ELSEVIER

Journal of Chromatography A, 786 (1997) 229–235

JOURNAL OF CHROMATOGRAPHY A

Application of 0.5-μm porous silanized silica beads in electrochromatography

Silke Lüdtke*, Thomas Adam, Klaus K. Unger

Institute of Inorganic and Analytical Chemistry, J.J. Becher Weg 24, 55099 Mainz, Germany

Received 12 March 1997; received in revised form 2 May 1997; accepted 26 May 1997

Abstract

Nonporous organo-silica composites as beads with 0.5 μm particle size were synthesized by means of a modified Stöber process. The organic constituent was burnt off by subjecting the particles to calcination at 550°C, leaving particles with a highly interconnected inner particle porosity. The particles were silanized with *n*-octyldimethylchlorosilane after rehydroxylation. The *n*-octyl-bonded particles were slurry packed into a fused-silica capillary of 85 mm length (380 mm total length) and 100 μm inner diameter. An outlet and inlet frit were prepared by fusing the particles under hydrothermal conditions using a thermal wire stripper. The column was tested with thiourea in acetonitrile–Tris–HCl (80:20, v/v) at pH 8. An electroendosmotic flow (EOF) of 3 mm/s was generated at a voltage of 30 kV. The reduced plate height–reduced velocity curve showed a minimum value of h~3.5–4 at an EOF of 2.5–3 mm/s. At a lower EOF, h increased linearly with decreasing EOF indicating that the plate height is diffusion controlled. The CE equipment (type 3DCE, Hewlett-Packard, Waldbronnm, Germany) was not optimized for minimum extra-column band broadening. The plate number N varied between 212 800 (thiourea) and 83 600–288 800 for a test solute of *n*-alkylbenzenes. The analysis time to separate a test mixture of five *n*-alkylbenzenes was less than 60 s. © 1997 Elsevier Science B.V.

Keywords: Stationary phases, electrochromatography; Organo-silica beads; *n*-Alkylbenzenes

1. Introduction

After the invention of capillary electro-chromatography (CEC) by Pretorius et al. [1] further pioneering work was performed by Jörgensen and Lukacs [2], Tsuda et al. [3] and Knox and Grant [4,5]. Based on the work of Rice and Whitehead [6], Knox and Grant [4] made some predictions on the ultimate minimum particle diameter of a packing material in a fused-silica capillary used in CEC. For slurry-packed capillary columns he calculated a significant loss of the electroosmotic flow (EOF) when the mean particle diameter d_p of the packing material is smaller than 40 times the thickness of the electrical double layer δ. At an electrolyte concentration of 1 mM and $\delta = 10$ nm the minimum particle diameter should be 0.4 μm. Using traditional silica manufacturing processes such fine particles cannot be made, nor is a sizing technique available to produce fractions of this submicrometer-size material. The solution is to use colloid silica chemistry and to generate colloidal silica particles of predetermined and controllable size in this range without applying any sizing. The Stöber process, based on the hydrolytic polycondensation of tetraethoxysilane in an aqueous ethanol/ammonia solution, allows one to

*Corresponding author.

PII S0021-9673(97)00600-6

synthesize monodisperse colloidal spherical silica particles at a particle size range between 0.1 and about 2 μm [7–10]. However, these colloidal particles are nonporous. To induce an inner particle porosity of the Stöber beads we modified the procedure in such a way that we used *n*-alkyltrimethoxysilane as an additive to the starting reaction solution [11]. The product obtained is a organosilica composite with *n*-alkylgroups chemically bonded in the bulk structure. Nonporous beads are still obtained. Their diameter can be adjusted following the Stöber protocol. To generate an inner particle porosity the particles are subjected to calcination at 550°C where the organic alkyl chains are completely burnt out. By using a specific *n*-alkyltrimethoxysilane as porogene and adjusting a certain content relative to tetraethoxysilane in the starting solution the pore structure of the final beads can be controlled over a wide range from microporous to mesoporous materials [12]. After rehydroxylation of the calcined beads the silica beads were surface silanized by a reaction with an appropriate silane following known procedures [13].

In other words the desired silica beads are now available to prove the statement of Knox and Grant [4]. Yet the packed capillary columns have to be prepared to run them under CEC conditions.

