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End-column electrochemical detection for inorganic and organic species in high-voltage capillary electrophoresis

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ABSTRACT

The electronics and construction are described for an end-column ultramicroelectrode (3–10 μm) detection system that permits the use of medium-sized capillaries (25 μm I.D.) without appreciable effects from the potential field at the end of the capillary. Normal peak-to-peak noise over 10 s was 0.01–0.1 pA. The background noise observed for a 200 × 10 μm carbon-fiber electrode placed either 180 μm within a 25-μm capillary or at a point 500 μm away from the capillary was essentially the same. A study of detector response as a function of the position of the electrode has shown that accurate location of the electrode is important for sensitive and reproducible detection. These studies also showed that differences between the density of the electrolyte exiting the capillary and the electrolyte in the detection cell could cause anomalous electrode response depending on the location of the electrode relative to the end of the capillary. Application of a carbon fiber or an Hg film electrode gave detection limits (twice the peak-to-peak noise over 10 s) of $2 \cdot 10^{-8}$ mol/l for Pb^{2+}, $1 \cdot 10^{-5}$ mol/l for NO_2^- and $5 \cdot 10^{-10}$ mol/l for catechol.

INTRODUCTION

Capillary electrophoresis (CE) has recently attracted considerable attention in many areas of analytical chemistry [1,2]. Due to its uniform flow profile, high separation efficiencies have been obtained for large molecules, such as proteins with small diffusion coefficients (close to $1 \cdot 10^6$ theoretical plates) [3] or for small species, such as inorganic ions (several hundred thousand theoretical plates) [4,5]. Another potential advantage of CE is that very small sample volumes can be analyzed; sample volumes below the attoliter range has been reported [6]. To make full use of these features, sensitive detection techniques are required. UV absorbance is widely employed in CE, but sensitivity is limited by the path length and detection volume, particularly when a small-I.D. capillary is used. Laser-based fluorescence detectors can provide more sensitive detection, but are normally limited to analytes which fluoresce. This is a disadvantage for inorganic species because very few inorganic ions exhibit fluorescence. Electrochemical detection can offer some advantages over these techniques. If ultramicroelectrodes (~10 μm) are used, detector response will not be limited by a small detection volume. Other attractive features of ultramicroelectrodes include high sensitivity, good selectivity and low cost. The first off-column electrochemical detector used in high-voltage CE was developed by Wallingford and Ewing [7,8]. Later similar designs were reported by Huang and Zare [9] and O'Shea *et al.* [10]. In these systems, porous glass or Nafion tubing was used to connect the separation capillary with a short piece of capillary used for detection. In this way the separation current passing through the capillary was separated from the detection cell, and this procedure was reported to be necessary to reduce the background noise. However, even in these systems it was observed that the background noise was proportional to the applied high voltage [7,10]. In spite of this, these systems worked well for the detection of organic compounds, and a detection limit of $6 \cdot 10^{-9}$ mol/l for hydroquinone was reported. The major drawback of these systems was the diffi-

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culty associated with making the porous joint. One alternative, termed 'end-column detection', has been reported [11]. In this technique the electrode is placed outside of the end of the separation capillary, and a porous joint is not used. It was stated that when a small capillary (5 μm) is used, the separation current will be very small and it will not significantly affect the detection current [11]. The use of a very small capillary increases experimental difficulties for routine work, because processes such as washing and drawing electrolyte through the capillary become very difficult. This paper will describe the results obtained for electrochemical detection with 10–25 μm capillaries without a porous-joint system. The detection of inorganic ions and catechol with carbon fiber or Hg-film electrodes was used to illustrate the potential of this electrode system.

Since very small currents (pA range) are produced with ultramicroelectrodes, careful consideration must be given to potential sources of noise. Important aspects that require attention in the design of such systems are discussed briefly, and the relationship between detector performance and positioning of the electrode has been studied. In previous reports it has been suggested that the position and area of the microelectrode relative to the bore of the capillary may be very important in amperometric detection [11,12]. It is expected that poor alignment of electrode with the capillary will significantly affect both column efficiency and detection limits, but to date no quantitative data have been reported on these effects.

