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Column switching in zone electrophoresis on a chip

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Abstract

This feasibility study deals with column switching in zone electrophoresis (ZE) separations on a column coupling (CC) chip. The column switching implemented into the ZE separations an on-chip sample clean up applicable for both the multicomponent and high salinity samples. In addition, complemented by different separation mechanisms in the coupled columns (channels), it provided benefits of two-dimensional separations. Properly timed column switching gave column-to-column transfers of the analytes, characterized by 99–102% recoveries, delivered to the second separation stage on the chip the analyte containing fractions contaminated only with minimum amounts of the matrix constituents. A diffusion driven transport of the matrix constituents to the second channel of the chip (due to direct contacts of the electrolyte solutions in the bifurcation region), representing 0.1–0.2% of the loaded sample constituents, was found to accompany the sample clean up performed on the CC chip. This source of potential disturbances to the separation in the second channel, however, is not detectable in a majority of practical situations. With respect to a 900 nl volume of the sample channel on the CC chip, the electric field and isotachophoresis (ITP) stackings were employed to minimize the injection dispersion in the separations and concentrate the analytes. Here, the column switching, removing a major part of the stacker from the separation system, provided a tool effective in a control of the destacking of analytes. Highly reproducible ZE separations as attained in this work also for the chip-to-chip and equipment-to-equipment frames can be ascribed, at least in part, to suppressions of electroosmotic and hydrodynamic flows of the solutions in which the separations were performed.

Keywords: Zone electrophoresis on chip; Column coupling chip; Column switching zone electrophoresis; Zone electrophoresis sample pretreatment on chip

1. Introduction

At present, electrophoresis methods are considered key separation tools of miniaturized (lab-on-a-chip) analytical systems. This status apparently reflects the fact that they fit very well to the concept of micro total analysis systems (see, e.g., [1] and references given therein) and from the point of view of miniaturization offer more benefits than chromatography methods [2]. Although all basic electrophoresis methods [3] can run on electrophoresis chips of various designs, recent reviews (see [1,4–9]) document a significant preference of zone electrophoresis (ZE).

For known reasons [1,4–8,10,11], conventional capillary electrophoresis (CE) detectors often cannot provide concentration sensitivities required in the ZE separations on chips. This fact stimulates, besides a broader use of laser induced fluorescence detection, developments of new detection devices reflecting specificities of the chip-based electrophoresis systems [1,5,11–19]. Efforts aimed at reaching favorable concentration detectabilities for the analytes on the CE chips via improved performances of the detectors are complemented by developments of sample pretreatment technologies compatible with electroseparations and applicable in miniaturized formats [6–8]. In this context, significant potentialities of electrophoresis based sample pretreatment techniques should be mentioned [5,6,8–10,20–37]. Here, for example, various alternatives of the electric field stacking (see, e.g.,

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[6,10,28,31,33,37]) and sweeping in micellar electrokinetic chromatography [27], were already proved to be concentrating techniques suitable for single column configurations of the miniaturized separation systems.

Recently, the use of CE chips provided with the column coupling (CC) configuration of the separation system attracts a significant attention [8,23–25,29,30,32,34,36,38–43]. This CE technology, introduced into CE by Everaerts et al. [44] to enhance concentration detectability in capillary isotachophoresis (ITP) of complex ionic mixtures, is in a straightforward way transferable to a chip format as shown in the quoted works. Although employed in some instances to the ITP separations [23–25,38–42], the CC chips typically serve as means integrating the ZE separation with a highly efficient ITP sample pretreatment. Benefits of such integration in miniaturized CE devices are already well documented [8,23–25,29,30,32,34,36].

For fundamental reasons [3,8], the CC chip is a general electrophoresis platform and as such not restricted in the use to the methods mentioned in the previous paragraph. Its specific functions (electrophoretically driven transport of the sample constituents from the column to column or out of the separation compartment), implemented via a well-defined column switching program during the run, are not limited to the ITP and ITP–ZE separations and can be extended to other electrophoresis methods as well. For example, it is reasonable to expect that the CC chip will provide in the ZE separations benefits in some respects analogous to those attained in the ITP–ZE combination along with specific advantages of ZE (e.g., spatially more favorable migration configurations of the separated constituents than in ITP). These facts stimulated our research linked with the use of the CC chip in the ZE separations and this introductory work was focused on some basic aspects relevant to the use of the column switching capabilities of this chip in ZE.

Conventional hydrodynamically opened CE separation systems, provided with the capillary tubes of 50–75 μm i.d.s, are assumed to operate with a negligible hydrodynamic flow (HDF) of the solution in the separation compartment. As shown, for example, by Wu and Pawliszyn [45] this need not be the case of short capillaries (these in dimensions correspond to the separation compartments of some chip designs) because HDF induced changes of the migration velocities of the separated constituents adversely affect performance of the electroseparations under such conditions. In addition, in the separations carried out on the CC chip they make a control of the run difficult, especially in instances when the column switching controlled sample clean up is a part of the separation protocol. Therefore, means effective in suppressing HDF are currently employed in the separations performed on the CC chips (see, a recent review on this topic [8]) and a technical solution of the fluidic part of the CE equipment as described in Section 2 eliminated HDF in experiments carried out in this work. Electroosmotic flow (EOF) of the solution in which the separation is performed can be a source of similar disturbances as HDF and it is advantageous to perform the separations on the CC chips with suppressed EOF. Means providing such electrokinetic conditions on the CE chips made of various materials were reviewed recently [46]. Of these, we preferred the use of the EOF suppressor that dynamically coats the chip channels.

