13
Capillary Electrochromatography

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13.1
Introduction

Capillary electrochromatography (CEC) is an emerging separation technique, which may eventually become the liquid-phase separation technique of the 21st century, as stated by Snyder [1], and has already received a mature status as described below. It is a powerful combination of high-performance liquid chromatography (HPLC) with the principles of capillary zone electrophoresis (CZE). It is conducted in capillary columns, across which an electric field is imposed, resulting in a movement of the mobile phase by electroosmotic flow (EOF) instead of a pressure-driven effect, which was first reported by Pretorius *et al.* [2]. CZE is a highly efficient separation technique when generating more than 500 000 plates per meter, with the disadvantage of not being able to separate neutral molecules. This was resolved by Terabe *et al.* [3] by using micellar mobile phases as stationary phases. Neutral molecules or ionic species easily dissolve in micelles made from ionic surfactants. An alternative to micellar electrokinetic chromatography (MEKC) is microemulsion EKC (MEEKC), first proposed by Berthold and De Carvalho [4]. The water-immiscible microemulsion droplets in nanometer-scale form stable pseudo-homogeneous translucent phases, which have a higher solubilization capacity and enlarged migration window compared with micellar phases [5]. Here the O/W emulsion is the usual type in comparison with inverted W/O emulsion MEEKC. Electrokinetic capillary chromatography (EKC) is a type of liquid–liquid partition chromatography, where a distribution is between an aqueous and a pseudo-phase (micelles, microemulsions, etc.). This is a distinct difference to reversed-phase micellar chromatography, where the distribution process is between three phases, which is a stationary-bounded, a water and a pseudo-phase.

CEC is predominantly used in the field of pharmacy and food science. The compounds can be divided into three main categories:

1. Lipophilic compounds without charges, where separation occurs due to different chromatographic affinities between the mobile and stationary phases.

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2. Lipophilic solutes with charged or ionizable groups (e.g. fatty acids, phenolic compounds). Their separation is due to solid–liquid distribution and different mobilities in the electric field. Additionally, external parameters such as pH and ionic strength of the eluent also contribute to the separation.
3. Hydrophilic substances possessing charges or ionizable groups rely on chromatographic affinity distribution and different movements in the electric field [6].

The first category comprise carotenoids, lipids and steroids, the second includes boswellic and fatty acids and vitamins and the last comprises flavones, alkaloids, cannabinoids, berberines and anthraquinones, to give just a few examples. Another method of classification is according to the separation mode, such as normal-phase, reversed-phase, ion-exchange, size-exclusion and affinity-based separations, likewise as in HPLC. In addition, it is worth mentioning that the instrumentation design incorporates pressurized CEC (PEC) and a microchip platform [7].

13.2 Theory

The advantage of EOF, compared with pressure-driven flow, is the lack of back-pressure in the column, which allows the use of smaller particles as with conventional HPLC, leading to higher efficiencies and number of theoretical plates. The EOF is the result of motion of a liquid induced by ionic species. The cause is an interfacial electric double layer at the solid–liquid interface, where counterions are strongly adsorbed in a monolayer at a charged surface, and give a linear decay of the surface potential, ψ_0. This rigid double layer (known as the Stern layer) is between the inner and outer Helmholtz layers, where this polarization phenomenon occurs (Figure 13.1)

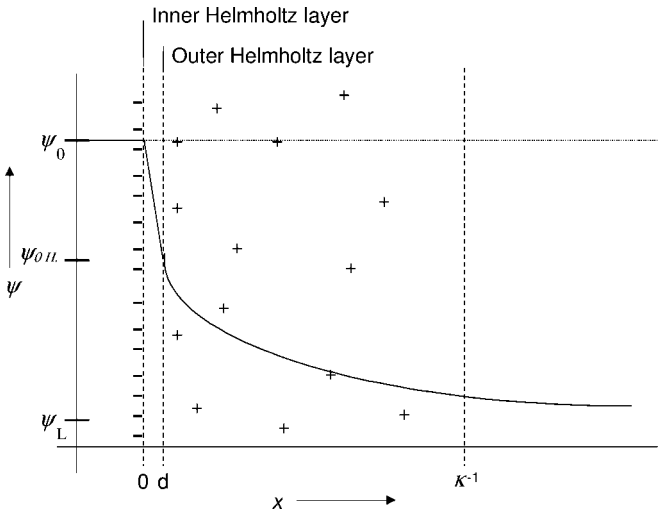

Figure 13.1 Schematic illustration of the electrostatic potential energy curve.

However, the surface potential is then further neutralized in the so-called "diffuse double layer". This can be described by the Gouy–Chapman statistical model, which accounts for the motion of ionic species according to their natural Brownian thermal mobility [8]. This model is only valid for highly diluted systems (<1 mM), since the molecular size of the ionic species is assumed to be zero. The Stern model is an extension for real ionic solutes, with the limiting case at the inner layer described by the Helmholtz model and the outer layer according to the Gouy–Chapman model. The Poisson equation gives the relation between potential ψ and charge density $\rho(x)$:

$$\Delta^2\psi(x) = -\frac{\rho(x)}{\varepsilon_0\varepsilon_r} \tag{13.1}$$

where ε_0 is the permittivity of vacuum and ε_r the dielectric constant. Solving this equation for the limiting case $x \to \infty$ gives the thickness of the diffusive double layer (Figure 13.1):

$$\kappa^{-1} = \sqrt{\left(\frac{\varepsilon_0\varepsilon_r kT}{2N_A e^2 I}\right)} \tag{13.2}$$

where k is the Boltzmann constant, T the absolute temperature, e the elementary charge, I the ionic strength and N_A Avogadro's number. As can be seen, an increasing ionic strength compresses this layer and decreases κ^{-1}.

