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Towards the column bed stabilization of columns in capillary electroendosmotic chromatography
Immobilization of microparticulate silica columns to a continuous bed

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Abstract

This article discusses a novel method generating a continuous bed inside the CEC column. The column bed composed of microparticulate reversed-phase silica is completely immobilized by a hydrothermal treatment using water for the immobilization process. This process eliminates the manufacture of frits of both ends of the column and all problems associated with their preparation. Fundamental studies on operational parameters will be presented such as the dependence of the immobilization on the column temperature, the type of stationary phase and the column back pressure. The immobilized CEC columns show the same high column efficiency as packed columns with frits.

Keywords: Stationary phases, CEC; Immobilization; Electrochromatography; Silica, microparticulate

1. Introduction

Capillary electrochromatography (CEC) has become a versatile, capillary separation technique which combines attractive features of micro-high-performance liquid chromatography (HPLC), regarding the control of retention and selectivity by mobile phase and stationary phase manipulation and of capillary electrophoresis (CE) regarding the high efficiency of separation [1–7]. In CEC the electro-osmotic flow (EOF) transports the mobile phase and the solutes through the column. This type of flow has favourable properties compared to the viscous flow used in an HPLC separation system. Flow velocity differences in the axial direction leading to band broadening by Eddy diffusion are much smaller in electrical driven solvent transport than in HPLC. Further, the EOF is generated by the charge of the silica particles, as well as by the charges at the capillary wall, in contrast to pressure-driven solvent transport. This allows the usage of much smaller particles or longer separation columns than in HPLC [3,8–11]. In CEC separation is achieved by solute–surface interactions or partitioning of the solute between the mobile and the stationary phase. When the solutes are charged, the separation is governed by

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differences in their electrophoretic mobilities in the mobile phase.

Recent papers have described applications of CEC in the pharmaceutical analysis for neutral and ionised analytes using reversed-phase and mixed-mode columns [12–20]. Much of the work has concentrated on the parameters effecting the speed and efficiency of separations including the applied voltage, pH value, ionic strength and content of organic modifier in the mobile phase [21–29]. A number of papers reported on methods to manufacture capillary columns and investigated the effect of particle size on column efficiency, column fragility and problems with bubble formation in the eluent [29–31]. CEC separations on slurry-packed and drawn-packed capillaries were performed by Knox and Grant [32]. Another alternative to pack columns using pressure is an electrokinetic packing method developed by Yan [33].

The main problem in the use of silica packed capillaries in CEC is that the packing material in the column bed needs to be retained; otherwise viscous or electrical forces drive the particles out of the fused-silica capillary column. Frit terminators like those used in conventional HPLC columns based on stainless steel tubes are not suited for this purpose because a relatively large stainless steel tube fitting is necessary to keep the frit terminator in place.

Several groups therefore have pursued the principal approach to immobilize part of the packed bed in the capillary by chemical means [34–37]. The problem with this approach is the lack of control on the porosity and length of this frits. In addition the fritted zone has another chemical composition than the packed bed which may adverse chromatography. Hjertén et al. reported a polyacrylamide gel which was polymerized in situ, creating a continuous porous bed [38]. Another continuous polymer bed was published by Nilsson et al. using molecular imprinting for the in situ preparation of chiral sorbents [39].

This article describes an alternative to generate a continuous bed consisting of a microparticulate reversed-phase silica packing. The column bed is completely immobilized in the capillary by a hydrothermal treatment. The decisive parameters controlling the immobilization were investigated and applications of such immobilized columns were shown.

2. Experimental

2.1. Chemicals

The chemicals used were tris(hydroxymethyl)aminomethane (Tris), 2-morpholinoethanesulfonic acid (MES), sodium acetate (NaOAc) and sodium dihydrogenphosphate (NaH_2PO_4) (Sigma, Deisendorf, Germany). The buffers were adjusted to the desired pH using either HCl or NaOH (E. Merck, Darmstadt, Germany). The sample compounds were thiourea, benzamide, naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, ethyl 4-hydroxybenzoate, propyl 4-hydroxybenzoate, butyl 4-hydroxybenzoate, pentyl 4-hydroxybenzoate, hexyl 4-hydroxybenzoate and the 16 polyaromatic hydrocarbon (PAH) standard (Sigma). The triazines were obtained from Dr. Ehrensdorfer (Augsburg, Germany) and dimethylphtalate, diethylphtalate, biphenyl and *o*-terphenyl as a gradient test mixture from Hewlett-Packard now Agilent Technologies (Waldbronn, Germany).

2.2. Capillaries

The capillaries were packed according to a conventional slurry packing procedure and immobilized through a hydrothermal treatment. The immobilization process and the setup of the laboratory-made heater were described in detail [29,40,41]. Polyimide coated fused-silica tubings were obtained from CS Chromatography Service (Langerwehe, Germany). The packed bed length was 25 cm, the total column length was 33.5 cm. Packing materials were obtained from Hypersil, Runcorn, UK (Hypersil ODS, 3 μm) and Waters Phase Separation, Clwyd, UK (Waters Sperisorb ODS I, 3 μm; Waters Sperisorb ODS II, 3 μm, Waters Spherisorb Mixed Moded SCX/C_{18}, 3 μm).

