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Galvanic decoupling of a postcolumn amperometric detector in capillary electrophoresis

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Abstract

A postcolumn amperometric detector for capillary electrophoresis techniques with galvanic decoupling of the detection electrodes and the detection circuit from the power unit of the detector (via a laboratory constructed d.c.–d.c. converter) and from the recording devices (via an opto-coupling device) was developed. The high insulating resistance achieved in this way (ca. 10^{11}–10^{12} Ω) reduced leak currents from the electrophoretic equipment through the detector to the low pA level. It was also effective in eliminating problems due to changes in the potential at the column outlet as found when current detection devices were used. The decoupling protected the detector in situations when the column outlet was at a potential of 4–5 kV. The detector was tested by isotachophoretic experiments carried out in a hydrodynamically closed separation compartment. Dispersions of the analyte zones in the detector were reduced about tenfold in comparison with a previous design of the detector. Experiments with a urine sample, however, revealed that the resolving power of the detector is limited in the isotachophoretic separations of complex mixtures of electrochemically active constituents. The use of a copper electrode was tested in the separation of triethylenetetramine and impurities present in its technical-grade preparation. This detection approach was shown to provide a high sensitivity for this amine and for impurities forming strong complexes with Cu(II) cations.

1. Introduction

Electrochemical methods combined with high-efficiency separation techniques offer remarkable selectivities and sensitivities for electrochemically active analytes (for a review see, e.g., Refs. [1–6]). Many of these analytes can be advantageously separated by capillary electrophoresis (CE), and capillary zone electrophoresis (CZE), capillary isotachophoresis (ITP) and micellar electrokinetic chromatography (MEKC) are CE techniques that can be mentioned in this respect. Since the first paper dealing with amperometric detection in CE appeared [7] increasing interest in this subject has been apparent [8–29].

In coupling amperometric detection with CE, three main problems were recognized [7]: (1) bipolar behaviour of the materials of which the working electrodes are made in the driving electric field accompanied by electrochemical phenomena as found in research on conductivity detection in ITP (Ref. [30], p. 176); (2) leaks of the driving current through the detection electrodes to the ground of the amperometric detector; and (3) transport of the separated constituents into the detection cell to achieve favourable hydrodynamic conditions for detection.

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The solution to the last of these problems is closely related to the concept of the separation compartment. While a hydrodynamically closed separation compartment requires the use of a suitable pump (see [7] and below), hydrodynamically opened separation systems use electroosmotically generated flow [14,17,18,20,22–24,26] or laminar flow induced by the electroosmotic pumping [8–13,15–17,19,21,25,27,28].

Leaks of the driving current through the detection cell are minimized by separating the detection cell from the end of the column by a capillary tube (detection capillary) filled with the electrolyte solution while the end of the column is coupled to the earth potential of the high-voltage power supply. Various types of the coupling devices have been proposed for this purpose [7,8,16,21,25]. These precautions need not be used for columns of 25 μm I.D. or less [28] working with low driving currents.

From the descriptions of the amperometric detectors used in CE [7–29], it is apparent that current electroanalytical measuring systems are preferred. These measuring systems were not designed for amperometric measurements under the conditions typical for electrophoretic separations. Therefore, their insulating resistances may not be sufficient to eliminate leak currents through the detection cell, e.g., when the working electrode is placed in the electrophoresis column or very close to the column outlet [29] or when the potential at the column outlet changes [7]. The column outlet can acquire a very high potential, e.g., when a bubble is formed at the end of the column. Although such a situation rarely occurs when the CE equipment is handled properly, a high potential on the input of the measuring system can cause damage to the detector when it is not designed with these facts in mind.

Recently, electrochemical phenomena occurring at the working electrode inserted into the solution in the column through which the driving current was flowing were demonstrated [28]. Associated with the bipolar behavior of the working electrodes, they give some implications concerning the proper construction of the working electrode for CE and implicitly stress a need to reconsider the design of the measuring electronics of the amperometric detector for CE.

The aim of this work was to develop an amperometric detector for CE meeting the following requirements: (i) minimum leak currents through the detection cell from the electrophoretic equipment; (ii) protection of the detector circuits and recording devices in situations when the column outlet acquires a very high potential; (iii) following our previous concept of the detection cell [7], to construct a cell with a reduced dead volume; and (iv) compatibility of the detector with both hydrodynamically closed and opened separation compartments.

This paper describes the construction of an amperometric detector in which the electrophoretic column and the measuring circuitry of the detector are galvanically decoupled from the recording devices and from the power unit of the detector. An improved detection cell of the detector is described. The detector was tested in a combination with a hydrodynamically closed CE separation unit. ITP separations were chosen for these tests because they have some advantages for these purposes: (1) well defined changes of the potential at the column outlet can be created easily as they are inherently linked with the ITP separation principles (see, e.g., Ref. [30]); (2) the dispersion in the postcolumn detector can be evaluated using appropriate spacing constituents in the samples; and (3) the dispersion due to the postcolumn detection can be related in a straightforward way to that due to the ITP separation itself which is available from the response of the on-column conductivity detector.