This paper reports on our first results in testing 0.5-μm *n*-octyl-bonded silica for CEC. Other groups are also engaged in the application of submicrometer silica beads in CEC using 1.5-μm nonporous ODS material [14,15] or 0.5-μm particles [16].

2. Experimental

2.1. Materials

The packing material was prepared by using *n*-octadecyltrimethoxysilane as porogene [12] and had the following characteristics in its native form: d_p= 0.5 μm, specific surface area a_s (BET)=534 m^2/g, specific pore volume v_p=0.62 ml/g, mean pore diameter p_d (BJH)=3.4 nm. The average particle size d_{pn}=0.56±0.6 μm was determined by counting the particles from images obtained by scanning electron microscope image (SEM). The pore size

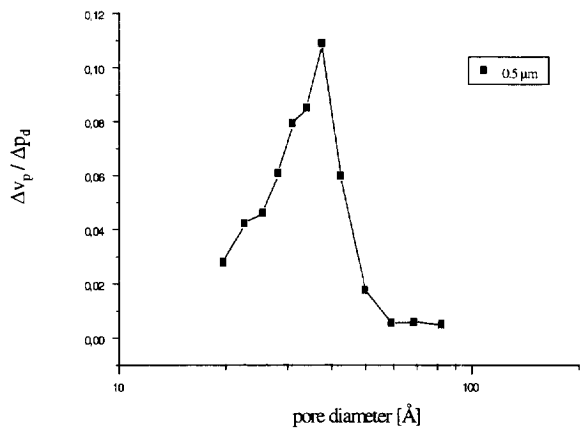

Fig. 1. Pore size distribution of the native 0.5-μm silica, determined by nitrogen sorption measurement at 77 K (BJH method).

distribution was derived from nitrogen sorption measurements at 77 K using the BJH-method (Fig. 1). After silanization with *n*-octyldimethylchlorosilane [17], a ligand density of 2.1 μmol/m^2 was yielded. The data measured by nitrogen sorption changed as follows: a_s (BET)=308 m^2/g, v_p=0.34 ml/g and p_d (BJH)=3.2 nm. The native silica was used as synthesized. From SEM no agglomeration of the particles was observed (Fig. 2).

The native Hypersil silica was supplied by Hewlett-Packard (Waldbronn) and had the following characteristics determined by nitrogen sorption measurement: specific surface area a_s (BET)=148 m^2/g,

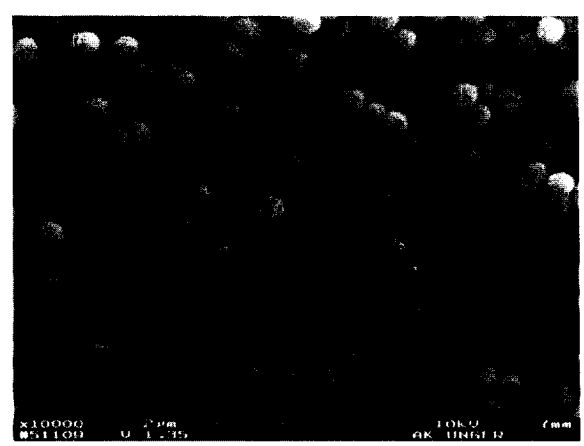

Fig. 2. SEM picture of the 0.5-μm silica beads.

specific pore volume v_p=0.61 ml/g, mean pore diameter p_d (BJH)=12.9 nm. The particle diameter was declared 3 μm. The silanization of this material was done in the same way as mentioned above, and led to the following changes concerning the data derived by nitrogen sorption measurement: a_s (BET)=121 m^2/g, v_p=0.52 ml/g, p_d (BJH)=10.5 nm. The ligand density was 1.7 μmol/m^2.

2.2. Packing of capillary columns and formation of frits

The particles were suspended in methanol at a concentration of 10% (w/w) using ultrasonic treatment. The packing of the capillary column (CS Chromatographie Service GmbH, Langerwehe, Germany) was performed on a home-made packing device. First a detection window was generated by burning off a small section of polyimide. The capillary was connected to the slurry chamber. The slurry was injected into the slurry chamber and a pressure of 700 bar was applied. At the lower end of the capillary a precolumn filter was placed to retain the packing material in the capillary during packing procedure. The frits were generated under pressure by a hydrothermal treatment of the packing material using a thermal wire stripper. First the outlet frit was generated. The column was allowed to decompress for 2 h. The capillary was removed and the excess packing material between the frit and the outlet of the column was flushed out. Next we generated the inlet frit using the same process as before. Fig. 3 schematically shows the process.