2. Experimental

2.1. Instrumentation

A poly(methylmethacrylate) CC chip employed in this work (Fig. 1) was manufactured using the procedures as described elsewhere [39]. The separations in this miniaturized device were performed with the aid of a laboratory constructed CE equipment. This equipment includes two units (Fig. 2):

(1) An electrolyte and sample management unit (E&SMU, in Fig. 2), provided with peristaltic micropumps (P-ZE1a, P-ZE1b, P-ZE2, P-S, in Fig. 2) and membrane driving electrodes (E1, E2, E3, in Fig. 2). Mutual connections of these devices and their connections to the inputs to the chip channels are apparent from a scheme shown in Fig. 2. Here, the rollers of a particular pump automatically close the corresponding inlet to the chip channel when the solution pumping is stopped (the rollers act as a valve). Excesses of the solutions pumped through the chip channels in the preparation of the run are trapped into a container (W, in Fig. 2) connected to a permanently opened outlet of the chip (W, in Fig. 1). The membrane driving electrodes are used to eliminate disturbances due to the bubble formation during the separation (their design concept is described elsewhere [8]).
(2) An electronic and control unit (E&CU, in Fig. 2) delivers the driving current either to the counter-electrode of the first channel (E1, in Fig. 2) or to the counter-electrode

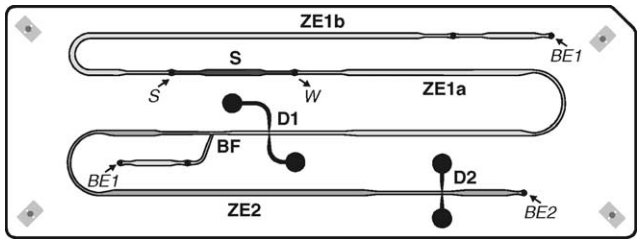

Fig. 1. A poly(methylmethacrylate) CC chip with conductivity detection. ZE1a, a front (separative) part of the first separation channel (a 4.5 μl volume; 59 mm × 0.2–0.5 mm × 0.14–0.2 mm [length × width × depth]) with a platinum conductivity sensor, D1; ZE1b, a rear (non-separative) part of the first separation channel (a 9.8 μl volume; 60 mm × 0.2–0.5 mm × 0.2–0.38 mm); S, sample injection channel (a 900 nl volume; 12 mm × 0.2–0.5 mm × 0.2 mm); ZE2, the second separation channel (a 4.3 μl volume; 56 mm × 0.2–0.5 mm × 0.14–0.2 mm) with a platinum conductivity sensor, D2; BF, bifurcation section. BE1, BE2, S: inlets for the background and sample solutions to the chip channels, respectively. W, an outlet hole from the chip channels.

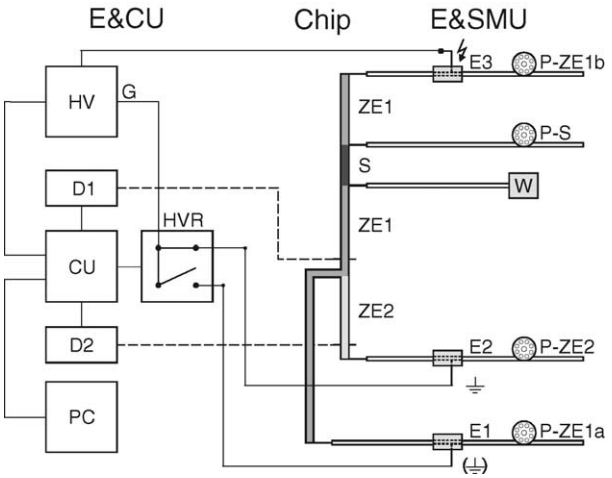

Fig. 2. A scheme of the CE equipment for the separations with the CC chips. Electronic and control unit (E&CU): CU, control unit; HV, high-voltage power supply (0–50 μA, 0–7 kV); D1, D2: conductivity detectors for the first and second separation channels, respectively; HVR, high-voltage relay switching the direction of the driving current in the separation compartment (moving reeds of this relay connect to the ground pole, G, of HV either E1 or E2). Electrolyte and sample management unit (E&SMU): P-ZE1a, P-ZE1b, P-ZE2, P-S: peristaltic pumps for filling the first (ZE1), second (ZE2) and sample (S) channels with the background electrolyte and sample solutions, respectively. W, waste container connected to the outlet hole on the chip (W, in Fig. 1). E1, E2: driving electrodes for the ZE separation channels, respectively; E3: the driving electrode connected to a high voltage pole of HV.

of the second channel (E2, in Fig. 2). The change of the direction of the driving current (the column-switching) is actuated via a relay (HVR, in Fig. 2).

The E&CU includes the measuring electronics of the A.C. contact conductivity detectors. The measuring electronics is galvanically decoupled from the platinum conductivity sensors on the chip (sputtered on the cover of the channels of the chip [39]) by transformers [47]. The E&CU drives the peristaltic pumps in the preparation step of the run. Its control unit (CU, in Fig. 2) also interfaces the CE equipment to a PC.

MicroCE Win software (version 2.4), written in the laboratory, controlled automated preparations of the runs (filling of the chip channels with the corresponding solutions in a required sequence), provided a time-programmed control of the ZE runs (including the column switching operation during the run derived from the signal of the conductivity detector in the first channel), acquired the detection data and provided their processing.

2.2. Chemicals, electrolyte solutions and samples

Chemicals used for the preparation of the electrolyte solutions and the solutions of model samples were obtained from Merck (Darmstadt, Germany), Sigma-Aldrich (Seelze, Germany) and Serva (Heidelberg, Germany).

Methylhydroxyethylcellulose 30 000 (Serva), purified on a mixed-bed ion exchanger (Amberlite MB-1, Merck), was used as a suppressor of electroosmotic flow. It was added to the electrolyte solutions or it was applied as a coating of the inner walls of the separation channels [48]. Compositions of the background (carrier) electrolyte solutions employed in the ZE separations on the chip are described in Table 1.

