CHAPTER 5

Capillary Electrochromatography with Open Tubular Columns (OTCEC)

MONIKA M. DITTMANN AND GERARD P. ROZING

1 Introduction

So far, the majority of work reported on capillary electrochromatography (CEC) has been carried out with fused silica capillaries packed with HPLC-type stationary phases. This is understandable as significant gains in separation efficiency and separation impedance are expected theoretically[1,2] if one compares a packed capillary column where the solvent is driven by hydraulic force (pressure-driven) with the electro-driven case where the solvent is driven by an electrical force. This improvement has been confirmed experimentally.[2–17] Moreover, in the electro-driven case, the solvent is dragged through the packed bed with equal force over the length of the column. Therefore, one is not penalized by increasing column backpressure, if the particle size is reduced or the column lengthened as in HPLC. With these benefits so obvious and the technological hurdles for packed column CEC in practice not too high, work in CEC has flourished since 1990.[18–48] The ultimate separation efficiency of the technique has not yet been predicted or achieved. Reports in which 1.5 μm, non-porous, reversed-phase type particles were used in CEC columns mention that 500 000 plates m^{-1} have been obtained.[49]

On the other hand, open tubular liquid chromatography (OTLC) provides the best efficiency and separation impedance under conditions for liquid chromatography. Knox predicted that under optimal conditions with respect to bandspreading and provided that the internal diameter of the capillary is smaller than 5 μm, very high efficiency can be achieved.[50,51] The added benefit of an open tube is its 20–30 times higher permeability. Therefore one can afford to lengthen the capillary without being penalized with insurmountably high column pressure, as in the case for packed columns. So a column efficiency of 500 000 plates m^{-1} achieved in minutes is conceivable.[51]

With these considerations in mind, one may question whether a combination

of electro-driven flow with liquid chromatography in open tubes – open tubular capillary electrochromatography (OTCEC) – leads to a further improvement of the performance of OTLC. It is our intention to establish the magnitude of this improvement based on the expansion of a theoretical model we developed a few years ago.[2] This will be addressed in Section 3.

Capillary electrophoresis (CE), and in particular capillary zone electrophoresis (CZE), has become a very useful separation technique for chemical analysis.[52] The attractive aspects of the method are a simple separation mechanism, based on differences in electrophoretic mobility, high separation efficiency and putatively simple manipulation of selectivity by mere manipulation of the mobile phase (or run buffer). The capillary wall, which is fused silica as in OTLC and CEC will not, in principle, contribute to retention and selectivity of separation, but only serves as a container for the run buffer and may contribute to overall solute and solvent transport if an electro-osmotic flow (EOF) is present. However, it readily becomes clear that the capillary wall does affect separation in CE to a noticeable extent, reflected by poor reproducibility of EOF (and therefore of migration times) and in particular the shape of the eluting zone. This problem becomes particularly apparent in the CZE separation of proteins. A broad array of chemically or dynamically modified fused silica capillaries have been prepared and made available commercially to counteract this problem.

From that observation, one may argue that chromatographic-type interactions are present and play a role in CZE-type separations, affecting migration and peak shape, although these may remain unnoticed because of the low values of the capacity ratio. So in a certain sense, one may see CZE as a mode of OTCEC although with very low k-values. Swedberg and McManigill quantified these effects before CEC became a prominent new separation technique.[53] The magnitude of the chromatographic effects in CZE-type separations based on this work will be reviewed in Section 2.

The open tubular format of a separation column has one main disadvantage compared with packed bed capillary column, namely the very low phase ratio (ratio of the volume of stationary phase and volume of mobile phase). This will limit loadability of OTLC columns and therefore readily compromise efficiency and, in combination with the small detection volume of the technique, the limit of detection of the system. Recently new approaches have been reported in which the surface layer of the capillary is provided with some porosity, which increases the phase ratio significantly without compromising the separation efficiency. These will be discussed in Section 4.

2 Chromatographic Effects in CZE

In CZE, the plate height is given by:

$$H = \frac{l^2}{12L} + \frac{2D_i}{u_i} \tag{5.1}$$

in which l is the length of the sample zone, L is the length of the capillary column from the inlet to the detector window, D_i is the diffusion coefficient and u_i the velocity of the solute. The solute's velocity is given by:

$$u_i = \mu_i E + u_{eof} \tag{5.2}$$

which rearranges to

$$u_i = \frac{\mu_i V}{L_{tot}} + u_{eof} \tag{5.3}$$

in which μ_i is the electrophoretic mobility of the solute, E is the field over the capillary, V is the applied voltage, u_{eof} is the electroosmotic flow velocity and L_{tot} is the overall length of the capillary. In equation (5.1) the first term describes the plate height due to the injection of a finite length sample zone and the second term the dispersion due to static diffusion in the axial direction. Substitution of real values for the sample zone length (1 mm) and the diffusion coefficient 5×10^{-5} cm^2 s^{-1}, at a velocity of 1 mm s^{-1} yields a plate height of 2 μm which leads to 500 000 plates m^{-1}.

In practice, lower plate numbers are often obtained in CZE. There may be several reasons for this. Electrophoretic effects will affect bandwidth, leading to triangular peak shapes. Poor capillary thermostating, leading to radial (or axial) temperature gradients in the capillary, will cause zone broadening. Also, dispersion at sample introduction owing to an inhomogeneous field strength distribution over the capillary inlet is known to affect the bandwidth negatively.[54]

In particular with high-molecular-weight substances such as peptides and proteins or with polar, basic solutes greatly reduced plate numbers and poor peak shapes are observed in CZE. Swedberg and McManigill have attributed this to the effect of both chromatographic retention and adsorption/desorption kinetics on the plate height.[53]

Supposing that the capillary wall in CZE behaves as a retentive layer, and that the adsorption/desorption process is not infinitely fast the HETP equation has to be expanded with two terms according to Giddings:[55]

$$H_{trans} = \frac{k^2}{(1+k)^2} \frac{r_c^2 u_i}{4D_i} \tag{5.4}$$

$$H_{kin} = \frac{2k}{(1+k)^2} \frac{u_i}{k_d} \tag{5.5}$$

Equation (5.4) gives the plate height contribution of the resistance to trans-channel mass transfer when the solvent is driven by EOF. Equation (5.5) describes the plate height contribution of the adsorption/desorption kinetics. In equations (5.4) and (5.5), k is the chromatographic capacity ratio, r_c is the capillary radius and k_d is the first order rate coefficient for the adsorption/

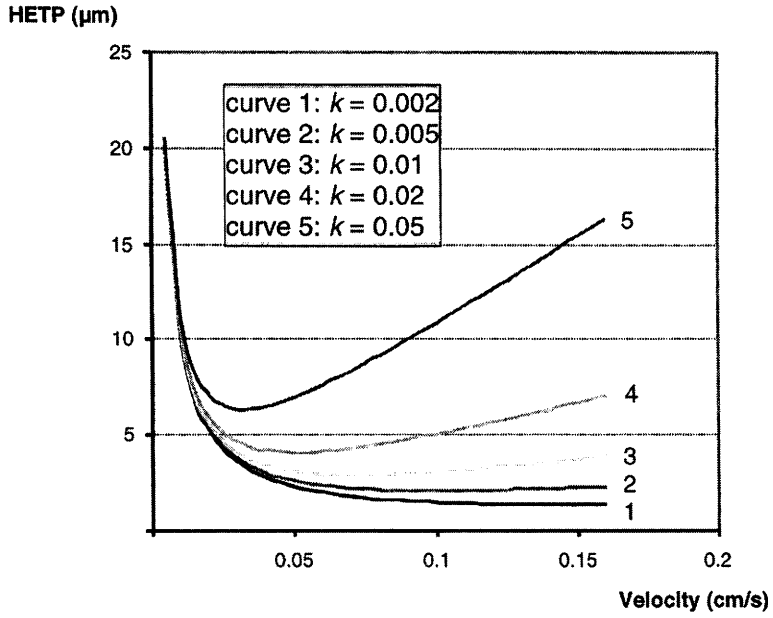

Figure 5.1 *HETP* versus *linear velocity calculated from equation (5.6). Diffusion coefficient is* 5×10^{-5}, *rate constant is* $10\ s^{-1}$ *(values typical for proteins)*

desorption process. In combination they lead to the following expression for the plate height:

$$H = \frac{l^2}{12L} + \frac{2D}{u} + \frac{uk}{(1+k)^2}\left[\frac{r_c^2 k}{4D_i} + \frac{2}{k_d}\right] \qquad (5.6)^*$$

With this expression, and substitution of meaningful values for diffusion and rate coefficients, the graphs shown in Figure 5.1 were obtained.

