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Influence of buffer zone concentrations on efficiency in partial filling micellar electrokinetic chromatography

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Abstract

The potential of counter pressure-moderated partial filling micellar electrokinetic chromatography (PF-MEKC) was investigated in this work. Plate numbers of homologous ω-phenylalcohols were measured in a two-plug PF-MEKC system varying the concentrations and hence the ionic strengths of the background buffer compared to the sodium dodecyl sulfate-containing separation buffer and the counter pressure on the cathodic buffer reservoir. It was observed that plate numbers are strongly influenced by both the buffer concentrations and the counter pressure. Highest plate numbers were obtained with a buffer system where the concentrations are adjusted such that the electroosmotic flow velocities in both zones are equal. Differences in the local electroosmotic flow velocities of the zones caused by different buffer concentrations are responsible for tremendously reduced plate numbers. The efficiency drop is explained in several models by the formation of an intersegmental pressure which produces a parabolically shaped laminar flow component in both zones. Thus, the electroosmotic plug-like flow profile is distorted and the efficiency is reduced. The effect of counter pressure on efficiency turned out to be very complex in dependence on the buffer system applied.

Keywords: Micellar electrokinetic chromatography; Partial-filling micellar electrokinetic chromatography; Band broadening; Buffer composition; Flow profiles; Phenylalcohols

1. Introduction

Micellar electrokinetic chromatography (MEKC) introduced by Terabe et al. [1], has proven to be a highly efficient technique for the separation of neutral and charged analytes in capillary electrophoretic systems. The separation is based on different partitioning of the analytes between the free buffer solution and the interior of the charged micelles (called pseudo stationary phase or separation additive) which move slower compared to the electroosmotic movement of the buffer due to their electrophoretic migration in opposite direction.

So far, sodium dodecyl sulfate (SDS) is mainly used as separation additive. In order to change selectivities other types of separation additives such as cyclodextrins [2,3], calixarenes or resorcarenes [4–7], dendrimers [8–11] and charge-transfer interacting additives [12–14] have been introduced. These additives do not form micelles but offer secondary chemical equilibria of different nature [15].

In the case of UV light-absorbing additives such as charge-transfer interacting compounds [12–14] or some proteins acting as chiral selectors [16–20], the

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commonly used direct UV detection is interfered. Detection is also impaired when coupling MEKC with mass spectrometry [21–26]. To overcome such detection problems a partial filling technique has been first applied by Valtcheva et al. [16] for the enantioseparation of β-blockers. This technique was modified and further developed for the separation of chiral compounds by different research groups [17–20,27–32] and for the separation of nitroaromatic compounds by Welsch et al. [12–14] using charged dyes as separation additives. The partial filling (PF) technique includes a filling of the capillary from the anodic side first with the pure buffer solution (background buffer zone, BB), followed by a buffer solution containing the separation additive (separation buffer zone, SB). Filling with the latter may be stopped at any length but before the border between the background buffer and the separation buffer reaches the detection window. The sample also injected on the anodic side is separated in the separation buffer zone by interactions with the separation additive. After changing into the background buffer zone the analytes migrate towards the detection window. That way, detection is not disturbed by the additive. Normally, the electroosmotic flow (EOF), μ_{eo}, exceeds the electrophoretic movement of the micelle, μ_{epMC}. Thus, during the run the separation buffer zone moves towards the detection window which results in a small migration time window. The window can be increased by combining the partial filling technique with the application of counter pressure from the cathodic side [12–14,33]. The counter pressure causes a delay of the separation additive in reaching the detection window.

The partial filling technique is inevitably connected with a boundary between the background buffer zone and the separation buffer zone which may give rise to additional band broadening. Several workers attributed this band broadening in partial filling and stepwise gradient (M)EKC to effects which result from analyte crossing of the zone boundaries [14,34–37]. On the other hand band broadening in PF-(M)EKC can be attributed to differences in the electroosmotic flow velocities of the zones caused by different buffer compositions, and/or ionic strengths and concentrations [30,35,38], and by differences in the capillary surface characteristics (axial non-uniform ζ-potentials) [39,40]. Electroosmotic flow differences produce an intersegmental pressure, p_i, at the zone boundary which may strongly effect the flow profiles and hence efficiencies. Some of these effects were studied in detail in MEKC [34,35], capillary zone electrophoresis (CZE) [38–40] and capillary electrochromatography (CEC) [41].

In this work, we studied the influence of buffer zone concentrations on efficiency in PF-MEKC without and with the application of counter pressure. In contrast to some papers mentioned above our investigations did not focus on effects at the zone boundary but on the flow profiles in the buffer zones. The results obtained with different buffer combinations in PF-MEKC are discussed on the basis of models for flow profile deformation and are compared with a conventional MEKC system using the same separation buffer.