EXPERIMENTAL

Apparatus

Polyimide-coated fused-silica capillaries, 10–25 μm I.D., were obtained from Polymicro Technology (Phoenix, AZ, USA). Before use the capillaries were washed with water–acetonitrile (50:50, v/v) and operating electrolyte. The high-voltage power supply, with reversible polarity (0 to *ca.* 30 kV), was obtained from Spellman (Model RHR30PN30, Plainview, NY, USA). The voltage input was housed in a Plexiglas box with an interlock on the access door to protect the operator. The detection cell and detector were housed in a faradaic cage to minimize the interference from external sources of noise. Electrochemical detection was based on a chronoamperometric mode with a three-electrode system. Detection was performed at the end of the separation capillary. The detection process was controlled via a 386DX/40MHz IBM personal computer equipped with PCL-818 or PCL-812 high-performance data acquisition card (B & C Microsystem, Sunnyvale, CA, USA). The computer programs, which controlled the application of potential to the electrode, the collection and display of the data, and the deposition of Hg for Hg-film electrodes, were written locally. In addition, this computer software includes programs for cyclic voltammetry and pulsed electrochemical techniques.

The electronic circuit diagram of the potentiostat for the home-made detector (cost about US$ 100) that was used in this work is shown Fig. 1. All resistors (metal film) had an error of ±1% and the amplifiers were LF353N wide-bandwidth dual-JFET operational amplifiers (Electronics, Toronto, Canada). This circuit is basically composed of a summing inverting amplifier (No. 1) and a differential amplifying circuit with a gain of 100 (Nos. 2, 3 and 4). A high-impedance circuit (amplifiers 5 and 6) was placed in the reference-electrode input circuit to ensure that the current flowing through the capillary did not pass through the reference electrode. This design minimized drift and noise in the reference system, and also helped to eliminate the formation of a ground loop between the reference electrode and ground [13]. To minimize electronic noise, low-pass filters were placed in the output circuit and the working-electrode input circuit. These filters reduced the noise with only a small loss in the separation efficiency (maximum time constant of the filters was 0.1 s). Other strategies used to further decrease background noise included computer software signal averaging, low-noise cables between the potentiostat and electrodes, faradaic cage screening and elimination of possible ground loops. A discussion of some of the factors important in the successful measurement of small currents (pA range) has been given elsewhere [14]. The voltage produced by the signal current was amplified via Nos. 2, 3 and 4 operational amplifiers and sent to the A/D convertor with the minimum step size corresponding to 0.015 pA. The data obtained were monitored in real time on the computer screen, and peak areas were determined as a summation of rectangular peak-area increments over the width of the peak.

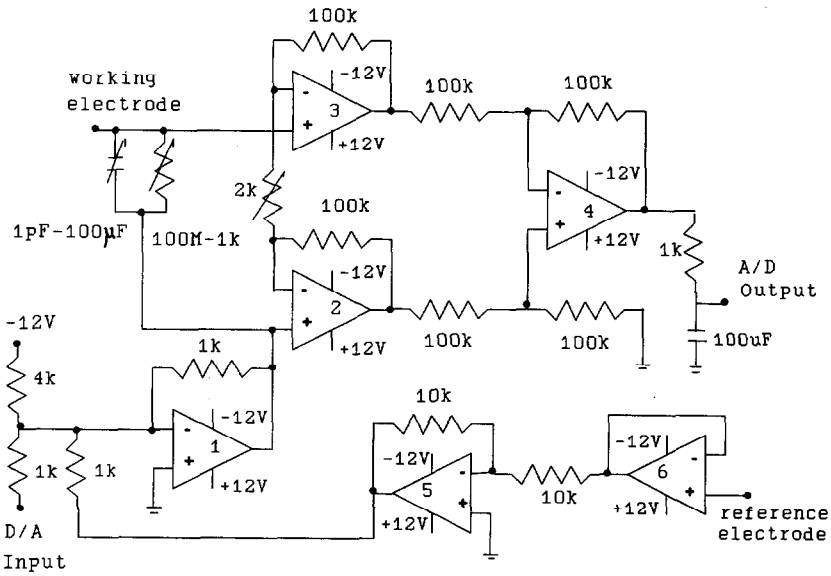

Fig. 1. Electronic circuit diagram for the detector. k = kΩ; M = 10^6 Ω.

