

CONDUCTIVITY DETECTION CELL FOR CAPILLARY ZONE
ELECTROPHORESIS WITH A SOLUTION MEDIATED
CONTACT OF THE SEPARATED CONSTITUENTS WITH
DETECTION ELECTRODES

RÓBERT BODOR, DUŠAN KANIANSKY, MARIÁN MASÁR

DEPARTMENT OF ANALYTICAL CHEMISTRY, FACULTY OF NATURAL SCIENCES, COMENIUS UNIVERSITY,

MLYNSKÁ DOLINA CH-2, SK-842 15 BRATISLAVA, SLOVAK REPUBLIC



INTRODUCTION AND AIMS OF THE WORK

Providing approximately 10-fold higher sensitivity than indirect UV detection, the conductivity detection can be considered as a general alternative to this, currently preferred, technique in the detection of ions that lack suitable chromophores.

From research linked with the developments of contact conductivity detectors for ITP it is known that reactions of some electrochemically active analytes on the detection electrodes and/or accompanying changes of the electrode surfaces may impair performances of the contact conductivity detectors significantly. Samples containing constituents exhibiting strong adsorptivities on the electrode surfaces may be sources of various problems as well. These disturbing phenomena occur also in CZE with the contact conductivity detection. Their impacts on the analytical data may be even more serious as in CZE often very small conductance changes due to the zones of the separated constituents are to be measured on a high conductance background of the carrier electrolyte.

The use of the contactless conductivity detection solves these problems in ITP. So far, however, current contact conductivity detectors provide, under comparable CZE working conditions, significantly higher detection sensitivities than their contactless counterparts.

A lack of an operational robustness of the contact conductivity detection in CE can be attributed to a direct contact of the separated constituents with the detection electrodes. Therefore, it seems reasonable to expect that by mediating this contact via an appropriately chosen electrolyte solution an improved robustness of the detector can be achieved. Following this general idea, an electrolyte solution mediated contact conductivity cell (ESMC-cell) for CZE was developed in this work.

INSTRUMENTATION

A CS Isotachophoretic Analyzer (Villa-Labeco, Spišská Nová Ves, Slovak Republic) was used in experiments performed in this work. It was assembled in a single-column configuration of the separation unit using the following modules: CZE injection valve with a 200 nl internal sample loop (Villa-Labeco), One of the columns provided with the the following detection sensors: (i) an ESMC-cell developed in this work (see Fig.2), connected to a 300 μm I.D. (500 μm O.D.) capillary tube made of PTFE of a 250 mm length to the cell; (ii) an on-column conductivity detection cell (Villa-Labeco), connected to a 300 μm I.D. (650 μm O.D.) capillary tube made of PTFE of a 160 mm length to the cell; (iii) a four-electrode, on-column contactless conductivity detection cell (Villa-Labeco), connected to a 300 μm I.D. (430 μm O.D.) capillary tube made of fused silica of a 130 mm length to the cell. Counter-electrode compartment with a hydrodynamically (membrane) closed connecting channel to the separation compartment (Villa-Labeco).

The signal from the conductivity detector was led to a Pentium PC via a Unilab data acquisition unit (Fitek, Šaľa, Slovak Republic). ITP Win software (version 2.31) obtained from Kascomp (Bratislava, Slovak Republic) was used for the acquisition and processing of the detection data.

TABLE 1: Electrolyte systems

Parameter	ES 1	ES 2
Solvent	Water	Water
Carrier ion	L-Aspartate	PEG-DC
Concentration (mmol/l)	10	14
Counter-ion	BTP	BTP
Concentration (mmol/l)	2.6	4.5
pH	4.0	3.6
Additive	m-HEC	m-HEC
Concentration (% w/v)	0.2	0.1
Complexing additive	α -CD	PVP K15
Concentration	70 mmol/l	5.1 % w/v

BTP = 1,3-bis[tris(hydroxymethyl)methylamino]propane; m-HEC = methylhydroxyethyl-cellulose; α -CD = α -cyclodextrin; PEG-DC = polyethyleneglycol dicarboxylic acid; PVP = polyvinylpyrrolidone

ELECTROLYTE SOLUTIONS AND SAMPLES

The solutions of the carrier electrolytes were prepared in demineralized water. Methylhydroxyethylcellulose 30 000 (m-HEC) added to the carrier electrolyte solutions served as a suppressor of the electroosmotic flow. The solutions were filtered through disposable membrane filters (a 1.2 μm pore size) before the use.

Mineral water (Budiš, Slovak Republic) tap water served as practical samples.

DESIGN OF THE DETECTION CELL

Schemes providing generalized views of the arrangements of the detection electrodes in contact conductivity detection cells as used in CZE are given in Fig. 1. The one proposed in this work (Fig. 1c) introduces an electrolyte solution mediated contact of the separated constituents with the detection electrodes.

A schematic drawing in Fig. 2 shows an actual arrangement of the detection cell based on this approach as developed in this work. The detection electrodes (c, in Fig. 2) are placed into channels (d, in Fig. 2) outside the separation capillary. The electrolyte solution filling these electrode channels mediates the electrical contact of the electrodes with the constituents migrating through the detection compartment of the cell (e, in Fig. 2).

