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Review

Advances in column technology and instrumentation in capillary electrochromatography

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Abstract

Capillary electrochromatography (CEC) is an emerging technique gaining increased interest. Improvement of instrumentation and column technology will be of prime importance for the further development of this technique and its use in validated methods. In this paper, developments in column technology and instrumentation for CEC are reviewed with emphasis on developments within the last 3 years. Attention is directed to the employment of stationary phases specifically designed for CEC, the use of soft and rigid gels in place of packings, fritless packed capillaries, column dimensions, the optimization of injection and detection parameters, and gradient elution CEC.

Keywords: Reviews; Electrochromatography; Capillary columns; Instrumentation; Stationary phases, electrochromatography

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1. Introduction

Capillary electrochromatography (CEC) can be defined as a liquid chromatographic method, in which the mobile phase is electroosmotically driven through the chromatographic bed [1–5]. The interest in CEC stems from the concept that the unique properties of the electroosmotic flow (EOF) make it possible to realize highly efficient liquid chromatographic separation systems that can overcome the peak capacity limitations of high-performance liquid chromatography (HPLC). Another point of interest in CEC is the possibility to combine chromatographic separation with separation due to electrophoresis making it possible to separate strongly basic, moderately basic, weakly basic, neutral, weakly acidic, moderately acidic, and strongly acidic compounds in one run [6].

The main driving force behind the development of CEC has been the fact that the electroosmotically driven flow in a porous plug (i.e. a capillary packed with particles) is virtually independent of the mean channel diameter. Smoluchowski [7] has shown that Eq. (1) retains its validity, if in the experiment of electroosmosis an open capillary is replaced by a porous plug [8]:

$$\frac{V}{i} = \frac{\varepsilon_D \varepsilon_0 \zeta}{\eta \lambda_0} \tag{1}$$

where V is the volume of liquid displaced per unit time; i the electric current; ε_D the dielectric constant of the bulk liquid; ε_0 the electric permittivity of vacuum; ζ the electrokinetic potential (zeta-potential); η the viscosity of the bulk liquid; and λ_0 the specific electric conductivity of the liquid.

Eq. (1) can be used to describe the electroosmotic flow through a porous plug, provided that the flow is laminar, the local radius of curvature of the particles and the size of the pores are large compared to the thickness of the double layer, and the effects of surface conductance are negligible. Porous plugs may be made of particles of quite irregular geometry, nearly spherical particles, fibres, more or less well orientated, or bundles of capillaries [9]. However, in CEC the velocity of the mobile phase is generally not given as flow-rate (volume/time) but as linear velocity (distance/time) determined via a non-retarded marker. It is assumed that this linear velocity is identical or at least proportional to the electroosmotic velocity v_{eo} (Eq. (2), constraints see Eq. (1)):

$$v_{eo} = \frac{\varepsilon_D \varepsilon_0 \zeta}{\eta} \cdot E \tag{2}$$

where E is the local electric field strength.

Provided the size of the pores is large compared to the thickness of the electric double layer, the linear velocity is virtually independent of the mean channel diameter and directly proportional to the local electric field strength. Consequently, separation columns can be employed in CEC that cannot be used (because of their high streaming resistance) in pressure driven LC. Another important feature of the electroosmotic flow is its flat streaming profile (compared to the parabolic streaming profile generated by pressure difference induced laminar flow) minimizing band broadening due to mass transfer resistance in the mobile phase, and reducing the A-term of the plate height equation.

Basic concepts of CEC have been reviewed in excellent papers by Crego et al. [10], Colón et al. [11,12], Robson et al. [13], and Cikalo et al. [14], addressing electroosmotic generation of mobile phase flow and theory of band broadening in CEC. Although first experimental studies have corroborated the theoretical predictions that the electroosmotic generation of the mobile phase flow results in a number of important advantages of CEC over conventional HPLC, it seems that many technical problems still hamper the widespread use of this potentially advantageous separation method. In order to exhaust the full potentials of this method, instrumentation is needed that is adapted to the specific needs of CEC. Currently, most of the work has been done with essentially the same instrumentation as used for capillary electrophoresis (CE). In general, this equipment cannot be used to generate gradients of the composition of the mobile phase, and often it cannot be used to equilibrate the separation capillary under pressure-induced flow.

While the preparation of capillaries by packing with reversed-phase silica gels (virtually exclusively octadecyl silica gel) has received the most attention in the early studies devoted to CEC, currently

various stationary phases specifically designed for use in CEC or with immobilized groups offering specific selectivities have been proven to be suited to solve separation problems in CEC. The crux of packed capillaries is the manufacture of inlet and outlet frits to retain the stationary phase. Consequently, there is increased interest in the production of fritless separation capillaries. Approaches to prepare fritless separation capillaries for CEC include polymerization of monomers in a capillary, tapered capillaries or to 'glue' the individual particles in the packed capillary. Advances in column technology has become a vigorous area of research in the last few years.

This review is primarily concerned with novel directions in producing highly efficient and robust separation capillaries for CEC, with concepts to optimize instrumental parameters, and with the experimental realization of gradient elution in CEC.

2. Bubble formation

In the early days of CEC, bubble formation during a chromatographic run was the most important practical problem that hampered the widespread use of this technique. In one of their pioneering works on CEC Knox and Grant [15] have stated that 'Drying out [of the packed capillaries] was particularly liable to occur with the wider capillaries and with the higher concentrations of electrolyte, indicating that self-heating was the primary cause.' They recommended thermostating of columns at temperatures close to ambient or operating the whole column under pressure as the most effective preventive measures.

There are, however, indications that self-heating is not the primary cause of the formation of bubbles in the packing. The first indication is that the electric current that is measured during separation in CEC is one to two magnitudes lower than that is measured under routine CE or MEKC conditions. Rebscher and Pyell [16] observed that the formation of bubbles invariably started with semi-packed column at the border between the packed and the unpacked section of the capillary. They interpreted this observation as follows: 'The following segment has a higher electroosmotic mobility than the preceding segment. Consequently, the second non-packed segment has the effect of a pump reducing the pressure in the packed section below the prevailing pressure.'

This interpretation is corroborated in a theoretical study by Rathore and Horváth [17]. They studied the interface of the packed and open segments of a semi-packed CEC column and discontinuities associated. They show that in order to satisfy the mass conservation law, in most cases a 'flow-equalizing intersegmental pressure', which is different from the pressure at the two ends of the column, develops at the interface of the packed and open segments.

A second reason for bubble formation is associated with frits. Rathore and Horváth [17] highlight that silica frits most likely have zeta potentials different from those of the bulk packings, and the discontinuity of zeta potential can lead to the development of flow-equalizing intersegmental pressure at such frits with concomitant bubble formation. Rebscher [18] observed that a completely packed capillary that invariably starts bubble formation at the inlet end during a chromatographic run, can be used without problems, if the direction of the electroosmotic flow is reversed. Rebscher and Pyell [19] observed that if a prepared column has a frit with low (mechanical) streaming permeability, bubble formation during a chromatographic run is very likely to occur directly after the frit. This problem could be completely circumvented if the frit with low permeability was replaced by a second frit. These observations suggest that not only differences in the zeta potential between frit and bulk packing but also an extremely low (mechanical) streaming permeability of the frit can result in bubble formation. Carney et al. [20] investigated factors affecting bubble formation. They conclude that bubble formation in CEC is a function of the length and nature of the frit. According to Carney et al. one possible solution of this problem is the recoating of octadecylsilane onto the silica frit minimizing differences in the zeta potential between the frit and the bulk packing material.

These studies suggest that for the design of a robust separation capillary suitable for CEC at ambient pressure it is important that the separation capillary is as homogeneous as possible (over the full length) in zeta potential (at the interface between the stationary phase and the mobile phase) and that it is

as homogeneous as possible (over the full length) in the cross sectional area of the inner volume of the capillary accessible to the mobile phase. These two constraints are fulfilled with fritless capillaries filled completely with stationary phase. Operating the whole column under pressure is not necessary with a robust separation capillary [21].

3. Open-tubular columns

From a technical point of view open-tubular (OT) columns are the simplest approach to fritless and robust columns in CEC. Tsuda et al. [3] are the first to work with OT-columns in CEC. Working with etched and porous silica layered silanized (monochlorodimethyloctadecylsilane) fused-silica capillaries with inner diameters in the range 5–25 μm, Bruin et al. [22] showed that the efficiency of electro-driven open-tubular liquid chromatography (ED-OT-LC) is by a factor of ca. 2 better than that of pressure-driven open-tubular liquid chromatography (PD-OT-LC) in agreement with theory.

The overall plate height equation is valid in ED-OT-LC and PD-OT-LC [22]:

$$H = \frac{2D_m}{v} + f(k)_m \cdot \frac{d_c^2 v}{D_m} + f(k)_s \cdot \frac{d_f^2 v}{D_s} \quad (3)$$

where H is the plate height; D_m or D_s the diffusion coefficient in the mobile or the stationary phase, respectively; v the linear velocity; d_c the inner diameter of the column; d_f the thickness of stationary phase layer; $f(k)_m$ or $f(k)_s$ are functions of k; and k is the retention factor.