Ultramicroelectrodes and detection cell

The ultramicro carbon working electrode was made from a single carbon fiber (10 μm I.D.), which was obtained from Amoco Performance Products (Greenville, USA). A fiber was put into a glass tube with an I.D. of about 0.5 mm at the tip. The tip was sealed with 5-min epoxy glue, and before the glue was dry the carbon fiber was pushed into the tube (under a microscope) until the desired exposed length was left. The length of the exposed fiber was normally in the range 200–400 μm. The carbon fiber was connected to a copper lead via a mercury junction. Ultramicro Hg-film electrodes were made as described elsewhere [15]. The auxiliary electrode was a platinum foil with an exposed surface area of 0.5 cm^2; this electrode also served as the ground for potential drop through the capillary. A saturated calomel electrode (SCE) (Miniature model, Fisher, Ottawa, Canada) was employed as the reference electrode.

To prepare a capillary-electrode detection system a 0.5 ml polyethylene vial was attached to a metal plate that was fastened to the same stand as a XYZ micropositioner (Model MR3, Klinger, Garden City, NY, USA). Fig. 2 shows the arrangement of the electrode cell and the capillary. The microelectrode was fixed above the tube with a small metal bar screwed onto the plate (see Fig. 2) with the exposed carbon fiber 0.2–1.5 mm above the top of the tube. The capillary was mounted on the micropositioner and its position was adjusted (under a microscope) against the end of the capillary. This arrangement allowed one to easily remove and realign both the capillary and the microelectrode. The exact position between the capillary and the electrode was measured with an optical scale under a microscope (to within 1–3 μm) from both a top view and side view direction. Electrolyte, which was added to cover both the capillary and the electrode, was held above the top of the polyethylene tube by surface tension. The reference and auxiliary electrodes were placed into the top of the solution as shown in Fig. 2. This assembly was used in the studies of the effects of alignment on detector performance. For more routine operation the capillary and the ultramicro working electrode were placed on a glass plate; the desired position between the capillary and the electrode was adjusted using the micropositioner (under a microscope), and a wall of epoxy was used to both fix the cell alignment and to function as an electrolyte reservoir for the reference and auxiliary electrodes.

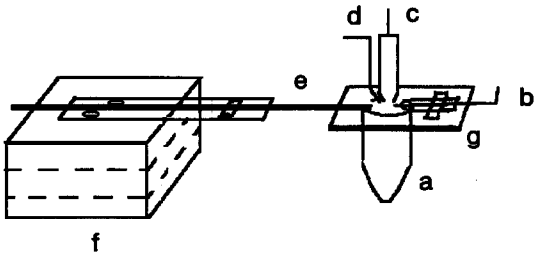

Fig. 2. View of the detection cell assembly. a = Plastic vial; b = carbon-fiber electrode; c = reference electrode; d = counter electrode; e = capillary; f = micropositioner; g = metal plate. The metal plate was fastened to the same heavy stand (not shown in diagram) used to hold the micropositioner.

