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RECEIVED for review February 20, 1990. Revised manuscript received July 9, 1990. Accepted September 21, 1990. This work was supported by the National Oceanographic and Atmospheric Administration under Cooperative Agreement No. NA85-WCH-06134.

End-Column Detection for Capillary Zone Electrophoresis

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INTRODUCTION

Zone electrophoresis in capillaries (*1–5*) has become an important technique in the repertoire of liquid-phase separations. Capillary electrophoresis has been used for separations of small and large molecules and comprises several subtechniques including capillary zone electrophoresis (CZE), capillary gel electrophoresis, micellar electrokinetic capillary electrophoresis, and capillary isoelectric focusing. CZE employs extremely high potential fields, resulting in highly efficient separations of ionic solutes.

A major aspect of CZE in need of new development is detection. Detection schemes developed to date include direct and indirect UV absorption (*6, 7*), fluorescence (*8, 9*), and radioisotope (*10*), as well as mass spectrometric (*11–14*), and electrochemical (*15–17*) detectors. Electrochemical detection schemes include methods involving conductivity measurements and amperometry. Existing electrochemical detectors for CZE use elaborate on-column and postcolumn detection schemes to prevent the high separation potentials used from interfering with the electrochemical process. One scheme involves construction of 40 μm diameter holes in the capillary using a laser (*16*). Small platinum wires are then placed in these holes to carry out on-column conductivity detection. Exact placement of small platinum electrodes on opposite sides of the capillary for on-column conductivity detection is critical to minimize background noise associated with the high potential field used for separation. Another scheme involves covering a crack in the capillary with a porous glass capillary (*17*) to provide off-column amperometric detection. Although these techniques work well with direct and indirect electrochemical detection, the problem arises of how to produce such structures reliably and inexpensively. This limitation has held back the routine application of both modes of electrochemical detection in CZE, although some reports of their use exist in the literature (*3, 5, 18–24*).

We describe here a new design for CZE conductimetric and amperometric detectors in which a sensing microelectrode is placed at the outlet of the fused-silica capillary. These "end-column detectors" are easy to construct. They do not suffer from electrical interference caused by the applied high voltage during the CZE separation. Amperometric detection has been accomplished by this method in narrow-bore (5 μm i.d.) capillaries where the electrophoretic current through the capillary is very small (1–15 nA) and electrical interference in minimized. Conductivity detection does not appear to be as sensitive to the electrophoretic current and associated potential fields. Hence, it has been carried out in larger diameter (80 μm i.d.) capillaries. End-column detectors demonstrate sensitivities approaching those of previous on-column conductivity and postcolumn amperometric detectors (*3, 16, 18*) with only a small sacrifice in resolution. Under typical operating conditions the use of an end-column CZE conductivity detector is found to cause extra zone broadening only about 25% larger than that associated with an on-column CZE conductivity detector. End-column amperometry provides detection limits as low as 56 amol with an efficiency of 143 000 theoretical plates for catechol.

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EXPERIMENTAL SECTION

Apparatus. *Conductivity Detection.* The end-column conductivity detector was placed directly at the outlet of the CZE capillary (Figure 1a). In these experiments the separation capillary was 80 μm i.d., 354 μm o.d., and made of fused silica (Polymicro Technologies, Inc., Phoenix, AZ) with a length of 60 cm. A reversible high-voltage power supply (Model R50B, Hipotronics Inc., Brewster, NY) provided a variable voltage of 0–30 kV with the outlet of the separation capillary at ground potential. Samples were introduced by gravity at the cathodic or the anodic end of the capillary by raising the inlet of known height (7–12 cm) with respect to the outlet for a fixed period of time (5–10 s).

Figure 1b shows a diagram of the end-column conductivity detector inside its protective plastic jacket; Figure 1c is an enlarged portion of Figure 1b. The sensing microelectrode (Pt wire, 50 μm in diameter, California Fine Wire Co., Grover City, CA) is centered in a 1 cm long fused-silica capillary (150 μm i.d., 355 μm o.d., Polymicro Technologies) and held in place by epoxy (Torrseal, Varian Corp., Lexington, MA). The surface facing the separation capillary outlet is sanded flat (with care). This assembly is held in place with a somewhat larger fused-silica capillary (approximately 355 μm i.d.). One end of this larger diameter capillary is sealed with epoxy to the sensing microelectrode holder. The other end extends about 1–2 mm so that the outlet end of the separation capillary can be nearly butted against the sensing microelectrode. This leaves a small path for the eluent, about 1–2 mm long with a wall separation of 1–2 μm.