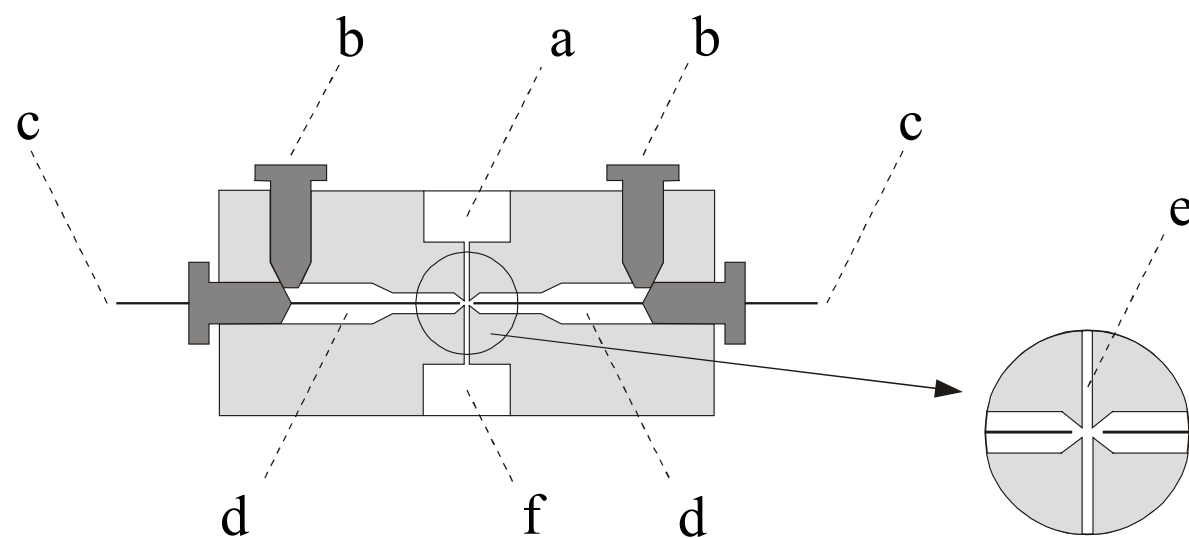


FIG. 2. A schematic drawing of the conductivity detection cell with an electrolyte solution mediated contact of the separated constituents with the detection electrodes.

a = a connection of the cell to the separation capillary, *b* = holes with screw plugs for filling the electrode channels (*d*) with the detection electrodes (*c*), *e* = the detection compartment, *f* = a connection of the cell to the counter-electrode compartment. A body of the cell was made of polymethylmethacrylate.

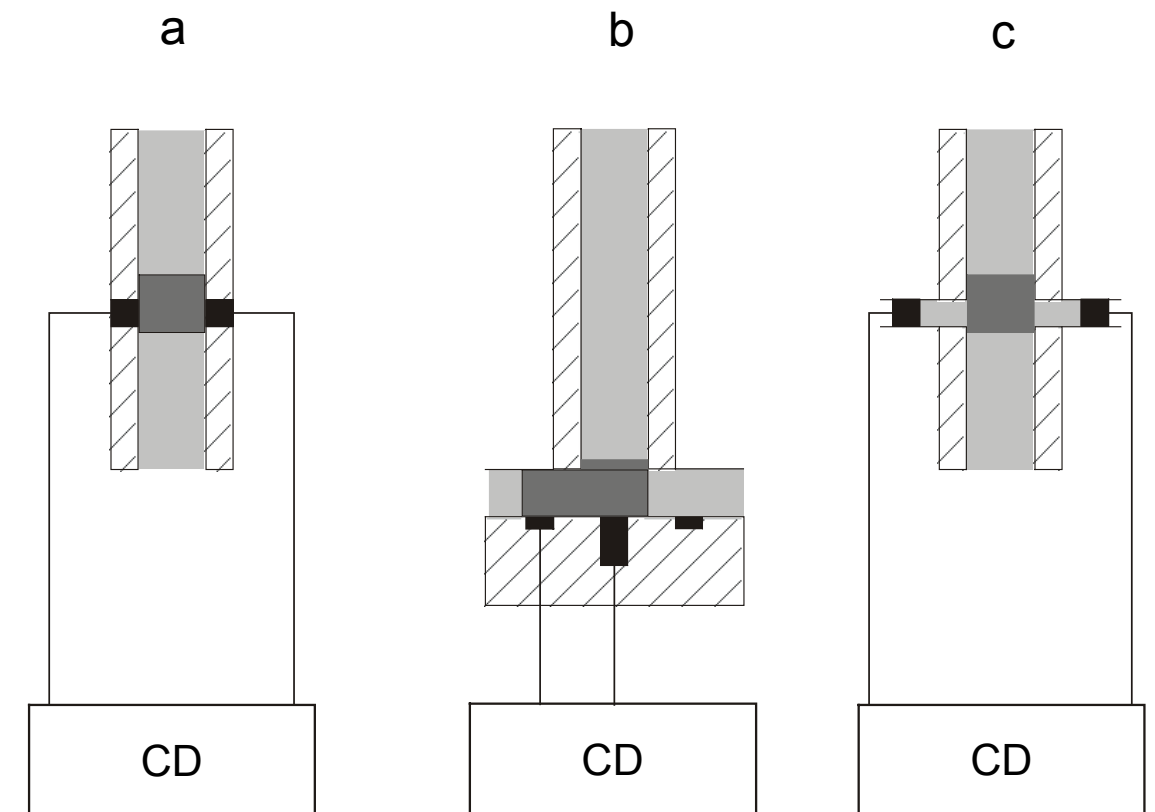


FIG. 1. General schemes of the arrangements of the detection electrodes in contact conductivity detection cells for CZE.

a = an in-column placement of the detection electrodes exposed to direct contacts with the separated constituents; *b* = a post-column placement of the detection electrodes exposed to direct contacts with the separated constituents; *c* = an on-column placement of the detection electrodes with an electrolyte solution mediated contact with the detection compartment of the cell. CD = measuring circuitry of the detector.

