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Application of a contactless conductometric detector for the simultaneous determination of small anions and cations by capillary electrophoresis with dual-opposite end injection

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Abstract

A contactless conductometric detection (CCD) system for capillary electrophoresis (CE) with a flexible detection cell was applied for the simultaneous determination of small anions and/or cations in rain, surface and drainage water samples. The applied frequency, the amplitude of the input signal, the electrolyte conductivity and electrode distance were found to be the most significant factors affecting the detection sensitivity. 2-(*N*-Morpholino)ethanesulfonic acid/histidine-based (MES/His) electrolytes were used for direct conductivity detection of anions and cations, while ammonium acetate was selected for indirect conductivity determination of alkylammonium salts. For the simultaneous separation procedure, involving dual-opposite end injection, an electrolyte consisting of 20 m*M* MES/His, 1.5 m*M* 18-crown-6 and 20 μ*M* cetyltrimethylammonium bromide provided baseline separation of 13 anions and cations in less than 6 min. The detection limits achieved were 7–30 μg/l for direct conductometric detection of various common inorganic cations and anions, excluding F^- (62 μg/l) and $H_2PO_4^-$ (250 μg/l), and 35–178 μg/l for indirect conductometric detection of alkyl ammonium cations. The developed electrophoretic method with conductometric detection was compared to ion chromatography.

Keywords: Contactless conductivity detection; Conductivity detection; Detection, electrophoresis; Inorganic anions; Inorganic cations

1. Introduction

Capillary electrophoresis (CE) has many attractive features, providing short analysis times and high separation efficiency, while requiring low sample volumes. Since its introduction by Mikkers et al. [1] and Jorgenson and Lukacs [2] CE has been used to analyze various species including small ions. Small highly charged ions are easily and rapidly separated in fairly simple electrolytic systems and they do not cause the significant problems that are often associated with larger molecules, such as adsorption, precipitation, and decomposition. However, the suitability of CE for small ions is restricted by their limited absorption in the UV–Vis region and the short optical path of the fused-silica capillary. In-

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direct detection in the visible range is commonly exploited in CE, but it is relatively insensitive and has a limited linear range [3–6]. There are several other approaches that can be used to enhance the sensitivity of detection, such as laser-induced fluorescence [7,8], electrochemical detection [9,10] or sample stacking [11,12], but conductometric detection is still a viable alternative for small ions, such as inorganic ions and small organic anions and cations.

The high electrophoretic mobility of small ions and the corresponding conductivities of the migrating analyte zones make this type of detection sensitive, regardless of the optical properties of the analytes. Even in the first, pioneering investigations of CE, the possibility of using modified conductometric detectors from isotachophoresis (ITP) systems [13] was explored [1,14]. These detectors required contact, i.e., the sensing electrodes were in contact with the separation electrolyte. However, their acceptance for routine use was limited, probably because of complications imposed by the small diameters (25–100 μm) of CE-separation capillaries, together with the fragility and difficulties of constructing such detectors [15–20], although their reported sensitivity was better than that of corresponding UV detectors [16,17].

Several other research groups focused on developing suppressed conductivity detection techniques for CE [21–24]. The possibility to suppress the background conductivity, thereby enhancing sensitivity, is an advantage of this approach. However, additional peak broadening might then occur. Since the introduction of conductometric detection for CE, more than 15 years elapsed before the first end-column contact conductometric detector was commercially released [25]. This device has a reasonably robust construction, but requires specially designed ConCap capillaries, which increases its costs. Furthermore, although very sensitive, contact between the electrodes and the electrolyte can be inherently disadvantageous due to the electrode polarization or redox reactions with the electrolyte that may occur, and the expensive instrumentation required.

Contactless conductometric detection (CCD) presents a more robust and easy to handle alternative to the contact methods. In this approach, the detector electrodes are not in direct contact with the electrolyte. A typical detector design comprises four electrodes placed towards the center of the separation capillary. This design is suitable for capillaries (PTFE or fused-silica) with an inner diameter not much less than 300 μm [26–29]. Such capillaries are not in common use in CE due to the large Joule heat formation associated with them. In the late 1990s Zemann and co-workers [30,31] and Fracassi da Silva and do Lago [32] published reports on contactless conductometric detectors with tubular electrodes, and several other authors have explored this type of detection system since then. The design of a CCD system remains virtually the same as in the cited publications [30–32] but with slight modifications of the detection cell and/or circuitry. For instance, Chvojka et al. constructed a dual photometric-contactless conductometric detector [33], Tůma et al. [34] showed that semi-tubular electrodes can be utilized instead of tubular ones and Kappes et al. [35] used this type of detector as an integral part of a multifunctional electrochemical detector in a portable CE instrument. Recently, Fracassi da Silva et al. [36] presented an improved, miniaturized version of their previous detector. The contactless conductometric detection mode is not restricted solely to capillary zone electrophoresis (CZE) and promising results have been obtained with micellar electrokinetic chromatography (MEKC) [37], anion-exchange capillary chromatography [38] and CE with nonaqueous solvents [39].

The potential of CE for the simultaneous determination of anionic and cationic compounds has been recognized. Anions and cations in a typical electrophoretic system, with one-end sample injection, can be separated in a single electrophoretic run provided their velocity vectors are in the same direction. This condition certainly applies for slow migrating molecules. It has been possible to separate anions and cations of large organic molecules and biomolecules in a reasonable time in this manner [40–42] when the migration velocities are low for both categories of ions. Based on the same principle, slowly migrating organic anions and inorganic cations (Li^+, K^+) could be separated in a system [43] with a high electroosmotic flow (EOF). Xiong and Li [44] used dual photometric detection for simultaneous determination of several inorganic ions.

The one-end injection approach is, however, not easily applicable for small, fast migrating analytes, such as inorganic anions and cations, because either anions or cations from the separated mixture will never reach the detector or they will arrive at the detector as a very broad peak after a long time. Therefore, several alternative approaches have been adopted for the simultaneous determination of small ions. Bächman et al. [45], for instance, have separated and determined some small chloro anions and inorganic cations simultaneously by using an indirect fluorescence detection technique. Their system employed a single, common electrolyte but two capillaries and two detectors, making their approach experimentally complicated.

Complexation of cationic species is another obvious choice when performing simultaneous separation of small ions. The cations are usually transformed into their negatively charged complexes and separated together with anions. This approach was applied by Krokhin et al. [46] for the separation of several anions and metal-4-(2-pyridylazo)-resorcinolato (PAR) complexes, while Soga and Ross [47] used direct complexation of metal cations with the electrolyte probe. 2,6-Pyridinedicarboxylic acid (PDCA) electrolyte allowed indirect UV detection of anions and also formed negatively charged complexes with cations, allowing their direct detection. Kobayashi et al. [48] tested complexation with EDTA and separation in a borate electrolyte for the analysis of anions and cations in mineral water, but the resolution of the separated peaks was rather poor. Kubáň et al. [49] exploited a similar principle using chromate electrolyte and EDTA-complexation, whereby separation of 19 anions and cations was achieved in about 6 min.

A recent publication by Kubáň and Karlberg [50] presented a new, very promising approach for the simultaneous determination of small ions. Simultaneous separation of 22 small ions within 5 min has been accomplished by injecting the sample from both capillary ends (dual-opposite end injection). Cations and anions migrate towards the capillary center and are detected approximately in the middle of the separation capillary (by indirect UV detection at 254 nm in the cited case). Independently, Padarauskas et al. [51] demonstrated the same principle for the separation of eight inorganic ions. Dual-opposite end injection techniques were later also applied by other workers [52–55]. Simultaneous determination of small ions has been reviewed by Padarauskas [56].

When using indirect UV detection to monitor the simultaneous separation of small anions and cations, the electrolyte composition reflects a compromise between suitability of the cationic/anionic background probes, their optical properties and pH [50,51]. Some cations cannot be detected since they tend to precipitate with the electrolyte counter-ions or at the high pH used, the choice of suitable UV-providing background probes is also rather limited and detection sensitivity may be compromised. Further, after the detection window is burned in the designated position in the center of the capillary, it is not easy to change its position without a risk of capillary breakage.