Because of the difference between the flat flow profile induced by electroosmosis compared to the parabolic flow profile observed for pressure difference induced laminar flow, there is a difference in $f(k)_m$ [22] resulting in a gain of efficiency for the same column if ED-OT-LC is performed instead of PD-OT-LC.

$$\text{ED-OT-LC:} \quad f(k)_m^{ED} = \frac{k^2}{16(1+k)^2} \quad (4)$$

$$\text{PD-OT-LC:} \quad f(k)_m^{PD} = \frac{1+6k+11k^2}{96(1+k)^2} \quad (5)$$

Bruin et al. [22] highlight that because of the flat flow profile in ED-OT-LC capillaries with wider inner diameter (up to 25 μm) can be used than in PD-OT-LC, having advantages concerning detection, loadability and column preparation.

For ED-OT-LC, developments in column technology have been reviewed in 1997 by Colón et al. [12]. These include coating of the inner surface with a polymeric material [23–27], coating of the inner surface with cellulose derivatives [28], silanization of the etched fused-silica surface [29–31], or adsorption of a surfactant bilayer or a protein layer [32].

Guo and Colón [33] fabricated an organic–inorganic hybrid material by the sol–gel method as a thin porous film attached to the inner wall of fused-silica capillaries. By this technique the phase ratio stationary phase/mobile phase could be improved compared with thin-film techniques. Under the experimental conditions used, efficiencies of 280 000–500 000 plates/m were observed with 10–13 μm I.D. capillaries. A similar approach has been used by Wu et al. [34] preparing reversed-phase open-tubular columns for ED-OT-LC–ion-trap storage–reflectron time-of-flight mass spectrometry separating a peptide mixture. The porous stationary phase was loaded onto the inner wall of fused-silica capillaries with an inner diameter of 9 μm.

Tan and Remcho [35] developed a new procedure for the preparation of thick polymethacrylate films bonded in 25 μm I.D. fused-silica capillaries. Sawada and Jinno [36] employed a similar approach, preparing polymer-coated 25 μm I.D. capillaries by in-capillary copolymerization of *N-tert.*-butylacrylamide with a charged monomer, after the pretreatment of the capillary inner surface with a bifunctional reagent. The capillaries prepared have been successfully applied in the separation of neutral analytes by ED-OT-LC. Tan and Remcho [37] bonded thin films of molecular imprinted polymers to the inner walls of 25 μm I.D. fused-silica capillaries using the in-situ polymerization technique published in 1997 [35]. With such a capillary a much higher resolution was achieved for a mixture of D- and L-dansyl phenylalanine by ED-OT-LC than by PD-OT-LC.

Although OT capillaries have some advantages over packed or filled capillaries, such as no liability to bubble formation and easy rinsing without the need to apply high pressure, OT capillaries are rarely

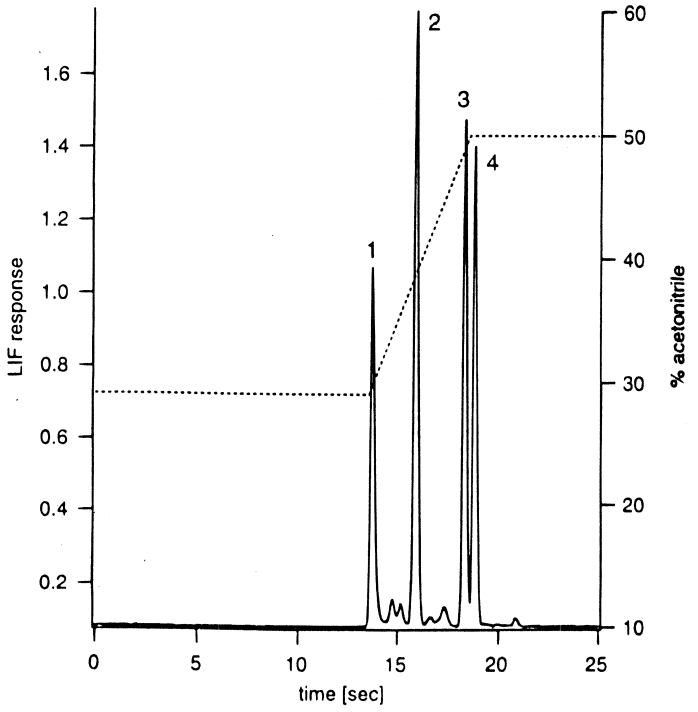

Fig. 1. Fast open-channel electrochromatography on a microchip. Separation of coumarin dyes. Channel depth, 5.2 μm; stationary phase, octadecylsilane; mobile phase, 10 m*M* borate buffer (pH 8.4) with a linear gradient of acetonitrile from 29 to 50%; peak identification, (1) C440, (2) C450, (3) C460, (4) C480; dotted line, gradient trace (From Ref. [38] with permission).

employed in CEC. This might be due to the relatively small inner diameters of such capillaries having disadvantages in combination with optical detection methods.

The miniaturization potential of ED-OT-LC has been used by Kutter et al. [38] for open-channel electrochromatography in combination with solvent programming using a microchip device. Fig. 1 shows the chromatogram for a four-component coumarin dye mixture with a channel depth of 5.2 μm and a film of stationary phase prepared by silanization of the pre-treated chip channel. The microchips were fabricated in-house from glass substrates [39].

4. Packed capillary columns

4.1. Production of frits

With traditional packing procedures, frits (mostly at the inlet and the outlet end) are necessary to stabilize the chromatographic bed. The physicochemical properties of these frits do not only determine the mechanical stability of the packed columns but also liability to bubble formation (see the Bubble formation section), band broadening during sample injection due to inhomogeneities in the flow through the inlet frit [16], band broadening during elution of an analyte zone from the packing into an open section of the capillary before detection [40], and decreased sensitivity due to adsorption of the analyte on the frit [41,42].

The following methods have been reported for the production of frits in CEC: (1) reaction of sodium silicate solution with formamide to form a porous silica plug [43,44]; (2) sintering of a plug of native silica gel wetted with an aqueous solution of potassium silicate [45]; (3) sintering of a plug of native silica gel wetted with pure water [19] and; (4) sintering of a portion of the chromatographic packing itself with a heated filament after having flushed the column with water [46,47]. Cikalo et al. [14] highlight that technique (4) relies on the stationary phase having a high sodium content, which may not be

found in the newer types of silica gel manufactured from tetraethoxysilane.

The problems outlined in this section and difficulties associated with the reproducible production of internal frits and their long-term stability make it very desirable to construct fritless capillary columns for CEC.

4.2. Packing techniques

Packed columns are prepared with the slurry technique. The retaining frit fixed at the outlet end during the packing process is either a sintered metal frit [14,48], connected via a union to the capillary, or a retaining frit prepared by sintering a plug of wetted native silica gel [45] directly in the capillary. The slurry of the packing material is either prepared in an organic solvent or in supercritical carbon dioxide [49]. Mostly the packing material is transported into the column with help of an external pump. Boughtflower et al. [50] immersed an ultrasound probe into the pressurized slurry reservoir to maintain homogeneity of the slurry during the packing process. Reynolds et al. [51] and Fermier and Colón [52] used columns packed by centripetal forces. Electrokinetic packing was described by Yan [53], and its use has been demonstrated by other workers [54–56].

4.3. n-Alkyl silica gels

Work in CEC has started with capillaries packed with porous octadecyl silica gel [1,2,4]. One prediction that can be derived from theoretical considerations is the independence of the velocity of the electroosmotically generated mobile phase flow from the mean channel diameter in the packing, hence the mean particle diameter of the packing. In order to verify this prediction, it was necessary to isolate fractions of silica gel material or to produce silica gels with smaller mean particle diameters (ca. 1.5 μm) than those that have been used at this time for HPLC.

In a first survey, Knox and Grant [15] compared capillaries packed with octadecyl silica gels of mean particle diameters ranging from 1.5 to 50 μm. One main conclusion of their work is that 'the electroosmotic velocity is essentially unaffected by particle size at least down to 1.5 μm'. Their work also shows that particle size has a tremendous impact on the obtainable efficiency.

Lüdtke et al. [57] were successful in preparing packed capillaries (100 μm I.D.) with 0.5-μm porous silanized silica beads (85 mm length of packing, 380 mm total length) and employing these capillaries in CEC. The porous sub-micron packing material had been prepared in their working group with a modified Stöber procedure [58]. No report was made on length of the capillary to the detector. The apparent reduced plate heights (not corrected for the non-packed section of the capillary) are relatively high [h(minimum) = 3.5–4]. It can be expected that with sub-micron packing material molecular diffusion is the dominating band broadening process, so that a further reduction in particle diameter will not result in significantly increased efficiency.