2.3. Instrumentation

All CEC experiments were carried out on a HP ^{3D}CE (Hewlett-Packard) instrument modified in such a way that a pressure of 1.0–1.2 MPa could be applied to the outlet and/or inlet vial. Before the first run the packed columns were put into the HP ^{3D}CE instrument and flushed with the mobile phase. The

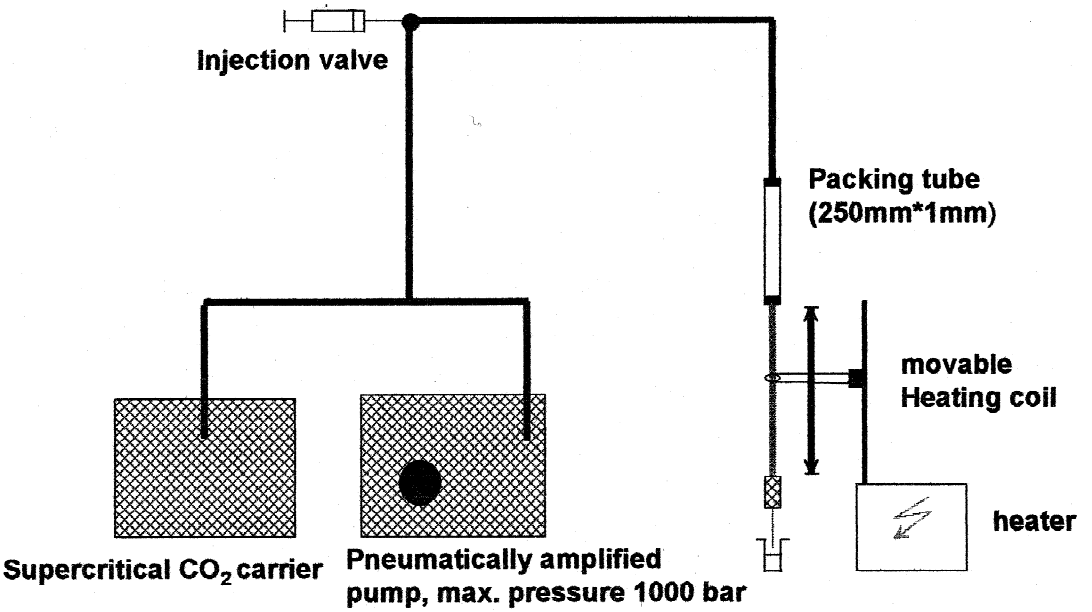

Fig. 1. Schematic set up of the packing apparatus with the immobilization heater.

detection wavelength was set at 254 nm. Samples were injected electrokinetically.

3. Results and discussion

Capillary separation methods are preferably performed in fused-silica tubing with internal diameters ranging from 25–100 μm I.D. Such capillaries provide an ideal material to serve as a container for packed beds of small porous or nonporous particles required for chromatography. In a conventional HPLC column the packed bed is typically kept in place under the high pressure that is applied (up to 40 MPa) by terminating sieves or frits, that are porous to the liquid but too narrow for the particles to move through.

Due to the narrow outer diameter of the fused-silica capillary tubing and the small volumes, it is not possible to use bulky fittings even if there are reduced in size accordingly. Therefore, attempts were made to form an in-column frit by chemical or thermal processes as described in the preceding section. The main objective of these processes was to ensure a sufficient chemical and physical adhesion to the capillary wall so that the fritted zone has sufficient stability to overcome, shrinking and cracking of the bed or fritted zone which occur, e.g., by drying. In such cases, gentle electrical or hydraulic force on the bed suffices to press out the bed and leave the column.

The most common way to prepare frits in narrow capillary columns is the hydrothermal treatment of the silica packing material. Benke et al. discussed the performance of frits fabricated by three different procedures [31].

The most commonly used technique is to make the frit from the packing by heating the capillary with a heating coil at the point at which the frit formation take place.

The temperature and duration of the heating must be determined experimentally for each individual packing. Under these conditions the particles are glued together and the chemical constitution of the zone that is fritted is not substantially altered.

The procedure can easily be performed at the inlet and outlet side of the packed capillary and the length of the fritted zone is controlled by the dimension of the external heating source. The permeability of the frit should not differ from that of the rest of the packing.