The formation of an inlet and outlet frit is essentially required to avoid a movement of the

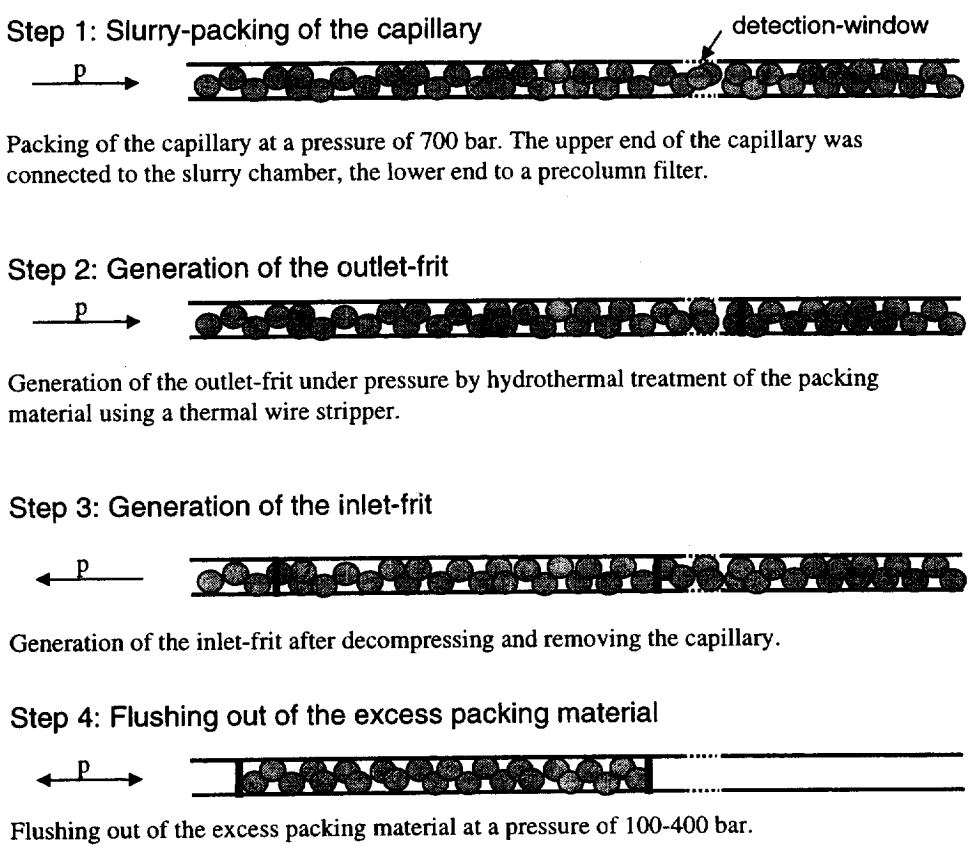

Fig. 3. Schematic representation of the packing procedure.

negatively charged particles to the anode when a field is applied.

2.3. Chemicals

All solvents were reagent grade and supplied by Merck KGaA (Darmstadt, Germany). The analytes and buffer salts were purchased from Sigma (Deisenhofen, Germany).

2.4. Equipment

Separations were performed using an HP^{3D} CE instrument (Hewlett-Packard, Waldbronn, Germany). Injections were performed electrokinetically by applying a voltage of 5 kV for 6 s. For the packing of the capillaries we used a Knauer pump. Frits were generated with a home-made electrical wire stripper.

3. Results and discussion

3.1. EOF measurements

The EOF was measured by using thiourea as a marker. The authors are aware of the fact that the choice of this substance does not provide the correct measurement of the EOF. Furthermore, the measured EOF might not only reflect the packed capillary. It might contain contribution by the frits and the unpacked part of the capillary. Fig. 4 displays the dependency of the EOF on the applied voltage. A linear relationship is obtained between 5 and 30 kV. The maximum EOF was close to 3 mm/s under these conditions. For comparison, the results obtained at a capillary packed with a C8-modified 3-μm Hypersil silica are also shown. The EOF on this column is lower at the same conditions.

It is known from literature data that reversed-phase silica generate a specific slope of the EOF vs. the voltage or field strength depending on the type of silica, the chemical bonding and the particle diameter.