The concentration of an ionic species at the interface, m_i^I, is related to the interfacial potential, ψ_0, by the Boltzmann equation:

$$m_i^I = m_i \exp\left(-\frac{ze\psi_0}{kT}\right) \tag{13.3}$$

The Poisson–Boltzmann equation then reads [8]

$$\frac{d^2\psi}{dx^2} = -\frac{1}{\varepsilon}\left[\sum_i z_i e c_i^0 \exp\left(\frac{-z_i e\psi}{kT}\right)\right] \tag{13.4}$$

where z_i is the ionic charge and c_i^0 its interfacial concentration. If the electric energy is small compared with the thermal energy, $|z_i e\psi| \ll kT$, the exponent in Equation (13.4) can be approximated with $e^{-x} = 1 - x$ to give

$$\frac{d^2\psi}{dx^2} = \frac{1}{\varepsilon}\left(\sum_i z_i e c_i^0 - \frac{\sum_i z_i^2 e^2 c_i^0 \psi}{kT}\right) \tag{13.5}$$

Because of electroneutrality in the bulk, the first term is zero, which gives a further simplification:

$$\frac{d^2\psi}{dx^2} = \kappa^2\psi \tag{13.6}$$

or, after integrating:

$$\psi = \psi_0 \exp(-\kappa x) \tag{13.7}$$

This result can be only applied to very dilute solutions of strong monovalent electrolytes, which is the basis of the Debye–Hückel theory. With concentrated solutions of higher valent ions, the linearization of the exponent function will not hold [9]. The exact formulation of the Poisson–Boltzmann equation for a symmetric $z{:}z$ electrolyte:

$$z_i = z_+ = -z_- = z \tag{13.8}$$

is

$$\tanh\left(\frac{ze\psi}{4kT}\right) = \tanh\left(\frac{ze\psi_0}{4kT}\right)\exp(-\kappa x) \tag{13.9}$$

For small ψ this equation and the approximation (13.7) are equal. However, this theory allows the prediction of interfacial concentrations with the knowledge of bulk concentrations, provided that the zeta potential is known. The exact position of the zeta potential is not known, but with a lack of anything better it is assumed to be at the outer Helmholtz layer at a distance d in Figure 13.1 and has to be determined experimentally.

However, when applying electric fields in such systems, the ionic species will respond to motion. The resulting effects are collectively called electrokinetic phenomena, giving rise to different cases, depending on the way in which motion is induced:

- *Electrophoresis* is the movement of a charged particle relative to a liquid under the influence of an electric field.
- *Electroosmosis* is the movement of a liquid relative to a stationary charged surface under an applied potential.
- *Streaming potential* is the field generated when a liquid is forced to move along a stationary charged surface.
- *Sedimentation potential* is the electric field observed when charged particles sediment.

The cause of electroosmotic flow is an electric double layer that forms at the stationary/solution interface. In capillary electrochromatography, the narrow channels are made up of silica and silanol groups form the inner surface of the capillary column. These silanol groups are ionized above pH 3. Hence the inner surface of the channel is negatively charged. In solutions containing ions, the cations will migrate to the negatively charge wall. This forms the electric double layer. When an electrical potential is applied to the column, with an anode at one end of the column and a cathode at the other, the cations will migrate towards the cathode. Since these cations are solvated and clustered at the walls of the channel, they drag the rest of the solution with them, even the anions [10]. A charged body, q, in an electric field, E, perceives a Coulomb force:

$$F_\mathrm{e} = qE \tag{13.10}$$

The resulting friction force is then

$$F_\mathrm{f} = uf \tag{13.11}$$

where f is the friction coefficient and u the velocity. The electroosmotic mobility, μ_e is then defined as

$$\mu_e = \frac{u}{E} = \frac{q}{f} \tag{13.12}$$

This is at infinite dilution in a non-conductive solvent. In reality, it depends on both particle properties (size, charge, etc.) and liquid properties (pH, ionic strength, etc.) and is approximated by the Smulochowski equation:

$$\mu_e = \frac{\varepsilon_r \varepsilon_0 \zeta}{\eta} \tag{13.13}$$

where η is the viscosity of the liquid and ζ the zeta potential. For a monovalent electrolyte and small potentials, we have [8]

$$\zeta = \frac{\sigma}{\varepsilon_0 \varepsilon_r \kappa} \tag{13.14}$$

where σ is the charge density of the surface of shear and κ is the Debye–Hückel parameter. The thickness of the double layer is

$$\delta = \kappa^{-1} = \sqrt{\frac{\varepsilon \varepsilon_r RT}{2cF^2}} \tag{13.15}$$

where R is the universal gas constant, F the Faraday constant and c the molar concentration. Combining Equations. (13.14) and (13.15) yields

$$u = \frac{\sigma}{\kappa \eta} E = \mu_e E \tag{13.16}$$

Consequently, a good eluent should generate a high ζ while processing a low electric conductivity to prevent excess Joule heat generation, which is an intrinsic conflict. However, EOF velocities of about 1–3 mm s^{-1} can easily be achieved and depend on capillary quality, pH value, co-solvents and other additives.

In comparison, in pressure-driven flow, the velocity in granular beds is according to the Kozeny–Carman equation [11]

$$u = 182 \frac{(1-\varepsilon_b)^2}{\varepsilon_b^3} \frac{d^2}{\eta L} \Delta p \tag{13.17}$$

where ε_b is the bed porosity, d the particle diameter, L the bed length with pressure drop Δp, η the viscosity of the liquid and 182 a factor for spherical particles [12].

On comparing Equation (13.16) with Equation (13.17), it is noticeable that the flow velocity in a pressure-driven system is proportional to the square of the particle diameter whereas it is independent under electroosmotic conditions. This is the reason why EOF allows very small diameter packing materials, and very efficient separations at high throughputs are possible, provided that the particle diameter $d > 20\sigma$, so that no double-layer overlap will occur [13].

Knox and Grant [14] calculated that with 1–10 mM electrolytes particles of about 0.5 µm could be used, generating up to 870 000 stages per meter without a significant loss in electroosmotic flow. It is important to note the linear relationship between

velocity and electrolyte concentration, since u is directly proportional to the zeta potential, which decreases with increasing electrolyte concentration.

When the operating constraints of conventional HPLC (maximum 400 bar) and CEC (30 kV) are taken into account, Equations (13.16) and (13.17) allow a comparison of efficiencies [15]. For a 3 µm packing material ($\varepsilon_b \approx 0.4$) in water ($\eta = 0.89$ mPa s, $\varepsilon = 80$), the zeta potential is assumed to be 40 mV. The plate number for an HETP calculation for a component with a retention factor $k' = 5$ was obtained for HPLC after 25 min in a 30 cm long column as 50 000. In CEC, an analysis time of 25 min could be obtained in a 50 cm long column, which then gives 150 000 plates. However, longer analysis times or use of smaller packing materials gives an even more pronounced benefit for CEC.

However, a combination of EOF and pressure-driven flow suppresses bubble formation at high voltage and permits unique control of the selectivity of ionic solutes [16]. The independent adjustment of pressure and applied voltage allows the setting of optimum selectivity and retention factors. Additionally, a medium pressure of about 6 bar can increase linear velocities considerably (up to 2.4 cm s^{-1}), which is a 10-fold increase compared with a pressureless separation.

