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Conductivity detection and quantitation of isotachophoretic analytes on a planar chip with on-line coupled separation channels

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Abstract

A poly(methylmethacrylate) chip, provided with two separation channels in the column-coupling (CC) arrangement and on-column conductivity detection sensors and intended, mainly, to isotachophoresis (ITP) and ITP–capillary zone electrophoresis (CZE) separations was developed recently. The present work was aimed at assessing its performance relevant to the detection and quantitation of the ITP analytes. Hydrodynamic (HDF) and electroosmotic (EOF) flows of the solution in the separation compartment of the CC chip were suppressed and electrophoresis was a dominant transport process in the ITP separations with model analytes carried out in this context. When the surfaces of the detection electrodes of the conductivity sensors on the chip were appropriately cleaned qualitative indices of the test analytes [relative step heights (RSHs)], provided by a particular detection sensor, agreed within 1% (expressed via RSDs of the RSH values). Their long-term reproducibilities for one sensor, as estimated from 70 ITP runs repeated in 5 days, were 2% or less. Sensor-to-sensor and chip-to-chip fluctuations of the RSH values for the test analytes were 2.5% or less. In addition, experimentally obtained RSH values agreed well with those predicted by the calculations based on the ITP steady-state model. Reproducibilities of the migration velocities attainable on the CC chips with suppressed EOF and HDF, assessed from the migration time measurements of the ITP boundary between well-defined positions on the separation channels of the chips (140 repeated runs on three chips), ranged from 1.4 to 3.3% for the migration times in the range of 100–200 s. Within-day repeatabilities of the time-based zone lengths for the test analytes characterized 2% RSDs, while their day-to-day repeatabilities were less than 5%. Chip-to-chip reproducibilities of the zone lengths, assessed from the data obtained on three chips for 100 ITP runs, were 5–8%.

Keywords: Conductivity detection; Chip technology; Coupled columns; Isotachophoresis; Detection, electrophoresis; Organic acids

1. Introduction

From recent articles reviewing electroseparations in lab-on-a-chip analytical systems (see, e.g., Refs. [1–5]) it is apparent that zone electrophoresis (ZE) is the dominantly used electrophoresis method in these miniaturized capillary electrophoresis (CE) devices (chips). So far, the use of other basic electrophoresis

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methods [6,7] attracted a limited attention and, for example, only very few works deal with isotachophoresis (ITP) separations on the chips [8–13].

A poly(methylmethacrylate) (PMMA) chip provided with two separation channels in the column-coupling (CC) configuration and on-column conductivity detectors was developed recently [10]. Following the column-coupling concept of some conventional CE equipment [14,15], this CC chip offers advantageous features for ITP and capillary zone electrophoresis (CZE) separations with on-line ITP sample pretreatment [11–13]. Connected to appropriately constructed electrolyte and sample management unit the CC chip can operate with suppressed hydrodynamic (HDF) and electroosmotic (EOF) flows in the separation channels (compartment) with electrophoresis being a dominant transport process in the separations performed by ITP and ITP–CZE [11–13]. Although not essential, this approach is favored in CE separations performed on the present chip [11–13] as it minimizes a number of sources contributing to run-to-run fluctuations of the migration velocities (times) of the separated constituents. In addition, such a transport concept can be assumed to reduce problems in quantitative analysis on the chip associated with EOF [16].

Conductivity detection is currently employed in conventional CE separation systems [6,7,17] and, for example, this detection technique plays a key role in monitoring of the ITP separations [6,7]. However, its use in microfabricated CE separation systems was introduced only recently [9–11]. From research linked with the developments of contact conductivity detectors for ITP it is known that reactions of some electrochemically active analytes on the detection electrodes and/or accompanying changes of the electrode surfaces may impair performances of the contact conductivity detectors significantly [6,7,18,19]. Samples containing constituents exhibiting strong adsorptivities on the electrode surfaces may be sources of problems in the detection of analytes as well [6,19]. Although the detection sensors for the conductivity detection on the chips are manufactured by different technologies [9,10] than those employed in conventional CE separation systems [6,7,17–25] in their use in the ITP separations occurrence of similar disturbances can be expected.

In this work some basic performance parameters of the CC chip relevant to its use in qualitative and quantitative ITP analysis were investigated. Using a series of the CC chips [10] we evaluated performances of their detection sensors relevant to the identification of the ITP analytes from the response of the conductivity detection. Here, succinate, acetate and benzoate served as test analytes in assessing short- and long-term reproducibilities on one chip and chip-to-chip reproducibilities of qualitative indices provided by the conductivity sensors. Means suitable for attaining reliable performances of the detection sensors were investigated in this context.

