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Review

(Micellar) electrokinetic chromatography: an interesting solution for the liquid phase separation dilemma

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Abstract

High-performance liquid chromatography (HPLC) is a well-established method in modern analysis. The method is simple, very robust and is applicable to the majority of components to be analyzed in contrast to gas chromatography. Low efficiency and small peak capacity are sore points of HPLC when complex mixtures have to be separated. The reason for this dilemma is the small diffusion coefficient of the analytes in the liquid mobile phase compared to a gaseous phase. This review, complemented by exemplary calculated data and some latest results of our own research, illustrates the dilemma of liquid phase chromatography to achieve high efficiencies under reasonable conditions. It is shown that (micellar) electrokinetic chromatography, offering fast and efficient separations, is a very promising solution for this dilemma. Additional features of this method are possibilities of on-line analyte concentration, coupling to mass spectrometry and the easy change of selectivities by applying various separation additives. The pros and cons of electrokinetic chromatography are pointed out and some application examples are given.
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Keywords: Reviews; Diffusion; Micellar electrokinetic chromatography; Electrokinetic chromatography

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1. Short historical background of modern separation methods

About one century ago the Russian botanist

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Mikhail Tswett pioneered chromatography. He succeeded in separating a plant pigment mixture in single colour zones using calcium carbonate as stationary phase [1]. Inspired from his observations and probably including his name (''tswett'' means ''color'' in Russian) he called the method ''chromatography''.

No attention was paid to chromatographic methods for a long time until Lederer's group separated carotinoids by adsorption chromatography [2]. After that, chromatography became of great interest and in 1941 Martin and Synge introduced liquid–liquid partition chromatography [3] already speculating that gas–liquid chromatography should also be possible. Ten years later this was realized and published by James and Martin [4]. In the following decades innovations on theory and practice of both gas (GC) and liquid chromatography (LC) spread out these techniques as most important analytical methods throughout the world.

In addition to this, zone electrophoresis came up in the 1960s and 1970s, first described by Hjertén [5] as free solution electrophoresis in 3 mm I.D. capillaries. Afterwards the use of smaller inner diameters to achieve higher efficiencies was proposed [6–8]. Capillary zone electrophoresis (CZE) with very high separation efficiency was demonstrated for the first time by Jorgenson and Lukacs [9–12]. A breakthrough for the separation of neutral analytes, not possible with ''normal'' CE, was made by Terabe et al. [13,14] who introduced micellar electrokinetic chromatography (MEKC). Since then, different kinds of electrokinetic chromatographic methods have been developed [15–21]. As a hybrid of capillary electrophoresis and high-performance liquid chromatography (HPLC), capillary electrochromatography (CEC) has been established since the 1980s [11,22–25].

Of course this is not a complete listing of chromatographic and electrophoretic separation techniques but is the main section of those analytical separation techniques we know today as playing an important role in the realm of modern analytical methods.

1.1. The liquid phase separation dilemma

After its introduction by James and Martin in 1952 [4], gas chromatography was carried out in packed columns, a technique especially used by the petrochemical industry. But the low separation efficiencies and long analysis times were unsatisfactory and so Golay's invention of the open-tubular column was a real pioneering work in the field of GC [26–28]. Based on the idea that a packed column is just a bundle of capillaries, he introduced the concept and theory of open-tubular capillary columns. The high diffusion coefficient of molecules in the gaseous mobile phase (three to four orders of magnitude higher compared to a liquid phase) enables the use of open-tubular columns with inner diameters as large as 0.5 mm as separation vehicle. Diffusion of the analytes to the capillary wall coated with the stationary phase is rapid enough to assure high plate numbers and peak capacities at moderate analysis times. In addition, open-tubular columns offer good permeability compared to packed columns.

Golay's work, unequaled until today, has opened a great potential for gas chromatography. Accompanied by important technical innovations, e.g. the flame ionization detector by McWilliam and Dewar suitable for the small peak volumes [29], the glass-drawing machine by Desty [30] and the introduction of the fused-silica capillary by Dandeneau and Zerrener [31], open-tubular capillary gas chromatography (CGC) has developed to a most powerful separation technique with high efficiency, high separation speed and high sensitivity. Many other workers promoted the development of GC to its high standards of today. After some years of slower growth, capillary gas chromatography now experiences its second advent thanks to the breakthrough of two-dimensional GC while still remaining the method of choice for fast and efficient separations of complex volatile mixtures.

The excellent performance of capillary gas chromatography should not deceive about the fact that most samples cannot be analyzed by GC because of insufficient volatility, thermal instability or decomposition under GC separation conditions. Without chemical modification only about 20% of the organic compounds are accessible to GC. However, those samples may be well suitable for liquid chromatography.

But in liquid chromatography (LC), the dilemma exists that the diffusion coefficient is three to four

orders of magnitude smaller compared to the gaseous phase and hence the diffusion speed of the solutes to the stationary phase is very limited [28]. Therefore, efficiency is low and open-tubular columns having the same dimensions as those in GC are inferior to packed columns [32,33]. In theory, shown by Knox and Gilbert [34], open-tube LC can only be brought to work by decreasing the capillary column inner diameters to values below 10 μm. Although theory predicts the superiority of open-tubular columns with very small inner diameters (<10 μm), they are not established in the practice of liquid chromatography. The small inner diameters are very exacting to injection, detection, sample capacity and the experimental set-up. Instruments for those small dimensions are commercially not available and so open-tubular liquid chromatography (OTLC) is predominantly reserved for a few specialists in universities and institutes. OTLC provides good efficiencies but because the technical realization remains difficult, the packed column type was developed according to the theory of modern liquid chromatography, thus providing acceptable separation efficiency [35–43]. This can be attained by using small particles with short diffusion paths. A simultaneous reduction of the analysis time is then possible by the application of high flow-rates. The decrease of particle diameters is, however, limited by the pressure drop in the column and a compromise between efficiency, speed and pressure drop has to be made. This problem was already discussed in 1969 by Knox and Saleem [44]. Therefore selectivity, mathematically expressed by α, is the decisive basis of successful liquid chromatographic separations. Whereas an increase of α by variation of the mobile and/or the stationary phase is a powerful tool to improve resolution of certain peak pairs [35,45,46], peak capacity of LC remains limited. The peak capacity, n_c, (Eq. (1)) a term for the number of ideal Gaussian peaks that can be theoretically separated with a resolution of 1.0 up to a defined maximum capacity factor, k_{max} [47–49], can only be increased by an increase of the plate number N. To separate complex mixtures, peak capacity must exceed by far the number of components because of statistical peak overlap [50–54]:

$$n_c = 1 + \frac{\sqrt{N}}{4} \cdot \ln(1 + k_{max}) \tag{1}$$

These limiting factors forced researchers to look for new developments in liquid phase separations. In the following some of these approaches will be discussed by means of some calculated data summarized in Table 1.

