Review

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Electrophoretic separations on chips with hydrodynamically closed separation systems

This review focuses on capillary electrophoretic separations performed on capillary electrophoresis chips (CE chips) with hydrodynamically closed separation systems in a context with transport processes (electroosmotic flow (EOF)) and hydrodynamic flow (HDF)) that may accompany the separations in these devices. It also reflects some relevant works dealing with conventional CE operating under such hydrodynamic conditions. The use of zone electrophoresis (ZE), isotachophoresis (ITP) and their on-line combination (ITP-ZE) on the single-column and column-coupling CE chips with the closed separation systems and related problems are key topics of the review. Some attention is paid to sample pretreatment in the separations performed on the CE chips. Here, mainly potentialities of the ITP-ZE combination in trace analysis applications of the miniaturized systems are discussed in a broader extent. Links between the ZE separation and detection provide a frame for the discussion of current status of the detection on the CE chips. Analytical applications illustrate potentialities of the CE chips operating with the closed separation systems (suppressed HDF and EOF) to the determination of small ions present in various matrices by ZE, ITP and ITP-ZE.

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Abbreviations: BE, background electrolyte; **CC**, column-coupling; **HDF**, hydrodynamic flow; **LE**, leading electrolyte; **μTAS**, micrototal analysis system; **TE**, terminating electrolyte; **ZE**, zone electrophoresis

1 Introduction

At present, electrophoretic methods are gaining a key position among the separation tools of lab-on-a-chip analytical systems. This is, apparently, due to the fact that they fit very well the concept of micrototal analysis sys-

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tems (μTASs) as introduced by Manz *et al*. (see, *e.g.*, a recent review on this topic [1] and references given therein) and in addition from the point of view of miniaturization they offer more benefits than chromatography methods [2]. Although electrophoresis on chips is developing very rapidly, especially from the point of view of fabrication procedures, its use in analytical practice is still limited. This documents recent review articles on the topic (see, *e.g.*, [1, 3–7]) and types of contributions presented at the μTAS 2002 symposium [8]. Such a situation is understandable because a broader use of CE in the miniaturized systems is closely linked with a progress in the fields of miniaturized sample pretreatment [6, 9] and detection [1, 4, 6, 10–15] technologies. This review focuses on the CE separations on the chips with hydrodynamically closed separation systems in a context with transport processes (electroosmotic flow (EOF) and hydrodynamic flow (HDF)) that may accompany the separations in the miniaturized systems. It also reflects some relevant works dealing with conventional CE operating under such hydrodynamic conditions.

Basic electrophoretic methods [16] (see also Section 2) differ in the spatial configurations of the separated constituents, sample loadabilities, concentrating effects, and in part in applicabilities for particular categories of the analytes [16–19]. Due to this, they offer tools that may be exploited in specific analytical situations and especially effectively on-line combined [20–26]. Undoubtedly, the combinations integrating several analytical functions give electrophoresis-significant potentialities in trace analysis applications of the miniaturized systems. Therefore, we pay a special attention to this topic with a focus on the combinations that integrate (electrophoretic) sample pretreatment with the electrophoretic separations. Current problems related to the detection in miniaturized CE are extensively treated in recent review articles [4, 10–14] and our discussion stresses links between the separation, sample pretreatment and detection.

Although conceptually close to conventional CE instruments working with the closed separation systems [17, 18], instrumentation currently used to the separations in the miniaturized CE format under such hydrodynamic conditions differ in some respects. Therefore, some instrumentation aspects are briefly discussed to provide a supporting frame for other topics dealt with in this review. Selected analytical applications of the CE chips operating with the closed separation systems illustrate potentialities of this instrumental concept to the determination of small ions present in various matrices. Here, the zone electrophoresis (ZE), ITP and ITP-ZE separations are included and advantages and limitations of these methods to solutions of analytical problems are outlined.

2 Electrophoretic methods and separations on chips

Classification of electrophoretic methods according to initial and marginal conditions of the separation distinguishes four basic methods [16]: (i) ZE, (ii) moving boundary electrophoresis (MBE), (iii) ITP, (iv) isoelectric focusing (IEF). This classification reflects the fact that at least in a general sense all these methods run in any device suitable to electrophoresis separations while a particular method is implemented *via* the composition(s) of the electrolyte solution(s) in which the separation is performed [16–19]. Therefore, any CE chip should give a possibility to perform the run with the sample using the most appropriate electrophoretic method. ITP and ZE separations carried out on a poly(methylmethacrylate) (PMMA) chip provided with conductivity detection [20] already illustrated such an approach. It is apparent that this feature gives electrophoresis a high operational flexibility and enhances its analytical utility in lab-on-a-chip systems.

As the basic electrophoretic methods *a priori* differ in the sample loadabilities, spatial configurations of the separated constituents, concentrating effects, and in part in applicabilities for particular categories of the analytes [16–19] they make tools that can be effectively on-line combined. Such a possibility was already demonstrated with CE chips provided with the column-coupling (CC) configuration of the separation channels [20–26]. The quoted works showed that the combinations offer means for reaching very low concentration detectabilities of the analytes present in complex ionic matrices also when short separation paths as typical for the CE chips are used.

3 Transport processes and electrophoretic separations on chips

Electrophoretic migrations of the separated constituents in CE may be superimposed on EOF and/or HDF flows of the solution in which the separation is carried out. Impacts of these transport processes on analytical performances of CE methods were studied in a context with developments of conventional CE systems (for an overview see, *e.g.*, [17–19, 27]). Although miniaturized CE systems do not represent new situations in these respects, dimensions of the separation compartments, new construction approaches and especially a new concept (μTAS) in which CE operates [1, 4–7, 9] bring certain specificities. Therefore, it is appropriate to overview roles of EOF and HDF in a context with the electrophoretic separations in these systems.

3.1 EOF in the opened and closed CE separation systems

EOF influences both the (apparent) migration velocities and zone profiles (peak dispersions) of the separated constituents in CE [17–19, 28]. Here, besides actual electrokinetic properties of the surface of the separation compartment, also a hydrodynamic arrangement of the separation system in which the CE separation is carried out plays a role. This is due to the fact that in two alternative arrangements, hydrodynamically opened and closed systems (Fig. 1), EOF contributes differently to both the migration velocities and zone profiles.

In the opened systems (Fig. 1a), as introduced into CE by Jorgenson and Lukacs [29], mainly the electrokinetic properties of the inner surface of the separation compartment, under the electrolyte conditions employed, determine the direction and size of EOF. In this instance (see also Fig. 2A), while equally contributing to the migration velocities of the separated constituents (a in Fig. 2A), EOF is usually assumed to have no impact on the peak dispersions [28]. However, differences in the ζ-potential along the separation compartment (caused, *e.g.*, by some of the sample constituents) cannot be excluded and these lead to a local pressure difference-driven flow of the solution (b in Fig. 2A) and consequently to a non-zero plate height contribution of EOF. Another contribution of EOF to the band broadening, caused by transversal nonhomogeneity of EOF velocity in the diffuse part of the double layer (for an overview see, *e.g.*, [30]), can be expected especially in the separations performed on the chips with the separation channels of dimensions comparable to the thickness of the diffuse layer on the phase boundary.

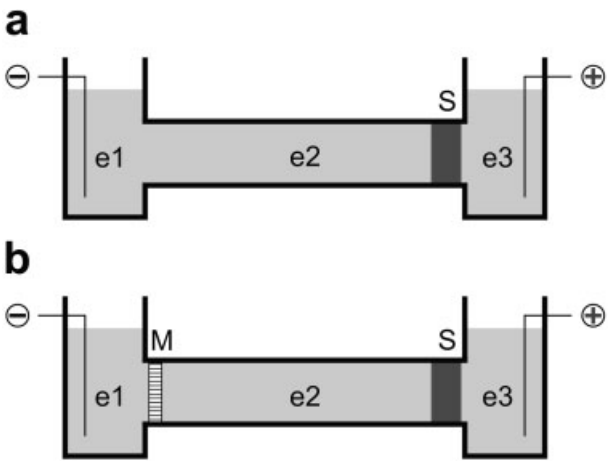

Figure 1. Basic concepts of hydrodynamic arrangements of the CE separation systems. (a) Hydrodynamically opened system (no hydrodynamic barrier acts against HDF of the solution between the electrode vessels); (b) Separation system hydrodynamically closed by a semipermeable membrane (M) preventing HDF of the solution between the electrode vessels. e1–e3 = electrolyte solutions in which the separation by one of the electrophoretic methods is performed; S = place for the sample.

In the separation system, hydrodynamically closed in the direction to the driving electrode by a semipermeable membrane [17–19], also electrokinetic properties of the membrane contribute to the resulting transport processes in the separation compartment (Fig. 2B) and consequently to the peak dispersions [28, 31]. Problems will occur when equal volume velocities of EOF in the capillary and the membrane pores are not reached (b in Figure 2B). This is a current situation in practice, and therefore in the ITP and ZE separations performed in the closed systems (see, *e.g.*, [17–19, 31–34]) it is preferred that $\zeta \approx 0$ and $\zeta_M \approx 0$ (c in Fig. 2B). EOF suppressors providing such electrokinetic conditions in the closed separation systems can be found in the literature [17–19, 31–34].

ITP migration of the sample constituents under non-zero EOF conditions in the opened separation compartment is characterized by changes of EOF during the separation [35–38]. In some instances these changes lead to a standstill operation (a migration transport of the sample constituents in the direction to the detector is compensated for by an oppositely acting EOF). This problem can be eliminated by rearranging the polarities of the driving electrodes and changing the positions of the leading and electrolyte solutions at the capillary ends so that the terminating zone is reaching the detector first [35–38]. On the other hand, ITP separations performed in the opened systems with suppressed EOF ($\zeta \approx 0$) are free from these disturbances and offer the same analytical advantages (see, *e.g.*, [39]) as the ITP separations performed in the closed systems with suppressed EOF [17, 18]. An amholytic gradient along the separation compartment can be very likely a source of fluctuations in EOF during the IEF separation. Therefore, in a pioneering work on capillary IEF by Hjertén *et al*. [40] performed in the opened system, suppressed EOF (*via* surface coating) was preferred.