Reproducibilities of the migration velocities (times) of the analytes attainable on the chip with suppressed HDF and EOF were determined for well defined migration paths in a context with investigations of short- and long-term and chip-to-chip reproducibilities of the ITP quantitation.

2. Experimental

2.1. Instrumentation

A schematic of the PMMA CC chip used in this work (design No. 1, in Ref. [10]) is given in Fig. 1. The ITP separations on the chip were performed in a laboratory designed and constructed CE equipment. This equipment includes two units:

(1) An electrolyte and sample management unit (E&SMU, in Fig. 2), connected via 300 μm I.D. FEP (fluorinated ethylene propylene copolymer) capillary tubes to the inlets of the channels on the chip. Valves of this unit (V1, V2, VT and VS, in Fig. 2) serve to open these inlets on filling the channels and they are closed during the CE runs. Pumping syringes (P1, P2, PS, PT, in Fig. 1), connected to the inlets of the corresponding valves, deliver appropriate electrolyte solutions and the sample to the channels before the separation. An outlet channel of the chip, connected to a waste container (W, in Fig. 2), is permanently opened.

(2) An electronic and control unit (E&CU, in Fig. 1), designed and constructed by Fitek (Šal'a, Slovak Republic), delivers the driving current, measures conductivity using platinum detection sensors sputtered on the channels of the chip and interfaces the

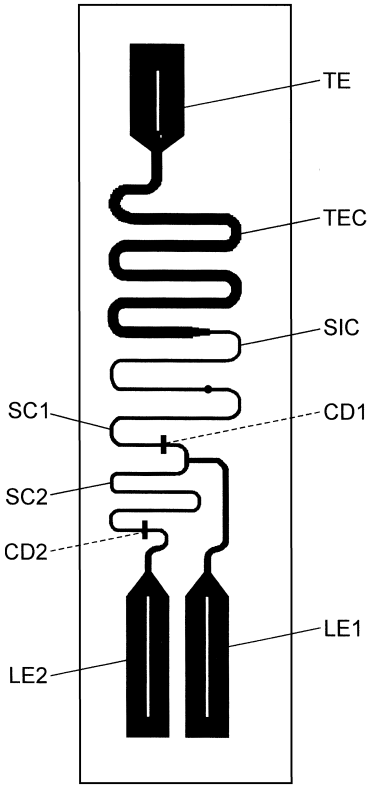

Fig. 1. A schematic of the CC chip. TE = Terminating buffer reservoir (10 μl); TEC = terminating channel (15 μl); SIC = a 1.2-μl sample injection channel [31×0.2×0.2 mm (length× width×depth)]; SC1 = the first separation channel [a 1.1 μl volume; 28×0.2×0.2 mm (length×width×depth)] with a platinum conductivity sensor; SC2 = the second separation channel [a 1.4 μl volume; 34.5×0.2×0.2 mm (length×width×depth)] with a platinum conductivity sensor; CD1, CD2 = conductivity detectors for SC1 and SC2, respectively; LE1, LE2 = leading buffer reservoirs (15 μl).

CE equipment with a personal computer. This unit includes the following modules (Fig. 2): (i) a high-voltage power supply (HVPS), delivering the stabilized driving current in the range of 0–50 μA with a maximum voltage of 5 kV connected to the chip. (ii) A high-voltage relay (HVR), for the column-switching operation of the equipment. (iii) Two conductivity detectors (CD1 and CD2), decoupled from the detection sensors on the chip by transformers with PTFE insulated coils. The detector for the first channel (CD1) is provided with a comparator circuit to identify a front boundary of the ITP zone of a selected effective mobility (needed in a control of the column-switching operation of the equipment). (iv) A control unit (CU), connecting the CE unit to a Pentium personal computer.

ITP Win software (version 2.31) obtained from Kascomp (Bratislava, Slovak Republic) was used for a time-programmed control of the CE runs and for the acquisition of the detection data and their processing.