In contrast, the choice of electrolyte for simultaneous separation with conductivity detection using the dual-opposite end injection approach is relatively straightforward, since virtually the same electrolytes are commonly used for the analysis of anionic and cationic species. There is no need to consider optical properties, therefore even non-UV-absorbing probes can be used, and a combination of direct and indirect detection methods is also feasible.

In this paper we describe the applicability of the contactless detector for the determination of small cations (inorganic or organic) and inorganic anions. Its potential is also demonstrated for the simultaneous determination of inorganic anions and cations in rain, surface and drainage waters. The flexible detection cell we describe allows precise adjustment of the detection point (i.e., of the effective lengths from both ends of capillary), as needed for efficient separation merely by positioning the fused-silica capillary inside it. No burning of detection windows is required and the detection point can be repositioned in an instant.

Important factors influencing the detector's sensitivity, such as input voltage and frequency as well as the detection cell parameters, were studied in detail and optimized. The results achieved by the method developed for simultaneous determination of anions and cations with CCD were compared to those achieved by ion chromatography (IC).

2. Experimental

2.1. Chemicals, solutions and samples, procedures

All chemicals were of reagent grade and deionized water was used throughout. Inorganic cation stock solutions (10 g/l) were prepared from the corresponding chloride salts except for the manganese stock solution, which was prepared from its sulfate salt. Inorganic anion stock solutions (10 g/l) were prepared from the corresponding potassium or sodium salts. Organic alkyl ammonium cation stock solutions (10 g/l) were prepared from the following salts: tetramethylammonium iodide (TMA^+), benzylammonium chloride (BA^+) and tetrabutylammonium bromide (TBA^+). All multi-ion calibration solutions were then prepared from these stock solutions. The rain, surface and drainage water samples were collected in the locality of Bílý Kříž in the Beskydy Mountains (Czech Republic) and were deep frozen prior to analyses.

The electrolytes were prepared from stock solutions of 150 m*M* 2-(*N*-morpholino)ethanesulfonic acid (MES), 150 m*M* L-histidine (His), 25 m*M* 18-crown-6, 5 m*M* cetyltrimethylammonium bromide (CTAB), 50 m*M* ammonium acetate and 50 m*M* 4-aminopyridine. Table 1 provides a complete list of the electrolytes used with their respective conductivity values. When required, the pH of the solutions was adjusted with 100 m*M* sulfuric or hydrochloric acid. All electrolyte solutions were filtered and degassed prior to use.

2.2. The CE components

The CE part of the system was accommodated in a Plexiglass box, built in the laboratory, equipped with a safety lock on the access door for protection. It included a high-voltage (HV) supply unit (Series 320, Bertan Associates, Hicksville, NY, USA) that provided a potential of up to 25 kV during all the runs. Polyimide-coated capillaries (Polymicro Technologies, Phoenix, AZ, USA; 50 μm I.D.×375 μm O.D.) of various lengths (typically: total lengths 70 cm and 67 cm) were used. Samples were introduced manually, in a hydrodynamic mode, from a height of 10–30 cm for 10–30 s. The experiments were done at the constant laboratory temperature of 23 °C, measured inside the CE protective box with the fused-silica capillary. No special temperature control device was present.

The electronic components of the contactless conductometric detector (see Fig. 1) were assembled as described by Fracassi da Silva and do Lago [32]. A function generator (Model GFG 8219A, Goodwill Instruments Co. Ltd., Taiwan), FG, providing a sinusoidal input signal of varying amplitude (typically 2–20 Vpp, peak to peak) is connected by a shielded cable to the first connector (IN) on the detection cell, which is attached, in turn, to the first

Table 1
Electrolytes used for direct and indirect conductivity detection

	Electrolyte	pH	Conductivity (μS/cm)
Direct conductivity detection of cations	10 m*M* MES/His	6.1	239
	20 m*M* MES/His	6.1	462
	35 m*M* MES/His	6.1	769
	60 m*M* MES/His	6.1	1237
	20 m*M* MES/His, 1.5 m*M* 18-crown-6	6.0	461
Direct conductivity detection of anions	20 m*M* MES/His, 30 μ*M* CTAB	6.0	463
Simultaneous separation of anions and cations	20 m*M* MES/His, 10 μ*M* CTAB,1.5 m*M* 18-crown-6,	6.0	462
	20 m*M* MES/His, 20 μ*M* CTAB, 1.5 m*M* 18-crown-6	6.0	462
Indirect conductivity detection	2 m*M* ammonium acetate	6.8	226
	6 m*M* ammonium acetate	6.8	649
	10 m*M* ammonium acetate	6.9	1054

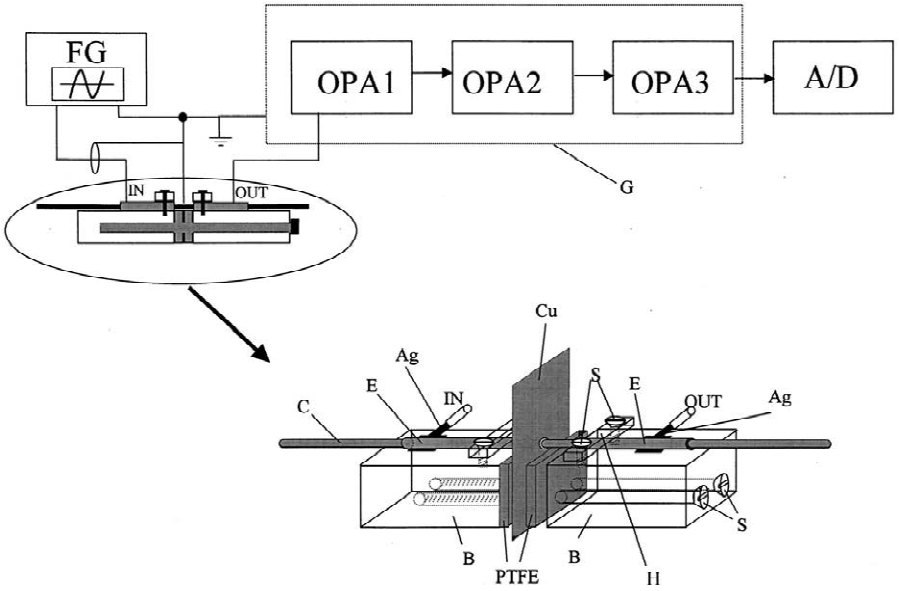

Fig. 1. Schematic diagram of the contactless conductometric detector with flexible detection cell. FG—Function generator, OPA1, OPA2, OPA3—current to voltage converter, rectifier and amplifier, respectively, A/D—analog to digital converter and PC, C—separation capillary, E—tubular electrode, Ag—silver paint layers, Cu—copper foil, PTFE—PTFE spacers, B—Plexiglas blocks, S—holding screws, H—holders, IN, OUT—connectors.

tubular electrode. The output signal from the second tubular electrode (OUT) is fed to the current-to-voltage converter (OPA1) with a 1 MΩ feedback resistor and the rectifier (OPA2). The resulting d.c. signal is amplified in the third unit (OPA3) and then registered by an A/D converter card (ELDSWin Pro 1.1 or Apex 1.7 laboratory data systems).

The flexible detection cell (see expanded view in Fig. 1) comprises two 20×20×25 mm Plexiglass blocks. The blocks enable precise adjustment of the detection gap between the two tubular electrodes, which are made from syringe cannulas (Terumo Europe, Belgium, 0.4 mm I.D.×0.6 mm O.D.). The electrode length was 20 mm. In addition, a 100-μm thick leaf of copper foil is sandwiched between the blocks. The capillary passes through a 400-μm hole drilled through the Cu foil.