2. Approaches to solve the liquid phase separation dilemma

2.1. Calculation of chromatographic parameters

In Table 1 different chromatographic parameters to achieve 100 000 plates for a component with $k=5$ are compared for capillary GC (CGC), pressure-driven open-tubular LC (PD-OTLC), electro-driven open-tubular LC (ED-OTLC), micro-high-performance LC (micro-HPLC), capillary electrochromatography (CEC) and micellar electrokinetic chromatography (MEKC). $k=5$ was selected because it is a reasonable value for all methods except for classical OTLC where it is somewhat too high. Calculated parameters are the column length, the retention time and the required pressure and voltage, respectively. In addition, the peak volume and the tolerated injection volume are specified. The latter is the maximum injection volume tolerated by the separation system in order to limit the band broadening to a certain degree. For the calculated values we defined 10% loss of efficiency with an injection quality factor of 2 [55]. All calculations were carried out using a flow-rate of 3 mm/s except for CGC where an optimum flow-rate of 38 cm/s for 250 μm I.D. and 95 cm/s for 100 μm I.D. were used. Field strengths in order to achieve a certain flow-rate in CEC and ED-OTLC were taken from practical measurements [56,57]. Values for MEKC are derived from typical experimental results as well [58,59] because the equations given by Terabe et al. [60] lead to unrealistic good values for the plate height, moreover these calculations necessitate parameters which are unknown or not available. Further values needed for the calculations are given in the table caption. Equations and symbol explanations used for the calculations are given in the Nomenclature.

For capillary GC, acceptable values are obtained for all parameters when operating the system at optimum flow velocities. For a component with a

Table 1
Calculated chromatographic parameters to achieve 100 000 plates for a component with $k=5$ for different separation methods

	Column diameter d_c	Particle diameter d_p	Column length L	Retention time t_R	Pressure/ voltage $\Delta p/\Delta U$	Peak volume V_p	Tolerated injection volume $V_{i,10\%tol}$
CGC	250 μm	–	21 m	5.5 min	0.4 bar	80 μl	15 μl
	100 μm	–	8.4 m	0.9 min	1.5 bar	5 μl	1 μl
PD-OTLC	10 μm	–	2.7 m	90 min	26 bar	20 nl	3 nl
	2 μm	–	0.17 m	6 min	42 bar	45 pl	7 pl
ED-OTLC	10 μm	–	1.4 m	46 min	137 kV	10 nl	2 nl
	2 μm	–	0.12 m	4 min	12 kV	30 pl	5 pl
Micro-HPLC	75 μm	5 μm	2.4 m	80 min	3100 bar	650 nl	100 nl
	75 μm	3 μm	1.1 m	36 min	3900 bar	300 nl	50 nl
	75 μm	1.5 μm	0.4 m	14 min	5900 bar	100 nl	20 nl
CEC	75 μm	5 μm	1.3 m	43 min	155 kV	350 nl	60 nl
	75 μm	3 μm	0.6 m	21 min	76 kV	170 nl	30 nl
	75 μm	1.5 μm	0.27 m	9 min	33 kV	70 nl	15 nl
MEKC	50 μm	–	0.3 m	5 min	24 kV	45 nl	7 nl

Values used for calculations: $k=5$, $N=100\ 000$, injection quality factor $K_i=2$, tolerated loss of efficiency for the calculation of $V_i=10\%$; GC, $D_m=2\times10^{-5}$ m^2/s (decane in H_2), $\eta=10.35$ μPa s (H_2/100 °C), $p_0=1.0\times10^5$ Pa, $\varepsilon=1$; OTLC, $D_m=1.0\times10^{-9}$ m^2/s, $\eta=1.0\times10^{-3}$ Pa s (ACN–H_2O=40:60), $u/u_{eo}=3$ mm/s, $\varepsilon=1$, $E=1000$ V/cm; micro-HPLC, $D_m=1.0\times10^{-9}$ m^2/s, $\eta=1.0\times10^{-3}$ Pa s (ACN–H_2O=40:60), $u=3$ mm/s, $\varepsilon=0.8$ (porous particles), $\vartheta=1.3$ (spherical porous particles), $A=1$, $B=2$; CEC, $D_m=1.0\times10^{-9}$ m^2/s, $\eta=1.0\times10^{-3}$ Pa s (ACN–H_2O=40:60), $u_{eo}=3$ mm/s, $\varepsilon=0.8$ (porous particles), $\vartheta=1.3$ (spherical porous particles), $A=0.7$, $B=2$, $E=1200$ V/cm; MEKC, values are derived from typical experimental results for $u_{eo}=3$ mm/s. Symbol explanations and formula used for calculations are given in the Nomenclature.

retention factor of 5, 100 000 theoretical plates are achieved within less than 6 min on both capillaries using moderate inlet pressures.

In pressure-driven and electro-driven OTLC, the use of a capillary with 10 μm I.D. results in unacceptable long analysis times and voltages as high as 137 kV which cannot be realized with common power supplies in an ordinary laboratory environment. By reducing the inner diameter to 2 μm, retention time, pressure and voltage remain in a realizable range. However, peak and injection volume then reach the pl-range, thus provoking problems with injection and optical detection. These problems can be overcome by using a chip [61].

In micro-HPLC using 5 μm particles, more than 3000 bar, a column length of 2.4 m and an analysis time of 80 min are required to achieve 100 000 plates. When reducing the particle diameter to 1.5 μm, 100 000 plates are achieved within 14 min. An only 0.4-m-long column is necessary but the inlet pressure rises to nearly 6000 bar. Those high pressures are not impossible as shown by Jorgenson and Lee [62–67] but a special apparatus had to be built.

Early attempts to reduce the column diameter of packed columns in HPLC were made because of the wish to analyse small sample volumes in order to save expensive solvents and in order to couple HPLC to mass spectrometry. However, despite a common belief, an increase of efficiency is not connected to a decrease of the column diameter. Higher efficiency is only attainable by a combination of very small particles and increased column lengths as discussed above. Unfortunately, this leads to an increase of the pressure drop. In order to realize very high separation efficiencies, the group of Jorgenson and later Lee's group applied very high pressures up to 8000 bar to pack and to operate packed capillaries in the reversed-phase mode [62–67]. The method was termed ultra-high pressure liquid chromatography (UHPLC). It offers improved efficiencies at moderate analysis times. For example a 30 μm I.D.×66 cm long fused-silica capillary packed with 1.5 μm non-porous particles was applied near the optimum flow-rate at 1400 bar inlet pressure. More than 200 000 plates were achieved for a component with $k=2$ within 30 min [62]. Higher than optimum flow-rates reduced the analysis time to 10 min still

resulting in 140 000–190 000 theoretical plates for this component.

Beside the challenging technical requirements, high pressures are connected to frictional heating at high flow-rates [62,68]. The heat is dissipated over the packing material and the mobile phase to the thermostated column wall. This results in radial and longitudinal temperature gradients within the column which generate viscosity, diffusivity and k gradients. These problems can be overcome by pre-cooling of the mobile phase [68,69] or by applying packed narrow bore capillaries as shown in UHPLC [62–67].

