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Isotachophoresis and isotachophoresis — zone electrophoresis separations of inorganic anions present in water samples on a planar chip with column-coupling separation channels and conductivity detection

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Abstract

The use of a poly(methylmethacrylate) chip, provided with two separation channels in the column-coupling (CC) arrangement and on-column conductivity detection sensors, to electrophoretic separations of a group of inorganic anions (chloride, nitrate, sulfate, nitrite, fluoride and phosphate) that need to be monitored in various environmental matrices was studied. The electrophoretic methods employed in this study included isotachophoresis (ITP) and capillary zone electrophoresis (CZE) with on-line coupled ITP sample pretreatment (ITP–CZE). Hydrodynamic and electroosmotic flows of the solution in the separation compartment of the CC chip were suppressed and electrophoresis was a dominant transport process in the separations performed by these methods. ITP separations on the chip provided rapid resolutions of sub-nmol amounts of the complete group of the studied anions and made possible rapid separations and reproducible quantitations of macroconstituents currently present in water samples (chloride, nitrate and sulfate). However, concentration limits of detection attainable under the employed ITP separating conditions ($2–3\cdot10^{-5}$ mol/l) were not sufficient for the detection of typical anionic microconstituents in water samples (nitrite, fluoride and phosphate). On the other hand, these anions could be detected at $5–7\cdot10^{-7}$ mol/l concentrations by the conductivity detector in the CZE stage of the ITP–CZE combination on the CC chip. A sample clean-up performed in the ITP stage of the combination effectively complemented such a detection sensitivity and nitrite, fluoride and phosphate could be reproducibly quantified also in samples containing the macroconstituents at 10^4 higher concentrations. ITP–CZE analyses of tap, mineral and river water samples showed that the CC chip offers means for rapid and reproducible procedures to the determination of these anions in water (4–6 min analysis times under our working conditions). Here, the ITP sample pretreatment concentrated the analytes and removed nanomol amounts of the macroconstituents from the separation compartment of the chip within 3–4 min. Both the ITP and ITP–CZE procedures required no or only minimum manipulations with water samples before their analyses on the chip. For example, tap water samples were analyzed directly while a short degassing of mineral water (to prevent bubble formation during the separation) and filtration of river water samples (to remove particulates and colloids) were the only operations needed in this respect.

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1. Introduction

Zone electrophoresis separations, performed either in free [capillary zone electrophoresis (CZE)] or micellar [micellar electrokinetic chromatography (MEKC)] solutions, are dominantly employed in laboratory-on-a-chip analytical systems (for a review see, e.g., Refs. [1–5]). So far, the use of other basic electrophoretic methods [isotachophoresis (ITP) and isoelectric focusing (IEF)] in these systems is very scarce in spite of the fact that they offer some specific analytical advantages [6–12].

A general concept followed in the miniaturization of capillary electrophoresis (CE) separation systems favors a short column (separation channel) of a very small inner diameter (cross-section) [13]. Therefore, the miniaturized (chip) systems accommodate only sub-nl sample volumes when employed in CZE and MEKC separations. The use of very sensitive detection techniques to monitor the separations performed on the chips is then essential when low concentration limits of detection (LODs) are to be achieved [1–5].

Enhanced sample loadabilities along with well-defined concentration capabilities are well known features of the ITP separations (see, e.g., Refs. [14,15]). Therefore, the use of ITP makes possible to increase significantly sample volumes loadable on the CE chip [8] and/or improve detectabilities of the CE analytes [6].

The column-coupling (CC) configuration of the separation compartment as proposed by Everaerts et al. [16] is analytically very beneficial in the CE separations performed in conventional instruments [16–26]. Using this instrumental approach the CE analysis can be divided into two stages in which specific advantages of electrophoretic methods (e.g., ITP and zone electrophoresis) are very effectively combined. For example, a well-defined sample pretreatment can be integrated into the CE separation performed in the CC equipment [18,25]. In addition, the use of ITP in the first separation stage significantly enhances an overall analytical effect as this electrophoretic method can increase the load capacity of the separation system by a factor of 10^3 or more in comparison to a current single-column CZE. As a result, some ITP [16–21] and ITP–CZE [22–26] combinations provide very favorable LODs also for the analytes present in complex ionic matrices.

A transfer of the column-coupling CE separation system to a chip format is a subject of our current research interest. A poly(methylmethacrylate) chip providing this CC technology in a miniaturized format was developed recently in our laboratories [27]. It was already shown to offer analytical advantages of its conventional counterparts in the chip based CE separations [8]. The present work was aimed at investigating its potential applicability to the CE separations and determination of inorganic anions (chloride, nitrate, sulfate, nitrite, fluoride and phosphate) that need to be currently monitored in water and various matrices of environmental origins [28,29]. Model samples taken into this investigation contained the anions at close concentrations and at concentrations reflecting many practical situations in water analysis (chloride, nitrate and sulfate as sample macroconstituents and nitrite, fluoride, and phosphate as microconstituents). Tap, river and mineral waters served as practical samples in this feasibility study. The separations and quantitation of the anions were carried out with two of the CE techniques running on the chip [8], viz., ITP and ITP–CZE.

