligible.

With columns packed with non-porous particles it was found previously that the presence of sodium dodecyl sulfate (SDS) in the mobile phase was critical to obtain a stable current and to avoid "air" bubble formation [16]. However, in this study it appeared that columns with porous Zorbax ODS particles could be operated without SDS in the mobile phase. In Table 1 the effect of the SDS concentration on μ_{eo} is compared for porous and non-porous particles. With the Zorbax particles, the presence of SDS in low concentrations (below the critical micelle concentration) appeared to increase

Table 1
The effect of the SDS concentration on the observed electro-osmotic mobility in CEC

SDS (mmol l^{-1})	μ_{eo} (10^{-8} m^2 V^{-1} s^{-1})	
	Porous	Non-porous
0	1.44	–
1	1.70	1.54
3	1.65	1.81
5	1.72	1.89

Packing materials: porous Zorbax ODS, 1.8 μm and non-porous Chromspher ODS, 1.5 μm. The mobile phase contained sodium tetraborate buffer (pH 9.2) and 80 and 60% (v/v) acetonitrile with the porous and non-porous particles, respectively.

the μ_{eo} by approximately 15%. With the non-porous particles the influence of the SDS concentration was stronger. However, this could be partly the result of the lower acetonitrile concentration in these experiments, causing a stronger adsorption of SDS on the surface of the particles.

The effect of the acetonitrile content of the mobile phase on μ_{eo} was small. An increase of the acetonitrile concentration from 70 to 80% (v/v) resulted in an increase of μ_{eo} by 10%. This is approximately in accordance with the decrease of the solution viscosity [19]. Therefore it may be concluded that the zeta potential is not strongly affected. Apparently, the adsorption of SDS on the surface of these particles surface, which is expected to depend strongly on the acetonitrile concentration, is of minor importance for the zeta potential.

The effect of the ionic strength on the EOF was studied with a mobile phase which contained 80% (v/v) acetonitrile. The observed electroosmotic mobilities with 1, 2 and 4 mmol l^{-1} sodium tetraborate were 1.47, 1.64 and 1.44·10^{-8} m^2 V^{-1} s^{-1}, respectively. The influence of the ionic strength on μ_{eo} can be attributed to two different, counteracting processes. First, an increase of the ionic strength will generally decrease the zeta potential of the particles, and thereby the velocity of the EOF. However, at the same time the thickness of the diffuse double layer is decreased. Possible double layer overlap, diminishing the EOF velocity inside the pores of the particles, will be reduced. Apparently, with the porous particles used in this study, there is an optimum value for the ionic strength (2 mmol l^{-1}) giving the highest μ_{eo}. It is interesting to note that with non-porous

particles, where obviously double layer overlap is not of importance, μ_{eo} was seen to be decreasing continuously with increasing ionic strength.

The influence of the pH on μ_{eo} was studied with porous and non-porous particles using different buffer solutions in the mobile phase. With both columns SDS was added to the mobile phase. The acetonitrile content of the mobile phase was 80% (v/v) with the porous and 60% (v/v) with the non-porous particles. The results are shown in Fig. 1. With the porous particles a (reproducible) steep increase of μ_{eo} was observed between pH 4 and pH 6; with the non-porous particles the change of μ_{eo} with the pH was more gradual. A possible explanation for this difference is that with the non-porous particles the surface charge is determined to a larger extent by adsorbed SDS, and less by the residual free silanol groups on the packing material. With the porous particles the higher acetonitrile content of the mobile phase will decrease the SDS adsorption.

3.2. Efficiency

The efficiency of the columns was studied using a series of PAHs as model compounds. Plate numbers and plate heights were calculated from the observed peak widths at half height. Fig. 2 shows plots of the plate heights as a function of the electroosmotic velocity of the mobile phase for acetone (unretained)
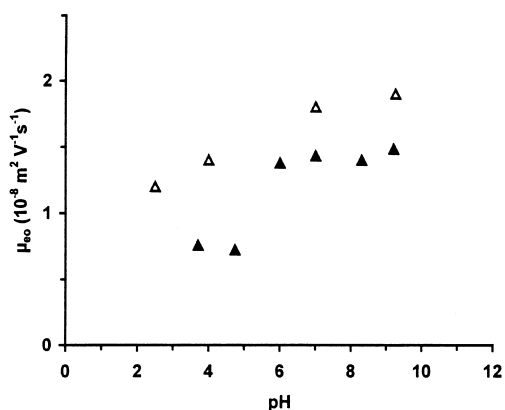