No firm conclusions can be yet drawn on the effect of mean particle diameter of reversed-phase silicas on the EOF under CEC conditions.

The results of the measurements, however, clearly indicate that an EOF is still generated with these 0.5 μm particles. The dependency of EOF on the pH of the mobile phase and on the content of the organic

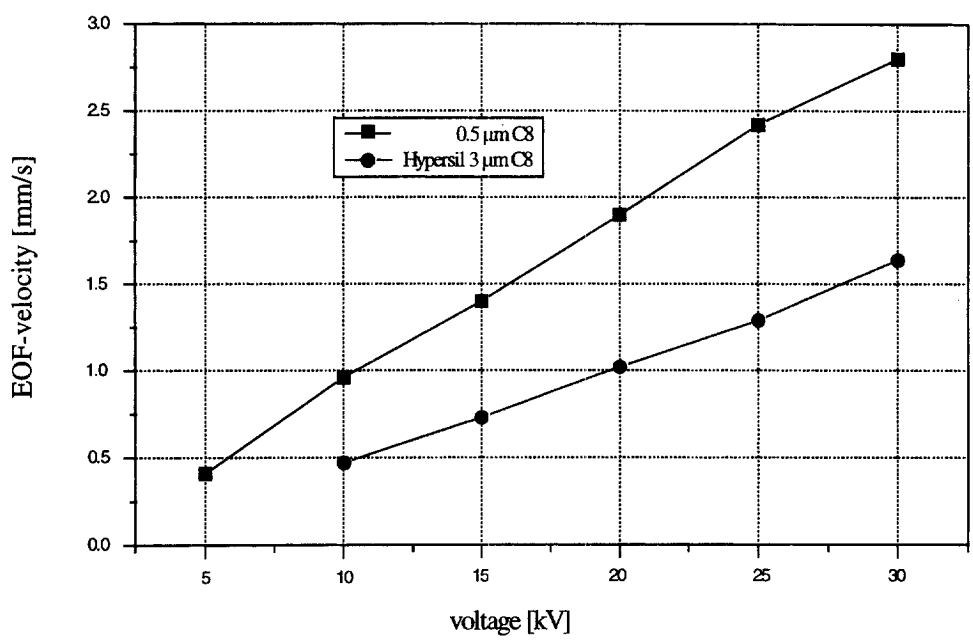

Fig. 4. EOF velocity versus the applied voltage: acetonitrile–Tris–HCl (80:20, v/v), pH 8, 20°C; capillaries, 8.5 (35)/38 cm and 100 μm.

modifier follows the same rules as observed with 3-μm commercial reversed-phase silicas [18].

3.2. Column performance

The dependence of the reduced plate height as a function of the reduced velocity was measured with the EOF-marker and compared to that obtained on a 3-μm Hypersil ODS-1 column under the same conditions. The results are shown in Figs. 5 and 6. With an increasing reduced velocity of $v > 1.5$ h drops down to about 2 and remains constant at higher reduced velocities. It appears that the first part of the curve is dominated by the diffusion of the solute as known from HPLC. There is no mass transfer effect seen on h of higher reduced velocities.

Fig. 6 shows the corresponding dependence on the capillary with the 0.5-μm particles. The reduced plate height decreases in a very similar way to the increase in the reduced velocity in comparison with Fig. 5. In a reduced parameter-plot the curves for different particle sizes should coincide. The lowest h values were $h = 3.5–4$ at v between 1.2 and 1.4. Higher reduced velocities could not be achieved with the equipment because the voltage was limited to 30 kV.

The reduced plate height of about 4 does not provide satisfying values. One must emphasize, however, that the equipment was not designed for such separations. To obtain more satisfying results it is necessary to reduce the total length of the capillary and to apply higher voltages to attain a better potential gradient. It is certain that extra column contributions will reduce the performance. Such contributions can arise from frits, the non-packed part of the capillary and inhomogeneities of the packed bed.

The separation of a synthetic mixture of six compounds obtained at a field strength of 790 V/cm is shown in Fig. 7. As expected, the analysis time is less than 1 min. The corresponding plate numbers N varied between 83 600 (thiourea) and 288 800 (*n*-pentylbenzene).