The silver layer on the surface of the grooved blocks provides electric connection from the cell to the function generator and to the electronic part of the detector. The electrodes were fixed in the desired position by two Plexiglass holders, 20×5×3 mm in size, which in turn were attached to the main block by two small screws. This allowed the detection cell diameter to be easily varied, since the tubular electrodes could be replaced within a couple of minutes by other electrodes of different lengths or inner diameters, if needed. All electronic components were accommodated in the grounded metallic case (G) to avoid any disturbance, and to minimize the noise. The detection cell was placed on top of the case and the copper foil was grounded.

An ISCO CV^4 UV–visible detector (ISCO, Lincoln, NE, USA) was employed for indirect UV detection at 262 nm. Electropherograms were registered using an ELDSWin Pro 1.1 laboratory data system (Chromatography Data Systems, Kungshög, Sweden) on a personal computer.

2.3. The IC components

A Dionex DX-600 ion chromatographic system equipped with a GP50 gradient pump, an ED50 electrochemical detector, an EG40 eluent generator and an AS40 autosampler (all parts Dionex, Sunnyvale, CA, USA) was used in all IC experiments. Anions were separated on an AS11 HC (250×2 mm) analytical column with an AG11 HC (50×2 mm)

guard column, and eluted by a linear gradient developed using 1–45 m*M* KOH, while cations were separated on a CS15 (250×2 mm) analytical column with a CG15 (50×2 mm) guard column, the eluent being isocratic 5 m*M* sulfuric acid–9% acetonitrile.

3. Results and discussion

3.1. Choice of the operational amplifier and applied frequency

The detection cell can be represented by an equivalent circuit consisting of two cylindrical capacitors and one resistor. The dielectric of the capacitors is formed by the fused-silica wall of the capillary, the outer polyimide coating and the air gap between this coating and the inner surface of the tubular electrode.

The geometry of the cell for contactless conductometric detection in CE is different from the design of the typical cells for batch conductometric measurements. The response of the detector is a complex function of many factors, for instance the cell geometry or electronic circuitry used and will vary with the applied frequency. This is clearly demonstrated in Fig. 2A, where the dependence of the signal measured at the OPA1 on frequency is shown for three different electrolyte solutions. It can be seen that the optimum frequency, i.e., the frequency giving the strongest signal, was constant (290 kHz) for all three solutions, although they differed in conductivity. In fact, the optimal frequency is constant over a much wider range of conductivities of the electrolyte solutions, as verified by measurements of KCl solutions ranging in concentration from 0.0001 to 0.1 *M* (results not shown).

The intensity of the signal increases with increases in the electrolyte concentration in accordance with Ohm's law. In CE, signal differences rather than the absolute values of the signals determine the sensitivity of the measurements, therefore for estimating sensitivity the graph in Fig. 2B is more relevant than that in Fig. 2A. When the difference in the signal resulting from a constant molar increase in the electrolyte concentration, e.g., from 10 to 15, 30 to 35 or 60 to 65 m*M*, is considered, the relationship between increases in resistance and the strength of

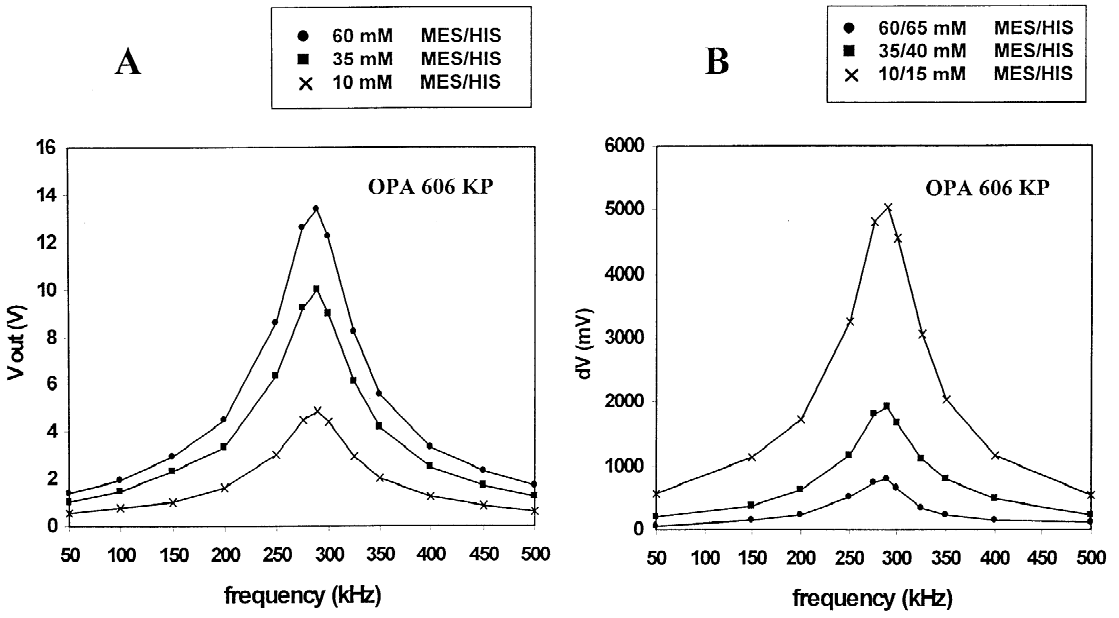

Fig. 2. Dependence of the maximum output signal on the concentration of the electrolyte solution for OPA606KP. (A) a.c. voltage measured at OPA1, (B) difference in the signal between two electrolyte solutions measured at OPA3. Conditions: V_{in}=20 Vpp, electrodes 2 cm, detection gap, 1.1 mm.

the signal is reversed, as shown in Fig. 2B. The optimized frequency of 290 kHz is regarded as a factor that is orthogonal to all other settings of the detector, implying that it can be optimized separately, as done here.

3.2. Analysis of factors influencing the sensitivity

The sensitivity of the detection provided by the contactless conductometric detector is not merely a function of the applied frequency. Other factors such as the applied input voltage and the conductivity of the separation electrolyte are also important. To investigate the effect of all these factors, an approach combining experimental design and chemometrics was adopted since it provides an efficient way of obtaining information about the influence, covariance and significance of the various factors.

Sensitivity was chosen as a response function, expressed in terms of output voltage at OPA1, the difference in the output voltage for two different concentrations of the electrolyte, the peak height and areas of the analytes separated in CE and, finally, the signal-to-noise ratio (S/N). Two variables, electrolyte concentration and the input voltage were varied in the ranges 10–60 m*M* MES/His and 5–20 Vpp, respectively, for direct conductivity detection and in the ranges 2–10 m*M* ammonium acetate and