The second aim of this work was to find a rule for the preparation of an optimum combination of background and separation buffers in order to obtain best efficiencies when using PF-MEKC.

2. Theoretical considerations

According to the relations expressed by Eqs. (1) and (2), the electroosmotic flow velocity, u_{eo}, decreases with increasing ionic strength, I, and therefore with increasing concentration, c:

$$u_{eo} \propto \frac{E}{4\pi\eta} \cdot \frac{1}{\sqrt{I}} \tag{1}$$

$$I = \frac{1}{2} \cdot \sum_i c_i z_i^2 \tag{2}$$

where E is the electric field strength, η is the viscosity of the buffer, c_i is the concentration and z_i is the charge number of an ion, i. Additionally, the electric field strength is lower in regions with higher ionic strength because of its lower electrical resistance.

In liquid filled open tubes, pressure induces a laminar flow, u_{hd}, showing a parabolic flow profile [42]:

$$u_{hd} = \frac{\Delta p}{4\eta L} \cdot (R^2 - r^2) \tag{3}$$

where Δp is the pressure drop along the capillary, L is the total capillary length, R is the radius of the capillary and r is the radial position.

In case the buffer zones in PF-MEKC move with different electroosmotic flow velocities, an intersegmental pressure, p_i, is formed at the boundary between separation buffer and background buffer due to the velocity mismatch of the buffer zones [34,35,38,40,41]. As the flow velocity in the capillary has to be uniform as a consequence of the non-compressibility of fluids at low pressures, p_i accelerates the slower moving buffer zone and delays the faster moving buffer zone to an average bulk velocity $\bar{u}_{eo}$. This is connected with the formation of a parabolic flow profile in each buffer zone according to Eq. (3) which superimposes the electroosmotic plug profile. The extent of the flow profile distortion depends on the value of the intersegmental pressure and the lengths of the buffer zones. While p_i is the same for the two buffer zones their lengths can differ and change during the run. As expressed in Eq. (3) the parabolic flow profile distortion decreases with increasing length of the buffer zone. This means, the longer the respective buffer zone the smaller the flow profile deformation.

On their way from the injection end to the detection window analytes are affected by the distorted flow profiles of one or both zones. It depends in which zone the analyte reaches the detection window. While the plate height at unique electroosmotic flows profits from the small longitudinal diffusion in a liquid and the low resistance to mass transfer at unique plug-like flow profiles [43] a parabolically shaped profile gives rise to an increased contribution of the mass transfer to plate height [44]. Thus, the degree of the distortion of the plug-like profile in the separation buffer zone will have a major influence on the efficiency drop.

The use of pressure on one side of the capillary induces a parabolic flow, u_{hd}, as well, which counteracts the electroosmotic flow when applied on the cathodic side of a conventional MEKC system (counter pressure, p_{cp}). In PF-MEKC, a parabolic flow profile generated by counter pressure superimposes the profiles discussed above. As a consequence plate numbers may be further reduced or restored in case of oppositely directed parabolic profiles. It is known that counter pressure can be applied to reduce the plug profile distortion caused by thermal effects (thermal band broadening) as reported by Gobie and Ivory [45] and Kutter and Welsch [12]. Kok [46] reported the compensation of band broadening caused by electrically decoupled detection capillaries with a hydrodynamic counter flow.

Based on these considerations the following three cases for counter pressure-moderated PF-MEKC were experimentally scrutinized and discussed:

In case 1 the EOFs of the buffer zones are equal [$\mu_{eo}(SB) = u_{eo}(BB)$]. In case 2 the EOF of the separation buffer $u_{eo}(SB)$ is lower than the EOF of the background buffer $u_{eo}(BB)$ [$u_{eo}(SB) < u_{eo}(BB)$]. In case 3 the EOF of the separation buffer exceeds the EOF of the background buffer [$u_{eo}(SB) > u_{eo}(BB)$].

To realize these cases experimentally the electroosmotic flows of the background buffers were varied by changing the buffer concentrations. These background buffers were combined with a separation buffer having a fixed concentration. All buffer combinations were tested without and with the application of counter pressure.

3. Experimental

3.1. Buffer systems and chemicals

In order to realize buffer systems matching the cases described above first we tried to calculate the needed buffer concentrations according to Eq. (2). Unfortunately, borate buffer systems have a lot of unknown dissociation equilibria which makes a calculation of the ionic strengths impossible. Additionally, the contribution of SDS micelles and monomers to the ionic strength are unknown.