2. Experimental

2.1. Instrumentation

A schematic diagram of the CE separation unit used in this work is given in Fig. 1. The unit was assembled from modules developed in this laboratory following our modular concept of the CE equipment [31]. An on-column conductivity detection cell [32] was used to monitor the

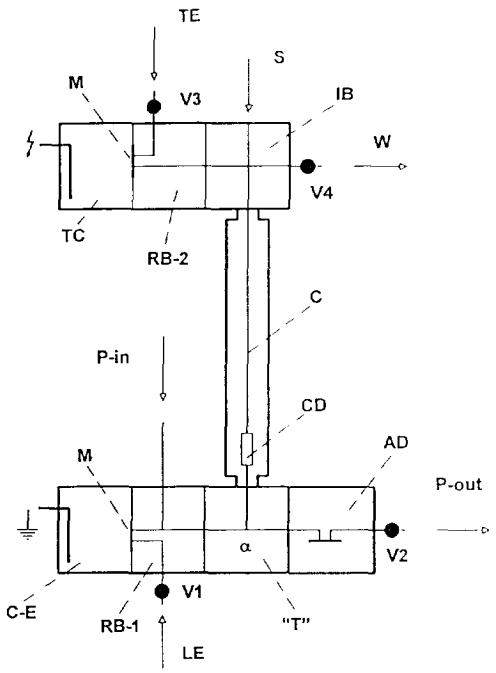

Fig. 1. Schematic diagram of the hydrodynamically closed CE separation unit with a postcolumn amperometric detector. IB = injection block for a microsyringe sample injection; C = capillary tube; CD = on-column conductivity detection cell; "T" = elution T-piece; α = bifurcation point; AD = amperometric detection cell; RB-1 = refilling block for the capillary tube (C); C-E = counter-electrode compartment; M = Cellophane membranes; LE = inlet for the leading electrolyte solution; P-in, P-out = inlet and outlet for the elution solution, respectively; RB-2 = refilling block for the terminating compartment (TC) and the injection block (IB); TE = inlet for the terminating electrolyte solution; W = waste; S = position for the injection of the sample; V1–V4 = valves.

separation in a 200 mm × 0.30 mm I.D. capillary tube made of a copolymer of fluorinated ethylene and propylene (FEP). The separation unit was used with the electronic units of a CS isotachophoretic analyser (Villa-Labeco, Spišská Nová Ves, Slovak Republic).

A laboratory-made syringe pump with a pumping head made of plastic material was used to deliver the electrolyte solution and the separated constituents to the detection cell. The pumping rate was 3.0 μl/min throughout this work.

A measuring unit of a PA-3 polarographic analyser (Laboratorní Přístroje, Prague, Czech Republic) was used in comparative experiments. Its mechanical earthing was disconnected and the insulating resistance of its power transformer determined the leak currents. This measuring unit was used in a combination with the detection cell described elsewhere [7]. All amperometric measurements were performed against a laboratory-made Ag–AgCl (1 *M* KCl) reference electrode.

A 3 mm diameter glassy carbon (Tokay Electrode, Nagoya, Japan) was glued by epoxy into a hole drilled into an epoxy rod serving as the body of the working electrode (see Fig. 5). The surface of the electrode was ground with sandpapers of fine grades and finally polished with a very fine grade of polishing powder. The copper electrode was constructed in the same way using a transformer copper wire of 3 mm diameter. Its surface was finally electrolytically coated with a layer of copper from a 1% aqueous solution of copper(II) sulphate at 20 μA for 2 h.

2.2. Operation of the CE equipment with the postcolumn amperometric detection

The separation unit used in this work (Fig. 1) is hydrodynamically closed by valves (V1–V4) and cellophane membranes (M). During the separation only the valve at the outlet from the detection cell (V2) is opened to collect the pumped (elution) solution into a waste vessel made of PTFE. The separated constituents, after detection in the conductivity detector (CD), are transported by the driving current into the stream of the pumped solution in a T-piece ("T"). The solution containing the transferred constituents from the column is transported into the detection cell (AD) attached to the T-piece.

2.3. Chemicals and sample handling

Chemicals used for the preparation of the solutions of the leading and terminating electrolyte solutions were brought from Serva (Heidelberg, Germany), Sigma (St. Louis, MO, USA), Reanal (Budapest, Hungary) and La-

chema (Brno, Czech Republic). Other chemicals were obtained from the same sources, except for triethylenetetramine (technical grade), which was from Spolchemie (Ústí nad Labem, Czech Republic).

The leading electrolyte solutions were prepared without anticonvective additives. To eliminate negative effects of electroosmosis [30] the walls of the capillary tube were coated with methylhydroxyethylcellulose 30 000 (Serva) by pushing a 0.5% aqueous solution of this cellulose derivative through the capillary tube [33].