CHOICE OF THE ELECTRIC CONTACT MEDIATING SOLUTION

A series of CZE experiments with identical and different mediator and carrier electrolyte solutions was carried out to investigate roles of their compositions on the response of the detector provided with the ESMC-cell. From typical electropherograms as obtained in these experiments (Fig. 3) we can see that the use of identical electrolyte solutions led to the best results (Fig. 3b) while the solutions of differing compositions characterized large baseline drifts of the detection signals (Figs. 3a and 3c).

To identify places and mechanisms of these conductance changes in the detection cell ITP separations of a model anionic mixture (Fig. 4) spiked with traces of anionic dyes (SPADNS and Bromphenol Blue) were monitored by the ESMC-cell. An ITP stack of the model analytes provided a well defined contiguous series of concentration pulses of different constituents that sequentially contacted the mediator solution (the leading

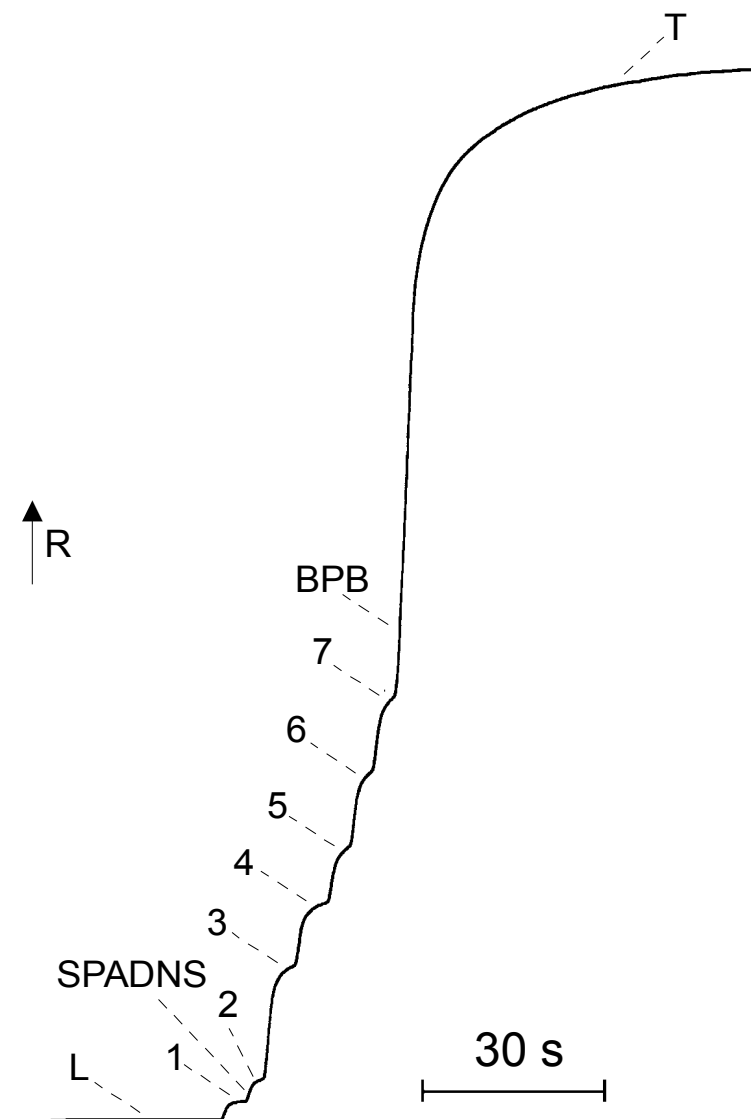


FIG. 4. An isotachopherogram from the separation of anions as obtained by an electrolyte solution mediated contact detection cell. The zone assignments: 1 = sulfate, 2 = chlorate, 3 = succinate, 4 = adipate, 5 = acetate, 6 = lactate, 7 = benzoate.. The concentrations of the anions in the injected sample (200 nl) were 5 mmol/l while the concentrations of the anionic dyes (SPADNS and BPB) were 100-fold lower. The leading electrolyte: 10 mmol/l hydrochloric acid buffered to pH = 6.0 with histidine (methylhydroxyethylcellulose present in the leading electrolyte solution at a 0.2 % [w/v] concentration served as a suppressor of the electroosmotic flow); the terminating electrolyte: 7 mmol/l 2-(N-morpholino) ethanesulfonic acid buffered to pH = 6.0 with histidine. The separation was carried out with a 50 μ A driving current.

electrolyte solution) present in the electrode channels (Fig. 2). The dyes, focused into very narrow bands in the stack, visualized processes occurring on these contacts. We observed that the constituents from the ITP stack entered the electrode channels, moved by electromigration in the direction to the detection electrodes and significantly contributed to changes in the composition of the mediator solution in the electrode channels. Relatively low rates of these changes caused that the plateau values of the detection signal for the ITP zones could be reached only very slowly (Fig. 4). The same transport processes were effective in the CZE separations and, therefore, changes in the compositions of the mediator solutions in the electrode channels explain large baseline drifts on the electropherograms in Figs. 3a and 3c. Therefore, identical compositions of the mediator and carrier electrolyte solutions a priori lead to favorable detection

conditions in the CZE separations (there is no driving force for diffusion and no change in the composition of the mediator solution accompany the electro-migration transport of the constituents through the electrode channels).