Columns packed with porous *n*-alkyl silica gel with a mean particle diameter of 1.8 μm have shown efficiencies of $>300\,000$ plates/m and $h<2$ [46,59].

Not only porous but also non-porous material has been employed for packed capillaries in CEC. Yamamoto et al. [60] successfully used 1.6 μm non-porous octadecyl silica gel for fast separations. However, the efficiency for retarded solutes was much lower than expected, probably due to mass overload. Seifar et al. [61] observed that, in order to obtain stable conditions in CEC with 1.5 μm non-porous octadecyl silica gel, it was necessary to add an anionic surfactant, sodium dodecyl sulfate (SDS), in a concentration below the critical micellar concentration to the mobile phase. Seifar et al. ascribe this effect to a dynamic modification of the non-polar alkylated surface. The columns produced by these authors were extremely efficient with ca. 500 000 plates/m and $h \approx 1.3$ [62]. With columns packed with 1.5 μm non-porous octadecyl silica gel Bailey and Yan [63] were able to separate a series of 14 nitroaromatic and nitramine explosive compounds in under 7 min (Fig. 2), featuring efficiencies of $>500\,000$ plates/m. Also Bailey and Yan recommend the addition of SDS to the mobile phase to prevent bubble formation.

Dadoo et al. [64] used columns packed electrokinetically with 1.5 μm non-porous octadecyl silica gel to achieve rapid separations with high efficiencies. A sample containing 16 polycyclic aromatic

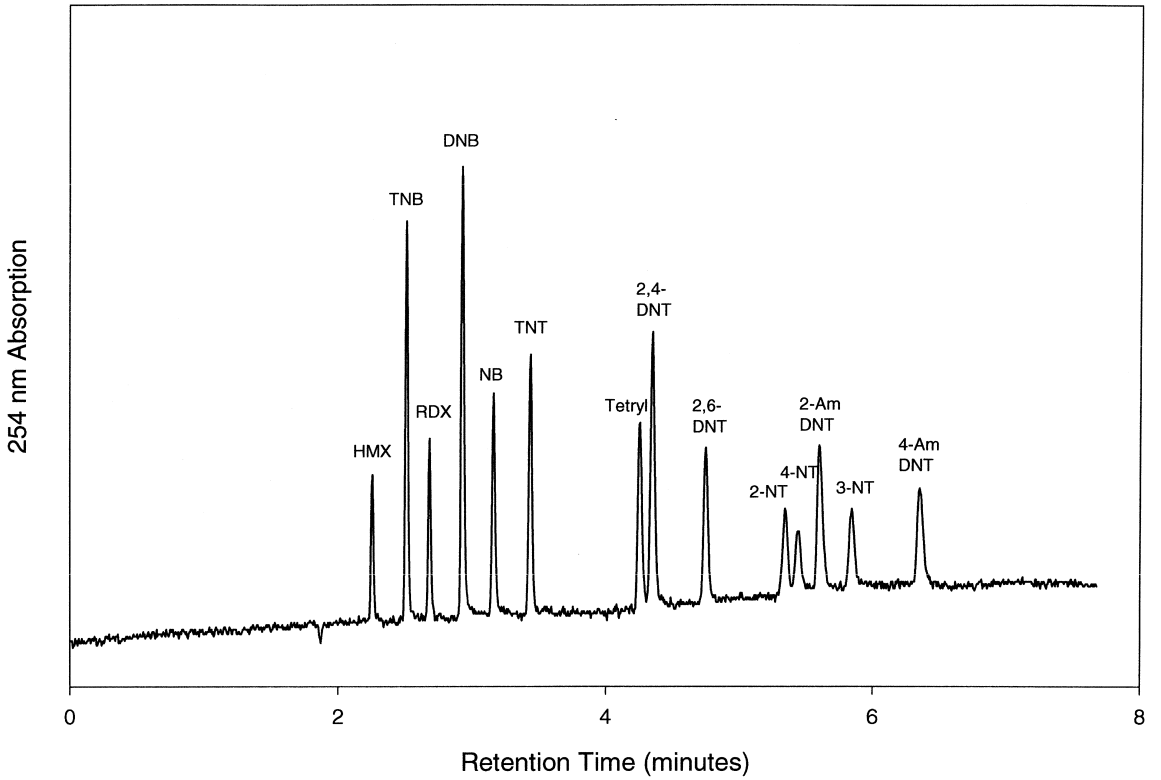

Fig. 2. CEC separation of explosives. Capillary, 34 (21) cm×75 μm; stationary phase, 1.5 μm nonporous octadecyl silica gel; mobile phase, 20% methanol, 80% aqueous buffer [10 m*M* 2-(*N*-morpholino)ethanesulfonic acid, 5 m*M* sodium dodecyl sulfate]; HMX, octahydro-1,3,5,7-tetranitro-1,3,5,7-tetrazocine; RDX, hexahydro-1,3,5-trinitro-1,3,5-triazine; DNB, 1,3-dinitrobenzene; TNB, 1,3,5-trinitrobenzene; NB, nitrobenzene; TNT, 2,4,6-trinitrotoluene; 2,4-DNT, 2,4-dinitrotoluene; tetryl, methyl-2,4,6-trinitrophenylnitramine; 2,6-DNT, 2,6-dinitrotoluene; 2-Am-DNT, 2-amino-4,6-dinitrotoluene; 2-NT, 2-nitrotoluene; 4-NT, 4-nitrotoluene; 4-Am-DNT, 4-amino-2,6-dinitrotoluene; and 3-NT, 3-nitrotoluene (From Ref. [63] with permission).

hydrocarbons (PAH's) (classified as priority pollutants by the US Environmental Protection Agency) was isocratically separated in under 10 min (Fig. 3). With detection in a packed section of the capillary >700 000 plates/m were obtained. In their experiments no anionic surfactant was added to the mobile phase.

While the use of spherical non-porous material with mean particle diameter <1.8 μm with very narrow size distribution constitutes one approach to obtain maximum efficiency in CEC, the use of wide-pore material and pore flow effects is another very interesting approach. Li and Remcho [65] report results obtained with octadecyl silica gels with mean pore diameters ranging from 6 to 400 nm. The authors assume that with large-pore material perfusive transport of the mobile phase through the pores is possible, reducing significantly the plate height of the chromatographic system. The experimental results, indeed, support this theory, indicating that the use of wide-pore packing material may provide for a notable increase in efficiency. They also showed that the reduced plate height (mean particle diameter 7 μm) is not only dependent on the mean pore diameter but also on the ionic strength of the mobile phase (Fig. 4). This result indicates according to Li and Remcho 'that the thickness of the electrochemical double layer obviously plays a significant role in perfusive transport through narrow channels'. With wide-pore octadecyl silica gel and a mobile phase of high ionic strength a reduced plate height of <1.4 was obtained.

