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Capillary electrophoresis and electrochemical detection with a conventional detector cell

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Abstract

The performance of a commercially available electrochemical detector cell has been evaluated for use in capillary electrophoresis (CE). It was shown that with proper installation of the capillary, the zone broadening caused by the cell is limited by the depletion of the analytes on the electrode surface. Despite the relatively large geometric volume of the cell, its contribution to zone widths was therefore only in the order of 1–2 s. When coupled to a CE set-up, plate numbers up to 100 000 could be obtained. With a 1-mm diameter glassy carbon electrode the detector noise was 3–5 pA, depending on the applied separation voltage. With this noise level detection limits of 10^{-8} mol l^{-1} could be obtained. The cell has been used for the determination of catecholamines, chlorophenols and *o*-phthalaldehyde derivatives of amino acids. © 1998 Elsevier Science B.V.

Keywords: Electrochemical detection; Detection, electrophoresis; Catecholamines; Chlorophenols; Amino acids

1. Introduction

Over the past two decades, one of the most active areas of electroanalytical research has been the development of amperometric detection systems for high-performance liquid chromatography (HPLC) and capillary electrophoresis (CE) [1]. During this period it has been amply shown that electrochemical detection (ED) offers a high sensitivity and selectivity for analytes that are electroactive at a modest potential. As a consequence, ED has become the method of choice for the determination of many easily oxidised compounds such as catecholamines, phenols, thiols and aromatic amines.

In CE, the most widely applied detection method is UV absorption, as this technique is straightforward and almost universally applicable. However, due to the short light path-length with on-line UV detection in CE (the inner diameter of the capillary), concentration detection limits are usually well above 10^{-6} mol l^{-1}. Laser-induced fluorescence (LIF) detection offers a high sensitivity in CE; however, it is expensive and lacks universality. Since the high sensitivity inherent to ED does not depend on the scale of measurement, ED could become an important alternative detection mode for CE.

The first implementation of CE–ED was shown in 1987 by Wallingford and Ewing [2]. After that, several research groups have also shown that CE–ED is in principle possible. Two major problems have to be considered in CE–ED. The first is the possible interference of the electrophoretic voltage with the detector current. Two strategies have been followed to minimise this interference. In end-column detection, where the sensing electrode of the detector is positioned in the same vial as the grounding electrode of the electrophoretic system, very close to the capillary end, the interference can

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be kept small by using very narrow capillaries [3–5]. With off-column detection, the electric field used for the separation is decoupled at some distance along the capillary from the detector. This decoupling can be realised by means of a conductive joint over a gap in the capillary [2,6–8], or with a palladium metal union, as developed in our laboratory [9].

The second problem to be considered was the small volume scale required for the detector. Zone volumes in CE are generally in the order of nanoliters; to preserve the separation efficiency, the detector to be used should also be extremely small. So far, only laboratory-made detector cells have been used for CE–ED, mainly with specially constructed micro-electrodes. With a single carbon fibre electrode positioned into or close to the capillary exit under a microscope, as introduced in microscale HPLC by Knecht et al. [10], the loss of separation efficiency can be kept to a minimum. For special analyses, e.g., in the determination of the content of single nerve cells [11], it is worthwhile to construct and install such a detector cell. However, for routine applications more robust and user-friendly instrumentation would be preferred. A number of integrated capillary–detector systems have been proposed recently [12–14], to avoid the cumbersome positioning of the working electrode. However, such instrumentation is still at the developmental stage.

In previous work we have shown that it is possible to use a detector cell of a somewhat larger scale in CE–ED with a still acceptable separation efficiency. With 0.5 mm diameter working electrodes made of a bundle of carbon fibres [15], conductive carbon cement [16,17] or graphite-epoxy [18], this laboratory-made cell could be used in CE giving plate numbers up to 100 000. Detection limits were obtained in the order of 10^{-7} mol l^{-1}, well below those with UV detection. However, when compared to HPLC–ED, these detection limits were still on the high side. This can be blamed on the electrode materials used; electrodes of the superior material glassy carbon, as generally used in HPLC–ED, were not available.