3.2 HDF in the opened and closed CE separation systems

Conventional opened CE separation systems (see, *e.g.*, [19, 27, 29]), provided with the capillary tubes of 50–75 μm ID, are assumed to operate with negligible HDF. This however need not be the case of short capillaries as even subtle differences in the solution levels in the driving

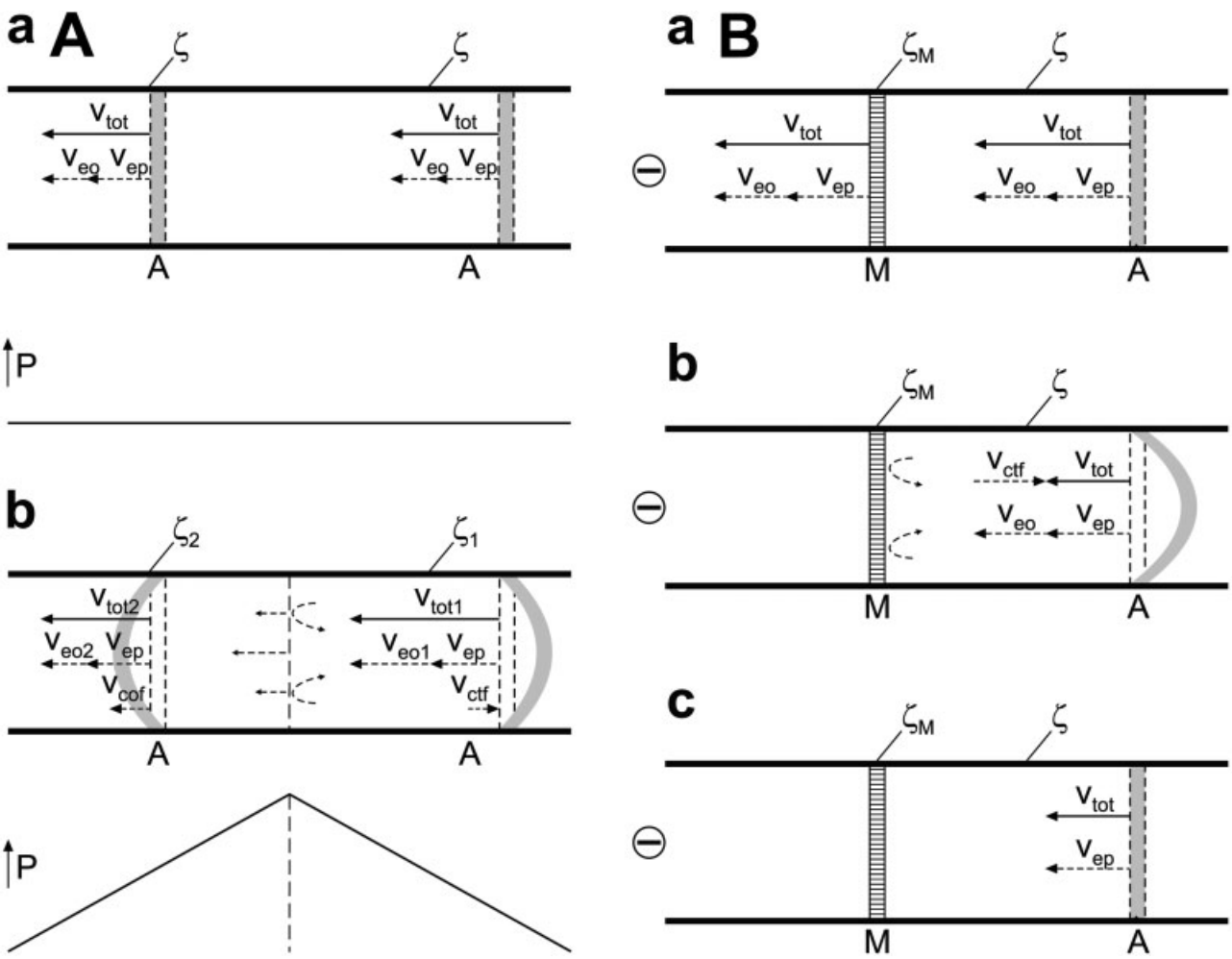

Figure 2. (A) Impacts of EOF on the migration velocities and zone profiles under selected electrokinetic conditions along the opened separation system. (a) Homogenous electrokinetic conditions along the separation system (a constant value of ζ-potential along the separation compartment); v_{ep}, v_{eo}, v_{tot} = electrophoretic, electroosmotic and total volume velocities, respectively. (b) Separation system consisting of two segments of differing electrokinetic properties (the boundary between the segments of different ζ-potentials ($\zeta_1 > \zeta_2$) is marked by a dashed line). v_{ep1}, v_{ep2}, v_{eo1}, v_{eo2}, v_{tot1}, v_{tot2} = electrophoretic, electroosmotic and total volume velocities, respectively (the numbers in the subscripts identify the segments); v_{cof}, v_{ctf} = velocities of coflow and counterflow of the solution due to the pressure drop developed on the boundary of the segments. P = course of pressure along the separation system. (B) Impacts of EOF on the migration velocities and zone profiles under selected electrokinetic conditions in the separation system closed by a semipermeable membrane. (a) Identical volume velocities of EOF in the separation compartment and membrane; (b) different volume velocities of EOF in the separation compartment and membrane ($v_{eo} \approx 0$ in the membrane; $v_{eo} > 0$ in the separation compartment); (c) suppressed EOF in the separation compartment and membrane ($v_{eo} \approx 0$ in both the separation compartment and membrane). v_{ep}, v_{eo}, v_{ctf}, v_{tot} = electrophoretic, electroosmotic, counterflow, and total volume velocities, respectively.

electrode vessels were shown to be sources of significant HDF [41]. In the CE chips, provided with the separation and sample loading channels of even smaller cross-sections, HDF may be a source of serious disturbances, *e.g.*, as recently reported by Crabtree *et al*. [42]. These authors demonstrated that HDF in the chip channels due to a hydrostatic pressure difference (siphoning effects) was accompanied by the one originating in a Laplace pressure difference (meniscus effects) developed during the run(s) in the driving electrode vessels. Especially, HDF due to the latter source was significant as it led to the flow rates comparable to those of EOF. A strict control of Laplace pressure in the opened chip channels seems difficult as it can be complicated by solvent evaporation from the driving electrode vessels. Although remedies to diminish these disturbing transport processes are given [42], their

contributions to within-run and run-to-run differences in the migration velocities of the separated constituents cannot be excluded.

In the ZE, ITP and ITP-ZE separations performed with the closed chip channels [20–24, 43–46] HDF is very small. However, when the driving electrodes are placed in the chip channels (for detail descriptions of such chip designs see, for example, the work of Grass *et al*. [23]) as employed in some works [20–23, 43, 44], small although reproducible HDFs due to gas formations at the driving electrodes remain, especially when the outlet from the chip channels is opened during the separation (see the Section 6.2). In addition, ionic products, formed from some buffers at the driving electrodes, can adversely affect the electrophoretic velocities of the separated constituents with such arrangements of the electrodes. The use of the closed separation system with the driving electrodes placed outside the chip channels, as employed in some application-related works [45–47], although eliminating impacts of the products of the electrode reactions on the migration velocities, does not eliminate HDF due to gas formation. This source of HDF can be eliminated in the way as employed in some conventional CE equipment for ITP [17, 18, 48] and CZE [31–34, 49] separations. Here, semipermeable membranes (see also Figs. 1 and 2B), closing the separation system in the direction(s) to the driving electrode(s), prevent a hydrostatic pressure difference-driven HDF in the separation compartment, and at the same time serve as hydrodynamic barriers for HDF linked with the gas formation. Especially, the use of mechanically supported membranes is effective in this respect [31]. This solution, hydrodynamic isolations of the driving electrodes from the separation compartment by mechanically supported membranes, is in some respects followed in the chip based CE separation system as described in the Section 6.

Usually, prolonging or shortening an effective length of the separation path in the CE column, EOF and HDF contribute to fluctuations of the migration velocities of the separated constituents ($\partial\nu_{tot}$) as outlined above. Here, in accordance with the law of propagation of errors [50], we can write:

$$\partial\nu_{tot} = \sqrt{(\partial\nu_{ep})^2 + (\partial\nu_{eo})^2 + (\partial\nu_{hd})^2} \quad (1)$$

where $\partial\nu_{ep}$, $\partial\nu_{eo}$, $\partial\nu_{hd}$ are symbols characterizing random fluctuations of the electrophoretic, electroosmotic and hydrodynamic velocities of the separated constituents. Equation (1) indicates that minimum disturbances to the migration velocity of the separated constituent can be expected in the CE runs with HDF and EOF close to zero values, as these are linked with small (negligible) $\partial\nu_{eo}$ and $\partial\nu_{hd}$ contributions to the migration velocity fluctuations.

We should note that the closed separation compartment does not exclude the use EOF and HDF to the solution handling (EOF pumping [51, 52] and a HDF-driven counterflow of the electrolyte solution in the CE separations [17, 18, 53]). Undoubtedly, the same applies for the chip-based CE equipment and several micropump designs based on electroosmotic pumping (see, *e.g.*, [51, 52, 54–57]) seem applicable also in combinations with the chips operating with the closed separation systems.

4 Links between the separation and detection on the CE chips

From recent reviews (see, *e.g.*, [1, 4, 6, 7, 9]) it is apparent that ZE separations, performed either in free or micellar solutions, are dominantly employed in the miniaturized systems. So far, the use of other electrophoretic methods attracted less attention. These facts are currently reflected in the designs of chip-based CE devices and short separation channels of very small cross-sections are favored as these provide rapid and high-efficiency ZE separations [1, 58]. On the other hand, favorable detectabilities of the analytes in the ZE separations carried out on the chips are required as well. Apparently, this, at least for some of the on-column CE detectors need not be met when the chip design favors only a maximum separation efficiency (for a current status of the detection in miniaturized CE see, *e.g.*, [1, 4, 10–14]). Therefore, efforts aimed at reaching adequate performances of some detectors may require certain compromises in this respect.

Considering an analogy between elution chromatography and ZE [59], the following relationship

$$c^*_{A,LOD} = \frac{3}{5}\frac{N_{p-p}}{S_A} \quad (2)$$

suitable to estimations of the limit of detection in elution chromatography [60], is appropriate for the same purposes in ZE. Here, $c^*_{A,LOD}$ is a symbol for a minimum concentration of the analyte (A) in its peak apex at which it is still detectable by the detector, N_{p-p} is the peak-to-peak noise of the detector in the signal units and S_A is its sensitivity for the analyte. When we assume the Gaussian shape of the analyte peak in ZE, the following relationship links $c^*_{A,LOD}$ with its concentration in the loaded sample, $c_{A,s,LOD}$, for a maximum sample load (expressed by a maximum length of the sample pulse, $l_{s,max}$):

$$c_{A,s,LOD} = \frac{\sqrt{2\pi}\sigma_A c^*_{A,LOD}}{l_{s,max}} \quad (3)$$

where σ_A is a symbol for the peak dispersion due to all dispersive phenomena to be expected in the ZE separations on the chips [19, 28, 30]. A combination of Eqs. (2) and (3) gives a relationship:

$$c_{A,s,LOD} = \frac{3}{5}\frac{\sqrt{2\pi}\sigma_A N_{p-p}}{I_{s,max} S_A} \quad (4)$$

in which contributions of performances of the separation and detection processes to the detection of the analyte are included.