Table 1
Electrolyte systems

	ES1	ES2	ES3
Carrier anion	MES	MES	MES
Concentration (mmol/l)	30	30	30
Counter-ion	Bis–tris	Bis–tris	Bis–tris
Concentration (mmol/l)	8	1	4
Co-counter-ion	–	Bis–tris propane	–
Concentration (mmol/l)	–	3	–
Additive	PVA	PVA	PVA
Concentration (%, w/v)	0.05	0.05	0.05
Complexing agent	–	α-Cyclodextrin	–
Concentration (mmol/l)	–	75	–
pH	5.6	5.6	5.2

MES, 2-(*N*-morpholino)ethanesulfonic acid; bis–tris, bis(2-hydroxyethyl)-amino-tris[hydroxymethyl]methane; bis–tris propane, 1,3-bis[tris(hydroxymethyl)-methylamino]propane; PVA, poly(vinyl alcohol) 100 000.

Water demineralized by a Pro-PS water purification system (Labconco, Kansas City, KS, USA), and kept highly demineralized by a circulation in a Simplicity deionization unit (Millipore, Molsheim, France), was used for the preparation of the electrolyte and sample solutions. The electrolyte solutions were filtered by disposable syringe membrane filters of 0.8 μm pore sizes (Millipore) before use.

Stock aqueous solutions of the test anions were prepared from analytical grades chemicals obtained from the above suppliers. Test samples as loaded onto the chips were prepared fresh daily.

3. Results and discussion

A scheme in Fig. 3 shows key working steps employed in the column switching ZE separations on the present CC chip. Apparently, this scheme reflects general guidelines formulated for an effective use of the coupled column separation system [49,50] as it includes means for applications of two different separation mechanisms in the coupled channels and, at the same time, makes possible a transfer of the analyte from the first to the second channel of the CC chip only with a limited number of accompanying sample constituents. Here, different separation mechanisms were implemented via appropriate compositions of the background electrolyte solutions placed into the separation channels prior to the ZE run (step a, in Fig. 3). On the other hand, the use of a proper program of the run (steps b–f, in Fig. 3) resulted in a transfer of the sample constituents of analytical interest to the second channel on the chip before their separation and detection in this channel (step f, in Fig. 3). A time-based program (see Section 2) that converts a well-defined scheme of the ZE run into particular running and switching steps was preferred in this work as it provided a high operational flexibility.

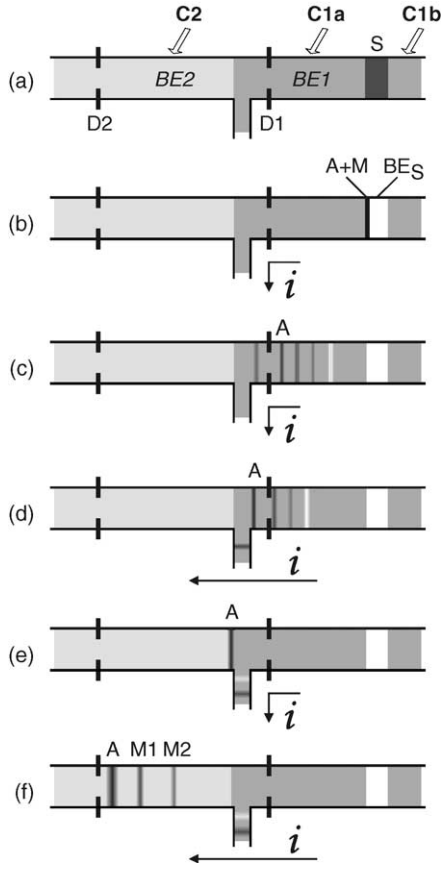

Fig. 3. A sequence of the working steps in column switching ZE on the CC chip. a, a starting arrangement of the solutions in the chip channels; b, an initial phase of the separation with the sample (S) stacking; c, the run in the first channel with removals of the matrix constituents migrating in front of the analyte (A); d, a transfer of the analyte to the second channel (the direction of the driving current switched); e, a continuation of the run in the first channel with removals of the matrix constituents migrating behind the analyte (the direction of the driving current switched); f, the separation and detection of the transferred constituents in the second channel (the direction of the driving current switched). C1a, C1b: front and rear parts of the first separation channel on the CC chip, respectively; C2: the second separation channel on the CC chip; D1, D2: detection sensors in the first and second separation channels, respectively; BE1, BE2: the background electrolyte solutions in the first and second separation channels, respectively; BE_S: the background electrolyte adapted to the composition of the sample; M, M1, M2: symbols for the sample matrix constituents; i: direction of the driving current.

3.1. Fluctuations of the migration velocities in the ZE separations on the CC chip

From the scheme in Fig. 3, it is apparent that reproducible migration velocities of the separated constituents contribute to an overall performance attainable in the column switching ZE separations on the CC chip. Therefore, an assessment of the reproducibility of the migration velocities under the preferred transport conditions (electrophoretic transport of the separated constituents with suppressed HDF and EOF) was one of the tasks of this work. Here, using seven CC chips of the design shown in Fig. 1 and two instruments constructed