The packed bed of the capillary often develops

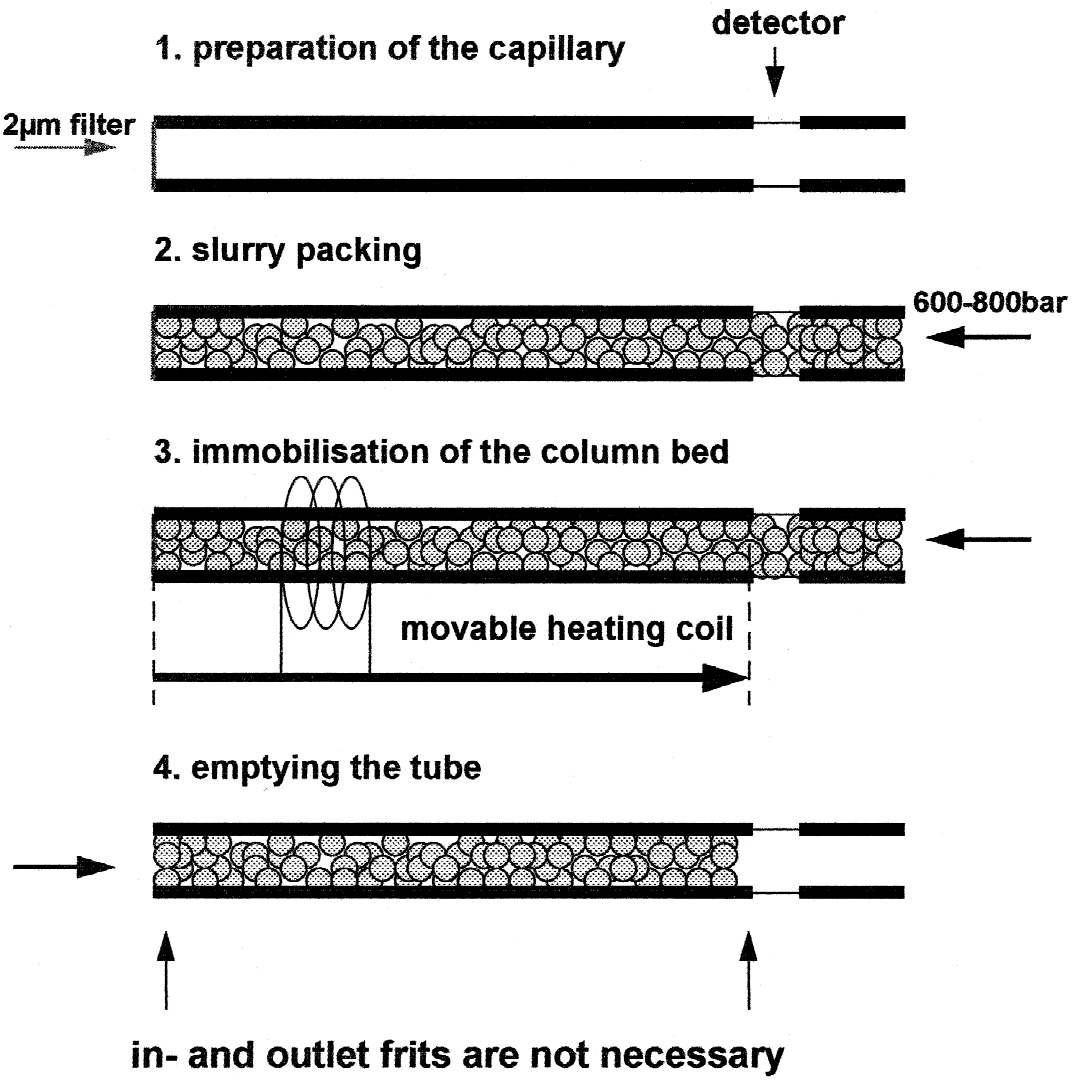

Fig. 2. Sequence of steps to achieve an immobilization of the packed bed.

fissures and cracks when used several times, caused by the movement of the negatively charged silica packing through their electrophoretic motion towards the anode. One solution to this problem is the immobilization of the whole packed bed in the capillary by a hydrothermal treatment. This process increases the lifetime of the packed capillary tremendously and avoids the formation of frits.

The immobilization should be performed under conditions, such that the polyimide layer on the outside of the capillary should be unaffected by the thermal treatment and the chromatographic properties such as retention and selectivity of the CEC column should remain unchanged.

The packing should be immobilized in such a way that it cannot be moved out of the column by pressure or electrophoresis. Also the permeability of the column should not be altered.

The capillaries were packed by two techniques: with the conventional slurry technique and with supercritical carbon dioxide. A microbore column was used for the slurry chamber with 1 mm I.D. in both cases (Fig. 1).

For the immobilization process a heater was

constructed with a processing motor to move the heating coil with a controlled speed and to maintain the heating coil at a constant temperature. The procedure of in situ hydrothermal treatment of the column bed is divided into four steps (Fig. 2). Firstly, the detection window is prepared by burning the polyimide layer and a precolumn filter is placed at the end of the capillary to retain the packing material during the process. A 10% (w/w) suspension of the packing material is made in acetone which is then placed in the reservoir of the slurry packer. A pressure of about 60 MPa is then applied to pack the material into the column. After the capillary is filled, a heating coil is moved along the desired length of the packed capillary with a constant velocity and at constant temperature in the range of 300 to 400°C. Several cycles are needed to achieve the column bed immobilization which depends on the type of reversed-phase packing. By the appropriate choice of the temperature of the heating coil and the number of heating cycle, the reversed-phase particles are glued together without plugging the capillary. The immobilized column is conditioned first with solvent purging and then by application of a low voltage before regular operation begins.

The immobilization of the microparticles in the packed bed is achieved as follows:

The particles of the bed are filled with water as well as the interstitial volume between the particles which have numerous contact sites to the neighbouring particles of the bed. When moving the heating coil along the packed capillary the high temperature in the heating zone leads to a dissolution of silica as polysilic acids at the other surface of the particles into the surrounding water. The solution is then assumed to saturated with respect to silica. After cooling off the heated zone the solution in the interstitial voids is supersaturated with respect to silica and redeposition of silica occurs at the interstices between the particles. Minute amounts of silica are required to chemically fuse the particles together. Fig. 3 shows a scanning electron micrograph of the immobilized bed and the immobilized particles. It is seen that the particles are glued together but retain their original shape.