The entire structure is protected by a plastic jacket, which is placed inside a reservoir containing the grounding electrode. A hole on the side of the plastic jacket lines up with the sensing

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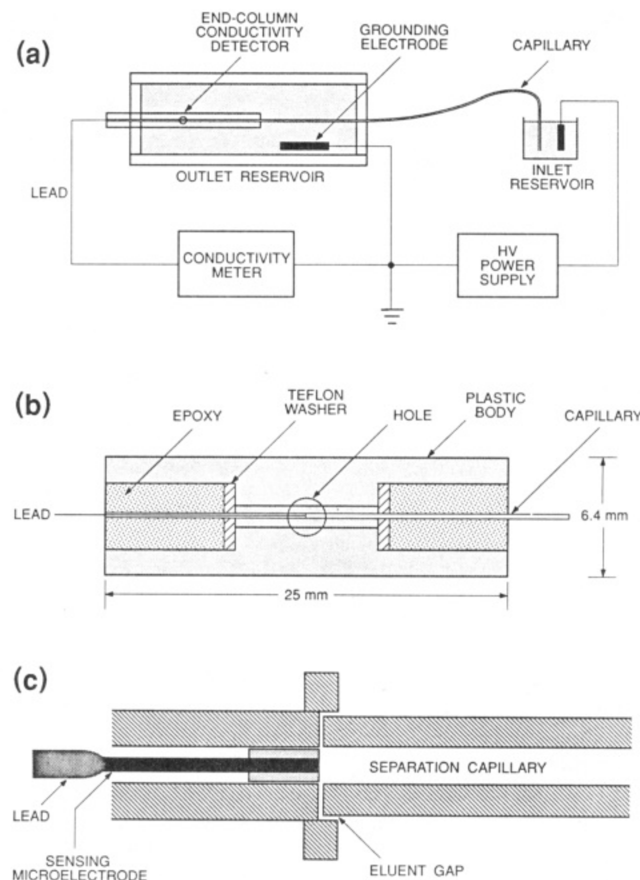

Figure 1. Schematic drawing of (a) the CZE separation device with an end-column conductivity detector, (b) a cross-section view of the plastic jacket assembly, and (c) an enlarged view of the end-column sensing microelectrode.

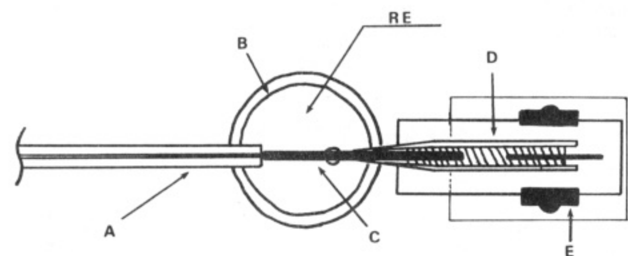

Figure 2. Schematic drawing of CZE with end-column amperometric detection: (A) capillary; (B) cathodic buffer reservoir and electrochemical cell; (C) carbon fiber electrode; (D) electrode assembly; (E) micromanipulator; (RE) reference electrode.

microelectrode so that fluid flows out of the separation capillary and into the reservoir. The conductivity measurement is made between the sensing microelectrode and the grounding electrode, using an ac circuit (*16*).

Amperometric Detection. The system used for amperometric detection with CZE is similar to that described by Wallingford and Ewing (*22*) with the exception that the porous glass joint is not used. Fused-silica capillaries having the dimensions of 5 μm i.d./140 μm o.d. were obtained from Polymicro Technologies (Phoenix, AZ). After a piece of capillary was cut to the desired length (50–70 cm), the capillary was positioned in the electrochemical cell with a stainless steel fitting that was epoxied (5-min epoxy gel, Devcon) in place. This fitting acted as the cathode for electrophoresis. The end of the microelectrode was manipulated through a slot cut into the opposite side of the cartridge and up against the end of the capillary with a micromanipulator (Newport, Model 422) while being viewed under a microscope. A top view of the detection apparatus is shown in Figure 2. The cell was then filled with 0.1 M KCl as supporting electrolyte.

In this work, 10 μm o.d. carbon fibers (Amoco Performance Products, Greenville, SC) with an exposed length of 100–250 μm were employed as working electrodes for electrochemical detection. Detection was performed in a two-electrode configuration with a sodium saturated calomel reference electrode (SSCE). Electrochemical detection was carried out at 0.8 V SSCE. The low currents measured required that the detection end of the system be housed in a Faraday cage in order to minimize the effects of external noise sources. Injection was by electromigration.