Fig. 1. Effect of the pH on the electroosmotic mobility in CEC with porous (▲) and non-porous (△) particles. Mobile phase: 5 mmol l^{-1} SDS, 80% (porous) or 60% (non-porous) acetonitrile and different buffer solutions. Data for the non-porous particles taken from Ref. [16].
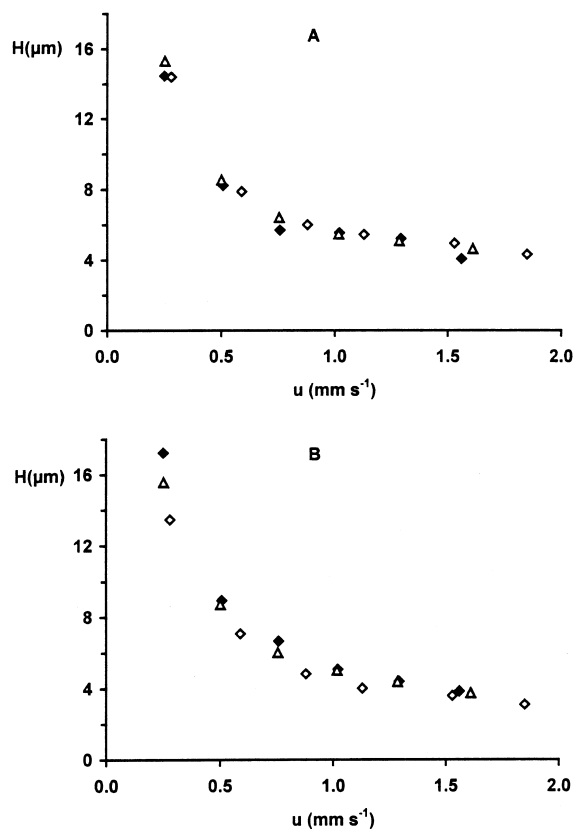

Fig. 2. Effect of the acetonitrile concentration on H–u curves for acetone (A) and pyrene (B). Acetonitrile content: mobile phase: 5 mmol l^{-1} SDS, 1.6 mmol l^{-1} sodium tetraborate (pH 9.2) and 70% (♦), 75% (△), or 80% (◇) acetonitrile.

and pyrene (retained) at different acetonitrile contents. As can be seen from this Figure, the plate heights decrease steeply with increasing linear velocity and then become more or less constant at larger velocities. Apparently, with low velocities the axial diffusion plays the major role, while mass transfer contributions to the plate height are small even at the highest velocities.

The effect of the acetonitrile concentration in the mobile phase was small. Only for strongly retained compounds (pyrene in Fig. 2B) a small decrease of the plate height with increasing acetonitrile concentration was observed at high linear velocities. This reflects the decreased contribution of the mass transfer term to the plate height with decreasing capacity factor. It is interesting to note that with non-porous particles a different behaviour had been

observed. With these particles an increase of the plate heights with increasing acetonitrile concentration was found, especially at lower linear velocities. With non-porous particles the mass transfer term is negligible in CEC; the increase of the plate height
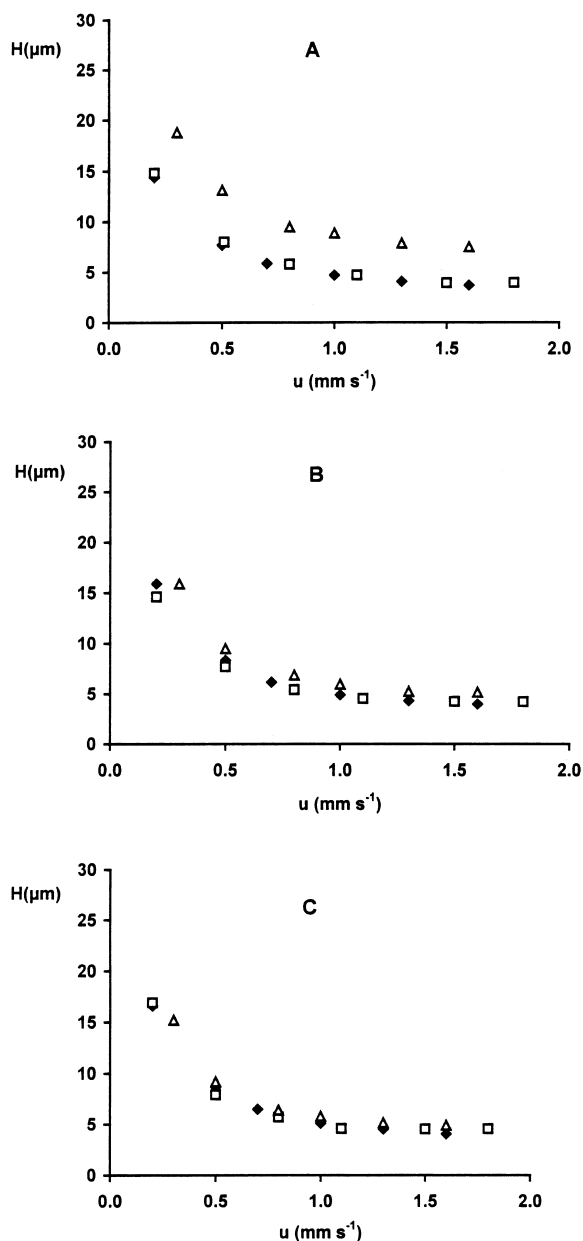
with the acetonitrile concentration at low velocities can be explained as the result of a higher axial diffusion in solutions with a lower viscosity.