Therefore we made an empirical approach: Instead of calculating the ionic strengths, the electroosmotic flow velocities of several buffers were measured using acetone as an EOF marker. The procedure is described below in Section 3.3. It is based on the assumption that buffers with a lower (higher) ionic strength show a higher (lower) EOF than buffers with a higher (lower) ionic strength (see Eq. (1)).

Buffer solutions were prepared from analytical-grade chemicals (Merck, Darmstadt, Germany). Borate buffer was exclusively used in this work. A

stock solution was prepared having a concentration of 0.1 *M* with boric acid and adjusted to pH 9.4 with 1 *M* sodium hydroxide solution. For all experiments, the separation buffer was prepared diluting the borate buffer to 0.015 *M* with water and methanol (10%, v/v), then SDS (Roth, Karlsruhe, Germany) was added to give a concentration of 0.015 *M*. For the background buffers the borate buffer was diluted to the desired concentration (0.01 *M*, 0.02 *M* and 0.03 *M*) with water and methanol (10%, v/v).

The homologous ω-phenylalcohols (benzylalcohol to ω-phenylhexanol, abbreviated 1–6) used as test compounds were obtained from Fluka and Aldrich (Deisenhofen, Germany) and used at concentrations of about 300 μg/ml in the separation buffer. Decanophenone (Aldrich) was used as micelle marker (MC). It was also employed as a marker for the separation buffer zone at a concentration of about 20 μg/ml.

All solutions were prepared using water purified by an Elgastat-UHQPS system (USF Elga, Ransbach-Baumbach, Germany).

3.2. CE system

All experiments were performed with a Prince system (injection device with high-voltage power supply; Prince Technologies, Emmen, The Netherlands). Accurate counter pressures were achieved using an ER 3000 electropneumatic pressure controller (Dräger Tescom, Lübeck, Germany).

For detection, a Jasco 875-CE UV detector (Jasco, Tokyo, Japan) and for data evaluation the personal computer-based integration software Gynkosoft version 4.22 (Dionex Softron, Germering, Germany) were used.

Plain fused-silica capillaries, 75 cm×50 μm I.D.×365 μm O.D. (MicroQuartz, Munich, Germany) were applied. The effective capillary length was 47 cm. Detection was carried out on-capillary at 254 nm (for acetone) and 205 nm (for the ω-phenylalcohols).

3.3. Procedures

Before each run, the capillary was rinsed with 0.2 *M* NaOH for 2 min. For the EOF measurements of the background buffers the capillary was rinsed with the respective background buffer for 3 min. In order to avoid undesirable zone boundaries, the background buffer was also used to dissolve the EOF marker and to fill the inlet and the outlet vials, respectively. The EOF of each background buffer was measured three times. A linear regression was carried out with the averaged EOF values (*x*-axis: migration time of acetone; *y*-axis: borate concentration). In the same way the EOF of the separation buffer was measured (separation buffer was used to fill the inlet and the outlet vials and to dissolve the EOF marker).

The required concentration of the borate background buffer having the same EOF as the separation buffer was determined from the regression plot. It turned out that the background buffer with a concentration of 0.02 *M* borate had the same EOF as the separation buffer. Based on these results three different buffer combinations were selected as listed in Table 1 to realize the three cases mentioned above.

For measurements in the partial filling mode, the capillary was rinsed with the desired background buffer for 3 min. After this, the separation buffer was injected for 1.2 min filling the capillary to 38% of its

Table 1
Buffer combinations used in PF-MEKC for this study

Case	Ionic strength	EOF	Buffer combination
1	$I(SB)=I(BB)$	$u_{eo}(SB)=u_{eo}(BB)$	SB: 0.015 *M* SDS, 0.015 *M* borate, 10% (v/v) MeOH BB: 0.02 *M* borate, 10% (v/v) MeOH
2	$I(SB)>I(BB)$	$u_{eo}(SB)<u_{eo}(BB)$	SB: 0.015 *M* SDS, 0.015 *M* borate, 10% (v/v) MeOH BB: 0.01 *M* borate, 10% (v/v) MeOH
3	$I(SB)<I(BB)$	$u_{eo}(SB)>u_{eo}(BB)$	SB: 0.015 *M* SDS, 0.015 *M* borate, 10% (v/v) MeOH BB: 0.03 *M* borate, 10% (v/v) MeOH

length which corresponds to a filling degree of 60% of the effective length (L_{eff}). During a run, the inlet vial contained the separation buffer and the outlet vial contained the appropriate background buffer in order to avoid further zone boundaries. The buffer in the outlet vial was replaced before each run. The separation buffer was marked with a small amount of the micelle marker decanophenone to illustrate the boundary between the separation buffer zone and the background buffer zone in the chromatograms. Measurements with and without the zone marker showed no differences in plate numbers.

