On-Column Electrochemical Detector with a Single Graphite Fiber Electrode for Open-Tubular Liquid Chromatography

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Construction and preliminary characterization of an on-column electrochemical (amperometric) detector for capillary LC columns is described. The working electrode is a single 9-μm carbon fiber which can be inserted with a micropositioner into the end of an open-tubular LC column (15 μm i.d.). This system was investigated in the oxidative mode by using a simple two-electrode arrangement. Coulometric efficiencies approach 100%, while detection limits for ascorbic acid, catechol, and 4-methylcatechol are on the order of 1 fmol or 10^{-7} M. Response is linear over the tested range of 10^{-6} to 10^{-3} M. The system's capability for handling real samples is demonstrated by separation and detection of electroactive components in a urine sample.

Open-tubular capillary columns for liquid chromatography offer a number of advantages over the larger diameter packed columns currently in widespread use. Chief among these advantages is the high separation efficiency that can be achieved with properly prepared capillary columns. Jorgenson and Guthrie (*1*) showed by theoretical calculations that the optimum column diameter for a wide range of operating pressures and analysis times is about 1 to 2 μm. They point out, however, that certain problems of sample injection, column fabrication, and especially detector design must be overcome for open-tubular capillary LC to become a useful and practical technique. It appears (*1, 2*) that sample injection can be accomplished quite simply and that column fabrication techniques will improve significantly in the near future. However, development of adequate detectors for such small columns appears to be a more serious problem, and the future of open-tubular liquid chromatography may depend critically on the availability of such detectors.

Prime requirements of a detector for open-tubular LC are low dead volume (requiring essentially "on-column" detection) and high sensitivity. This paper describes the design of a simple, yet very sensitive on-column electrochemical detector for capillary LC. Its main feature is an indicator electrode constructed from a single graphite fiber (9 μM diameter, ca. 0.7 mm length) inserted into the outlet end of the capillary column (15 μm diameter). The principle of operation is similar to the commercially available thin-layer flow-through electrochemical detectors for conventional (larger) packed columns, but the response of the graphite fiber detector is more nearly coulometric.

The use of graphite fibers as microvoltammetric electrodes has been reviewed by Wightman (*3*) and investigated by Wightman (*4*) and others (*5*), especially for application to in vivo studies of brain metabolism. There are no published reports, however, of their exploitation as single fiber electrodes for capillary LC/EC detection.

In a recent review of LC/EC detection for trace analysis Krull et al. (*6*) note that EC detection has been employed with microbore and capillary HPLC systems. It appears, however, that all reports (*7–12*) of electrochemical detectors for capillary LC involve postcolumn detection with miniaturized flow-through or "wall-jet" cells.

EXPERIMENTAL SECTION

Apparatus. An IBM EC 225 voltammetric analyzer was used along with a Fisher Recordall Series 5000 stripchart recorder to apply voltage to the cell and record measured current with time. A precision X-Y-Z three-axis translator with course/fine adjust, used to align and insert the graphite fiber electrode into the capillary, was obtained from the Oriel Corp., Stamford, CT (Catalog numbers 1622 and 1634). The chromatographic injection system and the procedures for capillary column preparation and sample injection have already been described (*1*). A planimeter was used to measure areas of chromatographic peaks. A Keithley Model 169 digital multimeter was used to measure the electrical resistance of the capillary column filled with mercury.

Graphite fiber electrodes were constructed by use of graphite fibers (Hercules Co., Magnamite Type AS-1) kindly supplied by Gilbert M. Gynn of Ashland Chemical Co., Columbus, OH. Graphite fibers, available in a variety of grades from several manufacturers, are generaly made by pyrolysis of acrylonitrile or from pitch. Some types have received surface treatment with a sizing material to facilitate embedding in various resins. We used the unsized type to obtain an electrode whose electrochemically active surface would not be limited to just its cross sectional end but extend over its entire axial surface.