With the Zorbax particles the highest plate number (75 000 per column or 310 000 plates m^{-1}) was observed for pyrene with 80% (v/v) acetonitrile in the mobile phase and an applied voltage of 30 kV. The minimum reduced plate height was 1.7, which is still higher than obtained previously with non-porous particles of approximately the same size. The cause of this is not clear; it could be the result of a poorer quality of the packing or it could be inherent to the porosity of the stationary phase.

The effect of the ionic strength of the mobile phase on the observed plate heights is shown in Fig. 3 for acetone and pyrene. The plate heights with ionic strengths of 2 and 4 mmol l^{-1} were almost the same at different voltages. However, as can be seen in the Figure, the plate heights were increased when the buffer concentration was decreased from 2 to 1 mmol l^{-1}. It is tempting to ascribe this effect to the increase of the double layer thickness and the development of a parabolic flow profile inside the pores of the particles. Such a parabolic flow profile would cause an increased mass transfer contribution
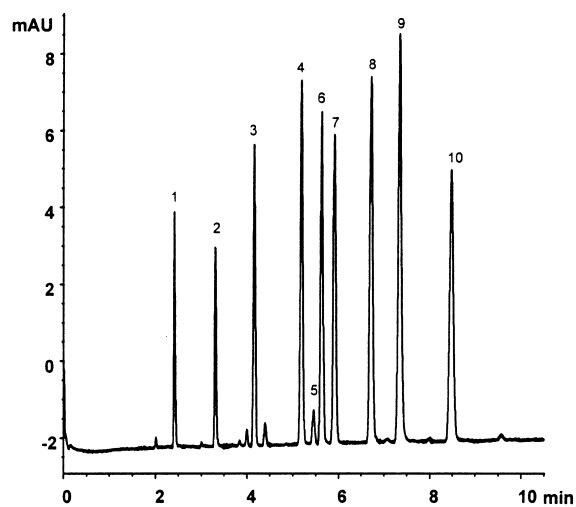

Fig. 3. Effect of the ionic strength on the H–u curve for acetone (A), naphthalen (B) and pyrene (C). Mobile phase: 80% acetonitrile and 1 ($\triangle$), 2 ($\square$) or 4 ($\blacklozenge$) mmol l^{-1} sodium tetraborate.

Fig. 4. CEC chromatogram of a mixture of PAHs. Mobile phase: 0.8 mmol l^{-1} sodium tetraborate, 85% acetonitrile. Applied voltage 30 kV. UV detection at 254 nm. Solutes: acetone (1), benzene (2), naphthalene (3), fluorene (4), acenaphthene (5), phenanthrene (6), anthracene (7), fluoranthene (8), pyrene (9), chrysene (10).
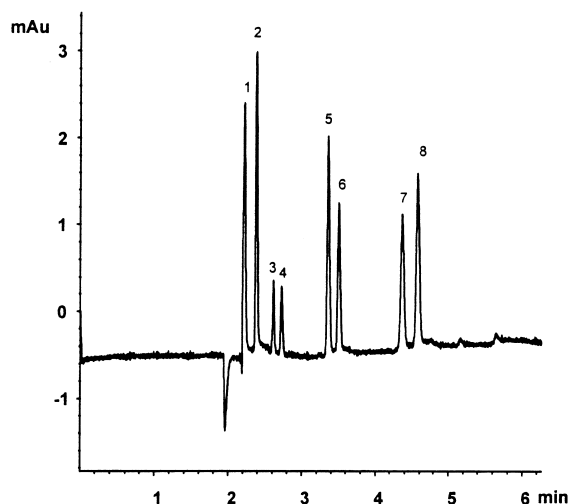
Fig. 5. CEC chromatogram of a mixture of steroids. Applied voltage 25 kV. UV detection at 254 nm. Mobile phase: 0.8 mmol l^{-1} sodium tetraborate, 80% acetonitrile, 5 mmol l^{-1} SDS. Solutes: estriol (1), hydrocortisone (2), estradiol (3), estrone (4), testosterone (5), 17-α-methyltestosterone (6), 4-pregnen-20α-ol-3-one (7), progesterone (8).