An alternative way to achieve high plate numbers is the electroosmotic movement of the mobile phase using the same packed capillaries as in micro-HPLC. The method, named electrochromatography (CEC), found great interest during the last decade. But as the data in Table 1 show, the use of 5 and 3 μm particles to achieve 100 000 plates within reasonable time cannot be realized with common CE equipment whose high voltage power supply outputs are limited to 30–40 kV. Voltages of 155 and 76 kV can only be realized with specially designed power supplies [70,71]. However, 30 kV are sufficient to achieve 100 000 plates for the $k=5$ peak within 9 min when using 1.5 μm particles in a 27-cm-long column. The use of such small particles is possible because the electroosmotic flow (EOF) generates no back pressure. In addition, the electroosmotic flow velocity is almost always independent of the channel diameter within the packing structure. Another advantage of electrochromatography is a dual separation mechanism for charged analytes: beside the different partitioning between the stationary phase and the mobile phase, the different electrophoretic mobilities of charged analytes influence the separation. These perspectives encouraged many workers to focus their research on CEC [11,22–25] and this method was developed quite fast [72–86]. But this technique includes many difficulties: for CEC as well as for micro-HPLC, it is known that the packing procedure of 1.5 μm particles is difficult and often offers poor packing qualities. This is a problem especially in CEC as the packing material can move when an electrical field is applied to the column. In order to keep the packing material in the capillary, frits have to be placed before and after the packing bed [56]. The consequences are local velocity differences in the sections of the stationary phase, the frits and the open-tubular detection capillary which can lead to internal pressures and flow profile distortions [85]. These velocity differences are also an explanation for the possible formation of bubbles. Another explanation is Joule heating especially when high buffer concentrations are used and thermostatization is not effective. Very small particles may also promote bubble formation. A solution to this dilemma can be the application of monoliths or silica rods as demonstrated by Tanaka and co-workers [87–89].

A summarizing view on Table 1 shows that micellar electrokinetic chromatography (MEKC) seems to be a good solution to obtain the required parameters: 100 000 plates are easily achieved within 5 min at moderate voltages of 24 kV and a capillary length of 30 cm. MEKC was introduced by Terabe et al. in several innovating papers [13,14,60]. Like CEC, this electrophoretic technique can separate neutral and charged compounds at the same time. A surfactant (typical anionic, but also cationic is possible) is added to the buffer solution that forms micelles above the critical micelle concentration (cmc). Separation occurs by different partitioning of the solutes between the mobile phase moving with the electroosmotic flow and the interior of the charged micelles moving slower than the EOF due to their electrophoretic migration in the opposite direction. Because the effect is similar to the action of a stationary phase the surfactant is called ‘‘pseudo-stationary phase’’. As CEC, MEKC profits from the flat electroosmotic flow profile which is independent of the capillary diameter. This is one reason why MEKC is a very efficient and fast separation technique.

The mentioned micro dimensions have important consequences. As also shown in Table 1, miniaturization results in very small peak volumes and tolerated injection volumes which means that all those micro techniques generate injection and detection problems compared to analytical HPLC [43,90,91].

Except for capillary GC, injection and detection volumes are in the nl- or even pl-range. Separate injection devices for those volumes are hard to realize unless integrated valves on a chip are used [61]. If possible, on-column injection is applied. The

commonly used UV-detection is only possible if the detector cell volume is below the peak volume in order to exclude extra column peak broadening. This is connected to a tremendous reduction of the optical path length and therefore to a reduced sensitivity because not any given optical path length can be combined with a certain detector cell volume. This means that UV detection is not the method of choice for micro- and nano-scale separation methods. Other detection methods such as electrochemical, laser-induced fluorescence and mass spectrometric detection are more suitable in case of small peak volumes.

2.2. Electrokinetic separation methods: interesting solutions

As it was shown in Table 1, MEKC represents an interesting alternative among the liquid phase separation techniques.

In MEKC, as in CE, longitudinal diffusion is the main contribution to plate height. The mass transfer contribution term which describes the inclusion–exclusion kinetics of the analyte with the micelle is much smaller compared to LC because the ‘‘stationary phase’’ micelle is in close vicinity to the analytes. As a result, very short diffusion distances occur and the equilibrium is reached extremely quickly. Efficiency therefore is very high. Micellar dispersity and intermicellar mass transfer can be neglected. Temperature effects are minimized by keeping the capillary diameter below 75 μm. A detailed discussion of band broadening effects in MEKC is given in Ref. [60].

Micellar electrokinetic chromatography offers many advantages. Very high plate numbers can be achieved in a short time. A 2-min separation of a homologous series of ω-phenylalcohols is shown in Fig. 1. MEKC separations are superior to HPLC separations especially for small k-values. An example is presented in Fig. 2 where some amino/nitroaromatics were separated with excellent efficiency within 9 min using MEKC (Fig. 2a). On the other hand, the same mixture cannot be fully separated using HPLC because efficiency is not sufficient (Fig. 2b).

The selectivity in MEKC systems can be easily changed by applying different surfactants (separation additives). The surfactant sodium dodecyl sulfate (SDS) has been mainly used so far but other SDS-like surfactants [92] were successfully introduced. But even a small change of the alkyl part of the surfactant can often help to improve the resolution of peak pairs unresolved in SDS–MEKC with still a high efficiency. Fig. 3 shows the separation of compounds with different functional groups using SDS and an in-house synthesized surfactant 4-cyclododecylmethoxy-butane-1-sodiumsulfonate (C-DMOBSS). The peak pair ω-phenylethanol/phenol not separated using SDS was resolved using CDMOBSS.

A special application of MEKC is microemulsion electrokinetic chromatography (MEEKC) [15,93–95]. In this technique the separation additive is not a pure micelle but a micellar aggregate with other supporting components. The core of the micelle (typically SDS) contains an oil droplet, e.g. n-octane, and is stabilized by a short-chain alcoholic co-surfactant such as 1-butanol. The nanometre-sized charged droplets are dispersed in the buffer solution. The

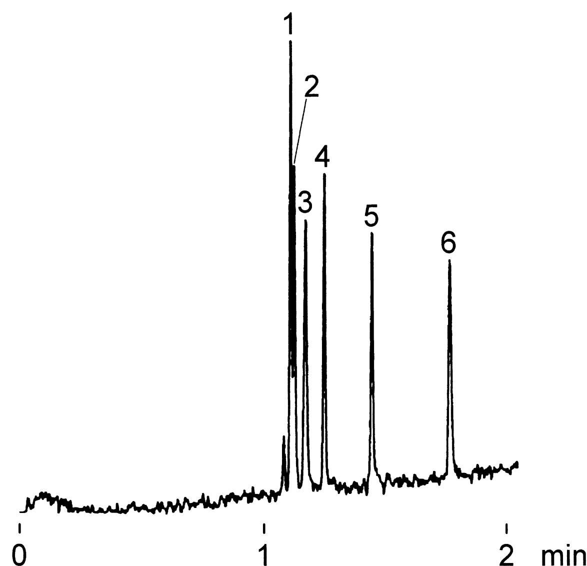

Fig. 1. Fast MEKC separation of homologous ω-phenylalcohols on a laboratory-made apparatus (for details see Ref. [56]). Separation buffer, 25 mM cholic acid, 100 mM Tris (pH 8.6); capillary (MicroQuartz, Munich, Germany), 35/19 cm×50 μm I.D.; field, 570 V/cm; detection, UV at 220 nm. Peak assignments (plate number in parenthesis): homologous series from (1) benzyl alcohol to (6) ω-phenylhexanol (80 000).