4. Conclusions

For the first time porous silanized submicrometer-size particles were applied in CEC. Particles of such

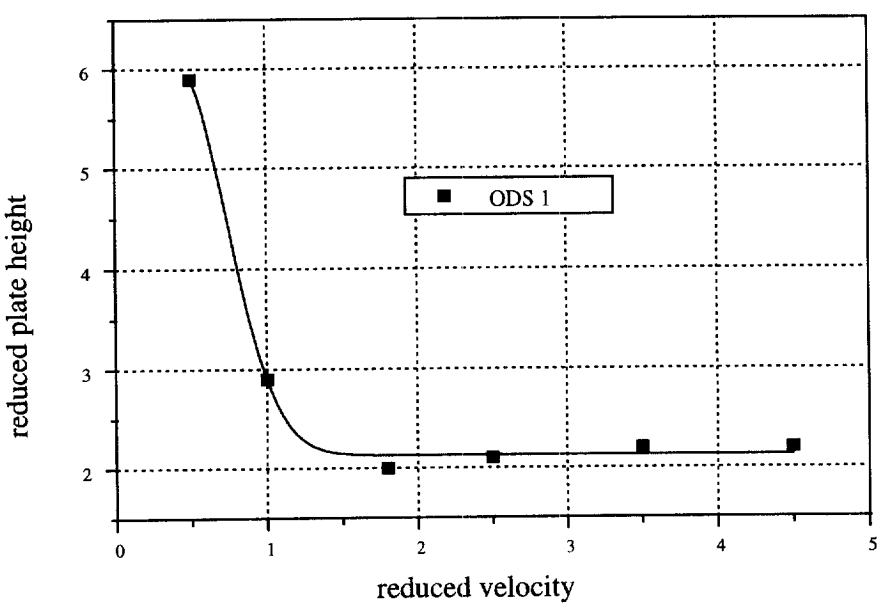

Fig. 5. Reduced velocity versus reduced plate height: acetonitrile–25 m*M* Tris–HCl (80:20, v/v), pH 8, 20°C; capillary, 35/38 cm and 100 μm, 3 μm ODS-1.

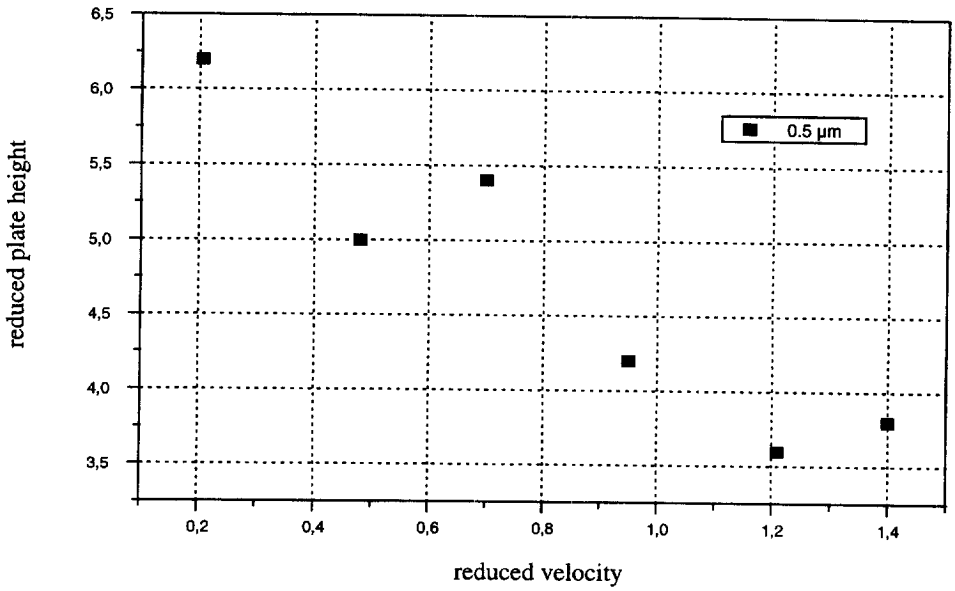

Fig. 6. Reduced velocity versus reduced plate height, acetonitrile–25 m*M* Tris–HCl (80:20, v/v), pH 8, 20°C; capillary, 8.5/38 cm and 100 µm, 0.5 µm C_8.

