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Short communication

Pseudo-electrokinetic packing of high efficiency columns for capillary electrochromatography

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Abstract

An improved and easy electrokinetic packing procedure is presented for the production of stable capillary columns suitable for capillary electrochromatography (CEC). In pseudo-electrokinetic packing a high electric field is used in conjunction with a hydrodynamic flow. The packing of silica-based reversed-phase columns can be achieved with basic, commercially available capillary electrophoresis (CE) equipment in approximately 15 min. The procedure is robust and a high success rate is achieved. No steps which might damage the stationary phase are involved and only a minimum amount of packing material is required. Columns packed according to the developed procedure are operated at high electric field strengths during the CEC separation, without the application of a stabilising pressure. Columns are stable for at least hundred runs and were tested using mixtures of polycyclic aromatic hydrocarbons and positively charged drugs. Separations were performed in a relatively high conducting ammonium acetate buffer, with efficiencies of up to 283 000 plates/m.

Keywords: Capillary columns; Stationary phases, electrochromatography; Electrochromatography; Polynuclear aromatic hydrocarbons; Clenbuterol; Salbutamol; Methadone

1. Introduction

Capillary electrochromatography (CEC) is a highly attractive separation technique in which the selectivity of liquid chromatography (LC) stationary phases is combined with electrophoretic migration. In CEC the mobile phase is driven through the column by electroosmosis, resulting in a flat flow velocity distribution over the cross-section of the column [1,2], reducing dispersion effects as present in hydrodynamically driven chromatography.

A critical aspect of CEC is the production of a column which does not deteriorate rapidly after applying an electric field. Currently, the preferred method for column fabrication is slurry packing [3–5]. However, in our experience slurry packing of capillary columns is time consuming and can have a limited success rate. Gas bubble formation, creation of voids and column breakdown during CEC operation are reported problems with slurry packed columns and are assumed to be related to frit and column quality [6,7]. In order to reduce gas bubble formation in the column during a CEC separation, 5–10 bar pressure is applied at both ends of the CEC

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column, complicating the equipment and limiting the combinations with extra-column techniques, like mass spectrometric detection. Several other approaches in column preparation have been reported, like the in situ polymerisation of continuous supports [8], supercritical column packing [9] and electrokinetic packing [10].

In electrokinetic packing, a high electric field is used to electrokinetically fill and tightly pack a column with charged stationary phase particles [10]. With electrokinetically packed columns up to 181 000 plates/m were obtained for polycyclic aromatic hydrocarbons (PAHs) [11]. In contrast to slurry packing, no pressure is used to fill the column, making electrokinetic packing a gentle technique and in potential applicable for column packing with very small-sized particles. The packing time and quality depends on the magnitude of the electrophoretic velocity of the stationary phase particles and the electroosmotic flow (EOF) through the column. However, when the migration direction is opposite to the EOF, as is the case with silica based stationary phases, the net migration velocity can be small which makes electrokinetic packing time consuming.

In this paper an improved electrokinetic packing procedure is described, in which the use of a very low hydrodynamic pressure is implemented in the packing procedure. Silica based reversed-phase columns, which were produced with this so-called pseudo-electrokinetic packing are considered in CEC separations of PAHs and positively charged drugs, using a regular electrophoresis background electrolyte without additional pressure at the capillary ends.

2. Experimental

2.1. Chemicals

Methanol and acetonitrile were obtained from Rathburn (Waterburn, UK). Isopropylalcohol came from Baker (Deventer, The Netherlands). Tris-(hydroxymethyl)methylamin (Tris) was purchased from Aldrich (Steinheim, Germany). Benzo[*a*]pyrene was obtained from Janssen (Beerse, Belgium) and anthracene, naphthalene, fluorene, pyrene, clenbuterol and methadone were purchased from Sigma (St. Louis, MO, USA). Salbutamol was kindly donated by the TNO Institute (Zeist, The Netherlands).

Buffer solutions were prepared freshly every day and degassed by 10 min of sonification in an ultrasonic bath. Standard solutions were prepared in the separation buffers and were sonificated prior to use. All solutions were prepared using water obtained from a Milli-Q system (Millipore, Bedford, MA, USA).