Chemicals. All chromatograms were obtained by using as mobile phase a pH 4.6 buffer that was 0.02 M in both acetic acid and sodium acetate and 1×10^{-3} M in EDTA. All sample solutions (except urine) were prepared by using this mobile phase buffer as solvent or diluent so that injected samples had virtually the same composition as the mobile phase except for the solutes to be separated. Because of its lower solubility in the aqueous mobile phase, helium was used to pressurize the mobile phase reservoir, thereby avoiding bubble formation due to pressure decrease near the end of the column.

Construction of Electrodes. Graphite-fiber electrodes were made by first embedding a single graphite fiber in a small bead of epoxy resin (Devcon, commonly available 5-min-curing epoxy glue) as follows. A small amount of epoxy that was beginning to harden was dropped onto a sheet of parafilm and allowed to solidify. The solid 4 mm diameter hemisphere was peeled from the parafilm and turned flat side up and a 2 to 3 cm length of graphite fiber was laid across its diameter. Additional epoxy was mixed, and as hardening began an amount similar to the original drop was carefully allowed to drop onto the flat surface where it sealed in the fiber and spread to the edge. After the bead had hardened, more epoxy was used to attach the bead to the end of the 10 cm length of 5 mm o.d. glass tubing, one end of the fiber extending into the tube. As the epoxy hardened, the bead was manipulated so that the fiber's orientation was parallel to the axis of the glass tube.

Electrical connection to the fiber was made by filling the tube with mercury and inserting a platinum wire. Finally, the length of the fiber extending from the epoxy bead was trimmed to the desired length (0.5 to 1.0 mm) with a surgical scissors.

Construction of the Electrochemical Cell. A glass cell was constructed to surround the fiber electrode with 0.1 M KCl electrolyte solution and to furnish the reference–counterelectrode. It consisted of a 2 to 3 cm length of 1 cm square glass tubing (for undistorted viewing through a microscope) one end of which was

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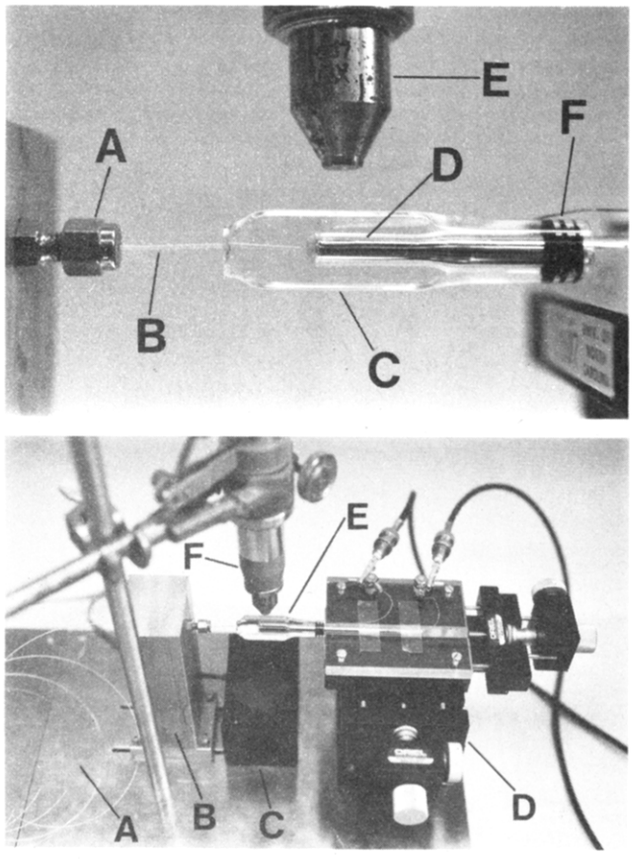

Figure 1. (Top) Close-up of cell assembly: capillary mounting connection (A), capillary (B), glass cell (C), mercury-filled electrode (D), microscope objective (E), O-rings (F). (Bottom) Detector module: coiled capillary column (A), capillary mounting block (B), microscope illuminator (C), precision X-Y-Z positioner (D), cell (E), microscope (F).

sealed to a 2 to 3 cm length of ordinary 10 mm o.d. tubing through which the graphite electrode was inserted and held by snug-fitting O-rings. The other end was drawn down to leave a 2 to 3 mm hole, large enough to admit the capillary with room for adjustment of the electrode/cell assembly relative to the capillary, but small enough so that solution was retained in the cell by surface tension. A silver wire, later anodized to coat it with silver chloride, was sealed (epoxy) into the cell through a small hole and served as the combined reference–counterelectrode. This glass cell is shown in Figure 1 (top).