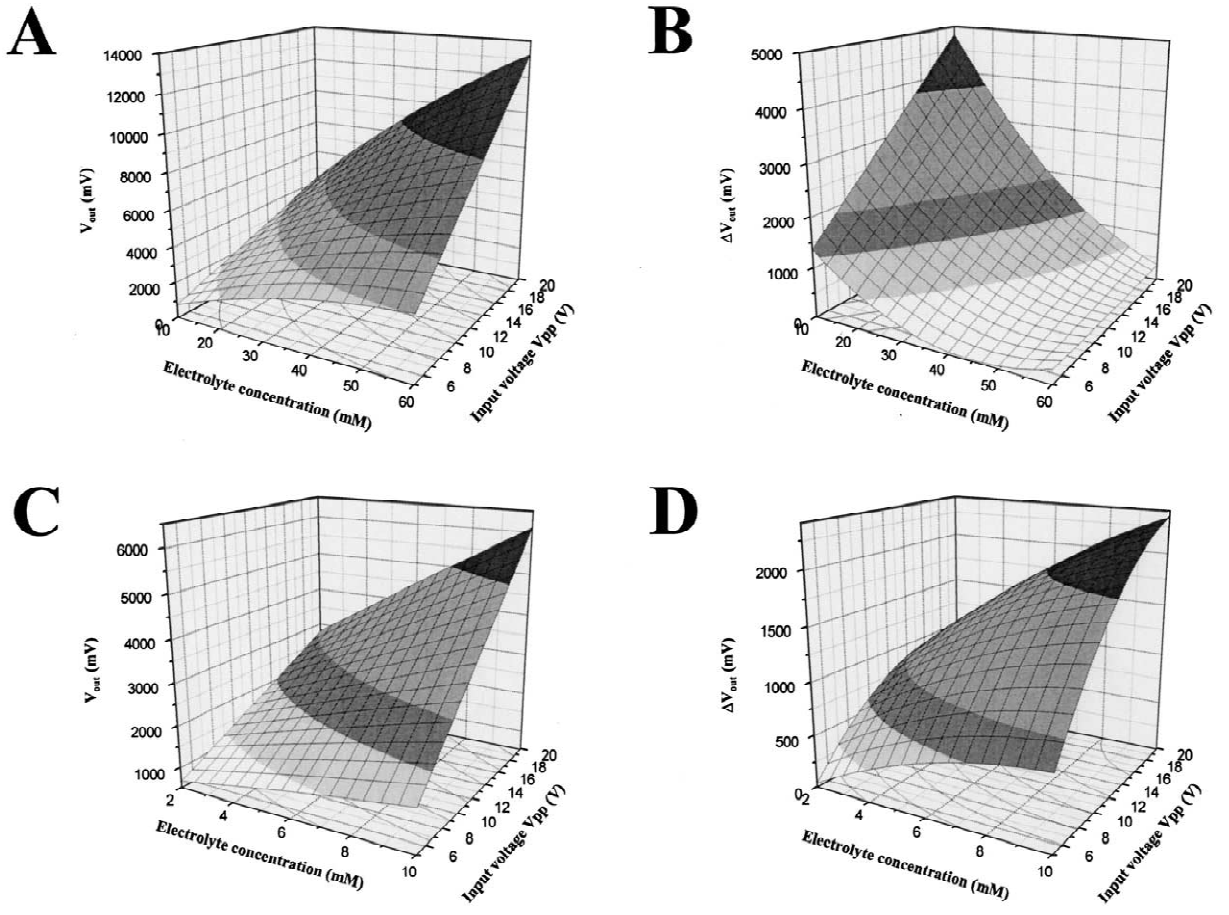

Fig. 3. Response surface plots (RSPs) for optimization of the detector response. A, C—RSPs for amplitude of the voltage measured at OPA1; B, D—RSPs for differences in the signal between two electrolyte solutions measured at OPA3. A, B—direct conductivity detection; C, D—indirect conductivity detection. Detector parameters: OPA 606 KP, f=290 kHz, electrodes 2 cm, detection gap 1.1 mm. Conditions: variable V_{in}=5–20 Vpp, electrolytes as in Fig. 2.

5–20 Vpp, respectively, for the indirect conductivity detection.

For direct conductometric detection, a mixture of six inorganic cations, each at 1 mg/l, and for indirect conductometric detection a mixture of three alkylammonium salts, each at 25 mg/l, were injected into the CE system for 10 s from an elevation of 300 mm. In total, 11 experiments with three center points were performed and the data were analyzed using the multilinear regression (MLR) algorithms of the Modde 4.0 program. In Fig. 3, some of the resulting response surfaces are depicted for direct (A, B) and indirect (C, D) conductivity measurements.

Graph A depicts the response surface of the output voltage measured directly at the OPA1. As explained previously, the signal intensity increases with an increase in the electrolyte concentration or in the input voltage, Vpp. However, in direct conductivity detection the response to changes in the electrolyte concentration was reversed, as shown in graph B. In this case, the response was the absolute value of the difference in the signal resulting from adding a constant molar increment (e.g., 5 m*M*) of the electrolyte, corresponding to a conductivity increase of approximately 100 μS, to the original electrolyte solutions. The difference in the signal resulting from an incremental increase in the electrolyte concentration, e.g., from 10 to 15, 30 to 35, or 60 to 65 m*M*, was measured. In direct conductivity detection, the signal of the analyte is measured relative to the low conductivity signal of the background electrolyte. Therefore, use of an electrolyte with low background conductivity improves the response of the detector to the equimolar addition of the measured compound.

In indirect conductivity detection (graph C), the response surface of the output voltage measured directly at the OPA1 is similar to that obtained in direct conductivity detection (graph A). Graph D shows the difference in the signal resulting from an incremental decrease in the electrolyte concentration, e.g., from 2 to 1, 6 to 5 or 10 to 9 m*M* ammonium acetate electrolyte solution. In each of these cases, the conductivity decreases by approximately 100 μS but, as shown in graph D, the signal intensity is reduced going from 2 to 1, 6 to 5 or 10 to 9 m*M* ammonium acetate electrolyte solutions. The reversed response of the signal (measured at OPA3) can be explained by the differences in the mechanism of the two detection modes. In indirect conductivity detection, a decrease in the signal intensity of the highly conductive background electrolyte is observed for analytes with low conductivity. In such cases, the sensitivity is improved by increasing the conductivity of the electrolyte.

The results illustrated in graphs B and D were confirmed by CE analysis of the cationic analyte mixtures. The response functions evaluated here were the peak height, peak area and S/N of the peaks in the electropherograms. Similar surface plots to those shown in graphs B and D were obtained for all three response functions for direct and indirect

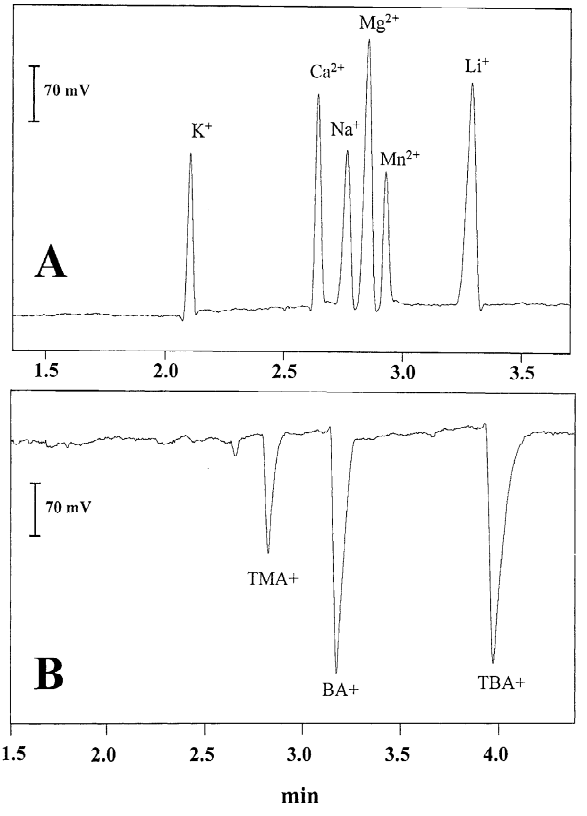

Fig. 4. Electropherogram of the separation of model mixtures for direct and indirect conductometric detection. Conditions: (A) CE conditions: electrolyte 20 m*M* MES/His, pH 6.1, T=23 °C, HV= 15 kV, hydrodynamic injection with capillary end elevated to a height of 30 cm for 10 s, effective capillary length 40 cm (total length 70 cm), analyte concentration, 1 mg/l. (B) Electrolyte 6 m*M* ammonium acetate, pH 6.8, other conditions as in (A) except analyte concentration, 25 mg/l.

conductivity detection, respectively. A slight plateau appears at ammonium acetate concentrations greater than 7 m*M* with indirect conductometric detection, implying that no further increase in sensitivity can be obtained at concentrations greater than 7–8 m*M* (results not shown). This might be due to the increase in signal noise generated with high concentrations of the electrolytes.