2.2. Apparatus

A programmable injection system for capillary electrophoresis (Prince, Prince Technologies, Emmen, The Netherlands) was used for both packing of the capillary columns as for the CEC runs. Columns were prepared using (endcapped) MOS Hypersil 3-μm particles (Hypersil, Runcorn, UK) as the packing material for the analytical column. Bare 7–9 μm silica particles, used to make a temporary frit, came from Merck (Darmstadt, Germany). Untreated fused-silica capillaries (75 μm I.D.×375 μm O.D) were obtained from SGE (Ringwood, Australia). A Jasco familic 300S pump (Jasco, Hachioji, Japan) was used for purging the column with degassed water and preconditioning of the column with the mobile phase. The connection of the column to the HPLC pump was made by a Rheodyne (Rheodyne, Berkeley, CA, USA) model 7000 switching valve. A 1/16 in. (1 in. =2.54 cm) polyether ether ketone (PEEK) fingertight nut and 25 mm PEEK tubing (400 μm I.D.) were used for the connection of the column to the switching valve.

Post-column detection was performed at 254 nm using a Spectroflow 757 variable wavelength UV detector (ABI Kratos, Ramsey, NJ, USA) equipped with a custom-made detection cell. The signal was registered using a model BD 40 flat bed recorder (Kipp & Zonen, Delft, The Netherlands).

Electrokinetic injections were realised by application of +5 kV for 5 s.

3. Results and discussion

In electrokinetic packing of capillary columns, stationary phase particles migrate electrokinetically into a capillary to form a packed bed. Often, silica-

based particles are used that contain weak acidic silanolic groups, resulting in a negatively charged surface when deprotonated in a basic pH environment. When brought in a capillary, the electrophoretic velocity of the particles depends on the electrophoretic mobility of the particle, the electroosmotic mobility of the background electrolyte and the applied field strength. In an untreated fused-silica capillary the EOF is directed opposite to the electrophoretic migration velocity of the silica particles. Consequently, electrokinetic introduction of the particles into a bare fused-silica capillary can be rather time consuming. The procedure could be accelerated significantly by a hydrodynamic introduction of a suspension of stationary phase into the capillary, which is finally packed with a high electric field. This pseudo-electrokinetic packing procedure is outlined schematically in Fig. 1.

In a fused-silica capillary of approximately 70 cm in length, a temporary frit was made by tapping the capillary outlet in a small amount of dense slurry of bare silica particles ($d_p = 7–9$ μm) and heating the capillary tip in a small gas flame until the silica particles were sintered [6] (step 1). This frit functions solely as a support for the stationary phase during the packing procedure and will be removed later on. A slurry of 20 mg MOS Hypersil was prepared in 300 μl of methanol–water containing 10 mmol/l Tris buffer pH 8 (75:25, v/v) as background electrolyte and placed in a 400-μl vial, which is sufficient for the production of several columns. As an organic additive, both acetonitrile and isopropylalcohol were tested. However, no significant differences in the quality of the produced columns were found.

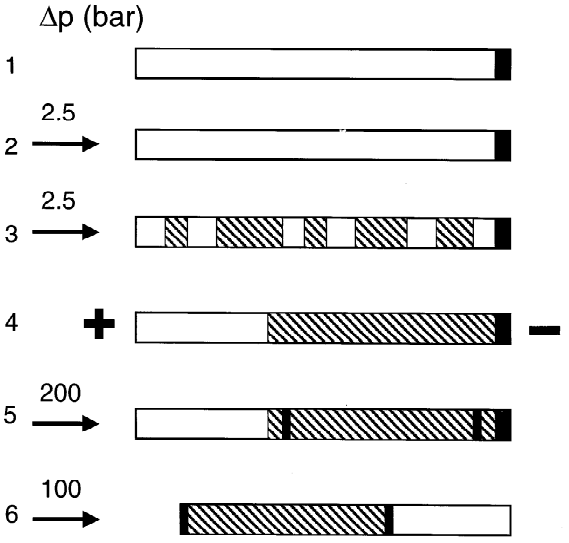

Fig. 1. Schematic overview of the pseudo-electrokinetic packing procedure in six steps: (1) fabrication of a temporary frit, (2) filling of the capillary with background electrolyte, (3) filling the capillary with stationary phase particles, (4) applying a high voltage for electrokinetic packing (it should be noticed that the polarity of the applied voltage is determined by the ζ-potential of the particles), (5) fabrication of the permanent frits and (6) removal of the excess of stationary phase particles.