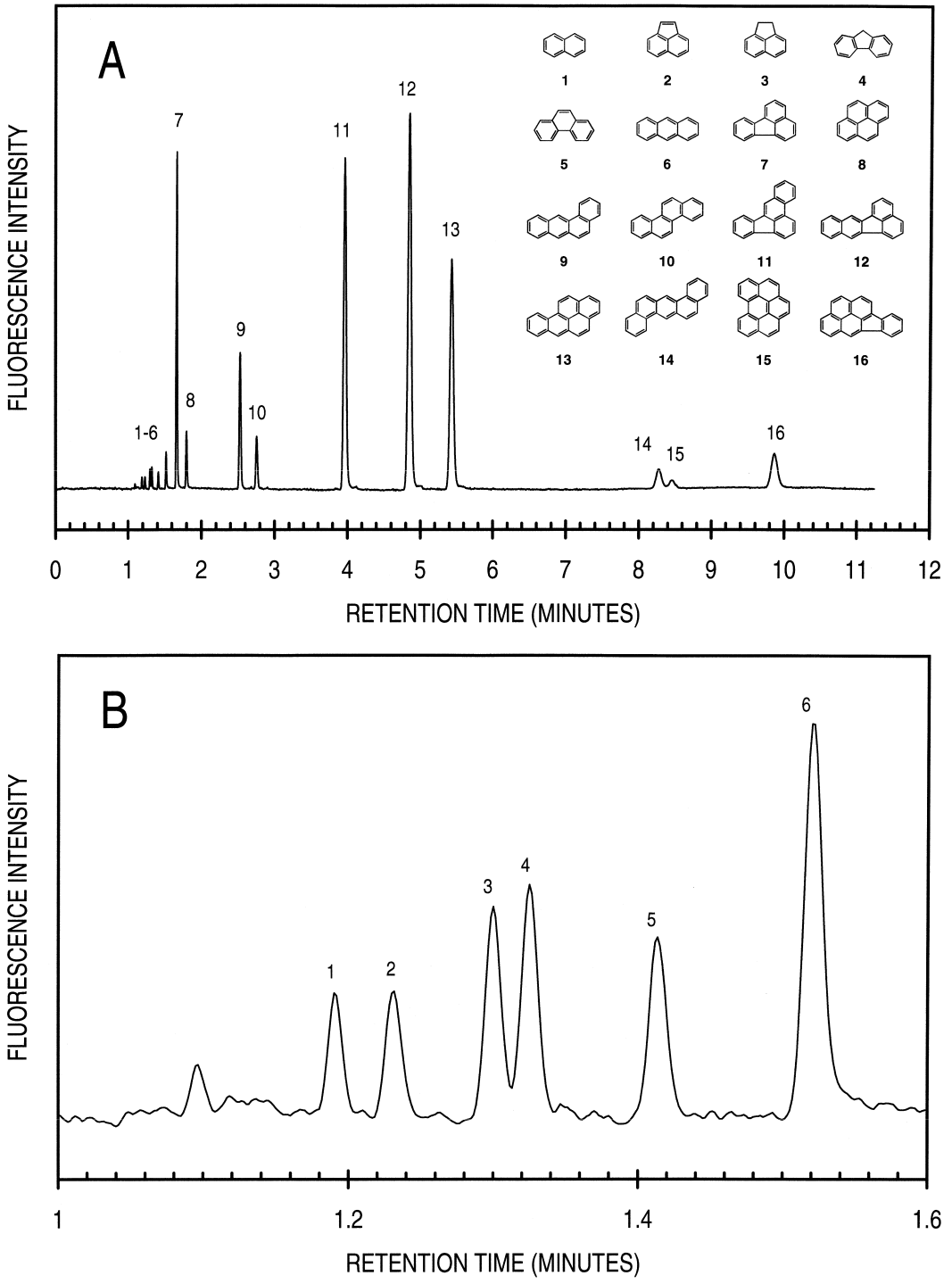

Fig. 3. CEC separation of 16 PAHs. Capillary, 30 (20) cm×100 μm; stationary phase, 1.5 μm nonporous octadecyl silica gel; mobile phase, 65% acetonitrile, 35% aqueous buffer (2 m*M* Tris), (A) full chromatogram; (B) expanded version of part A showing the peaks between 1.0 and 1.6 min (From Ref. [64] with permission).

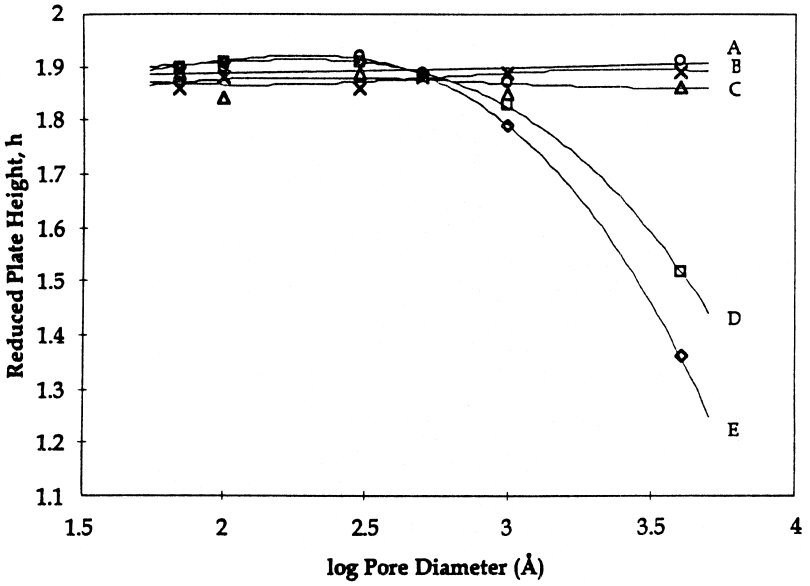

Fig. 4. Reduced plate height versus log (pore diameter) for various phosphate buffer concentrations; A, 1 m*M*; B, 5 m*M*; C, 10 m*M*; D, 30 m*M*; and E, 50 m*M*, (From Ref. [65] with permission).

The existence of pore flow was also assumed by Venema et al. [66] studying the possibilities of electrically driven size-exclusion chromatography (ED-SEC) with a capillary packed with wide-pore native silica gel and with dimethylformamide as mobile phase. In a later paper, the same authors [67] found that the intraparticle velocity with respect to the average interparticle velocity increased with increasing mean pore diameter of the packing and ionic strength of the mobile phase. They also observed that with increasing pore flow the plate heights obtained for a polymer standard decreased considerably.

Recently, Stol et al. [68] and Santalla-García et al. [69] report for columns, packed with octadecyl silica gel (mean particle diameter 7 μm) with mean pore diameter of 400 nm remarkably high efficiencies with mobile phases of moderate ionic strength. Up to 550 000 plates/m and reduced plate heights down to 0.26 were generated for retarded solutes [69]. It is demonstrated that in CEC, using columns with wide-pore stationary phases, the flow through the pores is substantial. The column is acting 'as if it is composed of several small particles' [68]. The use of reduced plate heights as a measure for column quality in CEC on wide-pore stationary phases can be questioned. The authors conclude that 'at high pore flow the separation efficiency is greatly increased due to faster mass transfer rate within the particles and higher flow homogeneity' [69].

From a practical point of view it should be emphasized that with wide-pore packing material of large particle size it is possible to obtain efficiencies that can be obtained with non-porous material only with particles of considerably smaller mean particle diameter. This is important, because the preparation of frits and the packing procedure are much easier with larger particles.

While the influence of the size, homogeneity, and porosity of the silica backbone on efficiency in CEC has been extensively investigated, not much is known about the influence of the alkylated surface. Zimina et al. [70] have measured the electroosmotic mobility with several octadecyl silica gels as stationary phase ($d_p = 5$ μm, composition of mobile phase kept constant) packed into capillaries of identical geometrical parameters. The electroosmotic mobility was largely dependent on the type of stationary phase. With two highly base-deactivated materials no electroosmotic flow was detected. However, there

was little correlation between the electroosmotic mobility and the silanol activity or carbon loading of the stationary phase.

Dittmann and Rozing [71] also determined the electroosmotic mobility with several octadecyl silica gels keeping the column dimensions and the composition of the mobile phase constant. They found that those stationary phases with a high surface concentration of silanol groups show high electroosmotic mobility, whereas phases with low surface concentration of silanol groups like 'base-deactivated' phases show a considerably lower electroosmotic mobility.

Adam et al. [72] determined the electroosmotic mobility dependence on the length of the alkyl group bonded to the silica gel and dependence on the coverage rate. They found that the electroosmotic mobility is independent of the length of the alkyl group and dependent on the molar coverage rate of the surface. Adam et al. concluded that the electroosmotic mobility is determined by the number of non-derivatized silanol groups per surface area.

4.4. Other stationary phases

One of the attractive features of CEC is the possibility to vary the selectivity of the chromatographic system by varying the stationary phase. Those stationary phases that have been used in the reversed-phase mode in HPLC, should be applicable in CEC provided that their surface properties permit the generation of a sufficiently high electroosmotic velocity.

Some workers have tested chiral stationary phases designed for HPLC in the CEC mode: α_1-acid glycoprotein silica gel [73], β-cyclodextrin silica gel [74], hydroxypropyl-β-cyclodextrin silica gel [75], vancomycin silica gel [76], or a *tert*.-butyl carbamoyl quinine based weak anion-exchanger [77]. Krause et al. [78] report the use of poly-*N*-acryloyl-L-phenylalanineethylester covalently attached to silica gel and of silica gel coated with cellulose tris(3,5-dimethylphenylcarbamate). While the feasibility of this approach was demonstrated, Krause et al. report that under the conditions of their study there was no gain in separation efficiency changing from pressure-driven to electrically-driven mode.

Excellent CEC chiral separations, however, were shown by Wolf et al. [79] using two brush-type chiral stationary phases, an (S)-naproxen-derived phase and (3R,4S)-Whelk-O, immobilized on 3 μm silica gel. Wolf et al. obtained efficiencies up to 200 000 plates/m.

Cahours et al. [80] employed capillaries packed with a 3 μm phenyl silica gel for the separation of benzodiazepines by CEC. The efficiency and the electroosmotic mobility reported are comparable to that reported for capillaries packed with 3 μm octadecyl silica gel. It can be expected, however, that selectivity is strongly dependent on the organic group bonded to the silica backbone. These first results support the assumption that in CEC the selectivity for neutral solutes can be varied by proper selection of the stationary phase in a similar fashion as known for HPLC.

4.5. Stationary phases specifically designed for capillary electrochromatography

In CEC the stationary phase does not only provide interaction sites for the solutes but also plays the dominant role in the generation of the electroosmotic flow, hence the propagation of the mobile phase through the chromatographic bed. Consequently, the design of stationary phases suited for CEC not only has to keep in mind the retentive properties of this material but also the electrokinetic properties. On the other hand, electrokinetic propulsion of the mobile phase does not require high inlet pressures. Therefore, it might be possible that material that was not attractive in HPLC, because it did not tolerate high pressure (like cellulose derivatives), can be successfully employed for high-efficiency separations in the CEC mode.