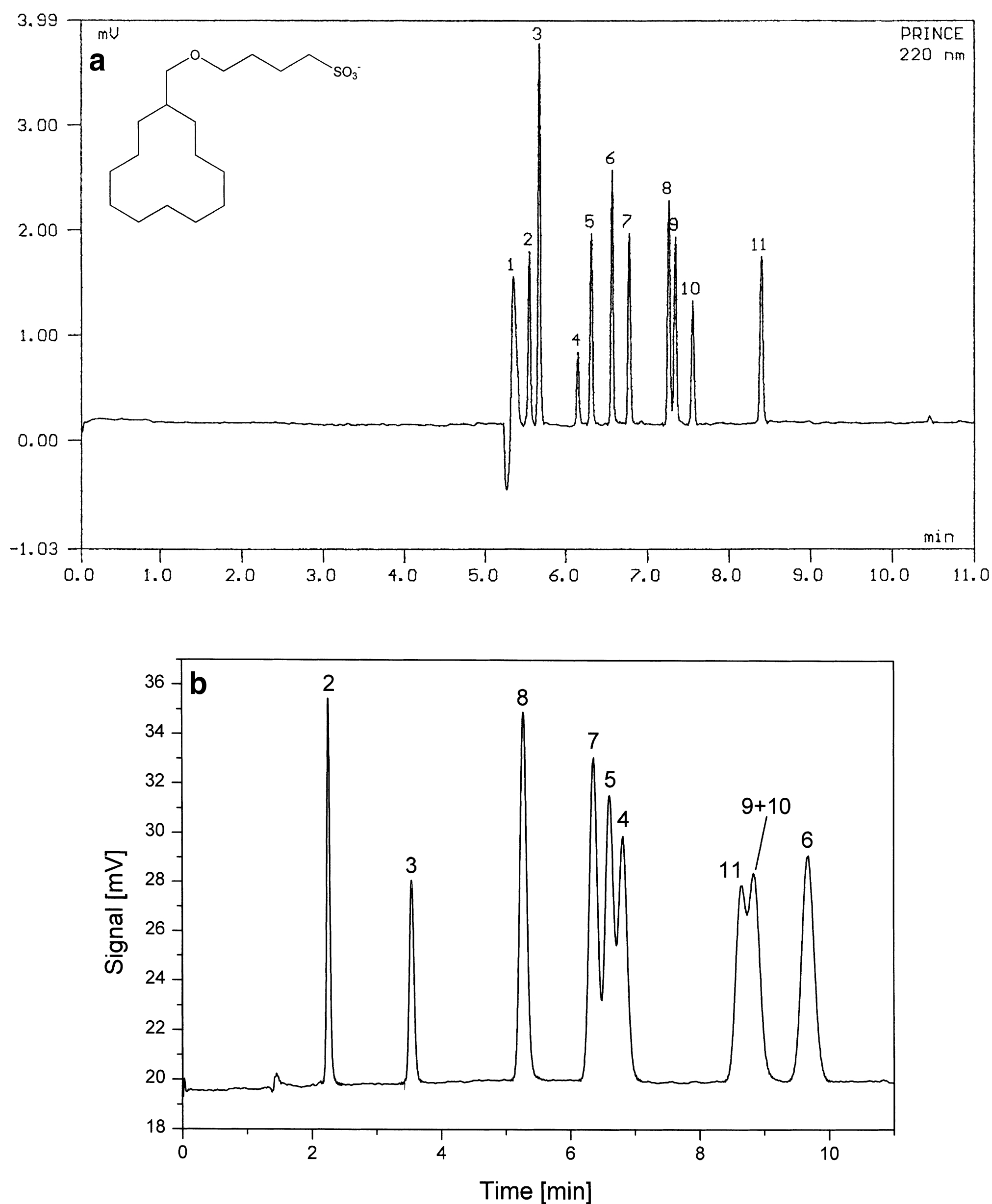

Fig. 2. Separation of some amino/nitroaromatic compounds using (a) MEKC and (b) HPLC. MEKC: CE equipment, as described elsewhere [58]; separation buffer, 25 m*M* CDMOBSS (in-house synthesized from cyclododecyl methanol according to Ref. [159], structure shown in the electropherogram), 20 m*M* borate (pH 9.4), 10% MeOH; capillary (MicroQuartz, Munich, Germany), 75/47 cm×50 μm I.D.; field, 400 V/cm; detection, UV at 220 nm. HPLC: HPLC equipment, Kontron pump 422 and UV-detector 535 with 1 μl-cell (Neufahrn, Germany); detection, UV at 220 nm; injection, 5 μl; column, 150×4.6 mm Phenomenex Luna 5 μm C_{18}(2) (Aschaffenburg, Germany); eluent, ACN–H_2O=50:50 (v/v); flow, 0.8 ml/min. Peak assignment (plate numbers for MEKC/HPLC in parentheses): (1) thiourea, (2) 2,4-diaminotoluene (166 000/10 600), (3) aniline (166 000/11 100), (4) 1,4-dinitrobenzene (160 000/not resolved), (5) 1,3-dinitrobenzene (198 000/9000), (6) 2,4,6-trinitrotoluene (238 000/11 500), (7) 1,2-dinitrobenzene (202 000/10 900), (8) 4-amino-2-nitrotoluene (200 000/11 100), (9) 2,4-dinitrotoluene (223 000/not resolved), (10) 2,6-dinitrotoluene (215 000/not resolved), (11) 2,3-dinitrotoluene (218 000/not resolved).