Some of the analytes studied are easily oxidized by oxygen. Therefore, their stock standard solutions (2–10 mM) in water were thoroughly deoxygenated with nitrogen. The solutions used for the analyses prepared daily by dilution with deoxygenated water. Solutions at 10^{-6} mol/l or lower concentrations were prepared immediately before injection into the equipment.

A Model 701N microsyringe (Hamilton, Bonaduz Switzerland) was used to inject sample solutions into the CE instrument. To minimize losses of the analytes by their interactions with metallic parts of the syringe, ethylenediaminetetraacetic acid (EDTA) was added at 10^{-5} mol/l to the sample solution. When this was not effective, a new syringe was used. Syringes were washed daily with demineralized water and with methanol and finally dried with nitrogen.

3. Results and discussion

3.1. Galvanic decoupling of the amperometric detector

The CE instrument with postcolumn amperometric detector (Fig. 1) can be represented by a simplified electrical equivalent as shown in Fig. 2. General requirements concerning the design of the detection circuit of the instrument are apparent from this scheme and they can be summarized as follows: (1) the value of R_{CE} should be minimized so that the bifurcation point in the connecting T-piece (end of the column) is close to the earth potential of the high-voltage power supply (connected to the counter electrode; see Fig. 1); (2) the resistance in the direction to the ground of the measuring electronics of the amperometric detector should be maximized so that the leak currents from the column are reduced to a minimum; and (3) the working electrode is placed in such a position that negative effects due to the bipolar behaviour of the working electrode (see below) are negligible.

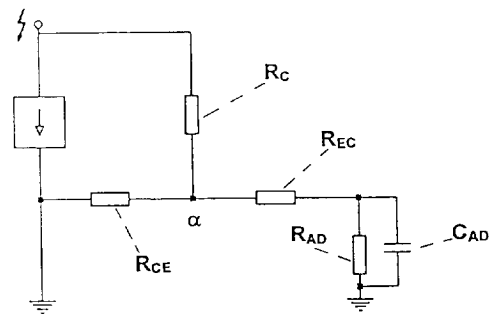

Fig. 2. Electrical equivalent scheme of the CE separation unit with a postcolumn amperometric detector. R_C = resistance of the column; R_{CE} = resistance of the channel to the counter electrode; R_{EC} = resistance of the elution channel (detection capillary); R_{AD} = resistance of the amperometric detection system relative to the earth potential; C_{AD} = capacitance of the detection system; α = bifurcation point.

An ideal solution to the first of these requirements ($R_{CE} \approx 0$) is to place the counter electrode at the column outlet. Although this is possible [21], such a solution introduces the risk of disturbances in the separation compartment due to migrating products of the electrode reactions. In this work, the value of R_{CE} was reduced by using a connecting channel between the bifurcation point and the counter electrode of 3.5 mm I.D. This gave an electric resistance of 40–50 kΩ for the leading electrolytes used in this work (Table 1) and, consequently, potentials of 1–3 V (against the earth potential of the power supply) at the bifurcation point for 30–50-μA driving currents. When used in ITP separation, this potential increased to 5–15 V on the entrance of the terminating zone when the solution in the direction to the detection cell was not pumped (these values were calculated using a published computer program [30]).

To meet the second requirement, we designed

Table 1
Electrolyte solutions

Parameter	System No.		
	1	2	3
Solvent	H_2O	H_2O	H_2O
Leading anion	Cl^-	Cl^-	Na^+
Concentration (mM)	10	10	10
Counter constituent	HIS	BALA	Borate
pH of the leading electrolyte	6.0	3.9	9.3
Terminating anion	MES	Acetate	$Tris^+$
Concentration (mM)	5	5	5

HIS = histidine; BALA = β-alanine; MES = 2-(N-morpholino)ethane sulphonate; Tris = tris(hydroxymethyl)aminomethane. The leading electrolyte solutions were used without anticonvective additives (electroosmosis was suppressed as described under Experimental).

the measuring electronics of the amperometric detector as shown in Fig. 3. The potentiostat itself (I in Fig. 3) is based on a current two-electrode arrangement for amperometric measurements [34]. Placed on a high-quality insulating plate (PTFE), it is powered by a perfectly insulated d.c.–d.c. converter (IV in Fig. 3) having leak currents of less than 10 pA when its secondary coil is kept at a 10 kV potential. This d.c.–d.c. converter was tested to withstand potentials as high as 20 kV at the column outlet without any damage to the other measuring and recording circuits operating at ground potential. The signal from the the potentiostat to the registration devices (line recorder, computer) is transferred via an interface (II in Fig. 3) based on the use of a photo-coupling device. The insulating resistance of this device is considerable higher than 10 GΩ and its operation was found to be reliable also when the potential at the column outlet was 4 kV.