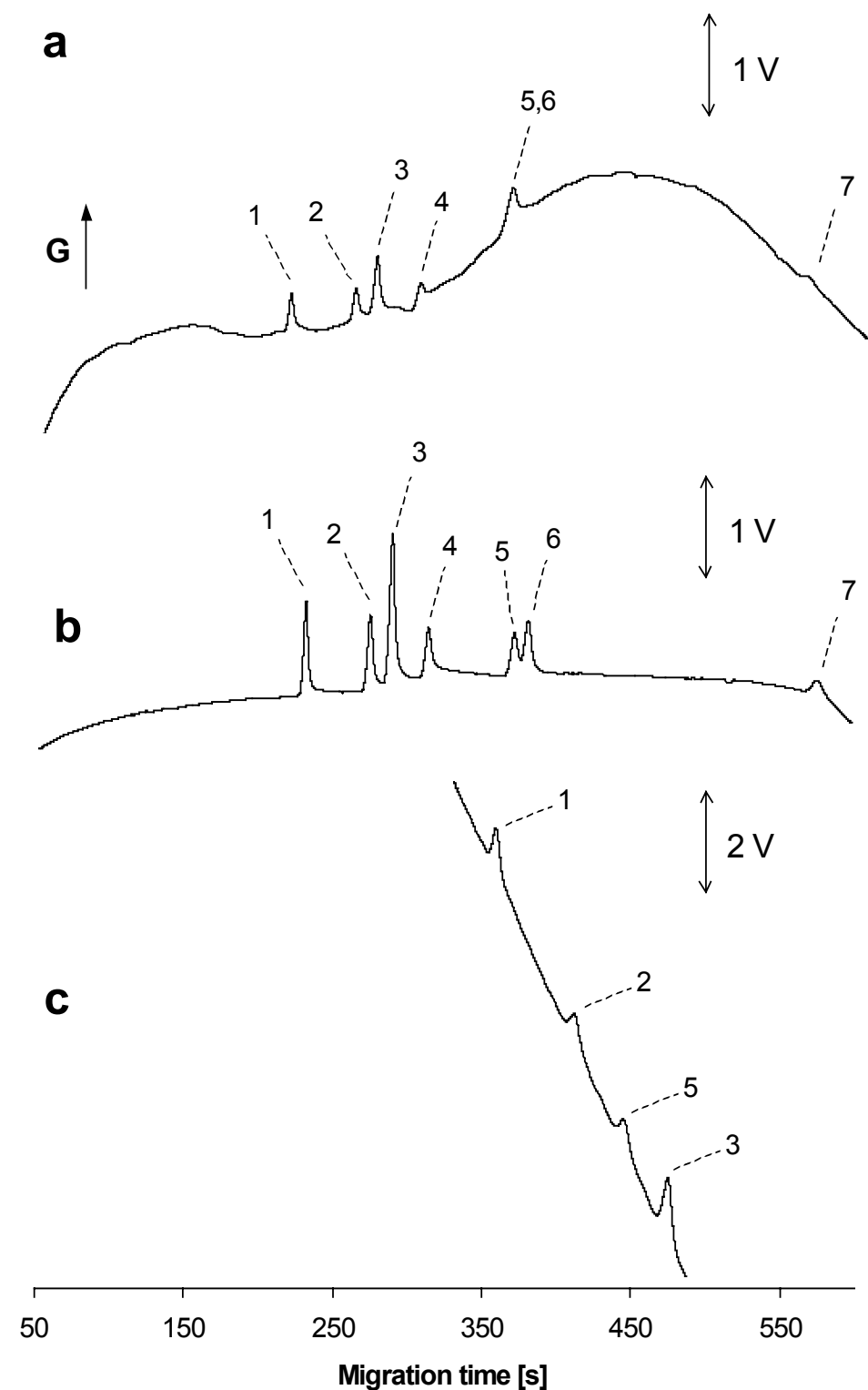


FIG. 3. A role of the composition of the mediator and carrier electrolyte solutions in the ESMC-cell on the response of the conductivity detector in CZE separations.

The compositions of the solutions were: (a) the carrier electrolyte solution-ES1 (Table 1), the mediator solution-ES2; (b) the carrier electrolyte solution-ES1, the mediator solution-ES1; (c) the carrier electrolyte solution-ES2, the mediator solution-ES1. The concentrations of the analytes in the injected sample were 20 μ mol/l. The separations were carried out with a 50 μ A driving current.

EVALUATION OF SOME PERFORMANCE PARAMETERS OF THE DETECTION CELL

CZE experiments with model analytes, monitored by the present ESMC-cell, were performed to obtain data characterizing its detection performance. Analogous data (Table 2), obtained for the same CZE experiments monitored by a contactless conductivity detector and a contact detection cell with the arrangement of the detection electrodes as shown in Fig. 1a, served as references.