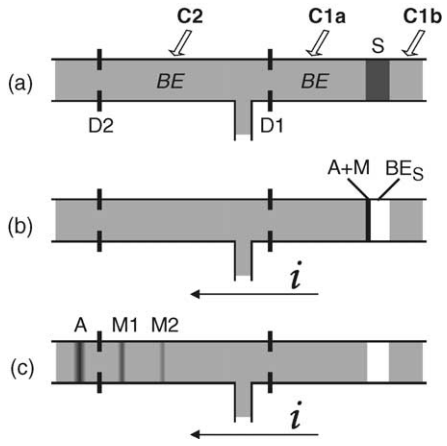

Fig. 4. A sequence of the working steps in a single column ZE separation on the CC chip. a, a starting arrangement of the solutions in the chip channels; b, stacking of the sample constituents; c, the separation and detection of the sample constituents. C1a, C1b: front and rear parts of the first separation channel on the CC chip, respectively; C2: the second separation channel on the CC chip; D1, D2: detection sensors in the first and second separation channels, respectively; BE_S: the background electrolyte adapted to the composition of the sample (S); A, analyte; M, M1, M2: symbols for the sample matrix constituents; i: direction of the driving current.

for the electrophoresis separations on the CC chips (Fig. 2), we carried out a larger series of relevant experiments. The separations, monitored by both conductivity detectors on the chip (see Fig. 2), followed the scheme as given in Fig. 4 [a single column mode of the ZE separation with the channels filled by an identical background electrolyte solution (ES1, in Table 1)]. The migration data obtained in this way (Table 2) made possible to evaluate not only fluctuations in the migration velocities of the test constituents on a particular CC chip but their fluctuations characteristic for chip-to-chip and equipment-to-equipment frames as well. Actual data for a group of the test anions show that very reproducible migrations of the separated constituents on the present CC chip were typical for both the short- and long-term time intervals. They show that the same statements apply also for the chip-to-chip and equipment-to-equipment frames (important for a chip-to-chip transfer of the analytical procedure). Electropherograms (Fig. 5) as obtained from the ZE separations of the test anions performed under identical working conditions on different CC chips with different electrophoresis instruments illustrate an attained migration performance.

3.2. Transfer of the analyte from the first to the second channel of the CC chip

For fundamental reasons [49,50], the use of column switching in the ZE separation of a multicomponent sample on the CC chip can be fully beneficial only for one or a very limited number of the sample constituents. Therefore, to perform a particular ZE run with column switching in an analytically favorable way, it is essential that the switching operations make possible to transfer from the first to the sec-

Table 2
Repeatabilities of the migration times of the test anions as attained in the ZE separations performed on the CC chips

Analyte	Migration time						*n*
	CD1[a]			CD2[a]			
	Mean (s)	S.D. (s)	R.S.D. (%)	Mean (s)	S.D. (s)	R.S.D. (%)	
Short-term repeatability[b]							
Tartrate	120	0.3	0.2	221	0.6	0.3	6
Aspartate	147	0.3	0.2	273	0.5	0.2	6
Acetate	176	0.3	0.2	328	0.7	0.2	6
Propionate	207	0.4	0.2	387	0.8	0.2	6
Glutamate	244	0.4	0.2	458	1.1	0.2	6
Long-term repeatability[c]							
Tartrate	120	0.4	0.3	220	0.9	0.4	23
Aspartate	147	0.3	0.2	272	1.6	0.6	23
Acetate	175	0.8	0.5	327	1.7	0.5	23
Propionate	206	1.1	0.5	386	2.2	0.6	23
Glutamate	244	1.2	0.5	457	2.4	0.5	23
Chip-to-chip repeatability[d]							
Tartrate	120	1.0	0.8	220	1.6	0.7	95
Aspartate	147	1.1	0.8	272	2.0	0.7	95
Acetate	176	1.5	0.9	327	2.5	0.8	95
Propionate	207	1.8	0.9	387	2.9	0.8	95
Glutamate	244	2.2	0.9	458	4.1	0.5	95
Equipment-to-equipment repeatability[e]							
Tartrate	120	1.1	0.9	221	1.7	0.8	142
Aspartate	147	1.3	0.9	273	2.2	0.8	142
Acetate	176	1.7	1.0	328	2.6	0.8	142
Propionate	208	1.9	0.9	387	2.9	0.8	142
Glutamate	245	2.3	0.9	458	3.9	0.9	142

[a] The data acquired from the detectors CD1 and CD2 of the chips (see Fig. 2), respectively.
[b] A series of the ZE runs performed on one chip in 1 day using an identical equipment.
[c] A series of the ZE runs performed on one chip in 5 days using an identical equipment.
[d] A series of the ZE runs performed on five chips in 18 days using an identical equipment.
[e] A series of the ZE runs performed in 18 days using seven chips and two CE instruments.

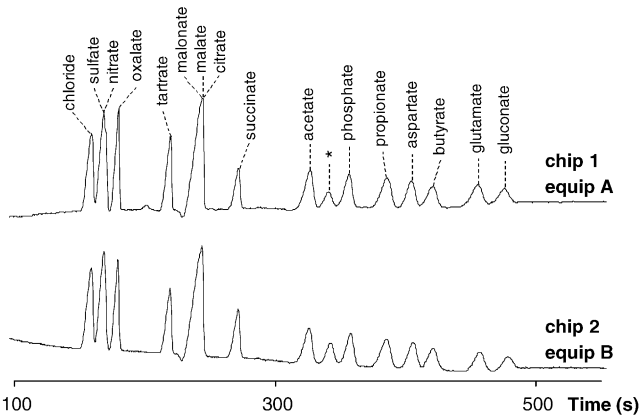

Fig. 5. Electropherograms from the ZE separations of a group of test anions as obtained under identical working conditions by two CE instruments. The separations, carried out in the electrolyte system ES1 (Table 1), followed the scheme in Fig. 4. In both instances, the driving currents during the runs were stabilized at 30 μA. The concentrations of the constituents in the loaded sample were: chloride, sulfate, nitrate, oxalate, tartrate, malonate, malate, citrate, succinate (each at a 10 μmol/l concentration), acetate, phosphate, propionate, butyrate (each at a 20 μmol/l concentration), aspartate at a 30 μmol/l concentration, glutamate at a 40 μmol/l concentration, and gluconate at a 60 μmol/l concentration.