Chemicals

All solutions were prepared from double-distilled, deionized water (Corning, Mega-Pure system, MP-6A and D2, New York, NY, USA). The background electrolyte for separation and detection of transition metal ions was similar to that used for the CE separation of the lanthanide series of metal ions [4]; this electrolyte was 0.005 mol/l N,N-dimethylbenzylamine, 0.0065 mol/l α-hydroxyisobutyric acid (HIBA) (98%, Aldrich, Milwaukee, WI, USA) and the pH value was adjusted with acetic acid to 4.90. The stock phosphate buffer was prepared by mixing 0.01 mol/l monosodium phosphate, phosphoric acid (BDH, Toronto, Canada) and 0.01 mol/l sodium dodecylsulfate (SDS) (99%, Sigma, St. Louis, MO, USA). The desired pH value (6.95) of the buffer was adjusted with NaOH. This solution was used in the separation and detection of catechol. The electrolyte in the separation reservoir was replaced every day to avoid chemical and pH changes in the electrolyte. The electrolyte used for the separation of NO_2^- was 0.005 mol/l sodium phosphate (BDH) plus 0.005 mol/l cetyltrimethylammonium chloride (CTAC) (Aldrich) at pH 6.52. All pH values were measured with a combination glass electrode calibrated at pH 4.0 and 7.0 (Aldrich, hydrion dry buffers). Thallium chloride, lead acetate, cadmium chloride, copper chloride and sodium nitrite (Aldrich) solutions were prepared as a 0.01 mol/l stock solutions. Catechol (Sigma) stock solutions were 0.01 mol/l, and were made 0.1 mol/l in perchloric acid. Samples were diluted to the desired concentration with operating electrolyte prior to use. All solutions, including electrolytes and samples, were filtered through a 0.2-μm Nylon-66 membrane syringe filter (Cole-Parmer, Chicago, IL, USA).

RESULTS AND DISCUSSION

Effect of electrode alignment

The dependence of the detector response (peak height) on the electrode alignment at the end of a 60 cm × 25 μm capillary was examined. The detector assymbly was arranged as shown in Fig. 2. The starting position of the tip of the carbon fiber (200 μm length) was at the center of the capillary and less than 10 μm from the end; at this position the measured signal current was assigned a value of 1. The error in determining the positions was *ca.* 1–3 μm. The position of the capillary was fixed and the position of carbon electrode was adjusted with the micropositioner. The signal obtained for a migrating sample zone was measured as the electrode was moved up, down, to the side, away in the axial direction and inside the capillary; currents was measured until its value decreased to about 10% of the maximum current. The results of this study are shown in Figs. 3 and 4; each point is the average of at least two measurements and the errors observed for the relative response was ~5%.

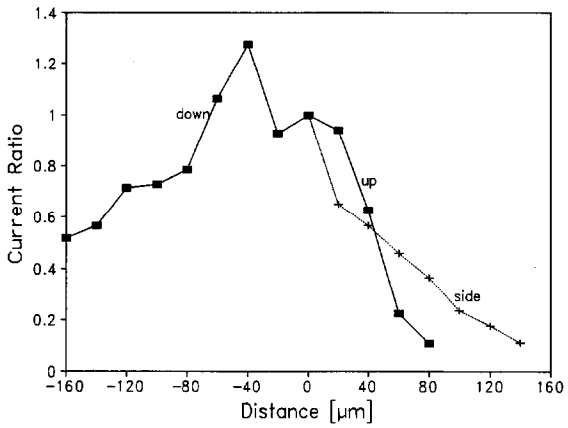

Fig. 3. Dependence of the relative detection sensitivity on the electrode alignment with the bore of a 25 μm I.D. capillary. The current obtained with the electrode at the center position just outside the capillary was given a value of 1.0. Experimental conditions: concentration of analyte, $1 \cdot 10^{-7}$ or $1 \cdot 10^{-5}$ mol/l; separation voltage, 30 kV; electrochemical detection potential, 700 mV; injection, 30 kV for 5 s; 60 cm × 25 μm capillary; electrode, 200 μm × 10 μm carbon fiber.

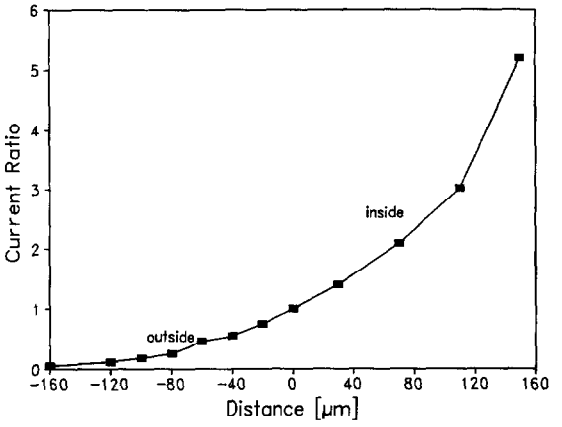

Fig. 4. Dependence of the relative detection sensitivity with axial position of the electrode. Conditions as for Fig. 3.