Figure 5.1 clearly reveals the effect of chromatographic retention on the HETP in CZE. At a velocity of $1\ mm\ s^{-1}$ a capacity ratio as small as 0.05 increases the HETP almost by a factor of 10 and therefore reduces the plate number by a factor of 10. If the rate constant of the adsorption/desorption process increases, the disastrous effect on HETP is mitigated, but still a factor 3 remains if the rate constant increases by a factor of 10. Lower diffusion coefficients, *e.g.* for macromolecules will also aggravate the effect.

McManigill and Swedberg have also tried to determine the relative contributions of the resistance to mass transfer and the rate constant terms to the overall HETP value obtained for a number of model protein substances. Their finding was, not unexpectedly, that for solutes with highest retention in CZE, the mass

*This equation is misprinted in the work by McManigill and Swedberg.[54] The above expression has been verified as correct with the authors.

transfer term dominates and for solutes with slow adsorption/desorption kinetics the kinetic term contributes.

3 Theory of Dispersion and Achievable Efficiency in OTLC and OTCEC

In the following treatment, the capillary will have a retentive layer and act as an open tubular capillary column. Two modes of operation will be conceived, *i.e.* pressure-driven and electro-driven mobile phase. In contrast to the previous section, where retention was an undesirable phenomenon, in this case retention is desired. Migration of the solutes owing to electrophoretic mobility is supposed to be absent when neutral solutes are used. Electrophoretic mobility of the solutes will complicate the description of zone dispersion. So this description applies to neutral solutes, which are separated by partitioning.

The plate height contributions for open tubular liquid chromatography are given by:

$$H = H_{\text{diff}} + H_{\text{film}} + H_{\text{trans}} + H_{\text{stat}} + H_{\text{kin}} \tag{5.7}$$

These terms have already been described in the previous section except for H_{film} and H_{stat}. These are the plate height increments caused by film resistance at the boundary of the retentive layer in the capillary and the contribution of resistance to mass transport by diffusion through the layer of stationary phase. The contribution by H_{film} can be neglected. The term H_{stat} is strongly dependent on the thickness of the retentive layer. For this discussion it is supposed that the retentive layer is a monomolecular layer of a bonded silane (C_{18}) to the FS. In this case also H_{stat} can be neglected. All kinetic effects are accounted in the contribution H_{kin}.

Therefore equation (5.7) reduces to

$$H = H_{\text{diff}} + H_{\text{trans}} + H_{\text{kin}} \tag{5.8}$$

The only factor in this equation that will be influenced by the flow velocity profile is H_{trans}. The expression for H_{trans} therefore will be different for pressure-driven and electrically driven flow.[56]

$$H_{\text{t,lam}} = \frac{(11k^2 + 6k + 1)}{(1+k)^2} \frac{r_c^2 u}{24 D_i} \tag{5.9}$$

$H_{\text{t,lam}}$ is the contribution to plate height from resistance to mass transfer for pressure-driven flow. The same contribution for EOF has been already given in equation (5.5). Thus, two HETP equations can be given (equations 5.6 and 5.10), for OTLC and OTCEC. Because the contribution to HETP by the injection zone width is also negligible, this term was eliminated and the

following two equations were used to calculate *H versus u* curves for OTCEC and OTLC:

$$H = \frac{2D_i}{u} + \frac{uk^2}{(1+k)^2}\frac{r_c^2}{4D_i} + \frac{2uk}{(1+k)^2 k_d} \tag{5.10}$$

$$H = \frac{2D_i}{u} + \frac{(11k^2+6k+1)}{(1+k)^2}\frac{r_c^2 u}{24D_i} + \frac{2uk}{(1+k)^2 k_d} \tag{5.11}$$

The results are given in Figure 5.2.

The minimum value of the *H versus u* curve is similar in both cases. For example for a solute with $k = 2$, the HETP minimum decreases by *ca.* 33% from 7.5 μm in OTLC to 5 μm in OTCEC. More pronounced though is the HETP improvement at higher velocities. For example the same solute at a velocity of 5 mm s^{-1} will have an HETP value of 24 μm in OTLC and 10 μm in OTCEC. With that observation in mind, one may consider the use of wider i.d. capillaries for OTCEC. This will definitely improve UV–Vis detection because of a longer path-length. Therefore in Figure 5.3, the *H versus u* curve in OTCEC mode is calculated for the case where the capillary is doubled to 20 μm. All other conditions were kept the same.

Comparing the *H versus u* curves in Figures 5.3 and 5.2(a) leads to the interesting observation that the two curves are very similar. Therefore, one may state that owing to the more favorable flow velocity distribution in the electro-driven case compared with the pressure-driven one – plug flow *versus* parabolic flow velocity profile – the capillary i.d. for OTCEC can be increased by a factor of almost two without severe penalty in HETP and therefore plate number. The benefits of EOF for capillary LC become most prominent at high velocities. The

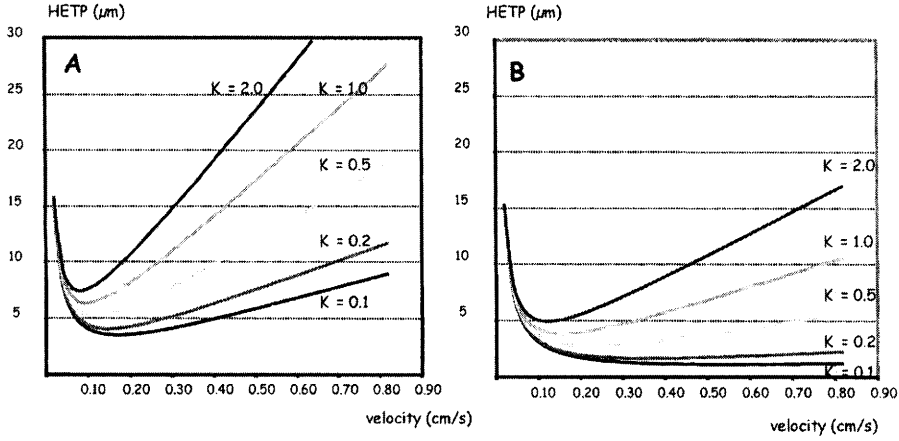

Figure 5.2 *Calculated* H versus u *curves* (A) *according to equation (5.11) for OTLC and* (B) *from equation (5.10) for OTCEC. Diffusion coefficient,* 1.5×10^{-5} cm^2 s^{-1}*; rate constant,* 2500 s^{-1}*; capillary i.d.,* 10 μm

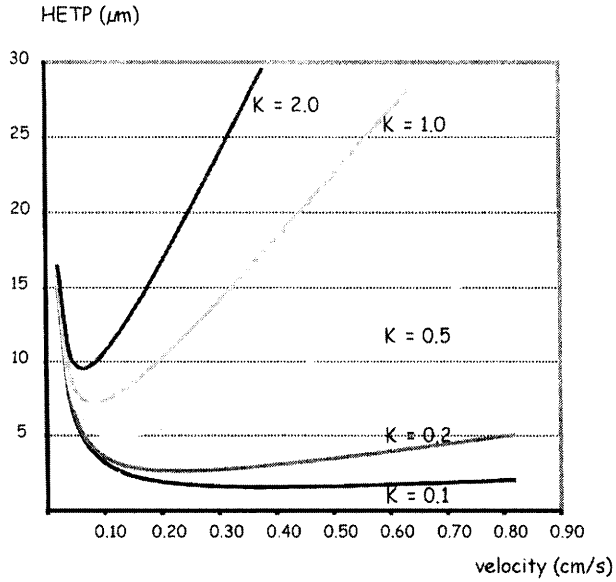

Figure 5.3 *Calculated* H versus u *curve for OTCEC with a capillary i.d. of 20 μm. All other conditions and parameters as for Figure 5.2*

question though is, what velocity can be achieved in OTCEC under the constraint that a maximum voltage of 30 kV is available in practice?