Two conclusions can be drawn from these results. Firstly, for an optimum frequency (in this case 290 kHz), given by the detection cell geometry and electronic parameters of the detector, the highest sensitivity is obtained at the highest possible input voltage (20 Vpp in this case). Secondly, the conductivity of the electrolyte is another significant factor contributing to the sensitivity of detection. Consequently, proper selection of the electrolyte and its conductivity should be made with respect to the detection mode used. For direct conductivity measurements, electrolytes of low conductivity should be preferred, while for indirect conductivity detection, high conductivity of the background electrolyte is advantageous. It should be mentioned that the peak height or area in CE is not a straightforward function of the electrolyte conductivity, because other factors such as electrolyte ionic strength, peak broadening, interactions between the analyte and electrolyte etc. have to be taken into consideration. However, the two detection modes have distinctly differing trends in their responses, as shown in Fig. 3 (graphs B and D). Electropherograms of model mixtures of the cations used to evaluate the detector's sensitivity for direct and indirect conductometric detection are shown in Fig. 4.

3.3. Effects of electrode and detection gap length on separation efficiency

Varying the electrode length from 0.5 to 3 cm had no significant effects on the separation efficiency, signal intensity or appearance of the electropherograms (data not shown). Short electrodes may be advantageous when separation in short capillaries is performed, because the total length of the detection cell can then be reduced to 0.5–1.5 cm. However, in our experiments electrodes 20 mm long were used, due to their ease of manipulation.

In contrast, the size of the detection gap had a significant effect on the separation efficiency. The detection gap was varied from 0.6 mm to 5.1 mm by varying the thickness of the PTFE spacers. Fig. 5 shows a plot of theoretical plate number, *N*, vs. the

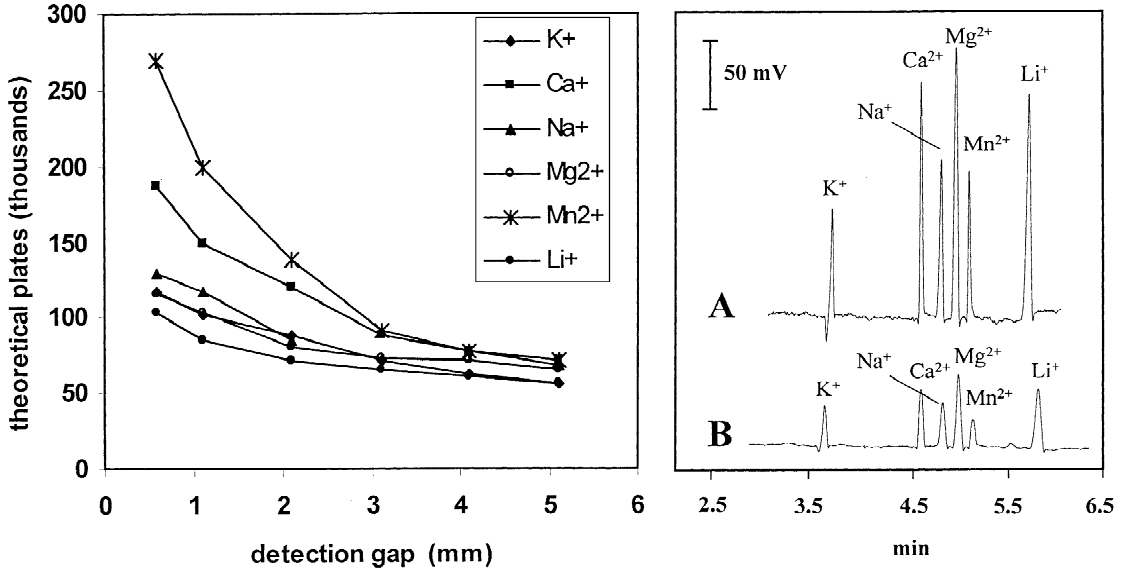

Fig. 5. Influence of detection gap width on the number of theoretical plates for several inorganic cations. A—Gap, 0.6 mm, B—gap, 5.1 mm. Detector parameters and CE conditions as in Fig. 4A. Variable detection gap: 0.6—5.1 mm.

width of the detection gap. An increase from about 60 000 theoretical plates to more than 100–250 000 is observed, when changing the detection gap from 5.1 to 0.6 mm. A corresponding increase in peak heights is also observed, as depicted on the right side of Fig. 5. This figure shows two electropherograms obtained for a mixture of six cations, each at a concentration of 1 mg/l, with different widths of detection gap. Note the slight increase in noise at a gap of 0.6 mm. To maximize resolution and sensitivity, the detection gap should be minimized. However, in all subsequent experiments a detection gap of 1.1 mm was used since the shortest possible length, 0.6 mm, yielded an increase in the baseline noise.

3.4. Separation of inorganic anions and cations, simultaneous separations

An electropherogram showing the separation of seven inorganic cations using a 20 m*M* MES/His electrolyte is presented in Fig. 6A. The separation of NH_4^+ and K^+ is achieved after addition of 1.5 m*M* 18-crown-6. Addition of 18-crown-6 does not significantly increase the background conductivity of the electrolyte, and thus does not compromise detection sensitivity. In Fig. 6B the analysis of a drainage water sample is shown, while Fig. 7 shows the separation of six inorganic anions and an analysis of tap water. The electrolyte contains the same concentration of background probes and 30 μ*M* CTAB for EOF reversal. Prior to each anion analysis, the separation capillary was rinsed with 1 *M* HCl for 20 s, followed by deionized water for 60 s, 40 μ*M* CTAB for 40 s and electrolyte for 180 s. The similarity in composition of the running electrolytes for both groups of ions suggests that a simple, dual-opposite end injection [50] of the samples might provide a suitable means for the simultaneous separation of anions and cations. Indeed, this is easily feasible. The concentration of CTAB was optimized and 10–20 μ*M* was found to be the most suitable concentration to provide slight reversal of the EOF. The separation length for the cationic analytes was shortened from 40 cm to 21 cm to compensate for the decrease in their migration velocities due to the EOF reversal. Sample was first injected by gravity from one capillary end, followed by the injection of

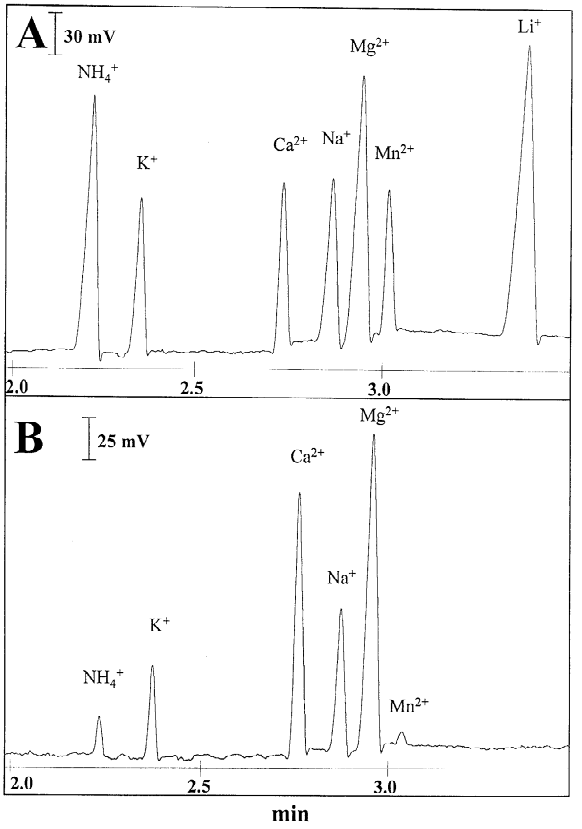

Fig. 6. (A) Determination of inorganic cations in standard solution containing 1 mg/l of each cation. (B) Determination of inorganic cations in drainage water sample. CE conditions: electrolyte; 20 m*M* MES/His+1.5 m*M* 18-crown-6, pH 6.0, *T*=23 °C, HV=20 kV, hydrodynamic injection with capillary end elevated to a height of 30 cm for 20 s, effective capillary length 40 cm (total length 67 cm).

an electrolyte plug. When the second injection of sample is made into the opposite capillary end, only the electrolyte plug is expelled. As a result, the two injected sample portions are maintained inside the capillary and the high voltage can be applied. A resulting electropherogram is depicted in Fig. 8A. In this case no need for a delay between the two injections was needed [50]. However, in more complicated separations this would be a further option to optimize the selectivity. For instance, when two additional ions, F^- and $H_2PO_4^-$, were to be determined in the same time frame, a delay of 35 s between the injections from the anionic and cationic ends produced the electropherogram shown in Fig.