The capillary is flushed with the background electrolyte (step 2) and the inlet of the capillary is placed in the dense sediment of stationary phase. Some slurry was pumped into the capillary using 2.5 bar pressure (step 3), which partially filled the capillary in a few minutes until the pressure build up, due to the blockage of the outlet frit by the stationary phase particles, is restricting the flow. During the hydrodynamic filling, several zones of column material with voids of buffer solution in between are observed. When the hydrodynamic filling is completed a voltage of +30 kV is applied at the capillary inlet (step 4). The application of the voltage results in the generation of an electroosmotic flow which is the driving force to continue and finally complete the packing of the column within 10 min. This process can be followed visually by the build up of the packed bed. Although the electrophoretic mobility of the particles is in opposite direction of the EOF, which is generated by the capillary wall, the net electrophoretic mobility of the particles is towards the capillary end. In case of reversal of the polarity, all particles would migrate towards the capillary inlet.

For the packing procedure a programmable injector for capillary electrophoresis (Prince) is used. However, it is anticipated that other commercial or laboratory-built systems are well suited for this purpose.

Following on, the column is connected to a conventional HPLC pump and flushed with degassed water for at least half an hour at 200 bar. Then, permanent frits are made from the packed bed column material, using a hot filament device [7] at a temperature of 600°C for 8 s, while maintaining the

pressure on the column (step 5). The support frit at the column end is then cut off and the excess of packing material is flushed away (step 6). Finally, an optical detection window is made by burning off the polyimide coating from the capillary at a distance of 2 cm from the outlet frit.

Using the described method, numerous ($n>40$) columns have been produced successfully, which were very stable under CEC operation conditions. This is in sharp contrast with slurry packing, by which we found a significant lower success rate.

To verify the quality of a column the separation of a test mixture of PAHs was performed. Prior to use, the column was flushed with the mobile phase for 15 min at 200 bar and preconditioned for CEC operation by applying +30 kV for 5 min. The PAHs were well separated in an acetonitrile–water (80:20, v/v) solution containing 2 mmol/l Tris, pH 8.0 buffer, and plate counts of up to 275 000 plates/m were obtained, as is shown in Fig. 2. The concentrations of the PAHs were 10–50 mmol/l and UV absorption detection was performed at 254 nm. Since the packing material is end-capped, the EOF generated by the column is relatively low, however the linear flow velocity that can be obtained is sufficiently high at an applied voltage of 30 kV.

The repeatability of the capacity factor (k'_{CEC}) on a pseudo-electrokinetically packed capillary column was tested over six runs for different PAHs, within 1 day and on one column (Table 1). For all compounds