2. Experimental

2.1. Instrumentation

Detail descriptions of the poly(methylmethacrylate) CC chips used in this work are given elsewhere (Ref. [27]). Schematic arrangements of the channels and their geometrical dimensions are given in Fig. 1. The separations on these chips were performed in a laboratory constructed CE equipment [8]. This equipment consisted of two units: (1) An electrolyte and sample management unit (E&SMU, in Fig. 1), connected via 300 μm I.D. FEP (fluorinated ethylene–propylene copolymer) capillary tubes to the

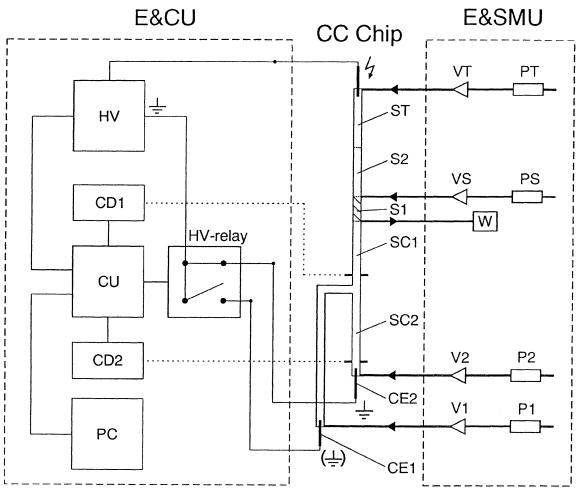

Fig. 1. A scheme of the CE equipment used in the ITP and ITP–CZE separations on the CC chips. Electronic and control unit (E&CU): CU=control unit; HV=high-voltage power supply (a high-voltage pole was connected to the driving electrode in the terminating channel of the chip, ST); CD1, CD2=conductivity detectors for the first and second separation channels, respectively; HV-relay=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 of HV either CE1 or CE2). CE1, CE2=counter-electrodes for the first and second separation channels, respectively. Electrolyte and sample management unit (E&SMU): V1,V2, VT=needle valves for the inlets of the separation and terminating channels of the chip; VS=a pinch valve for the inlet of the sample injection channel; W=waste container; P1, P2, PS, PT=syringes for filling the first, second, sample injection and terminating channels with the electrolyte and sample solutions, respectively. CC chip=a poly(methylmethacrylate) chip. Chip version 1.0: S1=a 1.2 μl sample injection channel [31× 0.2×0.2 mm (length, width, depth)]; S2=a 15.2 μl sample injection channel [76× 1.0×0.2 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 (connected to CD1); 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 (connected to CD2); Chip version 1.1: S1=a 0.96 μl sample injection channel [24× 0.2×0.2 mm (length, width, depth)]; S2=a 9.2 μl sample injection channel [46×1.0×0.2 mm (length, width, depth)]; SC1=the first separation channel [a 2.08 μl volume; 52×0.2×0.2 mm (length, width, depth)] with a platinum conductivity sensor (connected to CD1); SC2=the second separation channel [a 1.68 μl volume; 42×0.2×0.2 mm (length, width, depth)] with a platinum conductivity sensor (connected to CD2).

inlets of the channels on the chip. Valves of this unit (V1,V2,VT and VS, in Fig. 1) served to open these inlets on filling the channels and they were closed during the separations. Syringes (P1, P2, PS, PT, in Fig. 1), connected to the inlets of the corresponding valves, delivered electrolyte solutions and the sample to the channels before the CE run. An outlet channel of the chip, connected to a waste container (W, in Fig. 1), was permanently opened. (2) An electronic and control unit (E&CU, in Fig. 1) delivered the driving current, measured conductivity with the aid of platinum detection sensors, sputtered on the cover of the channels of the chip [27]. It also interfaced the CE equipment to a 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. Electrolyte solutions and samples

Chemicals used for the preparation of the electrolyte solutions and the solutions of anionic model mixtures were obtained from Sigma (St. Louis, MO, USA), Serva (Heidelberg, Germany) and Merck (Darmstadt, Germany). Methylhydroxyethylcellulose 30 000 (Serva), purified on a mixed-bed ion exchanger (Amberlite MB-1; BDH, Poole, UK), was used as a suppressor of electroosmotic flow (EOF). It was added to the leading and carrier electrolyte solutions or it was applied as a coating of the inner walls of the separation channels [30]. Compositions of the electrolyte solutions employed in the ITP and ITP–CZE separations on the chips are 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 separations were filtered by disposable membrane filters of 0.8 μm pore sizes (Sigma) connected to syringes used for filling the separation compartment (P_1, P_2, and P_T in Fig. 1).

Mineral (table) water (Budiš, Slovak Republic), bought in a local supermarket, was sonicated for 5 min before the analysis to remove free CO_2. Tap water samples were collected in the laboratory into polyethylene sample containers. They were appropriately diluted or injected directly into the sample channel of the chip without any sample preparation. A river water sample was taken from Danube in

Table 1
Electrolyte systems[a]

	ES1, ITP	ES2		
		ITP stage	CZE stage	
Leading anion	Dithionate	Chloride	Carrier ion	Aspartate
Concentration (mol/l)	$5 \cdot 10^{-3}$	10^{-2}	Concentration (mol/l)	10^{-2}
Counter ion	β-Alanine	β-Alanine	Counter ion	β-Alanine
Co-counter ion	Mg^{2+}	–	EOF suppressor	MHEC
Concentration (mol/l)	$1.8 \cdot 10^{-3}$	–	Concentration (%, w/v)	0.05
EOF suppressor	MHEC	MHEC	pH	3.35
Concentration (%, w/v)	0.1	0.05		
pH	3.5	3.2		
Terminating anion	Citrate	Aspartate		
Concentration (mol/l)	$5 \cdot 10^{-3}$	10		
Counter ion	–	β-Alanine		
pH	ca. 4	4.2		

[a] MHEC=methylhydroxyethylcellulose.