A major disadvantage is the self-heating during CEC operation, which limits the inner capillary diameter to less than 0.32 mm, otherwise bubble formation will occur. The heat generated in a packed bed is

$$Q = E^2 \lambda c \varepsilon_b \tag{13.18}$$

where λ is the molar conductivity and c the electrolyte concentration. The maximum temperature in the center of the capillary is then [14]

$$\Delta T = Q d_c^2 / 16k \tag{13.19}$$

where d_c is the capillary diameter and k the thermal conductivity of the electrolyte. As a result, the heat production with CEC is about 1500 times higher than with HPLC, with the additional problem that having organic solvents present will result in a lowering of the effective boiling point of the eluent. This is why pressures up to 50 bar are applied in CEC, when operating at high voltages using high organic or high buffer concentrations [17].

The flow characteristic of EOF in an empty tube is about plug flow and only a small laminar shear layer exists, the thickness of which of several micrometers depends on the conductance of the mobile phase (Figure 13.2). This is in contrast to pressure-driven flow with a parabolic flow profile (Hagen–Poiseuille-flow), which increases resistance to mass transfer in the mobile phase and therefore also the plate height. However, with stationary phases in CEC band broadening will occur, which is smaller but from a similar cause as in conventional liquid chromatography. Therefore, Knox and Grant [14] adapted the Van Deemter plate height equation:

$$H = A u^{\frac{1}{3}} + B/u + Cu \tag{13.20}$$

taking into account u^n ($0.25 < n < 0.35$) in the A-term, and with $n = 1/3$ is known as the Knox equation. The A-term describes eddy diffusion and dispersion effects, B the

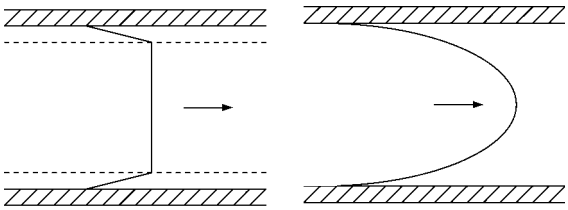

Figure 13.2 Comparison of EOF (a) and pressure-driven flow (b).

molecular diffusion and C the mass transfer resistance, which is usually dominant in HPLC applications. However, according to Grant [18], the HETP with small 0.5 µm particles adds about 0.5 µm for the A-term, 0.025 µm for the C-term and 1.475 µm for the molecular diffusion. From this, with particles smaller than 1 µm, the molecular diffusion term is the dominant contribution factor for band broadening, resulting in the well-known plate height equation used in CZE:

$$H = 2D/u \tag{13.21}$$

Nowadays, non-invasive tomographic methods, such as NMR spectroscopy, are used to reveal hydrodynamics [19] and temperature profiles [20] in CEC capillaries. The EOF in CEC is in the microliter or nanoliter per second range and can be measured by weighing the mass of eluent transferred, by determining the zeta potential or the current under different EOF conditions and evaluating the residence time of neutral markers (e.g. alcohols). Otherwise, miniaturized flow sensors comprising Prandtl tubes, piezoelectric elements, etc., can be used [21].

13.3 Stationary Phases

There are three principal modes of CEC possible, depending on the column format, which may be open-tubular (o-CEC), packed or monolithic. The o-CEC columns can be easily prepared and there is a variety of surface immobilization chemistry. Their advance is in their absence of bubble formation, arising with packed columns at the frit, thus affecting the column performance [22]. In order to enhance the unfavorable wall-to-volume ratio in o-CEC, narrow capillaries (<50 µm i.d.) are used. This is advantageous in respect of peak efficiencies and HETP but reduces detection pathlengths and resolution according to the Lambert–Beer law. In comparison with a packed column, the loadability of the o-CEC is very low and lower retention factors are the consequence. Over recent years, much effort has been devoted to the development of monolithic stationary phases. Their porosity and functionality can be tailored to any specific separation problem. Additionally, the column design is frit free and there is no problem with inhomogeneous packing as reported with conventional packed-bed CEC.

13.3.1 o-CEC Phases

The o-CEC technique was first reported by Tsuda *et al.* [23] and the stationary phase is either adsorbed/attached or chemically bonded to the capillary wall. In order to increase the surface area, the wall may be etched [24], which gives a 1000-fold higher area to be covered. The inner wall of the silica capillary is negatively charged, which causes problems when processing proteins, peptides or basic solutes, resulting in peak tailing, an unstable baseline and non-reproducibility [25]. Applications of weakly attached or strongly adsorbed stationary phases [26–31] or permanent coatings (covalently bonded/cross-linked polymers) [32–35] to shield the negatively charged silanol groups on the capillary wall have demonstrated that the protein–wall interaction can be effectively reduced. Recently, sol–gel chemistry for o-CEC columns has been used to obtain a thin stationary layer (thickness about 1 μm) with appropriate fine-tuned ligand chemistry matching the separation problem [32, 36]. Alternatively, functionalized polymeric porous layers grafted to the inner capillary wall are also in use [37].

The use of bilayer coatings was reported from Kapnissi *et al.* [31], where a permanently adsorbed coating of a cationic polymer salt [poly(diallymethylammonium chloride)] was covered with a dynamically adsorbed polymeric surfactant [poly (sodium undecylenic sulfate)]. In contrast to the stable coatings, the adsorbed layers can be easily prepared. Traditionally, polymeric surfactants have been used in MEKC [38] and the separation principle can therefore be transferred to o-CEC. However, several other types of dynamically attached pseudo-stationary phases (PSPs) exist, such as cyclodextrins [39], dendrimers [40], proteins [41], liposomes [42], ionenes [43], siloxanes [44] micelles [3, 38] and microemulsions [45]. Comparisons between MEEKC and MEKC are often made, as their separation basis is similar [46–48]. In MEKC, surfactant molecules form micelles and solutes dissolve in them, which facilitates separation. Solutes can penetrate a microemulsion droplet more easily than a more rigid micelle and the loadability of a droplet compared with a micelle is much higher.

However, an alternative to using surfactant systems is to use nanoparticle-based PSPs directly. They are more compatible with mass spectrometric (MS) detection and do not hamper electrospray ionization (ESI) [49]. In that respect, nanoparticles from silica [50], gold [51] and polymers [52] and even molecularly imprinted nanoparticles [53] have been used. Imprinting is based on a technique for tailor-making network polymers, where templates for a specific solute give a high separation affinity.