ond channel, besides the analyte, only a minimum number of the accompanying sample constituents. Our experiments performed along this line were directed, mainly, on a situation in which the constituent of interest migrates in the first channel only partially resolved from the neighboring constituents. Aspartate, meeting this migration requirement (see Fig. 6), served as a test analyte in these experiments. Here, our focus was on a transfer providing its full recovery along with a maximum matrix removal [via the steps c and e of the run (Fig. 3)]. The transfer step, commenced when the front edge of the aspartate peak reached the bifurcation region on the chip (see Figs. 1 and 3), was time controlled. Therefore, its duration determined the recovery rate for aspartate and, at the same time, the amounts of the neighboring constituents (propionate and butyrate) transferred. An impact of the transfer time on these parameters illustrates electropherograms in Fig. 6 and relevant recovery data (Table 3). These data, based on the response from the detector in the second channel, show that a certain interval of the transfer time (8–10 s) led to an optimum transfer of aspartate under the working conditions employed. A typical repeatability of the transfer step as achieved in our experiments with an identical transfer program illustrates electropherograms in Fig. 6D.

A transfer of a group of the sample constituents of close effective mobilities from the first to the second separation channel was investigated to assess an extent of mutual diffusion driven mixing of the transferred constituents when these did not migrate for a certain time after the transfer to the second channel. Such a situation in column switching ZE occurs, for example, when less mobile sample constituents are

Table 3
Recoveries of aspartate in its transfer from the first to the second separation channel on the CC chip

Transfer time[a] (s)	Mean value of the peak area of the constituent[b] (mV s)			Recovery[c] (%)
	Aspartate	Propionate	Butyrate	Aspartate
6	26.5 (n = 5)	0	0	70.1
8	37.2 (n = 5)	0	0	98.5
10	38.6 (n = 5)	0	0	102.1
12	38.2 (n = 5)	0	6.5 (n = 5)	101.0

[a] A start of the transfer was derived from the detection of the aspartate peak apex in the first channel (see also Fig. 6).
[b] The data are based on the response of the detector in the second channel of the chip.
[c] A mean value of the aspartate peak area (37.8 mV s) obtained from the response of the detector in the second channel in five runs with complete transfers of the loaded sample served as a value corresponding to a full recovery.

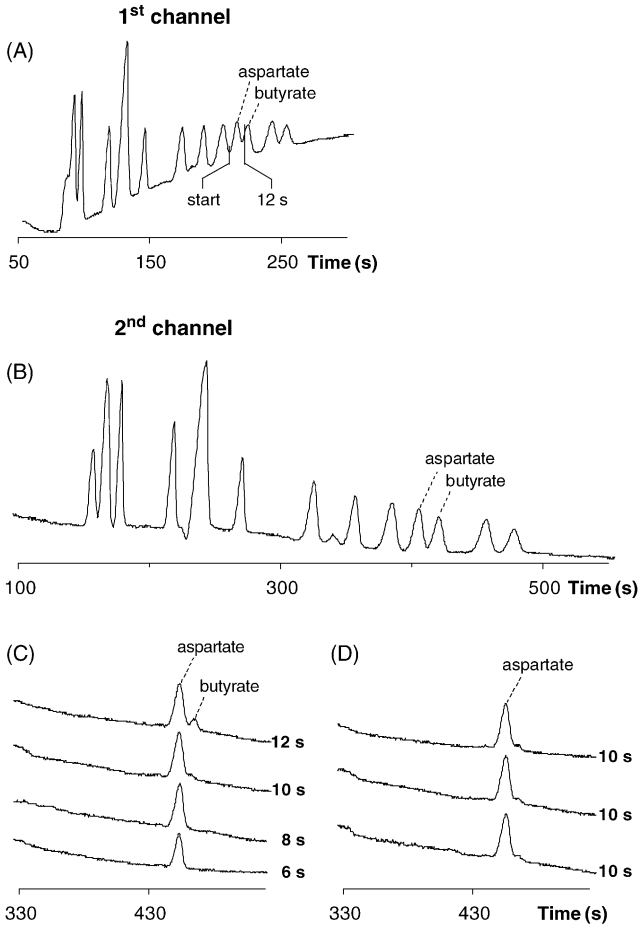

Fig. 6. Time controlled channel-to-channel transfers of the sample constituents in the ZE runs on the CC chip. The same sample as in Fig. 5 was used. (A) An electropherogram as obtained from the conductivity detector in the first channel (D1, in Fig. 2) in the run with the test sample; (B) a complete transfer of the loaded sample to the second channel (the run was performed using the program as in Fig. 5); (C) transfers of aspartate for different transfer times [a full span of the transfer times is marked in (A)]; (D) a repeatability of the aspartate transfer as attained for a 10 s transfer time. The separations were carried out in the electrolyte system ES1 (Table 1) with the driving current stabilized at 30 μA. In the separations under (C) and (D), the scheme as shown in Fig. 3 was followed.