Electropherograms in Fig. 5 illustrate detectabilities of the model analytes by the detection systems employed in our performance tests. From these electropherograms we can see that the drift of the baseline of the detection signal characteristic for the present ESMC-cell was comparable to the drifts of the reference detection systems when identical mediator and carrier electrolyte solutions were used.

A comparison of the LOD data for the contact conductivity detection cells, obtained with the same CE equipment, shows (Table 2) that the ESMC-cell provided 3-4 times higher LOD values than the one serving as a reference. Dispersive disturbances linked with the transport processes in the cell as discussed above appear to be responsible for this. On the other hand, the LOD values of the present detector were 20-25 times lower than those attainable under identical working conditions by the contactless conductivity detector. This indicates that an elimination of the contact of the separated constituents with the detection electrodes via an electrically conductive mediator offers a competitive alternative to the contactless detection as far as the detectability is concerned.

TABLE 2: Some performance parameters of the ESMC and reference detection cells

Anion	peak area* R.S.D. (%)	cLOD (nmol/l)			Separation efficiency (N/m)		
		ESMC-cell	Reference contact	Contactless	ESMC-cell	Reference contact	Contactless
Chloride	3.1	38	9	1060	118000	186000	168000
Sulfate	2.9	26	5	540	123000	211000	165000
Nitrate	3.2	45	10	1200	143000	294000	210000
Nitrite	3.1	97	20	2580	87000	191000	172000
Iodide	3.9	63	19	1700	126000	234000	167000
Fluoride	4.9	75	17	2230	123000	187000	147000
Phosphate	4.4	155	34	3750	117000	229000	156000

*calculated from ten parallel determinations of a model sample, containing the anions at 10 $\mu\text{mol/l}$ concentrations, in the electrolyte system ES 1 (Table 1) with a 50 μA driving current. CLOD = the concentration limit of detection; N/m = the number of theoretical plates per metre, calculated from the data obtained for 10 $\mu\text{mol/l}$ concentrations of the anions in the electrolyte system ES 1 using a 50 μA driving current (the data for the contactless detector were obtained with 30 $\mu\text{mol/l}$ concentration of the anions).