2.2. Chemicals and electrolyte solutions

Chemicals used for the preparation of the electrolyte solutions and the solutions of anionic model mixtures were bought from Sigma (St. Louis, MO, USA), Serva (Heidelberg, Germany), Merck (Darmstadt, Germany) and Lachema (Brno, Czech Republic). Methylhydroxyethylcellulose 30 000 (Serva), purified on a mixed-bed ion exchanger (Amberlite MB-1; BDH, Poole, UK), was used as a suppressor of EOF. It was added to the leading electrolyte solution or it was applied as a coating of the inner walls of the separation channels [26]. A detailed composition of the electrolyte system employed in our ITP experiments is given in Table 1. Water purified by a Pro-PS water purification system (Labconco, Kansas City, KS, USA) was used for the preparation of the solutions. The electrolyte solutions used in the ITP separations were filtered by disposable membrane filters of 0.8-μm pore size (Sigma) connected to syringes used for filling the separation compartment (P1, P2 and PT, in Fig. 2).

3. Results and discussion

3.1. Elimination of sources of disturbances in the conductivity detection on the CC chip

Each of the separation channels of the present CC chip (Figs. 1 and 2) is provided with a conductivity sensor (sputtered Pt-sensing electrodes) to monitor the separation in a particular channel. The sensor in the first separation channel (SC1 in Figs. 1 and 2), in addition, serves, as a part of a comparator circuit (see Experimental), in a control of the column-switching operation of the CE equipment. Therefore, its proper performance is essential not only for the detection of

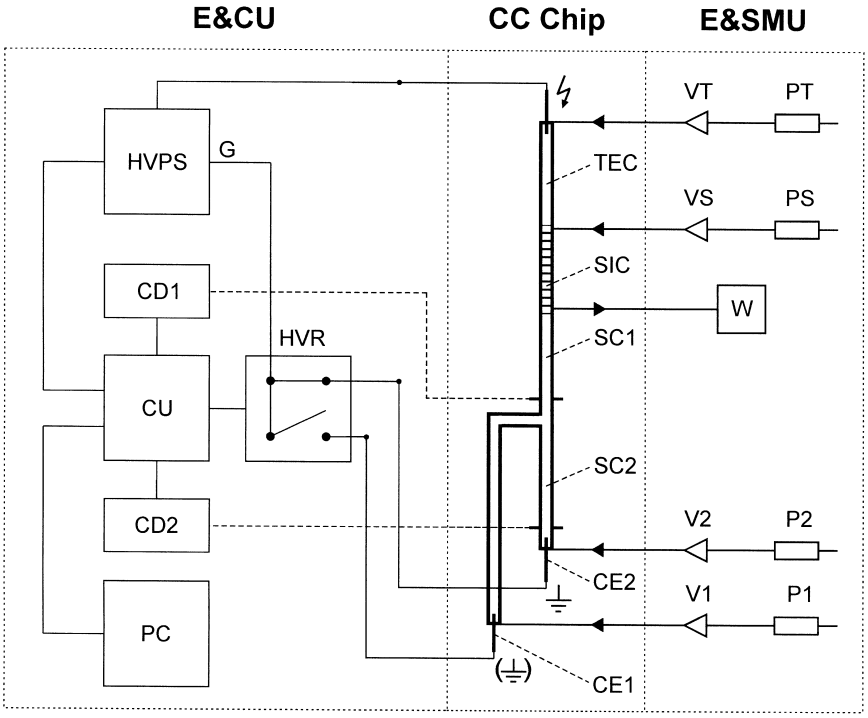

Fig. 2. A block scheme of the CE equipment to the separations with the closed separation compartment of the CC chip. E&CU = Electronic and control unit; HVPS = high-voltage power supply [its high-voltage pole is connected to the driving electrode in the high voltage (terminating) channel of the chip, TEC]; CD1, CD2 = conductivity detectors for the first and second separation channels, respectively; HVR = a high-voltage relay switching the direction of the driving current in the separation compartment [moving reeds of this relay connect to the ground pole (G) of HVPS either CE1 or CE2]. TEC = A high-voltage (terminating) channel; SIC = a 1.2-μl sample injection channel; SC1 = the first separation channel with a conductivity sensor (connected to CD1); SC2 = the second separation channel with a conductivity sensor (connected to CD2); CE1, CE2 = counter-electrodes for the first and second separation channels, respectively; E&SMU = electrolyte and sample management unit; V1, V2, VT = needle valves for the inlets of the separation and terminating channels; VS = a pinch valve for the inlet of the sample injection channel; W = waste collector. P1, P2, PS, PT = Syringes for filling the first, second, sample injection and terminating channels with the electrolyte and sample solutions, respectively.

Table 1
Electrolyte system

Parameter	
Solvent	Water
Leading anion	Chloride
Concentration (mmol/l)	10
Counter-ion	Histidine
pH	6.0
EOF suppressor	Methylhydroxyethylcellulose
Concentration (%, w/v)	0.2
Terminating anion	Glutamate
Concentration (mmol/l)	5
Counter-ion	Histidine
pH	6.0

the analytes but also for a reliable operation of the equipment provided with the CC chip.