As expected, as the electrode was moved away upwards or to the side, the detection current decreased with the distance from the center point (see Figs. 3 and 4). When the analyte zone moves out of a capillary the influence of the electroosmotic and electrophoretic forces will stop immediately, and the analyte zone will then be pushed by the electroosmotic flow of the electrolyte still inside the capillary. This action will produce some convection and mixing of the analyte zone as it migrates into the solution in the detection cell. In addition to convection, analyte will diffuse in all directions away from the end of the capillary. When the electrode was positioned in the front of the capillary and then moved into the capillary, the detection sensitivity increased approximately five-fold (Fig. 4), because more electrode surface was exposed to the analyte, which gave improved electrochemical efficiency.

When the electrode was moved down from the center point (Fig. 3), the detection current first passed through a maximum value and then decreased continuously. It was found that the location of this current maximum could be changed to above or below the capillary depending on the density (change in electrolyte concentration) of the solution in the detection cell. The decrease in current past the maximum was slower than that observed in other directions, and was still 10% of the maximum value at a distance of 500 μm. As the analyte zone moves away from the tip of the capillary under the influence of a density difference, its velocity will increase with distance, and thus analyte transfer to the electrode surface will increase with electrode displacement from the center of the capillary. As the distance increases, however, convective mixing begins to dominate, and thus the signal will eventually begin to decrease. The results in Figs. 3 and 4 show that careful alignment of the electrode assembly is important for reproducible results. Once an electrode assembly is aligned it must be fixed firmly in that position.

Under optimum conditions a peak-to-peak noise of 0.01 pA (10 s) was observed when the amplifier was set at its highest sensitivity setting. These noise levels, which are better than or equal to those reported recently elsewhere [1,10], were not obtainable without the high-impedance reference input (see Experimental). These noise levels are of the same order of magnitude as that expected from the thermal noise in resistors, and further reductions in noise levels may require special low-noise circuits. The noise level observed with an electrochemical detection system depends on a number of factors such as electrode material, electrode size, potential applied to the electrode and electrolyte composition. Consequently the normal range observed for noise levels varied from 0.1 to 0.2 pA, depending on experimental conditions. When the effect of electrode position on the signal-to-noise ratio was evaluated with a 200 μm × 10 μm carbon fiber electrode, it was found that there was no obvious trend in the noise level as the electrode was moved down to 360 μm from the center point of a 25-μm capillary. In addition, the background noise observed when the electrode was placed 180 μm inside the end of the capillary was <0.1 pA with and without the application of a 30-kV separation voltage. Smaller capillaries (10 μm) were also used, but no significant decrease in the noise was observed. This is in contrast to other detection systems where the small capillaries (5 μm) were used to reduce background noise [11]. Changes in signal-to-noise ratio within the capillary should follow the same trend as the current ratio shown in Fig. 4. It was observed that for all the electrode positions in this study, the value of the separation voltage had small effects on the absolute level of the background signal, but had no discernable relationship with the value of the peak-to-peak noise.

The effect of electrode alignment on the column

efficiency was also measured. As expected column efficiency decreased as the electrode was moved away from the capillary. For example, when the electrode was moved from the center point downward by 200 μm the efficiency decreased about 80% due to convection and diffusion effects. The same trend was observed for other directions except when the electrode was moved into the capillary; from 0 to 150 μm inside the capillary, the efficiency increased by *ca.* 30%.

The detection sensitivity of a disk-shaped carbon electrode, made by breaking the carbon fiber on a cylindrical electrode, was also determined. Under similar experimental conditions, the sensitivity decreased approximately 40 times with a disk carbon electrode in comparison with cylindrical carbon fiber electrodes (about 250 μm in length). Since the ratio of surface areas of the two electrodes is about 100, this suggests that only about half of the area of the cylindrical electrode is in contact with the analyte. The peak-to-peak background noise observed with the disk electrode was essentially the same as for the cylindrical electrode. The dependence of detection sensitivity and signal-to-noise ratio on the position of the disk-shaped carbon electrode followed the same trend as that for cylindrical electrodes.