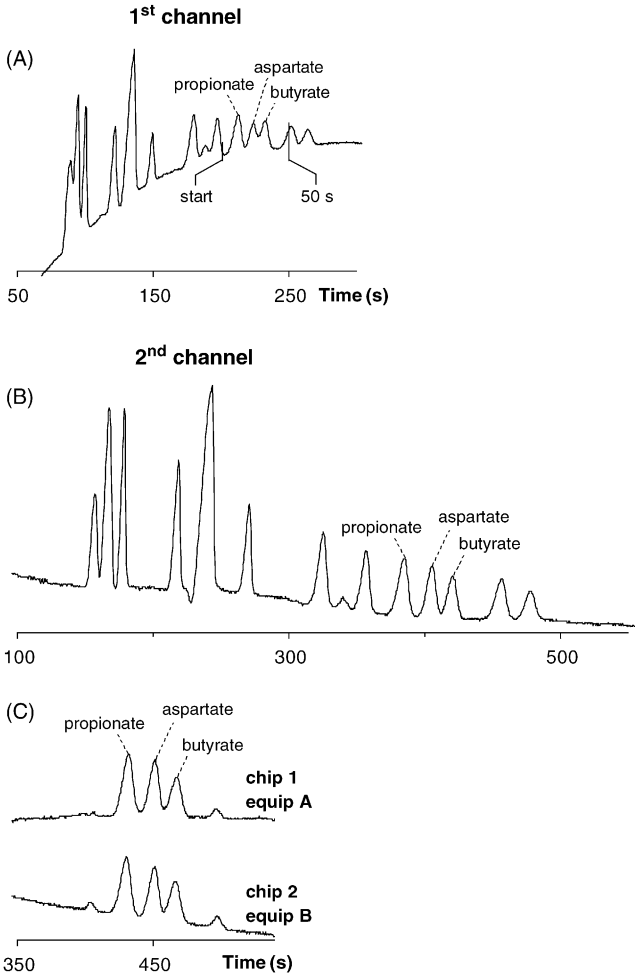

Fig. 7. An impact of interruption of the delivery of the driving current on the peak dispersions of the constituents transferred to the second channel. (A) An electropherogram as obtained from the conductivity detector in the first channel (D1, in Fig. 2) in the run with a test sample (for the sample composition, see Fig. 5); (B) a complete transfer of the loaded sample to the second channel (the run was performed using the time program as in Fig. 5); (C) only the propionate, aspartate and butyrate peaks were transferred to the second channel during a 50 s transfer time [see marks on the electropherogram in (A)]. The separations grouped under (C), carried out in the electrolyte system ES1 (Table 1) with the driving current stabilized at 30 μA, followed the scheme shown in Fig. 3.

removed from the separation system (step e, in Fig. 3). Illustrative electropherograms (Fig. 7), obtained from ZE runs on two chips with the aid of two instruments, show that this dispersive process was not critical, at least, when the transferred constituents were left for 60 s in the second channel before their separation continued. In addition, these electropherograms illustrate an excellent equipment-to-equipment reproducibility of both the transfer and dispersive processes as achieved in our experiments.

An electrophoretic removal of the matrix constituents from the separation compartment in column switching ZE (steps c and e, in Fig. 3) directly contacts their zones with the background electrolyte solution present in the second channel. Therefore, diffusion driven transfers of the matrix constituents from the bifurcation region to the second channel a priori accompany this electrophoretically performed sample clean up. Although the presence of the matrix constituents, transferred to the second channel in this way, was not detected in the runs carried out with the multicomponent test sample (Figs. 6 and 7), this only indicates that this undesired transfer was not detectable for such a sample composition. On the other hand, when the test sample contained chloride in a very large excess (at about 10^3 higher concentration in comparison to the runs shown in Figs. 6 and 7) the presence of chloride in the second channel was detected also in instances when the program of the run was set to reach its complete removal (Fig. 8A). ZE experiments with such a program of the run for different contact times of the chloride zone with the BE solution in the second channel (the driving current was

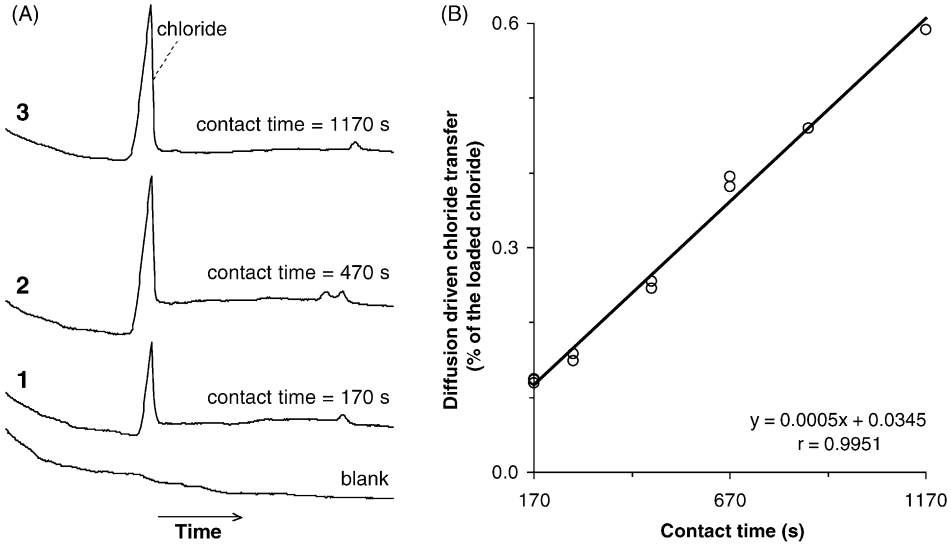

Fig. 8. An estimation of extent of diffusion driven transfer of a matrix constituent to the second channel during its electrophoretic removal on the CC chip. Chloride, serving as a matrix constituent, was present in the loaded sample at a 14 mmol/l concentration. (A) Electropherograms obtained for different contact times of the chloride peak with the electrolyte solution filling the second channel of the chip. The contact times included: (1) only the time during which the chloride peak was electrophoretically led out of the separation compartment (170 s); (2) the contact time relative to (1) was prolonged by interrupting the delivery of the driving current for 300 s when the chloride peak apex reached the bifurcation region; (3) the same as in (2), only the delivery of the driving current was interrupted for 1000 s. The runs, carried out in the electrolyte system ES1 (Table 1) with the driving current stabilized at 30 μA, followed the scheme in Fig. 3. (B) A plot of the fraction of the loaded chloride transferred to the second channel by diffusion against the contact time in the bifurcation region.

switched off for a certain time when the apex of the chloride peak reached the bifurcation region) revealed a contact time dependent size of the chloride peak as detected in the second channel (see a plot in Fig. 8B). These data show that the chloride removal accompanied a diffusion driven transfer corresponding to 0.1–0.2% of chloride loaded onto the chip. This also indicates that 99.8–99.9% of the loaded chloride was removed by ZE sample clean up.