The quality of immobilization is controlled by three operational parameters at a given reversed-phase packing material: the temperature of the heating coil, the speed by which the coil is moved along the packed columns and the number of cycles of this operation.

It was found that one cycle was not sufficient to immobilize the bed. The number of cycles and the two other parameters were specific for each reversed-

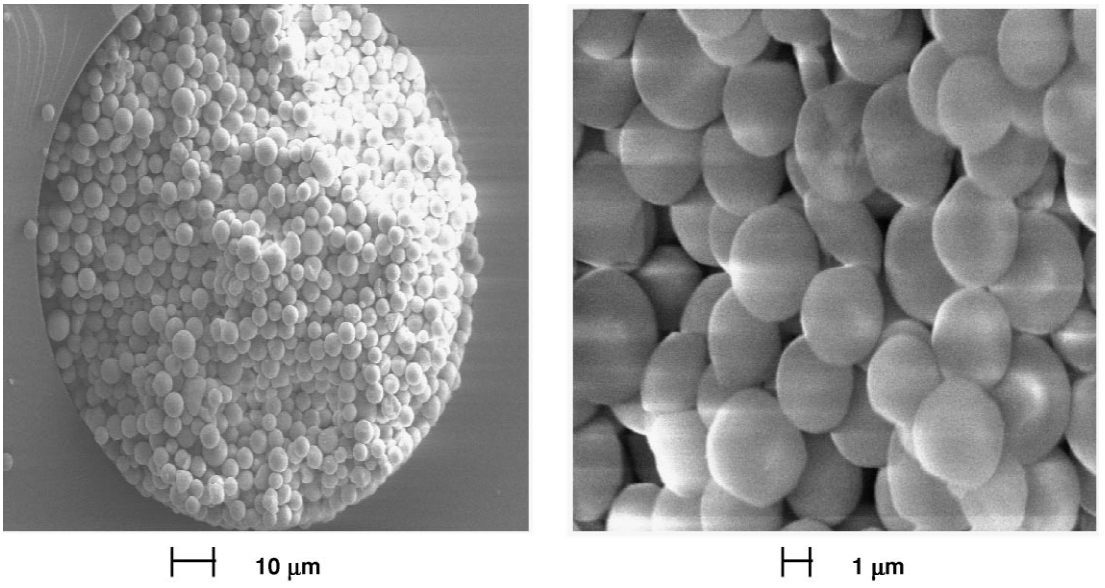

Fig. 3. Scanning electron microscope shots of the immobilized packing material.