Bratislava into polyethylene sample containers. The sample was filtered through a 0.45 μm pore size filter (Sigma) before the ITP–CZE analysis.

3. Results and discussion

3.1. ITP separations of inorganic anions on the CC chip

ITP separations of inorganic anions of our interest were carried in the electrolyte system ES1 (Table 1) using the version 1.0 of the CC chip (Fig. 1). The use of this electrolyte system, combining two separation mechanisms in the separations of inorganic anions (differences in acid–base properties of the analytes and their different complexations with Mg^{2+}), was preferred as, contrary to other alternatives suitable to the ITP separations of inorganic anions (for a review see, e.g., Refs. [28,29]), it made possible a direct determination of chloride [31].

A tandem coupled configuration of the separation channels of the chip with a 62 mm separation path, providing a maximum load capacity [32,33] attainable on the CC chip [8], was preferred in the ITP separations performed in this work (Fig. 2). An isotachopherogram from the separation of a model mixture of anions in Fig. 3 shows that rapid ITP separations of the studied anions were possible under such working conditions. These anions could be detected by the conductivity detector on the chip when their concentrations in the loaded sample (a 1.2 μl sample channel; S1, in Figs. 1 and 2) were $2–3 \times 10^{-5}$ mol/l. Repeatabilities of their quantitations characterized ca.10% RSD values when the loaded sample contained the anions at 10^{-4} mol/l concentrations. Higher concentrations of the analytes, corresponding to their time-based zone lengths of 10 s or more, led to improved reproducibilities (repeatabilities) of the determination (2–5% RSD values). Volume fluctuations with which the sample was loaded on the chip in repeated runs very likely set the reproducibility limits [34] in the quantitations performed in our experiments.

An isotachopherogram as obtained from the analysis of tap (drinking) water (Fig. 4) show that under the preferred working conditions we could detect and quantitate in this sample only the anionic macroconstituents (chloride, nitrate and sulfate). ITP experiments performed in this context with model mixtures, resembling in the anionic compositions typical water samples [35], revealed that chloride and nitrate and sulfate and nitrate can be expected as critical pairs in the analysis of water samples on the chip when the concentrations of chloride and/or sulfate are higher than ca.10^{-3} mol/l. Appropriate dilutions of water samples were needed in such instances to achieve the ITP resolutions of analytes.

Attempts aimed at detecting anionic microconstituents in the above water sample using a 15.2 μl

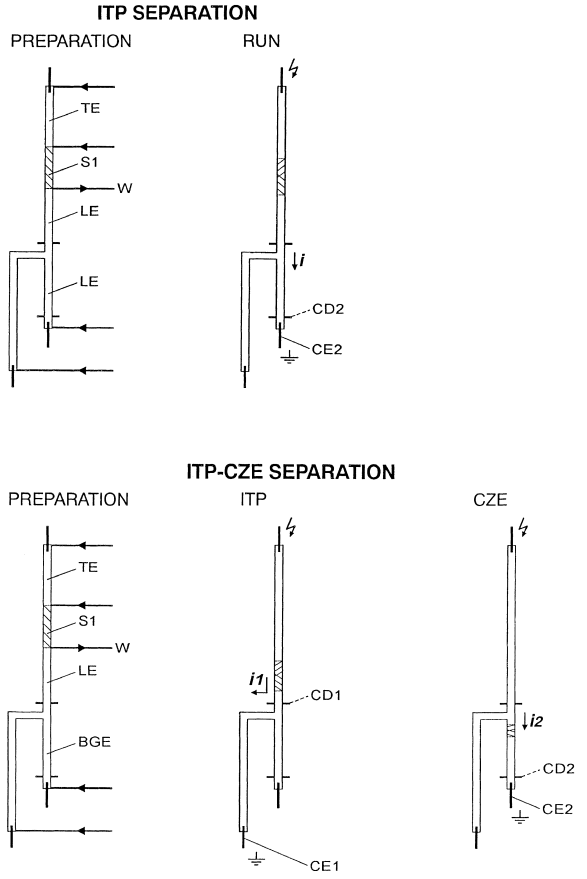

Fig. 2. Schemes of the ITP (the tandem-coupled separation channels) and ITP–CZE separation modes as employed in the separations of inorganic anions on the CC chip. Preparation= arrangements of the solutions in the channels of the chip at the start of the separation. S1=sample; LE=leading electrolyte solution; TE=terminating electrolyte solution; BGE=background (carrier) electrolyte solution. CD1, CD2=on-column conductivity sensors in the first and second separation channels, respectively; CE1, CE2=driving electrodes for the first and second separation channels, respectively; i, i1, i2=symbols for the driving currents (arrows at the symbols indicate the directions of the driving currents).