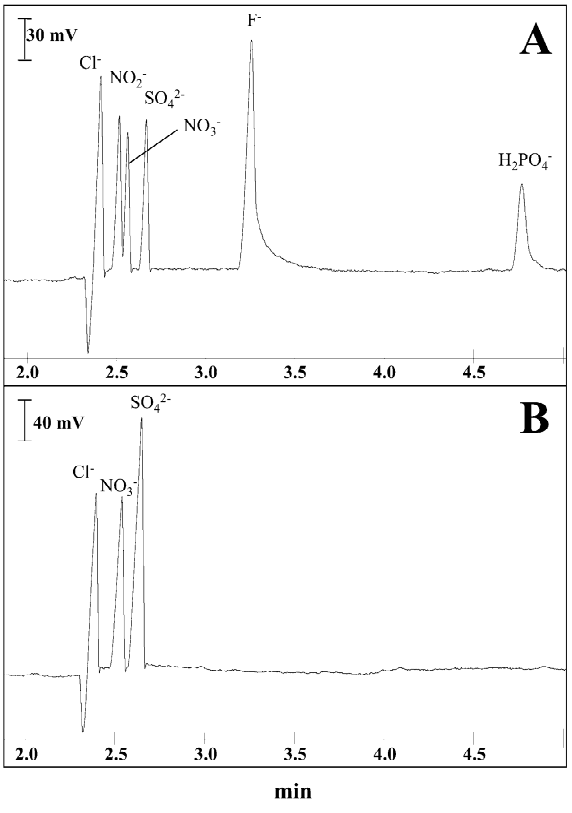

Fig. 7. (A) Determination of inorganic anions in standard solution containing 1 mg/l of each anion except for F^- (2 mg/l) and $H_2PO_4^-$ (3 mg/l). (B) Determination of anions in tap water sample (dilution 1:10). CE conditions: electrolyte 20 m*M* MES/His+30 μ*M* CTAB, pH 6.0, *T*=23 °C, HV=−20 kV, hydrodynamic injection with capillary end elevated to a height of 20 cm for 20 s, effective capillary length 35 cm (total length 67 cm).

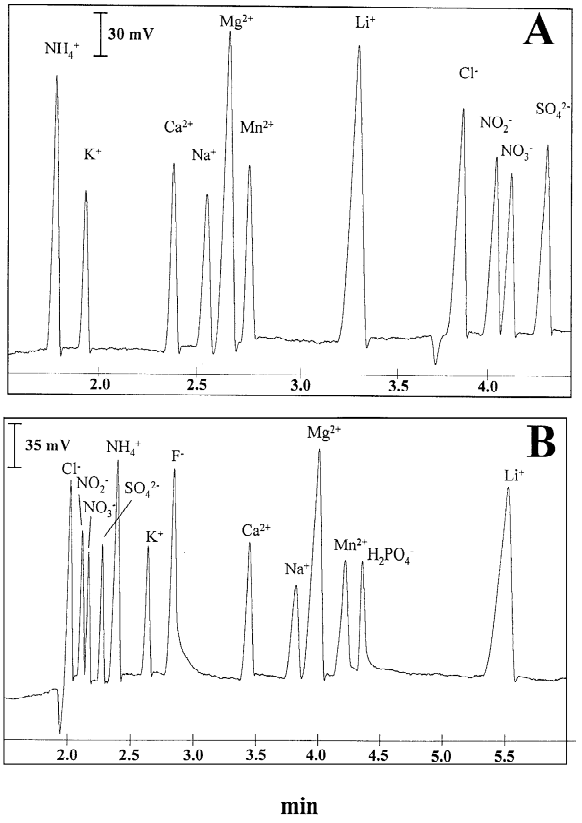

Fig. 8. (A) Simultaneous determination of 11 inorganic cations and anions in standard solution containing 1 mg/l of each ion. CE conditions: electrolyte: 20 m*M* MES/His+1.5 m*M* 18-crown-6+10 μ*M* CTAB, pH 6.0, *T*=23 °C, HV=−20 kV, hydrodynamic injection with capillary ends elevated to a height of 30 cm for 30 s (cathodic end) and to 10 cm for 30 s (anodic end), effective capillary length (anions) 46 cm and (cations) 21 cm (total length 67 cm). (B) Simultaneous determination of 13 inorganic cations and anions using timely displaced injection in standard solution containing 1 mg/l of each ion (except for F^- 2 mg/l and $H_2PO_4^-$ 3 mg/l). CE conditions: electrolyte: 20 m*M* MES/His+1.5 m*M* 18-crown-6+20 μ*M* CTAB, pH 6.0, *T*=23 °C, HV=−22.5 kV, hydrodynamic injection: capillary ends elevated to a height of 30 cm for 20 s (cathodic end) and to 20 cm for 30 s (anodic end), effective capillary length (anions) 41 cm and (cations) 26 cm (total length 67 cm). Time between injections, 35 s.

8B, where up to 13 ions were determined simultaneously in ca. 5.5 min. In this case no introduction of the electrolyte plug was necessary. The same rinsing procedure as for analysis of anions was needed for the simultaneous separation. In Table 2 limits of detection (LODs) (three times signal-to-noise ratio) for the separate analysis of cations and anions and their simultaneous analysis are compared. The detection limits in the former case range from 7 to 25 μg/l for most of the ions (except for F^- (125 μg/l) and $H_2PO_4^-$ (250 μg/l)), and for simultaneous determination the LODs range from 10 to 30 μg/l (F^- (62 μg/l) and $H_2PO_4^-$ (250 μg/l)). Thus, there is a slight increase (a factor of 0.5–2.0) in the LODs for both anions and cations compared to the separate analysis, but the increase is not particularly significant. The detection limits achieved for the simultaneous separation of anions and cations were better by a factor of 10 than corresponding LODs using dual-opposite end injection and indirect UV detection [57], making the CCD detection system very attractive. The calibration was linear from 50 μg/l to 5

Table 2
Comparison of limits of detection (LODs, μg/l) and calibration linearity for separate and simultaneous determination of anions and cations by CE and IC

	LOD, CE		LOD, IC	r^2, CE[d]		r^2, IC[e]
	a	b	c	a	b	c
Ammonium	10	12	16	0.9986	0.9991	0.9993
Potassium	13	20	35	0.9994	0.9992	0.9991
Sodium	11	20	18	0.9990	0.9992	0.9995
Calcium	15	25	8	0.9996	0.9995	0.9993
Magnesium	8	10	10	1.0000	0.9988	0.9999
Manganese	25	30	–	1.0000	0.9992	–
Lithium	7	10	2	1.0000	0.9993	1.0000
Chloride	7	12	3	0.9999	0.9986	0.9994
Nitrite	10	20	7	0.9993	0.9996	0.9999
Nitrate	10	20	10	0.9995	0.9999	0.9997
Sulfate	14	20	6	0.9990	0.9997	0.9998
Fluoride	125	62	3	0.9999	0.9990	0.9990
Phosphate	250	250	15	0.9990	0.9993	0.9999

[a] Determined for the analysis of separate group of anions and cations, gravity injection 30 cm/20 s.
[b] Determined for the simultaneous analysis of anions and cations, gravity injection: anions 30 cm/30 s, cations 10 cm/30 s.
[c] Determined for the analysis of separate group of anions and cations, injection volume 25 μl.
[d] Calibration based on five points, in the range 50 μg/l to 5 mg/l, except for fluoride and phosphate (500 μg/l to 15 mg/l).
[e] Calibration based on five points, in the range 50 μg/l to 10 mg/l.

mg/l. The detection limits for indirect conductometric detection of alkylammonium salts ranged from 35 to 178 μg/l. The data for inorganic anions and cations were compared to results obtained with the ion chromatographic method (see Table 2).