In recent years a number of electrochemical detectors have been brought on the market intended for micro HPLC. The cell volume with these detectors is well below 1 μl. When regarding their geometric volume as such, their scale still seems too large for application in CE. However, the effective volume and response time of an electrochemical cell depends on the volumetric flow-rate through it [19]. Therefore, with the very low flow-rates encountered in CE, the response rate of such a detector may well be appropriate to monitor the narrow zones emerging from a capillary.

In the study described here we have evaluated the performance of a commercially available electrochemical cell when used in CE. The flow dependency of its response rate was investigated under typical CE conditions. The cell was coupled to a CE system in the off-line mode. With several exemplary analyte types the performance of this CE–ED system, in terms of available separation efficiency, sensitivity and repeatability, was investigated.

2. Experimental

2.1. Apparatus

The electrochemical cell used was a Unijet cell (Bioanalytical Systems, West Lafayette, IN, USA) with a custom-made 1-mm diameter glassy carbon working electrode, and an (in-flow) Ag/AgCl reference electrode. The stainless-steel half of the cell body served as the auxiliary electrode. Spacer gaskets were 16 μm thick.

A Prince programmable injector for CE, including a high-voltage source, was obtained from Prince Technologies (Emmen, Netherlands). Voltages of 25–30 kV were applied. Fused-silica capillaries (Composite Metal Service, Hallow, UK) with inner diameters of 50 and 75 μm were used. In CE experiments the capillaries were 85 cm long. During electrophoresis, a pressure of 20 mbar was applied on the front-end buffer vial to compensate for the back-pressure of the detector system [20]. Samples were injected hydrodynamically, with 75 μm capillaries at 40 mbar for 6 s.

The outlet of the capillary was connected to the electrochemical detector by means of a palladium decoupler, with a design as described previously [17], and a polyether ether ketone (PEEK) coupling tubing of 6 cm×63 μm I.D. A Bioanalytical Systems Petit Ampere LC-3C potentiostat was used. Signals were recorded with a BD 112 model strip chart

recorder (Kipp and Zonen, Delft, Netherlands) and via a HP 35900 A/D convertor board to a Hewlett-Packard Chemstation. For UV detection, a UV–Vis 200 instrument (Linear, Reno, NV, USA) was used.

2.2. Chemicals and solutions

Chemicals used were of analytical grade purity. They were obtained from standard suppliers and used as received. Except when stated otherwise, a 20 mmol l^{-1} ACES–10 mmol l^{-1} NaOH solution with pH 6.7 was used as background electrolyte. All buffer solutions used contained 1 mmol l^{-1} NaCl to stabilize the in-flow reference electrode of the cell. Solutions were filtered over a 0.45 μm filter and deaerated with helium before use.

For the derivatization of amino acids with *o*-phthalaldehyde (OPA) a reagent solution was used containing 7 mmol l^{-1} OPA, 3 mmol l^{-1} 2-mercaptoethanol (2-ME), 5 mmol l^{-1} borax and 20% (v/v) methanol. The reagent was mixed with the amino acid solution in a ratio of 1:1. After 3 min reaction time, the mixture was injected.

3. Results and discussion

3.1. Zone broadening behaviour of the cell

In preliminary experiments it was found that it was very difficult to operate the Unijet cell under CE conditions with a single 16 μm gasket as spacer. Often, the back-pressure of the cell was too high to allow an undisturbed flow (induced by pressure or by the electroosmotic process) through the capillary. Moreover, the back-pressure tended to increase during use of the cell. This resulted in a steadily decreasing apparent electroosmotic (or pressure induced) flow during experiments. Therefore, a double spacer was routinely used. With this, the back-pressure problems were eliminated. The nominal geometric volume of the cell with two spacers is 25 nl.

The cell used in our studies is of the thin-layer type. The response rate of such a cell may depend on the flow regime within the volume close to the electrode surface. The actual behaviour of the cell may be thought of as somewhere between two extremes: that of an ideal mixing chamber and that of a true thin-layer cell. In the first case the response rate will be mainly determined by the time required to flush the electroactive components out of the volume over the working electrode. The contribution of the cell to the width of a zone passing through it can be expressed in this case as:

$$\Delta\sigma^2 = \frac{V_{\text{cell}}^2}{F^2} \quad (1)$$

where $\Delta\sigma^2$ is the increase of the variance of the zone (in squared time units), V_{cell} the effective volume of the cell and F the volumetric flow-rate of the solution.