3.3. Two-dimensional ZE separations on the CC chip

A well-defined transfer of the analyte containing sample fraction to the second channel of the CC chip provides means for 2D ZE separations when the transfer is combined with the use of different separation mechanisms in the channels. Here, as in other column coupling separations, a full benefit of the 2D approach can be reached only for one or a very limited number of the sample constituents [49,50]. Electropherograms in Fig. 9 illustrate these 2D possibilities of the CC chip. One of them (Fig. 9B) documents a perfect column switching transfer of the peak of the constituents that could not be resolved under the separating conditions employed in the first channel (malate, malonate and citrate). Then, a subsequent ZE separation in the second channel, using the background electrolyte differentiating the effective mobilities of the transferred constituents via an ionic strength effect [51] and host-guest complexations with α-cyclodextrin (ES2, in Table 1), led to their rapid baseline resolutions (Fig. 9C). When we consider the migration performance and reproducibility in the column switching operation as attained under our working conditions (see above), then excellent repeatabilities of two-dimensional ZE runs, documented by data in Table 4 and illustrated by electropherograms in Fig. 9C, were not surprising.

With respect to a 900 nl volume of the sample injection channel of the present CC chip (Fig. 1) the use of the electric field or ITP stacking techniques [52] is needed to keep the injection dispersion in the ZE separations performed on this chip on acceptable levels. In fact, their use is assumed in the scheme of the ZE run (step b, in Fig. 3) and, for example, the electric field stacking was effective in the all experiments as

Table 4
Repeatabilities of 2D ZE separations on the CC chips

Analyte	Migration time[a]			Peak area[a]			*n*
	Mean (s)	S.D. (s)	R.S.D. (%)	Mean (mV s)	S.D. (mV s)	R.S.D. (%)	
Short-term repeatability[b]							
Malate	420	1.6	0.4	62.0	0.5	0.9	5
Malonate	442	1.3	0.3	63.8	0.6	0.9	5
Citrate	473	2.4	0.5	40.4	0.4	0.9	5
Chip-to-chip repeatability[c]							
Malate	422	2.4	0.6	66.6	4.9	7.4	10
Malonate	443	2.2	0.5	65.0	1.5	2.3	10
Citrate	476	5.4	1.1	42.4	2.1	5.0	10

For the working conditions, see the legend to Fig. 9.

[a] The data were obtained from the conductivity detector in the second channel of the CC chip.

[b] A series of the ZE runs performed on one chip in 1 day.

[c] A series of the ZE runs performed on two chips using an identical CE equipment.

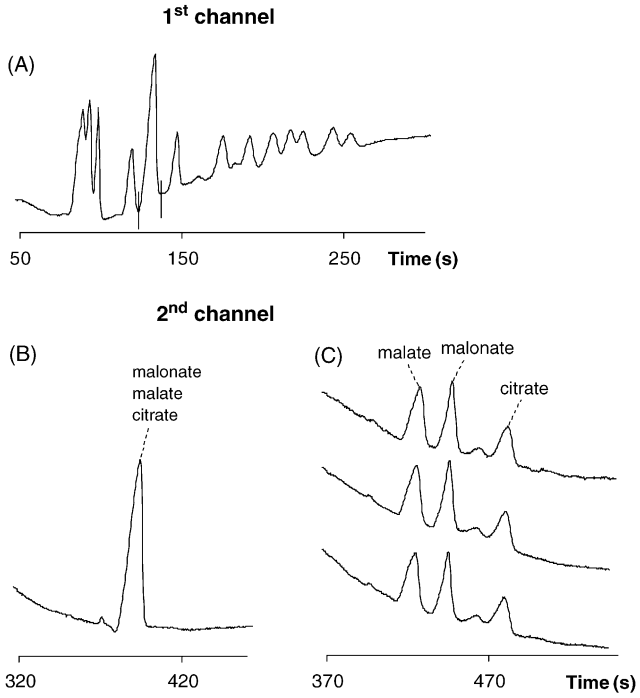

Fig. 9. A 2D ZE separation on the CC chip. An electropherogram obtained from the conductivity detector: (A) in the first channel (D1, in Fig. 2) in the run with a test sample (see, Fig. 5) using the background electrolyte ES1 (Table 1) and (B) in the second channel (D2, in Fig. 2) documenting a transfer of the peak under which migrated in the first channel malonate, malate and citrate (the separation in the second channel was carried out in the electrolyte system ES1 [Table 1] with the driving current stabilized at 30 μA); (C) the resolutions of malonate, malate and citrate in the second channel using the background electrolyte ES2 (Table 1) with the driving current stabilized at 30 μA. The separations in (B) and (C) followed the scheme in Fig. 3.