The voltage that is needed to reach a certain velocity in a capillary column of length L is given by:

$$V = \frac{uL\eta}{\varepsilon_0 \varepsilon_r \zeta} \tag{5.12}$$

and for a pressure-driven case the pressure is given by

$$\Delta P = \frac{32ul\eta}{d_c^2} \tag{5.13}$$

These calculations were done for the same capillary column as used in Figure 5.2. Therefore the HETP values for this capillary column in the pressure- and electro-driven mode at the particular velocity are also available. These data are summarized in Figure 5.4.

A zeta potential of 35 mV was used in the calculation. It was assumed that the fused silica capillary has a mono-molecular C_{18} silane layer bonded to its surface. The value of the zeta potential is close to experimental values measured by Dittmann and Rozing for silica-based RP column[56] and lower than the 100 mV that one may expect for a bare fused silica tube.

Figure 5.4 shows again that at the same velocity the capillary column in

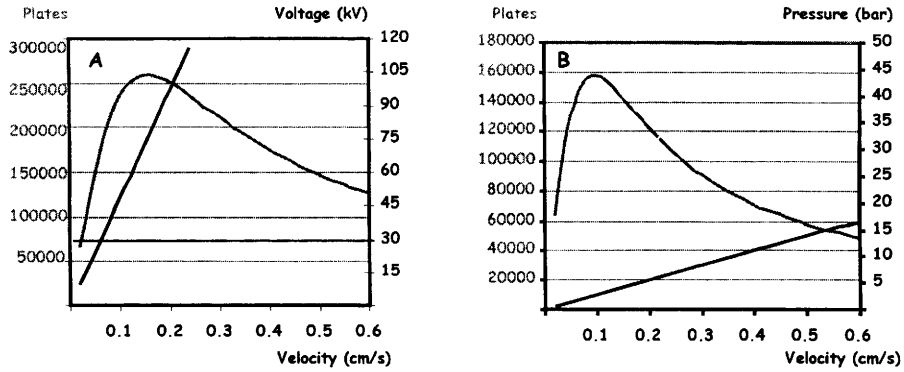

Figure 5.4 *Calculation of* (A) *the voltage or* (B) *the pressure required to drive the mobile phase, and the plate number obtained.* k = *1; diffusion coefficient* D_i = *2500* s^{-1}*; viscosity* η = *0·87 cP; capillary length* L = *1 m; capillary i.d.* = *10* μ*m, zeta potential* ζ = *35 mV*

electro-driven mode, delivers 2–2.5 times more plates. On the other hand, the figure also shows that the maximum speed that can be obtained in electro-driven mode under these conditions is 0.06 cm s^{-1}. In the pressure-driven mode, though, only 16 bar is required to drive the mobile phase through the column. So in the pressure-driven mode one has the option to increase the length of the column and the velocity of the mobile phase by increasing the pressure and thus obtain the same number of plates in the same time in OTLC as in OTCEC.

This has been calculated for the following example. Suppose one wants to obtain 200 000 plates in a 10 μm i.d. open capillary coated with an ODS layer. The solvent contains 70% acetonitrile and the solute has k = 0.1. The values for all other parameters used to calculate the HETP value at a particular velocity are as given in Figures 5.2–5.4. In this calculation the column is elongated so that, with the desired velocity and the resulting HETP value, 200 000 plates are achieved. Further the voltage and pressure to drive the solvent through the column are calculated as well as the time it takes for the solute with k = 0.1 to elute. The results of these calculations are given in Figure 5.5.

A clear observation can be made. Under these conditions in OTCEC mode, one obtains the required plate number, in the following way. In order to generate 200 000 plates per column, for example, at 0.2 cm s^{-1}, a capillary length of 34 cm is required. Under these hypothetical conditions, one needs 30 kV to obtain this velocity. A migration time of 3.1 min is predicted for the solute with k = 0.1 [see right hand y-axis in Figure 5.5(a)].

In the OTLC mode of operation one needs a capillary length of *ca.* 72 cm to obtain the required plate number but only a pressure of *ca.* 31 bar to obtain the velocity of 0.2 cm s^{-1}. The migration time for the solute with k = 0.1 doubles to 6.6 min.

However, a capacity ratio 0.1 is not a very practical value in liquid chromatography. The next example uses a capacity ratio 1.0. As was seen

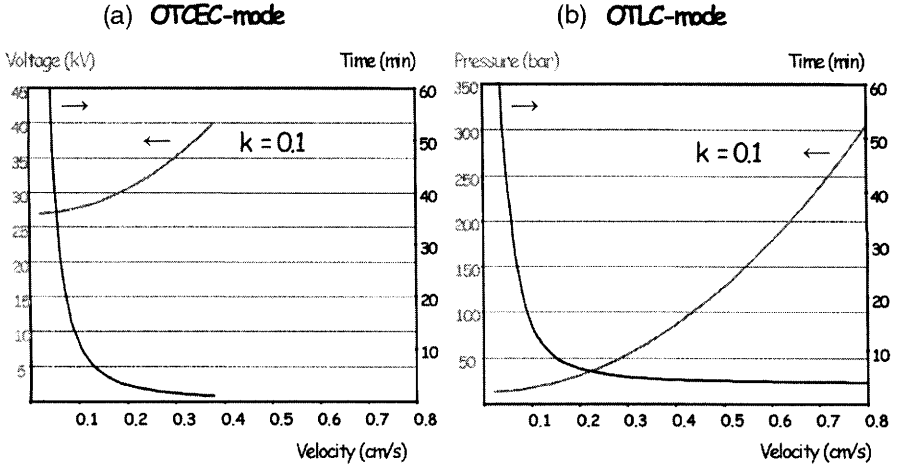

Figure 5.5 (a) *Plot of voltage required in OCTEC and* (b) *plot of pressure required in OTCL to obtain 200 000 plates, and analysis time for a solute with* k = *1* versus *velocity of the solvent. Capillary i.d. – 10 μm; solvent is 70% acetonitrile in dilute buffer. Other parameters as given in Figures 5.2–5.4*

before in Figures 5.2 and 5.3, the HETP values quickly increase owing to the growing contribution of trans-channel diffusion to the HETP. In Figure 5.6 $k = 1$ has been inserted in the equations.

As seen before, with an increase in k value, the HETP values also increase. As a consequence one needs a longer capillary column to achieve the required plate number, in this case 200 000. Now the 30 kV constraint becomes an obstruction for OTCEC. With maximum applied voltage 30 kV, the field strength in the long capillary decreases and a low velocity is obtained. For example, at 30 kV a velocity of approx. 0.05 cm s^{-1} occurs in a capillary with sufficient length to generate 200 000 plates. The migration time for the solute with $k = 1$ will be close to two hours.

On the other hand, in OTLC mode, one can operate the capillary column at 400 bar. With a capillary length of 357 cm, a velocity of 0.5 cm s^{-1} can be achieved with the required plate number. The migration time of a solute with $k = 1$ will be *ca.* 23 min under these conditions.

The intrinsically higher efficiency of open tubular liquid chromatography in the electro-driven mode will allow using shorter capillaries to obtain the required efficiency in shorter time than in OTLC at low k values. On the other hand, the OTCEC system is constrained by the availability of the maximum voltage that can be delivered (30 kV in commercial CEC instruments). Further lengthening of the column will lead to a reduction in velocity and therefore increase of migration time.

