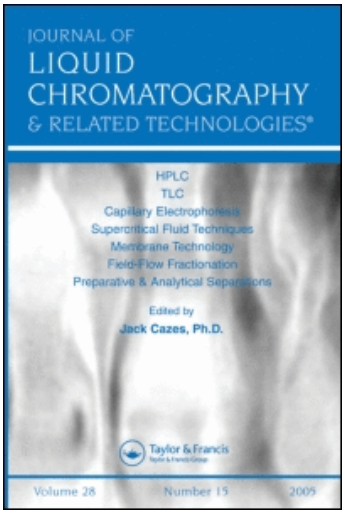

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RAPID SCAN MICROVOLTAMMETRIC DETECTION FOR LIQUID CHROMATOGRAPHY

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ABSTRACT

A carbon microvoltammetric electrode has been used as the working electrode in a rapid scan detector for high-performance liquid chromatography (HPLC). Analytes are detected by the 9 μm carbon fiber as they elute into a cell. Effects of scan rate and flow rate are discussed. Detection limits of 10^{-6} M (1.1 ng, injected) have been obtained for hydroquinone, and the detector is linear over four orders of magnitude in concentration. The two-dimensional resolving power is demonstrated using a mixture of six standards and the detector is used for the identification of caffeine in tea.

INTRODUCTION

Rapid scan electrochemical detection for liquid chromatography is becoming increasingly popular. Commonly used techniques such as amperometric detection offer high sensitivity but are less selective and less informative. Scanning electrochemical

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detection improves selectivity at the expense of higher background due to charging current. Several detectors have been developed which discriminate against charging current including square wave voltammetry (1-6) and coulostatic voltammetry (7-12). Staircase voltammetry combined with post-run background subtraction has also been reported (13,14).

The detector described in this paper is based on the scanning microvoltammetric detector developed in our laboratory for open-tubular liquid chromatography (15-18), and is similar to that used by Baur et al. at constant potential (19). The 9 μm carbon fiber working electrode is placed in the eluent as it flows into a cell. The detector has been used successfully for the separation of a standard mixture of catechols and amines, for the identification of caffeine in tea, and to separate aminoacids in individual neurons.

MATERIALS AND METHODS

The chromatographic system included an Altex Model 110A pump (Altex Scientific Inc., Berkeley, CA) and a six-port injection valve with a 10 μL injection loop (Valco Instruments, Houston, TX). A C18 column (4.5 mm X 25 cm) packed with 5.0 μm particles was used (IBM Instruments Inc., Danbury, CT). Stainless steel tubing and fittings were used for all connections.

Fabrication of the carbon fiber working electrodes has been discussed elsewhere (20). The detection cell (Figure 1) is a U-shaped glass tube (1 cm i.d.) with a fine frit divider. Sealed into one side of the working electrode compartment is a 1 mm i.d. glass tube. Part of the tube extends inside the compartment and bends so that the tip is accessible from the top of the cell. The remaining segment of the tube, outside the cell, connects to the column via stainless steel tubing. The 9 μm carbon fiber working electrode is inserted into the outlet end of the glass tube, allowing detection of analytes as they elute. A 10X magnifier is used to view the fiber as it is lowered into the tube. The walls of the glass compartment are square to allow a clear view of the electrode. The entire compartment is filled with mobile phase, and a drain tube maintains a constant fluid level. The second compartment holds the Ag/AgCl reference electrode and is filled with 0.1 M potassium chloride.

The scanning electrochemical system, used previously with open-tubular columns, has been described elsewhere (15,16). All voltammetric data shown here have been background subtracted, and the data were smoothed before and after derivatization.