Detection of inorganic and organic ions

The performance of the electrode systems was tested for various inorganic ions and catechol; catechol was chosen since this compound was used previously in evaluations of electrochemical detectors for CE, and its use here permits a comparison with previous results [7,8,11]. Fig. 5 shows the electropherogram for the separation and detection of four transition metal ions at an ultramicro Hg-film electrode, 5 μm in radius with a thickness of 3 μm on a gold substrate. Electroreduction was used to detect these metal ions at −1100 mV *versus* SCE. The electrode was placed at the center of the outlet of the capillary. Separations were performed on 60 cm × 25 μm capillaries. A buffer of N,N-dimethylbenzylamine with HIBA was used as the background electrolyte; HIBA was employed as a complexing counter-ion to improve separation selectivity for the metal ions [4,5]. The sample mixture was injected by electromigration at 10 kV for 2 s; the concentrations were $1 \cdot 10^{-6}$ mol/l for Pb^{2+} and $1 \cdot 10^{-5}$ mol/l for other ions. The signal-to-noise ratio for Tl^{+}, Pb^{2+} and Cd^{2+} was larger than 100, and for Cu^{2+} was about 30. The detection limits for these transition metal ions were up to 100 times lower than those reported [16] for UV detection (*ca.* 8 fold for Cd^{2+} and *ca.* 100 fold for Pb^{2+}). The number of theoretical plates (calculated from peak width measured at the half peak height) was approximately $8.5 \cdot 10^{4}$ for Tl^{+}, $1.5 \cdot 10^{5}$ for Pb^{2+}, $3.6 \cdot 10^{5}$ for Cd^{2+} and $7.0 \cdot 10^{4}$ for Cu^{2+}. It should be noted that at the potential used (−1100 mV) oxygen was reduced at the electrode, and consequently high background currents were observed (note background current in Fig. 5 relative to that in Figs. 6 and 7). Thus the detection limits for these elements would be improved if the effects of oxygen were removed. The efficiency for Cd^{2+} is approximately twice that reported previously for end-column conductivity detection with 50-μm capillaries [17].

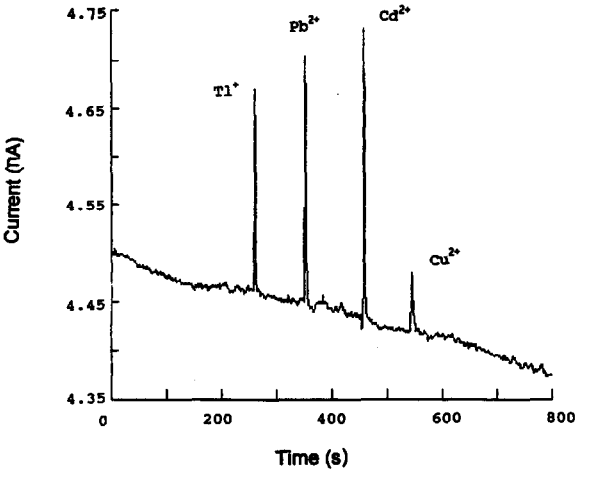

Fig. 5. Electropherogram of transition metal ions with detection at an ultramicro Hg-film electrode (10 × 3 μm). Experimental conditions: concentration of analyte, $1 \cdot 10^{-6}$ mol/l for Pb^{2+} and $1 \cdot 10^{-5}$ mol/l for other ions; separation voltage, 30 kV; electrochemical detection potential, −1100 mV; injection, 10 kV for 2 s.