One important disadvantage of octadecyl silica gel, when used as stationary phases in CEC, is the dependence of the electroosmotic mobility on the pH of the mobile phase. There are applications, where mobile phases with low pH have to be used. In these cases the electroosmotic velocity at low pH of the mobile phase may be unfavorably low.

Assuming that with a strong cation-exchanger the electroosmotic velocity will be high even at low pH, Smith and Evans [81] tested the separation of basic

drugs by CEC with a strong cation-exchanger as stationary phase. The exchanger was made specifically for CEC, based on a specially prepared silica gel ($d_p = 3$ μm) onto which is bonded a propyl sulfonate group. In their experiments with the cation-exchanger, Smith and Evans found extremely high efficiencies (>8 000 000 plates/m). The formal reduced plate height would be 0.04. The data material presented proves the existence of a focusing effect that has neither been observed in chromatography nor in capillary electrophoresis. To-date, the mechanism of this focusing effect is still under investigation [82–84].

Recently, Ståhlberg [85] presented a theory for zone migration in capillary electrochromatography, taking into account that in case of charged solutes migration is determined by an electrophoretic and a chromatographic transport mechanism. He shows that in the general case, the mixing of chromatographic and electrophoretic transport mechanisms gives rise to strong non-linear effects. These affects may cause strong band broadening. However, there are conditions, where the form of the eluting peak is stabilized during the migration of the zone through the column by a combination of electrical field and adsorption effects. According to Ståhlberg, 'this effect may offer one possible explanation for the appearance of the extremely sharp peaks that have been observed for elute ions when a cation-exchanger was used as stationary phase'.

While the use of strong cation-exchangers demonstrates that the existence of sulfonic acid groups bound to the surface of the stationary phase significantly enhances the electroosmotic velocity at low pH, strong cation-exchangers have the disadvantage that they do not allow separation in the reversed-phase mode. In order to make it possible to perform reversed-phase CEC with relatively high electroosmotic velocity quasi-independent of the pH, Zhang and El Rassi [86] introduced a novel silica-based multilayered stationary phase. This stationary phase comprises a relatively hydrophilic and charged sub-layer covalently attached to the silica support and a retentative top layer of octadecyl groups chemically bound to the sub-layer. The charge in the sub-layer is ensured by the permanent ionization of sulfonic acid groups. The separation of ten standard purine and pyrimidine bases and their corresponding nucleosides was obtained with a capillary packed with this novel mixed-mode phase and with a mobile phase pH 4.5 containing 40% (v/v) acetonitrile [87]. The selectivity exhibited under these conditions was significantly different from that obtained with an octadecyl silica gel.

The synthesis and use of mixed-mode phases possessing sulfonic acid groups along with hydrophobic alkyl groups were also reported by Dittmann and Rozing [71], Smith and Evans [88] and Adam et al. [72]. It is important to note, however, that other workers demonstrated the possibility to perform rapid analysis in CEC with capillaries packed with commercial octadecyl silica gel and mobile phases buffered at pH 2.5 [89].

Huang et al. [90] have chosen another approach to perform CEC with mobile phases of low pH. They have used a mixed-mode phase that contains octadecyl and dialkylamino groups (reversed-phase/anion-exchange phase). The amino groups determine at low pH the charge density at the surface. In the range of pH 2–5 the electroosmotic mobility is independent of the pH and is reduced above pH 5.2. At low pH the direction of the electroosmotic flow is reversed with respect to the direction found with bare silica gel. Huang et al. have demonstrated that this phase is ideally suited for the separation of (positively charged) peptides, as the positively charged protonated secondary amino groups of the stationary phase do not act in this case as ion-exchange sites.

Yang and El Rassi [91] report the synthesis of an octadecyl silica gel with light surface coverage of alkyl groups. This stationary phase was designed to allow a relatively high electroosmotic velocity due to a high concentration of non-reacted silanol groups at the surface. Although 75% of the surface silanol groups remained intact, the produced stationary phase exhibited a reversed-phase chromatographic behavior in CEC with polar mobile phase.

Alicea-Maldonado and Colón [92] demonstrated the potential of a fluoropolymer as stationary phase in CEC. Maruška and Pyell [93,94] developed cellulose-based micro-beads as stationary phases for CEC. These phases have been employed in the normal-phase and in the reversed-phase mode. Lin et al. [95] packed capillaries with ground and sieved polymers produced by the molecular imprinting method and used these capillaries for CEC.

5. Fritless columns

Generally, the crux of packed capillaries is the neccessity to manufacture reproducibly inlet and outlet frits to retain the stationary phase. Properties of the frits may cause bubble formation (Section 2). Inhomogenities in the flow of the mobile phase through the inlet frit are discussed as being responsible for extra-column band broadening [16], and the mechanical properties of the frits may be the weak point of a separation capillary limiting its lifetime. Taking these negative points into regard, the production of fritless capillary columns for CEC is highly desirable and has been the object of very active research in the last few years.

5.1. Soft gels

Fritless capillaries for CEC have been prepared by the in-situ synthesis of highly swollen crosslinked hydrophobic hydrogels in fused-silica capillaries. Fujimoto et al. [96] realized continuous beds by radical copolymerization of *N*-isopropylacrylamide and 2-acrylamido-2-methylpropanesulfonic acid with *N,N′*-methylenebisacrylamide as cross-linking agent in capillaries pretreated with (γ-methacryloxypropyl)-trimethoxysilane (inner diameter: 50–75 μm). On the application of an electric field, an EOF is developed in the synthezised bed without forcing the gel out of the capillary. The sulfonic acid groups have been introduced to increase the charge density of the gel and consequently the electroosmotic velocity. With hydrogels containing hydrophobic groups the separation mechanism for non-charged solutes is predominantly reversed-phase chromatography. While the stability of these filled capillaries is reported to be satisfying, the chromatographic efficiency is relatively low (ca. 100 000 plates/m).

In a similar approach Liao et al. [97] introduced hydrophobic groups into the gel through the co-polymerization of stearyl methacrylate (or *n*-butyl methacrylate) with piperazine diacrylamide, methacrylamide, and vinylsulfonic acid. In order to create continuous beds with high chromatographic efficiency, Ericson et al. [98] developed a three-step procedure for the preparation of continuous polymer bed columns with high density of the chromatographically active hydrophobic group and a more rigid gel matrix. By adding poly(ethylene glycol) into the polyacryl amide matrix, Palm and Novotny [99] produced macroporous gels as stationary phases for CEC (Fig. 5). The efficiency of separation capillaries (inner diameter 100 μm) filled with the macroporous gel competes with that of packed capillaries (>300 000 plates/m for retarded neutral solutes). Chiral separations have been achieved in CEC with continuous beds via the preparation of monolithic molecularly imprinted flow-through polymers [100–103].

In capillary gel electrophoresis recent studies focus on the use of capillaries filled with replaceable linear non-cross-linked polymers instead of capillaries filled with an immobilized cross-linked polymer. If hydrophobic groups are introduced into the linear polymer matrix those media can be used to manipulate the selectivity in capillary electrophoresis due to hydrophobic interaction of the solutes with the polymer matrix [104]. Schure et al. [105] suggested the use of polyelectrolytes with grafted hydrophobic ligands as replaceable ‘pseudostationary’ phases for ‘high-performance capillary gel electrochromatography with replaceable media’. It is quite obvious that this technique can be considered as a hybrid of capillary electrochromatography (with an immobilized second phase, the stationary phase) and electrokinetic chromatography (with a dissolved ‘separation carrier’ added to the mobile phase).

5.2. Rigid gels

In contrast to soft gels, capillaries filled with a rigid monolith permit rapid mobile phase exchange, thus easy regeneration of the separation capillaries by hydrodynamic pumping. Rigid monoliths are not compressible, do not change their size on swelling, and do not require chemical anchoring to the walls of a capillary.