This treatment demonstrates that because of the more favorable flow velocity distribution in capillary columns where the solvent movement is propagated by electrical field force, the overall HETP value does indeed decrease. However,

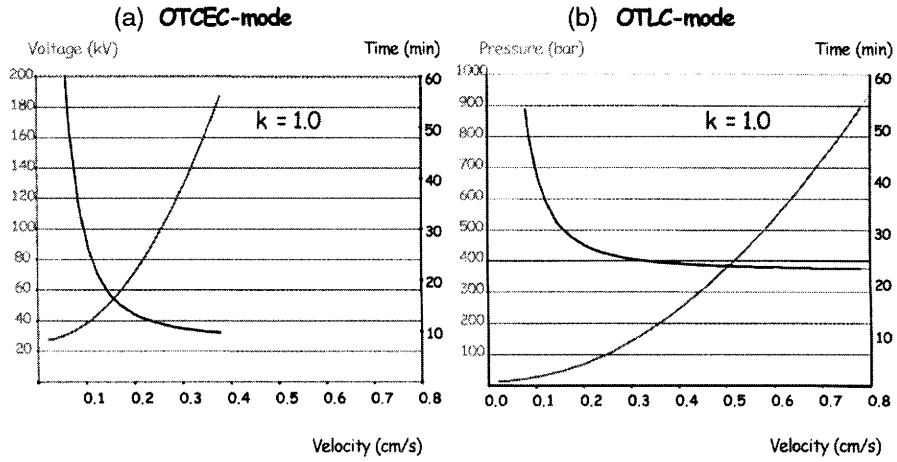

Figure 5.6 *Plots as for Figure 5.5, but with* k = *1*

owing to the 30 kV constraint in practical and commercial CEC equipment, this higher efficiency can be exploited only in relatively short capillaries and at low k values. In this case, the analysis time will be consequently very short. So one may argue that OTCEC will be eventually best suited for high-speed, high-efficiency capillary separations. In this mode the capillary i.d. can even be doubled compared with a pressure-driven mode. Because a high electrical field is more easily achieved in planar, microfluidic separation systems, one may anticipate that this field will eventually benefit.

OTLC on the other hand will eventually be a technique equivalent to capillary GC when long capillary columns at high mobile phase velocity are used. Decreasing the capillary i.d. will favor the OTLC mode.

It should, however, be kept in mind that this treatment applies to solutes that are uncharged and separate by partition chromatograph. When the solutes are charged, their electrophoretic mobility will add another component to their overall velocity. Also, the inevitable electrophoretic contributions to zone broadening affect the above treatment. It is easy to predict that systems that combine both modes of driving the solvent through a separation capillary or microfluidic channel will lead to the most powerful separation devices. The high separation efficiency and additional manipulation of selectivity by the application of the electric field will extend the capabilities of such devices beyond what is currently achievable.

4 Improving the Phase Ratio for OTCEC

The low phase ratio of the column, besides the low sensitivity of UV–Vis absorption detection, remains the main problem in OT capillary LC separation methods and leads to low sample capacity and loadability. This problem has been described and discussed by several authors in conjunction with OTLC.

Poppe and co-workers have addressed the issue by using thick layer polymeric phases on the capillary wall.[57–59] The drawback of such a phase, however, is the low diffusion coefficient of the solute in the retentive layer. This leads to a significant contribution to the overall HETP for retained solutes (just the term that was eliminated in the discussion at the beginning of Section 3). However it is questionable whether such a capillary column will have the surface charge necessary to generate a zeta potential and therefore an EOF.

To date, three different approaches have been published to increase the phase ratio of a capillary column and at the same time maintain an EOF. These approaches will be discussed here.

The phase ratio β of an open tubular capillary column can be estimated from:

$$\beta = \frac{r_c^2}{(r_c - \delta)^2} - 1 \tag{5.14}$$

in which δ is the stationary phase film thickness. For a 10 μm i.d. OT capillary column with a coating that is a monomolecular layer of an alkylsilane (2.5 nm) a phase ratio of 0.001 is calculated. For a packed column, a phase ratio of 0.1 is typical. If a thick stationary phase layer of say 0.1 μm is deposited on the surface of the capillary the phase ratio increases to 0.04. However, as mentioned before, such thick retentive layers will contribute significantly to band spreading because of the low diffusivity of the solutes in the layer.

Colon and co-workers[60,61] deposited a copolymer of tetraethoxysilane (TEOS) and *n*-octyltriethoxysilane (C_8-TEOS) on the inner surface of 13 μm i.d. fused silica capillaries. The copolymer was generated by acid hydrolysis of a mixture of TEOS/C_8-TEOS by which a sol (a colloidal suspension of very small particles) is formed. The sol gelates and is then transferred into the capillary. After a short time the gel is forced out of the capillary, leaving a film of TEOS/C_8-TEOS copolymer on the surface of the capillary. Overnight thermal treatment at 120 °C hardens the hydrogel and leaves a C_8-modified sub-micron particle silica glass layer on the capillary surface.

An illustration that a truly RP-type retentive layer has been obtained is given in Figure 5.7. Panel A shows the separation obtained for the solutes in a neutral test mixture on a capillary prepared by this method with 13 μm i.d. and a length of 50 cm. Conditions typical for reversed-phase chromatography were applied *i.e.* methanol/aqueous buffer 2/1. Panel B shows the result obtained under the same conditions with a capillary coated with TEOS. The absence of separation demonstrates the effect of the retentive layer in the coated capillary.

The EOF obtained in this case is *ca.* 0.1 cm s^{-1} at 30 kV, which is significantly lower than predicted from Figure 5.4. Therefore one must suppose that under these conditions the interface had a low surface charge. (The pH of the buffer was not specified by these authors.) This also confirms the theoretical finding in Section 3 that the available voltage quickly becomes the limiting factor for speed in OTCEC.

The layer thickness of these capillaries and therefore the phase ratio can be

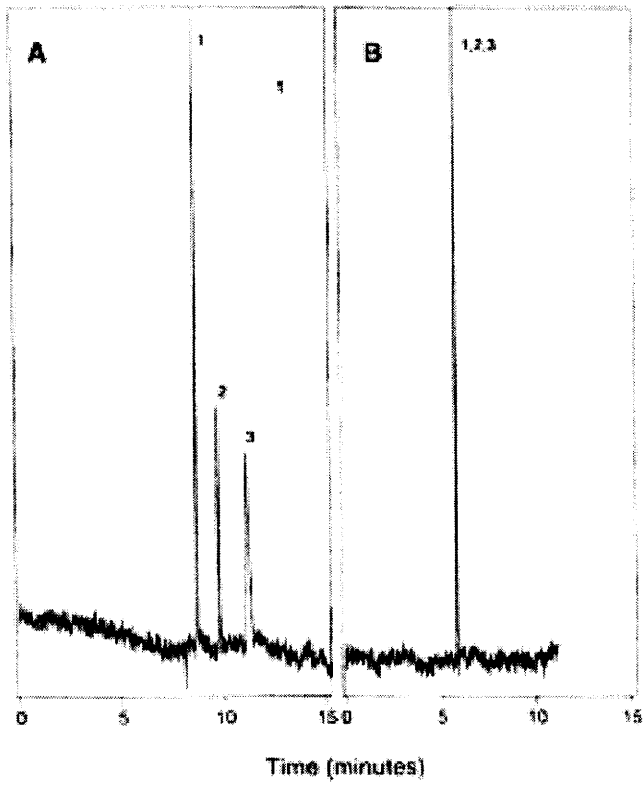

Figure 5.7 *Separation of a text mixture containing three polyaromatic hydrocarbon (PAH) compounds by OTCEC in two different capillaries:* (A) *capillary coated with copolymer C_8-TEOS/TEOS, ratio 0·2;* (B) *capillary coated with TEOS only. Separation conditions: column i.d. 13 μm, length 50 cm (to detector window); mobile phase methanol/1 mM phosphate buffer 2/1, voltage 30 kV; detection 220 nm. Peak identification:* 1, *naphthalene;* 2, *phenanthrene;* 3, *pyrene*
(Reproduced from Y. Guo and L.A. Colon[60] with permission of the publishers of *Anal. Chem.*)

influenced by variation of the ratio TEOS/C_8-TEOS. A linear increase of the capacity ratio of neutral solutes is found when this ratio increases. In practice the increase of retention can be offset by increasing the proportion of the organic solvent in the mobile phase without affecting the efficiency.