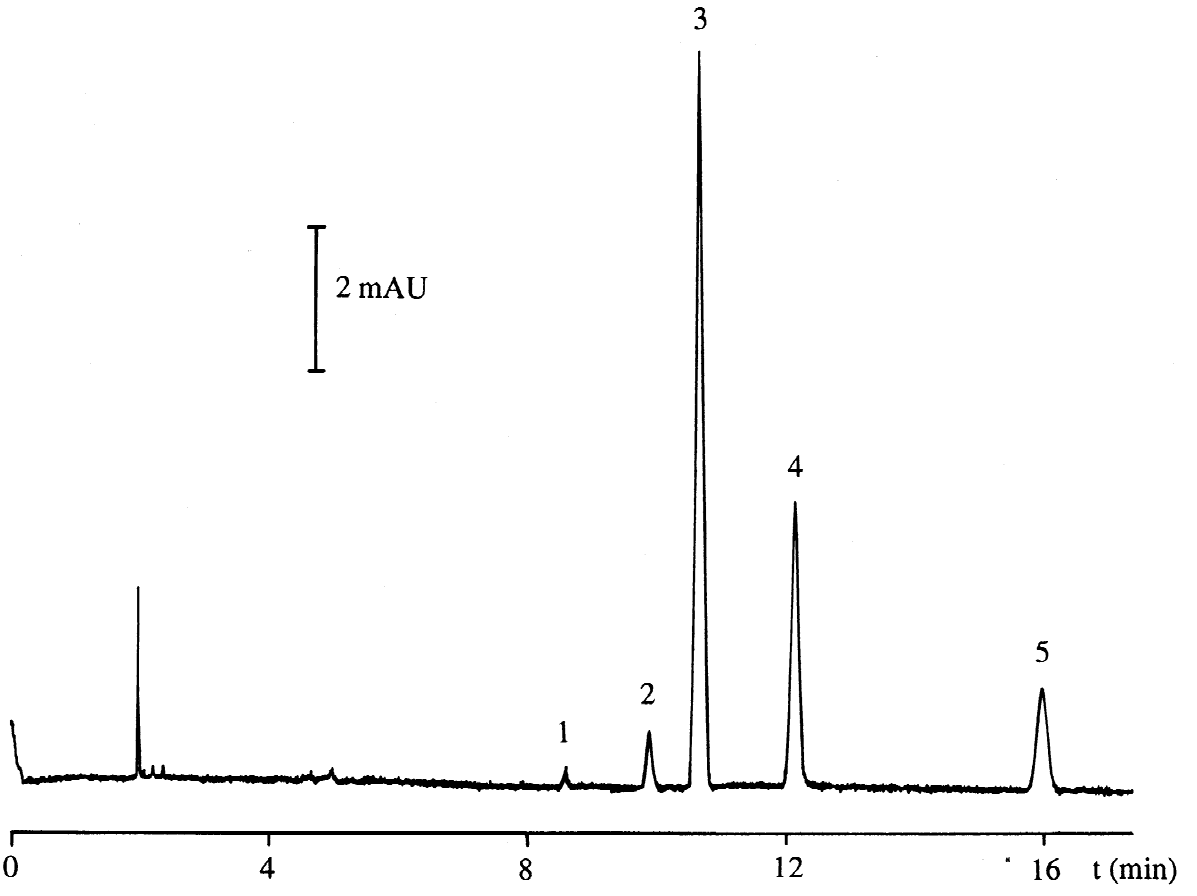

Fig. 2. Separation of some PAHs on a pseudo-electrokinetically packed column (16 cm×75 μm I.D.). Peaks: 1=naphthalene; 2=fluorene; 3=anthracene; 4=pyrene; 5=benzo[*a*]pyrene. Mobile phase composition: 2 mmol/l Tris, pH 8.0, in acetonitrile–water (80:20, v/v). Electrokinetic injection 5 s at +5 kV. Applied voltage: +30 kV. The separation efficiencies are up to 283 000 plates/m.

Table 1
Repeatability of the k'_{CEC} of several PAHs ($n=6$)

PAH	k'_{CEC}	RSD (%)
Naphthalene	0.19	4.4
Fluorene	0.24	3.5
Anthracene	0.27	2.8
Pyrene	0.34	3.3
Benzo[*a*]pyrene	0.48	3.1

Table 2
Separation efficiency of fluorene as tested on ten different pseudo-electrokinetically packed columns[a]

Column	Efficiency (plates/m)
1	255 000
2	221 000
3	246 000
4	237 000
5	283 000
6	196 000
7	276 000
8	225 000
9	238 000
10	264 000
Mean	244 000
RSD (%)	~11

[a] Separation was performed in 10 mmol/l ammonium acetate buffer, pH 8.0, in acetonitrile–water (80:20, v/v). Electrokinetic injection 5 s at +5 kV. The applied CEC voltage was +30 kV.

the relative standard deviations of the capacity factor are between 3.0 and 4.5%, which is comparable with separations on slurry packed columns [12]. In Table 2 the efficiency of the fluorene signal using ten pseudo-electrokinetically packed columns is shown. All tested columns with a $d_p = 3$ μm were prepared using 10 mmol/l ammonium acetate buffer, pH 8.0, in methanol–water (75:25, v/v). On average, a relatively high efficiency was obtained (244 000 plates/m) with a relative standard deviation of approximately 11%.