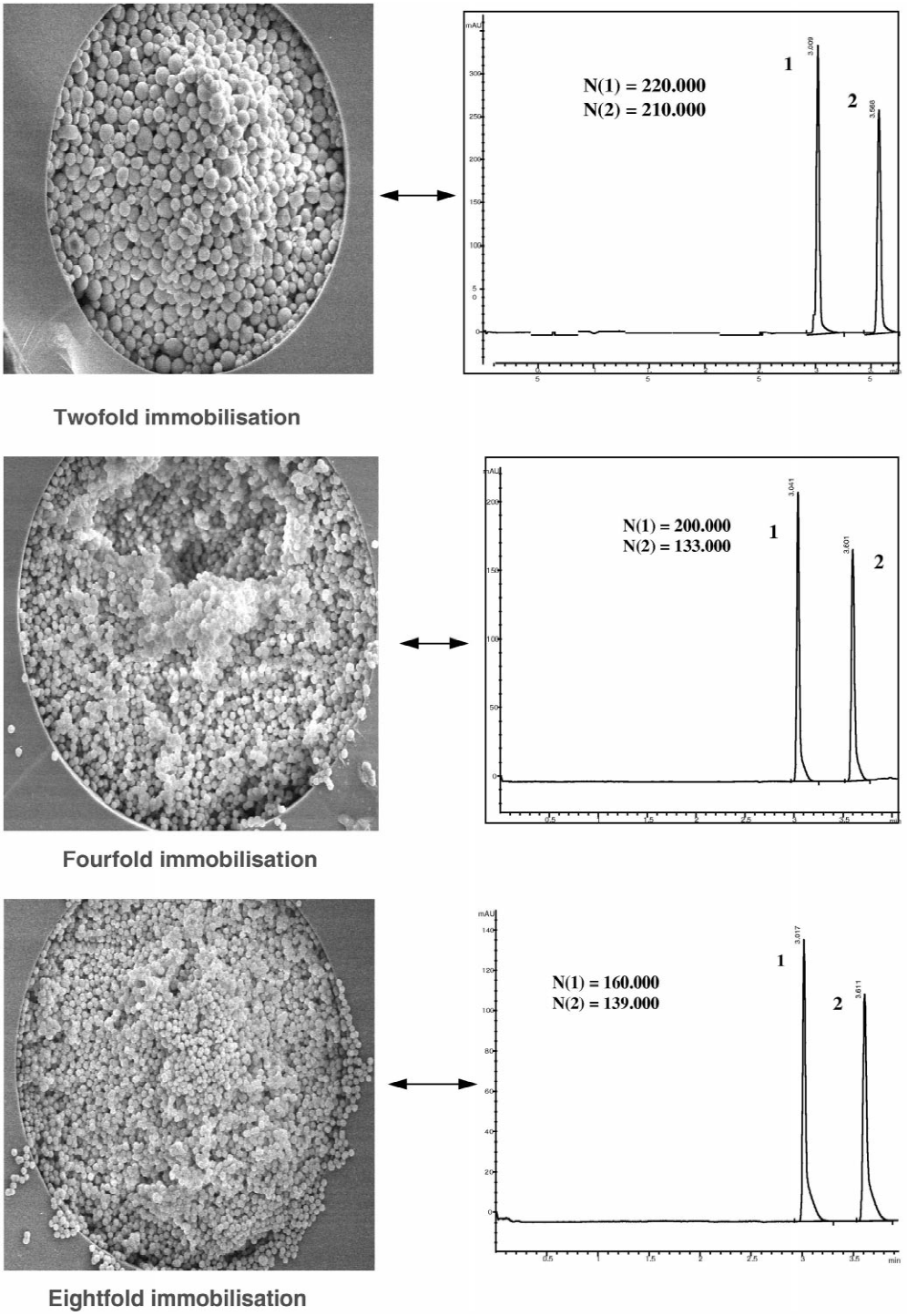

Fig. 4. Influence of the number of immobilization cycles on the column efficiency. CEC conditions: capillary: 250 mm effective length×0.1 mm I. D. packed with ODS I, 3 μm and immobilized as in figure; mobile phase: acetonitrile–25 m*M* Tris–HCl (pH 8) (80:20, v/v); applied voltage: 25 kV; injection: 5 kV, 6 s; detection: UV absorbance at 254 nm; temperature: 20°C; sample: 1=thiourea, 2=benzamide.