sample channel on the chip (S2, in Fig. 1) were not effective as the load capacity of the separation channels was not sufficient to the ITP resolutions of the anions present in such a sample volume. Therefore, it seems reasonable to assume that the use of the present CC chip to the ITP determination of typical anionic microconstituents in water samples (nitrite, fluoride and phosphate) is restricted to

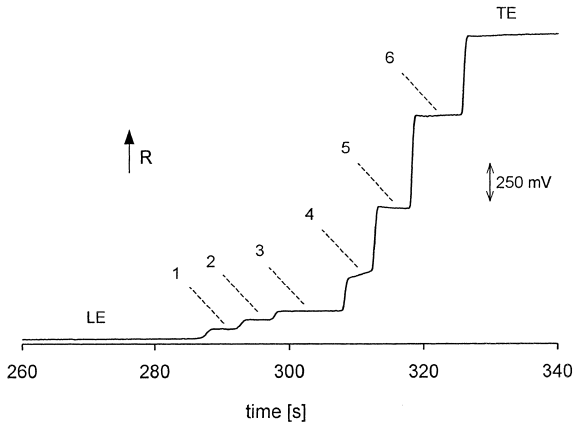

Fig. 3. ITP separation of inorganic anions (each at a $4 \cdot 10^{-4}$ mol/l concentration) present in a model sample. The isotachopherogram was registered from the detection sensor in the second channel (CD2, in Figs. 1 and 2). The zone assignments: 1=chloride, 2=nitrate, 3=sulfate, 4=nitrite, 5=fluoride, 6=phosphate. LE, TE=leading and terminating zones, respectively. The separation was performed on the chip version 1.0 (Fig. 1) and followed an ITP scheme as shown in Fig. 2. The electrolyte system ES 1 (Table 1) was used in both channels of the chip. The driving current was stabilized at 20 μA.

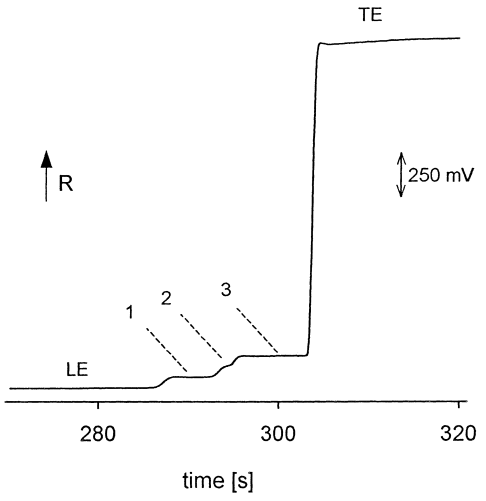

Fig. 4. ITP separation of inorganic anions present in a tap water sample. The isotachopherogram was registered from the detection sensor in the second channel (CD2, in Figs. 1 and 2). The zone assignments: 1=chloride, 2=nitrate, 3=sulfate. LE, TE=leading and terminating zones, respectively. The separation was performed on the chip version 1.0 (Fig. 1) and followed an ITP scheme as shown in Fig. 2. The electrolyte system ES 1 (Table 1) was used in both channels of the chip. The driving current was stabilized at 20 μA.

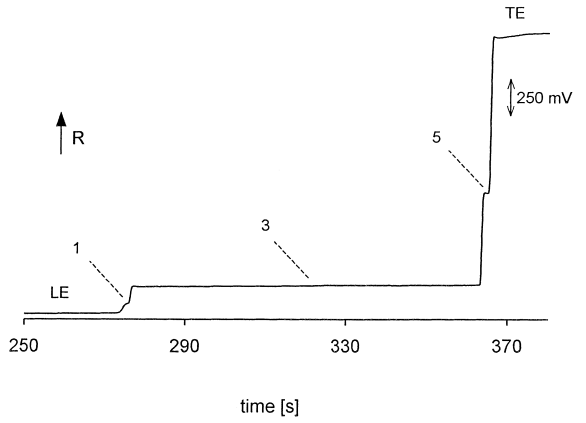

Fig. 5. ITP separation of inorganic anions present in a mineral (table) water sample. The isotachopherogram was registered from the detection sensor in the second channel (CD2, in Figs. 1 and 2). The zone assignments: 1=chloride, 3=sulfate, 5=fluoride. LE, TE=leading and terminating zones, respectively. The separation was performed on the chip version 1.0 (Fig. 1) and followed an ITP scheme as shown in Fig. 2. The electrolyte system ES 1 (Table 1) was used in both channels of the chip. The driving current was stabilized at 20 μA.

samples containing these constituents at relatively high concentrations (see, e.g., an isotachopherogram in Fig. 5) unless a more favorable approach to the ITP separation is elaborated (see, e.g. Refs. [28,29,36]). In a general sense, these limitations of ITP on the chip with the conductivity detection of zones can be ascribed to a small performance index of this combination (a ratio of the volume of the separation channels to the volume of the detection cell [37]).

3.2. ITP–CZE separation of nitrite, fluoride and phosphate present in high ionic strength matrices

CZE on-line coupled with ITP in a conventional column-coupling CE separation system offers a very convenient alternative to the determination of nitrite, fluoride and phosphate when these are present in matrices with high concentrations of chloride, sulfate and nitrate [26]. Therefore, the use of this combination on the CC chip (version 1.1, in Fig. 1) was investigated in details as it offers a means to overcome the above limitations of ITP in the determination of anionic microconstituents in water samples.