3.2. Amperometric detection cell and its use with galvanically decoupled detection circuit

In CE with postcolumn amperometric detectors, mainly fibre and disc types of working electrodes are used (see, e.g., [7,8,28,29]). From the point of view of the bipolar properties of electrically conductive materials placed in the electric field in the column (Ref. [30], 176), it can be deduced that these electrodes will exhibit different behaviour under such experimental conditions. These differences are clear from the schematic illustrations in Fig. 4. Here, the fibre electrode inserted into the solution in the column will be polarized on application of the driving current and a potential difference between the two sides of the fibre exposed to the solution will be the result. When this potential difference is sufficient to drive an oxidation reaction at the anodic side and at the same time a reduction reaction at the cathodic side, the driving current will flow through the electrode as shown schematically in Fig. 4A. For example, in this way water can be electrolysed with the production of oxygen and hydrogen on the anodic and cathodic sides of the electrode, respectively. This process cannot be eliminated even when the electrode is disconnected from the measuring circuitry ($R_D \approx \infty$).

The disc electrode inserted into the solution in the column makes direct contact with it only through a front plane (Fig. 4B). On application of the driving current only one end of the electrode is available for the electrode reaction in the solution and the electrode reaction loop cannot be closed via the electrode itself. This indicates that the disc electrodes offer a more convenient alternative as far as the bipolar effects are concerned. However, this assumes in some respects an idealized situation (the contact plane of the disc electrode is placed in an equipotential of the driving field, no local potential gradients on the surface of the electrode, e.g., due to its roughness) and in practice some negative effects probably cannot be eliminated to a certain extent.

A schematic view of the detection cell used in this work is given in Fig. 5. The cell was mechanically fixed to the T-piece (2 in Fig. 5) and the distance of the surface of the working electrode from the outlet of the elution channel (4 in Fig. 5) was defined by PTFE spacers (5 in Fig. 5). Although the cell was designed to accommodate various types of working electrodes (simple fibres, strand of fibers [35], disc), we preferred in this work the disc type as shown