In addition, some positively charged compounds were analysed. For the basic drugs clenbuterol, salbutamol and methadone plate counts up to 150 000 plates/m were obtained (Fig. 3). From Fig. 3 it can be seen that particularly for clenbuterol and salbutamol the peaks are almost symmetric and that methadone shows only a minor tailing. The improved peak shape is probably due to the end-capped nature of the stationary phase, since weakly basic drugs can show severe tailing on silica-based stationary phases [13].

The pseudo-electrokinetic packed columns are stable for at least a hundred runs and can be operated at high voltages without the need of additional stabilising pressure to prevent gas bubble formation. In addition, the use of a highly conductive 25 mmol/

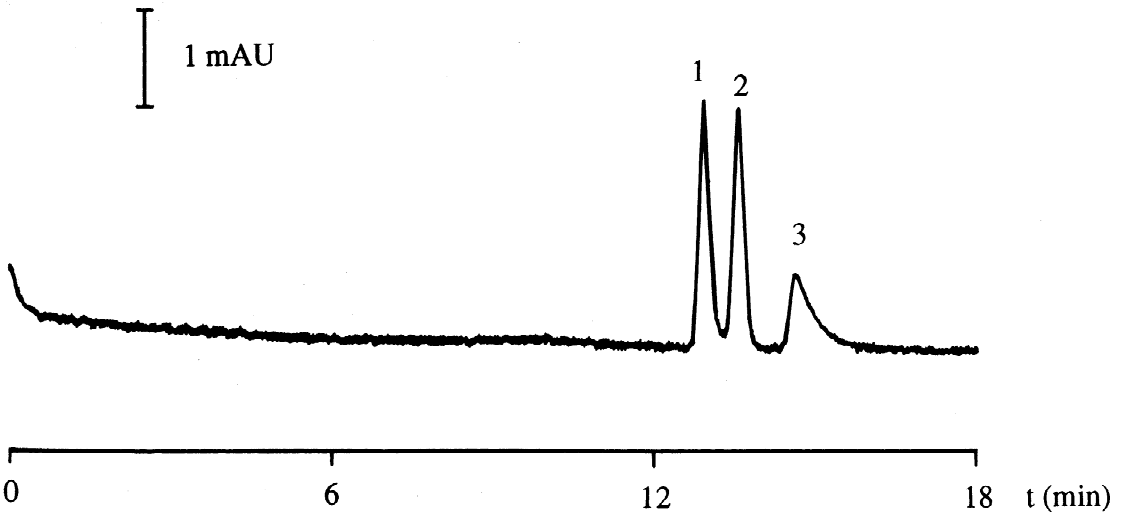

Fig. 3. Separation of some positively charged compounds on a pseudo-electrokinetically packed column (16 cm×75 μm I.D.). Peaks: 1=clenbuterol; 2=salbutamol; 3=methadone. Background electrolyte: 25 mmol/l ammonium acetate buffer, pH 5.0, in acetonitrile–water (90:10, v/v). Electrokinetic injection 5 s at +5 kV; applied voltage: +30 kV.

l ammonium acetate pH 5.0 buffer (+30 kV, 9 μA) in methanol–water (72:25, v/v) did not affect either the column stability or the detector signal. Furthermore, the creation of voids did not occur at all.

4. Conclusions

The presented method for capillary column production is fast and easy and requires only basic commercially available equipment or a simple custom-build system. The columns are stable for over hundred runs and do not need additional pressure to prevent gas bubble formation during CEC operation. Further, the method is robust and generates columns which provide high separation efficiencies of up to 150 000 and 283 000 plates/m for positively charged drugs and PAHs, respectively. The procedure is a gentle packing procedure in which no steps are involved, which might damage the stationary phase and only limited amounts of stationary phase are needed for the production of a column.

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