A clear demonstration of the effect of the increased phase ratio is given in Figure 5.8. Here the separation of a mixture of five PAH compounds was compared using a capillary column coated *via* the sol–gel process and a capillary column where a monomolecular layer of *n*-octyldimethylsilane was coated in a conventional manner. Under similar conditions to Figure 5.7 the capacity ratios of the solutes were 4–5 times higher with the sol–gel coated capillary (panel A) than with the conventional coated capillary (panel B) even

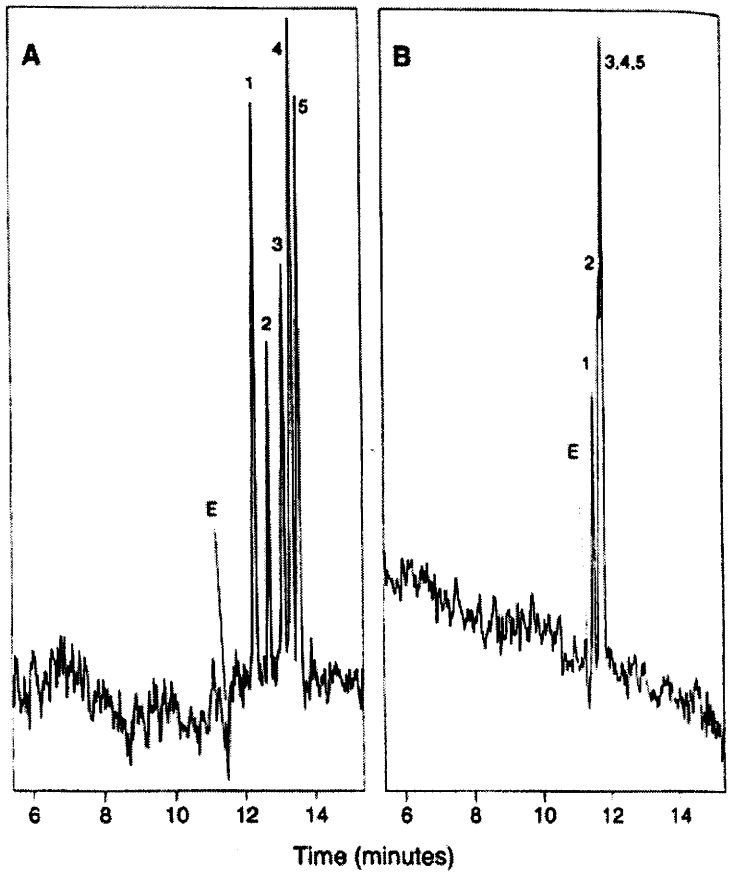

Figure 5.8 *Electrochromatograms of a text mixture of five PAHs.* (A) *Capillary coated by sol–gel method (C_8-TEOS/TEOS ratio 0·4), mobile phase ratio (methanol/buffer) 70/30;* (B) *capillary coated with* n-*octyldimethyloctylchlorosilane, mobile phase ratio (methanol/buffer) 60/40. Separation conditions are given in Figure 5.7. Peak identification:* 1, *naphthalene;* 2, *biphenyl;* 3, *fluorene;* 4, *2-ethylnaphthalene;* 5, *2,6-dimethylnaphthalene. E indicates electroosmotic mobility*
(Reproduced from Y. Guo and L.A. Colon[60] with permission of the publishers of *Anal. Chem.*)

with a lower proportion of methanol used in the mobile phase to increase retention.

The efficiency of the capillaries prepared in this way agrees with the graph in Figure 5.4(A), which supports the theoretical treatment used.

Capillary columns prepared by the sol–gel method showed good longevity and stability after treatment with low and high pH mobile phases for extended periods. This can be attributed to the fact that the retentive layer is not bonded to the surface *via* siloxane bonds as in the conventional bonding procedures but is incorporated in the bulk of the retentive layer.

Pesek and co-workers took a somewhat different approach.[62–64] This group etched the surface of the capillary by hydrolytic treatment of the bare silica with ammonium hydrogen difluoride. After prior treatment with concentrated hydrochloric acid, the capillary was etched at varying temperatures and durations. These parameters determine the morphology of the surface that is obtained in the etching process. The authors found that conditions leading to a spongy, sand-dune kind of surface obtained in the treatment provided the best compromise between an increase of the phase ratio and the robustness of the surface structures obtained (Figure 5.9). The spongy silica surface obtained in this way was modified to a reversed-phase-type layer by a silanization/hydrosilanization procedure pioneered by this group. In this process, first, almost all silanol groups on the fused silica surface are covered by modification with triethoxysilane. This leaves a silicon hydride layer on the surface, which serves as a hook to connect an alkane group *via* a hydrosilation reaction.

The initial work of this group was done with 50 μm i.d. fused silica capillaries. An example is given in Figure 5.10. In Figure 5.10(a) the lack of separation of two proteins, turkey (1) and chicken (2) lysozyme on a bare fused silica capillary at a low pH of the run buffer is demonstrated. The low pH was selected to

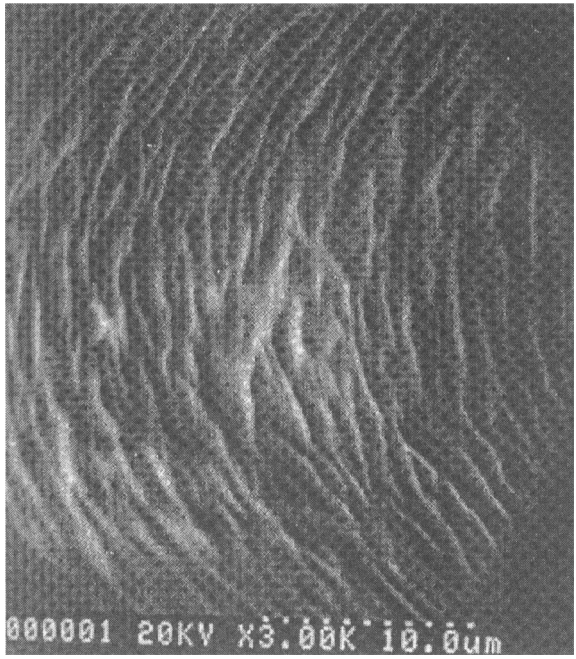

Figure 5.9 *Scanning electron photograph of the inner wall of a fused silica capillary etched with ammonium hydrogen difluoride at 300 °C for 4 h*
(Reproduced from J.J. Pesek and M.T. Matyska[64] with permission of the publishers of *J. Cap. Electrophoresis*)

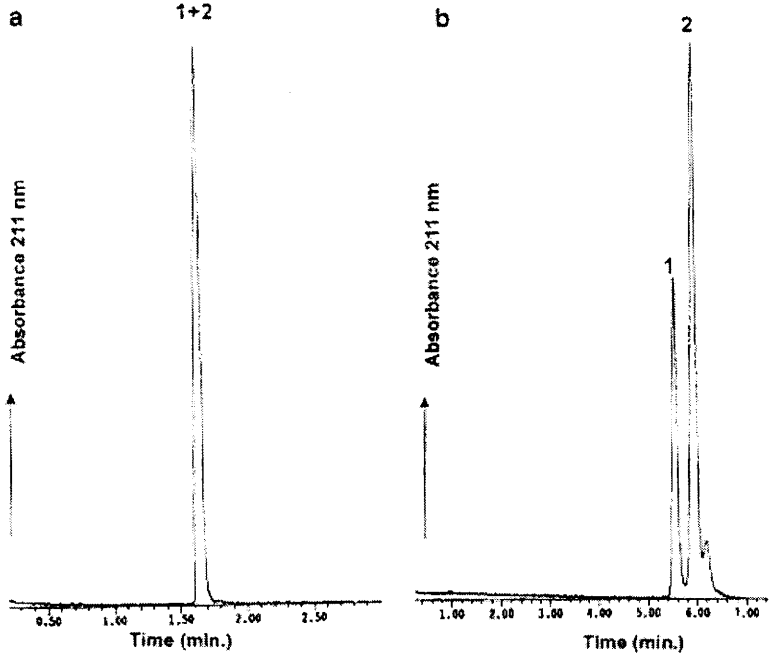

Figure 5.10 *Separation of turkey* (1) *and chicken* (2) *lysozyme on* (a) *a bare capillary and* (b) *a C_{18}-modified capillary. Capillary i.d. = 50 μm, length = 45 cm; run buffer pH = 2.14*
(Reproduced from J.J. Pesek and M.T. Matyska[64] with permission of the publishers of *J. Cap. Electrophoresis*)

suppress the electroosmotic flow so that the selectivity of separation is only related to electrophoretic mobility differences and, in Figure 5.10(b), to chromatographic interactions. The lack of separation in the CE mode is not unexpected because the two proteins differ only in one amino acid. In Figure 5.10(b) the separation was obtained under the same conditions and therefore can only be due to the chromatographic interactions. So in this case, one is dealing with selectivity enhancement of an electrophoretic (CZE)-type separation assisted by chromatographic interaction. As argued in Section 2, the price for retention in CE is loss in efficiency as illustrated in Table 5.1.