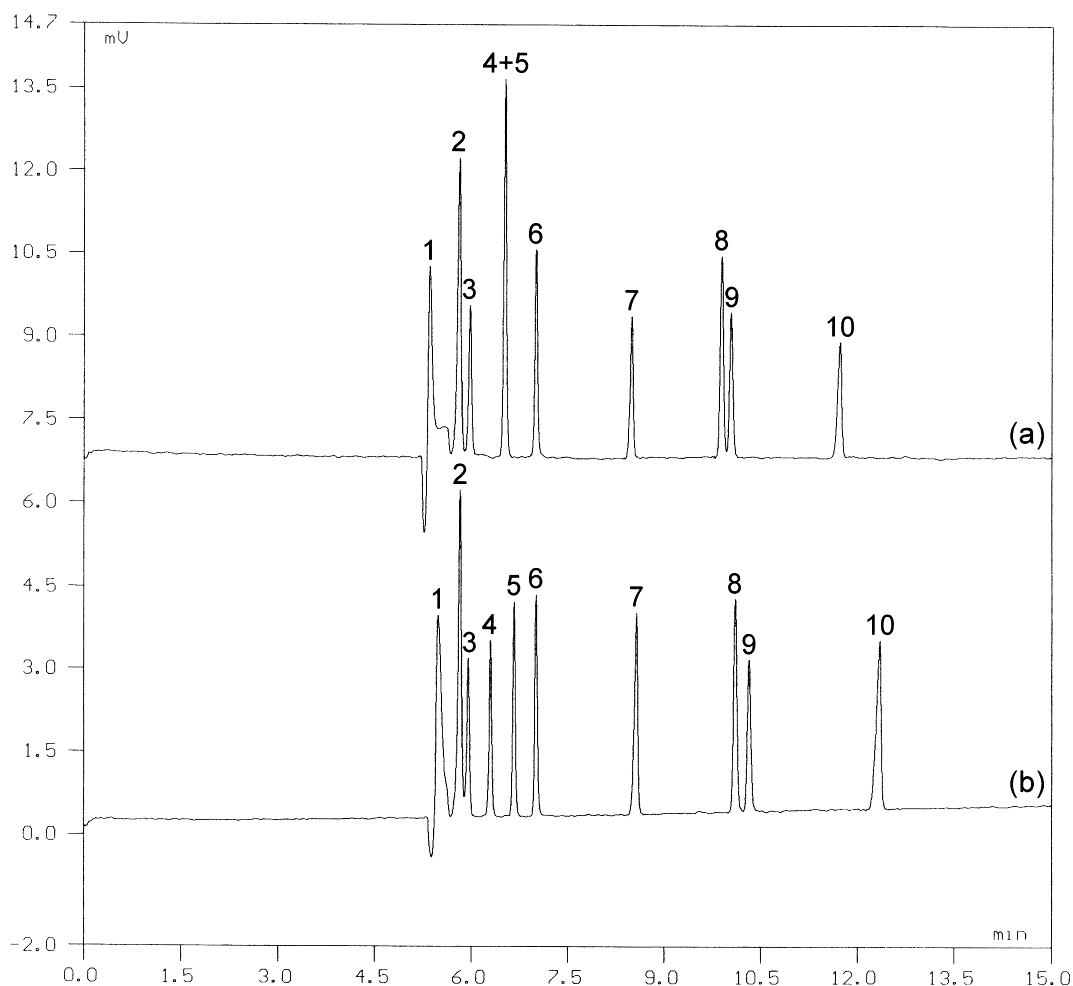

Fig. 3. MEKC separation of compounds with different functional groups with (a) SDS and (b) CDMOBSS (for details see Fig. 2). Separation buffer, 25 m*M* surfactant, 20 m*M* borate (pH 9.4), 10% MeOH; capillary, 75/47 cm×50 μm I.D.; field, 400 V/cm; detection, UV at 205 nm. Peak assignments (plate numbers for SDS/CDMOBSS in parenthesis): (1) thiourea (EOF marker), (2) aniline (55 000/74 000), (3) benzyl alcohol (74 000/90 000), (4) ω-phenylethanol (not resolved/121 000), (5) phenol (not resolved/123 000), (6) 1,2-dinitrobenzene (123 000/134 000), (7) toluene (126 000/115 000), (8) methyl parabene (155 000/154 000), (9) ethyl parabene (151 000/150 000), (10) *m*-xylene (133 000/110 000).

separation mechanism is similar to MEKC. The microemulsion droplet is larger than a pure micelle resulting in higher sample capacity. Detection problems are therefore reduced.

Beside SDS and similar surfactants [92], other types of additives can be used to achieve special selectivities. The spectrum of potential separation additives is very wide including exotic and expensive ones because the needed amount of separation additive is small. Examples are anionic siloxane polymers and other polymeric additives, cyclodextrines, dendrimers and charge-transfer interacting additives [96–113]. Selectivities offered by these additives are often completely different to those offered by typical alkyl chain surfactants. Of special interest are chiral additives for the separation of enantiomers, e.g. chiral surfactants [114,115], cyclodextrines [107,116,117], crown ethers [118,119], polysaccharides [120,121], proteins [16,17,122] and antibiotics [123,124]. These separations profit from

the small consumption of a chiral selector and from a superior separation performance. As MEKC needs no bonding chemistry to fix a stationary phase, very different separation systems can be realized just by flushing the capillary with the desired separation buffer. The method is therefore very flexible and column packing procedures are unnecessary.

A useful feature of electrokinetic chromatography is the possibility to study molecular interactions. Binding and association constants are estimated using the partial filling technique. Different separation zone lengths of the chiral selector were evaluated by the resulting electrophoretic mobilities for the estimation of association constants between enantiomers and human α_1-acid glycoprotein (AGP) [122] and between enantiomers and cyclodextrines [125]. The mobility ratio of peptides relative to a non-interacting standard was measured as a function of the concentration of vancomycin to estimate binding constants [126]. The method is very easy and the resulting values match those of other methods.

Of course (M)EKC also has some disadvantages but researchers are currently looking for solutions. One major drawback of all capillary electrophoretic techniques is detection sensitivity. UV detection, generally carried out on-column, is the most common detection method. As in CE the capillary inner diameters are below 100 μm, the optical path length is very small and unsatisfactory sensitivities result. But sensitivity in CE and (M)EKC can be improved in an elegant way by applying stacking and sweeping techniques [127–134]. Especially for biomedical and environmental samples a concentration of the analyte zone is necessary. Briefly, the stacking process is based on focusing effects on boundaries that separate regions with different ionic strengths. In MEKC neutral analytes can be swept by a stacked micelle zone that enters the analyte zone. Depending on the injection method, sensitivity can be increased by at least one order of magnitude up to a 100 000-fold increase in peak height. The spectrum of different stacking and sweeping methods is very large. For further information the reader should refer to the references listed at the end of this paper.

A serious problem is the limited migration time window expressed by t_{MC}/t_0. Only these components can be separated which elute in the window between the neutral marker (t_0) and the micelle marker (t_{MC}). Although (M)EKC offers good efficiencies, especially peaks eluting close to t_{MC} undergo a relatively high band broadening and hence resolution gets worse. There are several possibilities to enlarge the migration time window. All possibilities are based on slowing down the electroosmotic flow. Beside the use of buffers with high ionic strengths one possibility to slow down the electroosmotic flow is capillary surface modification [57]. An example for an increased migration time window on a surface modified capillary is given in Fig. 4. It shows a separation of homologous ω-phenylalcohols using a polymerized micelle [polymerized sodium 10-undecenyl-1-oxybutane sulfonate (poly-SUOBS)] as separation additive. By enlarging the migration time window, peak capacity, calculated according to Ref. [135], increases from 71 to 107. Another possibility is applying moderate counter pressures at the cathodic side [58,59,111–113,135]. With this method the migration time window can be enlarged simply without changing the chemistry of the system. Unfortunately, efficiency is diminished when applying counter pressure because the plug profile is disturbed. The enlargement of the migration time window partly compensates this effect. Consequently, the peak capacity goes through a maximum [135]. Especially the resolution of late eluting analytes can be improved at moderate counter pressures. This was shown for a partial filling system where a late eluting unresolved peak pair could be resolved with a resolution of 2.43 at 25 mbar counter pressure [113] (see Fig. 5).