The ITP–CZE separations on the chip were carried out in the electrolyte system (ES 2, in Table 1) in the composition similar to that used in a conventional CE separation system [26]. Its leading electrolyte served to resolve the macro- (chloride, sulfate and nitrate) and microconstituents (nitrite, fluoride and phosphate) via their differences in acid–base properties. This group separation was accompanied by removals of the substantial parts of the macroconstituents from the separation compartment to the counter-electrode of the ITP stage (CE1, in Fig. 2). On the other hand, the carrier electrolyte solution used in the CZE stage provided sufficient separation selectivity for the resolutions of the microconstituents, maintained differences in the effective mobilities of macro- and microconstituents and favored sensitive detections of the microconstituents by the conductivity detector (CD2, in Fig. 2).

The LODs for the microconstituents in the CZE stage of the combination were estimated from the runs with model samples. Actual LOD values as attained under the preferred working conditions on the chip for nitrite ($5\cdot10^{-7}$ mol/l), fluoride ($5\cdot10^{-7}$ mol/l) and phosphate ($7\cdot10^{-7}$ mol/l) for a 960 nl sample volume are remarkable. They were about 50 times lower than those achieved for these analytes by the above ITP procedure. Their further reductions by injecting the sample via a 9.2 μl sample channel (S2, in Fig. 1) of the CC chip used in the ITP–CZE experiments (version 1.1, in Fig. 1) seem possible. This solution, however, may be relevant only in situations when the accompanying anionic sample macroconstituents do not overload the ITP stage of the separation system and the microconstituents can be separated as a group in this stage.

With respect to the LOD attained for the microconstituents we could quantitate them also in instances when they were present in the loaded sample at $2\cdot10^{-6}$ mol/l concentrations (Fig. 6). However, from the practical point of view it is important, that their reproducible separations and quantitations were possible also in instances when the samples contained the macroconstituents at 10^4 higher concentrations (see illustrative electropherograms in Fig. 7). Such a performance can be apparently ascribed to a high resolution rate [32] attainable in the ITP group separation.

The analysis times were determined, mainly, by

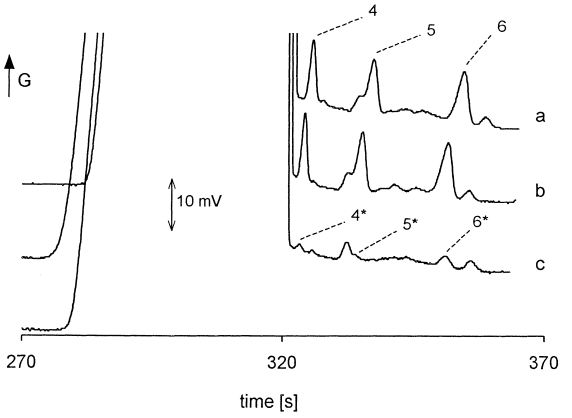

Fig. 6. Electropherograms from the ITP–CZE separations of inorganic anions present in a model sample containing nitrite (4), fluoride (5) and phosphate (6) at $2 \cdot 10^{-6}$ mol/l concentrations and, at the same time, chloride, sulfate and nitrate at $2 \cdot 10^{-3}$ mol/l concentrations. The concentrations of the microconstituents corresponded to their limits of quantitations. a, b=repeated runs with the model sample, c=blank run (4*–6* mark migration positions of the analytes). The separations were carried out on the chip version 1.1 (Fig. 1) in the electrolyte system ES 2 (Table 1) and followed an ITP–CZE scheme as shown in Fig. 2. The driving currents were 20 and 18 μA in the ITP and CZE channels, respectively.

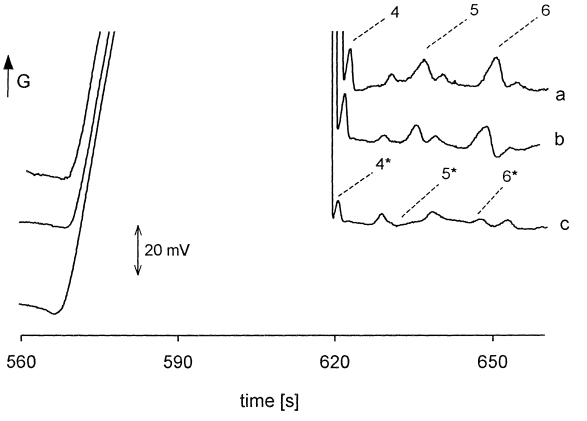

Fig. 7. ITP–CZE runs with a sample containing nitrite (4), fluoride (5) and phosphate (6) at $4 \cdot 10^{-6}$ mol/l concentrations and chloride at a 10^4 higher concentration. a,b=repeated runs with the same sample, c=blank run (4*–6* mark migration positions of the analytes). The separations were carried out on the chip version 1.1 (Fig. 1) in the electrolyte system ES 2 (Table 1) and followed an ITP–CZE scheme as shown in Fig. 2. The driving currents were 20 and 18 μA in the ITP and CZE channels, respectively.

the separations (sample pretreatment) performed in the ITP stage. In extreme instances [$4–5 \cdot 10^{-2}$ mol/l (0.8–5.0 g/l) concentrations of the macroconstituents] this sample pretreatment step required about 9 min to be accomplished. In this context, it should be noted that this time can be probably reduced by appropriately modifying the ITP working conditions (e.g., by increasing the concentration of the leading anion and/or maximizing the driving current). However, ITP concentration of impurities originating from the electrolyte system, enhanced due to a prolongation of the ITP pretreatment (compare blank runs in Figs. 6 and 7), cannot be eliminated in this way [14,15].