3.5. Parameters of the method

In Table 3, the relative standard deviations (RSDs, %, $n=8$) for migration times and peak areas for anions and cations are shown. Due to the manual

Table 3
Relative standard deviations of migration time [RSD(t_M), %] and peak area [RSD(PA), %] for separate and simultaneous determination of anions and cations by CE and IC, $n=8$

	RSD(t_M), CE		RSD(PA), CE		RSD(t_R), IC	RSD(PA), IC
	a	b	a	b	c	c
Ammonium	0.77 (0.17)	0.87 (0.59)	2.9 (2.3)	5.3 (1.2)	0.42	2.1
Potassium	0.86 (0.13)	0.89 (0.52)	3.9 (1.3)	6.1 (3.2)	0.25	3.5
Sodium	0.85 (0.09)	0.99 (0.37)	4.2 (3.7)	7.1 (2.2)	0.28	1.9
Calcium	0.83 (0.07)	0.93 (0.27)	4.0 (2.2)	5.0 (3.2)	0.29	1.3
Magnesium	0.86 (0.05)	0.98 (0.24)	4.0 (4.0)	4.3 (1.5)	0.25	1.3
Manganese	0.83 (0.06)	0.99 (0.29)	5.1 (4.7)	6.8 (4.1)	–	–
Lithium	0.86 (I.S.)	1.11 (I.S.)	3.4 (I.S.)	4.2 (I.S.)	0.57	1.2
Chloride	0.81 (0.14)	0.36 (0.12)	7.7 (3.4)	8.1 (4.7)	0.27	1.1
Nitrite	0.85 (I.S.)	0.35 (I.S.)	5.1 (I.S.)	3.0 (I.S.)	0.08	0.9
Nitrate	0.84 (0.03)	0.35 (0.02)	4.6 (1.2)	2.9 (1.3)	0.04	0.9
Sulfate	0.79 (0.15)	0.37 (0.04)	4.7 (2.3)	2.7 (1.6)	0.06	0.5
Fluoride	0.99 (0.25)	1.10 (0.81)	6.3 (5.8)	4.6 (1.9)	0.43	1.3
Phosphate	1.40 (0.88)	1.01 (0.89)	8.6 (6.6)	5.0 (2.7)	0.05	0.9

The values in the parentheses are the values obtained after correction by internal standard (I.S.).
[a] Determined for the analysis of separate group of anions and cations, gravity injection 30 cm/20 s.
[b] Determined for the simultaneous analysis of both anions and cations, gravity injection: anions 30 cm/30 s, cations 10 cm/30 s.
[c] Determined for the analysis of separate group of anions and cations, injection volume 25 μl.

gravity injection, the RSDs are rather poor. Correction with internal standards (Li^+ for cations, NO_2^- for anions) significantly improves the values and the migration time reproducibility falls within 0.9%, while the peak area RSDs decrease to less than 6.6%, which is acceptable for this injection method. The data were compared to results obtained using an ion chromatographic method. Ion chromatography provides better reproducibility, but the LODs and calibration linearity of the two techniques are comparable. Further automation of the entire injection procedure might significantly improve the reproducibility of CE analysis, thus making the method a suitable substitute for the more expensive, elaborate and time-consuming ion chromatography. Total ionic analysis of the various samples can be achieved with CE in less than 6 min, while two separate ion chromatographic systems are needed for obtaining the same data by IC. Furthermore, IC requires approximately 42 min (20 and 22 min for the two separations), so CE gives a sevenfold reduction in analysis time.

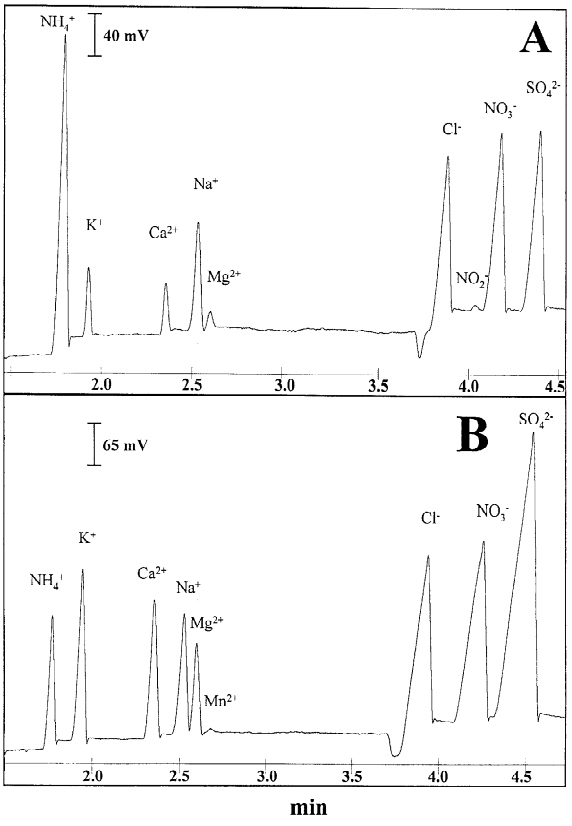

Fig. 9. Analysis of rain water sample (A) and surface water sample (B). CE conditions as in Fig. 8A.

3.6. Analysis of real samples

The method for simultaneous determination of anions and cations was applied to various water samples. In Fig. 9 the simultaneous determination of anions and cations in rain and surface water samples is shown. The total ionic analysis of the samples took only about 4.5 min. The quantitative results obtained, with reference to a calibration curve, were compared with data derived from the ion chromatographic methods and are summarized in Table 4. Excellent agreement between the methods was obtained, demonstrating the suitability of the simultaneous electrophoretic analysis of small anions and cations.

4. Conclusions

It has been shown that contactless conductometric detection is a powerful method, which can be easily used with capillary electrophoresis. The flexible design of the detection cell enables rapid and versatile modification of the cell parameters, according to the analytical requirements. The replacement of the capillary takes only a few seconds, with no need for burning detection windows or any other capillary treatment. Such flexibility is especially important for method development and optimization, when changing the position of the detection window is required, for instance in dual-opposite end injection approaches for the simultaneous separation of small anions and cations.

CCD not only provides a flexible solution for method optimization, but the choice and selection of the electrolytes for simultaneous separations is easier than with UV-absorption detection, and detection sensitivity is better by a factor of 10. The method developed for simultaneous total ionic analysis proved to be fast, sensitive and reliable, as well as giving comparable results to well-established ion chromatographic methods. Further improvements in injection reproducibility are however needed. This

Table 4
Quantitative analysis of real samples (mg/l)

	NH_4^+	K^+	Ca^{2+}	Na^+	Mg^{2+}	Mn^{2+}	Li^+	Cl^-	NO_2^-	NO_3^-	SO_4^{2-}
Surface water											
CE	1.10	4.75	2.65	2.45	0.75	0.25	ND	3.80	ND	8.15	11.10
IC	1.02	4.60	2.81	2.51	0.78		ND	3.71	0.02	8.01	10.90
CE simultaneous	1.25	4.45	2.60	2.35	0.55	0.30	ND	3.60	0.03	7.90	10.70
Rain water											
CE	1.20	0.45	0.30	0.65	0.10	ND	ND	1.15	ND	2.35	1.90
IC	1.09	0.38	0.37	0.58	0.15		ND	1.02	0.005	2.47	1.95
CE simultaneous	1.30	0.35	0.40	0.55	0.10	ND	ND	1.10	ND	2.40	1.85
Drainage water 1											
CE	ND	0.05	0.90	0.55	1.10	0.20	ND	0.65	ND	0.10	5.75
IC	ND	0.05	0.99	0.49	1.18		ND	0.68	ND	0.08	5.99
CE simultaneous	ND	0.05	0.90	0.40	0.90	0.20	ND	0.65	ND	0.10	5.75
Drainage water 2											
CE	ND	0.10	1.25	0.40	1.35	0.15	ND	0.50	ND	0.05	9.30
IC	ND	0.12	1.11	0.40	1.28		ND	0.48	ND	0.06	9.44
CE simultaneous	ND	0.10	1.05	0.35	1.20	0.15	ND	0.40	ND	0.05	9.20
Drainage water 3											
CE	0.02	0.35	1.20	0.50	0.80	0.20	ND	0.45	ND	1.05	6.10
IC	0.02	0.30	1.26	0.43	0.88		ND	0.47	ND	1.18	6.27
CE simultaneous	0.03	0.30	1.15	0.40	0.60	0.20	ND	0.40	ND	1.00	6.10
Lysimetric water											
CE	ND	3.15	3.25	0.50	0.45	0.25	ND	1.45	ND	1.95	6.90
IC	ND	3.36	3.28	0.59	0.47		ND	1.38	ND	1.83	7.08
CE simultaneous	ND	3.15	3.30	0.50	0.30	0.30	ND	1.40	ND	1.75	6.85

Each value is the mean value of three measurements, RSD ($n=3$)≤11%.

and progress in developing of alternative separation electrolytes for total ionic analysis with CCD will be reported in a subsequent publication.