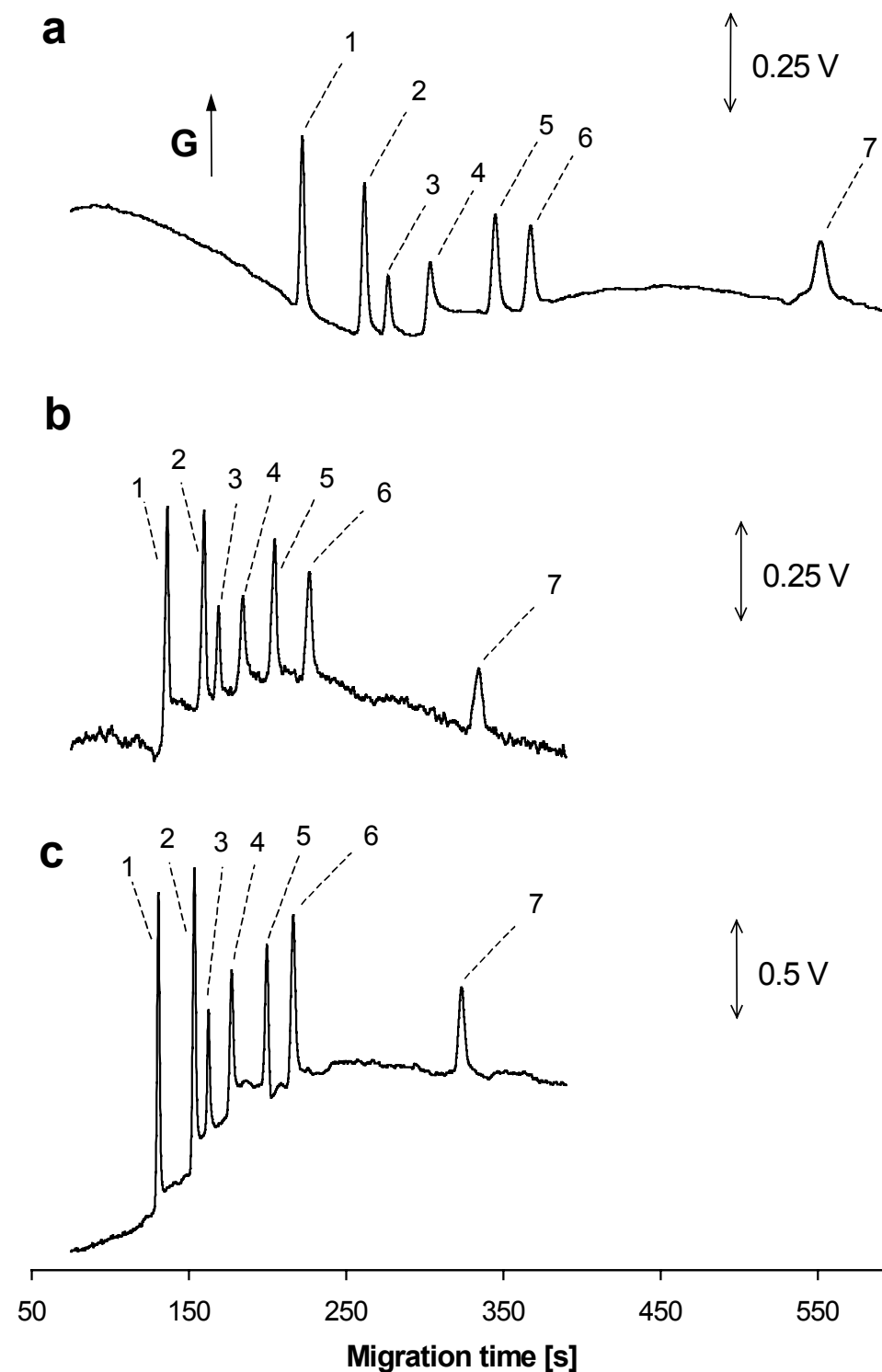


FIG. 5. Detectabilities of the test analytes with the aid of the ESMC-cell (a), contactless (b) and reference contact (c) detection cells.

Peak assignments: 1 = chloride, 2 = nitrate, 3 = sulfate, 4 = nitrite, 5 = iodide, 6 = fluoride, 7 = phosphate. The concentrations of the anions in the injected samples in the CZE runs (a) and (c) were 10 $\mu\text{mol/l}$ (the concentration of sulfate was 2 $\mu\text{mol/l}$). Their concentrations in the CZE run (b) were 40 $\mu\text{mol/l}$ (the concentration of sulfate was 8 $\mu\text{mol/l}$). The separations were carried out in the carrier electrolyte ES 1 (Table 1). In the run (a) ES 1 served also as the mediator solution. The driving current was stabilized at 50 μA .

Reproducibilities of the determination of the analytes using the present ESMC-cell, expressed via the RSD values, spanned from 2.9 to 4.4 % for 10 $\mu\text{mol/l}$ concentrations of the analytes in the injected sample (Table 2) and agreed well with those obtained by the reference contact detection system.

Lower separation efficiencies for the test analytes were typical for the present ESMC-cell (Table 2). About 10-times larger nominal volume of the cell, in comparison to the volumes of the reference cells, offers a partial explanation of this fact. A major role in this respect can be ascribed to the rates of the transport processes in the cell as discussed above.

Parameters of the regression equations of the calibration graphs as obtained with the present cell for the model anions in two series of calibration experiments are given in Table 3. Relative differences in the numerical values of the slopes (analytical sensitivities) indicate a good long-term stability of the response of the detection cell (detection sensitivity) as these differences include, in addition to the fluctuations in the detection sensitivity, at least, small deviations in the compositions of the carrier electrolyte solutions in which the calibration data were obtained and small differences in the compositions of the calibration solutions.

We found that the detection of iodide in the electrolyte system containing PVP (ES 2, in Table 1) is disturbed by its electrode reaction (see Fig. 6b) on the detection electrodes exposed to direct contacts with

TABLE 3: Parameters of the regression equations ($Y = a + bX$) for the calibration graphs of the test anions for 5-50 $\mu\text{mol/l}$ concentrations

Anion	a [mV.s]	b [mV.s/ $\mu\text{mol.l}^{-1}$]	r
Chloride	-32.5 ^a	100.557 ^a	0.9967 ^a
	146.9 ^b	113.466 ^b	0.9985 ^b
Nitrate	-25.9 ^a	95.247 ^a	0.9985 ^a
	33.1 ^b	107.795 ^b	0.9980 ^b
Iodide	-5.3 ^a	92.606 ^a	0.9981 ^a
	76.8 ^b	100.844 ^b	0.9989 ^b
Sulfate	-97.8 ^a	186.850 ^a	0.9986 ^a
	78.9 ^b	201.998 ^b	0.9995 ^b
Nitrite	-52.0 ^a	91.543 ^a	0.9935 ^a
	70.3 ^b	94.022 ^b	0.9962 ^b
Fluoride	-112.3 ^a	82.976 ^a	0.9987 ^a
	-72.3 ^b	97.819 ^b	0.9970 ^b
Phosphate	-103.6 ^a	74.519 ^a	0.9937 ^a
	-5.5 ^b	66.940 ^b	0.9951 ^b

Y = peak area; X = concentration of the test anion in the injected sample ($\mu\text{mol/l}$); a = intercept; b = slope; n = number of data points; r = correlation coefficient.

^a Data based on initial calibration experiments (n = 15);

^b Calibration experiments after a 1 month use of the cell (n = 15).

the separated constituents (a, in Fig. 1). As expected, such disturbances did not appear in any of the comparative experiments performed with the ESMC-cell (see a typical electropherogram in Fig. 6a obtained under identical working conditions). These results indicate a positive role of the mediated contact of the analytes with the detection electrodes in enhancing an operational robustness of the contact detection.