13.3.2 Granular Packed Columns

The traditional operating mode of CEC with a conventional packed column is the use of commercially available chromatographic resins, as used for HPLC or μHPLC. Examples are RP C_{18}-modified silica particles of typical diameter 3–5 μm, ion-exchange resins and stationary phases for chiral separations. The latter include

selectors such as proteins and polysaccharides, cyclodextrins, macrocyclic antibiotics, chiral crown ethers, small donor–acceptor (Pirkle-type) selectors, chiral ion exchangers and ligand-exchange selectors [54]. In that respect, the use of dual selector systems to enhance selectivity is noteworthy [55]. However, as in o-CEC, one can make use of chiral stationary phases or of chiral mobile phase additives to achieve separation. As enantiomeric separations have steadily increased over recent years, relevant reviews can be found in the literature [56–60].

As already mentioned, packed columns have the disadvantage of bubble formation, especially at the outlet frits [61], hence fritless design is a challenge and becoming state-of-the-art in CEC [62, 63]. Completely fritless design is possible when relying on the keystone effect [64]. This makes it possible to retain, e.g., spherical particles 40 μm in diameter at a tapered opening of the capillary, with an i.d. of 65 μm of the orifice. A fritless setup is a prerequisite for CEC with, e.g., non-silica-based particles or chiral stationary phases, and is promising for coupling with high-resolution detection methods, such as is NMR spectroscopy or MS [65].

In order to improve robustness and suppress bubble formation [23], pressurized flow generated by an LC pump was introduced as pressurized flow electrochromatography (PEC). Additionally it has a benefit with respect to retention time, since EOF is now superimposed by pressurized flow. Applied voltage and pressure are two tunable parameters for the adjustment of selectivity in PEC [18, 66]. Most important, it is amendable for the gradient elution technique similarly to conventional HPLC, hence with PEC the promises of CEC can be fully exploited [67].

13.3.3 Monolithic Phases

Rapid developments in genomics and proteomics have pushed the introduction of capillaries, filled with monoliths. Generally, they provide higher performance than conventional particle-packed columns in HPLC and can be easily prepared by *in situ* polymerization [68, 69]. Their advantage is that the packing of columns can be avoided and homogeneous, fritless beds with low pressure drops are available. The simplicity of their preparation, and also good control over their porous properties and surface chemistry, promote research and application [70]. Additionally, the capillary diameter are similar to stationary granular phases, and monoliths have a higher surface area compared with o-CEC, making the capacity and detection sensitivity fairly high.

Monolithic CEC columns are formed from both organic polymers and silica and the first application involved a swollen hydrophilic polyacrylamide gel, similar to that used in capillary gel electrophoresis [71]. Polymeric monoliths based on acrylamide, methacrylate and styrene, etc., are prepared in a mold by thermally or UV-initiated polymerization of the monomers, and new developments regarding their preparation have been reported in recent years [25].

The first application of silica-based monoliths for HPLC was reported by Tanaka's group [72]. Polymer-based monoliths usually possess micropores, resulting in a decrease in efficiency for small molecules. The major advance with silica-based

monoliths is an independent design of macro- and mesopores and micropores can be leached with alkali (pH > 8) [73, 74]. The intrinsic problem with all silica-based stationary phases for CEC is the controversial effect of the ligands attached. On the one hand, they allow the chromatographic separation and, on the other, they decrease the number of ionizable groups and reduce the EOF, which could be circumvented by a modified functionalization procedure [70, 75]. Generally, silica-based monoliths exhibit better mechanical stability and separation efficiency than organic monoliths. A homogeneous bed design is more tricky when trying to achieve good adhesion on the capillary wall and trying to avoid cracks due to shrinkage in the manufacturing procedure. However, silica is not inert at high or low pH and is sensitive to high temperatures. Alternatively, inorganic materials, including metal oxides such as ZrO_2, TiO_2 and HfO_2, have attracted attention because of their chemical and thermal stability [25]. Hybrid organic–inorganic monoliths, prepared by the sol–gel technique, which exhibit higher stability than a silica sol–gel monolith and have high enantioselectivities, such as molecularly imprinted stationary phases, are also of interest [76].

13.4 Chip Electrochromatography

CEC is usually performed in silica capillaries under pure EOF or mixed conditions, when additional pressure is applied. The development of microanalytical systems, also called "lab-on-a-chip", has witnessed explosive growth in recent years [77, 78]. However, highly selections stationary phases are required to compensate for the loss of resolution due to the short column length and small plate numbers. With plate heights of about 1 μm [79], an additional problem is the need for high-resolution detection techniques, such as laser-induced fluorescence (LIF) and ESI-MS [80]. The most commonly used chip configuration comprised straight channels with simple cross, T or double T injection. Figure 13.3 shows a design with two solvents, where the mixing ratio can be kept constant (isocratic conditions) or be varied with time (gradient conditions). On-chip mixing is a great advantage since with conventional CEC gradient elution is a complex issue [81].

One of the earliest examples of a micro total analysis system (μTAS) was on an etched glass chip [83]. The use of glass chips requires a cover-plate, typically attached by high- or low-temperature bonding. In order to avoid this high-temperature treatment, the use of polymer-based substrates, such as polydimethylsiloxane (PDMS), opened up new avenues for chip production [84]. Like glass, it is transparent in the visible light range, so LIF detection is applicable. However, the most commonly used materials are still glass and quartz, which allow the construction of very complex channel structures [85].

The stationary phases are likewise conventional CEC open-channel (o-μCEC), packed-channel (p-μCEC) and monolithic (m-μCEC) types. In o-μCEC, a C_{18} or C_8 reversed-phase coating is most popular [86]. An alternative is to incorporate selectors during PDMS polymerization to achieve appropriate surface modification [87]. The p-o-μCEC has some difficulties with homogeneous filling, and frits, weirs,

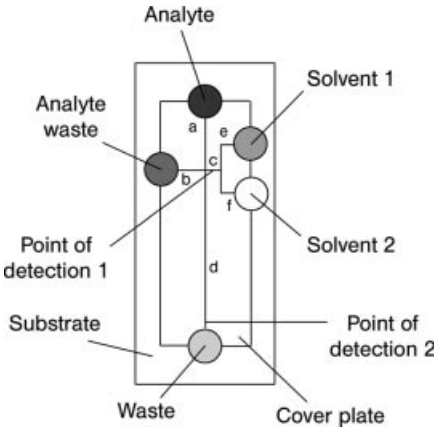

Figure 13.3 Schematic of a chip with channels and reservoirs shown. The channel dimensions are (a) 12.3, (b) 6.0, (c) 3.2, (d) 36.3, (e) 11.4 and (f) 11.1 mm. The effective length of the main channel from the cross to "point of detection 2" is approximately 25 mm. The channel depth is 9 µm and its width at half-depth is 50 µm. Reprinted from [82] with permission.