Plate numbers of the ω-phenylalcohols were measured for every combination of the background buffer and the separation buffer without (0 mbar) and with the application of counter pressures of 5, 10 and 20 mbar, respectively, at a field strength of 400 V/cm. The analytes were dissolved in separation buffer.

For the measurement of plate numbers in the conventional MEKC mode, the capillary was rinsed with the separation buffer for 3 min. During a run, the inlet and outlet vials were filled with separation buffer. Plate numbers were measured without (0 mbar) and with 10 and 20 mbar counter pressures at a field strength of 400 V/cm.

Rinsing procedures (1000 mbar), injection of the separation buffer (450 mbar) and injection of the sample (10 mbar/0.1 min) were carried out hydrodynamically.

The discussed values are the mean of three measurements. The data for a particular buffer combination at different counter pressures were evaluated by graphs plotting the theoretical plate number N vs. the effective velocity u_{eff} of the analytes (Figs. 2, 4 and 6). For better comparison the same scale was chosen for all graphs. For comparison with conventional MEKC (Fig. 8a and b) the data for all buffer combinations and conventional MEKC at a particular counter pressure were plotted in similar graphs. For the latter the connection of data points has no physical reason but is only for better survey. N was directly taken as calculated by the Gynkosoft program, u_{eff} was calculated using Eq. (4) (L_{eff} is the length to the detection window, t_M is the migration time of the respective analyte):

$$u_{eff} = \frac{L_{eff}}{t_M} \text{ (mm/s)} \quad (4)$$

4. Results and discussion

Efficiencies of a series of ω-phenylalcohols were determined using three different buffer combinations in the PF-MEKC mode without and with the application of counter pressures (see Table 1).

4.1. Discussion of the three cases of buffer combinations on the basis of models for flow profile deformation

4.1.1. Case 1: $u_{eo}(SB) = u_{eo}(BB)$

The uniformity of the electroosmotic flows of both zones could be met by a combination of a background buffer consisting of 0.02 *M* borate buffer and 10% (v/v) methanol and a separation buffer consisting of 0.015 *M* borate buffer, 0.015 *M* SDS, and 10% (v/v) methanol (Table 1). To illustrate the separation performance achieved in this partial filling system four chromatograms are shown in Fig. 1. The upper track was obtained without counter pressure. The lower tracks were recorded at counter pressures

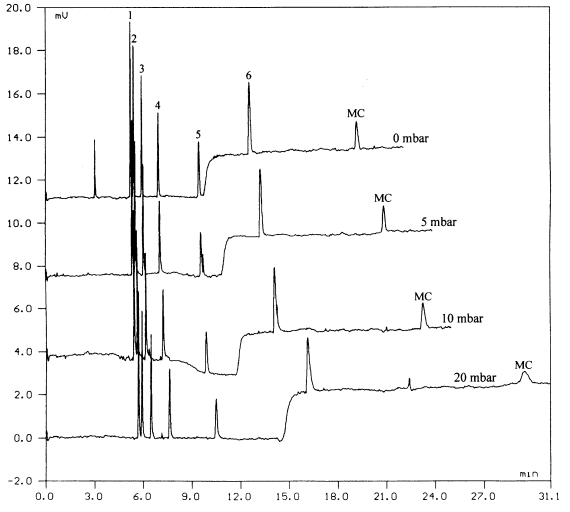

Fig. 1. Chromatograms of six homologous ω-phenylalcohols obtained by PF-MEKC with buffer combination 1 and application of counter pressures of 0, 5, 10 and 20 mbar, respectively, on the cathodic buffer reservoir. The baseline step indicates the breakthrough of the separation buffer zone which is marked with a small amount of decanophenone. Conditions: buffers: see Table 1; field: 400 V/cm; detection: UV at 205 nm; filling degree: 38% of L (60% of L_{eff}); compounds 1–6: homologous ω-phenylalcohols from (1) benzylalcohol to (6) ω-phenylhexanol, (MC) micelle marker decanophenone.

of 5, 10, and 20 mbar. Without counter pressure all alcohols reach the detection window within the background buffer zone except for ω-phenylhexanol. The chromatograms illustrate that migration times and peak widths increase with increasing counter pressure.