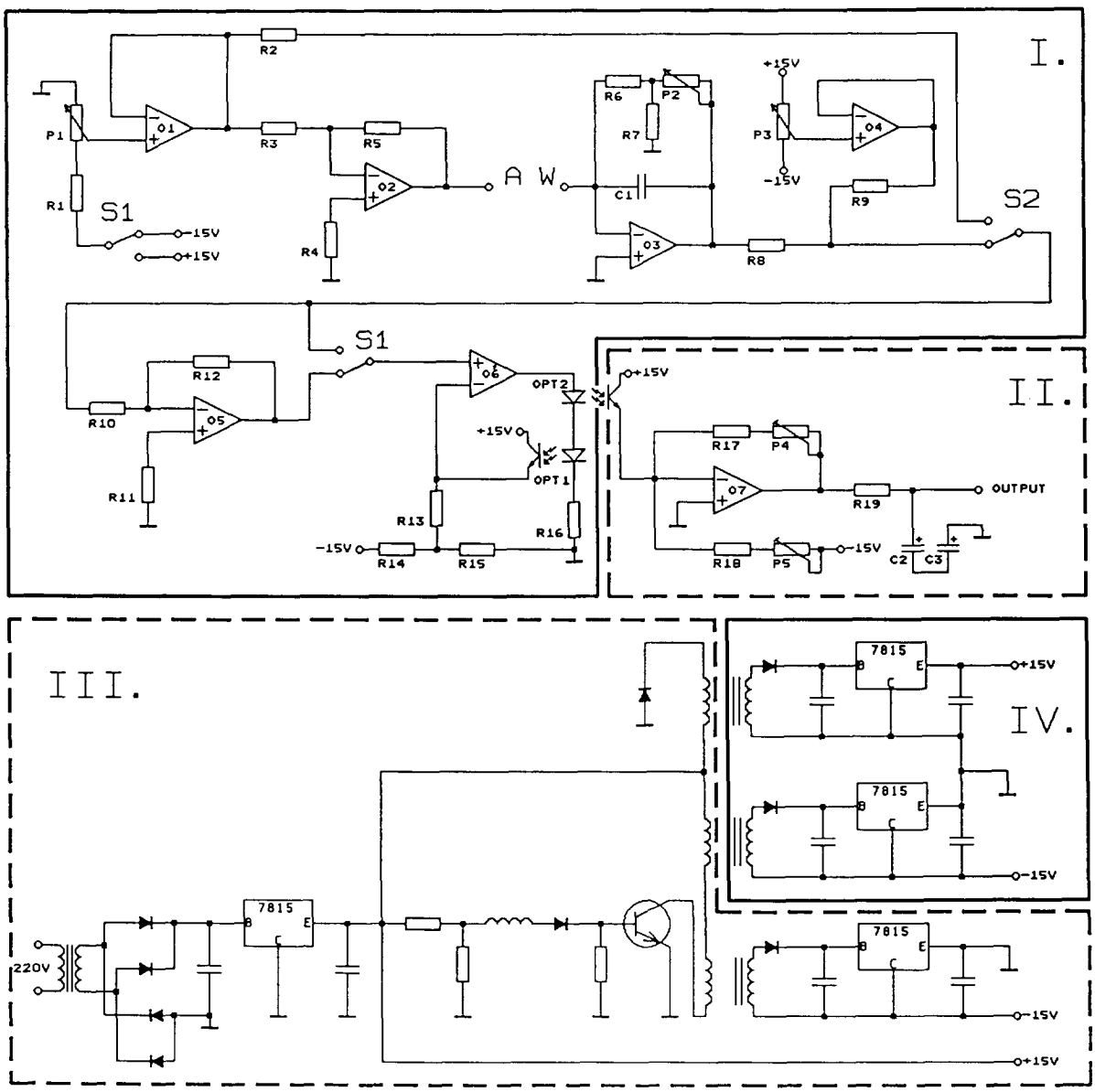

Fig. 3. Electronic circuit of the amperometric detector. I = potentiostat in a two-electrode arrangement; II = interface between the potentiostat and the registration devices; III = power unit of the detector; IV = d.c.–d.c. converter. Semiconductor components: OA_1, OA_2, OA_4–OA_7 = MAA 741 (AD 741); OA_3 = MAC 155 (LF 155); OPT1, OPT2 = WF MB 101. Resistors: R_1, R_4, R_{11}, R_{14} = 47 kΩ; R_2, R_3, R_8, R_9, R_{10}, R_{12}–R_{14} = 10 kΩ; 5 kΩ; R_6 = 3 MΩ; R_7 = 1 kΩ; R_{15} = 510 Ω; R_{16} = 22 Ω; R_{17} = 180 kΩ; R_{18} = 15 kΩ. Potentiometers: P_1, P_3 = 100 kΩ; P_2 = 5 MΩ. Capacitors: C_1 = 150 pF; C_2, C_3 = 50 μF. S_1 = polarity switch; S_2 = switch for the measurements of potential on the working electrode or the detection current. Full-line frames contain the circuits operating on the high-voltage side (I and IV) and which are galvanically decoupled from the power unit (III) and from the interface (II).

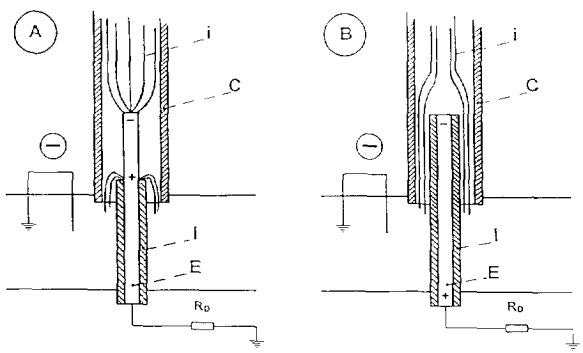

Fig. 4. Bipolar behaviour of (A) fibre and (B) disc electrodes inserted into the capillary tube (C) through which the driving current flows. Current lines (i) indicate the paths of the driving current. E = electrode; I = insulating layer; R_D = insulating resistance of the detector to the ground.

in Fig. 5 for the following reasons: (i) inherently better properties in terms of the bipolar effects as discussed above; (ii) we could compare the present detection system with that developed in our previous work [7]; and (iii) the working electrode could be aligned easily without using micropositioners.

The optimum solution in this respect requires that the choice considers mainly the signal-to-nose ratio [36]. A detailed investigation of various types of working electrodes along this line is the subject of our current research.

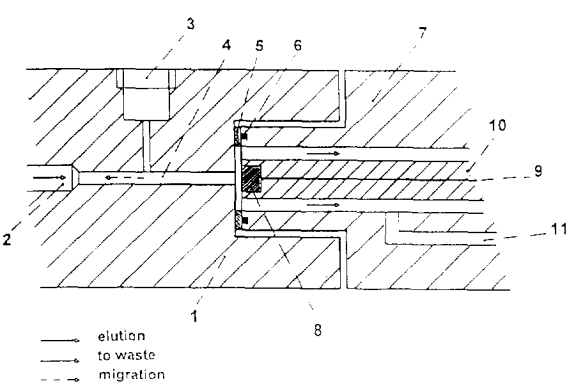

Fig. 5. Amperometric detection cell attached to the T-piece. 1 = T-piece; 2 = channel to the counter electrode; 3 = connection for the column; 4 = elution channel (detection capillary) of 4 mm × 0.2 mm I.D.; 5 = PTFE spacer (0.05 mm); 6 = O-ring for a leak-proof connection; 7 = amperometric cell; 8 = working electrode; 9 = connecting cable to the electronics of the detector; 10 = epoxy body of the electrode; 11 = connecting channel for the reference electrode.