In the second case (true thin-layer behaviour) the depletion of the electroactive compound by the reaction on the electrode surface will be the rate-limiting factor. The response rate is then not dependent on the size of the electrode or the cell volume. For the depletion process in a one-sided thin layer cell the time constant is equal to $b^2/3D$, where b is the thickness of the cell and D the diffusion coefficient of the analyte. This time constant can be regarded to a close approximation as the standard deviation related to the detection process. Therefore, the increase of a zone variance in this case is approximately given by:

$$\Delta\sigma^2 = \frac{b^4}{9D^2} \quad (2)$$

Eq. (2) shows that with true thin-layer behaviour of the cell the electrode size nor the flow-rate are expected to have an influence on the response rate.

To study the behaviour of the cell, a 50 μm capillary was directly mounted to the detector. Narrow plugs of a solution of an electroactive compound (catechol) where injected and propelled through the capillary by means of pressure. The detection potential was set at +0.6 V, which was found to give the limiting sensitivity for catechol. From the peak widths at half height the peak standard deviations σ were estimated. Peaks were recorded with varying applied pressures giving various residence times. Two series of measurements were performed. For the first series, the mounting of the capillary to the detector cell was done without special care. Visual inspection of the cell under a

magnifying glass showed that the capillary end receded slightly (<0.1 mm) from the surface of the cell half. For the second series, the capillary end was carefully aligned with the face of the cell body half, by pressing them together on the surface of a glass plate, to minimize the dead volume between the capillary end and the electrode surface. The experimental peak variance (σ^2) is shown as a function of the peak time in Fig. 1. Under pressure driven conditions in a thin capillary, the variance of an (initially narrow) band of an analyte when it reaches the detector can be estimated as:

$$\sigma^2 = \frac{d_c^2}{96D} \cdot t \qquad (3)$$

where d_c is the capillary inner diameter and t the residence time of the analyte plug in the capillary. Included in Fig. 1 is the calculated contribution to the peak variance caused by the Poiseuille flow in the capillary. For this, Eq. (3) was applied with an estimated value for the diffusion coefficient of catechol of $7 \cdot 10^{-10}$ m^2 s^{-1}.

In the first series of experiments, performed after installation of the capillary without special care, the detector contribution to the peak width appeared to increase strongly with the peak time. This is an indication of mixing chamber behaviour, as described in Eq. (1). However, after proper aligning of the capillary the peaks measured were narrower and the influence of the flow-rate on the detector contribution to the peak variance was considerably smaller (series b in Fig. 1). In this series of measurements the cell approached a true thin-layer behaviour (Eq. (2)).

In the following experiments, the capillary was connected to the detector cell via the palladium decoupler and the PEEK coupling tubing. Since it was clear from previous studies [9,16,17] that the importance of extra-column zone broadening in CE–ED decreases with increasing diameter of the separation capillary, now a 75 μm I.D. capillary was used. The different parts of the set-up were connected a number of times and after each reinstallation a series of measurements at varying pressures, applied to propel the sample plug, was carried out. The results of these measurements are shown in Fig. 2. The mounting of the various parts of the system appeared to be well reproducible. Standard deviations of the peaks, measured with comparable flow-rates, differed less than 5% between the measurement series. Fig. 2 shows that with 75 μm I.D. capillaries the behaviour of the cell approaches even more that of a thin-layer cell. The response time of the detector could be limited to 1–2 s. With typical migration times in CE of 5–10 min, it seemed possible to obtain plate numbers in the order of 100 000 with this detector cell.

The coulometric yield of the electrode process

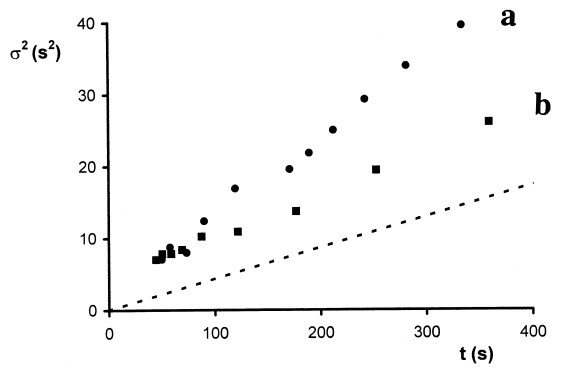

Fig. 1. Influence of the positioning of the capillary on the detector contribution to the peak variance. For experimental conditions see Section 2. The different marks indicate different measurement series after reinstallation of the cell. The dotted line gives the calculated variance contribution of the laminar flow in the 50 μm I.D. capillary as a function of the residence time of the analyte.