The chromatographic interactions of the solute bradykinin with the C_{18}-coated layer on the fused silica inner wall decreases the efficiency and peak shape dramatically. If methanol is added to the run buffer, this reduces the chromatographic interactions by increased solvating power, and increases the efficiency and improves the peak symmetry (last line of Table 5.1).

The EOF velocity found for the etched, C_{18}-modified capillary was very low, and, as in packed column CEC, depends on the mobile phase composition. Figure 5.11 shows the EOF velocity dependence on the proportion of methanol in the run buffer. The plot closely follows the expected track based on the

Table 5.1 *Efficiency and peak symmetry for bradykinin on different capillaries*

Capillary	*Buffer pH*	*Plate number*	*Symmetry*
Bare fused silica	3.7	400 000	1.00
Etched, 300 °C, 3 h	3.7	337 000	0.73
Etched, Si–H modified	3.7	542 000	0.73
Etched, C_{18} modified	3.7	68 000	1.12
Etched, C_{18} modified	3.0	33 600	2.47
Etched, C_{18} modified	3.0 with 10% methanol	79 000	2.22

Efficiency calculated from the width at half peak height. Symmetry calculated at 10% of peak height. Buffer lactic acid/β-alanine pH 3.7. Voltage 25 kV. Capillary length 50 cm (to the detector window), overall length 70 cm, i.d. 50 μm.

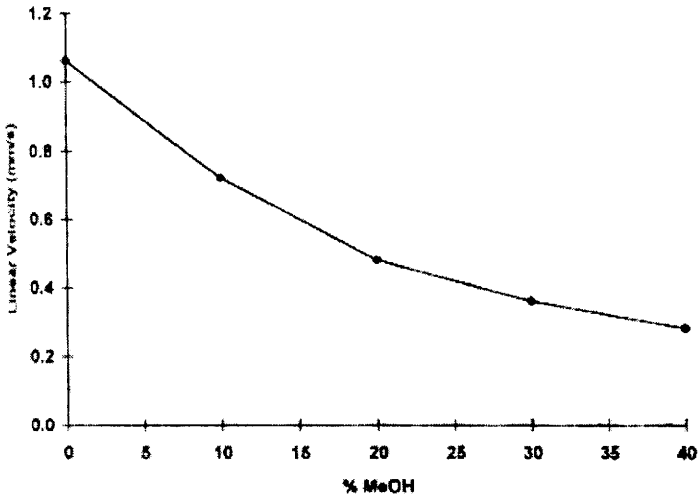

Figure 5.11 *EOF velocity measured with a neutral marker, dimethyl sulfoxide (DMSO), in an etched, C_{18}-modified capillary as a function of the proportion of methanol in the run buffer at pH 2.14*
(Reproduced from J.J. Pesek and M.T. Matyska[62] with permission of the publishers of *J. Chromatogr.*)

change in the quotient of the dielectric constant and the viscosity as reported for packed column CEC.[47]

Nevertheless, this example, and others provided by this group cannot be regarded as an OTCEC experiment but rather as the mode of separation described in Section 2, *i.e.* CE with chromatographic interaction. Because of the large internal diameter of the capillaries used, 50 μm, the phase ratio for chromatography becomes very small. With the same assumptions as at the beginning of this section, phase ratios of 0.0002 and 0.008 are calculated from equation (5.14) for a retentive layer of 2.5 and 100 nm respectively. In addition because of the very low value or absence of EOF there is barely any flow-driven

transport of the solutes through the retentive tube, which is characteristic for chromatographic separation.

In another paper the authors describe the same capillary modification technique applied to much narrower i.d. fused silica capillaries.[65] In this work fused silica capillaries with 20 μm i.d. were used. Quite remarkably, at low values of the pH of the solvent, an anode EOF was observed. This finding indicates the presence of positive charges on the surface at low pH. The authors postulated the presence of ammonium sites responsible for the flow reversal. In practice though this means again that in such kinds of capillaries there will be very low EOF, and therefore a low flow transport contribution for the solutes.

The i.d., lower than that used in the previous work, should however improve the efficiency of separation. The separation of the two lysozymes from turkey and chicken performed under the same conditions as in Figure 5.10 led to the chromatogram in Figure 5.12. A clear improvement of efficiency is indeed observed.

Although, because of the very low (or absent) flow transport, this approach in a pure sense may not be regarded a OTCEC, it is a very promising approach to separations. In particular it may become the method of choice for the separation

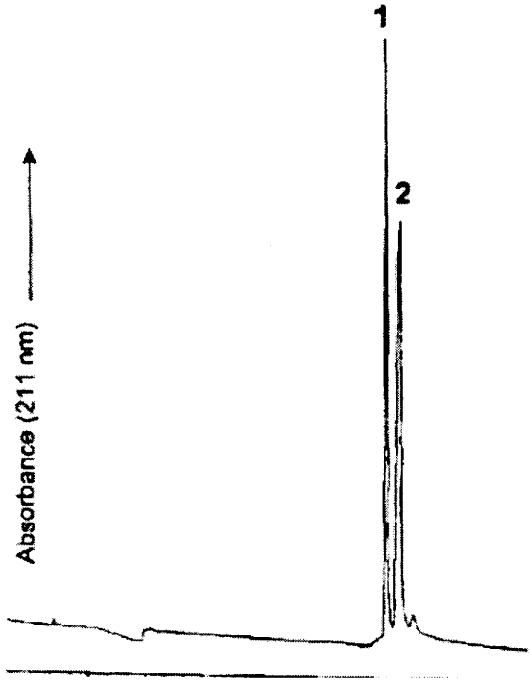

Figure 5.12 *Separation of turkey* (1) *and chicken* (2) *lysozyme on a 20 μm i.d. etched, C_{18}-modified fused silica capillary. Voltage 30 kV; capillary length 51.5 cm; other conditions as in Figure 5.10*
(Reproduced from J.J. Pesek and M.T. Matyska[65] with permission of the publishers of *J. Chromatogr.*)

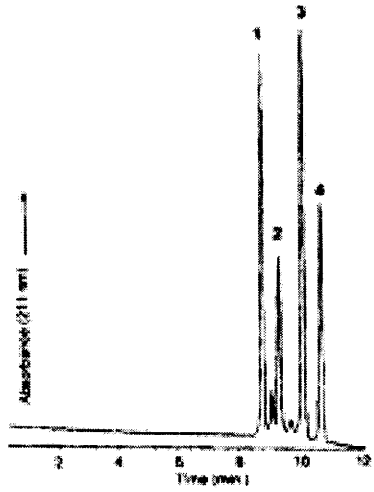

Figure 5.13 *Separation of a mixture of cytochrome c's on 20 μm i.d. etched C_{18}-modified fused silica capillary. Voltage 15 kV; other conditions as for Figure 5.12. Peak identification:* 1, *horse;* 2, *bovine;* 3, *chicken;* 4, *tuna*
(Reproduced from J.J. Pesek and M.T. Matyska[65] with permission of the publishers of *J. Chromatogr.*)

of proteins. This is well illustrated by the chromatogram in Figure 5.13, which shows the separation of 4 cytochrome c's on an etched, C_{18}-modified capillary. Conditions are similar to those in Figure 5.12, though the voltage applied was reduced to 15 kV.

From the EOF *versus* pH plot one would expect an anodic flow at the pH 3.7 that is used in this experiment. So flow transport present will actually oppose the direction of the solutes. This may be a very attractive property of this system, as it keeps the solutes longer in the separation capillary and therefore allows more time for separation.