Leaks of the driving current through the detection electrodes, electrode reactions due to bipolar behaviors of the detection electrodes in the electric field, and contaminated surfaces of the detection electrodes are key sources of disturbances in the conductivity detection in CE [6]. To keep leaks of the driving current through the detection electrodes of the conductivity sensors on the chip to a minimum the sensors were decoupled from the measuring electronics of the detector by insulating transformers as currently used in conventional CE equipment. In this way the leak currents could be suppressed to values of 10–20 pA or less also when the driving

potential in the detection cell reached 5 kV (a maximum provided by the high-voltage unit of the equipment used in this work).

The widths of the Pt-detection electrodes contacting the electrolyte solutions in the separation channels of the chip are 50 μm [10] to minimize negative impacts of the electrode reactions due to bipolar behavior of the electrodes in the driving electric field [6,18]. Our experiments revealed that even when the electric field strengths as high as 700–800 V/cm were applied to the separation channels of the chip no visible deterioration of the performances of the detectors, attributable to the bipolar behavior of the detection electrodes in the electric field, occurred.

Despite the above precautions in the construction of sensing and measuring parts of the conductivity detector we found that in some instances the detection in the ITP runs on a new chip was accompanied by an enhanced noise of the detection signal (Fig. 3a). Here, thorough washing of the separation channel with demineralized water (cleaning of the surfaces of the detection electrodes) eliminated these disturbances in some instances (Fig. 3b). However, even extensive washing procedures with various types of surface active agents were not effective in eliminating some detection disturbances (Fig. 3c). We found that an electrochemical cleaning of the detection electrodes as recommended for some conventional conductivity detection cells [15,25] provided a simple and effective solution in such instances. Here, a pair of 100-V pulses of reversed polarities was applied between the electrodes of a particular sensor while the leading electrolyte solution (Table 1) was pumped through the separation compartment. A stream of the solution removed the products formed by the cleaning pulses and at the same time prevented overheating of the sensor by high currents flowing between the electrodes on the application of these pulses. An overall effect of this cleaning procedure is clearly illustrated by isotachopherograms in Fig. 3c and d.

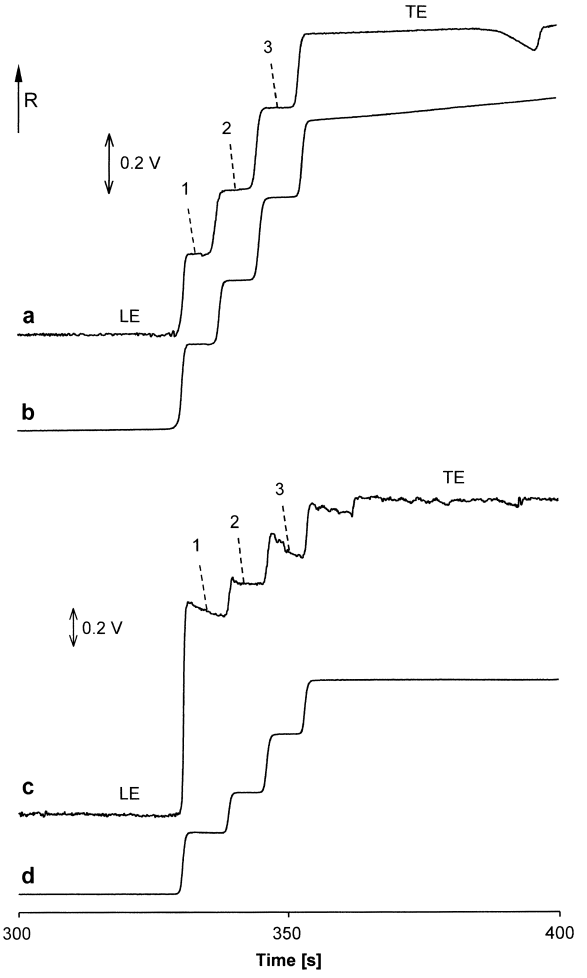

Fig. 3. Impacts of cleaning of contaminated surfaces of the detection electrodes on the responses of the conductivity sensors in the ITP separations on the CC chips. Isotachopherograms from the separations of a three-component test mixture of anions (a) before and (b) after cleaning of the detection electrodes of the sensor by deionized water. Records from the separations of the same test mixture as obtained by a particular sensor (c) before and (d) after electrochemical cleaning of its electrodes (for details, see the text). LE = Leading anion; 1 = succinate; 2 = acetate; 3 = benzoate; TE = terminating anion. The concentrations of the analytes in the test sample were 300 μmol/l. For the composition of the electrolyte system see Table 1. The driving current was 10 μA.