The detection techniques and detectors differ in their N_{p-p} and S_A values, and therefore Eq. (4) has to be treated specifically for each of the CE detectors. Nevertheless, this equation shows that a low $c_{A,s,LOD}$ (a typical analytical target) need not be reached even for a minimum attainable value of σ_A in ZE [61, 62] once N_{p-p}/S_A ratio of the detector (detection technique) is not appropriate. For example, from a general treatment of principles of the UV-absorbance detection in CZE [63, 64] and current research on this topic in a context with the ZE separations on chips [65] it is apparent that geometrical dimensions of the chip channel have to reflect also this when an adequate performance of this key on-column CE detection technique in the chip-based separation systems is to be reached.

5 Sample pretreatment in electrophoretic separations on chips

Equation (3) shows that the ZE detectability of the analyte in a particular sample ($c_{A,s,LOD}$) can be enhanced by increasing the length of the sample pulse (the sample load). It also shows that this may be analytically beneficial only when an increased sample load does not degrade the separation efficiency (*e.g.*, by a significant contribution of the injection dispersion to σ_A [19, 28]). A loss of the resolution of the analyte, *e.g.*, due to electromigration dispersion(s) of the matrix constituent(s), is another factor that may prevent an increase of the sample load. On the other hand, the length (volume) of the sample pulse loadable into the ZE column is rather unrestricted when the electric conductivity of the sample is significantly lower relative to that of the background (carrier) electrolyte solution. In this instance, the sample stacking [34, 66–69] reduces the volume in which the loaded constituents are subjected to the ZE separation. Therefore, it is reasonable to expect that any precolumn sample pretreatment that reduces the electric conductivity of the original sample solution while providing a full recovery of the analyte on the pretreatment, is in fact effective in reducing the $c_{A,s,LOD}$ value in ZE.

Enhanced risks of the peak overlaps are common in the separations of multicomponent mixtures by column chromatography techniques [70]. Undoubtedly, this is a general problem of the column separations and it is relevant to electroseparations as well. It may become critical especially in short columns, *i.e.*, under the conditions typical for the separations performed on the chips. This illustrates (Fig. 3) plots of probabilities in obtaining pure peaks of the analytes for varying numbers of the sample constituents in the ZE runs in the columns of 10 and 100 mm lengths. Here, the probabilities were calculated using the relationships as derived by Davis and Giddings [70] for elution chromatography. Although these relation-

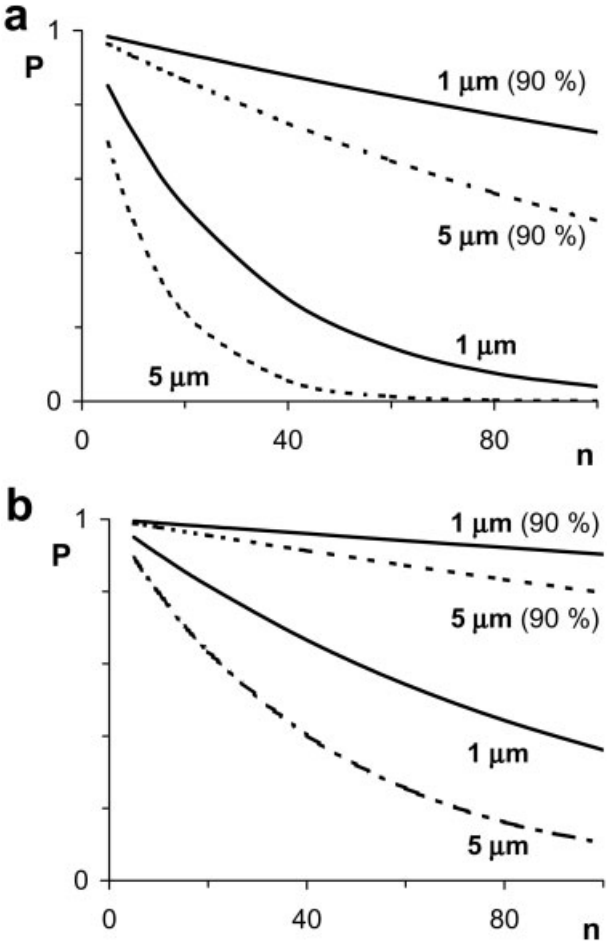

Figure 3. Probabilities of obtaining pure peaks of the ZE analytes as calculated for varying numbers of the sample constituents and for 1 and 5 µm plate height values as parameters. (a), (b) Plots for 10 and 100 mm lengths of the separation path, respectively. The numbers in parentheses identify the plots, obtained for the same lengths and plate height values when a 90% reduction of the number of the sample constituents by sample clean up was assumed. For further details see Section 5.

ships reflect a certain simplification of the problem, linked with the model employed, the plots in Fig. 3 serve as clear indications of difficulties in obtaining pure peaks in the separations of multicomponent mixtures on the chips also under the working conditions favoring very high separation efficiencies. In addition, they indicate that the sample pretreatment should also reduce the number of matrix constituents accompanying the analyte in the sample.

5.1 Characterization of the sample pretreatment in ZE

When we consider the above facts it is apparent that the sample pretreatment in ZE can be well characterized by an accompanying change of the electric conductivity of the sample solution. This is due to the fact that its value is a sum of contributions of the (ionic) constituents present in the sample, and for the original sample κ_s can be expressed by (for the sake of simplicity only monovalent sample constituents are assumed):

$$\kappa_s = F\left(c_{A,s}\bar{m}_A + \sum_{M,i} c_{M,i}\bar{m}_{M,i}\right) \quad (5)$$

where c and $\bar{m}$ are symbols for the concentrations of the ionic sample constituents and their effective mobilities (under particular ZE separating conditions), respectively. The subscripts identify the original sample solution (s), the analyte (A) and the sample matrix constituents (M,i). The counterionic constituents present in the original sample are included into the matrix constituents in Eq. (5). When identical volumes of the original and pretreated sample solutions are assumed, the electric conductivity of the sample (κ_{s^*}), reached after an ideal sample pretreatment (only the analyte and the counterionic constituent, identified by the subscript C, are present in the sample), is given by:

$$\kappa_{s^*} = F\left(c_{A,s^*}\bar{m}_A + c_{C,s^*}\bar{m}_C\right) \quad (6)$$

Equations (5) and (6) show that the difference between κ_s and κ_{s^*} includes both the reduction of the number of the matrix constituents (needed to reduce the risks of the analyte peak overlap) and desalting of the sample (needed to increase the sample load without sacrificing the separation performance of ZE). Obviously, such a characterization cannot reflect an impact of the sample pretreatment on disturbances to the separation process, *e.g.*, linked with changes of the ζ-potential of the separation compartment caused by adsorption of some (trace) sample constituents.

5.2 Electrophoretic sample pretreatment on the CE chips

When we consider the sample amounts currently handled in conjunction with the separations on the CE chips it is clear that direct couplings of the sample pretreatment procedures to the separation stages of the analysis are almost a must. In fact, such a requirement is inherently included in a basic concept of μTAS [1, 58]. A recent review by Verpoorte *et al*. [9], dealing in details with sample pretreatment in these systems, documents a general preference of such an approach. This review also shows that many of the sample pretreatment techniques compatible with electrophoresis separations are already transferred to a chip format. Of these, the highest degree of compatibility very likely offers electrophoresis methods. From the analytical point of view it is important that they provide, mainly: (i) different separation mechanisms in the pretreatment and separation stages of the analysis (to benefit from a 2-D separative effect [71]); (ii) an electrophoretically driven removal of the matrix constituents from the separation system on the pretreatment (to desalt the sample and reduce the number of the sample constituents); (iii) processing of an adequate amount of the sample (to make the analyte detectable in the separation stage of the analysis); (iv) a nondispersive transfer of the analyte after the pretreatment to the separation stage.

As already mentioned above, analytically relevant electrophoretic methods (ZE, ITP, IEF) differ in the sample loadabilities, spatial distributions of the separated constituents and concentrating effects. This gives a practical frame for their functional combinations, especially when we realize that the methods are implemented *via* the compositions of the electrolyte solutions in which the separations are performed (see the Section 2). Although all electrophoretic methods can be mutually on-line combined, so far some attention was paid only to the ITP-CZE coupling. This combination, introduced 13 years ago [72], makes the use of the CC configuration of the separation system as originally proposed by Everaerts *et al*. [73] for an enhanced concentration detectability in ITP. Its analytical benefits in conventional CE seem already well documented [74–98]. In addition, an extension of this combination to ITP-capillary electrochromatography (ITP-CEC) as reported by Mazereeuw *et al*. [99] should be mentioned here. The number of separation stages into which the CC system is split is not restricted once the use of multiple stages has an analytical sense [100, 101]. A transfer of the ITP-CZE combination to the chip-based CE systems [20–26, 102] was in fact inspired by analytical performance of this combination in conventional CE (see also below).

6 Some instrumentation aspects

6.1 Basic electrophoretic methods and their combinations on the chips

Glass and quartz, preferred fabrication materials in the early period of developments of the miniaturized systems, seem at present less attractive and the use of plastic materials prevails. This preference of plastics and polymers supports several arguments as discussed in details in recent reviews on this topic [103–105]. There are many CE chip designs described in the literature (for a review see, *e.g.*, [1, 4, 6, 9] and references given therein). Although the designs in some respects reflect the materials of which the chips are made and the fabrication procedures employed, from the point of view electroseparations, however they follow general concepts as known from conventional CE systems [16, 18, 19, 34, 53, 59]. Therefore, only a limited number of basic configurations of the separation channels on the CE chips can be distinguished. Schematic drawings of the single-column and CC-CE chips in Fig. 4 illustrate two of them. The former configuration (Fig. 4a) provides means for running basic electrophoretic methods (ZE, ITP, IEF), although in some instances with transient phases due to differences in the electrolyte and sample compositions (*e.g.*, ZE with a transient ITP induced by the sample composition [68, 69, 106–109]). On the other hand, the latter configuration (Fig. 4b), suitable mainly, for on-line combinations of the electrophoretic methods (*e.g.*, ITP-ZE, 2D ITP [110]), may be used in the runs with basic electrophoretic methods once the coupled channels are filled with the proper electrolyte solution(s) [20–22]. Its use can be very likely extended to comprehensive ITP-ZE separations in the way as proposed by Lee *et al*. [90, 93] and ITP-CEC [99] by packing the C2 channel (Fig. 4b) with a suitable chromatography sorbent.