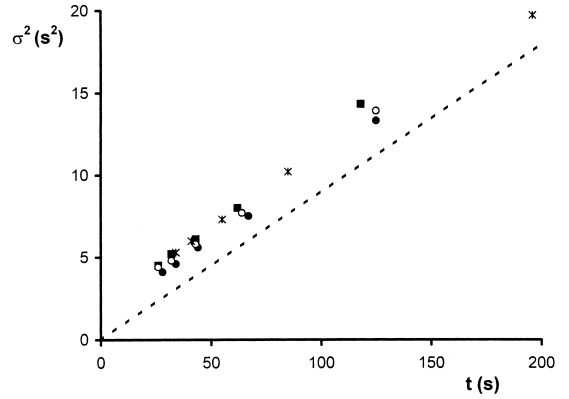

Fig. 2. Zone variance contribution of the detection system including decoupler. The different marks indicate different measurement series after reinstallation of the cell. The dotted line gives the calculated variance contribution of the 75 μm I.D. capillary.

was, within the uncertainty of the injection volume, at or close to 100% for all solutes studied. This is another indication for the thin-layer behaviour of the cell, since apparently the depletion of the electroactive compounds at the electrode surface is complete.

3.2. Baseline stability and noise

The electrochemical reaction taking place in the decoupler during electrophoresis causes an upward pH shift of the background electrolyte (BGE) [9]. When such a pH shift is excessive, the detector baseline signal may increase strongly after switching on the high voltage source, because the oxidative background current is expected to increase with the pH of the solution. It appeared that the baseline could be kept in control better: (i) with a relatively high electroosmotic flow (EOF), i.e., with a high pH of the BGE; (ii) with a relatively high concentration of the acidic component of the buffer in the BGE, i.e., with a BGE pH below the pK_a of the buffer, and (iii) when high concentrations of non-buffering salts such as sodium dodecyl sulfate (SDS) are avoided. A few seconds after the voltage source was turned on, a transient rise of the baseline was generally observed due to the changing pH at the electrode surface, but the signal returned to the original level within a few minutes.

As previously with other detector cells and electrode materials [9,16], it was found that the detector noise increased with the applied separation voltage (see Table 1). Apparently the decoupling of the separation voltage was not complete. Another explanation of the influence of the electrophoresis voltage on the detector noise may be that a ripple in the separation voltage is transferred to the detector through a fluctuation of the EOF. The noise level depended only marginally on the detection potential in the range from +0.4 to +0.9 V.

The frequency of the noise was fairly high; as the time constant of the measuring circuitry was increased, a reduction in the noise level could be obtained (see Table 1). In further measurements a damping constant of 0.5 s was used as a compromise between low detection limits on one hand and a high efficiency on the other.

Table 1
Influence of the separation voltage and the damping time constant on the detector noise

Separation voltage (kV)	Time constant (s)	Noise[a] (pA)
0[b]	0.5	1.5
10	0.5	2
20	0.5	3
30	0.5	5
30	0.2	8
30	0.5	5
30	1.0	3

[a] Peak-to-peak noise.
[b] With pressure driven flow.

3.3. Applications

The performance of the detector in CE was evaluated with a number of standard applications. In Fig. 3 an electropherogram is shown obtained in the separation of catechol compounds. A borate buffer with a pH of 10 was used as BGE, so that the catechol compounds carried a small negative charge and migrated against the EOF. EDTA (0.5 mmol l^{-1}) was added to the BGE to prevent the oxidation