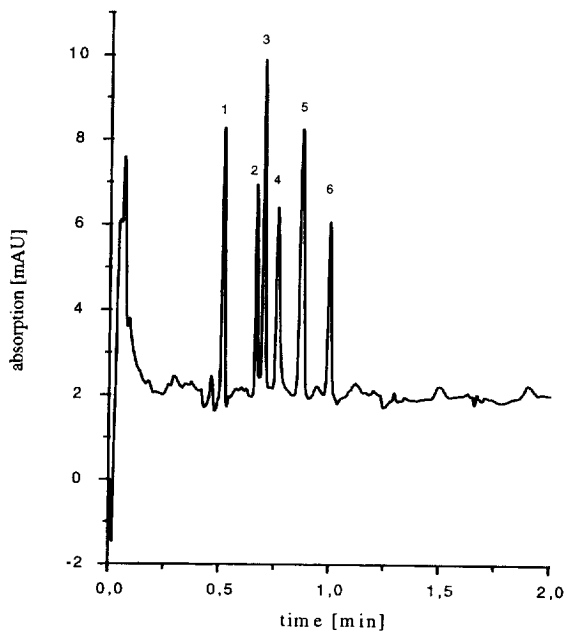

Fig. 7. Separation of a mixture of *n*-alkylbenzenes: 1, thiourea; 2, benzene; 3, ethylbenzene; 4, propylbenzene; 5, *n*-butylbenzene; 6, *n*-pentylbenzene acetonitrile–25 m*M* Tris–HCl (80:20, v/v), pH 8, 20°C, 790 V/cm; capillary, 8.5/38 cm and 100 µm, 0.5 µm C_8.

small diameter cannot be applied in traditional HPLC due to the high back-pressure.

An EOF is observed on this column comparable to that observed on reversed-phase silicas with larger particle diameter. There is a clear indication that with these particles diffusion is the dominant contribution to plate height. With commercial equipment the best performance is obtained at the highest field strength. i.e. full benefit of the capillaries can be obtained at a voltage >30 kV.

As expected analysis time is drastically reduced to less than 60 s while maintaining a high efficiency.

Further work will include the investigation of a series of silica packings with a mean particle diameter $0.1<d_p<1.0$ µm. We will also investigate porous and nonporous silanized submicrometer-size silica beads of identical particle diameter in CEC.

Acknowledgements

We gratefully acknowledge Hewlett-Packard GmbH, Waldbronn, Germany and the Deutsche Forschungsgemeinschaft, Bonn, Germany for supporting this work.

References

[1] V. Pretorius, B.J. Hopkins, J.D. Schieke, J. Chromatogr. 99 (1974) 23.
[2] J.W. Jorgenson, K.D. Lukacs, J. Chromatogr. 218 (1981) 209.
[3] T. Tsuda, K. Nomura, G.J. Nakagawa, J. Chromatogr. 248 (1982) 241.
[4] J.H. Knox, I.H. Grant, Chromatographia 24 (1987) 135.
[5] J.H. Knox, I.H. Grant, Chromatographia 26 (1988) 329.
[6] C.L. Rice, R. Whitehead, J. Phys. Chem. 69 (1965) 401.
[7] W. Stöber, A. Fink, E. Bohn, J. Colloid Interface Sci. 26 (1968) 62.
[8] K.K. Unger, H. Giesche, US Patent 4,775,520 (1988).
[9] E.sz. Kováts, L. Jelinek, C. Erbacher, European patent EPO,574,642A1 (1992)
[10] T. Bader, P.D. Dubois, Patent No. WO 91/07349 (1991).
[11] C. Kaiser, K.K. Unger, Patent Application No. P19530031.9 (1995).
[12] G. Büchel, I. Lauer, K.K. Unger, Proceedings of COPS V, Sept. 1996, Bath, UK.
[13] K.K. Unger, in: K.K. Unger (Ed.), Packings and Stationary Phases in Chromatographic Techniques, M. Dekker, New York, 1990, p. 365.
[14] R. Dadoo et al., LC·GC 10 (1995) 164.
[15] F.T. Hafner, H. Engelhardt, poster presentation HPCE '97.
[16] L. Colon et al., Lecture HPCE '97.
[17] K.D. Lork, J.N. Kinkel, K.K. Unger, J. Chromatogr. 352 (1986) 199.
[18] M.M. Dittmann, K. Wienand, F. Bek, G.P. Rozing, LC·GC 10 (1995) 800.