Remcho and co-workers[66,67] used an approach originally reported by Poppe and co-workers[57–59] to prepare thick layers of polymethacrylate on the inside of a fused silica capillary in an attempt to increase the phase ratio of a capillary column. After hydroxylation of the surface by acid treatment, 3-trimethoxysilylpropyl methacrylate is bonded to the capillary wall. The vinyl group of the acrylate provides a chemical hook for subsequent polymerization with *n*-butyl methacrylate or with a mixture of *n*-butyl methacrylate with 1,4-butanediol methacrylate. The last monomer functions as a cross-linker. In this way, a layer of linear or cross-linked polyacrylate was obtained. This layer was carefully dried and cured at 120 °C in order to obtain a stable film of polyacrylate on the inside of the capillary serving as a reversed-phase type retentive layer.

Varying the concentration of monomer and cross-linker influences the retentive properties of this layer. Increase of the monomer concentration leads to higher capacity factors, although beyond a certain concentration of the

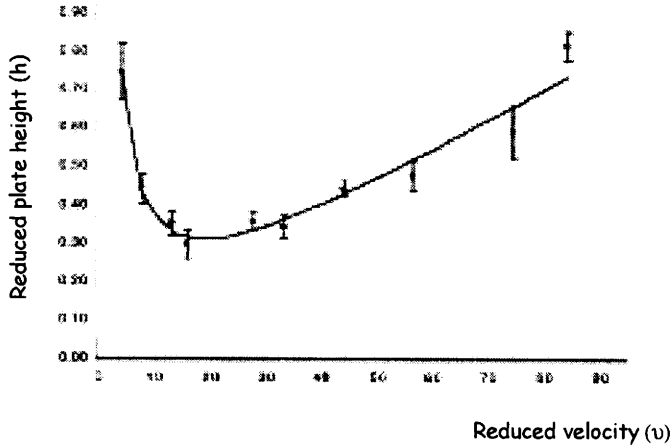

Figure 5.14 *Effect of flow velocity on plate height for an unretained solute (acetone). Capillary with a linear polyacrylate layer prepared with 45% monomer (n-butyl acrylate) concentration; i.d. 25 μm, length to detector 20 cm* (Reproduced from Z.J. Tan and V.T. Remcho[66] with permission of the publishers of *Anal. Chem.*)

modifier retention on linear polyacrylate films started to decrease. The same observation was made when using cross-linked polyacrylate.

Unfortunately, these capillaries also show a relatively low EOF. Values range between 0.1 and 0.2 cm s^{-1} at 30 kV on a 45 cm capillary with a mobile phase of phosphate buffer (pH 7) acetonitrile 4/1.

The work was done with 25 μm i.d. capillaries. A reduced parameter plot (h/υ curve or Knox plot) was obtained and is given in Figure 5.14.

A minimum reduced plate height of 0.3 was found which is an HETP value of 8 μm which is higher than our prediction in Figure 5.3 (although conditions are not very comparable).

The cross-linked phases appeared to have better efficiency for the unretained solute. Plate heights of 3–4 μm were obtained in a 25 μm i.d. capillary with a cross-linked layer of *n*-butyl methacrylate and 1,4-butanediol dimethacrylate. This is in agreement with the predictions of Figure 5.3 for a 20 μm i.d. capillary. However, as expected, for retained solutes the efficiency decreases rapidly by a factor of 2–4 depending on the k of the retained solute. The k itself depends on the monomer/cross-linker concentration in the coating mixture.

In a final experiment, these authors quantitated the effect of pressurized flow on the HETP and indicated the potential of such capillaries when one uses a combination of pressure-driven and electro-driven flow to separate solutes. This is shown in Figure 5.15.

The upper two traces allow a direct comparison of pressure-driven OTLC and electro-driven OTLC. Pressure was set so that the velocity in both cases is the same, illustrated by the equivalence of retention times in both traces. It is clear though that the efficiency of the peaks in the pressure drive case is a factor of 2–3

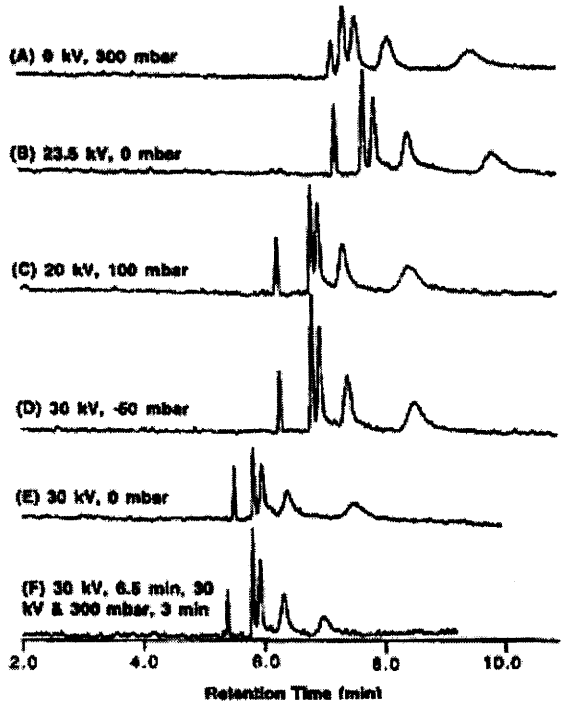

Figure 5.15 *Comparison of OTCEC and OTLC separations. Capillary: cross-linked poly-(*n*-butyl methacrylate), total length 60 cm, length to detector 45 cm. Solutes:* 1, *acetone;* 2, *methyl paraben;* 3, *ethyl paraben;* 4, n-*propyl paraben;* 5, n-*propyl paraben. Solvent: phosphate buffer (pH 7)/acetonitrile 4/1* (Reproduced from Z.J. Tan and V.T. Remcho[66] with permission of the publishers of *Anal. Chem.*)

worse than in the OTCEC mode. In combining pressure and voltage one can have both transport mechanisms cooperating as in trace 3, and gain in speed, or opposing so that one may obtain a longer time for separation.

Also, as expected from the discussion in Section 3, the pressure required to drive the solvent at a particular speed is very low.

5 Conclusions

A few striking conclusions can be derived from the treatment in Sections 2 and 3 and the experimental results described in Section 4.

In OTCEC the flat, plug flow velocity profile generated by electromotive forces has a large impact on zone broadening compared with pressure-driven flow. A significant reduction of the HETP values by a factor of 2–3 for non- or slightly retained solutes is expected theoretically and demonstrated experimentally. As theory predicts, with increase of retention the HETP increases rapidly,

particularly when the capillary i.d. is larger than 10 μm. Nevertheless, one can safely state that because of the more favorable zone broadening in the OTCEC case, one may be able to use a two times larger i.d. capillary than in OTLC, alleviating the poor UV–Vis detection sensitivity caused by the short light path.

On the other hand, the voltage used as a driving force for OTCEC quickly reaches its practical limit of 30 kV when capillaries are lengthened for high efficiency. In this respect, OTLC will easily outperform OTCEC. Electro-driven separations therefore will only be practical with short (<50 cm) capillary columns where high fields are achievable and therefore good velocities are achieved. To achieve very high plate numbers, long capillary columns that run at high velocity in OTLC mode are necessary.

Attempts to improve the phase ratio of capillary columns are penalized by loss of EOF. The attempts described in this chapter all lead to values <0.2 cm s^{-1}. Different coatings may be required.

Finally, a combination of OTLC and OTCEC seems the way to go. In particular because low pressure, <10 bar, is sufficient to obtain a speed of a few millimetres per second in a capillary of 10 μm i.d. Because this capability is available in modern CE instruments, the technique can be exploited without large technical and practical hurdles. The combination of separation mechanisms, namely partitioning and electromigration, promises an excellent way to manipulate selectivity of separation.

Charged solutes will add an electrophoretic velocity component to their transport mechanism. The combination of pressure-driven and electro-driven transport with the solute's electrophoretic migration, in an appropriate way, will provide a very powerful way to tune the selectivity of separation. Practical models to predict retention in such systems will be required for this method to be used in practice. Planar, microfluidic channels seem predestined for such systems. One can expect a large interest in this field in the near future.

Acknowledgement

The authors want to thank their colleague Gordon Ross of the Hewlett-Packard Waldbronn Analytical Division, for helpful comments and corrections.