3.2. Reproducibility of the response of the conductivity sensors in the ITP separations on the CC chips

Despite reproducible productions of the conductivity sensors on the CC chip [10] and the use of the above cleaning procedures, the heights of plateaus of the ITP zones of the test analytes as registered on the isotachopherograms by different sensors scattered in some extents. In some instances, this scatter reflected

differences in the conductance cell constants of the detection sensors (attributable to differences in contact areas of the detection electrodes with the electrolyte solution and small differences in distances between the electrodes in the sensors). However, the signal amplitudes (plateaus) for a particular ITP analyte slightly deviated also in repeated runs monitored by the same sensor and in the runs performed on different chips monitored by the sensors exhibiting only very small differences in their conductance cell constants. Here, run-to-run temperature differences could be responsible for the deviations. To reduce the impacts of these sources of detection disturbances on the qualitative characterizations of the ITP analytes we preferred the use of relative step heights (RSH values) as proposed, for the same reasons, in the ITP separations performed in conventional equipment [6,15]. Here, the amplitude of the detection signal of a particular analyte (h_A) is related to the amplitude as obtained for a reference constituent, h_R (see Fig. 4):

$$\mathrm{RSH}_{A,R} = \frac{h_A - h_L}{h_R - h_L} \quad (1)$$

where h_L is the signal amplitude for the leading zone.

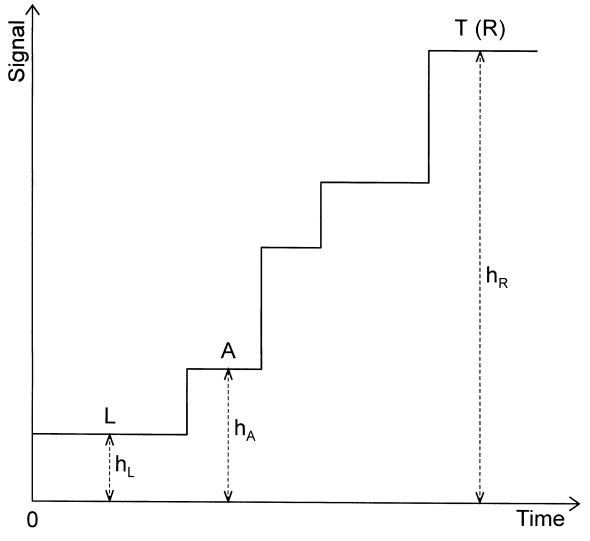

Fig. 4. A schematic isotachopherogram defining the heights of the plateaus of the leading (h_L), analyte (h_A) and terminating (reference) (h_R) zones as used in the calculations of the RSH values from the response of the conductivity detector.

When the detection cell operates correctly its conductance cell constant does not vary during the ITP run and the RSH of the analyte ($\mathrm{RSH}_{A,R}$), in fact, relates its effective mobility ($\bar{m}_A$) with the effective mobilities of the leading ($\bar{m}_L$) and reference ($\bar{m}_R$) constituents [15]:

$$\mathrm{RSH}_{A,R} = \frac{\bar{m}_R}{\bar{m}_L - \bar{m}_R} \cdot \left(\frac{\bar{m}_L}{\bar{m}_A} - 1 \right) \quad (2)$$

From Eq. (2) it is apparent that due to close temperature coefficients of the mobilities of ions [6,7] the RSH values are not significantly influenced by small run-to-run temperature fluctuations.

Average RSH values as, typically, obtained for the test analytes in short- (1 day) and long-term (5 days) time frames on a particular sensor show remarkable agreements (Table 2). Low scatters of these qualitative indices are also apparent from the data in Table 2. In addition, the average RSH values agree very well with those including the RSH data as obtained from the ITP runs performed on various chips (Table 2).

The data in Table 2 include also the RSH values calculated for the test analytes with the aid of the steady-state ITP model [6]. Agreements of the experimental and calculated data are noteworthy. They indicate that the RSH values may be considered as suitable input data in obtaining ionic mobilities and p*K* values of the ITP analytes from the response of the conductivity detector on the chip when the separations are carried out under appropriately chosen separation conditions.