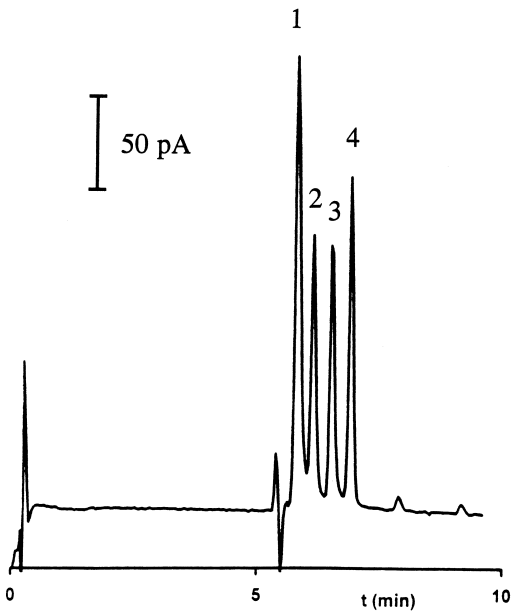

Fig. 3. The separation of catecholic compounds. Applied voltage: 25 kV. Detection potential: +0.8 V. For other experimental conditions see Section 2. Sample concentration: $5 \cdot 10^{-7}$ mol l^{-1} for each compound. Peaks: (1) DA; (2) E; (3) NE and (4) DOPAG.

of the solutes during electrophoresis. The peaks for the basic compounds (catecholamines) showed some tailing, probably by adsorption of these compounds on the capillary wall. Plate numbers were in the order of 35 000 to 50 000. This is somewhat lower than in other applications (see below). This can be explained partly as the result of adsorption, and partly because of the relatively short migration times of these analytes. In such a case the time constant of the detection process is relatively of more importance. Detection limits for these compounds were between $1 \cdot 10^{-8}$ and $2 \cdot 10^{-8}$ mol l^{-1}. This is lower by a factor of 50 compared to what was obtained previously, for chemically similar compounds, with a laboratory-made electrochemical detector cell [9].

Fig. 4 shows the separation of a number of chlorophenols, separated in an ACES buffer of pH 6. To the BGE 20 mmol l^{-1} of SDS was added to obtain additional separation selectivity by the micellar distribution process. Detection was performed at +0.8 V. In this application plate numbers up to 60 000 were obtained. The higher conductivity of this BGE, because of the presence of SDS, resulted in an increased noise level. Detection limits for the chlorophenols were in the order of $2 \cdot 10^{-8}$–$3 \cdot 10^{-8}$ mol l^{-1}, which is lower by a factor of 5 compared to previous results obtained with a laboratory-made cell and a graphite–epoxy electrode [18].

Another application studied was the determination of OPA derivatives of amino acids. This application was chosen because it enabled us to compare the performance of various detection principles in CE: UV detection, fluorescence detection and ED. The OPA derivatives were separated with a BGE containing a borate buffer (pH=9.5) and 20 mmol l^{-1} SDS. A detection potential of +0.5 V was applied. Fig. 5 shows a typical electropherogram. Plate numbers in the order of 80 000 to 100 000 were obtained. The excess of 2-ME in the reaction mixture caused a high, broad peak in the electropherogram

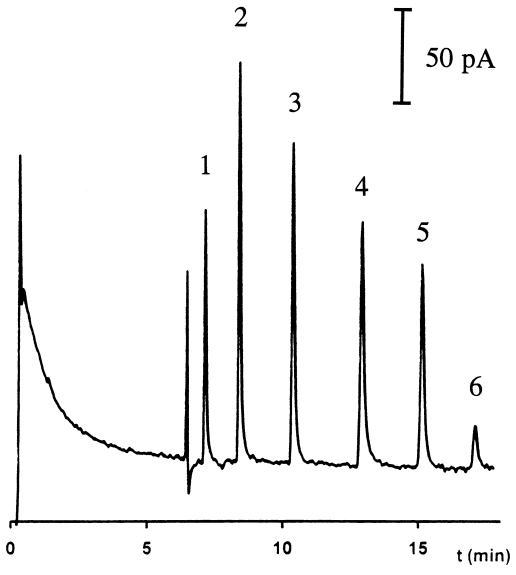

Fig. 4. The separation of chlorophenols. Applied voltage: 30 kV. Detection potential: +0.8 V. For other experimental conditions see Section 2. Sample concentration: $5 \cdot 10^{-7}$ mol l^{-1} for each compound. Peaks: (1) phenol; (2) 2-CP; (3) 2,6-diCP (4) 2,4-diCP; (5) 2,4,6-triCP and (6) pentaCP.