membranes or a fritless design ("keystone effect") [88] is used to entrap the beads (1–5 µm diameter). The monolithic separation phases are prepared *in situ* by either chemical initialization [89] or photopolymerization [90]. The need for positively charged monoliths when separating proteins and peptides was reported using suitable surface modification using ethylbutylamines [91]. Microfabrication to give

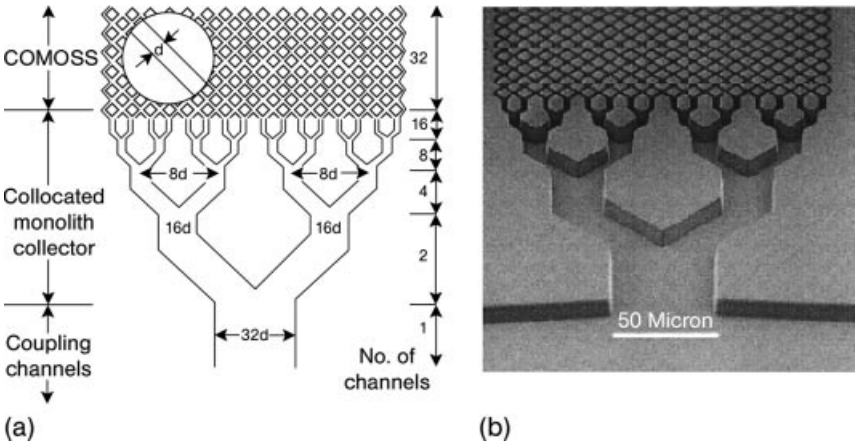

Figure 13.4 Configuration if the inlet splitter: (a) design layout reflecting the 2^n architecture of the splitter with constant cross-sectional area and (b) an SEM image showing the microfabricated inlet splitter. The similarity between these two images demonstrates the fidelity with which a computer model may be microfabricated. Reprinted from [85] with permission.

an array of C_{18}-modified collocated monolith support structures (COMOSS) was first introduced by Regnier and co-workers in the 1990s [85]. As can be seen from Figure 13.4, the channel dimensions are independent of any packing process and their width and length can be varied independently, maintaining extremely good uniformity. A diamond-like geometry was found to be the optimum shape [92] to achieve a plate height of 1.6 μm.

13.5 Conclusions and Perspectives

Capillary electrochromatography has experienced rapid progress during the last decade, expanding from 17 publications in 1994 to 191 in 2007. This has also led to several books and reviews [93–104] and analytical instrumentation is readily commercially available [105]. The developments in CEC include research on optimum stationary phases (polymer or silica based, adsorbed or imprinted, etc.), mobile phases (aqueous electrolytes with/without admixture of organic solvents or pseudophases) and apparatus design (open-tubular, packed or monolithic capillaries) up to lab-on-a-chip devices for μTAS [107].

CEC is a high-resolution technique and has found applications in biochemical analysis (amino acids/amines, peptides, proteins, nucleosides/nucleotides, carbohydrates, etc.), pharmaceuticals (acidic/basic drugs, vitamins/food components, etc.) and in the industrial and environmental field [inorganic anions/cations, synthetic polymers, (poly)aromatics, pesticides/insecticides/herbicides, etc.]. Affinity-based separation methods including kinetic affinity methods, biospecific interactions, immunoaffinity and chiral and molecularly imprinted recognition and are topics of special monographs [106–108]. Modern microfabrication techniques for lab-on-a-chip applications and the use of microfluidic devices for μ-CEC separation are still in the state of growth with high potential. Although first commercial instruments for DNA and RNA analysis with o-μ-CEC are available, the problems encountered with sample preparation, analytics and proper integration of control and automation promotes further research and development.

References

1 L. R. Snyder, HPLC: past and present, *Anal. Chem.*, **2000**, *72*, 412–420.

2 V. Pretorius, B. Hopkins, J. D. Schieke, Electroosmosis. New concept for high speed liquid chromatography, *J. Chromatogr.*, **1974**, *99*, 23–30.

3 S. Terabe, K. Otsuka, K. Ichikawa, A. Tsuchiya, T. Anolo, Electrokinetic separations with micellar solutions and open-tubular capillaries, *Anal. Chem.*, **1984**, *56*, 113–116.

4 A. Berthold, M. De Carvalho, Oil in water microemulsions as mobile phases in liquid chromatography, *Anal. Chem.*, **1992**, *64*, 2267–2272.

5 A. Marsh, B. J. Clark, K. D. Altria, A review of the background operating parameters and applications of microemulsion liquid