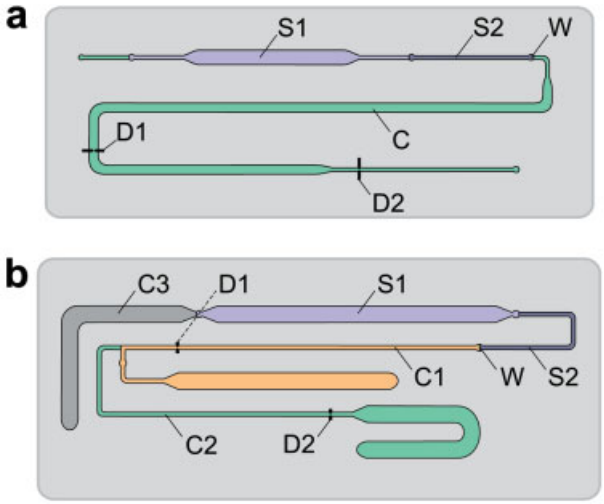

Figure 4. (a) Single-column and (b) CC poly(methylmethacrylate) chips provided with the conductivity detection cells. Details of (a): S1 and S2 = 4500 and 500 nL sample injection channels, respectively; W = an outlet hole from the chip channels to a waste container (not shown); D1, D2 = platinum conductivity sensors. C = the separation channel (W – D2) consists of two sections: (i) a 5700 nL channel between W and D1 ($59.4 \times 0.5 \times 0.2$ mm (length, width, depth); the volume is corrected for a transient part behind W) and (ii) a 2800 nL channel between D1 and D2 ($31.4 \times 0.5 \times 0.2$ mm; the volume is corrected for a transient part in front of D2). Details of (b): C3 = terminating electrolyte channel; S1 and S2 = 9000 and 950 nL sample injection channels, respectively; W = an outlet hole from the chip channels to a waste container; C1 = first separation channel (3050 nL volume; $76 \times 0.2 \times 0.2$ mm (length, width, depth)) with a platinum conductivity sensor (D1); C2 = second separation channel (1680 nL volume; $42 \times 0.2 \times 0.2$ mm) with a platinum conductivity sensor (D2).

6.2 Closing the separation compartment of the chip

The CE chips can be usually employed in both hydrodynamic arrangements of the separation system (see the Section 3) and an actual arrangement is provided by an external fluidic system (electrolyte and sample management unit, E&SMU) employed in filling the chip channels with the electrolyte and sample solutions. For example, in the separations carried out with the closed separation system we prefer the use of the E&SMUs as shown in Fig. 5. While the construction of E&SMU in Fig. 5a includes (needle or pinch) valves as inlet devices for the solutions with which the chip channels are filled, the one in Fig. 5b makes the use of microperistaltic pumps. Here, the pump rollers themselves automatically close the inlet to a particular chip channel when the solution pumping is stopped. Alternative electrolyte solutions with which the channels of the CC chip are filled before the runs (Fig. 5) indicate key differences associated with its use to the ITP, ZE and ITP-ZE separations.

The chip and E&SMU are connected to the electronic and control unit (E&CU) of the equipment in the way shown in Fig. 6. The power supply of the E&CU (HV in Fig. 6) delivers the driving voltage of a required polarity to the electrodes (E1–E3 in Figs. 5 and 6) placed between the inlets to the corresponding chip channels and the valves or the pumps (when the rollers close the separation compartment). Here, the driving electrode E3 is permanently connected to the high-voltage pole of the power supply while the driving electrodes E1 and E2 (the counterelectrodes for the separation channels) are connected to its ground

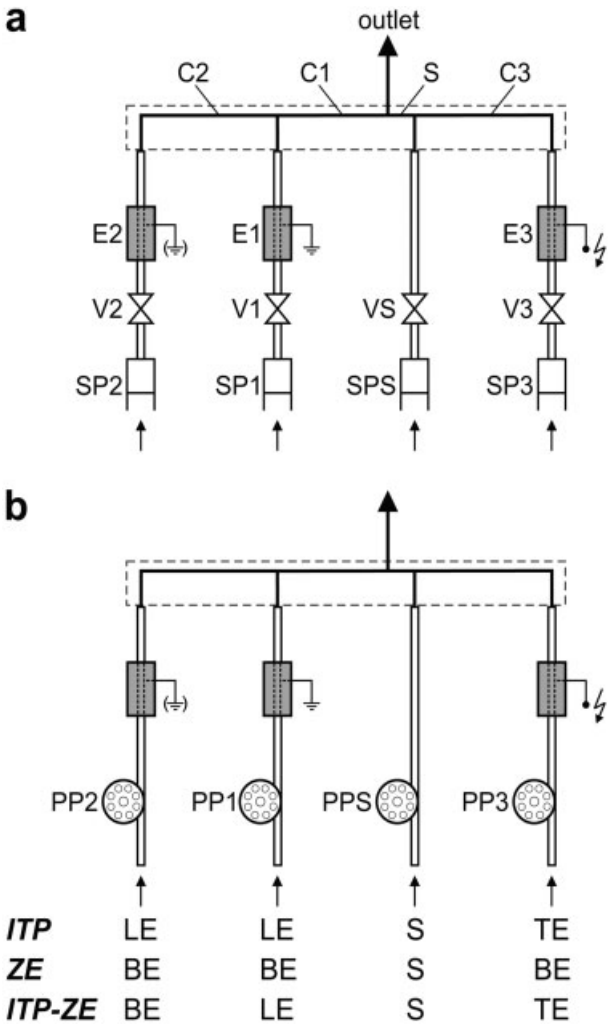

Figure 5. E&SMUs hydrodynamically closing the separation system on the chips during the CE runs. (a) E&SMU closing the inlets to the chip channels (C1–C3, S) by needle or pinch valves (V1–V3, VS). The electrolyte and sample solutions are delivered sequentially to the channels by syringes (SP1–SP3, SPS); excesses of the solutions are led *via* "outlet" to a waste container (not shown). (b) E&SMU closing the inlets to the chip channels by rollers of the peristaltic pumps (PP1–PP3, PPS). The driving electrodes (E1–E3) are connected to the power supply as shown in Fig. 6. The chip channels for the ITP, ZE and ITP-ZE separations are in both instances filled with the solutions as shown in (b). LE, TE, BE = the leading, terminating and background (carrier) electrolyte solutions electrolytes, respectively. For further details see Section 6.2.

via a high-voltage relay (HVR in Fig. 6). Such connections of the electrodes make possible to transport the separated constituents either to the electrode E1 or E2. Benefits from such an operation are apparent from illustrative schemes describing the ZE, ITP and ITP-ZE separations in the Section 7.

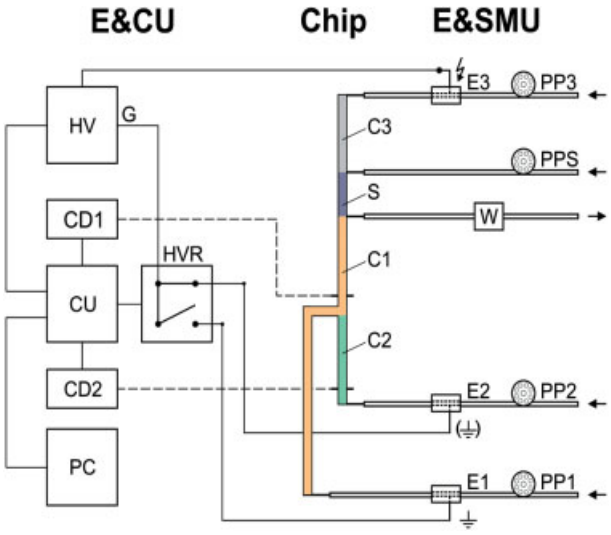

Figure 6. Scheme of the equipment used in the CE separations on the CC chips. E&CU: CU = control unit; HV = high-voltage power supply; CD1, CD2 = conductivity detectors for the first (C1) and second (C2) separation channels, respectively; HVR = high-voltage relay switching the direction of the driving current in the separation compartment (moving reeds of the relay connect either E1 or E2 to the ground pole, G, of HV). E&SMU closing the inlets to the chip channels by rollers of the peristaltic pumps (see Fig. 5b): E1, E2 = driving electrodes for the first (C1) and second (C2) separation channels, respectively; E3 = the driving electrode connected to the high-voltage pole of the power supply (HV). PP1, PP2, PP3, PPS = peristaltic pumps for filling the first (C1), second (C2), third ("terminating", C3), and sample (S) channels with the electrolyte and sample solutions, respectively; W = a tube connected to the outlet hole on the chip (W in Fig. 4).

As discussed in the Section 3.2, gas bubbles formed by electrochemical reactions at the driving electrodes are sources of HDF in the chip channels. In the separation system closed in the way as shown in Figs. 5 and 6, HDF is directed to the waste collector *via* a tube (W in Fig. 6) connected to the outlet hole on the chip (W in Fig. 4). These sources of HDF can be eliminated in the way as favored in some conventional CE equipment [17, 18, 48, 111], *i.e.*, by closing the separation system in the directions to the driving electrodes with the aid of semipermeable membranes (see also Figs. 1 and 2). Although eliminating HDF, this solution is less convenient for the miniaturized CE devices as it requires electrode vessels and regular replacements of the electrolyte solutions in these vessels (at least when the electrolyte system in which the separation is performed is changed). A more convenient solution in this respect is provided by the design of the driving electrode as

shown in Fig. 7. Here, a metallic electrode (made, *e.g.*, from a platinum net) is in a direct contact with a semipermeable membrane (wetted by the electrolyte solution) that mediates the electric contact of the electrode with the electrolyte solution in the separation compartment. At the same time, the membrane prevents entrance of the gas bubbles from the electrode to the separation compartment (the bubbles are released *via* the holes in the Pt electrode to the surrounding, see also Fig. 7). In addition, the Pt electrode mechanically supports the membrane so that disturbances due to its movement [31] are prevented. More than two years lasting testing of the membrane driving electrodes in our laboratories revealed their excellent performances in eliminating disturbances linked with gas formation in the separations carried out on the CE chips of various designs.