Peters et al. [106–108] presented a technique for the one-step preparation of ‘molded’ rigid polymer monoliths in non-pretreated fused-silica capillaries (inner diameter 100–150 μm). The porous polymeric material was prepared by co-polymerization of ethylene dimethacrylate, butyl methacrylate, and 2-acrylamido-2-methyl-1-propanesulfonic acid in the presence of a porogenic solvent. The composition of the porogenic solvent allowed the fine-tuning of the

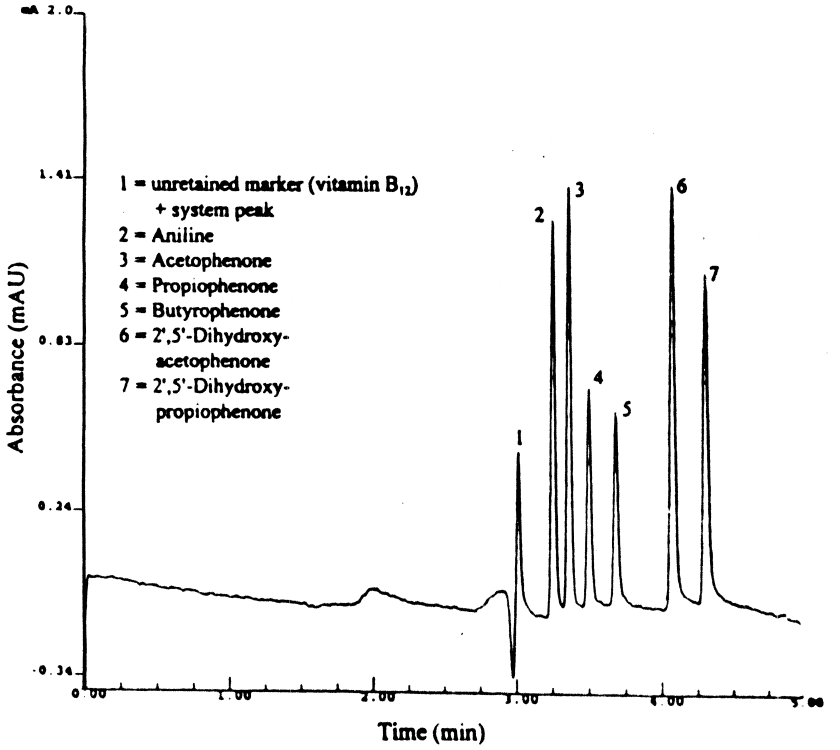

Fig. 5. CEC separation of alkyl phenones in a capillary filled with a macroporous polyacrylamide/poly(ethyleneglycol) matrix. Capillary, 25 (20.5) cm×100 μm; mobile phase, 20% acetonitrile, 80% aqueous buffer (10 m*M* Tris–15 m*M* boric acid, pH 8.2) (From Ref. [99] with permission).

porous properties of the resulting polymer over a broad range. The so-produced separation capillaries can be used over a very large pH range (2–12). These capillaries have been employed in the reversed-phase mode for the separation of neutral solutes (Fig. 6). The efficiency under optimized composition of the polymeric stationary phase is not much higher than in HPLC (120 000 plates/m). Peters et al. showed that this efficiency is achieved for capillaries of identical composition and porosity of the monolith, regardless of their length, that varied from 30 to 120 cm.

Minakuchi et al. [109] prepared octadecylated porous silica rods (surface-modified inorganic polymer) for HPLC by hydrolytic polymerization of tetramethoxysilane accompanied by phase separation in the presence of water-soluble organic polymers and subsequent silanization. The same approach was used for the preparation of continuous rods directly in fused-silica capillaries [110]. The possibility to use such capillaries in CEC was discussed. Fujimoto [111] reports the use of such a miniaturized silica rod column in CEC, however, with very limited efficiency.

5.3. Tapered capillaries

Tapered capillaries offer a viable alternative to frits in CEC with packed capillaries. External tapers used as capillary outlets provide high sensitivity in low-flow sheathless electrospray ionization mass spectroscopy [112]. A taper of ca. 10 μm inner diameter (internal or external taper) at the outlet end of a capillary obviates the need for an outlet end frit to retain 3 μm particles of the packing [112,113]. When using a taper instead of a frit upon packing, the particles arrange themselves in such a way that a porous plug is formed at the capillary outlet. Mayer et al. [114] report the production of fritless capillaries (tapered end at the inlet side and neither frit

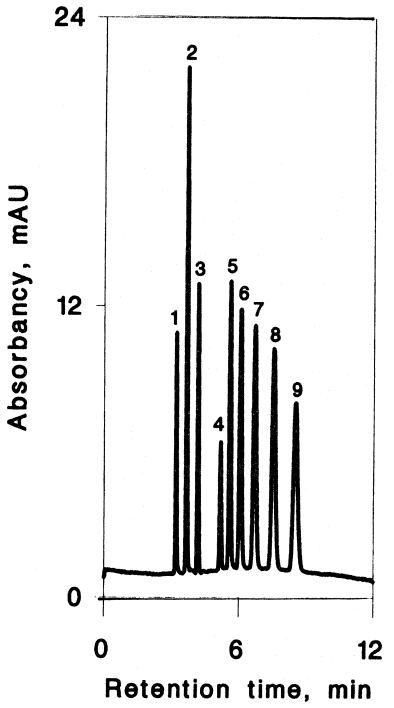

Fig. 6. CEC separation of benzene derivatives in a capillary filled with a monolith. Capillary, 30 cm×100 μm; mobile phase, 80% acetonitrile, 20% aqueous buffer (5 m*M* phosphate, pH 7); peak identification (1) thiourea, (2) benzyl alcohol, (3) benzaldehyde, (4) benzene, (5) toluene, (6) ethylbenzene, (7) propylbenzene, (8) butylbenzene, (9) amylbenzene (From Ref. [106] with permission).

nor taper at the outlet side), packed with 1.5 μm nonporous octadecyl silica gel. These capillaries permitted the separation of steroids with very high efficiency (>500 000 plates/m for retarded solutes). Also Mayer et al. report that the addition of an anionic surfactant to the mobile phase was necessary in case of reversed-phase nonporous packing material to obtain stable working conditions for CEC.

5.4. 'Glueing' of individual particles

Monoliths can be prepared by converting a conventionally packed column into one with monolithic structure. Asiaie et al. [115] succeeded in sintering packings of capillary columns. The effect of sintering is shown in Fig. 7. In the sintering process the compacted powder is converted into a monolith in which the particles are joined to each other by grain

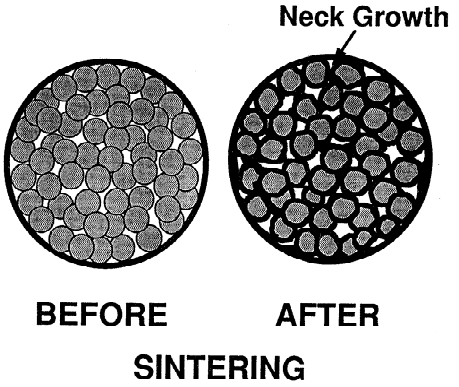

Fig. 7. Schematic illustration of the effect of sintering on the octadecylated silica packing in fused-silica capillaries (From Ref. [115] with permission).

boundaries. Asiaie et al. highlight that much better results were obtained with sintered octadecyl silica gel than with sintered beds of native silica gel. During sintering the organic layer is thermolyzed. After sintering the octadecylated surface has to be renewed by treatment with a solution containing dimethyloctadecylchlorosilane. The so-produced separation capillaries showed separation characteristics comparable to those of conventionally packed ones.

Another possibility for the stabilization of packings and thus eliminating the need for frits is the embedding of packed particles in a rigid sol–gel matrix. These monolithic beds containing embedded octadecyl silica gel particles can be prepared in a one-step procedure. Tetraethoxy silane [116] or a mixture of tetramethoxysilane and ethyltrimethoxysilane were used as sol–gel precursors [117]. Also the trapping of particles in a silicate matrix was used for the production of frittless packed capillaries [118].

6. Optimization of instrumental parameters

6.1. Column dimensions

Two important dimensions of the separation capillary are its total length and its inner diameter. Although there is no restriction of the length of the packed bed due to back-pressure as in pressure-

driven liquid chromatography, there is a practical limitation of the total length of the separation capillary. Approximating the chromatographic bed as an homogeneous electric field, the field strength inside corresponds to voltage applied at both ends divided through total length of the capillary. The high voltage sources used in most experimental set-ups permit a voltage up to 30 kV. If the mobile phase velocity at the minimum of the Van-Deemter-curve is known as well as the electroosmotic mobility for the packing wetted with a specific mobile phase, the maximum total length can be calculated, taking into account that the mobile phase velocity equals the electroosmotic mobility multiplied by the electric field strength.

The maximum inner diameter in CEC is controversially discussed. Capillaries used in CEC have usually an inner diameter of 100 μm. For comparison, in CE and MEKC capillaries have mostly inner diameters of 50–75 μm. Because of the high content of organic modifier and the low concentration of buffering salts in mobile phases employed for CEC, the electric current that is measured during a chromatographic run in CEC is one to two orders of magnitude lower than in capillary electroseparative techniques with open capillaries. From this point of view, it should be possible to work in CEC with capillaries of much larger inner diameter (than in CE or MEKC) without loss in chromatographic efficiency. The advantage of wider capillaries is: (1) the possibility to use larger detection volumes without inducing additional instrumental band broadening; and (2) an increase in the volume flow-rate making easier introduction of the eluate into a mass spectrometer without the need to use a supporting sheath flow. Larger detection volumes provide a gain in sensitivity with most detection techniques and thus a reduction of the detection limits for analytical methods using CEC.