In Fig. 2 the measured plate numbers are plotted vs. the effective velocities for the homologous ω-phenylalcohols 1–6. Without counter pressure plate numbers come to 90 000–100 000 for the first three homologues (data points from right to left). With increasing counter pressure plate numbers drop by 15 000 to 20 000. Because there are no differences in the electroosmotic flow velocities of the separation buffer and the background buffer zone no flow-equalizing pressure is generated. Neglecting temperature effects, the flow profile of the buffer zones can be supposed to be ideal plug-like as demonstrated in Fig. 3, which explains the high plate numbers. Counter pressure causes a parabolic distortion of the existing plug-like profile in anodic direction (see Fig. 3). The distortion increases with increasing counter pressure. As a consequence plate numbers decrease. ω-Phenylhexanol which does not pass the boundary between the buffer zones shows slightly higher plate numbers compared to ω-phenylpentanol. This can be attributed to band broadening effects connected with the cross over of the latter from the separation buffer zone into the background buffer zone.

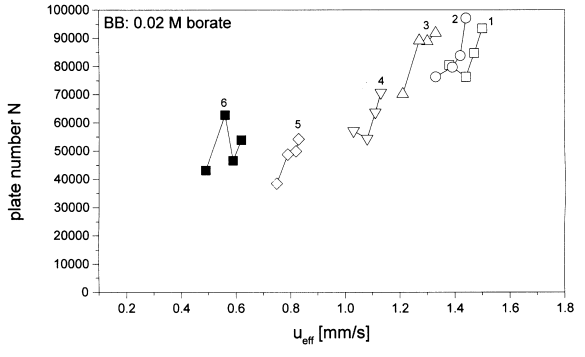

Fig. 2. Plate numbers vs. effective velocities for buffer combination 1 applying different counter pressures. Conditions: buffers, field, detection and filling degree: see Table 1 and Fig. 1; compounds (from right to left): ■ benzylalcohol (1), ● ω-phenylethanol (2), ▲ ω-phenylpropanol (3), ▼ ω-phenylbutanol (4), ♦ ω-phenylpentanol (5), ■ ω-phenylhexanol (6); symbol fillings: full symbols: detection in the separation buffer zone, open symbols: detection in the background buffer zone; counter pressure: increasing counter pressure (0, 5, 10, 20 mbar) is represented by decreasing u_{eff} in each data set.

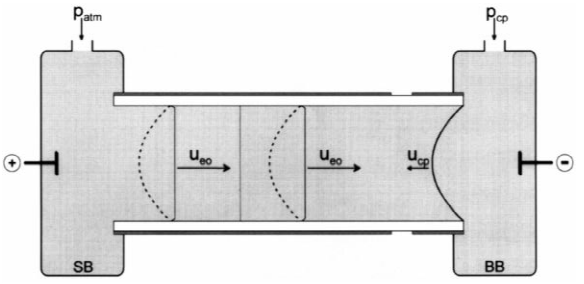

Fig. 3. Scheme of the buffer constellation for case 1: $u_{eo}(SB) = u_{eo}(BB)$. (⌒) plug-like flow profile in the capillary; (—) profile of the laminar flow component, u_{cp}, caused by counter pressure; (- - -) flow profile formed by the superposition of the plug-like electroosmotic profile with u_{cp}.

4.1.2. Case 2: $u_{eo}(SB) < u_{eo}(BB)$

This ratio of the electroosmotic flow velocities was realized by a lower concentrated background buffer (see Table 1). It is probably most often the case in practical PF-MEKC because the separation buffer is usually prepared by adding the separation additive to the background buffer. As it becomes clear from Fig. 4 already the plate numbers obtained without counter pressure are very low for all homologous ω-phenylalcohols. The highest value of

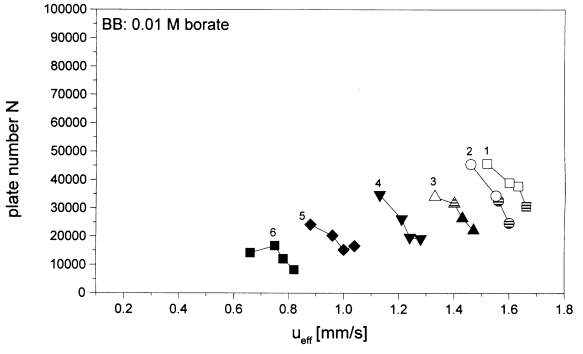

Fig. 4. Plate numbers vs. effective velocities for buffer combination 2 applying different counter pressures. Conditions: buffers, field, detection and filling degree: see Table 1 and Fig. 1; compounds: see Fig. 2; symbol fillings: see Fig. 2, striped symbols: detection together with the boundary between separation and background buffer; counter pressure: see Fig. 2.