It may sometimes be advantageous to use higher contents of organic modifiers, e.g. for better solvation of highly hydrophobic analytes. However, using micelle forming additives this is limited because the micellar structure, necessary for efficient separations, is disturbed at higher contents of organic modifiers. Besides the aim of obtaining new selectivities, new pseudostationary phases were introduced because of this problem. Stable structures were developed which cannot break down when using higher contents of organic modifiers. This is the case for example for resorcarenes as additives where polyaromatic hydrocarbons (PAHs) were separated in a buffer containing a resorcarene and 50% acetonitrile [136]. Dyes as separation additives which offer charge-transfer in-

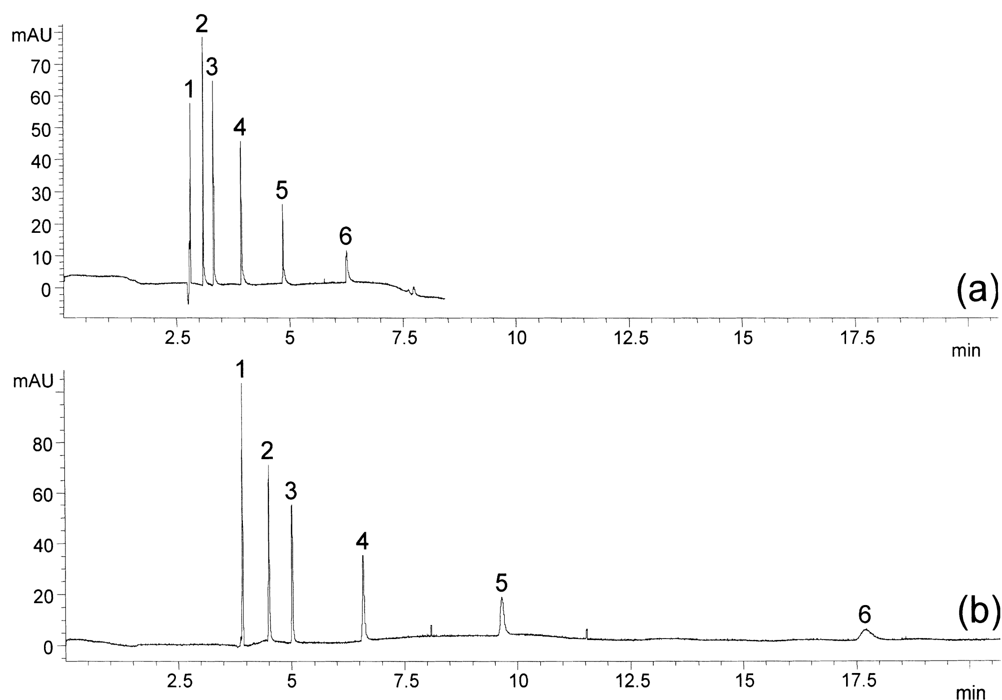

Fig. 4. MEKC separation of ω-phenylalcohols on (a) bare fused-silica and (b) Tentacle-8 [57] coated fused-silica. Separation buffer, 1% poly-SUOBS (in-house synthesized from 10-undecene-1-ol according to Ref. [159] and polymerized according to Ref. [105]), 10 m*M* phosphate (pH 9); capillary, 60/51.7 cm×50 μm I.D.; field, 500 V/cm; detection, UV at 205 nm. Peak assignment: (1)–(6) homologous series of ω-phenylalcohols. Reproduced with permission from Ref. [57].

teractions with the analytes [111–113] are also very suitable with higher contents of organic modifiers. A polymeric dye [poly(vinylamine) sulfonate anthrapyridone, (Poly R-478)] was used with buffer methanol contents up to 70%. The separation of some amino/nitro aromatics with Poly R-478 and 40% methanol is shown in Fig. 5 [113]. Micellar structures, stable in buffers with high organic modifier content, have also been developed [104–106,137–140]. SDS-analogous monomeric surfactants with a double bond at the alkyl chain end are polymerized to micelle polymers assuming a resulting structure similar to micelles formed by monomers. These micelle polymers were used with high contents of acetonitrile or methanol for the separation of PAHs [139] and polychlorinated biphenyl (PCB) congeners [140]. The separation of six fat-soluble vitamins, not possible in common SDS–MEKC, is shown in Fig. 6 using polymerized sodium 10-undecenyl sulfate (poly-SUS) in a buffer with 40% 1-propanol.

But most common separation additives cause problems with mass spectrometric (MS) detection because surfactants can contaminate the ion source and produce a high background whereby sensitivity is lowered tremendously. Nevertheless, direct coupling of MEKC to MS has been reported using SDS as surfactant [141,142]. In most cases, however, special techniques were used to overcome this problem. One possibility is the application of special surfactants as high-molecular-mass surfactants [143], polymerized surfactants as the mentioned poly-SUS [144,145] and vaporizable fluorinated surfactants [146]. Other possibilities are the use of a special atmospheric pressure chemical ionization (APCI) interface [147,148], anodically migrating micelles [149] or the partial filling (PF) technique [119,150–154]. A survey of MEKC-MS coupling is given by Yang and Lee [155].

Similarly, the partial filling technique can be used to overcome UV-detection interferences. Because UV-detection is a widespread detection method, a lot