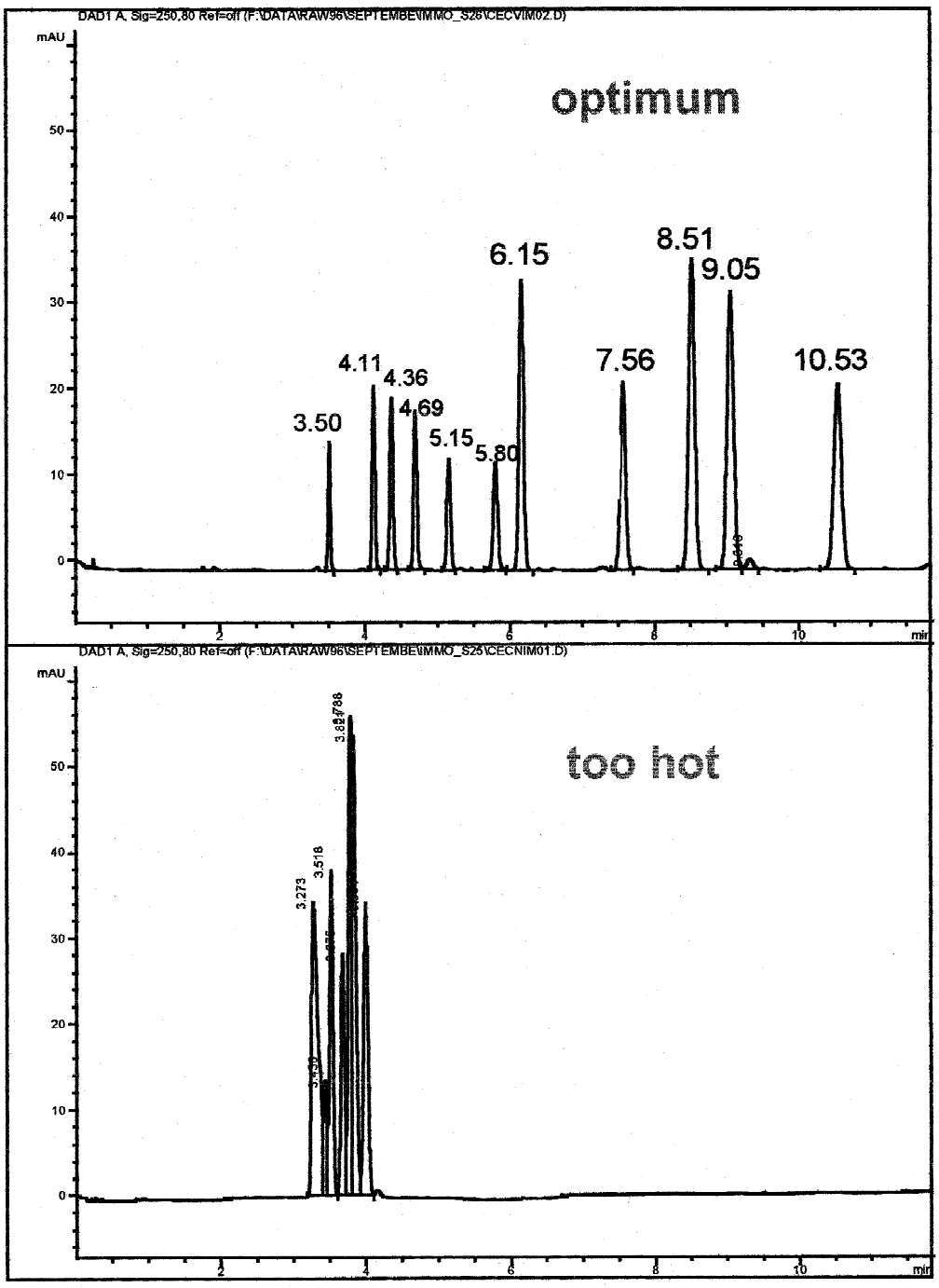

Fig. 5. Influence of the immobilization temperature on the chromatographic properties of the CEC column. CEC conditions: capillary: 250 mm effective length×0.1 mm I. D. packed with Hypersil ODS 3 μm and immobilized; mobile phase: acetonitrile–25 m*M* Tris–HCl buffer (pH 8) (80:20, v/v); applied voltage: 25 kV; injection: 5 kV, 6 s; detection: UV absorbance at 254 nm; temperature: 20°C; sample: 1=thiourea, 2=ethyl-, 3=propyl-, 4=butyl-, 5=pentyl-, 6=hexyl-parabene, 7=naphthalene, 8=fluorene, 9=phenanthrene, 10=anthracene, 11=fluoranthene.

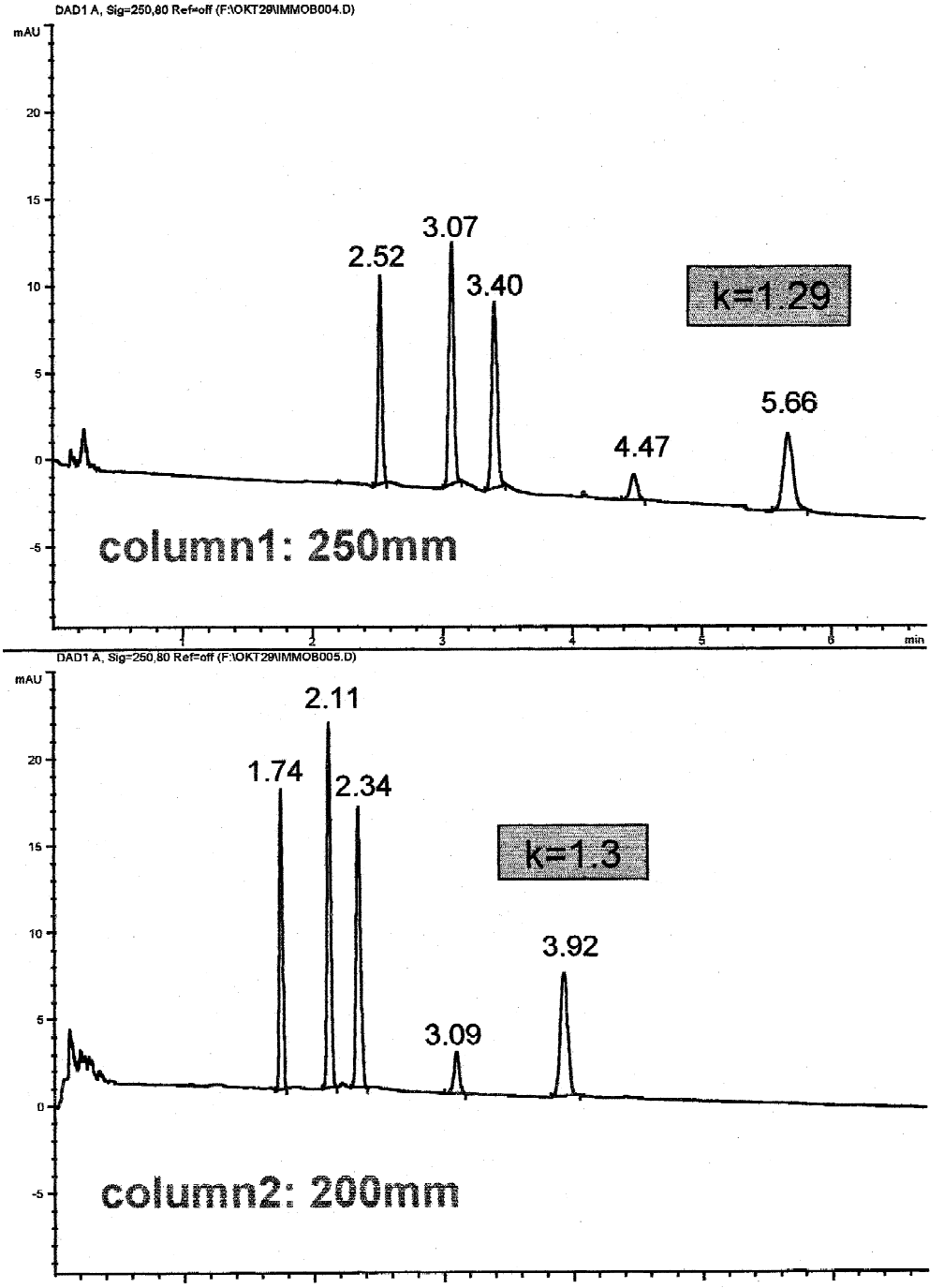

Fig. 6. Comparison of different segments of a continuous bed column. CEC conditions: capillaries: (1) 250 mm effective length, (2) 200 mm effective length×0.1 mm I. D. packed with ODS I, 3 μm and immobilized; mobile phase: acetonitrile–25 m*M* Tris–HCl (pH 8) (80:20, v/v); applied voltage: 30 kV; injection: 5 kV, 6 s; detection: UV absorbance at 254 nm; temperature: 20°C; sample: 1=thiourea, 2=dimethylphthalate, diethylphtalate, biphenyl, *o*-terphenyl.