30 000 plates for benzylalcohol is 65 000 plates lower than the value obtained in case 1 (equal electroosmotic flows). The low plate numbers can be explained by a parabolic distortion of the electroosmotic plug profiles which is outlined in Fig. 5a: the difference between the electroosmotic flow velocities of the zones results in a flow-equalizing intersegmental pressure, p_i (see Section 2). It must be lower than the ambient pressure, p_{atm}, in order to accelerate the slower moving separation buffer and to delay the faster moving background buffer to an average bulk velocity $\bar{u}_{eo}$. This is connected with a parabolic flow profile distortion in each buffer zone according to Eq. (3). In the separation buffer zone the parabolic distortion is directed towards the cathode, in the background buffer zone it is directed towards the anode (the opposite direction of such parabolically distorted flow profiles caused by a non-uniform ζ-potential distribution in the capillary has been reported by Herr et al. [40]).

There are several reasons that for the chosen experimental conditions the flow profile deformation in the separation buffer zone is the main source for the dramatic loss of efficiency in this buffer system: (i) the separation buffer zone is shorter than the background buffer zone. Thus, the parabolic distortion is more pronounced in the separation buffer zone. (ii) Because mass transfer occurs only in the separation buffer zone its flow profile has a major influence on efficiency. (iii) Without counter pressure all ω-phenylalcohols migrate in the separation buffer zone or together with the zone boundary. Hence, in this example the flow profile of the background buffer is anyway ruled out to contribute to peak broadening. (iv) Efficiency is increased by 15 000–20 000 plates with increasing counter pressure up to 20 mbar. As illustrated by Fig. 5b counter pressure, p_{cp}, induces a laminar flow component, u_{cp}, the parabolic shape of which is directed in parallel to the distorted flow profile in the background buffer zone but oppositely directed to the distorted flow profile in the separation buffer zone. This compensation seems to be decisive for the observed improvement of plate numbers with increasing counter pressure.

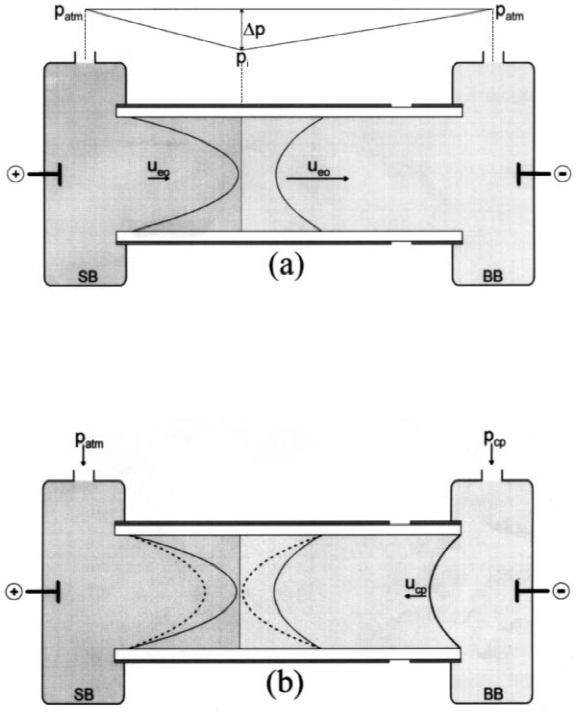

Fig. 5. Schemes of the buffer constellation for case 2: $u_{eo}(SB) < u_{eo}(BB)$. (a) (—) Flow profiles in the buffer zones without counter pressure (p_i = intersegmental pressure); (b) (- - -) flow profiles formed by the superposition of the profiles in (a) with the profile of the laminar flow component, u_{cp}, which is caused by counter pressure.

4.1.3. Case 3: $u_{eo}(SB) > u_{eo}(BB)$

This type of mismatch of the electroosmotic flow velocities was realized by a 0.03 *M* borate background buffer combined with the standard separation

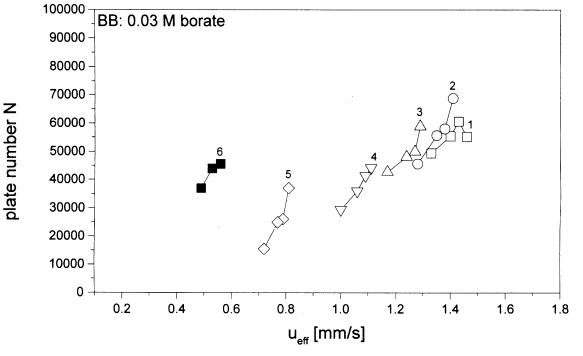

Fig. 6. Plate numbers vs. effective velocities for buffer combination 3 applying different counter pressures. Conditions: buffers, field, detection and filling degree: see Table 1 and Fig. 1; compounds, symbol fillings and counter pressure: see Fig. 2.