3.3. Transport processes and CE separations on the CC chip

EOF and HDF of the solution in which the CE separation is carried out may accompany electrophoretic migrations of the separated constituents. Usually employed to prolong or shorten an effective length of the separation path in the CE column [6,7,17], EOF and HDF also contribute to overall run-to-run fluctuations of the migration velocities of the separated constituents (∂v_{tot}) in accordance with the law of propagation of errors [27]:

$$\partial v_{tot} = \sqrt{(\partial v_{ep})^2 + (\partial v_{eo})^2 + (\partial v_{hd})^2} \quad (3)$$

Table 2
Reproducibilities of qualitative indices for the test analytes on the CC chip

Analyte	RSH values obtained from the response of the detection sensors					
	CD1[a]			CD2[a]		
	Average	RSD (%)	*n*	Average	RSD (%)	*n*
Short-term reproducibility[b]						
Succinate	0.277	0.68	17	0.275	0.72	21
Acetate	0.486	0.64	17	0.482	0.53	21
Benzoate	0.752	0.66	17	0.751	0.43	21
Long-term reproducibility[c]						
Succinate	0.276	2.09	59	0.276	1.63	80
Acetate	0.486	1.66	59	0.483	1.00	80
Benzoate	0.750	1.02	59	0.749	0.68	80
Chip-to-chip reproducibility[d]						
Succinate	0.278	2.51	76	0.276	1.51	101
Acetate	0.488	1.68	76	0.483	0.90	101
Benzoate	0.752	1.12	76	0.750	0.65	101
Calculated RSH values[e]						
Succinate	0.287					
Acetate	0.499					
Benzoate	0.737					

[a] Data obtained from the conductivity sensors CD1 and CD2 of the CC chip, respectively (see Figs. 1 and 2).

[b] Obtained from the measurements performed on one chip in 1 day.

[c] Obtained from the measurements performed on one chip in 5 days.

[d] Obtained from the measurements performed on three chips (chosen at random) in 10 days. The data were provided by three detection sensors in the first channel (CD1) and by two sensors in the second channel (CD2). n = Number of repeated ITP runs (for the composition of the electrolyte system see Table 1) with a 10 μA driving current. The concentrations of the analytes were 300 μmol/l.

[e] The calculations were carried out with the aid of a program based on the ITP steady-state model [6]. The following physicochemical constants were used in the calculation [$pK_{a,1}$, $pK_{a,2}$ = pK values for the dissociations into the first and second steps, respectively; m_1, m_2 = absolute values of the limiting mobilities ($cm^2\ V^{-1}\ s^{-1}\ \cdot 10^{-5}$) of particular ionic forms of the constituent]: acids: hydrochloric: $m_1 = 79.08$, $pK_a = 0$; histidine: $m_1 = 29.6$ $pK_a = 6.04$; succinic: $m_1 = 33.0$, $pK_{a,1} = 4.21$, $m_2 = 64.5$, $pK_{a,2} = 5.50$; acetic: $m = 42.4$, $pK_a = 4.76$; benzoic: $m_1 = 33.6$; $pK_{a,1} = 4.20$; glutamic: $pK_{a,1} = 4.37$, $m_1 = 27.9$, $pK_{a,2} = 9.96$, $m_2 = 49.6$.

where, ∂v_{ep}, ∂v_{eo}, ∂v_{hd} are symbols characterizing random fluctuations of the electrophoretic, electroosmotic and hydrodynamic velocities in repeated separations, respectively.

Eq. (3) indicates that CE separations carried out without EOF and HDF offer, in general, the highest reproducibility of the migration velocities (times) of the separated constituents. Therefore, such transport conditions favor both highly reproducible CE quantitations and reliable runs on the chip in the separations using its column-switching capabilities [11,13] (see also Experimental). From geometrical dimensions of the channels on the CC chip (Fig. 1) it is apparent that its separation compartment has a relatively small hydrodynamic resistance and, therefore, only a small pressure difference between the inlet channels can cause an undesired HDF of the solution in this compartment. To prevent such, hardly controllable, flows during the CE runs the inlet channels to the separation compartment were closed with the aid of the valves (V1, V2, VT and VS, in Fig. 2). This solution, the separation in a hydrodynamically closed separation compartment, in fact, prevented HDF in the same way as employed in conventional CE instruments designed, mainly, for the ITP separations [6,7]. The EOF in our experiments was suppressed by a dynamic coating of the separation channels by methylhydroxyethylcellulose present in the leading electrolyte solution (Table 1).