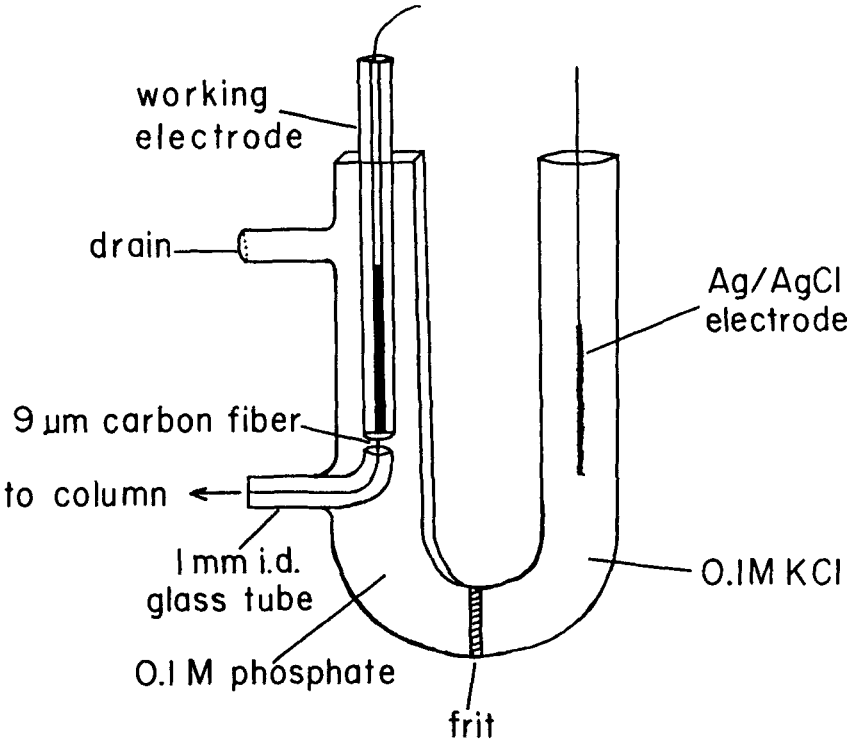

FIGURE 1. Schematic of the electrochemical cell.

Unless specified otherwise, the scan rate was 1 V/s, the flow rate was 1 mL/min, and the electrode length was 2 mm.

The mobile phase was 10% methanol/90% 0.1 M phosphate (pH 4.5). All sample solutions (except tea) were made using the mobile phase as the solvent. Phosphoric acid was obtained from Fisher Scientific (Fair Lawn, NJ) and caffeine from Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were obtained from Sigma Chemical Co. (St. Louis, MO).

Tea samples were prepared using Lipton Tea (Englewood Cliffs, NJ). Tea leaves were placed in 40 mL boiling 0.1 M phosphate solution for about 5 minutes. After the solution cooled, it was filtered through a Whatman No. 42 filter (Maidstone, England) and then through a 0.45µm filter (Gelman Sciences, Ann Arbor, MI).

TABLE 1

Analysis of Detector Linearity

	Slope	Correlation Coefficient
Chromatographic Peak Height	1.005	0.9983
Chromatographic Peak Area	0.998	0.9979
Voltammetric Area	0.950	0.9983

RESULTS AND DISCUSSION

Chromatographic flow leads to hydrodynamic transport of analytes, resulting in voltammograms with a sigmoidal shape at scan rates less than or equal to 1 V/s. At scan rates greater than 1 V/s, however, scan rate-dependent peaking occurs in the voltammograms because the experimental time scale is too short to permit establishment of steady-state concentration gradients at the electrode.

The shape of the voltammogram and the magnitude of the current are independent of flow rate over the tested range of 0.5 to 2.5 mL/min.

Detection limits and linearity were evaluated by making triplicate injections at seven concentrations over the range of 10^{-2} to 10^{-6} M hydroquinone. The detection limit for hydroquinone is 10^{-6} M (1.1 ng or 10 pmole, injected). Although the detection limit is higher than that obtained using a typical amperometric detector, it is comparable to other scanning detectors (13). In work reported previously, lower detection limits (10^{-8} for hydroquinone) were obtained using on-column detection in open-tubular columns, mainly due to less dilution of the sample in the column (16).

The linearity of the detector can be evaluated by use of a log-log plot of response vs. concentration (21). Three different methods were used to determine the response (Table 1). The chromatographic peak height and area both have near-unity slopes (Table 1), indicating that the response varies in direct proportion to the change in concentration. The voltammetric peak area does not vary directly with concentration as shown by the lower slope.

Figure 2 shows the single potential chromatogram (E = +1.0 V vs. Ag/AgCl) and the three-dimensional plot (3-D) of a mixture of ascorbic acid, epinephrine,