Precisions of the determination of the microconstituents were evaluated from experiments with model samples containing them at $4 \cdot 10^{-6}$ mol/l concentrations while $1–40 \cdot 10^{-3}$ mol/l concentrations of chloride in these samples represented variable ionic matrices due to the macroconstituents. The RSD values, characterizing the precision of the quantitation of the microconstituents for such sample compositions are given in Table 2. These values reflect, mainly, the use of different electrolyte solutions, preparations of fresh sample solutions (from the same stock solutions) and a day-to-day variability of the response of the detector. In addition, small systematic biases due to the microconstituents originating from the stock solution of chloride, especial-

Table 2
Reproducibilities of the peak areas in the ITP–CZE determination of inorganic anionic microconstituents present in model samples with a variable concentration of chloride[a]

Anion	A[b], RSD (%)	B[b], RSD (%)	C[b], RSD (%)
Nitrite[c]	3.1	14.4	15.2
Fluoride	4.0	26.0	24.9
Phosphate	6.8	14.1	20.0

[a] The data were obtained on the same chip.

[b] A=chloride at $1–7.5 \cdot 10^{-3}$ mol/l concentrations (the RSD data calculated for a series of 6 runs performed in one day); B=chloride at $1–40 \cdot 10^{-3}$ mol/l concentrations (the RSD data calculated for a series of 15 runs performed in the next day); C=chloride at $1–40 \cdot 10^{-3}$ mol/l concentrations (the RSD data calculated for both series of the CE runs).

[c] The data for concentrations of chloride in the sample higher than $4 \cdot 10^{-2}$ mol/l are not included (a destacking of nitrite in the CZE stage became critical, see Fig. 9).

ly, when this anion was present in the injected sample at a high concentration should be taken into account as well.

Parameters of the regression equations describing the calibration graphs for the determination of microconstituents in the CZE stage of the ITP–CZE combination on the chip are given in Table 3. The calibration graphs covered the concentration spans from the limits of quantitation of the analytes up to $2 \cdot 10^{-5}$ mol/l concentrations (maximum concentrations at which they are currently expected in water samples [35]).

3.3. ITP–CZE of anionic microconstituents present in water samples

A practical applicability of the ITP–CZE procedure as described above was tested in the analysis of different types of water samples and, here, we paid attention to the separation and detection of nitrite, fluoride and phosphate. Electropherograms in Fig. 8 illustrate its use to the separation of these constituents in a tap water sample. Tentative assignments of the identities to the microconstituent peaks on the electropherograms were carried out from the ITP–CZE runs with the sample spiked with the microconstituents at $4 \cdot 10^{-6}$ mol/l concentrations. This simple procedure showed that a constituent having the effective mobility close to that of nitrite (4a, in Fig. 8), in fact, oxidized nitrite added to the tap water sample. Therefore, its presence in this sample (probably as a result of water chlorination), at the same time excluded the presence of nitrite at a detectable concentration.

Repeatabilities attainable in the CZE quantitation

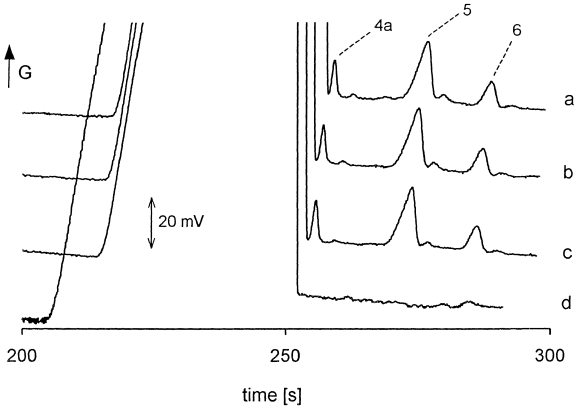

Fig. 8. Electropherograms from the CZE stage of the ITP–CZE combination in the separations of inorganic anionic microconstituents present in a tap water sample. a, b = repeated ITP–CZE runs with the sample from the same sampling, c = the same sample as in (a) and (b) from a parallel sampling, (d) = blank run (the terminating electrolyte solution injected into the sample channel). The peak assignment: 4a = an unidentified microconstituent migrating in the neighborhood of nitrite ('nitrite oxidant'); 5 = fluoride and 6 = phosphate. The separations were carried out on the chip version 1.1 (Fig. 1) in the electrolyte system ES 2 (Table 1) and followed an ITP–CZE scheme as shown in Fig. 2. The driving currents were 20 and 18 μA in the ITP and CZE channels, respectively.

of the anionic microconstituents of our interest in the tap water sample characterize the data given in Table 4. These data, illustrated by the eletropherograms in Fig. 8, clearly outline potentialities of the ITP–CZE procedure on the CC chip for reproducible determinations of nitrite (or 'nitrite oxidant'), fluoride and phosphate in drinking water.