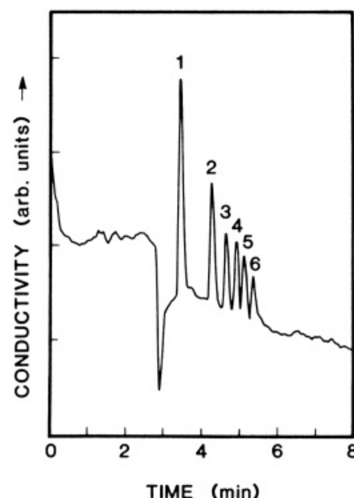

Figure 3. Electropherogram obtained with an end-column conductivity detector showing the baseline resolution of a mixture of six carboxylic acids at 5×10^{-5} M each: (1) formate; (2) acetate; (3) propanoate; (4) butanoate; (5) pentanoate; (6) hexanoate. The capillary is 80 μm i.d., 60 cm to detector, and is operated at 20 kV and 88 μA. The buffer is MES/HIS (20 mM each) at pH 6 with 1 mM TTAB. Injection is by gravity.

Chemicals. All chemicals were from Sigma Chemical Corp. (St. Louis, MO) and were used without further purification. Conductivity detection runs were carried out with a buffer solution consisting of 20 mM morpholinoethanesulfonic acid (MES)/histidine at pH 6.0 to which a cationic surfactant, tetradecyltrimethylammonium bromide (TTAB), was added (1 mM final concentration). Water used to prepare solutions was freshly deionized and distilled with a water purifier (Model LD-2A coupled with a Mega-Pure automatic distiller, Corning Glassworks, NY).

Amperometric detection runs were also carried out with MES, but these buffers were adjusted to the desired pH by addition of solid NaOH. Catecholamine stock solutions were prepared as 0.01 M solutions in 0.1 M perchloric acid and diluted to the desired concentration with operating buffer.

RESULTS AND DISCUSSION

End-Column Conductivity Detection. Figure 3 shows an electropherogram obtained when a mixture containing six different carboxylic acids at 5×10^{-5} M each is injected onto the CZE setup illustrated in Figure 1. The sensitivity and the resolution are quite similar to what has been observed previously (see Figure 1 of ref 18). The TTAB in the buffer reverses the electroosmotic flow direction (*18*); therefore, we also reverse the polarity of the power supply so that the most mobile anion arrives first at the detector. Because of the sensitivity, universality, ease of operation, and ability to quantitate on an absolute basis with the use of an internal standard (*18*), CZE separations employing conductivity detection appear to be of wide possible use.

Of course, this end-column conductivity detector has some additional dead volume compared to an on-column conductivity detector. This dead volume arises from the "eluent gap" (see Figure 1c). We estimate this dead volume to be about 5–6 nL. To investigate the effect of this dead volume on the separation efficiency, the following experiment was carried out. Benzoate anion ($C_6H_5COO^-$) was injected at 1×10^{-5} M, and the peak profile was recorded by using on-column UV absorption (*25*) and end-column conductivity detection. The

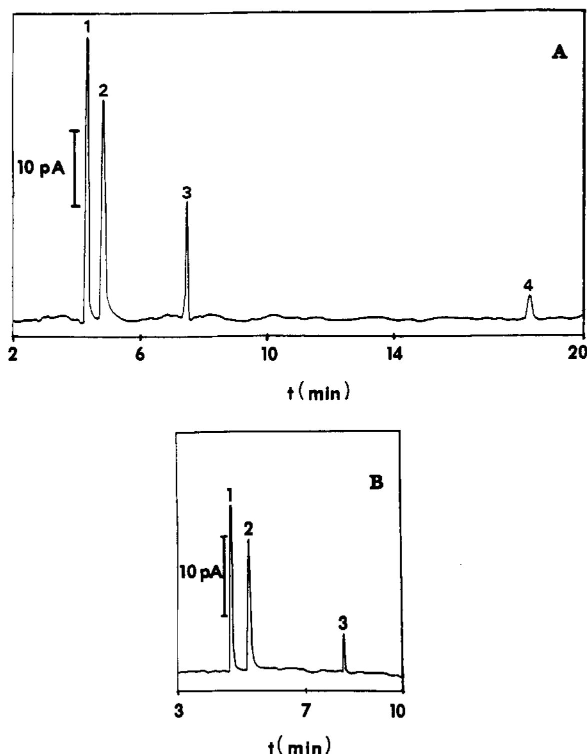

Figure 4. Electropherograms obtained with end-column amperometric detection. (A) Analytes: (1) dopamine (1×10^{-5} M); (2) isoproterenol (1×10^{-5} M); (3) catechol (1×10^{-5} M); (4) 3,4-dihydroxyphenylacetic acid (2×10^{-5} M). (B) Analytes (5×10^{-6} M each): (1) dopamine; (2) isoproterenol; (3) catechol. Conditions: separation potential, 20 kV; electrochemical detector potential, 0.7 V; injection, 20 kV for 5 s.