3.3. Evaluation of the performance of the detector

The change in the potential at the column outlet was found to be a source of undesirable disturbances in the amperometric detector coupled to the ITP separation [7]. The isotachopherograms in Fig. 6 illustrate this problem. When the measuring unit of the current amperometric system (see Experimental) was used in conjunction with the CE equipment, a 5 V change in the potential at the column outlet due to the entry of the terminating zone into the elution channel (see Fig. 5) caused a response of

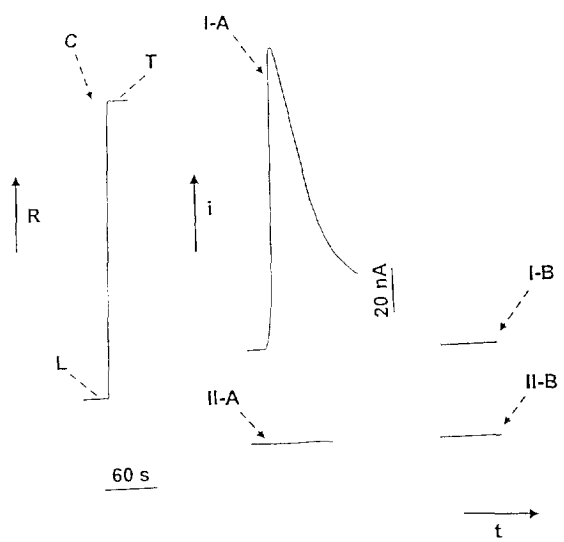

Fig. 6. Responses of the amperometric detectors on the entrance of the terminating zone into the elution channel in the bifurcation point. A = modified PA-3 detector (see Experimental); B = present detector (Figs. 3 and 5); I and II = without and with pumping of the elution solution, respectively. C = isotachopherogram from the conductivity detector. Electrolyte system No. 1 (Table 1) was used for the separations and the leading electrolyte served also as the elution solution. A glassy carbon electrode was set at +400 mV vs. an Ag–AgCl (1 *M* KCl) reference electrode. The driving current was 45 μA. L and T = leading and terminating zones, respectively; *R*, *i* and *t* = increasing resistance, detection current and time, respectively.

the detector equal to ca. 100 nA (I-A in Fig. 6). It must be stressed that in this instance the elution solution was not pumped and the detection cell had a conductive connection with the column outlet via the elution channel (capillary tube of 100 mm × 0.25 mm I.D.). The electric resistance of this connection was ca. 16 MΩ. This false response did not occur when the composition of the solution at the column outlet and in the elution channel was kept constant by pumping the elution solution (II-A in Fig. 6). From these experiments, it is apparent that even small fluctuations in the potential at the column outlet can contribute significantly to the overall noise of the detector. Isotachopherograms I-B and II-B (Fig. 6) were obtained, under conditions otherwise identical with those for their counterparts (I-A and II-A), with the present detector. These experiments show that the galvanic decoupling of the electrophoretic equipment and the potentiostat from the rest of the detector circuits (Fig. 3) eliminated the problems with the false response of the detector. In the experiments illustrated by the isotachopherogram I-B in Fig. 6 this false response could not be resolved from the noise of the detector (ca. 90 pA). This is, at least, a 10^3-fold improvement when related to the response of the detector without such a strict decoupling (I-A in Fig. 6).

Leak currents through the detection cell were very significantly reduced using the present detector. While in runs I-A and II-A in Fig. 6 the background current increase due to the driving current was 30–35 nA, it could not be resolved from the detector noise (ca. 90 pA) with the galvanic decoupling as developed in this work.

These improvements in the performance of the measuring electronics of the detector enables us to decrease significantly the dead volume of the detection cell {a 100-nl volume of the elution channel with the present detection cell (Fig. 5) vs. 4900 nl with the previous detector [7]}. The overall dispersion of the analyte zones in the detection cell was evaluated in the ITP separation of nitrite and ascorbate in the spike mode of analysis [7] using succinate as a spacing constituent. Isotachopherograms from these experiments are given in Fig. 7. Here, the analytes