phase material. Fig. 4 shows the effect of immobilization of two, four and eight cycles, respectively, on the column efficiency of the capillary. The best result with respect to column efficiency is obtained after two cycles in this specific case. More cycles lead to a drastic decrease of the column plate number and to severe peak tailing. In this context it is also mandatory to control the retention and selectivity of the CEC column with a test mixture as exemplified in Fig. 5. A too high immobilization temperature adjusted by the resistance of the heating coil can lead to a degradation of the surface of the reversed-phase silica in a way that *n*-alkyl bonds are cleaved at the surface and the column looses partially its retention characteristic.

Under optimal conditions no remarkable difference of the retention time and the resolution was observed before and after the immobilization.

The column stability was checked with a stress test of over 300 injections with a mixture of neutral compounds. The retention time and the plates remained constant.

One advantage of immobilized columns other than stability is that when column breakage occurs the parts can be further used for separation. Fig. 6 displays chromatograms of two parts of such an immobilized column. The chromatograms indicate that the resolution is maintained after cutting off a segment.

Usually, the detection window at the packed column is burnt directly after the packing bed to avoid additional band broadening. To study the influence of the distance between the detection window and the packed bed on the column efficiency, detection windows were burnt at different distances to the packed bed. Fig. 7 shows that the efficiency of compounds with and without retention starts to decrease after a distance of 4 cm. We assume, that the plug like profile of the EOF is responsible for the maintenance of the high efficiency in the unpacked part of the capillary up to this length.

The following examples demonstrate the versatility and efficiency of CEC immobilized column. Fig. 8 displays a separation of a mixture of triazines on a 25 cm CEC Hypersil ODS column.

In conventional HPLC, this separation be performed using gradient elution.

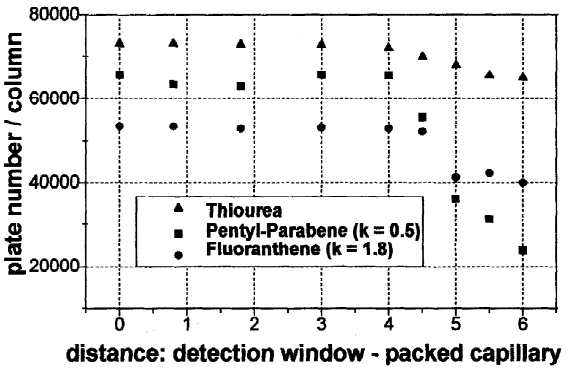

Fig. 7. Influence of distance of the packed capillary to the detection window on the efficiency. CEC conditions: capillary: 250 mm effective length×0.1 mm, packed with Hypersil-ODS, 3 μm, distance: packed capillary – detection window as in figure; mobile phase: acetonitrile–25 m*M* Tris–HCl (pH 8) (80:20, v/v); voltage: 20 kV; injection: 5 kV, 5 s; detection: UV absorbance at 254 nm; temperature: 20°C; sample: thiourea, pentyl-parabene, fluoranthene.

A separation that demonstrates the capability of isocratic CEC to replace gradient HPLC is the separation of the US Environmental Protection Agency (EPA) 16 PAH standard. Fig. 9 shows the separation of the PAHs on a CEC Hypersil C_{18} column. A complete separation of all 16 PAHs was achieved in about 15 min. In HPLC, this separation

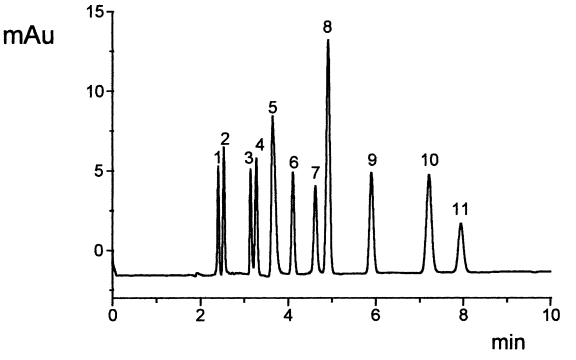

Fig. 8. Separation of herbizides on an immobilized CEC column. CEC conditions: capillary: 250 mm effective length×0.1 mm, packed with ODS I, 3 μm immobilized, mobile phase: acetonitrile–25 m*M* Tris–HCl (pH 8) (56:44, v/v); voltage: 30 kV; injection: 5 kV, 5 s; detection: UV absorbance at 220 nm; temperature: 15°C; sample: 1=desisopropylatrazine, 2=desethylatrazine, 3=simazine, 4=cyanazine, 5=atrazine, 6=sebutylazine, 7=propazine, 8=terbutylazine, 10=2-hydroxy-terbutylazine, 11=2-hydroxyatrazine.