buffer (Table 1). A plot of plate numbers for the ω-phenylalcohols vs. effective mobilities is given in Fig. 6. A similar course as in Fig. 2 is seen but the absolute plate numbers are lower by 20 000–45 000. Again, the velocity mismatch gives rise to a flow-equalizing intersegmental pressure which in this case is higher than the ambient pressure, p_{atm}, in order to accelerate the background buffer zone and to slow down the separation buffer zone to an average bulk velocity $\bar{u}_{eo}$. By analogy with case 2, the arising laminar flow component distorts the plug-like electroosmotic flow profile in each buffer zone. But contrary to case 2 the parabolic distortion in the separation buffer zone is directed towards the anode, and in the background buffer zone it is directed towards the cathode (Fig. 7a). As discussed for case 2 (see Section 4.1.2) the flow profile distortion in the separation buffer zone is assumed to govern peak broadening. This interpretation is supported by the fact that for this buffer combination plate numbers are further reduced when applying increased counter pressure. As outlined in Fig. 7b the application of counter pressure enlarges the parabolic deformation of the flow profile in the separation buffer zone into anodic direction. At the same time the parabolic flow profile distortion in the background buffer zone which has a secondary importance on the efficiency is diminished.

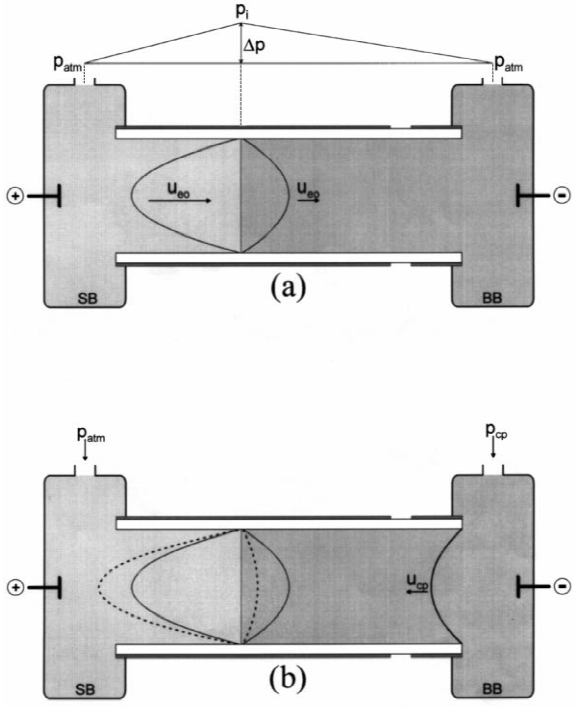

Fig. 7. Schemes of the buffer constellation for case 3: u_{eo}(SB)> u_{eo}(BB). (a) (—) Flow profiles in the buffer zones without counter pressure (p_i = intersegmental pressure); (b) (- - -) flow profiles formed by the superposition of the profiles in (a) with the profile of the laminar flow component, u_{cp}, which is caused by counter pressure.

4.2. Comparison of plate numbers of the three cases with one another and with conventional MEKC

To allow a direct comparison of the influence of the different buffer combinations on efficiency and to give an impression on the performance of PF-MEKC compared to conventional MEKC in the same capillary totally filled with separation buffer, the theoretical plate numbers obtained without the application of counter pressure and with the application of a counter pressure of 20 mbar are plotted vs. the effective velocities in Fig. 8a and 8b, respectively. The graphs clearly show that maximum efficiency for each ω-phenylalcohol is to be seen in case of classical MEKC regardless whether a counter pressure is applied or not. Efficiencies are lower in the partial filling modes. This is understandable because the homogeneous buffer in conventional MEKC ensures an optimum plug-like flow profile, and the analytes have not to cross a zone boundary. The higher length of the separation zone in our conventional MEKC experiments may also play a role. Fig. 8a and 8b also show that efficiency in PF-MEKC is strongly dependent on the ionic strengths and hence the concentrations of both the separation buffer and the background buffer. If the concentrations are adjusted such that the electroosmotic flow velocities of the two zones are very similar, plate numbers are highest and may reach nearly those of conventional MEKC if the analyte has not to pass the zone boundary (e.g., ω-phenylhexanol). This is also true when counter pressure is applied.