References

1. J.H. Knox and I.H. Grant, *Chromatographia*, 1987, **24**, 135.
2. M.M. Dittmann, F. Bek, K. Wienand, and G.P. Rozing, *LC-GC*, 1995, **13**, 800.
3. J.W. Jorgenson and K.D. Lukacs, *J. Chromatogr.*, 1981, **218**, 208.
4. J.H. Knox and I.H. Grant, *Chromatographia*, 1991, **32**, 317.
5. H. Yamamoto, H. Baumann, and F. Erni, *J. Chromatogr.*, 1992, **593**, 313.
6. C. Yan, D. Schaufelberger, and F. Erni, *J. Chromatogr.*, 1994, **670**, 15.
7. T. Tsuda, *LC-GC*, 1992, **5**, 26.
8. S. Kitagawa and T. Tsuda, *J. Microcol. Sep.*, 1994, **6**, 91.
9. S. Kitagawa and T. Tsuda, *J. Microcol. Sep.*, 1995, **7**, 59.
10. N.W. Smith and M.B. Evans, *Chromatographia*, 1994, **38**, 649.
11. N.W. Smith and M.B. Evans, *Chromatographia*, 1995, **41**, 197.

12. R.J. Boughtflower *et al.*, *Chromatographia*, 1995, **41**, 398.
13. R.J. Boughtflower *et al.*, *Chromatographia*, 1995, **40**, 329.
14. B. Behnke and E. Bayer, *J. Chromatogr.*, 1994, **680**, 93.
15. B. Behnke, E. Grom, and E. Bayer, *J. Chromatogr.*, 1995, **716**, 207.
16. H. Rebscher and U. Pyell, *Chromatographia*, 1994, **38**, 737.
17. T. Eimer, K.K. Unger, and T. Tsuda, *Fresenius' J. Anal. Chem.*, 1995, **352**, 649.
18. J.L. Liao, N. Chen, C. Ericson, and S. Hjerten, *Anal. Chem.*, 1996, **68**, 3468.
19. C. Yan, R. Dadoo, R.N. Zare, D.J. Rakestraw, and D.S. Anex, *Anal. Chem.*, 1996, **68**, 2726.
20. A.S. Rathmore and C. Horvath, *J. Chromatogr. A*, 1996, **743**, 231.
21. M.R. Euerby, C.M. Johnson, K.D. Bartle, P. Myers, and S.C.P. Roulin, *Anal. Commun.*, 1996, **33**, 403.
22. H. Rebscher and U. Pyell, *J. Chromatogr. A*, 1996, **737**, 171.
23. M.M. Robson, S. Roulin, S.M. Shariff, M.W. Raynor, K.D. Bartle, A.A. Clifford, P. Myers, M.R. Euerby, and C.M. Johnson, *Chromatographia*, 1996, **43**, 313.
24. M.T. Dulay, C. Yan, D.J. Rakestraw, and R.N. Zare, *J. Chromatogr. A*, 1996, **725**, 361.
25. F. Lelievre, C. Yan, R.N. Zare, and P. Gareil, *J. Chromatogr. A*, 1996, **723**, 145.
26. C. Fujimoto, Y. Fujise, and E. Matsuzawa, *Anal. Chem.*, 1996, **68**, 2753.
27. H. Minakuchi, K. Nakanishi, N. Soga, N. Ishizuka, and N. Tanaka, *Anal. Chem.*, 1996, **68**, 3498.
28. J.-L. Liao, N. Chen, C. Ericson, and S. Hjerten, *Anal. Chem.*, 1996, **68**, 3468.
29. M.M. Dittmann and G.P. Rozing, *J. Chromatogr. A*, 1996, **744**, 63.
30. S.E. van den Bosch, S. Heemstra, J.C. Kraak, and H. Poppe, *J. Chromatogr. A*, 1996, **755**, 165.
31. M.R. Taylor, P. Teale, S.A. Westwood, and D. Perrett, *Anal. Chem.*, 1997, **69**, 2554.
32. A. Maruska and U. Pyell, *Chromatographia*, 1997, **45**, 229.
33. S. Nilsson, L. Schweitz, and M. Petersson, *Electrophoresis*, 1997, **18**, 884.
34. M.R. Taylor and P. Teale, *J. Chromatogr. A*, 1997, **768**, 89.
35. L. Schweitz, L.I. Andersson, and S. Nilsson, *Anal. Chem.*, 1997, **69**, 1179.
36. T.M. Zimina, R.M. Smith, and P. Myers, *J. Chromatogr. A*, 1997, **758**, 191.
37. J.M. Lin, T. Nakagama, X.Z. Wu, K. Uchiyama, and T. Hobo, *Fresenius' J. Anal. Chem.*, 1997, **357**, 130.
38. C. Wolf, P.L. Spence, W.H. Pirkle, E.M. Derrico, D.M. Cavender, and G.P. Rozing, *J. Chromatogr. A*, 1997, **782**, 175.
39. M.M. Robson, M.G. Cikalo, P. Myers, M.R. Euerby, and K.D. Bartle, *J. Microcol. Sep.*, 1997, **5**, 357.
40. M.R. Euerby, D. Gilligan, C.M. Johnsson, S.C. Roulin, P. Myers, and K.D. Bartle, *J. Microcol. Sep.*, 1997, **5**, 373.
41. P. Sandra, A. Dermaux, V. Ferraz, M.M. Dittmann, and G.P. Rozing, *J. Microcol. Sep.*, 1997, **5**, 409.
42. J.M. Ding and P. Vouros, *Anal. Chem.*, 1997, **69**, 379.
43. J.H. Miyawa, M.S. Alasandro, and C.M. Riley, *J. Chromatogr. A*, 1997, **769**, 145.
44. G.A. Lord, D.B. Gordon, P. Myers, and B.W. King, *J. Chromatogr. A*, 1997, **768**, 9.
45. C. Ericson, J.-L. Liao, K. Nakazato, and S. Hjerten, *J. Chromatogr. A*, 1997, **767**, 33.
46. M.M. Dittmann and G.P. Rozing, *J. Microcol. Sep.*, 1997, **5**, 399.
47. K.D. Altria, N.W. Smith, and C.H. Turnbull, *Chromatographia*, 1997, **46**, 664.
48. A. Palm and M. Novotny, *Anal. Chem.*, 1997, **69**, 4499.
49. R.M. Seifar, W.Th. Kok, J.C. Kraak, and H. Poppe, *Chromatographia*, 1997, **46**, 131.
50. J.H. Knox and M.T. Gilbert, *J. Chromatogr.*, 1979, **186**, 405.
51. J.H. Knox, *Chromatographia*, 1980, **18**, 453.
52. J.W. Jorgenson and K. Lukacs, *Anal. Chem.*, 1981, **53**, 1298.
53. S. Swedberg and D. McManigill in 'Techniques in Protein Chemistry', ed. T. Hugli, Academic Press, 1989, p. 472.

54. G.P. Rozing, *Am. Lab.*, December 1998, 33.
55. J.C. Giddings in 'Dynamic of Chromatography, Part 1, Principles and Theory', Marcel Dekker, New York, 1965.
56. M.M. Dittmann and G.P. Rozing, *J. Microcol. Sep.*, 1997, **5**, 399.
57. R. Swart, J.C. Kraak, and H. Poppe, *J. Chromatogr.*, 1994, **670**, 25.
58. R. Swart, J.C. Kraak, and H. Poppe, *Chromatographia*, 1995, **40**, 587.
59. R. Swart, J.C. Kraak, and H. Poppe, *J. Chromatogr.*, 1995, **689**, 177.
60. Y. Guo and L.A. Colon, *J. Microcol. Sep.*, 1995, **7**, 485.
61. Y. Guo and L.A. Colon, *Anal. Chem.*, 1995, **67**, 2511.
62. J.J. Pesek and M.T. Matyska, *J. Chromatogr.*, 1996, **736**, 255.
63. J.J. Pesek, M.T. Matyska, and L. Mauskar, *J. Chromatogr.*, 1997, **763**, 307.
64. J.J. Pesek and M.T. Matyska, *J. Cap. Electrophoresis*, 1997, **5**, 213.
65. J.J. Pesek and M.T. Matyska, *J. Chromatogr.*, in press.
66. Z.J. Tan and V.T. Remcho, *Anal. Chem.*, 1997, **69**, 581.
67. Z.J. Tan and V.T. Remcho, *J. Microcol. Sep.*, 1998, **10**, 99.