Although the advantages of wider separation capillaries are very obvious, this topic has rarely been addressed in experimental studies. Yan et al. [119] and Vissers et al. [120] demonstrated the possibility to perform CEC with a capillary of 320 μm inner diameter packed with 5 μm octadecyl silica gel.

While the possibility to use packed capillaries with an inner diameter $\gg 100$ μm has been clearly demonstrated, very few data are available on the dependence (or independence) of the achievable chromatographic efficiency on the capillary inner diameter. Rebscher and Pyell [19] estimated the influence of the capillary inner diameter on the achievable chromatographic efficiency independent of the influence of the quality of the frit and other randomly distributed parameters by packing several capillaries (inner diameter 75, 100, and 150 μm) with 3 μm octadecyl silica gel according to the same protocol. The comparison of the plate heights determined did not exhibit significantly lower plate heights for the capillaries with I.D. = 75 μm than for the capillaries with I.D. = 150 μm. Similar results have been obtained by Steiner et al. [121], however, reporting a loss in efficiency of 50–60% when increasing the inner diameter from 100 to 180 μm.

Typically, columns used for CEC consist of a packed and an open section, each contributing to the electroosmotic flow. Studies performed with packed capillaries of varying fraction of the packed section (25–100% of the total length) showed that there is a dependence of the overall observable mobile phase velocity on the length of the non-packed fraction of the separation capillary [122].

With ‘duplex’ columns having a pre-detection open segment between the end of the packing and the detection window, the selectivity for charged solutes can be fine-tuned by varying the length of the packed segment [123]. This impact of the geometry of the separation capillary on separation selectivity is due to the fact that separation in the open section is exclusively obtained by electrophoresis, while in the packed section separation is obtained through chromatographic and electrophoretic effects.

Additionally, ‘duplex’ columns (short packed section) can be used for very rapid separations. Extremely short elution times have been obtained via ‘short-end injection’ with the detection window directly after the end of the short (7.5 cm) packed section [124]. Capillaries consisting of two segments (‘segmented capillaries’) each packed with a different packing material can be used for the manipulation of the electroosmotic flow [125]. In this case one segment is packed with the stationary phase for the chromatographic separation, the other segment is packed with native silica gel serving as the EOF accelerating segment.

6.2. Detection mode

Two types of capillaries are used in CEC. In most cases the capillary is composed of a segment containing the stationary phase and of another segment that is empty. In this case, detection is performed in the empty or open section of the capillary. This detection mode is commonly called on-column detection (OCD). The disadvantage of this approach is the appearance of a discontinuity at the interface between the filled and the open section. These discontinuities may result in intersegmental pressure differences changing the nature of the mobile phase flow [17] from purely electroosmotically driven to partly pressure driven (without an external pressure difference). For an extreme intersegmental pressure difference even bubble formation may result, especially in case of internal frits, that may have electrokinetic properties completely different from the bulk (packing) material.

A second type of capillary used in CEC is completely filled with the packing or a thin layer of stationary phase or a monolith. In this case, detection has to be performed in the chromatographic bed itself. This detection mode was referred to as in-column detection (ICD) [126]. In case of packed capillaries, the detection volume is filled with packing material. Typically, completely filled capillaries are more robust than partly filled capillaries, because of the absence of axial nonuniformities and discontinuities [16,86]. Some workers report higher achievable chromatographic efficiencies with ICD, compared to OCD [15,54,127], observing that the internal frit at the end of the chromatographic bed can be a source of substantial dispersion of analyte zones as they migrate through the frit. With a separation capillary packed with nonporous 1.5 μm octadecyl silica gel, Dadoo et al. [64] obtained extremely high efficiencies only with ICD.

Fluorimetric and photometric ICD has been reported for CEC. The presence of a second (solid) phase (aside from the mobile phase or eluate) in the detection window results in increased diffuse scattering of light at the irregular interfaces between the two phases. Also if the stationary phase does not absorb at the detection wavelength, in the detection volume along the optical path of the detector there is (nonspecific) intensity loss of the incident beam due to diffuse scattering. The extent of intensity loss is dependent on the difference in diffraction index between the two phases. In the ideal case (match of diffraction indices of stationary and mobile phase) the detection window is transparent, becoming opaque or even nontransparent in case of differences in the diffraction index.

Banholczer and Pyell [128] compared ICD with OCD photometric detection (UV detection) performed with fused-silica capillaries of 180 μm inner diameter, packed with 3 μm octadecyl silica gel. They determined the influence of the detection mode on linearity of the calibration function, precision and detection limit. In their experiments the baseline noise for ICD is about twice that for OCD. This negative impact on the detection limit is mitigated by a signal enhancement in case of ICD. This signal enhancement is due to enrichment of the solute in the stationary phase and can be quantitatively described with following equation:

$$S_I/S_O = (1 + k)\varphi_M \quad (6)$$

where S_I is the sensitivity for ICD; S_O the sensitivity for OCD detection; k the retention factor; and φ_M the volume fraction of mobile phase in the chromatographic bed.

The validity of this equation was shown experimentally [128]. In Fig. 8 chromatograms of the same sample obtained with ICD or OCD are shown. All other experimental conditions except the location of the detection window were kept constant. The visible change in peak height ratios is due to the dependence of the enhancement effect on the retention factor (Eq. (6)).

Eq. (6) was derived (in analogy to the considerations of Guthrie and Jorgenson [129]) for fluorescence ICD (packed capillary CEC) in order to explain effects observed when comparing results obtained for fluorescence detection in a packed section of completely packed capillaries to results obtained for fluorescence detection in the open section of partly packed capillaries [127]. In deriving Eq. (6) additional effects (although often observed) due to instrumental band broadening and due to variations of the fluorescence quantum yield (environmental effects) are neglected.

Taking Eq. (6) and the increase in baseline noise into account, the relative limit of detection (LOD) for a method employing ICD (in comparison to a method employing OCD) can be calculated as:

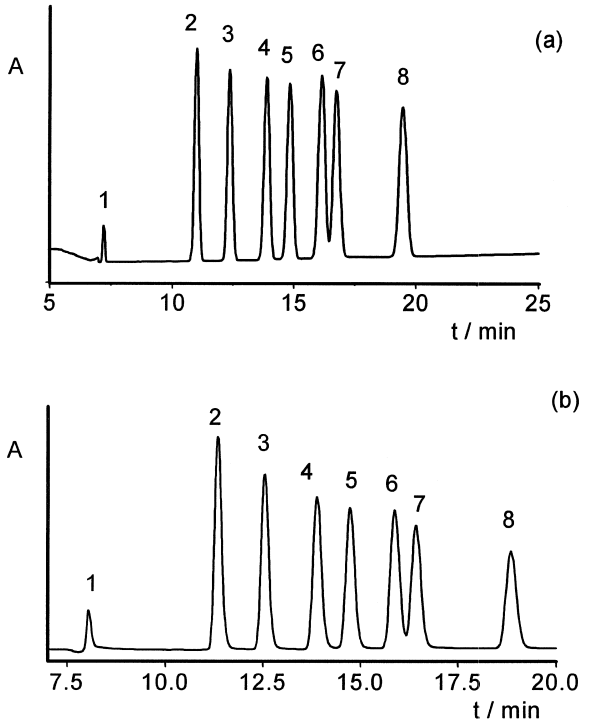

Fig. 8. Comparison of chromatograms obtained with (a) in-column and (b) on-column photometric detection ($\lambda = 230$ nm). Separation of alkyl benzoates (identical samples) (1) thiourea, (2) methyl benzoate, (3) ethyl benzoate, (4) phenyl benzoate, (5) benzyl benzoate, (6) *p*-tolyl benzoate, (7) butyl benzoate, (8) isopentyl benzoate. (a) Capillary, 32.5 (27.0) cm×180 μm I.D., (b) capillary, 40.0 (35.0) cm×180 μm I.D.; stationary phase, 3 μm porous octadecyl silica gel; mobile phase, 80% acetonitrile, 20% aqueous buffer (2 m*M* phosphate, pH 7.3) (From Ref. [128] with permission).

$$\mathrm{LOD\ (ICD)} = \frac{\mathrm{LOD(OCD)} \cdot F(\mathrm{noise})}{(1+k)\varphi_{\mathrm{M}}} \tag{7}$$

where F(noise) is the factor by which baseline noise in ICD is increased compared to baseline noise in OCD.

The validity of Eq. (7) has been verified for photometric detection [128]. From this equation follows that when $F(\mathrm{noise}) = 2$ and $\varphi_{\mathrm{M}} = 0.7$, the LOD with ICD is lower than with OCD provided $k > 2$. Consequently, there may be an improvement of the LOD for late eluted solutes when employing ICD instead of OCD. Improvement of detection limits for late eluted solutes by the use of packed flow cells was also reported for microcolumn liquid chromatography [130].