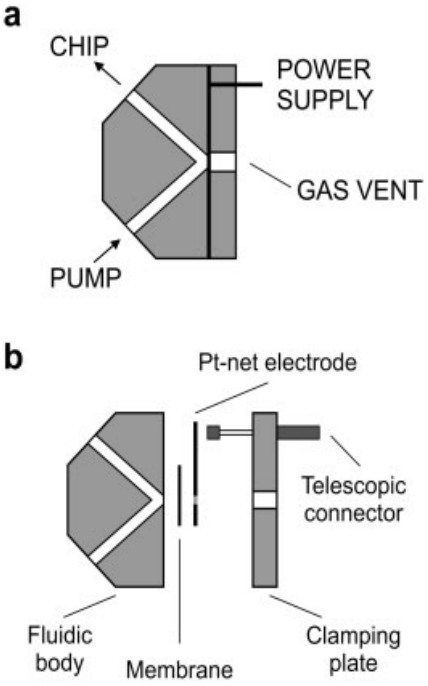

Figure 7. A membrane driving electrode for the CE separations on the chips. (a) Assembled electrode: chip, pump = connections of the electrode assembly to the chip inlet and pump, respectively; power supply = connection of the electrode to the power supply; gas vent = a hole for gas evolved at the electrode (the size of the hole is significantly magnified). (b) Electrode components: fluidic body = a poly(methylmethacrylate) body of the electrode with the hole for the solution pumped to the chip channel; membrane = a semipermeable membrane; Pt-net electrode = a strip of a platinum mesh; telescopic connector = a connection of the electrode to the power supply; clamping plate = a poly(methylmethacrylate) plate tightly clamping the Pt-electrode and membrane to the fluidic body.

6.3 Closed separation compartment of the chip and the detection of analytes

General links between the separation and detection processes in miniaturized CE as discussed in the Section 4 outline well-known problems regarding the detection sensitivity that brings miniaturization especially to photometric UV-absorbance detection (undoubtedly a key detection technique in conventional CE). Although attempts to solve these problems of photometric detection are apparent [65, 112–116], at the same time we can see a significant increase of interest in the use of conductivity and amperometry detection techniques in the miniaturized CE as documented by recent reviews relevant to these topics [11–14, 117].

Efforts aimed at minimizing fluctuations in the migration velocities of the separated constituents on the CE chips (see the Section 3) are in fact closely linked with the ones focused on an enhanced reliability of the analytical data as obtained from these devices. The closed separation system with suppressed EOF, providing working conditions that are associated with minimum fluctuations in the migration velocities (see Eq. (1) and an accompanying discussion), has therefore inherent advantages from the point of view of the qualitative and quantitative analysis. While having no special requirements regarding the use of the on-column detectors its use in combinations with the postcolumn detectors may require a pump-driven flow of the solution that transports the analytes to such a detector. A general solution to this problem can be found in conventional CE with the closed separation system, for example, as proposed in the work that for the first time coupled amperometric detection to CE [118].

The CE runs on the chips provided with the single column configuration of the separation compartment are usually monitored by one of the detectors compatible with these devices (see, *e.g.*, [1, 4, 10–14]). The situation is in some respects more demanding with the CC chips [20–24]. This is due to the fact that one of the detectors (CD1 in Fig. 6), besides the acquisition of the analytical data from the corresponding separation channel, serves also as a sensing element in an automated control of the column switching operation during the run (a switching of the direction of the driving current to the electrode E1 or E2, as shown in Figs. 5 and 6). Here, the use of conductivity detection is beneficial when ITP is employed in the first separation channel (C1 in Figs. 4–6). It is a universal ITP detection technique of a high resolving power [17, 18] and provides a staircase-like course of the detection signal along the ITP stack. Such a signal course is convenient for the column-switching sensing as it identifies the presence of a front (or rear) boundary of a particular zone from the ITP stack in the detection cell. The use of the conductivity

detection in the second separation channel of the CC chip (C2 in Figs. 4–6) is more restrictive when the ZE separation is to be monitored in this channel. Nevertheless, once the electrolyte system used in this stage is in a harmony with a sensitive conductivity detection of the ZE separands [33, 34, 119], its detection performance can be very impressive [21, 22, 24].

A recent work by Wainright *et al*. [26] showed that the ITP-ZE separation of fluorescently labeled ACLARA eTag reporters on the CC chip, combined with their laser-induced fluorescence detection in the ZE channel, led to sub-pmol/L limits of detection for these constituents (a 400-fold reduction in the detectability in comparison to what was possible by ZE). Undoubtedly, such concentration detectabilities demonstrate analytical benefits attributable to a combination of highly sensitive detection technique with a powerful separation technique on the chip and indicate potentialities of miniaturized CC devices in (ultra)trace analysis. These results also clearly indicate that efforts aimed at coupling the CC chips with other detection techniques of adequate detection performances (*e.g.*, UV absorbance, amperometry, potentiometry, chemiluminescence, and MS) may significantly contribute in extending (ultra)trace analysis applications of miniaturized CE.

The use of Raman spectroscopy and NMR to the detection (identification) of the constituents separated by CE requires that these are present in the detection (measuring) cell at high concentrations. Such requirements need not be easily met by ZE. Considering this, Walker *et al*. [120] preferred the use of ITP and showed that a concentrating power of ITP makes possible spectral identification of ppb concentrations of the analytes by normal Raman spectroscopy on a CE chip. Similarly, recent works dealing with CE-NMR combinations [121–123] demonstrated that ITP provides means effective in acquiring ^{1}H NMR spectra for nmol amounts of the analytes in about 10 s [123]. As nmol amounts of the sample constituents can be easily separated by ITP on the CC chip [20–22] this CE device can be considered as a promising sample pretreatment and separative tool for NMR.

7 ZE, ITP and ITP-ZE separations on chips with the closed separation system

Examples of the ZE, ITP and ITP-ZE separations discussed in this section were obtained mainly on the chips shown in Fig. 4 and their versions that did not differ in architectures of the separation compartments and key geometrical dimensions of the channels. From the schemes shown in Fig. 4 it is apparent that the use of the chips is not restrictive as far as the hydrodynamic arrangement of the separation system is concerned. However, their geometrical dimensions are responsible for relatively small hydrodynamic resistances of the channels, and therefore already small pressure differences between the chip inlets lead to HDFs that deteriorate the separations [20]. The chip designs reflect their electrophoretic multifunctionalities and the geometrical dimensions of their channels also enhanced sample loadabilities. The chip in Fig. 4a is intended to the single-column ZE and ITP separations while the one in Fig. 4b to the CC separations using ZE, various alternatives of ITP and mainly ZE with on-line ITP sample pretreatment.

7.1 ZE separations

Both chips shown in Fig. 4 are provided with two sample injection channels. Their volumes (see the legend to Fig. 4) give possibilities of loading (very) diluted sample solutions. On the other hand, such sample volumes require that in the ZE separations the electric field stacking [34, 66, 68] or sample-induced ITP stacking [68, 69, 106–109] are effective as otherwise significant injection dispersions [19, 28] are unavoidable. Therefore, these focusing steps are included into a general scheme of the ZE separation on these chips (Fig. 8b). An electropherogram (Fig. 9) as obtained from the ZE separation of oxalate loaded in the sample at a 2×10^{-7} mol/L concentration in the presence of a large excess of chloride (a 3.5×10^{-3} mol/L concentration in the loaded sample) shows an analytical effectiveness of this approach. This electropherogram also shows that the separation path available on the chip (Fig. 4a) was sufficient for a complete destacking [108] of oxalate from chloride. The use of the single-column chip to rapid ZE determination of oxalate in urine [46], oxalate in beer [47] and the ZE separations of proteins [124] documents some of its application potentialities.

7.2 ITP separations

A pioneering era in the developments of CE is closely associated with ITP [17, 18]. So far however, the use of this electrophoresis method to the separations in the miniaturized CE systems is rather scarce. The works by Walker *et al*. [120] and Prest *et al*. [125] can be very likely considered as the first attempts aimed at using ITP to the separations in these systems. While the former of these works employed ITP in the opened separation system, mainly as a concentration tool for normal Raman spectroscopy, the latter transferred conventional ITP with the conductivity detection to a miniaturized format.

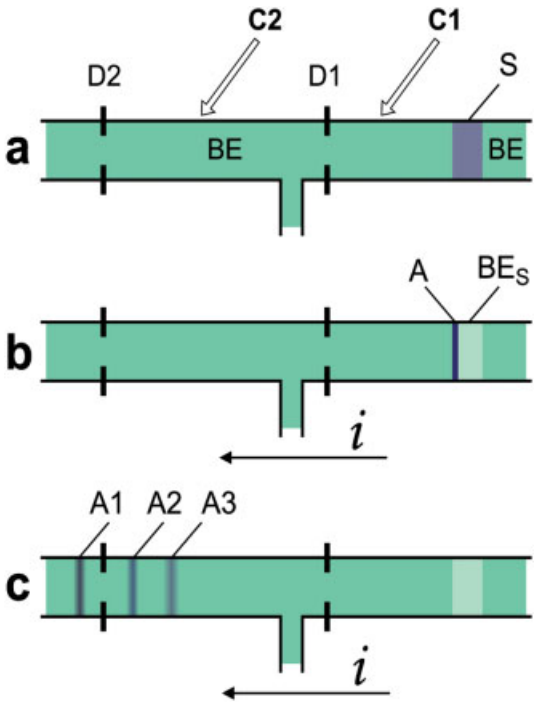

Figure 8. Characteristic phases in the ZE separation on a CC chip with the closed separation system. (a) Initial arrangement of the solutions in the chip channels; (b) electric field stacking of the sample constituents; (c) detection of the separated sample constituents. C1, C2 = first and second separation channels on the chip; D1, D2 = detection sensors in the separation channels; BE_S = background electrolyte adapted to the composition of the sample; S = sample; A = stacked sample constituents; A1–A3 = resolved sample constituents, i = direction of the driving current.