to the plate heights [11]. However, the plate height increments appear to be constant with varying mobile phase velocities. Moreover, the effect is weaker for more retained compounds (viz. Fig. 3A–C). So far we could not find a plausible explanation for the decreased column efficiency at low ionic strength.

The performance of the capillary packed with 1.8-μm ODS-modified Zorbax particles is illustrated in Figs. 4 and 5, that show the separation of a mixture of PAHs and of a series of steroids, respectively.

3.3. Capacity factors and loadability

In order to compare the retention behaviour of porous and non-porous particles, naphthalene and pyrene were injected as model compounds. Table 2 shows the k' values of naphthalene and pyrene observed with different acetonitrile content of mobile phase, on columns with porous and non-porous particles. The ratio of the k' values with the two types of particles was in the order of 12 to 15 for all solutes and mobile phase compositions. Unfortunately, exact data on the physical properties of the two experimental packing materials were not available. Based on data given for comparable materials, however, the specific surface area for the Zorbax packing can be estimated as 200 m^2 ml^{-1}, and for the non-porous packing as 2.7 m^2 ml^{-1}. With porosities estimated as 0.7 and 0.4 for the porous and non-porous particles, respectively, one would predict capacity factors to differ by a factor of 40–50. This factor is considerably larger than what was found experimentally. Apparently, there is also a difference in the chemical properties of the bonded phases. The difference in acetonitrile content to obtain the same capacity factors on both columns should be more than 20%.

Fig. 6 shows the effect of the solute concentration on the observed plate numbers for pyrene on a non-porous particle column and for naphthalene on a column with Zorbax particles. The experimental conditions were chosen such that the solutes had comparable capacity factors on the two columns; the mobile phase contained 60% (v/v) of acetonitrile with the non-porous and 80% (v/v) acetonitrile with the porous particles. No drop in efficiency was

Table 2
Effect of the acetonitrile content of the mobile phase on k' values of naphthalene and pyrene on columns with porous and non-porous particles

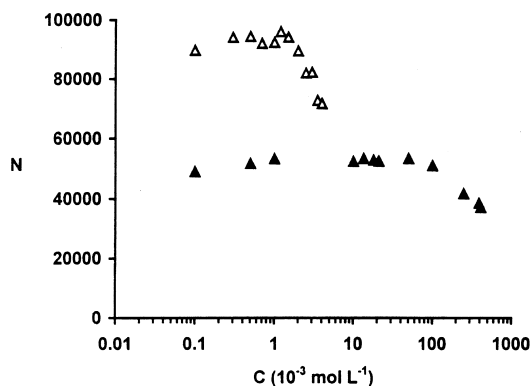| AcCN (%, v/v)	Solute	k'_{porous}	$k'_{non\text{-}porous}$	$k'_{porous}/k'_{non\text{-}porous}$
60	Naphthalene	3.1	0.20	15.5
	Pyrene	10.2	0.71	14.4
70	Naphthalene	1.4	0.12	11.6
	Pyrene	4.1	0.32	12.8
80	Naphthalene	0.8	0.06	13.3
	Pyrene	2.3	0.19	12.1

Fig. 6. Effect of the solute concentration on the observed plate numbers. Mobile phase: 5 mmol l^{-1} SDS, 2 mmol l^{-1} sodium tetraborate and acetonitrile. Applied voltage 20 kV. ($\triangle$): Pyrene on a column with 1.5 μm non-porous particles; 60% acetonitrile; ($\blacktriangle$): naphthalene with 1.8 μm porous particles; 80% acetonitrile.

observed for naphthalene with porous particles up to $1 \cdot 10^{-1}$ mol l^{-1}, while the upper limit was in the order of $2 \cdot 10^{-3}$ mol l^{-1} for pyrene with non-porous particles. Therefore, it can be concluded that the loading capacity of the porous particles was about 50-times higher than that of the 1.5-μm non-porous particles. According to the difference in estimated specific surfaces between the two packing materials, a difference by a factor of 75 would have been expected.

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