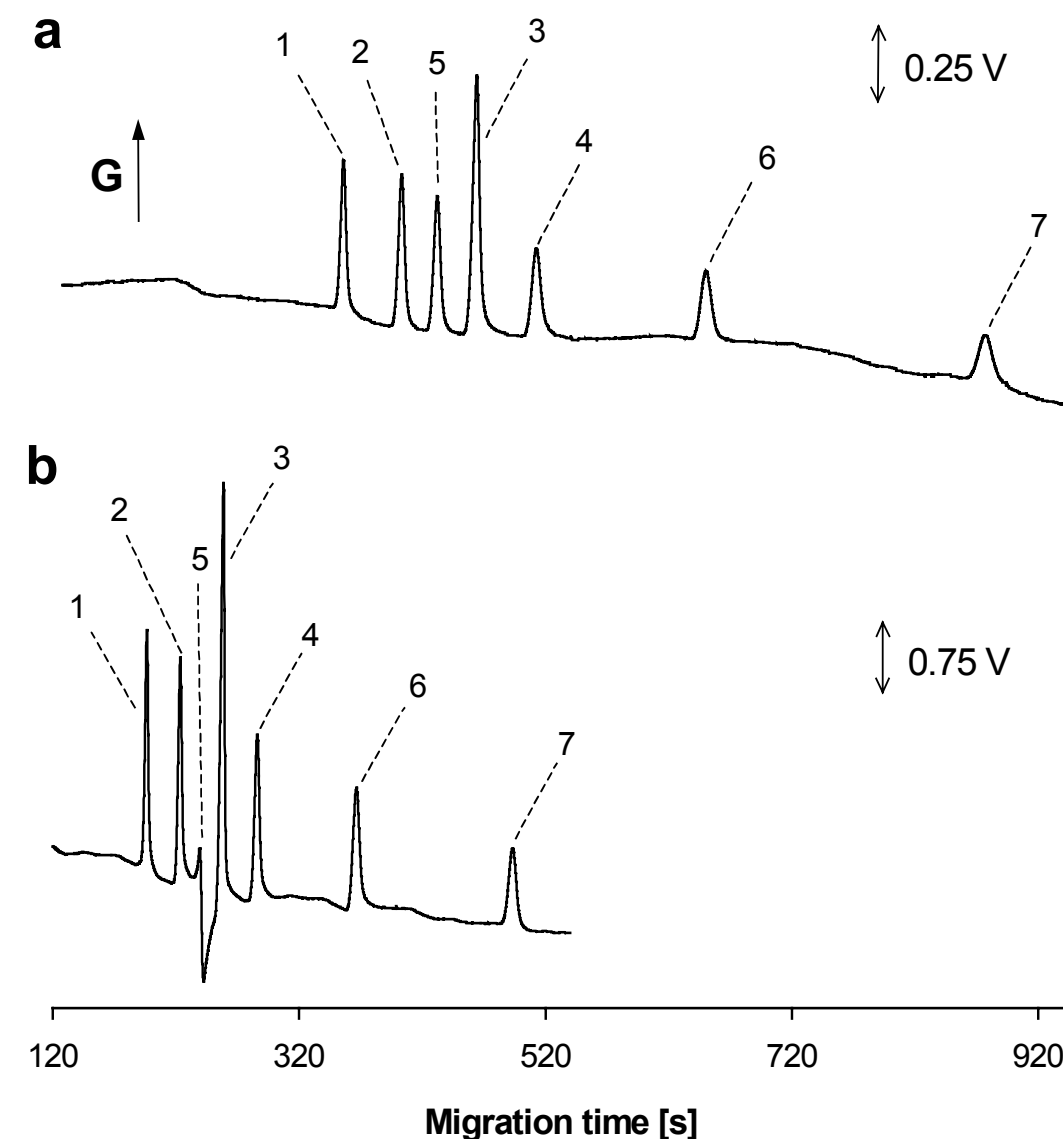


FIG. 5. A comparison of the detection of iodide in the aid of the ESMC (a) and reference contact (b) detection cells.

Peak assignments: 1 = chloride, 2 = nitrate, 3 = sulfate, 4 = nitrite, 5 = iodide, 6 = fluoride, 7 = phosphate. The concentrations of the anions in the injected samples were 30 $\mu\text{mol/l}$. The separations were carried out in the carrier electrolyte ES 2 (Table 1) The carrier electrolyte served also as the mediator solution in the run (a). The driving current was stabilized at 50 μA .

The use of the detection cell in the analysis of practical samples

A practical utility of the present ESMC-cell was assessed in experiments carried out with tap and mineral water samples. The separations were performed in the carrier electrolyte solution ES 2 (Table 1) as this was shown to provide favorable sample loadabilities in the analysis of water and soil samples. An electropherogram as obtained from the analysis of an undiluted sample of tap water (Fig. 7a) shows that under the preferred separating conditions the detector was sensitive enough to detect not only the macroconstituents (chloride, sulfate and nitrate) but also fluoride (present in the sample at about 4 $\mu\text{mol/l}$ concentration). Although other anionic microconstituents to be expected in water samples

were also resolved from the macroconstituents (Fig. 7b) their concentrations in the actual sample were too low to be detectable by the conductivity detector under the employed electrolyte conditions.

Electropherograms in Figs. 7c and 7d, obtained from the analysis of mineral water, illustrate detection capabilities of the ESMC-cell in situations when high ionic strength samples (an actual sample contained the anionic macroconstituents at a total concentration about 10 times higher than the tap water sample) were loaded onto the CZE column. Here, besides the macroconstituents (chloride and sulfate) also fluoride (the only anionic microconstituent listed on the label of the bottled mineral water) could be reliably detected.

CONCLUSIONS

An electrolyte solution mediated contact of the separated constituents with the detection electrodes is feasible and offers a practically applicable detection approach for CZE. Our experiments revealed that the use of identical mediator and carrier electrolyte solutions is essential when an acceptable performance of the detector is to be attained.