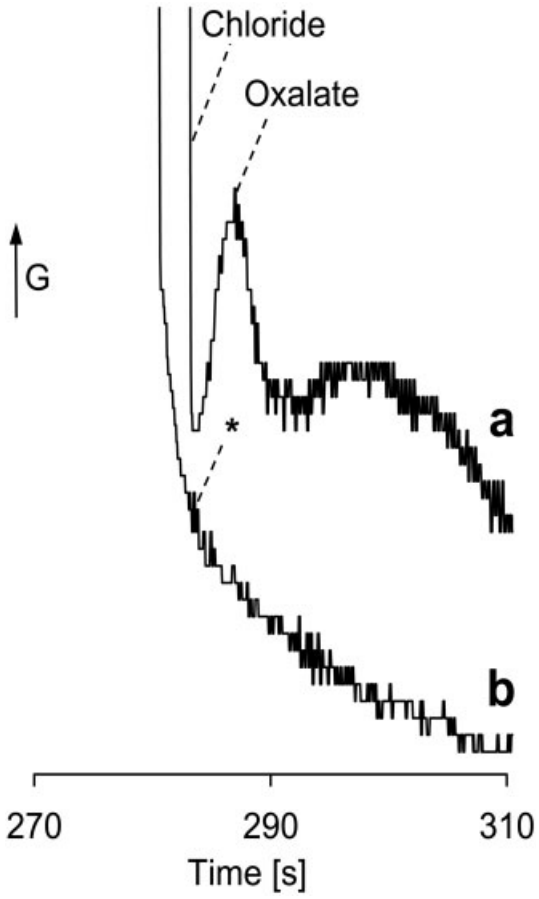

Figure 9. ZE separation of oxalate from a large excess of chloride on a CE chip. The separation was performed on the single-column chip (Fig. 4a) using the equipment as shown in Fig. 6 (the pump PP1 and the driving electrode E1 were not connected). (a) Loaded sample (500 nL volume) contained oxalate at a 2×10^{-7} mol/L concentration while chloride was present at a 3.5×10^{-3} mol/L concentration; (b) blank run (only chloride at a 3.5×10^{-3} mol/L concentration present in the loaded sample). The separations were carried out at pH 4.0 (BE: 15 mmol/L propionic acid adjusted to pH 4.0 by ε-aminocaproic acid; methylhydroxyethylcellulose, present in the solution at a 0.05% w/v concentration served as an EOF suppressor). The samples were prepared in a 20% v/v BE solution. The separations were carried out with a 12 μA driving current. G = increasing conductance.

As already mentioned above, the chips shown in Fig. 4 are CE devices suitable also for the ITP separations (see a scheme in Fig. 10 illustrating key phases in the ITP separation). The one in Fig. 4a, intended for the single-column separations, is provided with two conductivity cells (D1 and D2 in Fig. 4a) in the separation channel. The cell placed closer to the sample injection channels (D1 in Fig. 4a) is intended to monitor the ITP separations in which the sample macroconstituents are to be detected and/or determined. The separation channel/detection cell volume ratio is *ca.* 1700 for the cell D2. This makes the use of advantages of the volume coupling [126] in enhancing the sensitivity of conductivity detection for the ITP microconstituents (the zones of microconstituents are prolonged immediately before the detection in the cell D2). An electropherogram as obtained from the ITP separation of organic acids present in a white wine sample (Fig. 11) illustrates sample load capabilities (the sample loaded on the chip corresponded to *ca.* 50 nL of undiluted wine) and analyte detectabilities (the acids could be detected down to 1 mg/L concentrations) of such a chip design.

The CC chip (Fig. 4b) provides a broader choice of the ITP techniques than the single-column chip. Here, the techniques known from its conventional counterpart [73, 111] can be employed when appropriate electrolyte systems are used in the coupled separation channels. The CC

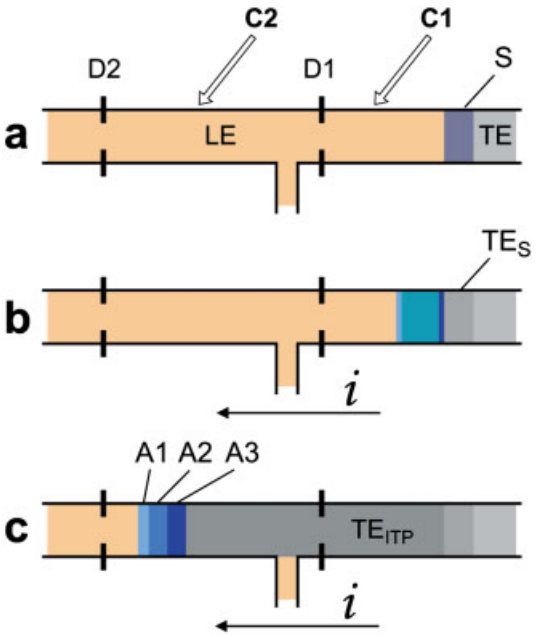

Figure 10. Characteristic phases in the ITP separation on a CC chip with the closed separation system. (a) Initial arrangement of the solutions in the chip channels; (b) ITP separation process; (c) detection of the separated constituents under the ITP steady-state conditions. C1, C2 = first and second separation channels on the chip, respectively; D1, D2 = detection sensors in the separation channels; TE_S = terminating electrolyte adapted to the composition of the sample; T_{ITP} = terminating electrolyte adapted to the composition of the leading electrolyte solution; S = sample; A1–A3 = sample constituents, i = direction of the driving current.

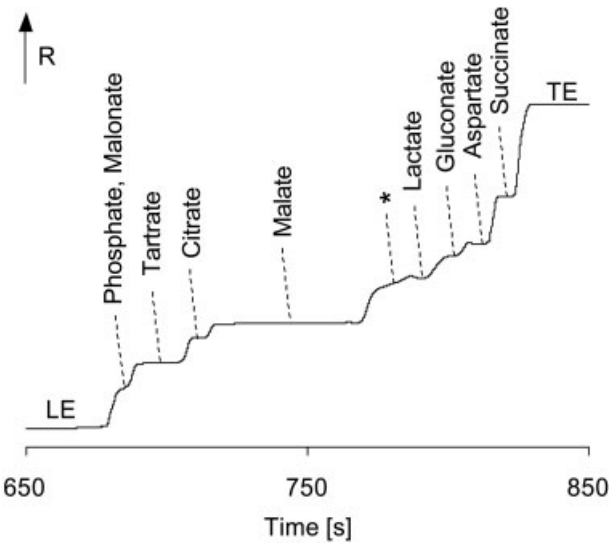

Figure 11. ITP separation of anions present in a white wine sample on a CE chip. The separation was performed on the single-column chip (Fig. 4a) using the equipment as shown in Fig. 6 (the pump PP1 and the driving electrode E1 were not connected). The sample, loaded by a 500 nL sample injection channel, was 10 times diluted with water before the analysis. The ITP separation was carried out at pH 2.9 (LE: 10 mmol/L HCl, adjusted with β-alanine to pH 2.9; TE: 5 mmol/L glutamic acid, adjusted with histidine to pH 5.0; EOF was suppressed by methylhydroxyethylcellulose, present in both solutions at a 0.1% w/v concentration). The driving current was 20 μA and it was reduced to 10 μA before the detection (D2, in Fig. 4a). (*) an unidentified anionic wine constituent.

chip, for example, was shown [20–23, 43, 45] to provide an equivalent to the single column ITP separation (when performing the separation in the first separation channel of the chip (C1 in Fig. 4b)) and ITP in the tandem-coupled columns (when performing the run in both separation channels of the chip (C1 and C2 in Fig. 4b) filled with the same leading electrolyte). Specific advantages are attainable when the separation channels are filled with the leading electrolytes of differing concentrations [20, 45]. This technique, the ITP separation in a concentration cascade, introduced into conventional CE by Boček *et al*. [127], enhances the detectabilities of the separated constituents from the response of the conductivity detection due to well-known links between the concentration of the leading electrolyte and the lengths (volumes) of the zones. Undoubtedly, the use of the CC chip can be extended to 2-D ITP separations by combining different separation mechanisms in the channels [110]. An interesting approach to the ITP separations on the chip was presented recently by Prest *et al*. [128]. These authors, transferring bidirectional ITP to the chip format, made possible determination of cationic and anionic constituents in one run. Practical potentialities of this approach can be assessed also from the works dealing with this topic in a context with the developments of conventional CE [129–131].

7.3 ITP-ZE separations

ITP and ZE, differing in the sample loadabilities, spatial configurations of the separated constituents and concentrating capabilities, can be on-line combined on the CC chip in two general ways [20–26]: (i) ITP, concentrating the sample constituents into a narrow pulse is intended, mainly, as a sample injection technique for ZE; (ii) ITP, while concentrating the analyte and some of the matrix constituents into a narrow pulse, serves mainly as a sample cleanup technique and removes a major part of the sample matrix from the separation system before the final ZE separation.

The scheme in Fig. 12 illustrates the use of ITP to the sample injection into the ZE channel on the CC chip. Here, to prevent overloading of the ZE stage, the sample load is

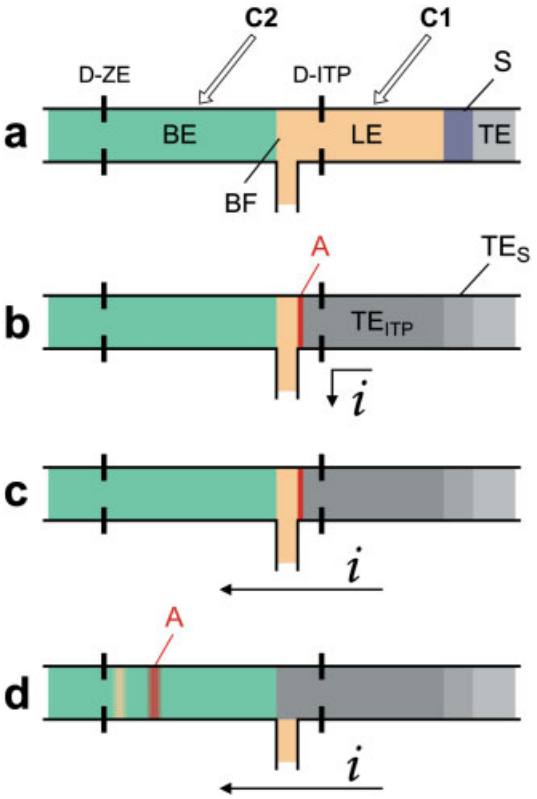

Figure 12. ITP as a sample injection technique for ZE on a CC chip with the closed separation system. (a) Initial arrangement of the solutions in the chip channels; (b) End of the run in the ITP channel; (c) electrophoretic transfer of the focused sample constituents to the ZE channel (by switching the direction of the driving current); (d) separation and detection of the sample constituents in the ZE channel. BF = bifurcation region; C1, C2 = the ITP and ZE separation channels on the CC chip, respectively; D-ITP, D-ZE = detection sensors in the ITP and ZE separation channels, respectively; TE_S = terminating electrolyte adapted to the composition of the sample (S); T_{ITP} = terminating electrolyte adapted to the composition of the leading electrolyte solution; A = analyte, i = direction of the driving current.