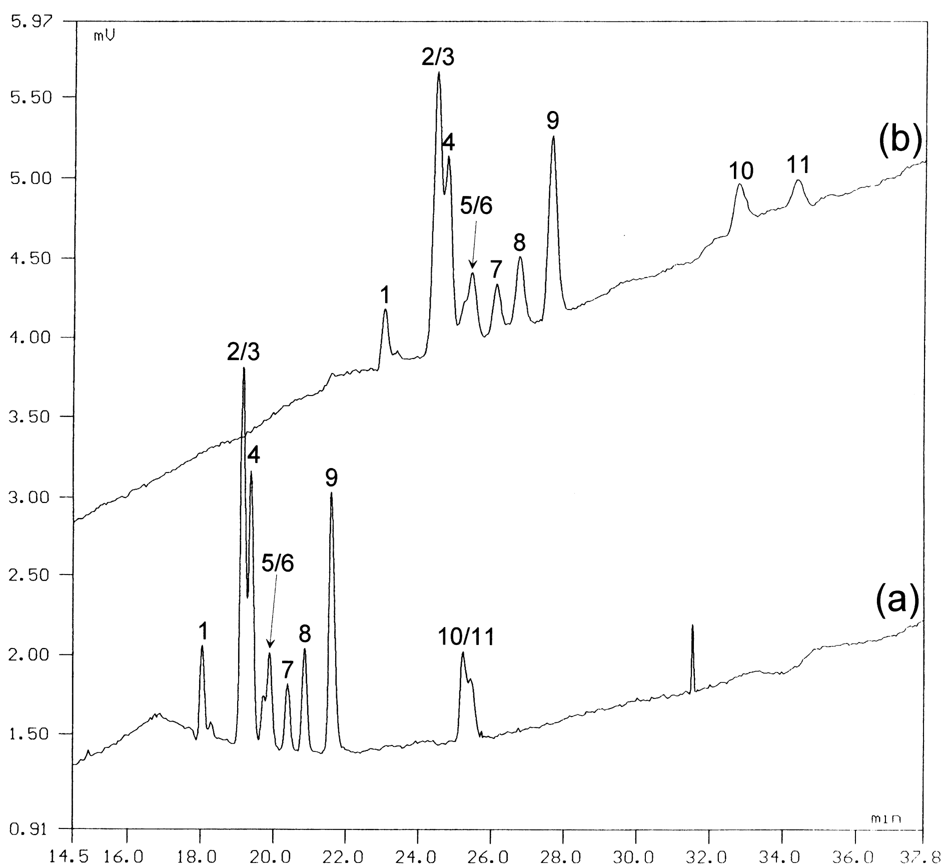

Fig. 5. Effect of increased counter pressure on the separation performance of a mixture of amino/nitro aromatics using the polymeric dye Poly R-478 as separation additive in PF-EKC. (a) 10 mbar and (b) 25 mbar counter pressure. Separation buffer, 10 mg/ml Poly R-478 (Sigma, Steinheim, Germany), 20 m*M* borate (pH 9.4), 40% MeOH; filling degree, 100% of L_{eff}; background buffer, 25 m*M* borate, 10% MeOH; capillary, 75/47 cm×50 μm I.D.; field, 400 V/cm; detection, UV at 254 nm. Peak assignment: (1) 2,6-diaminotoluene, (2) 2,3-dinitrotoluene, (3) 3-nitroaniline, (4) 2,6-dinitrotoluene, (5) 2-nitroaniline, (6) 4-nitroaniline, (7) 4-nitrotoluene, (8) diphenylamine, (9) 2,4-dinitrotoluene, (10) 2-amino-4,6-dinitrotoluene, (11) 2-aminonaphthalene. Reproduced with permission from Ref. [113].

of potential separation additives are excluded as they are UV-active. For example proteins [16,17,122,156], calixarenes or resorcarenes [136,157,158] and charge-transfer interacting additives [111–113] belong to this group. The partial filling (PF) technique was first applied by Valtcheva et al. [156] for the enantioseparation of β-blockers. The method was modified and further developed for the separation of chiral compounds [16,17,119,122,124,125] and the separation of nitroaromatic compounds by our group [111–113] using charge-transfer interacting additives as separation additive. In this technique the capillary is first filled with the pure buffer solution (background buffer, BB) which does not interfere detection followed by the injection of a buffer solution containing the separation additive (separation buffer, SB). The movement of the separation buffer zone towards the detection window can be slowed down or counterbalanced by an appropriate counter pressure, p_{cp}. In practice, efficiency is often dramatically reduced when applying the PF technique which can be explained by the boundary between the separation buffer and the background buffer. On one hand, band broadening can occur at analyte crossing of the zone boundary. On the other hand efficiency is effected by distorted flow profiles in the buffer zones in case of different electroosmotic flow velocities in the two buffer zones. These distorted flow profiles are caused

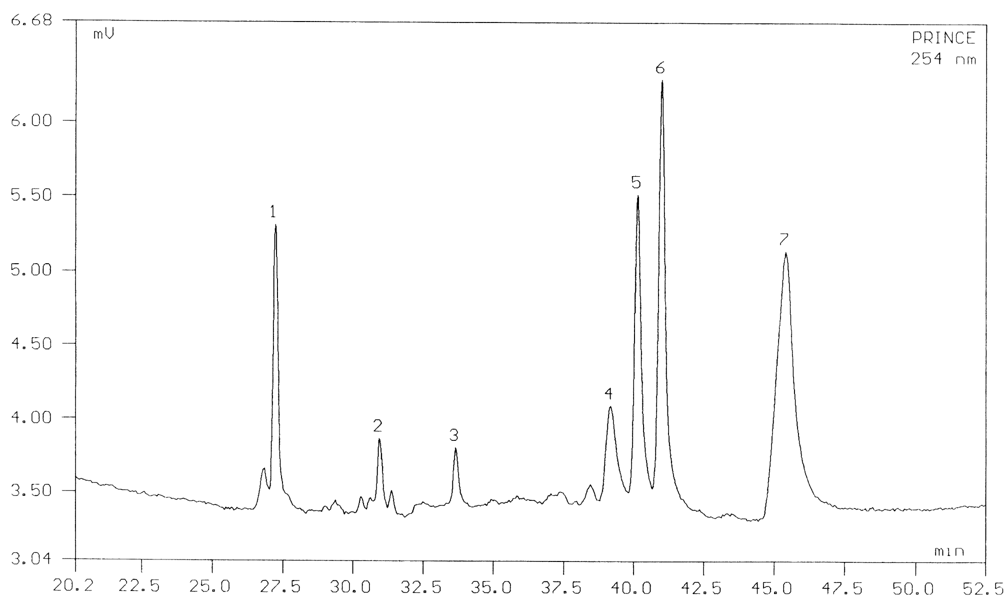

Fig. 6. MEKC separation of fat-soluble vitamins using a micelle polymer in a buffer with high content of organic modifier. CE-equipment, as described elsewhere [58]; separation buffer, 0.5% poly-SUS (in-house synthesized from 10-undecene-1-ol by reaction with chlorosulfonic acid and polymerized according to Ref. [105]), 10 m*M* borate (pH 9.4), 40% 1-propanol; capillary, 75/47 cm×50 μm I.D.; field, 400 V/cm; detection, UV at 254 nm. Peak assignment (plate numbers in parentheses): (1) acetone (EOF-marker), (2) vitamin A alcohol (147 000), (3) vitamin A acetate (101 000), (4) vitamin E (42 000), (5) vitamin D_2 (132 000), (6) vitamin D_3 (144 000), (7) vitamin K_1 (26 000).

by a flow-equalizing intersegmental pressure, p_i, arising at the boundary. In practice, the separation buffer is often prepared by just adding the separation additive to the background buffer, resulting in a higher ionic strength and a lower electroosmotic flow velocity of the separation buffer zone. The schemes for such a buffer combination and a buffer combination with equalized EOFs are depicted in Fig. 7. The velocity difference between the buffer zones causes a small intersegmental pressure (Fig. 7a) which accelerates the slower moving separation buffer and slows down the faster moving background buffer. This generates a parabolic distortion of the electroosmotic plug profile in each buffer zone as shown in Fig. 7a. Compared to the case with equalized EOFs (no intersegmental pressure, Fig. 7b), efficiency is here dramatically reduced (see Fig. 8a). But plate numbers can be improved by the application of weak counter pressures (see Fig. 8b) because the counter pressure reduces or even compensates for the parabolic distortion in the separation buffer (Fig. 7a). Of course, counter pressure reduces the efficiency in case of equalized EOFs (see Fig. 8b) because of the parabolic distortion of the plug profile (Fig. 7b). A detailed discussion is given in Ref. [58]. The consequence drawn out of these experiments is that it is important to equalize the electroosmotic flows of the buffers to gain maximum efficiency. But the intersegmental pressure does not only depend on the magnitude of the velocity difference but also on the relative buffer zone lengths and the total length of the capillary [59].