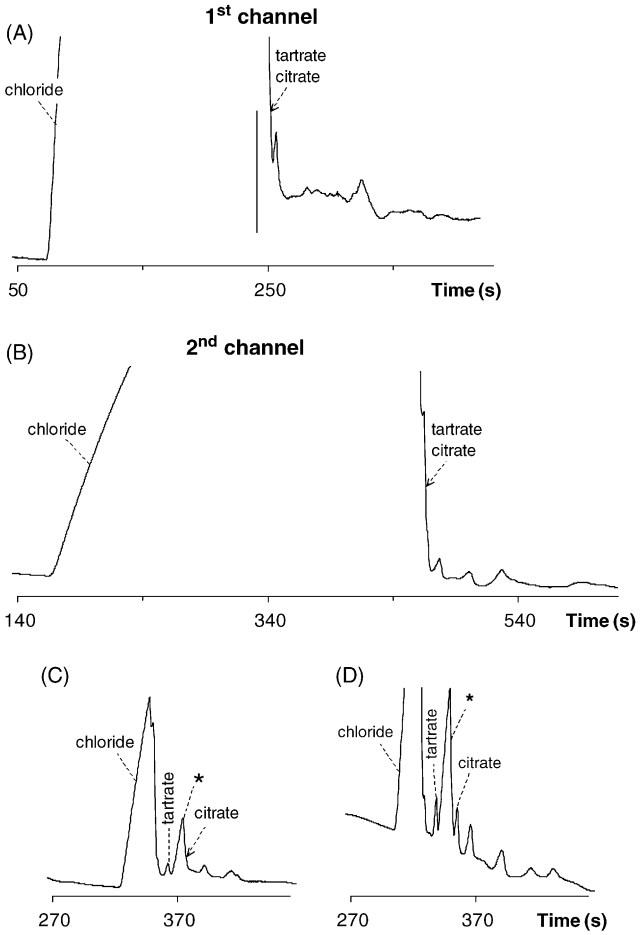

Fig. 10. A column switching enhanced destacking in the ZE separation on the CC chip. (A) an electropherogram as obtained from the conductivity detector in the first channel (D1, in Fig. 2) in the run with a test sample containing chloride at a high concentration (the sample composition: chloride at a 14 mmol/l concentration; tartrate at a 3.5 μmol/l concentration and citrate at a 2.6 μmol/l concentration); (B) an electropherogram from the same run as in (A) registered from the conductivity detector in the second channel (D2, in Fig. 2) for a complete transfer of the loaded sample; (C) the same sample as in (A) only a main part of chloride was removed from the separation system [see a mark in (A)] before the transfer of the analytes to the second channel; (D) the same as in (C), only the separation in the second channel was carried out in the background electrolyte ES3 (Table 1).

discussed above. In this work, we paid attention to the use of the ITP stacking as well. Here, our model experiments with anionic constituents were focused on samples characterized by high matrix/analyte ratios (typical situations in the analysis of samples of biological origins). It was assumed that the use of the CC chip could be beneficial in a control of the destacking of analytes via a proper column switching program during the ZE run. Analytical potentialities of such an approach illustrate electropherograms in Fig. 10. The electropherograms in Fig. 10A and B (registered from the detection sensors in the first and second separation channels of the chip, respectively) clearly show that a high concentration of the stacker (chloride) in the loaded sample adversely affected the destacking rate [53]. Due to this, the test analytes (tartrate and citrate) were not resolved from chloride before their detections in the second channel (Fig. 10B). Here, a removal of the main part of the stacker (the step c, in Fig. 3) apparently solved this problem (Fig. 10C and D). In addition, we could also make the use of the 2D capabilities of the CC chip and enhance the resolution rate of the analytes by performing the run in the second channel under slightly differing electrolyte conditions (Fig. 10D).

4. Conclusions

This feasibility study showed that column switching provides means that significantly enhance resolving power attainable in the ZE separations performed on the CC chip. These, mainly, include (1) on-column sample clean up of both the multicomponent and high salinity samples and (2) different separation mechanisms applicable in the coupled channels (2D features). In a general sense, these means are characteristic for the coupled column separation systems and column switching ZE on the CC chip can be considered as a CE analogy of the column switching chromatography separation system [49,50]. Experiments carried out in this work indicate that ZE in the column coupling separation system

is simpler to operate than its chromatography counterpart as no column conditioning is required (2–3 min lasting replenishments of the electrolyte solutions in the channels between the runs were the only operations needed to start the run with a new sample).

Reliable and high recovery column-to-column transfers of the analytes were possible also in instances when the analyte migrated in the first channel with the effective mobility close to those of the matrix constituents. Here, a proper timing of the transfer provided for the second separation stage the analyte fraction containing only minimum amounts of the matrix constituents of close migration properties (see, e.g., Fig. 6). Undoubtedly, a very reproducible transfer process, a well-defined and highly efficient removal of the matrix constituents from the separation compartment and the use of different separation mechanisms in the channels are features that makes column switching ZE on the CC chip a very promising tool for a miniaturized analysis of multicomponent samples.

The electric field and ITP stacking techniques can be employed in column switching ZE on the CC chip in the same way as in current single column ZE separation systems. Our experiments clearly document (Fig. 10) that column switching offers a tool for a control of the destacking process when transient ITP is effective in an initial phase of the separation.

A diffusion transport of the matrix constituents to the second channel of the CC chip, associated with the direct contacts of the electrolyte solutions in the bifurcation region, is an inherent disturbance to the separation in the second channel. Our experiments performed with the samples containing chloride at a high concentration (Fig. 8) indicate that this undesired transfer of the matrix constituents to the second column (representing 0.1–0.2% of the loaded sample) can be in many instances ignored.

The use of the present CC chip is not restrictive as far as the hydrodynamic arrangement of the separation system is concerned. However, the chip, as any other CE device, offers higher reproducibilities of the migration velocities of the separated constituents when used with a hydrodynamically closed separation system [8]. High reproducibilities as attained in this work in the ZE runs on the CC chip with and without column switching can be, at least in part, attributed to a preference of the closed system (eliminating HDF) with suppressed EOF.

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