To reach resolutions of the microconstituents as shown in Fig. 8 required that a transfer of the sample

Table 3
Regression equations of the calibration lines (y = a + bx) for the determination of microconstituents[a] by ITP–CZE on the CC chip

Anion	*a* (V s)	*b* (V s l mol^{-1})	*r*
Nitrite	$5 \cdot 10^{-4}$	$7.2 \cdot 10^{3}$	0.9956
Fluoride	$6 \cdot 10^{-4}$	$9.3 \cdot 10^{3}$	0.9971
Phosphate	$1.5 \cdot 10^{-3}$	$10.1 \cdot 10^{3}$	0.9940

[a] y = peak area, a = intercept, b = slope, x = concentration (mol/l), r = correlation coefficient; the regression equations were calculated from 12 determinations of the anions present in the calibration solutions at $2–20 \cdot 10^{-6}$ mol/l concentrations.

Table 4
Repeatabilities of the peak heights and peak areas of inorganic anionic microconstituents as obtained in their ITP–CZE determination in a tap water sample[a]

Anion	RSD (%)	
	Peak heights	Peak areas
Nitrite oxidant[b]	3.6	6.2
Fluoride	2.4	2.4
Phosphate	5.5	5.0

[a] The data obtained from a series of 15 CE runs performed with the same sample in one day.

[b] See electropherograms in Figs. 8 and 9.

macroconstituents into the CZE stage be minimized. When the CZE stage was overloaded by the macroconstituents, at least, the most mobile microconstituent could not be destacked or it was destacked only partially (see Ref. [38] for a detailed theoretical treatment of this process). Electropherograms (a)–(c) in Fig. 9 illustrate fluctuations in the resolution of this microconstituent when a transfer of the macroconstituents into the CZE stage reached a critical level.

Electropherograms in Fig. 10 illustrate the use of the ITP–CZE procedure to the detection of microconstituents present in a mineral (table) water sample (the same sample as analyzed by ITP, Fig. 5). A chemical composition of this sample was somewhat specific as it contained an enhanced concentration of fluoride (10^{-4} mol/l). Nevertheless, the electropherograms show that despite a significant electromigration dispersion of the fluoride peak the separation in the CZE stage (a 42 mm separation channel) was efficient enough and the constituents transferred into this stage could be rapidly resolved into their peaks (Fig. 10b).

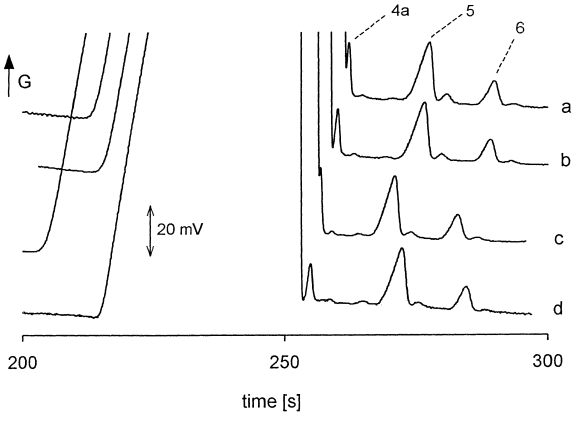

Fig. 9. An impact of a transfer of a variable amount of the macroconstituents into the CZE stage on the resolution of the most mobile microconstituent (4a). a–c = ITP–CZE runs of the same sample as in Fig. 8a and b with insufficient removals of the macroconstituents from the separation compartment after the separation in the ITP stage. d = the same as in a–c only the removal of the macroconstituents was enhanced. The separations were carried out on the chip version 1.1 (Fig. 1) in the electrolyte system ES 2 (Table 1) and followed an ITP–CZE scheme as shown in Fig. 2. The driving currents were 20 and 18 μA in the ITP and CZE channels, respectively.

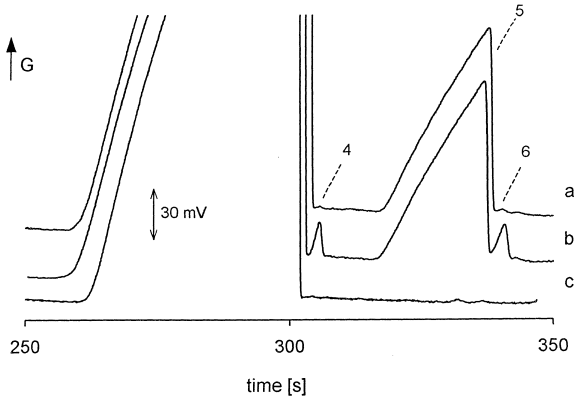

Fig. 10. Electropherograms as obtained by the conductivity detector in the CZE stage of the ITP–CZE runs with a mineral water sample 'Budiš'. a = ITP–CZE run with an undiluted sample; b = the same sample as in (a) spiked with nitrite (4), fluoride (5) and phosphate (6) at $4 \cdot 10^{-6}$ mol/l concentrations; (c) = blank run (the terminating electrolyte solution injected into the sample channel). The separations were carried out on the chip version 1.1 (Fig. 1) in the electrolyte system ES 2 (Table 1) and followed an ITP–CZE scheme as shown in Fig. 2. The driving currents were 20 and 18 μA in the ITP and CZE channels, respectively.