3. Summary

It was shown that the small diffusion coefficient is the biggest hindrance for efficient separations in an acceptable time in HPLC. Alternatives such as OTLC and CEC are often limited by long analysis times and demanding apparative requirements. On the other hand, MEKC is an interesting alternative among the liquid phase separation methods. Easy to perform, it offers fast and efficient separations

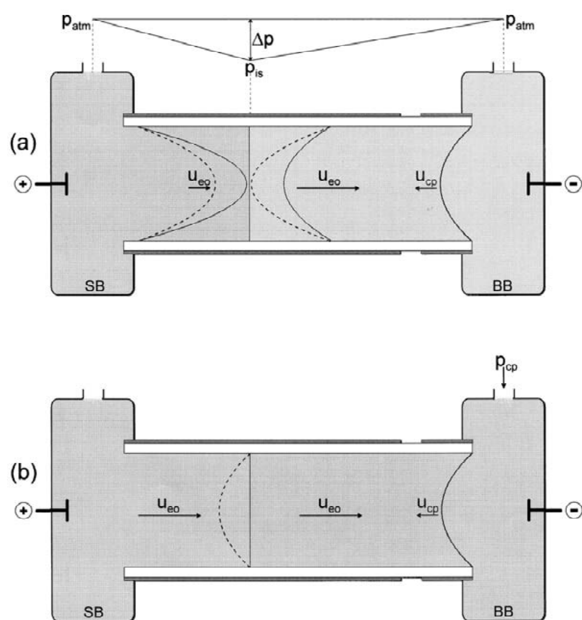

Fig. 7. Schemes of two partial filling systems with (a) u_{eo} (SB) < u_{eo} (BB) and (b) u_{eo} (SB) = u_{eo} (BB). (———) Initial flow profiles without counter pressure; (- - -) flow profiles formed by the superposition of the initial flow profiles with the profile of the laminar flow component, u_{cp}, which is caused by counter pressure. Reproduced with permission from Ref. [58].

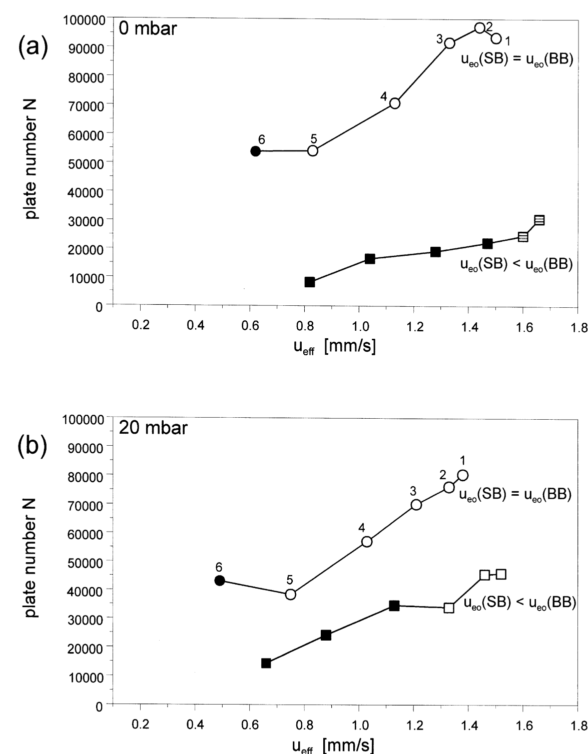

Fig. 8. Comparison of plate numbers obtained from the partial filling systems shown in Fig. 7 (a) without counter pressure and (b) with a counter pressure of 20 mbar. Separation buffer, 15 m*M* SDS, 15 m*M* borate (pH 9.4), 10% MeOH; filling degree, 60% of L_{eff}; background buffer, ■ 10 m*M* borate (pH 9.4), 10% MeOH and ● 20 m*M* borate (pH 9.4), 10% MeOH; capillary, 75/47 cm × 50 μm I.D.; field, 400 V/cm; detection, UV at 205 nm; symbol fillings, full symbol, detection in the separation buffer zone; open symbols, detection in the background buffer zone; striped symbols, detection together with the boundary between separation and background buffer; (1)–(6) homologous series of ω-phenylalcohols. Reproduced with permission from Ref. [58].

without technical expense. The disadvantages of this method are rather small and scientists are currently working on improving the method.

It is hoped that further research and development progresses (M)EKC to a method which will find its place in different fields of science and industry.

4. Nomenclature

Symbol explanations

A	non-dimensional constant characterizing the packing quality
B	non-dimensional constant characterizing longitudinal diffusion contribution
C	mass transfer term
d_c	capillary inner diameter
d_p	particle diameter
D_m	diffusion coefficient in the mobile phase
E	electrical field strength
$f(k)$	function of the retention factor
$f(k)_{eo}$	function of the retention factor (electro-driven)
$f(k)_{hd}$	function of the retention factor (pressure-driven)
h	reduced plate height
H	plate height
H_{min}	minimum plate height
k	retention factor
K_i	constant describing the quality of the injection (injection quality factor)
L	column/capillary length

N	plate number
p_0	inlet pressure
Δp	pressure drop
t_R	retention time
u	mobile phase velocity
u_{opt}	optimum mobile phase velocity
ΔU	voltage
$V_{i,10\%tol}$	tolerated injection volume (10% band broadening allowed)
V_p	peak volume
ε	porosity
η	viscosity of the mobile phase
ϑ	tortuosity factor
ν	reduced mobile phase velocity
Θ	tolerated band broadening

Formula used for calculations
General formula

$$f(k)_{hd} = \frac{1 + 6k + 11k^2}{96(1+k)^2}$$

$$f(k)_{eo} = \frac{k^2}{16 \cdot (1+k)^2}$$

$$L = H \cdot N$$

$$t_R = \frac{L}{u} \cdot (1+k)$$

$$\Delta U = L \cdot E$$

$$V_p = \frac{\pi \cdot d_c^2 \cdot \varepsilon \cdot L \cdot (1+k)}{\sqrt{N}}$$

$$V_{i,10\%tol} = \frac{\pi \cdot d_c^2 \cdot \varepsilon \cdot L \cdot \Theta \cdot K_i \cdot (1+k)}{4\sqrt{N}}$$

$$\Theta = \sqrt{\text{tolerated loss of efficiency}}$$

Special formula used for GC

$$u_{opt} = \frac{D_m}{d_c} \cdot \sqrt{\frac{2}{f(k)}}$$

$$H_{min} = 2 \cdot d_c \cdot \sqrt{2 \cdot f(k)}$$

$$\Delta p = \frac{8}{d_c} \cdot \sqrt{p_0 \cdot (u \cdot L \cdot \eta + p_0)} - p_0$$

Special formula used for OTLC

$$H = \frac{B}{u} + C \cdot u = \frac{2 \cdot D_m}{u} + f(k) \cdot \frac{d_c^2}{D_m} \cdot u$$

$$\Delta p = \frac{32 \cdot u \cdot \eta \cdot L}{d_c^2}$$

Special formula used for micro-HPLC and CEC

$$C_{HPLC} = \frac{0.37 + 4.69k + 4.04k^2}{24(1+k)^2}$$

$$C_{CEC} = \frac{0.37 + 4.69k + 4.04k^2}{24(1+k)^2} \cdot \frac{1}{3}$$

$$\nu = \frac{u \cdot d_p}{D_m}$$

$$h = A \cdot \nu^{1/3} + \frac{B}{\nu} + C \cdot \nu$$

$$H = h \cdot d_p$$

$$\Delta p = \frac{800 \cdot u \cdot L \cdot \varepsilon \cdot \eta \cdot \vartheta^2}{d_p^2}$$

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