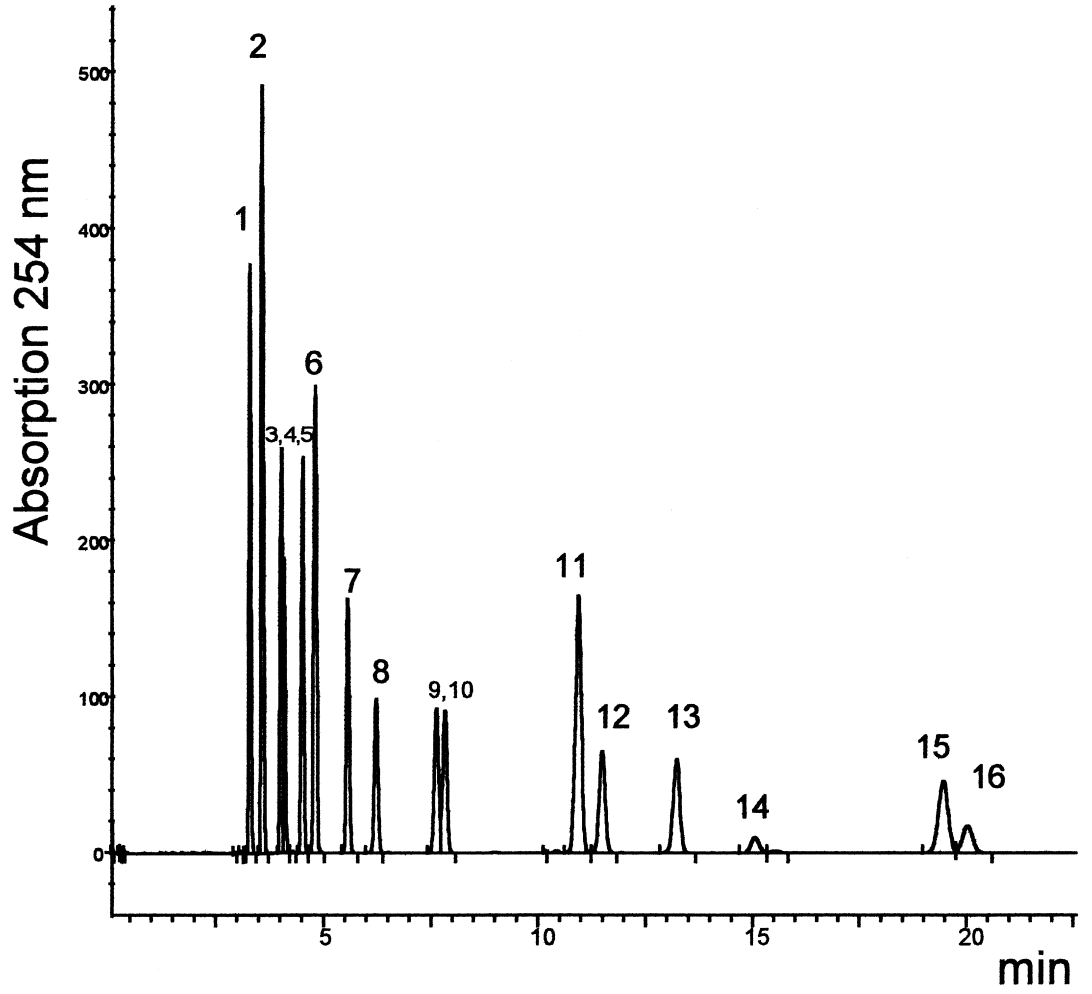

Fig. 9. CEC separation of a 16 PAH mixture in an isocratic mode. CEC conditions: capillary: 250 mm effective length×0.075 mm, packed with ODS I, 3 μm immobilized, mobile phase: acetonitrile–50 m*M* Tris–HCl (pH 8.5) (80:20, v/v); voltage: 30 kV; injection: 5 kV, 6 s; detection: UV absorbance at 254 nm; temperature: 30°C; sample: 1=naphthalene, 2=acenaphthalene, 3=acenaphthene, 4=fluorene, 5=phenanthrene, 6=anthracene, 7=fluoranthene, 8=pyrene, 9=benz(*a*)anthracene, 10=chrysene, 11=benzo(*b*)fluoranthen, 12=benzo(*k*)fluoranthene, 13=benzo(*a*)pyrene, 14=dibenz(*a,h*)anthracene, 15=benzo(*ghi*)perylene, 16=indeno(1,2,3-*cd*)pyrene.

can only be achieved using gradient elution and applying a stationary phases with special selectivity.

4. Conclusion

In this paper, a novel procedure of column immobilization is presented. It is possible to routinely pack and immobilize the packed bed in the capillary with reversed-phase silica packings. The advantages are that the immobilized packing cannot be moved out of the column by pressure or electrophoresis, the polyimide layer is unaffected by thermal treatment and the chromatographic properties (retention and selectivity) of the stationary phase remain unchanged. The procedure consists of a hydrothermal in situ process and needs only water for the immobilization. The process increases the lifetime of the packed capillary drastically and makes the manufacture of frits unnecessary. The immobilization of the packed bed leads to more stable CEC columns than these with frits. In the future improvement in

column technology and packing materials will be of prime importance for the continued growth and development of CEC.

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