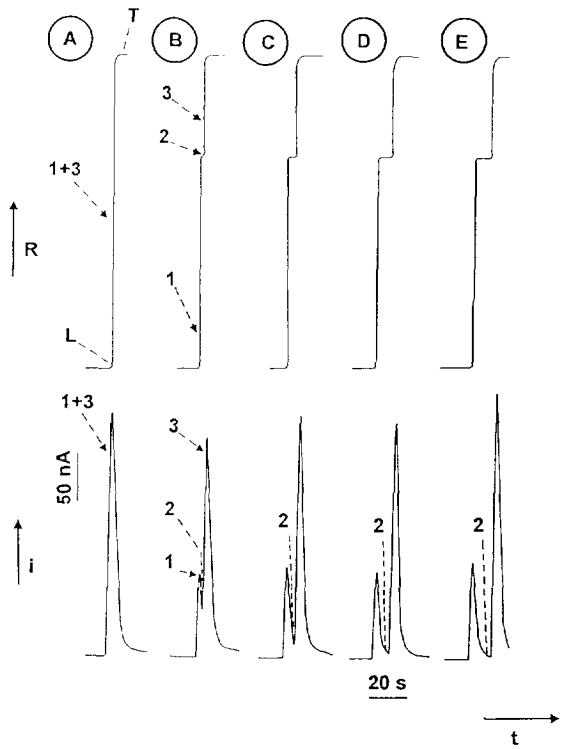

Fig. 7. Resolution of nitrite (1) and ascorbate (3) by the present amperometric detector. Succinate (2) was used as a spacing constituent and its zone lengths in the column were (A) 0, (B) 1, (C) 2, (D) 3.5 and (E) 4.5 mm. The amounts of nitrite and ascorbate injected were always 80 and 8 pmol, respectively. The sample volume was 1 μl. A glassy carbon electrode was set at +800 mV vs. an Ag–AgCl (1 *M* KCl) reference electrode. The separations were carried out in electrolyte system No. 2 (Table 1) and the leading electrolyte was used also for elution. The driving current was 50 μA. Other symbols as in Fig. 6.

were injected in amounts not sufficient for the formation of their own zones detectable by the conductivity detector. They migrated focused in the interzonal boundary layer between the leading and terminating zones (Fig. 7A) or spaced by the succinate zone in the corresponding interzonal boundary layers (Fig. 7B–E). Under our working conditions the boundary layer can be estimated to have a 0.1–0.2 mm thickness [30]. With a migration velocity of ca. 0.5 mm/s the analytes were transferred into the elution solutions at the column outlet within 0.2–0.4 s. This corresponded to their transfers into 10–20nl

volumes of the elution solution. From the isotachopherograms obtained from the amperometric detector we can see that they passed through the detection cell in ca. 500-nl volumes. This dispersion can be conveniently expressed via the length of the spacing zone giving a baseline resolution of the analytes by the amperometric detector [7]. The isotachopherogram in Fig. 7 show that this was achieved for a 2-mm length of the succinate zone. This is almost one order of magnitude improvement in the dispersion compared with what was possible with out previous detector [7]. However, a comparison with conductivity detection (see Fig. 7) shows that the present detection cell is not exploiting fully the resolving power of the ITP separation and a further improvement of its construction is desirable.

The noise of the detector (peak-to-peak) was typically in the range 70–100 pA, depending on the electrolyte system, pumping rate and state of the surface of the working electrode. Considering the signal-to-noise ratio of 3 and the working conditions as used in experiments in Fig. 7, this gives an estimate of the limit of detection of 5–7 fmol for ascorbate or a 5×10^{-9}–$7 \cdot 10^{-9}$ mol/l concentration of this analyte for a 1-μl sample injection volume. However, in experiments aimed at confirming this estimated value we could not detect ascorbate when its concentration in the sample was lower than $5 \cdot 10^{-8}$–$8 \cdot 10^{-8}$ mol/l. This is probably due to the fact that the injection of the sample with the aid of a microsyringe having both needle and plunger made of stainless steel can be accompanied by an oxidation reaction of the labile analytes due to traces of Fe(III) ions [37] and the precautions taken to minimize this effect (see Experimental) may not be sufficient for such low concentrations. Experiments carried out with ITP with UV detection (254 nm) and with proportionally higher sample injection volumes using a PTFE-lined injection valve gave identical conclusions.

Human urine containing hundreds of acidic constituents [38], many of which can be expected to be oxidizable electrochemically, was briefly studied to show the possibilities of the present detector in the analysis of complex mixtures of electrochemically active constituents. Isotachopherograms obtained in these experiments are given in Fig. 8, from which it is apparent that the resolution of the separands was the main problem for both detectors. On the other hand, from the response of the conductivity detector we can estimate that the concentrations of the oxidizable analytes in the sample were 10^{-4} mol/l or less while the amperometric detector indicated a high sensitivity. Solving the resolution problems, e.g., by using a mixture of appropriately chosen

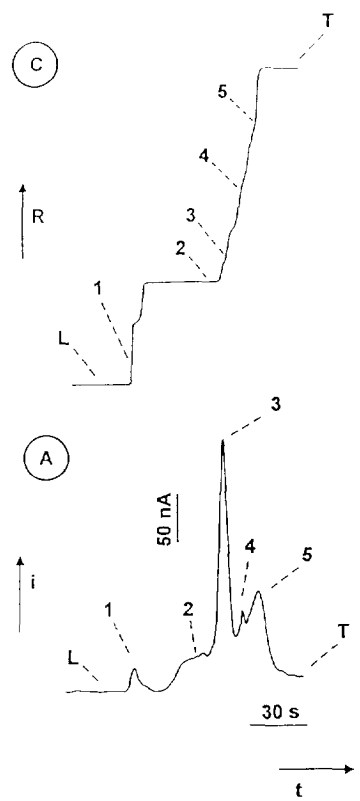

Fig. 8. ITP separation and postcolumn amperometric detection of anionic human urine constituents at pH 3.9. A 2-μl volume of the sample [a mid-stream fraction diluted 1:10 (v/v) with water] was injected. (C and A) = isotachopherograms from the conductivity and amperometric detectors, respectively. The numbers (1–5) indicate identical regions on the isotachopherograms. The separations were carried out in electrolyte system No. 2 (Table 1) using the leading electrolyte also for elution. The driving current was 50 μA. The working electrode (glassy carbon) was set at 800 mV vs. an Ag–AgCl (1 *M* KCl) reference electrode. Other symbols as in Fig. 6.