CZE experiments with iodide containing samples demonstrated (see Fig. 6) an enhanced robustness with respect to disturbances originating in a direct contact of the separated constituents with the detection electrodes. The present ESMC-cell, contrary to the one used as a reference contact cell in our experiments, required no special maintenance (e.g., cleaning of the surfaces of the detection electrodes) to reach a reliable long-term performance.

Our results indicate that diffusion and electromigration between the electrode channels and the detection compartment of the ESMC-cell, due to changes in the composition of the electrolyte solution in the detection compartment during the separation, set performance limits of the detector. Therefore, improved LOD for the CZE analytes and reduced band broadening in the cell can be expected, mainly, via an improved construction of the ESMC-cell.

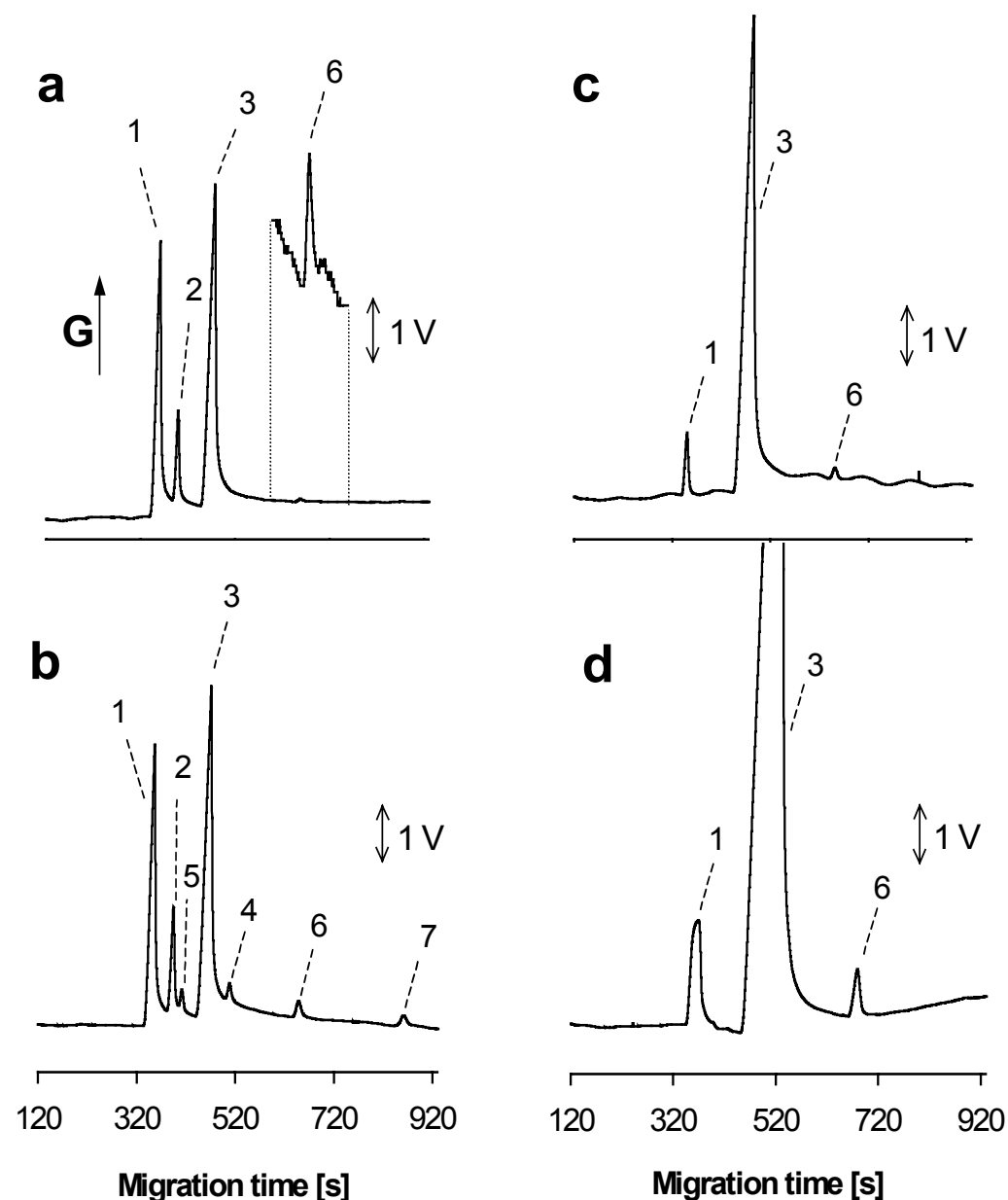


FIG. 7. Electropherograms from the analyses of water samples using the ESMC-cell. a) CZE run with an undiluted tap water sample; b) the same sample as in (a) spiked with the test anions at 30 $\mu\text{mol/l}$ concentrations; c) mineral water “Budiš” 5 times diluted with demineralized water; d) the same as in (c) only the undiluted sample was injected. Peak assignments: 1 = chloride, 2 = nitrate, 3 = sulfate, 4 = nitrite, 5 = iodide, 6 = fluoride, 7 = phosphate. The separations were carried out in the carrier electrolyte ES 2 (Table 1). The driving current was stabilized at 50 μA .