UV absorption detector is 9 cm upstream from the end-column conductivity detector, so that the additional zone broadening in traveling this distance is minimal (*25*). We find that the peak from the end-column conductivity detector is 23% broader than that for the UV absorption detector, at an injection volume of 23 nL. This extra zone broadening caused by the additional dead volume of the end-column conductivity detector fits well what we estimated for the magnitude of the extra broadening.

Thus, the end-column conductivity detector has some disadvantage in efficiency. However, for typical injection volumes (20 nL) and for an 80 μm i.d. capillary this extra zone broadening is less than 25%, which is a modest penalty to pay for the advantage of such a simple construction. Note that the end-column conductivity detector can be readily fitted on the outlet of any capillary electrophoresis system, suggesting its use as a simple universal second detector for CZE separations.

End-Column Amperometric Detection. The CZE system for amperometric detection consists only of a fused-silica capillary and a carbon fiber microelectrode. There are two major differences between this design and that used with earlier direct amperometric detection schemes (*3, 22*). First, there is no porous glass junction to serve as an electrical connector between a separation capillary and a detection capillary. In the end-column detector, there is no need to isolate the sensing electrode from the high electric field needed for electrophoresis because the internal diameter of the separation capillary is so small (5 μm) that very little current is passed. Second, the carbon fiber microelectrode is not placed directly into the separation capillary. It is aligned with the bore of the capillary and positioned up against but not into the capillary, thereby creating a thin-layer cell at the capillary outlet. Because the diameter of the electrode is twice the internal diameter of the capillary, good oxidation efficiency is obtained. Detector sensitivity is further increased by approximately 1 order of magnitude when a cylindrical carbon fiber electrode is used (as shown) relative to a disk-shaped electrode. Apparently, the eluent from the capillary forms a sheathlike flow around the electrode, providing more efficient oxidation of solutes at the detector.

The electropherogram in Figure 4A shows the separation of 2.0 fmol of dopamine, 1.8 fmol of isoproterenol, 1.2 fmol of catechol, and 1.0 fmol of 3,4-dihydroxyphenylacetic acid obtained with a 5 μm i.d. capillary that was 56.6 cm long. It is clear that cations, neutrals, and anions are readily separated by this technique. Figure 4B shows an electropherogram obtained in a separate study of the linearity of the detection system. An equimolar (5×10^{-6}) mixture of dopamine, isoproterenol, and catechol was injected onto a 5 μm i.d., 56.6 cm long capillary. The peaks correspond to 960 amol of dopamine, 870 amol of isoproterenol, and 560 amol of catechol. No attempt was made to optimize these separations.

Standard calibration curves for test solutes have been computed. In one set of experiments, detection of catechol was examined for total injection amounts ranging from 1.13 fmol to 0.113 pmol (10^{-5}–10^{-3} M). Linear regression analysis provides values for the slope and correlation coefficient of 7.7×10^{-3} and 0.991, respectively.

In another set of experiments, detection of dopamine, isoproterenol, and catechol has been examined for average total injection amounts ranging from 80 amol to 4.0 fmol (5.0×10^{-7} to 2.5×10^{-5} M). Linear regression analysis provides correlation coefficients of 0.998 for dopamine, 0.999 for isoproterenol, and 0.993 for catechol. The detection limits computed for these compounds are 64 amol for dopamine, 70 amol for isoproterenol, and 56 amol for catechol ($S/N = 2$, peak-to-peak noise evaluated over 10 peak widths). These values were calculated on the basis of the lowest amount injected, which was 5×10^{-7} M (80 amol).

CONCLUSION

End-column conductimetric and amperometric detection methods provide an easy and convenient method for eliminating detection problems associated with the high voltage drop across the capillary in CZE separations. The primary advantage of end-column detection, as described in this paper, is its simple design and construction. Therefore, electrochemical detection may be applied to CZE with greater ease and reliability. The only limitation to this technique appears to be that small internal diameters must be used for the amperometric mode. End-column detection, as described in this paper, should make CZE with electrochemical detection accessible to a wider range of investigations and investigators.

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RECEIVED for review July 26, 1990. Accepted October 16, 1990. This work was supported, in part, by grants from the National Institutes of Health (A.G.E) and Beckman Instruments (R.N.Z. and A.G.E.). A.G.E. is a National Science Foundation Presidential Young Investigator, an Alfred P. Sloan Fellow, and a Camille and Henry Dreyfus Teacher-Scholar.