Separation efficiency was also studied for an inorganic anion, NO_2^-; phosphate with CTAC was used as the operating electrolyte. CTAC was used to modify the internal wall of the capillary and reverse the direction of electroosmotic flow. A cylindrical carbon fiber electrode was held at 700 mV *versus* SCE to detect NO_2^- by electrooxidation. Fig. 6

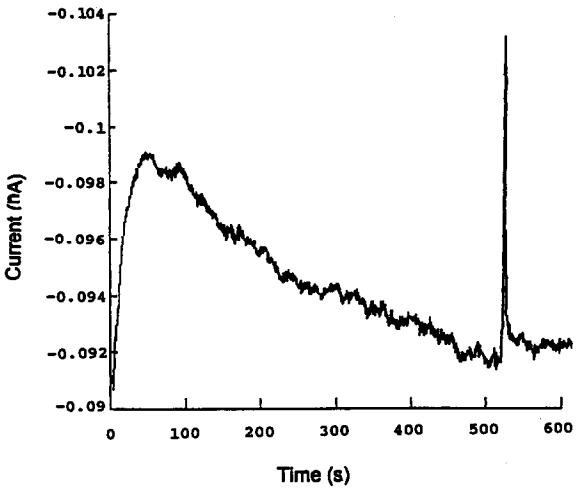

Fig. 6. Electropherogram of NO_2^- with detection at a carbon-fiber electrode. Experimental conditions: concentration of analyte, $1 \cdot 10^{-4}$ mol/l; separation voltage, 8 kV; electrochemical detection potential, 700 mV; injection, 8 kV for 1 s; 55 cm × 25 μm capillary; electrode, 200 × 10 μm carbon fiber.

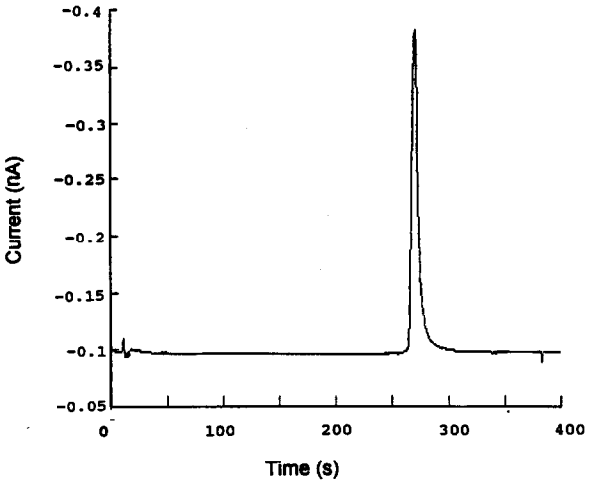

Fig. 7. Electropherogram of catechol with detection at a carbon-fiber electrode. Experimental conditions: concentration of analyte, $1 \cdot 10^{-7}$ mol/l; separation voltage, 30 kV; electrochemical detection potential, 700 mV; injection, 30 kV for 5 s; 60 cm × 25 μm capillary; electrode, 200 × 10 μm carbon fiber.

shows an example of the NO_2^- separation. The signal-to-noise ratio was about 20. The number of theoretical plates was $1.5 \cdot 10^6$, which corresponds to $2.8 \cdot 10^6$ plates per meter. It was observed that the column efficiency increased with a decrease in the separation voltage: plate numbers were $1.9 \cdot 10^5$ at 30 kV, $2.7 \cdot 10^5$ at 20 kV and $6.0 \cdot 10^5$ at 10 kV. This is opposite to the trend expected from longitudinal diffusion effects, and it is unlikely that Joule heating [18] would cause such large effects in a 25-μm capillary. Surface adsorption of the negative charged NO_2^- onto the positively charged surface of the capillary (due to presence of CTAC) may be a factor, but further studies with different concentrations of CTAC would be required to establish if this is the cause of this pattern.

The detection of catechol was tested with a cylindrical carbon-fiber electrode with an electrolyte containing 0.01 mol/l sodium phosphate and 0.01 mol/l SDS at pH 6.95. Fig. 7 shows an electropherogram for catechol with a signal-to-noise ration of ~1000. When a more dilute solution (10^{-8} mol/l) was sampled it was found that the detection limit (twice the peak-to-peak noise) was $5 \cdot 10^{-10}$ mol/l. The sample volume used here was *ca.* 2.2 nl (8 kV for 10 s), which corresponds to a detection limit of 1.1 amol. This compares favourably with a value of 56 amol reported recently for catechol [11] where a 5-μm capillary was used to reduce noise and enhance mass detection limits.

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