Construction of the Detector Assembly. Figure 1 (botton) shows the detector module including the stand that was constructed to hold the end of the capillary, the precision positioner with the electrode and surrounding cell attached to it, and the 100× microscope used to view the capillary while adjusting the position of the electrode for insertion of the fiber. A Vespel ferrule was used in the fitting to hold the capillary snugly where it emerges from the hole drilled through the aluminum block. For convenience, the block is movable on slotted tracks in the baseplate.

Procedure for Setting Up the Detector. The detector is readied for use by first rinsing and filling the cell with 0.1 M KCl, stopping the small hole with a finger. As the fiber electrode with its O-ring seals is inserted, the stoppering finger is released and the electrode is pushed into position within the square section. The mercury-filled tube extending from the cell–electrode assembly is mounted horizontally in a V-shaped groove on the positioner stage and taped in place so that the fiber is just within the microscope's viewing area. Electrode connections are made to the IBM Instrument. Next, the block holding the horizontal capillary is advanced along the tracks in the baseplate until the end of the capillary is centered in the microscope's viewing area; then, it is locked in place. The positioner is then adjusted so that the capillary and fiber are both in sharp focus (nearly vertically aligned) and correctly aligned laterally for insertion. Insertion generally requires several trials, making small adjustments of the positioner fine controls. The resilient fiber bends visibly when it misses the hole and runs into the glass, but it returns to its original position when backed away again. With practice, electrode insertion requires less than 5 min. The fiber is finally inserted up to the point where the glass shelf of the capillary end nearly touches the epoxy bead. If the capillary and fiber are not perfectly parallel, there is danger that the fiber will break off during insertion since the misalignment gets worse the farther the fiber is inserted. Obviously, both proper straightening of the capillary end and proper (parallel) mounting of the fiber on the glass tube during electrode construction are important considerations.

All chromatograms (except that of urine) were obtained with the fiber electrode potential at +0.8 V (vs. Ag, AgCl), a commonly chosen potential at which many substances are oxidizable, yet at which the background current is small. Generally, the electrode was preconditioned by holding it at +1.3 V for a minute or more before setting it at +0.8 V.

Procedure for Measuring Column Dimensions. The average diameter, d, and the total volume, V, of the capillary column were determined by a novel method. The column was filled with mercury and its electrical resistance was measured. Then the mercury was forced from the column and weighed. The measured resistance, R, is proportional to the ratio of the column length, L, to its cross sectional area, A

$$R = \rho\frac{L}{A} \tag{1}$$

ρ = specific resistance of mercury

whereas the measured mass of mercury, M, is proportional to the column volume, i.e., to the product of its length and cross sectional area

$$M = d_{Hg}V = d_{Hg}LA \tag{2}$$

d_{Hg} = density of mercury

By use of literature values of ρ and d_{Hg}, and the measured values R and M, simultaneous solution of these two equations yields values for both L and A, and therefore for V and d. By this method the column used for all chromatographic measurements was found to have a diameter of 14.86 μm, a length of 265.2 cm, and a volume of 0.460 μL.

RESULTS AND DISCUSSION

Detection Limits and Linearity. Figure 2 shows chromatograms of an equimolar mixture of ascorbic acid (AA), catechol (CAT), and 4-methylcatechol (MCAT) at sample concentrations of 1.00×10^{-4} M and 1.00×10^{-6} M. In both cases the sample injection time was 5.0 s and the mobile phase was pressurized at 400 psig. Since AA is an unretained solute, it serves as a marker of the dead time (t_m = 344 s). Accordingly, the capacity factors, k', for CAT and MCAT are 0.338 and 1.07, respectively.