At any divergence of the electroosmotic flows, a parabolic flow profile distortion reduces efficiency. Compared to MEKC and PF-MEKC with 0.02 *M* borate as background buffer (case 1) a disastrous

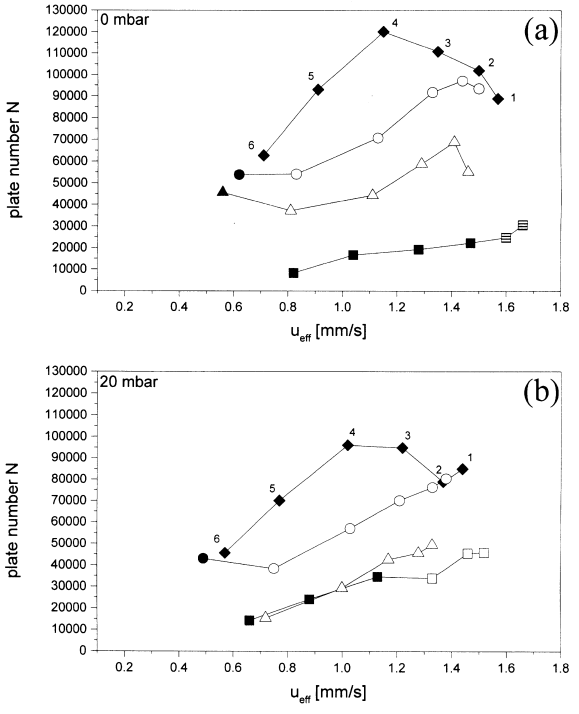

Fig. 8. Comparison of plate numbers obtained in conventional MEKC and in the PF-MEKC mode using different buffer combinations (a) without counter pressure (0 mbar) and (b) with a counter pressure of 20 mbar. Conditions: buffers for PF-MEKC: see Table 1; buffer for conventional MEKC: only separation buffer as in Table 1; field, detection and filling degree in PF-MEKC: see Fig. 1; symbols: ♦ conventional MEKC, PF-MEKC with ■ 0.01 *M* borate, ● 0.02 *M* borate, ▲ 0.03 *M* borate and 10% (v/v) methanol in the background buffer; compounds: homologous ω-phenylalcohols from (1) benzylalcohol to (6) ω-phenylhexanol; symbol fillings: see Figs. 2 and 4.

collapse of efficiencies is observed for the partial filling system with the lower concentrated (0.01 *M* borate) background buffer whereas the decrease is not as strong using the higher concentrated (0.03 *M* borate) background buffer. One reason therefore might be a stacking effect of the micelles at the zone boundary in the latter case because they are slowed down due to the lower electric field strength in the background buffer zone.

The application of a counter pressure of 20 mbar (Fig. 8b) decreases the efficiency by about 10 000–25 000 plates in MEKC as well as in PF-MEKC with 0.02 *M* borate as background buffer due to the parabolic distortion of the plug-like flow profile.

In case of varying electroosmotic flows of the zones counter pressure leads to a superposition of the induced flow profile with the already distorted flow profiles. As discussed above this results in increased plate numbers in case of a lower concentrated background buffer and in decreased plate numbers in case of a higher concentrated background buffer. Thus, plate numbers become similar on a low level for the buffer combinations 2 and 3 when applying a counter pressure of 20 mbar.

5. Conclusions

PF-MEKC is an interesting alternative to conventional MEKC in case detection is interfered by the separation additive. A high efficient conventional chromatographic system can be imitated by counter pressure moderated PF-MEKC.

Our studies showed that efficiency in PF-MEKC is strongly influenced by the flow profile in the separation buffer zone. An optimum plug-like flow profile which results in maximum plate numbers can be achieved by an adaptation of the electroosmotic flow velocities of the separation buffer zone and the background buffer zone by adjusting appropriate buffer concentrations. Even with the application of counter pressure which affects the efficiency by a parabolic distortion of the plug-like profile efficiency is maximum compared to other buffer combinations.

Differences in the concentrations (ionic strengths) of separation buffer and background buffer result in a mismatch of the electroosmotic flow velocities. This is connected with a considerable reduction of plate numbers. The reason is a parabolic deformation of the plug-like flow profiles due to a laminar flow component as a result of an intersegmental pressure. The efficiency drop is especially high in case of a lower concentrated background buffer. In this case plate numbers can be slightly increased by the application of counter pressure. In case of a higher concentrated background buffer the efficiency drop is not as high as in the former case, however, plate numbers are diminished by counter pressure. Both types of buffer combinations should be strictly avoided at buffer preparation, especially the former one.

The results are in good agreement to the discussed models and result in an important conclusion: if

PF-(M)EKC has to be used, e.g., because of detection interferences, it is important to equalize the electroosmotic flows of the two buffers.

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