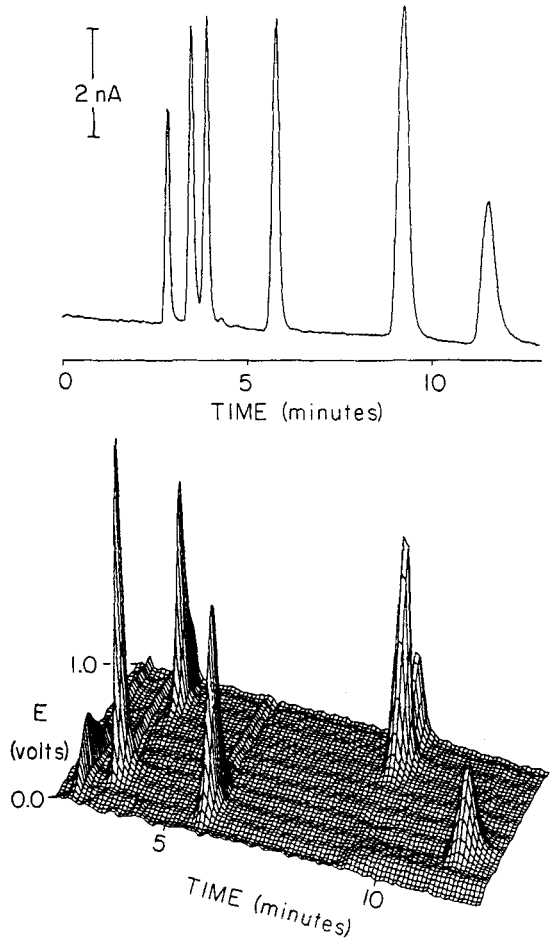

FIGURE 2. (Top) Single potential chromatogram at +1.0 V vs. Ag/AgCl of six standards, all 0.1 mM: ascorbic acid, epinephrine, tyrosine, hydroquinone, resorcinol and catechol. Flow rate was 1.0 mL/min, scan rate was 1 V/s, mobile phase was 10% methanol/90% 0.1 M phosphate, and electrode length was 2 mm.
(Bottom) Partial three-dimensional chromatovoltammogram of same mixture.

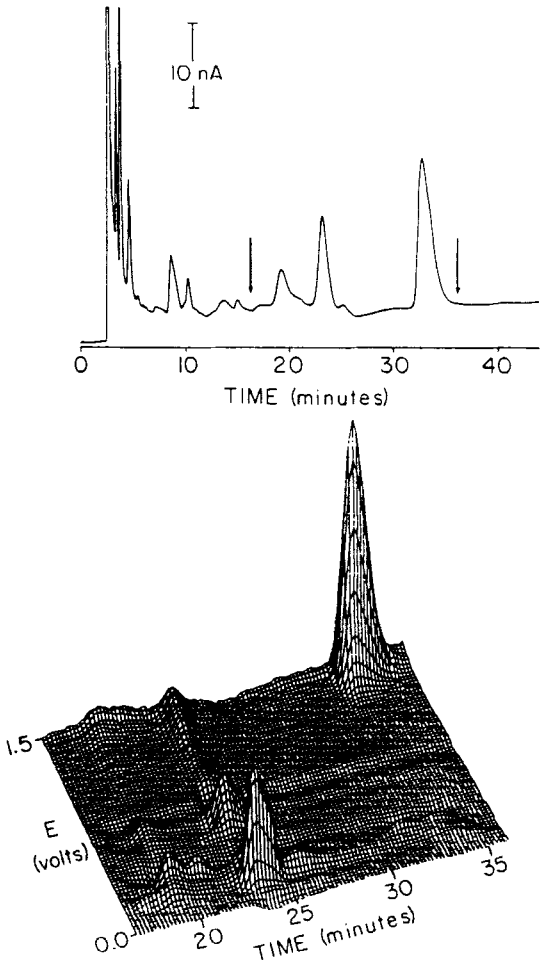

FIGURE 3. (Top) Single potential chromatogram at +1.5 V vs. Ag/AgCl of tea. Elution time of caffeine is 33 min. Flow rate was 1.0 mL/min, scan rate was 1 V/s, mobile phase was 10% methanol/90% 0.1 M phosphate, and electrode length was 2 mm.
(Bottom) Partial three-dimensional chromatovoltammogram of tea (segment designated by arrows in single potential chromatogram).

tyrosine, hydroquinone, resorcinol and catechol (all 0.1 mM). The sigmoidal voltammograms have been smoothed and mathematically derivatized as previously described (16). The scanning technique obtains electrochemical information about the analytes and provides an additional dimension of resolution. Peaks such as epinephrine and tyrosine which are overlapped chromatographically are completely resolved electrochemically. Derivatization of the voltammetric waves further improves the resolution in the electrochemical domain.

Scanning detection is particularly useful in the analysis of complex mixtures. In *Figure 3*, the single potential chromatogram (E = +1.5 V vs. Ag/AgCl) and a partial 3-D plot of tea are shown. Identification of caffeine (retention time = 33 min) is easily made by comparison of the retention time and $E_{1/2}$ from a standard run to the tea sample.

CONCLUSION

These preliminary studies show that scanning electrochemical detection employing carbon fibers can be useful for analyses with conventional HPLC columns as well as open-tubular columns. Scanning provides both electrochemical and chromatographic information about analytes; the two dimensions of resolution are particularly advantageous in analyses of complex mixtures.

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