spacing constituents or via a combination of ITP with CZE [39], we can expect very sensitive analytical procedures for the oxidizable analytes present in urine.

CE techniques are convenient alternatives to the separation of cationogenic amines. At present, their selective detection (not considering chemical derivatization reactions), especially for trace analysis applications, is not available. The use of a copper electrode polarized anodically as proposed for HPLC and flow analysis systems [1–3] and for CZE of amino acids, peptides [15] and sugars [17] was tested in conjunction with the present amperometric detector. Triethylenetetramine of a technical grade served as an analyte in these tests. From the isotachopherograms obtained (Fig. 9), we can see that the polyamine contained seven impurities, four of which gave a response to the amperometric detector. The staircase-like appearance of the response for the main constituent is unexpected as a straight plateau should ideally be achieved. A possible explanation for this reproducible response may lie in the kinetics of the chelate formation at the electrode [3, 15] and in the presence of borate in the electrolyte solution (Table 1), which is reported to have a negative influence on the response of the copper electrode [3]. Nevertheless, considering the noise of the detector (50–80 pA), our results clearly indicate that this detection approach could be very sensitive, e.g., for polyamines. A detailed study focused on the use of an amperometric detector with a copper electrode for the trace determination of amines by CE techniques will be a subject of our future research.

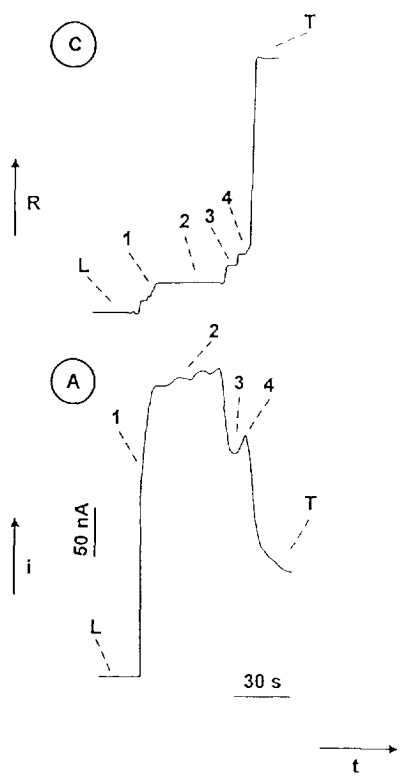

Fig. 9. ITP separation of cationic constituents present in triethylenetetramine of technical grade with amperometric detection at a copper electrode. A 2 μl volume of the polyamine (10^{-3} mol/l concentration) was injected. The numbers (1–4) indicate identical regions on the isotachopherograms. The separations were carried out in electrolyte system No. 3 (Table 1) using the leading electrolyte also for elution. The driving current was 40 μA. The copper electrode was polarized at 200 mV vs. an Ag–AgCl (1 *M* KCl) reference electrode. Other symbols as in Fig. 6.

4. Conclusions

The described postcolumn amperometric detector for CE with galvanic decoupling of the detection electrodes and the detection circuit from the power unit of the detector and from the recording devices alleviates problems due to leak currents flowing from the electrophoretic equipment through the detector. This solution also eliminated false responses of the detector caused by potential changes at the column outlet.

Components used for the decoupling (d.c.–d.c. converter and opto-coupler) serve as devices that protect the detector in situations when a high voltage occurs at the column outlet (e.g., bubble formation in the column). In the detector described here, parameters of the opto-coupler restricted this function to 4–5 kV. Nevertheless, this protective function can be improved by using a more appropriate opto-coupling device and a detector withstanding the full voltage of the high-voltage power supply can be constructed.

In this work the detector was used with a

detection cell with a dead volume ca. 50 times smaller than that described previously [7]. Although a tenfold improvement in the dispersion of the analyte zones could be achieved, the ITP experiments with practical samples indicate that a further decrease is desirable. This, however, requires that the optimum construction of the cell will consider also the signal-to-noise performance of the detector [36].

Although the detector was tested in ITP experiments carried out in a hydrodynamically closed separation compartment, it is apparent that it can be used in CZE and MEKC. It is obvious that the hydrodynamic concept of the separation compartment plays only a minor role in this respect.

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