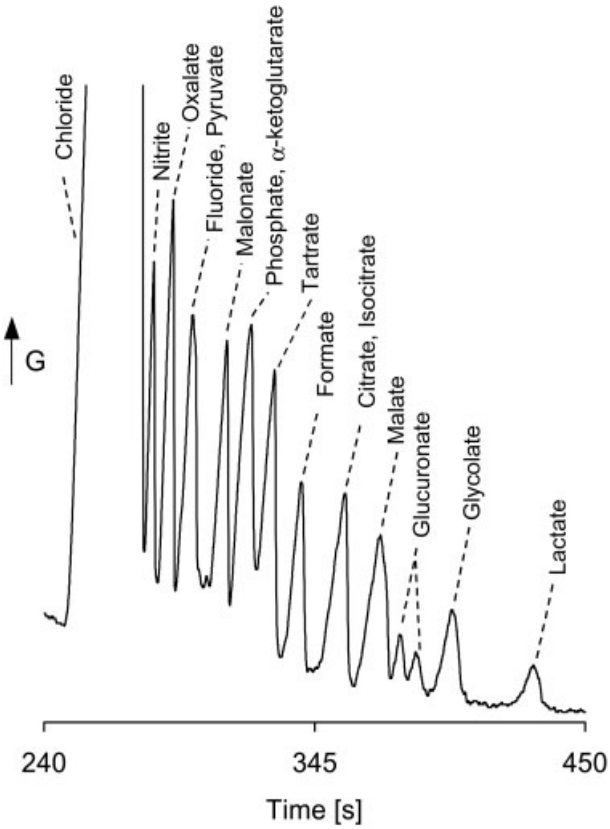

Figure 13. ZE separation of a mixture of anions using ITP as a sample injection technique on a CC chip with the closed separation system. The scheme in Fig. 12 was followed and the separation was performed on the CC chip (Fig. 4b) using the equipment as shown in Fig. 6. The sample containing the anions at 10^{-5} mol/L concentrations was loaded with the aid of a 950 nL sample injection channel. LE: 10 mmol/L HCl, adjusted to pH 3.2 by β-alanine; TE: 10 mmol/L aspartic acid adjusted to pH 4.2 by β-alanine; BE: 10 mmol/L aspartic acid adjusted to pH 3.6 by β-alanine; methylhydroxyethylcellulose, present in the solutions at a 0.1% w/v concentration served as a EOF suppressor. The separation in the ITP stage was carried out with a 15 μA driving current while the transfer of the sample pulse into the ZE channel and the ZE separation were carried out with a 20 μA driving current. G = increasing conductance.

restricted. Therefore, the sample constituents can be concentrated between the leading and terminating zones in a very short ITP channel. To prevent losses of the sample constituents, the direction of the driving current to the ZE channel must be switched before the sample pulse crosses the outlet from the ITP channel (Fig. 12b). Due to this, besides the sample pulse, also the leading electrolyte, filling the bifurcation region (BF in Fig. 12) before the switching of the columns, is transferred to the ZE channel. The transferred leading electrolyte acts as a stacker [68, 69, 108] for the sample constituents in the ZE stage and, at the same time, determines the rates at which they are destacked in this stage (see, *e.g.*, [108]). An electropherogram in Fig. 13 shows that the destacking rates need not be critical in reaching the ZE resolutions of multicomponent mixtures of the separated constituents on the CC chip.

The scheme in Fig. 12 indicates that the use of the CC chip may be beneficial, for example, in situations when the sample contains a large excess of very mobile ions (*e.g.*, Cl^- or Na^+ in biosamples). This is due to the fact that these are removed from the separation system before the pulse, containing the sample constituents migrating in

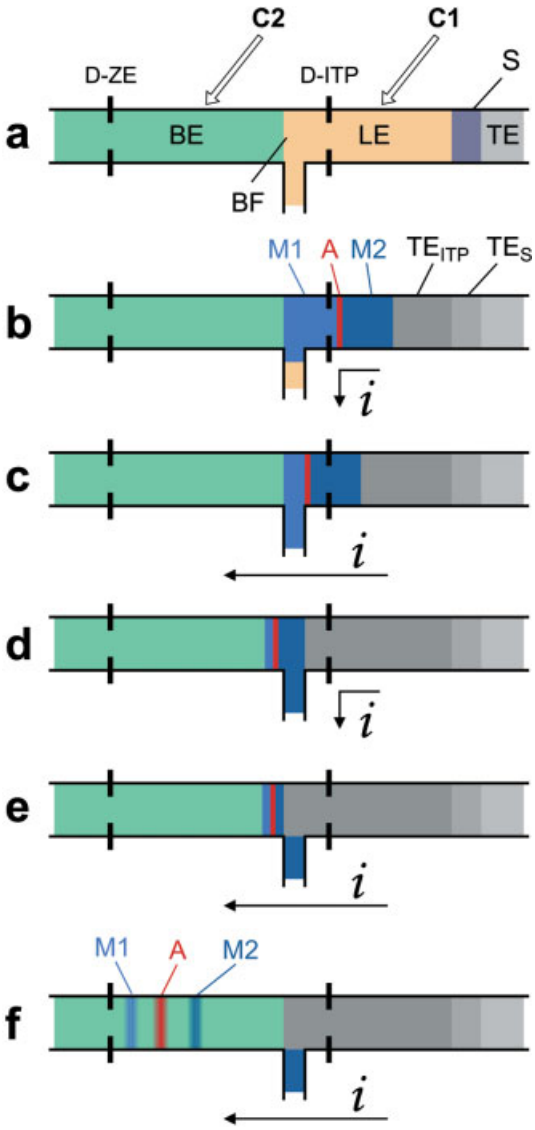

Figure 14. ITP sample clean up for ZE on a CC chip with the closed separation system. (a) Starting arrangement of the solutions in the chip channels; (b) ITP separation with the analyte (A) trapped into the boundary layer between the zones of front (M1) and rear (M2) spacers; (c) end of the run in the ITP channel followed by an electrophoretic transfer of the analyte containing fraction to the ZE channel (by switching the direction of the driving current); (d) removal of the sample constituents migrating behind the transferred fraction (by switching the direction of the driving current); (e) starting situation in the separation performed in the ZE channel (the direction of the driving current was switched); (f) separation and detection of the transferred constituents in the ZE channel. For abbreviations see Fig. 12.

the ITP stack, reaches the column outlet (Fig. 12b). Such a sample cleanup can be significantly enhanced when the ITP-ZE run follows the scheme shown in Fig. 14. Here, a trap of the analyte into the boundary layer between the zones of a pair of the spacing constituents (M1 and M2 in Fig. 14) makes possible removals of the matrix constituents stacked in front of the front spacer (M1) and behind the rear spacer (M2) in the ITP stage of the run. This matrix removal, performed by switching the direction of the driving current, complements a transfer of the analyte into the ZE channel (Fig. 14c–e). The boundary layer in which the analyte is trapped may contain also other sample constituents of the corresponding effective mobilities [88]. Due to this, ITP can only approach requirements defining an ideal sample pretreatment for ZE (see Eqs. (5) and (6) and an accompanying discussion in the Section 5).

The repeatability with which the analyte-containing fraction is transferred from the ITP stack to the second channel of the closed separation system on the CC chip is, in general, high (see, *e.g.*, the data given in [20]). This gives a practical frame to the use of the ITP-ZE combination on this chip and several works [20–22, 24, 25, 102] already demonstrated its analytical benefits. In this context we should note that the results of these works are in some respects restricted as they are based only on the use of the conductivity detection in the ZE stage of the run. Nevertheless, this need not be disadvantageous for some low-molecular weight analytes and, for example, one of the quoted works [21] demonstrated low sub-μmol/L detectabilities of nitrite, fluoride and phosphate in tap, table and river water samples. Such detectabilities, linked in part with a 960 nL sample load on the CC chip, were attained also with the samples containing chloride and sulfate (typical anionic macroconstituents in waters) at 10^4 higher concentrations. A feasibility study dealing with the use of the ITP-ZE combination on the CC chip to the determination of bromate in drinking water was published recently [24]. Here, by loading a 9000 nL volume of the sample on the chip it was possible to detect this anion (a by-product formed on disinfection of drinking water) at a 2×10^{-8} mol/L concentration while its determination at a 8×10^{-8} mol/L (10 ppb) concentration was still possible when the sample contained chloride at a 2 mmol/L concentration. Electropherograms in Fig. 15, obtained from the ITP-ZE analysis of chlorinated tap water, illustrate general potentialities of this combination on the CC chip to a rapid detection and determination of anionic disinfection by-products in drinking water.

The ITP-ZE combination on the CC chip can be convenient to the determination of preservatives and taste-intensifying components in foodstuff as shown by Bodor *et al*. [22]. Here, minimum sample preparation needed also for highly complex food matrices before the run on the chip is a key practical benefit of the combination while its detec-

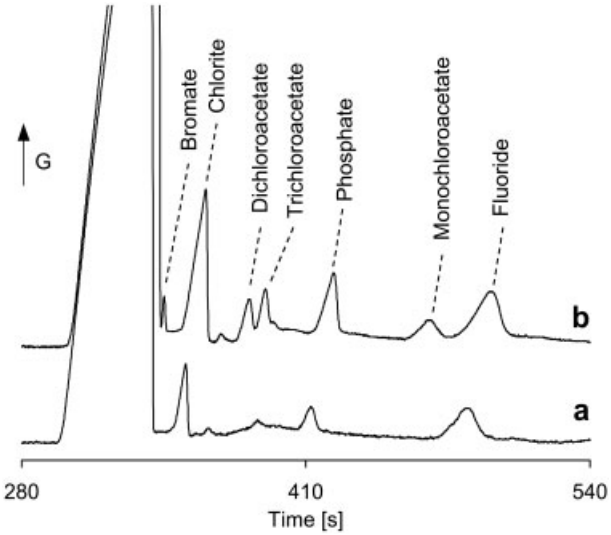

Figure 15. ITP-ZE of anionic disinfection by-products in drinking water on a CC chip with the closed separation system. The scheme in Fig. 14 was followed in the separation performed on the CC chip (Fig. 4b) using the equipment as shown in Fig. 6. (a) Tap water (the sample loaded by a 9000 nL sample injection channel); (b) same sample as in (a) only spiked with the anions at the following concentrations (mol/L): bromate, 2×10^{-7}; chlorite, 3×10^{-6}; dichloroacetate, 10^{-6}; trichloroacetate, 10^{-6}; monochloroacetate, 2×10^{-6}; phosphate, 2×10^{-6}; fluoride, 3×10^{-6}. LE: 10 mmol/L HCl, adjusted to pH 3.05 by β-alanine; TE: 10 mmol/L citric acid adjusted to pH 4.2 by β-alanine; BE: 6 mmol/L citric acid adjusted to pH 2.9 by β-alanine; methylhydroxyethylcellulose, present in the solutions at a 0.1% w/v concentration, served as a EOF suppressor. The separation in the ITP stage was carried out with a 25 μA driving current while the transfer of the sample pulse into the ZE channel and the ZE separation were carried out with a 21 μA driving current. G = increasing conductance.

tion sensitivity is still maintained. Electropherograms in Fig. 16 serve as an illustration of the potentialities of the ITP-ZE combination in this application area.