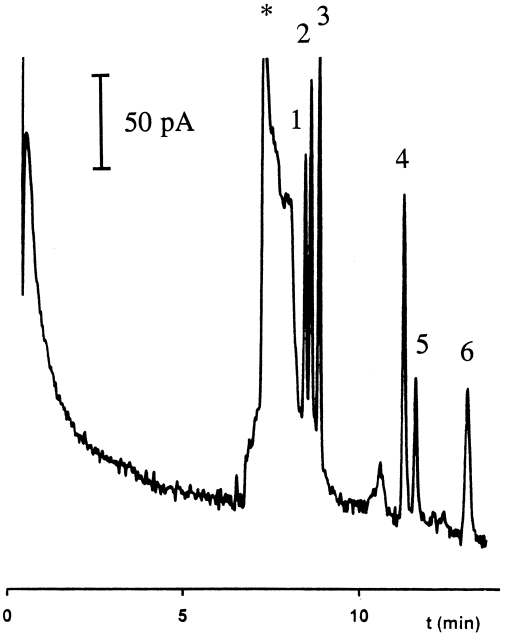

Fig. 5. The separation of OPA-derivatised amino acids. Applied voltage: 28 kV. Detection potential: +0.5 V. For other experimental conditions see Section 2. Sample concentration: 10^{-6} mol l^{-1} for each compound in the reaction mixture. Peaks: (1) His; (2) Ser; (3) Gly; (4) Glu; (5) Asp and (6) Lys. The peak indicated with an asterix stems from the excess of 2-ME in the reaction mixture.

Table 2
Comparison of detection limits obtained for OPA-derivatized amino acids with different detection modes

Detector	LOD[a] (mol l^{-1})	Ref.
UV, 335 nm	$5 \cdot 10^{-6}$–$10 \cdot 10^{-6}$	This work
UV, 220 nm	$5 \cdot 10^{-7}$–$10 \cdot 10^{-7}$	This work
Modified LC fluorescence detector	$1.5 \cdot 10^{-6}$–$3 \cdot 10^{-6}$	[21]
CE fluorescence detector	$1.5 \cdot 10^{-8}$–$3 \cdot 10^{-8}$	[22]
Electrochemical	$1 \cdot 10^{-7}$–$2 \cdot 10^{-7}$	This work

[a] Concentration detection limits obtained with a 75 μm I.D. capillary, $S/N = 2$.

Table 3
Performance of the CE–ED system studied

	Plate number	LOD[a] (mol l^{-1})	Peak area repeatability[b]
Catecholamines	35 000–50 000	$1 \cdot 10^{-8}$–$2 \cdot 10^{-8}$	<1%
Chlorophenols	50 000–60 000	$2 \cdot 10^{-8}$–$3 \cdot 10^{-8}$	1–2%
OPA derivatives	80 000–100 000	$1 \cdot 10^{-7}$–$2 \cdot 10^{-7}$	3%[c]

[a] Concentration limit of detection, $S/N = 2$.
[b] Relative standard deviation, $n = 5$.
[c] Including derivatization.

that interfered with early amino acid peaks (see Fig. 5). The noise level under these conditions was considerably higher than in other applications. This was probably related to the combination of a high pH and a high conductivity of the BGE. Detection limits were in the order of $1 \cdot 10^{-7}$–$2 \cdot 10^{-7}$ mol l^{-1} for most of the derivatives.

Detection limits for the OPA derivatives were also assessed using UV detection, at the selective wavelength of 335 nm, as well as at 220 nm, where a higher sensitivity was obtained. The results of the present study and those obtained previously with fluorescence detection can be compared in Table 2. Only with fluorescence detection specially designed for CE the detection limits are lower than with ED.

The repeatability of the CE–ED system was investigated by repeated injection of standard solutions and measuring peak areas. Except for the OPA derivatives of amino acids, the relative standard deviations were in the order of 1% ($n = 5$). With the OPA derivatives the repeatability was less; however, in this application the error by the derivatization procedure was included in the results. The performance parameters of the CE–ED system obtained in the various applications are summarized in Table 3.

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