The volume of sample injected, calculated from the injection time, dead time, and total column volume (0.460 μL) is 0.67 nL, corresponding to a sample size of 0.67 pmol for the 10^{-4} M sample and 6.7 fmol for the 10^{-6} M sample. From the signal-to-noise ratio observable in the lower part of Figure 2 it appears that, even without electrical shielding of the detector, the detection limit is on the order of a femtomole (10^{-7} M). Proper electrical shielding should reduce the noise level so that a detection limit of 0.1 or even 0.01 fmol (10^{-8} or 10^{-9} M) may be achievable.

Linearity of the detector's response was verified, within experimental error, by measuring peak areas on chromatograms obtained under identical conditions at 400 psig using 5.0-s injections of sample solutions of different concentration. The uncertainty in injection time, and hence in sample volume, was estimated to be about 10%.

Coulometric Efficiency. The small distance separating the graphite fiber from the capillary wall together with the fiber's length and the high sensitivity exhibited by the detector suggest a thin-layer cell of high coulometric efficiency (moles detected/moles injected). To ascertain the coulometric ef-

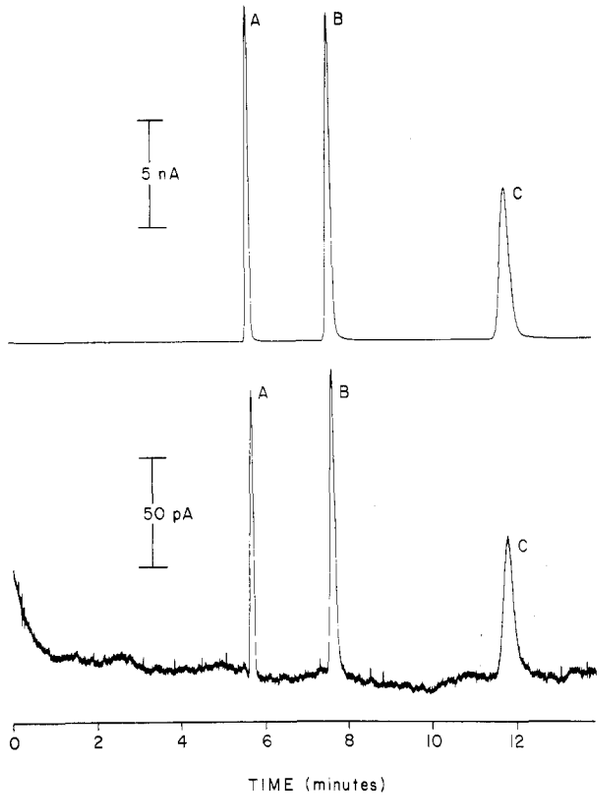

Figure 2. Chromatograms of equimolar mixtures of ascorbic acid (A), catechol (B), and 4-methylcatechol (C) using 5-s sample injections and a column head pressure of 400 psig: (upper) 1×10^{-4} M; (lower) 1×10^{-6} M.

ficiency as a function of flow velocity, known amounts of an equimolar 1.00×10^{-4} M mixture of AA, CAT, and MCAT were injected at different flow velocities, and the amounts detected were calculated from measured chromatographic peak areas by using the Faraday, known chart speed, current sensitivity, and the knowledge that these electrooxidations involve 2 faradays/mol. Different flow velocities were achieved by adjusting the applied pressure between 200 and 800 psig. The amount of sample injected was accurately calculated from the injection time, dead time, column volume, and known sample concentration, but the sample amount was kept sensibly constant at all flow velocities by changing the injection time from 80 s at 200 psig down to 20 s at 800 psig. Extremely long injection times (about 10 times longer than normal) were chosen deliberately to minimize the relative error due to the uncertainty in the injection time (0.5–1.0 s). The resulting chromatographic peaks, though very broad and flat-topped, were well resolved and of large area, which made peak integration also very accurate. The results of this coulometric efficiency study are shown in Figure 3. As expected, the coulometric efficiency is higher at low flow velocities where eluting molecules have a longer time to contact the electrode surface. For CAT and MCAT, coulometric efficiency appears to be 100% at linear flow velocities below about 4 to 5 mm/s (200 psig). The combination of several measurement errors may cause coulmetric efficiencies to be in error as much as 5%. Nevertheless, coulometric efficiencies are remarkably high, even at the high flow velocities. The lower response for AA may be attributed to slower electrode kinetics (*4*).