River water samples currently contain organic anions at concentrations comparable to those of the inorganic anionic microconstituents [35]. These are, at least, in part linked with a dissolved humic material. Therefore, this sample type was used to assess capabilities of the ITP–CZE procedure on the chip to the detection (and quantitation) of nitrite, fluoride and phosphate in such a complex organic matrix. A filtration of the sample through a 0.45 μm pore size filter (to eliminate particulates and colloids) was the only sample preparation step carried out before the ITP–CZE separation. Relevant electropherograms from the runs with the river water samples are given in Fig. 11. The electropherograms (a) and (b) in this figure correspond to a parallel sample collection carried out at the same sampling site. When these are compared to the ones obtained for the tap water sample (Fig. 8) a slightly enhanced matrix background in the migration interval of the microconstituents is visible in the river water sample. Nevertheless, these electropherograms indicate that the ITP–CZE combination on the CC chip has practical potentialities for rapid determinations of nitrite, fluoride and phosphate in river water matrices. Here, however, in some instances [35] dis-

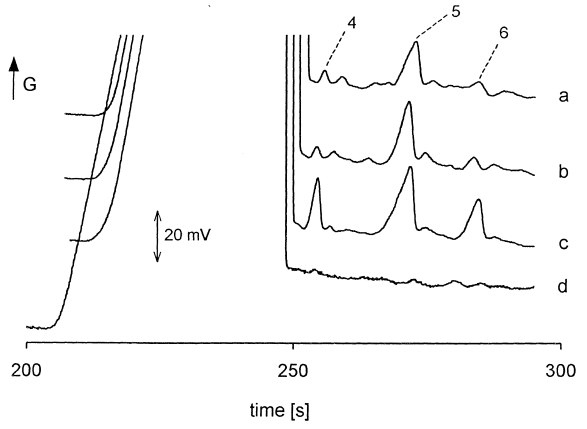

Fig. 11. Electropherograms from the conductivity detector in the CZE stage of the separation as obtained in the ITP–CZE runs with river water samples. a, b = ITP–CZE runs with the samples collected, in parallel, from the same sampling site; c = the same sample as in (b) spiked with nitrite (4), fluoride (5) and phosphate (6) at $4 \cdot 10^{-6}$ mol/l concentrations; d = blank run (the terminating electrolyte solution injected into the sample channel). The separations were carried out on the chip version 1.1 (Fig. 1) in the electrolyte system ES 2 (Table 1) and followed an ITP–CZE scheme as shown in Fig. 2. The driving currents were 20 and 18 μA in the ITP and CZE channels, respectively.

solved organic anionic constituents should be expected as potential sources of (small) systematic errors when their co-migrations with the inorganic microconstituents are not prevented, e.g., by appropriately modifying the electrolyte system [39].

4. Conclusions

This feasibility study outlines promising analytical potentialities of the CC chip in the ITP and ITP–CZE separations and determination of some inorganic anions (chloride, nitrate, sulfate, nitrite, fluoride and phosphate) that need to be currently monitored in water samples. Although all methodological possibilities offered by the chip for the ITP separations [8] were not investigated in this work, our results imply that this CE method has limitations in the detection of nitrite, fluoride and phosphate on relevant concentration levels. On the other hand, these anions could be detected at sub-μmol/l concentrations by the conductivity detector in the CZE stage of the ITP–CZE combination performed on the same chip. A sample pretreatment performed in the ITP stage of the combination effectively complemented such a detection sensitivity and nitrite, fluoride and phosphate could be reproducibly quantified also in samples containing the macroconstituents at 10^4 higher concentrations. Here, it seems appropriate to state that our ITP–CZE experiments carried out in this context demonstrated (Fig. 7), so far, the largest macro-/microconstituent concentration ratio at which CE on the chip separated and quantified trace constituents. However, they also imply that, mainly, the use of a more sensitive detector can further enhance this ratio.

ITP–CZE analyses of tap, mineral and river water samples showed that the CC chip offers means for very rapid procedures in the determination of the microconstituents in water (280–350 s analysis times under our working conditions). Although the ITP sample pretreatment itself required 150–240 s to be accomplished it should be noted that this operation meant a removal of nanomol amounts of the macroconstituents from the separation compartment of the chip. In this context, we should stress minimum requirements linked with the sample preparation before the CE analysis. For example, tap water samples could be loaded onto the chip directly, while a short degassing of mineral water (to prevent bubble formation during the CE run) and a filtration of river water sample (to remove particulates and colloids) were the only pre-column operations to be performed with the samples.

In general, the determination of both macro- and microconstituents in one ITP–CZE run on the CC chip should be possible. However, it can be easily deduced that the electrolyte systems used for these purposes in conventional CC equipment [26] offer only limited possibilities in this respect. Therefore, a search for the electrolyte system providing the ITP resolutions of the macroconstituents in the first separation channel of the chip without sacrificing detectabilities of the microconstituents in the CZE stage seems essential to achieve such a goal.

From recent reviews dealing with the CE separations of inorganic anions [28,29] we can judge that the present ITP and ITP–CZE procedures on the CC chip are probably applicable also to other environmental matrices (e.g., soil, aerosols) or matrices of biological (e.g., body fluids, food products) origins.

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