chromatography (MELC). *J. Sep. Sci.*, **2005**, *28*, 2023–2032.
6 H. Scherz, C. W. Huck, G. K. Bonn, CEC and EKC of natural compounds, *Electrophoresis*, **2007**, *28*, 1645–1657.
7 I. Miksik, and P. Sedlakova, Capillary electrochromatography of proteins and peptides. *J. Sep. Sci.*, **2007**, *30*, 1686–1703.
8 R. J. Hunter, *Zeta Potential in Colloid Science*, Oxford University Press, Oxford, **1981**.
9 M. J. Schwuger, *Lehrbuch der Grenzflächenchemie*, Georg Thieme, Stuttgart, **1996**.
10 http://en.wikipedia.org, **2007**.
11 P. C. Carman, Fluid flow through granular beds, *Trans. IChemE*, **1997**, *75*, 32–48.
12 F. Durst, R. Haas, W. Interthel, The nature of flows through porous media. *J. Non-Newtonian Fluid Mech.*, **1987**, *22*, 169–189.
13 N. Smith,Capillary electrochromatography, www.CE and CEC.com, **1999**.
14 J. H. Knox, I. H. Grant, Miniaturization in pressure and electroendosmotically driven liquid chromatography: some theoretical considerations. *Chromatographia*, **1987**, *24*, 135–143.
15 M. M. Dittmann, G. P. Rozing, *Theory and Practice of Capillary Electrochromatography*, CRC Press, Cleveland, OH, **1997**, Vol. 5, pp. 139–152.
16 M. Ye, H. Zou, R. Wu, H. Fu, Z. Li, Modeling and optimization of separation of ionic solutes in pressurized flow capillary electrochromatography. *J. Sep. Sci.*, **2002**, *25*, 416–426.
17 F. Steiner, B. Scherer, Instrumentation for capillary electrochromatography. *J. Chromatogr. A*, **2000**, *887*, 55–83.
18 I. H. Grant, Capillary electrochromatography. *Methods Mol. Biol.*, **1996**, *52*, 197–209.
19 U. Tallarek, E. Rapp, H. van As, E. Bayer, Using NMR displacement imaging to characterize electroosmotic flow in porous media. *Magn. Reson. Imaging*, **2001**, *19*, 453–456.
20 M. E. Lacey, A. G. Webb, J. V. Sweedler, On-line temperature monitoring in a capillary electrochromatography frit using microcoil NMR. *Anal. Chem.*, **2002**, *74*, 4583–4587.
21 A. Vegari, A. Guttmann, Theoretical and nomenclatural considerations of capillary electrochromatography with monolithic stationary phases. *Electrophoresis*, **2006**, *27*, 716–725.
22 A. Malik, Advances in sol–gel based columns for capillary electro-chromatography: sol–gel open-tubular columns. *Electrophoresis*, **2002**, *23*, 3973–3992.
23 T. Tsuda, K. Nomura, G. Nakagawa, Open-tubular microcapillary liquid chromatography with electro-osmosis flow using a UV detector. *J. Chromatogr. A*, **1982**, *248*, 241–247.
24 Z. Liu, K. Otsuka, S. Terabe, Evaluation of extended light path capillary and etched capillary for use in open tubular capillary electrochromatography. *J. Chromatogr. A*, **2002**, *961*, 285–291.
25 J. Ou, J. Dong, X. Cong, Z. Yu, M. Ye, H. Zou, Recent progress in polar stationary phases for CEC. *Electrophoresis*, **2007**, *28*, 148–163.
26 J. E. Melanson, N. E. Baryla, C. A. Lucy, Double-chained surfactants for semipermanent wall coatings in capillary electrophoresis. *Anal. Chem.*, **2000**, *72*, 4110–4114.
27 C. Wang, C. A. Lucy, Mixed cationic/anionic surfactants for semipermanent wall coatings in capillary electrophoresis. *Electrophoresis*, **2004**, *25*, 825–832.
28 F. B. Erim, A. Cifuentes, H. Poppe, J. C. Kraak, Performance of a physically adsorbed high-molecular-mass polyethyleneimine layer as coating for the separation of basic proteins and peptides by capillary electrophoresis. *J. Chromatogr. A*, **1995**, *708*, 356–361.
29 H. Katayama, Y. Ishihama, N. Asakawa, Stable cationic capillary coating with successive multiple ionic polymer layers

for capillary electrophoresis. *Anal. Chem.*, **1998**, *70*, 5272–5277.

30 S. Ullsten, L. Soederberg, S. Folestad, K. E. Markides, Quaternary ammonium substituted agarose as surface coating for capillary electrophoresis. *Analyst*, **2004**, *129*, 410–415.

31 C. P. Kapnissi, C. Akbay, J. B. Schenoff, I. M. Warner, Analytical separations using molecular micelles in open-tubular capillary electrochromatography. *Anal. Chem.*, **2002**, *74*, 2328–2335.

32 R. Freitag, S. Constantin, Investigation of factors in fluencing the performance of open-tubular stationary phases in capillary electrochromatography. *J. Sep. Sci.*, **2000**, *23*, 835–843.

33 D. Belder, A. Deege, H. Husmann, F. Kohler, M. Ludwig, Crosslinked poly(vinyl alcohol) as permanent hydrophilic column coating for capillary electrophoresis. *Electrophoresis*, **2001**, *22*, 3813–3818.

34 H. Wan, M. Ohman, L.G. Blomberg, onded dimethylacrylamide as a permanent coating for capillary electrophoresis. *J. Chromatogr. A*, **2001**, *924*, 59–70.

35 G. Kleindienst, C. G. Huber, D. T. Gjerde, L. Yengoyan, G. K. Bonn, Capillary electrophoresis of peptides and proteins in fused-silica capillaries coated with derivatized polystyrene nanoparticles. *Electrophoresis*, **1998**, *19*, 262–269.

36 Y. Zhao, R. Zhao, D. Shangguan, G. Liu, A new type of capillary column for open-tubular electrochromatography. *Electrophoresis*, **2002**, *23*, 2990–2995.

37 X. Huang, J. Zhang, C. Horvath, Capillary electrochromatography of proteins and peptides with porous-layer open-tubular columns. *J. Chromatogr. A*, **1999**, *858*, 91–101.

38 C. P. Palmer, S. Terabe, micelle polymers as pseudostationary phases in MEKC: chromatographic performance and chemical selectivity. *Anal. Chem.*, **1997**, *69*, 1852–1860.

39 S. Fanali, Separation of optical isomers by capillary zone electrophoresis based on host–guest complexation with cyclodextrins. *J. Chromatogr.*, **1989**, *474*, 441–446.

40 N. Tanaka, T. Tanigawa, K. Hosoya, K. Kimata, T. Araki, S. Terabe, Starburst dendrimers as carriers in electrokinetic chromatography. *Chem. Lett.*, **1992**, *6*, 959–962.

41 L. Valtcheva, J. Mohammad, G. Pettersson, S. Hjerten, Chiral separation of β-blockers by high-performance capillary electrophoresis based on non-immobilized cellulase as enantioselective protein. *J. Chromatogr.*, **1993**, *638*, 263–268.

42 M. Bonoli, S. J. O. Varjo, S. K. Wiedmer, M.-L. Riekkola, Cationic liquid vesicles as coating precursors in capillary electrochromatography: separation of basic proteins and neutral steroids. *J. Chromatogr. A*, **2006**, *1119*, 163–169.

43 K. Kopecka, E. Tesarova, A. Pirogov, B. Gas, Ionenes acting as pseudostationary phases in capillary electrokinetic chromatography. *J. Sep. Sci.*, **2002**, *25*, 1027–1034.

44 T. Chen, C. P. Palmer, Evaluation of polymers based on a silicone backbone as pseudostationary phases for electrokinetic chromatography. *Electrophoresis*, **1999**, *20*, 2412–2419.

45 A. Marsh, B. Clark, M. Broderich, J. Power, S. Donegon, K. Altria, Recent advances in microemulsion electrokinetic chromatography. *Electrophoresis*, **2004**, *25*, 3970–3980.