Role of EDTA. The presence of EDTA in the mobile phase buffer, as recommended in the literature (*13*), was necessary to eliminate undesirable phenomena, including a gradually increasing background current, nearly total disappearance of the AA peak, and diminution of the MCAT peak, all attributable to slow chemical attack of the stainless steel parts of the injection system by the aqueous buffer. Presumably ferrous iron thus introduced interferes by complexing with AA, and to a smaller extent MCAT, and is itself oxidizable at the applied potential. The latter accounts for the gradual increase in background current if EDTA is not present. Flushing the injection system (by opening the drain valve) with fresh non-EDTA-containing mobile phase causes the background current to decrease abruptly at the dead time. However, with the drain valve closed (as during the recording of a chromatogram) the mobile phase within the injector parts is nearly stagnant (extremely small flow into the column) and slow attack of the stainless steel results in a gradual increase in ferrous iron concentration of the mobile phase at the head of the column. Apparently complexation of the iron with EDTA renders it electroinactive at the graphite fiber electrode at +0.8 V, and the greater stability of the iron–EDTA complex precludes reaction of the iron with AA and MCAT.

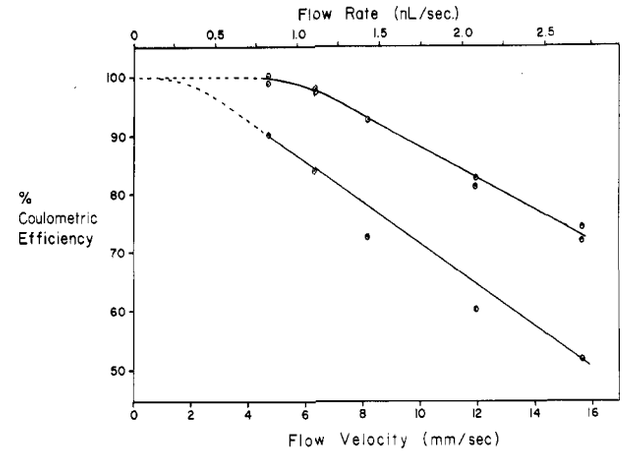

Figure 3. Coulometric efficiency as a function of mobile phase flow velocity: (upper line) catechol and 4-methylcatechol; (lower line) ascorbic acid.

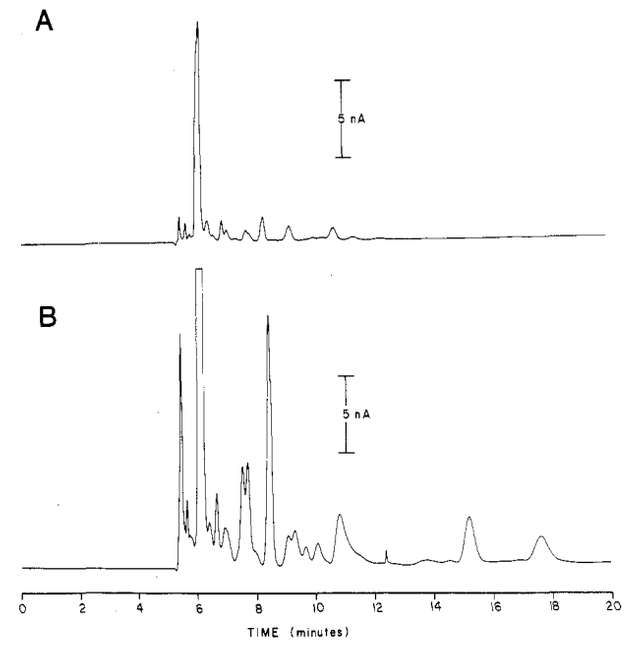

Figure 4. Chromatograms of human urine, using 4-s injections and a column head pressure of 400 psig: indicator electrode and potential of (A) +0.40 V and (B) +1.00 V vs. Ag/AgCl reference electrode.