6.3. Sample injection

The extremely high efficiencies obtainable in CEC, primarily a micro(or nano)-column separation technique, can only be fully exploited in the absence of overloading and extra-column band broadening effects. It is obvious that avoidance of intolerable extra-column band broadening will become more difficult with increasing efficiency and increasing degree of miniaturization. In-column detection in combination with voltage switching techniques permits the experimental determination of extra-column band broadening effects in CEC [16].

One source of extra-column band broadening is the process of sample injection. Sample injection is in most cases done electrokinetically, i.e. by controlled application of a voltage ramp during a defined time period. The instrumental realization of this injection mode, making it possible to inject reproducibly few nanoliters of a sample, is relatively easy compared to injection devices developed for pressure-driven micro-LC. The selection of optimum injection parameters is important in CEC, in order to benefit from increase in sensitivity with larger injection volumes without intolerable loss in efficiency due to volume overload. In Fig. 9 the peak width at half height w_{h} for retarded solutes is given dependent on the length of the injected sample plug. At a low injected plug length w_{h} is independent from the injection parameters, while at high volume overload w_{h} is proportional to the length of the injected sample plug.

For CEC, Pyell et al. [131] investigated whether band broadening due to sample injection is within a tolerable range for standard sample injection procedures. They derived equations that permit the user of CEC to calculate optimum injection parameters:

$$U_{\max} = 0.7\,\frac{LL_{\mathrm{T}}(1+k_{\mathrm{S}})}{\mu_{\mathrm{eo}} t_{\mathrm{I}}\ \sqrt{N}} \tag{8}$$

$$t_{\max} = 0.7\,\frac{LL_{\mathrm{T}}(1+k_{\mathrm{S}})}{\mu_{\mathrm{eo}} U_{\mathrm{I}}\ \sqrt{N}} \tag{9}$$

where $U_{\max}$ is the maximum injection voltage; $t_{\max}$ the maximum injection time; L the length of the column to the detection window; L_{T} the total length; k_{S} the retention factor for the solute with the sample solvent as mobile phase; μ_{eo} the electroosmotic

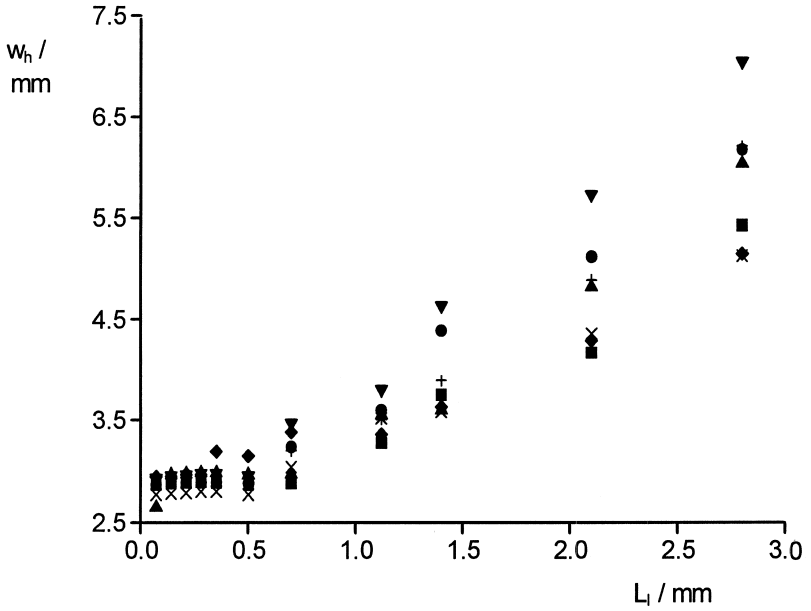

Fig. 9. Dependence of the peak width at half height on the injected plug length (electrokinetic injection) for various alkyl benzoates. Capillary, 31.0 (25.8) cm×180 μm; stationary phase, 1.5 μm porous octadecyl silica gel; mobile phase, 80% acetonitrile, 20% aqueous buffer (2 m*M* phosphate, pH 7.3); ■, methyl benzoate; ●, ethyl benzoate; ▲, benzyl benzoate; ▼, *p*-tolyl benzoate; ♦, isopentyl benzoate; +, phenyl benzoate; ×, butyl benzoate (From Ref. [131] with permission).

mobility; t_I the injection time; U_I the injection voltage; and N the number of theoretical plates determined in the absence of volume overload.

These equations operate with magnitudes easily accessible in CEC. Zone sharpening due to enrichment of solutes in the stationary phase in the first section of the column is taken into account (with the parameter k_S). The calculations show that with standard injection procedures ($t_I = 5$ s, $U_I = 5$ kV) in CEC with octadecyl silica gel as stationary phase in many cases the criterion for the tolerable extra-column band broadening (an increase in w_h by 5%) is not fulfilled in absence of focusing effects.

7. Gradient elution

To realize the full potential of CEC it is necessary to develop the capability of gradient elution. Gradient elution improves the peak capacity of a chromatographic system via zone compression. Gradient elution will be indispensable for analyzing very complex samples. The following gradients may be applied: gradient of the composition of the mobile phase, temperature gradient, voltage gradient.

Although with the small dimensions of a separation capillary in CEC temperature gradients (temperature between the melting and the boiling point of the mobile phase) can be realized, this approach has not been reported to the best of the author's knowledge. Temperature gradients permit a gradual decrease in the retention factors of the solutes and will have an effect analogous to that of gradients of the composition of the mobile phase with increasing elution strength.

Gradients of the composition of the mobile phase have mostly been realized with the help of a gradient delivering HPLC pump. In the early days of CEC, pressure-supported CEC (also called pseudo-CEC) was very attractive because of the ability of the high inlet pressure to suppress unwanted bubble formation. The combination of electroosmotically and pressure-induced mobile phase flow, however, will have a negative effect on the chromatographic efficiency. Behnke and Bayer [132] were the first to report pressurized gradient CEC. This approach was

used by many workers [133], in particular to build-up hyphenated systems (CEC–electrospray ionization–MS [134], CEC–electrospray ionization ion-trap–reflection time-of-flight–MS [135], CEC–NMR [136]).

Yan et al. [137] developed an experimental set-up for gradient CEC with electrokinetic generation of the gradient of the composition of the mobile phase. Two high-voltage power supplies are used to generate two electroosmotic flows in two channels (connected to two mobile phase reservoirs) that are merged in front of the column head. The voltage of the two high-voltage power supplies is controlled by a computer. The ratio of the electroosmotic flow-rate between the two channels delivering the mobile phase is gradually changed, thus generating a gradient of the composition at the mixing tee. The shortcomings of this approach are the need to disconnect the column from the mixing tee before each sample injection, and difficult control on the exact composition of the mobile phase as it enters the column. The inherent miniaturization potential of this gradient formation technique was used by Kutter et al. [38] for gradient CEC on a chip (Fig. 1).

In case of using commercial automated CE equipment for CEC, the realization of step-wise gradients of the composition can be performed easily and reproducibly by changing the (buffer) vial at the inlet side at a pre-defined time interval [138,139]. In case of photometric detection, the removal and re-application of voltage is associated with a strong spike, and the drastic change of composition of the mobile phase results in baseline disturbances, when this zone passes the detector. Consequently, this technique needs the placing of these disturbances between sufficiently base line separated peaks.

For the generation of linear gradients, most workers used a gradient-delivering pump. If a pressure-induced additional flow is to be avoided, the head of the separation capillary ‘merely dips into the stream of mobile phase passing by’. Sample is injected via a normal type or micro-injection valve [140–144]. Also automation via the use of an (HPLC) autosampler is reported [145–147].

Other workers used a miniaturized titration device for gradient CEC [34,148]. Also voltage programming is reported as alternative to mobile phase composition gradient programming [149]. It has to be emphasized, however, that with this technique no variation (during a chromatographic run) in the retention factor is affected. In addition, in many cases it is difficult to obtain a sufficiently high electroosmotic flow in CEC, so that for many separations the maximum voltage of the high voltage power supply has to be taken for the complete chromatographic run. Voltage programming as an alternative to mobile phase composition gradient programming might therefore require stronger high voltage power supplies than those usually employed.

8. Concluding remarks

In the last few years, column technology and instrumentation for CEC have made large steps forward. Extremely high efficiencies were obtained with nonporous octadecyl silica gel and wide-pore packing material. The disadvantages of frits make fritless capillaries desirable. Many alternatives to capillaries packed with the stationary phase have been developed, including gel-filled capillaries, open-tubular columns and monoliths.

While these factors affecting the separation have been under intensive investigation, factors improving the detection sensitivity seem to be somewhat neglected. The hyphenation of CEC with MS and NMR-spectroscopy was successfully realized. Most of the researchers employed photometric detection in spite of the unfavorably low detection volumes in CEC. In order to widen the scope of applications more sensitive and possibly more selective detection techniques are needed.

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