The reproducibilities of the migration velocities

(times) attainable for the separated constituents on the chip under the above transport conditions were evaluated from the measurements of the migration times needed for the ITP boundary formed by the leading and terminating ions (Table 1) to migrate between well defined positions in the separation compartment of the chip (L_1 and L_2, in Fig. 5). The average values of the migration times obtained in these evaluations in short- and long-term time frames show very good agreements for both migration paths (Table 3). They also show that the migration data for the L_1 migration path exhibited a larger scatter than those obtained for the L_2 migration path. This fact is understandable as the RSD values for the former path reflect besides the fluctuations in the migration velocity of the boundary [6,7,28] also run-to-run differences in its initial position [a position of the contact plane of the leading and terminating electrolyte solutions on the chip (SC1/SIC in Fig. 5)]. This source of random errors did not contribute to the RSD data characterizing the L_2 path as this, defined by the positions of the detection sensors (see Fig. 5), was filled with the leading electrolyte solution before the start of the ITP runs. Here, the migration times of the ITP boundary scattered, mainly, due to factors influencing its electrophoretic velocity [6,7,28].

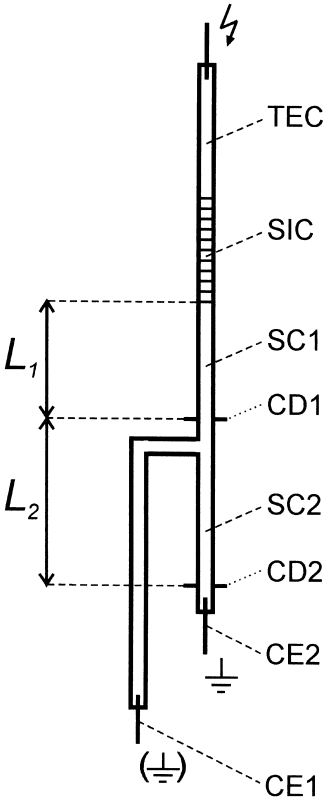

Fig. 5. Migration paths (L_1, L_2) as used in evaluations of the reproducibility of the migration velocity of the ITP boundary on the CC chips. TEC = Terminating channel; SIC = sample injection channel; SC1 = the first separation channel with a conductivity sensor (connected to CD1 in Fig. 2); SC2 = the second separation channel with a conductivity sensor (connected to CD2 in Fig. 2); CE1, CE2 = counter-electrodes for the SC1 and SC2 channels, respectively.

Table 3
Reproducibilities of the migration times for the chloride/glutamate boundary for the L_1 and L_2 migration paths on the chip[a]

Migration times					
L_1 path			L_2 path		
Average (s)	RSD (%)	n[e]	Average (s)	RSD (%)	n[e]
Short-term reproducibility[b]					
131.46	1.02	10	196.32	0.57	10
Long-term reproducibility for the same electrolyte solutions[c]					
132.67	1.40	27	193.91	1.20	27
Long-term reproducibility for repeatedly prepared electrolyte solutions[d]					
133.52	3.32	140	195.06	1.71	140

[a] The migration paths on the chip (L_1 and L_2) are defined in Fig. 5.
[b] Short-term reproducibility for the measurements performed on the chip in 1 day.
[c] Long-term reproducibility for the measurements performed in 3 days using identical leading and terminating electrolyte solutions.
[d] The data obtained from the measurements performed in 7 days on the same chip with a series of the leading electrolyte solutions (Table 1) slightly differing in pH (5.9–6.1) and in the concentration of methylhydroxyethylcellulose (0–0.17%, w/v).
[e] n = Number of the repeated ITP runs (for the composition of the electrolyte system see Table 1) with a 10 μA driving current.

3.4. Reproducibilities of the time-based zone lengths of the analytes in the ITP separations on the CC chip

Typical data, as obtained from the measurements of the migration times of the ITP boundary (Table

3), show that highly reproducible migration conditions are attainable on the CC chip without EOF and HDF. Very good agreements of the average zone lengths of the test analytes, characteristic for the CD1 and CD2 sensors in short- (1 day) and long-term (5 days) time frames on one chip and also on various chips (Table 4), are, undoubtedly, linked with such transport conditions. Small systematic deviations of the average zone lengths as provided by both sensors can be very likely ascribed to minute geometrical differences of the CD1 and CD2 sensors (due to a hot embossing production of the channels and/or slight differences in geometries of the sputtered detection electrodes [10]).