Ölvecká *et al*. [102] reported direct determination of valproate (an antiepileptic drug) in serum by ITP-ZE on the CC chip with conductivity detection. Here, the ITP-ZE procedure covered a therapeutic range of the drug (50–100 mg/L) and provided its 90–94% recoveries when filtration of the serum samples (typically, 70-fold diluted before the filtration) was the only precolumn sample handling operation. Electropherograms in Fig. 17, obtained for the concentration of valproate corresponding to the lower value of the therapeutic range, illustrate capabilities of ITP-ZE on the CC chip in this particular application. It seems logical to expect that its use can be extended to the (direct) determination of other drugs in

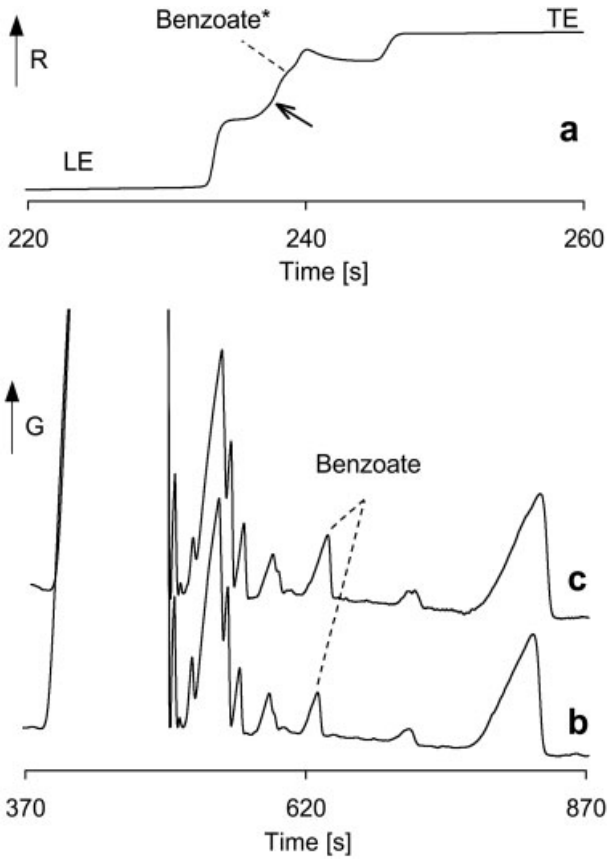

Figure 16. Electropherograms from the ITP-CZE separation of benzoate present in a ketchup sample on a CC chip with the closed separation system. The scheme in Fig. 14 was followed and the separation was performed on the CC chip (Fig. 4b) using the equipment as shown in Fig. 6. The loaded sample (950 nL) contained 1 g of ketchup dissolved in a 500 mL volume. (a) Record from the ITP stage (an arrow indicates a start of the ITP stack transferred into the ZE stage); (b) record for the sample from the ZE stage; (c) same as in (b) only the injected sample was spiked with benzoate at a 10 μmol/L concentration. LE: 10 mmol/L HCl, adjusted to pH 3.9 by β-alanine; TE: 10 mmol/L propionic acid adjusted to pH 4.7 by ε-aminocaproic acid; BE: 10 mmol/L propionic acid adjusted to pH 4.2 by ε-aminocaproic acid; methylhydroxyethylcellulose, present in the solutions at a 0.2% w/v concentration served as a EOF suppressor. The separation in the ITP stage was carried out with a 8 μA driving current while the transfer of sample pulse into the ZE channel and the ZE separation were carried out with a 7 μA driving current. G, R = increasing conductance and resistance, respectively.

serum. This however requires enhanced detection sensitivity in the ZE stage of the combination as the therapeutic ranges for many drugs need not be covered by the conductivity detection when a high serum dilution is taken into account.

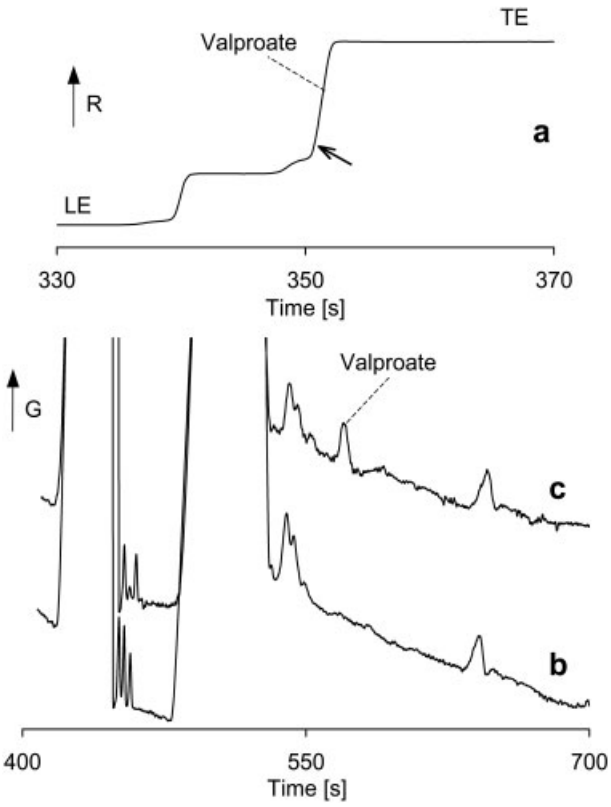

Figure 17. Electropherograms from a direct ITP-ZE determination of valproic acid in serum on a CC chip with the closed separation system. The scheme in Fig. 14 was followed and the separation was performed on the CC chip (Fig. 4b) using the equipment as shown in Fig. 6. The sample was diluted 70-fold with water and filtered (0.45 μm pore size filter) before the ITP-ZE run and it was loaded by a 950 nL sample injection channel of the chip. (a) Record from the ITP stage (an arrow indicates a start of the ITP stack transferred into the ZE channel); (b) trace from the ZE stage in the run with a serum sample; (c) same as in (b) only the sample was spiked with valproic acid at a 5 μmol/L concentration. LE: 10 mmol/L HCl, adjusted to pH 6.1 by histidine; TE: 5 mmol/L 2-(*N*-morpholino)ethanesulfonic acid adjusted to pH 6.0 by histidine; BE: 10 mmol/L 2-(*N*-morpholino)ethanesulfonic acid adjusted to pH 5.5 by histidine; methylhydroxyethylcellulose, present in the solutions at a 0.2% w/v concentration served as a EOF suppressor. The separation in the ITP stage was carried out with a 7.5 μA driving current while the transfer of the sample pulse into the ZE channel and the ZE separation were carried out with a 5 μA driving current. G, R = increasing conductance and resistance, respectively.

The use of ITP-ZE in the open separation system of the CC chip was demonstrated recently by Wainright *et al*. [26]. Suppressing EOF by adding poly(ethylene oxide) to the leading electrolyte solution, these authors eliminated variable migration velocities of the ITP stack during the run [35–37]. In addition, increased viscosities of the electrolyte solutions due to the presence of the polymer, reduced HDF in the separation system. Concentrating capabilities of ITP, acting cooperatively with an ITP desalting of the sample, contributed to sub-pmol/L concentration limits of detection for a group of fluorescent labels (eTag reporters) as provided by laser-induced fluorescence detection.

8 Concluding remarks

HDF in the separation channels on the CE chips with the opened separation systems may originate at least from the following sources: (i) siphoning; (ii) Laplace pressure differences (meniscus effects); (iii) solvent evaporation from the driving electrode vessels. Although remedies to minimize (eliminate) some of the sources are proposed [42] it appears that convincing experimental results in this respect are still missing. Therefore, fluctuations of HDF in the CE runs on the chip operating with the opened separation system cannot be overlooked and their contributions to overall fluctuations of the migration velocities of the separated constituents (see Eq. 1) neglected.

An effective use of the closed separation system on the chip requires that HDF due to gas formation at the driving electrodes is eliminated. This can be achieved, for example, in the way as currently employed in conventional CE equipment working in this hydrodynamic arrangement or using the driving electrodes of the construction as shown in Fig. 7. The CE runs in the closed separation systems, however reach a desired performance only when EOF is suppressed. This is, in fact, a required electrokinetic approach when we consider EOF as a source of fluctuations of the migration velocities in CE (Eq. 1). Very reproducible electrophoretic determination as attained on the chips with the closed separation system (see references discussed in the Section 7) seem, at least in part, attributable to the use of this hydrodynamic arrangement. On the other hand, some practical benefits linked with the use of EOF transport of the solution in which the CE separation is performed [27] are undoubtedly lost in this way.

Conventional CE is, in general, rather demanding as far as the sample pretreatment is concerned, especially when low concentration limits of detection for the analytes are to be reached. Miniaturization of CE further enhances these demands as documented by current efforts aimed at developing miniaturized sample preparation and sample handling techniques [1, 4, 8, 9]. On-line combinations of electrophoretic methods can be considered as promising means in integrating sample pretreatment with the electrophoretic separations on the chips. The results

achieved by the ITP-ZE combination on the CC chip with the closed separation system [20–22, 24, 25, 102] and the ones provided by the same combination on the CC chip with the opened separation system and EOF and HDF reduced by a polymeric additive in the leading electrolyte solution [26] apparently justify such expectations.

Dimensions of the chip channels (favoring very high efficiencies in the ZE separations) are restrictive in implementing some of the key CE detection techniques. For example, the detection techniques based on light absorption (single- and multiwavelength photometry absorbance detectors) are, in fact, not used in the CE separations on the chips. From the application point of view this is a clear limitation of current miniaturized CE devices. On the other hand, many works dealing with implementations of conductivity (both contact and contactless) and amperometric detectors on the CE chips can be found in the literature (see, *e.g.*, [11–14] and references given therein). These detectors, having inherent predispositions to the detection of analytes present in very small volumes, are simple in the constructions and their dimensions fit well the sizes of the CE chips. Nevertheless, it seems logical to assume that further developments of the detection techniques may have a key impact on a broader use of miniaturized CE systems.

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