46 H. Huang, Y. C. Lai, C. W. Chiu, J. M. Yeh, Comparing micellar electrokinetic chromatography and microemulsion electrokinetic chromatography for the analysis of preservatives in pharmaceutical and cosmetic products. *J. Chromatogr. A*, **2003**, *993*, 153–164.

47 J. M. Sanchez, V. Solvado, Comparison of micellar and microemulsion electrokinetic chromatography for the analysis of water- and fat-soluble vitamins. *J. Chromatogr. A*, **2002**, *950*, 241–247.

48 S. H. Hansen, C. Gabel-Jensen, S. Pedersen-Bjergaard, Comparison of microemulsion electrokinetic chromatography and solvent-modified

micellar electrokinetic chromatography. *J. Sep. Sci.*, **2001**, *24*, 643–650.

49 C. Nilsson, S. Nilsson, Nanoparticle-based pseudostationary phases in capillary electrochromatography. *Electrophoresis*, **2006**, *27*, 76–83.

50 K. Bächmann, B. Göttlicher, I. Haag, K.-Y. Han, W. Hensel, A. Mainka, Capillary electrokinetic chromatography with a suspension of chromatographic particles. *J. Chromatogr. A*, **1994**, *688*, 283–292.

51 W.-L. Tseng, M.-F. Huang, Y.-F. Huang, H.-T. Chang, Nanoparticle-filled capillary electrophoresis for the separation of long DNA molecules in the presence of hydrodynamic and electrokinetic forces. *Electrophoresis*, **2005**, *26*, 3069–3075.

52 R. A. Wallingford, H. G. Ewing, Capillary electrophoresis. *Adv. Chromatogr.*, **1989**, *29*, 1–76.

53 M. Yan, O. Ramstrom, *Molecular Imprinted Materials – Science and Technology*, Marcel Dekker, New York, **2005**.

54 B. Preinestorfer, M. Lämmerhofer, Recent accomplishments in the field of enantiomers separation by CEC. *Electrophoresis*, **2007**, *28*, 2527–2565.

55 G. Gubitz, M. G. Schmid, Advances in chiral separation using capillary electromigration techniques. *Electrophoresis*, **2007**, *28*, 114–126.

56 C. M. Johnson, P. A. McKeown, M. R. Enerby, Modes of CEC separation. *J. Chromatogr. Library*, **2001**, *62*, 87–110.

57 C. Fujimoto, Enantiomer separation by capillary electrochromatography using frittless packed columns. *Anal. Sci.*, **2002**, *18*, 19–25.

58 J. Kang, D. Wistuba, V. Schuring, Recent progress in enentiomerse separation by capillary electrochromatography. *Electrophoresis*, **2002**, *23*, 4005–4021.

59 F. M. Okanda, Z. El Rassi, Biospecific interaction (affinity) CEC and affinity nano-LC. *Electrophoresis*, **2007**, *28*, 89–98.

60 D. Mangelings, M. Maftouh, Y. van der Heyden, Capillary electrochromatographic chiral separations with potential for pharmaceutical analysis. *J. Sep. Sci.*, **2006**, *28*, 691–709.

61 R. A. Carney, M. M. Robson, K. D. Bartle, P. Myers, Investigation into the formation of bubbles in capillary electrochromatography. *J. High Resolut. Chromatogr.*, **1999**, *22*, 29–32.

62 E. Rapp, E. Bayer, Improved column preparation and performance in capillary electrochromatography. *J. Chromatogr. A*, **2000**, *887*, 367–378.

63 U. Dyell, Advances in column technology and instrumentation in capillary electrochromatography. *J. Chromatogr. A*, **2000**, *892*, 257–278.

64 G. A. Lord, D. B. Gordon, P. Myers, B. W. King, Tapers and restrictors for capillary electrochromatography and capillary electrochromatography–mass spectrometry. *J. Chromatogr. A*, **1997**, *768*, 9–16.

65 A. von Brocke, G. Nicholson, E. Bayer, Recent advances in capillary electrophoresis/electrospray mass spectrometry. *Electrophoresis*, **2001**, *22*, 1251–1266.

66 T. Tsuda, Advances in capillary chromatography. *Chromatography*, **2000**, *21*, 1–10.

67 C. Yao, S. Tang, R. Gao, C. Jiang, C. Yan, Enantiomer separations on a vancomycin stationary phase and retention mechanism of pressurized capillary electrochromatography. *J. Sep. Sci.*, **2004**, *27*, 1109–1114.

68 J. L. Liao, S. Hjerten, High-performance liquid chromatography of proteins on compressed, non-porous agarose beads: II. Anion-exchange chromatography. *J. Chromatogr. A*, **1988**, *457*, 175–182.

69 E. Klodzinska, D. Moravcova, P. Jandera, B. Buszewski, Monolithic continuous beds as a new generation of stationary phase for chromatographic and electro-driven separation. *J. Chromatogr. A*, **2006**, *1109*, 51–59.

70 F. Svec, Recent developments in the field of monolithic stationary phases for capillary electrochromatography. *J. Sep. Sci.*, **2005**, *28*, 729–745.

71 Y. Baba, M. Tsuhako, Gel-filled capillaries for nucleic acid separations in capillary electrophoresis. *Trends Anal. Chem.*, **1992**, *11*, 280–287.

72 N. Ishizuka, H. Minakuchi, K. Nakanaishi, N. Soga, H. Nagayama, K. Hosoya, N. Tanaka, Performance of a monolithic silica column in a capillary under pressure-driven and electrodriven conditions. *Anal. Chem.*, **2000**, *72*, 1275–1280.

73 K. Nakanishi, H. Shikata, N. Ishizuka, N. Koheiya, N. Soga, Tailoring mesopores in monolithic macroporous silica for HPLC. *J. High Resolut. Chromatogr.*, **2000**, *23*, 106–110.

74 D. Allen, Z. El Rassi, Capillary electrochromatography with monolithic silica column: I. Preparation of silica monoliths having surface-bound octadecyl moieties and their chromatographic characterization and applications to the separation of neutral and charged species. *Electrophoresis*, **2003**, *24*, 408–420.

75 W. Li, D. P. Fries, A. Malik, Sol–gel stationary phases for capillary electrochromatography. *J. Chromatogr. A*, **2004**, *1044*, 23–52.

76 M. Kato, H. Saruwatari, K. Sakai-Kato, T. Toyo'oka, Silica sol–gel/organic hybrid material for protein encapsulated column of capillary electrochromatography. *J. Chromatogr. A*, **2004**, *1044*, 267–270.