The RSD values (Table 4) reflect, besides factors that contribute to the precision attainable in the zone-length measurement in ITP in general [6,7,29], also run-to-run fluctuations in the length (volume) of the injected sample pulse (SIC, in Fig. 2). An impact of this factor on the lengths of the zones of the test analytes can be estimated from accompanying RSD data (see, Table 4) in which these fluctuations were corrected for by using the length of the acetate zone as a reference [30]. Although small sample injection volume scatters occurred in our experiments (see the relevant data in Table 4), such an estimation indicates that a ''double-T'' sample injection channel of the present chip design provides good volume reproducibility in loading the sample on the chip. Therefore, 2–3-times higher RSD values obtained for a long-time frame can be, in part, linked with changes in the compositions of the test samples (prepared

Table 4
Reproducibilities of the zone lengths of the test analytes on the CC chips

Analyte	Zone length					
	CD1[a]			CD2[a]		
	Average (s)	RSD (%)	n[f]	Average (s)	RSD (%)	n[f]
Short-term reproducibility[b]						
Succinate	5.90	2.5	11	6.39	2.3	11
Acetate	6.38	1.5	11	6.74	2.1	11
Benzoate	7.08	1.7	11	7.23	2.5	11
Succinate/acetate[e]	0.924	1.5	11	0.948	2.6	11
Benzoate/acetate[e]	1.111	1.8	11	1.074	2.5	11
Long-term reproducibility[c]						
Succinate	6.17	4.8	66	6.38	7.4	73
Acetate	6.33	5.4	66	6.83	5.6	73
Benzoate	6.83	6.3	66	7.28	6.3	73
Succinate/acetate[f]	0.976	4.4	66	0.935	5.6	73
Benzoate/acetate[f]	1.087	4.0	66	1.068	5.7	73
Chip-to-chip reproducibility[d]						
Succinate	6.24	4.9	92	6.58	8.1	105
Acetate	6.37	5.0	92	6.95	6.1	105
Benzoate	6.87	5.8	92	7.24	5.8	105
Succinate/acetate[e]	0.981	3.8	92	0.946	5.1	105
Benzoate/acetate[e]	1.086	3.8	92	1.043	6.2	105

[a] Data for the conductivity sensors in the first (CD1) and second (CD2) separation channels, respectively (see Figs. 1 and 2). The ITP runs evaluated either by CD1 or CD2 were carried out in the electrolyte system described in Table 1 with a 10 μA driving current. The concentrations of the analytes in the test sample were 300 μmol/l.

[b] Calculated from the data obtained with one chip in 1 day.

[c] Calculated from the data obtained with one chip in 5 days.

[d] Calculated from the data obtained with three chips in 10 days.

[e] The lengths of the analyte zones relative to that of the acetate zone.

[f] n = number of ITP runs.

daily by diluting a stock solution of the test sample). Here, we should note that short zone lengths of the analytes, characteristic for our experiments (the sample loads reflected the load capacity of the first separation channel to perform the zone length measurements from the response of the CD1 sensor), do not provide higher precisions in the quantitations performed in conventional ITP equipment [6,7,29].

The RSD values characterizing the zone length data as obtained from the ITP runs performed on different chips of the present design (Table 4) include, besides the above factors, small volume differences of the injection channels on various chips as well. By taking the length of the acetate zone as a reference [30] these differences could be eliminated and relevant RSD data reflect very good agreements in the quantitations of the test analytes attainable under our working conditions on different chips.

4. Conclusions

Our results show that the conductivity detection offers a promising alternative to the detection of zones in the ITP separations performed on the CC chip. Its reliable short- and long-term performance in obtaining qualitative characteristics (RSH values), however, required that the surfaces of the detection electrodes were appropriately cleaned. Some of the cleaning procedures as developed for these purposes for conventional CE conductivity detection cells [6,15,25] were found effective also for the sensors on the present chip.

Employed with suppressed EOF and the closed separation compartment (to prevent HDF), the CC chip provided working conditions under which only the electrophoretic transport of the separated constituents was effective. Very good agreements of the average zone lengths of the test analytes as attained on different chips of the present design (see Table 4) can be attributed, at least partially, to the use of this approach. In addition, these results indicate its promising potentialities as far as a chip-to-chip transferability of the ITP procedures to the determination of the ionogenic analytes is concerned. This is important from the point of view of routine applications of the chip when we consider that it is a disposable CE device intended for a short-term use [10].

The CC chip itself is compatible also with other detection techniques as currently employed in conventional CE equipment. The use of these on-column and/or post-column techniques in combinations with the conductivity detection can, undoubtedly, enhance an overall utility of the chip in ITP analysis [6,7].

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