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References

[1] F.E.P. Mikkers, F.M. Everaerts, T.M.E.P. Verheggen, J. Chromatogr. 169 (1979) 1.
[2] J.W. Jorgenson, K.D. Lukacs, Anal. Chem. 53 (1981) 1298.
[3] P. Jandik, W.R. Jones, J. Chromatogr. 546 (1991) 431.
[4] W.R. Jones, P. Jandik, J. Chromatogr. 546 (1991) 445.
[5] W. Beck, H. Engelhardt, Chromatographia 33 (1992) 313.
[6] W. Beck, H. Engelhardt, Fresenius J. Anal. Chem. 346 (1993) 618.
[7] L. Gross, E.S. Yeung, J. Chromatogr. 480 (1989) 169.
[8] L. Gross, E.S. Yeung, Anal. Chem. 62 (1990) 427.
[9] W. Lu, R. Cassidy, A. Baranski, J. Chromatogr. 640 (1993) 433.
[10] J. Wen, R.M. Cassidy, Anal. Chem. 68 (1996) 1047.
[11] R.L. Chien, J.C. Helmer, Anal. Chem. 63 (1991) 1354.
[12] D.S. Burgi, R.L. Chien, Anal. Chem. 63 (1991) 2042.
[13] F.M. Everaerts, T.P.E.M. Verheggern, J. Chromatogr. 73 (1972) 193.

[14] F.E.P. Mikkers, F.M. Everaerts, T.P.E.M. Verheggen, J. Chromatogr. 169 (1979) 11.
[15] X. Huang, T.K.J. Pang, M.J. Gordon, R.N. Zare, Anal. Chem. 59 (1987) 2747.
[16] X. Huang, R.N. Zare, S. Sloss, A.G. Ewing, Anal. Chem. 63 (1991) 189.
[17] X. Huang, R.N. Zare, Anal. Chem. 63 (1991) 2193.
[18] H. Zhao, R. Dadoo, R.J. Reay, T.A. Kovacs, R.N. Zare, J. Chromatogr. A 813 (1998) 205.
[19] S. Kar, P.K. Dasgupta, H. Liu, H. Hwang, Anal. Chem. 66 (1994) 2537.
[20] D. Muller, I. Jelínek, F. Opekar, K. Štulík, Electroanalysis 8 (1996) 722.
[21] P.K. Dasgupta, L. Bao, Anal. Chem. 65 (1993) 1003.
[22] P.K. Dasgupta, S. Kar, Anal. Chem. 67 (1995) 3853.
[23] N. Avdalovic, C.A. Pohl, R.D. Rocklin, J.R. Stillian, Anal. Chem. 65 (1993) 1470.
[24] M. Harrold, J. Stillian, L. Bao, R. Rocklin, N. Avdalovic, J. Chromatogr. A 717 (1995) 371.
[25] C. Haber, W.R. Jones, J. Soglia, M.A. Surve, M. McGlynn, A. Caplan, J.R. Reineck, C. Krstanovic, J. Cap. Electrophoresis 3 (1996) 1.
[26] B. Gaš, M. Demjanenko, J. Vacík, J. Chromatogr. 192 (1980) 253.
[27] J. Vacík, J. Zuska, I. Muselasová, J. Chromatogr. 320 (1985) 233.
[28] D. Kianianský, V. Zelenská, M. Masár, F. Ivanyi, S. Gazdíková, J. Chromatogr. A 844 (1999) 349.
[29] D. Kianianský, M. Masár, J. Marak, R. Bodor, J. Chromatogr. A 834 (1999) 133.
[30] A. Zemann, E. Schnell, D. Volgger, G.K. Bonn, Anal. Chem. 70 (1998) 563.
[31] K. Mayrhofer, A.J. Zemann, E. Schnell, G.K. Bonn, Anal. Chem. 71 (1999) 3828.
[32] J.A. Fracassi da Silva, C.L. do Lago, Anal. Chem. 70 (1999) 4339.
[33] T. Chvojka, I. Jelínek, F. Opekar, K. Štulík, Anal. Chim. Acta 433 (2001) 13.
[34] P. Tůma, F. Opekar, I. Jelínek, Electroanalysis 13 (No. 12) (2001) 989.
[35] T. Kappes, B. Galliker, M.A. Schwarz, P.C. Hauser, Trends Anal. Chem. 20 (2001) 133.
[36] J.A. Fracassi da Silva, N. Guzman, C.L. do Lago, J. Chromatogr. A 942 (2001) 249.
[37] J.A. Fracassi da Silva, C.L. do Lago, Electrophoresis 21 (2000) 1405.
[38] E.F. Hilder, A.J. Zemann, M. Macka, P.R. Haddad, Electrophoresis 22 (2001) 1273.
[39] J. Muzikár, T. van de Goer, B. Gaš, E. Kenndler, J. Chromatogr. A 924 (2001) 147.
[40] S.A. Shamsi, N.D. Danielson, Anal. Chem. 67 (1995) 4210.
[41] P.A. Gallagher, N.D. Danielson, J. Chromatogr A 781 (1997) 533.
[42] P.A. Gallagher, C.M. Oertel, N.D. Danielson, J. Chromatogr. A 817 (1998) 31.
[43] F. Foret, S. Fanali, L. Ossicini, P. Boèek, J. Chromatogr. 470 (1989) 299.
[44] X. Xiong, S.F.Y. Li, Electrophoresis 19 (1998) 2243.
[45] P. Bachmann, I. Haumann, T. Groh, Fresenius J. Anal. Chem. 343 (1992) 901.
[46] O.V. Krokhin, H. Hoshino, O.A. Shpigun, T. Yotsuyanagi, J. Chromatogr. A 776 (1997) 329.
[47] T. Soga, G.A. Ross, J. Chromatogr. A 834 (1999) 65.
[48] J. Kobayashi, M. Shirao, H. Nakazawa, J. Liq. Chromatogr. 21 (1998) 1445.
[49] P. Kubáň, P. Kubáň, V. Kubáň, J. Chromatogr. A 836 (1999) 75.
[50] P. Kubáň, B. Karlberg, Anal. Chem. 70 (1998) 360.
[51] A. Padarauskas, V. Olsauskaite, G. Schwedt, J. Chromatogr. A 800 (1998) 369.
[52] A. Padarauskas, V. Olsauskaite, V. Paliulionyte, J. Chromatogr. A 829 (1998) 359.
[53] A. Padarauskas, V. Paliulionyte, B. Pranaityte, Anal. Chem. 73 (2001) 267.
[54] D. Durkin, J.P. Foley, Electrophoresis 21 (2000) 1997.
[55] V. Unterholzner, A. Zemann, E. Schnell, P.R. Haddad, presented at the Ion Chromatography Symposium 2000, Nice, September 2000, poster 94.
[56] A. Padarauskas, Rev. Anal. Chem. 20 (2001) 271.
[57] P. Kubáň, unpublished results (1998).