77 M. Pumera, Microchip-based electrochromatography: designs and applications. *Talanta*, **2005**, *66*, 1048–1062.

78 L. Szekely, A. Guttmann, New advances in microchip fabrication for electrochromatography. *Electrophoresis*, **2005**, *26*, 4590–4604.

79 C. T. Culbertson, S. C. Jacobson, J. M. Ramsey, Microchip devices for high-efficiency separations. *Anal. Chem.*, **2000**, *72*, 5814–5819.

80 G. J. M. Bruin, Recent developments in electrokinetically driven analysis on microfabricated devices. *Electrophoresis*, **2000**, *21*, 3931–3951.

81 M. J. Sepaniak, D. F. Swaile, A. C. Powell, Instrumental developments in micellar electrokinetic capillary chromatography. *J. Chromatogr. A*, **1989**, *480*, 185–196.

82 J. P. Kutter, S. C. Jacobson, J. M. Ramsey, Integrated microchip device with electrokinetically controlled solvent mixing for isocratic and gradient elution in micellar electrokinetic chromatography. *Anal. Chem.*, **1997**, *69*, 5165–5171.

83 D. J. Harrison, K. Fluri, K. Seiler, Z. Fan, C. S. Effenhausen, A. Manz, Micromachining a miniaturized capillary electrophoresis-based chemical analysis system on a chip. *Science*, **1993**, *261*, 895–897.

84 C. S. Effenhauser, G. J. M. Bruin, A. Paulus, M. Ehrat, Integrated capillary electrophoresis on flexible silicone microdevices: analysis of DNA restriction fragments and detection of single DNA molecules on microchips. *Anal. Chem.*, **1997**, *69*, 3451–3457.

85 B. He, N. Tait, F. Regnier, Fabrication of nanocolumns for liquid chromatography. *Anal. Chem.*, **1998**, *70*, 3790–3797.

86 J. W. Jorgenson, E. J. Guthrie, Liquid chromatography in open-tubular columns. Theory of column optimization with limited pressure and analysis time and fabrication of chemically bonded reversed-phase columns on etched borosilicate glass capillaries. *J. Chromatogr.*, **1983**, *255*, 335–348.

87 W. Xu, K. Uchiyama, T. Shimosaka, T. Hobo, Fabrication of polyester microchannels and their applications to capillary electrophoresis. *J. Chromatogr. A*, **2001**, *907*, 279–289.

88 L. Ceriotti, N. F. de Rooij, E. Verpoorte, An integrated fritless column for on-chip capillary electrochromatography with conventional stationary phases. *Anal. Chem.*, **2002**, *74*, 639–647.

89 C. Ericson, J. Holm, T. Ericson, S. Hjerten, Electroosmosis- and pressure-driven chromatography in chips using continuous beds. *Anal. Chem.*, **2000**, *72*, 81–87.

90 S. M. Ngola, Y. Fintschenko, W. Y. Choi, T. J. Shepodd, Conduct-as-cast polymer

monoliths as separation media for capillary electrochromatography. *Anal. Chem.*, **2001**, *73*, 849–856.

91 I. M. Lazar, L. Li, Y. Yang, B. L. Karger, Microfluidic device for capillary electrochromatography–mass spectrometry. *Electrophoresis*, **2003**, *24*, 3655–3662.

92 B. E. Slentz, N. A. Penner, F. Regnier, Geometric effects of collocated monolithic support structures on separation performance in microfabricated systems. *J. Sep. Sci.*, **2002**, *25*, 1011–1018.

93 S. A. Rathore, A. Guttmann, *Electrokinetic Phenomena – Principles and Applications in Analytical Chemistry and Microchip Technology*, Marcel Dekker, New York, **2004**.

94 I. S. Krull, R. L. Stevenson, K. Mistry, M. E. Schwartz, *Electrochromatography and Pressurized Flow Capillary Electrochromatography – an Introduction*, HNB Publications, New York, **2000**.

95 Z. Deyl, F. Svec, Capillary electrochromatography. *J. Chromatogr. Library*, **2001**, *62*, 1.

96 K. D. Barthe, P. Myers, *Capillary Electrochromatography*, RSC Chromatography Momographs, Royal Society of Chemistry, Cambridge, **2001**.

97 J. Simal-Gandara, The place of capillary electrochromatography among separation techniques. A review. *Critical Reviews in Analytical Chemistry*, **2004**, *34*, 85–94.

98 E. Kenndler, A. Rizzi, Electrokinetic chromatography. *J. Chromatogr. Library*, **2004**, *69A*, 297–318.

99 V. T. Remcho, S. L. Clark, A. Coneau, G. S. Chirica, Applications of capillary electrochromatography. *Electrokinetic Phenomena*, **2008**, 345–425.

100 C. Demesmay, G. Puy, F. Progent, J.-L. Rocca, Electrokinetic and electrochromatographic techniques, Part II. Capillary electrochromatography: Diversity of stationary phases. *Spectra Analyse*, **2005**, *34*, 26–31.

101 M. P. Henry, C. K. Ratnayake, Electrochemical properties of columns in capillary electrochromatography I. Ohm's law, resistivity, and field strength. *J. Chromatogr., A*, **2005**, *1079*(1–2), 69–76.

102 S. G. Weber,Capillary seperations prove likely. A report on 29th International Symposium on capillary chromatography (and 3rd GC × GC symposium) at Riva del Garda, Italy, 30. Mai 2006. *Trends in Analytical Chemistry*, **2006**, *25*, 629–632.

103 G. Gubitz, M. G. Schmid, Chiral Separations. *Krik-Othmer Sep. Techn.*, (2nd Edition), John Wiley & Sons, Hoboken, NJ, **2008**, 1, 553–579.

104 S. G. Weber, Solid-phase microextraction and solid phase extraction with capillary electrophoresis and related techniques. *Handbook of Capillary and Microchip Electrophoresis and Associated Microtechniques*, CRC Press LLC, Boca Raton (3rd Edition), **2008**, 811–823.

105 G. P. Rozing, A. Dermaux, P. Sandra, Instrumentation for capillary electrochromatography. *J. Chromatogr. Library*, **2001**, *62*, 39–85.

106 Z. El Rassi, CEC and EKC. *Electrophoresis*, **2006**, *27*, 727.

107 Z. El Rassi, CEC and EKC. *Electrophoresis*, **2007**, *28*, 1643.

108 Z. El Rassi, CEC and EKC. *Electrophoresis*, **2008**, *29*, 751.