Analysis of Urine. A 24-h human urine sample was used to test the practical application of this chromatographic detector (and system) to "real" samples. Pretreatment of the urine sample included adjusting the pH to 1.0 with HCl, heating at 80 °C for 30 min, cooling, readjusting the pH to 3.0 with NaOH, and centrifuging. Figure 4 shows chroma-

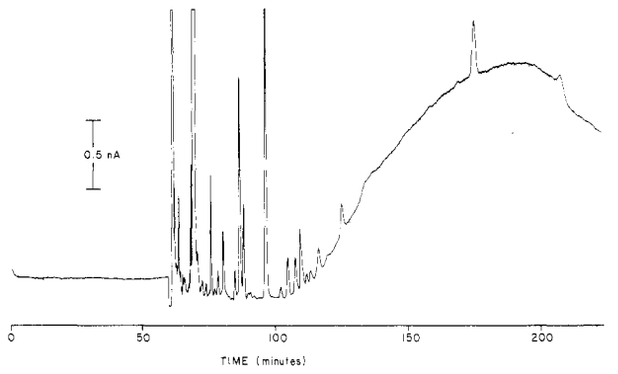

Figure 5. Chromatogram of human urine, using 12-s injection and a column head pressure of 40 psig; indicator electrode potential of +1.00 V vs. Ag/AgCl reference electrode.

tograms obtained at fairly high flow velocity (400 psig, 4.0-s injection) at two different electrode potentials, +0.4 V and +1.0 V. Differences between these two chromatograms demonstrate the possibility of electrochemically selective detection to aid in the identification of chromatographic peaks based on differences in the redox potentials of various substances. One can envision the use of more sophisticated voltammetric techniques (*14*) to obtain both potential and time resolution. Figure 5 shows a chromatogram of the same urine sample at slow flow velocity (40 psig, 12-s injection) at +1.0 V. Comparison with Figure 4B shows clearly the increased chromatographic resolution achieved at slower flow velocity. A high background current "hump" was only observed in the slow chromatogram of the urine sample. This hump was not eliminated by the inclusion of EDTA and may be attributable to more serious corrosion of the inlet system by some component of the urine sample.

Additional Considerations. The background current at the graphite fiber electrode is approximately 300 to 400 pA at +0.8 V (vs. Ag, AgCl) in aqueous solution. The potential range over which the background current remains reasonably flat extends from about +1.2 V to −1.2 V, depending somewhat on the electrochemical pretreatment procedure and the nature of the aqueous solution. The background current is potential dependent but flow independent; i.e., with no potential applied, no "streaming current" was detectable. Its wide potential range suggests that this electrode may be suitable for reductive mode EC detection, especially, perhaps with an electrodeposited thin mercury film, although this has not yet been investigated.

Like any solid electrode the graphite fiber electrode's response characteristics can be expected to change if its surface is "fouled", for example by insoluble reaction products. Although no such behavior was observed during our chromatographic experiments, even following chromatograms of the complicated pretreated urine sample, other types of samples (e.g., phenols) may adversely affect electrode performance. In such cases electrochemical "cleaning" similar to the electrochemical pretreatment may be effective in restoring electrode performance. More complicated electrochemical treatment procedures have been reported (*14–17*) for dealing with this problem.

Obviously, for simple two-electrode amperometric detection the IBM instrument can be replaced by a simpler apparatus—a mercury cell, voltage divider, and a picoammeter. We plan to employ such an apparatus along with a shielded detector to extend our study of the characteristics of this sensitive on-column electrochemical detector and its application to a variety of situations.

Note Added in Proof: An on-column potentiometric detector employing an ion-selective microelectrode inserted into the end of an open-tubular LC column was recently described by Manz and Simon (*18*).

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Registry No. AA, 50-81-7; CAT, 120-80-9; MCAT, 452-86-8.

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