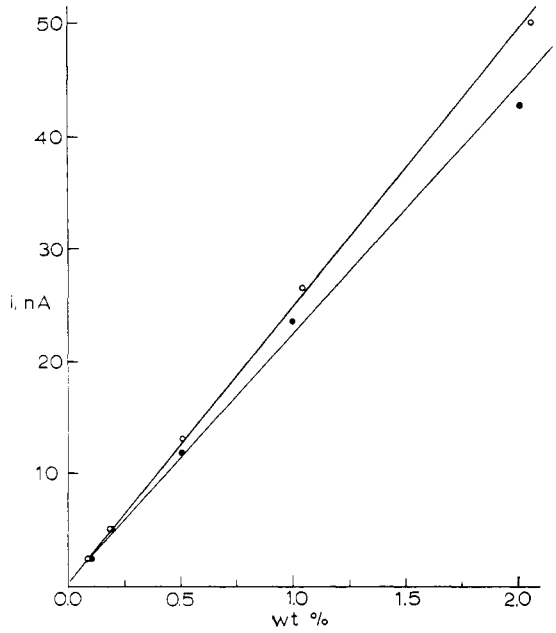

Figure 4. Calibration curve for PTZ and PANA in TMP. Data were collected for solutions prepared by addition of 1.000 g of antioxidant/TMP oil mixture to 5.00 mL of acetonitrile. A 10-μm (diameter) platinum ultramicroelectrode in cell configuration 3 was used. Scan rate was 25 mV/s. PTZ data points, open circles; PANA data points, filled circles. Slope/intercept information (units as given on figure): PTZ (24.39 ± 0.40; 0.45 ± 0.43) PANA (20.99 ± 0.83; 1.09 ± 0.86).

both PTZ and PANA were linear (Figure 4), giving r values of 0.9996 and 0.997, respectively. The method is characterized by rather good precision (1.2% relative standard deviation), leading to detection limits of approximately 0.010% (as expressed for antioxidants in actual oil samples). Continuing studies have shown that interferences encountered in used oil samples are slight and occur as an increase in background current at potentials more positive than +0.95 V.

CONCLUSIONS

This work demonstrates the feasibility of electrochemical determination of antioxidants in lubricating oils. The procedure is greatly simplified by mixing the oil with a suitable cosolvent, thereby providing sufficient conductivity to obtain voltammetric measurements on the solution. Further improvement can be achieved through the use of a compartmented cell which provides small amounts of additional electrolyte by leakage into the ultramicroelectrode compartment. Ultramicroelectrodes allow the use of very simple measuring equipment and will allow construction of a reliable unit for field determination of additives in lubricating oils. In addition, the steady-state responses obtained with ultramicroelectrodes are easily measured and interpreted.

Registry No. PTZ, 92-84-2; DPA, 122-39-4; PANA, 90-30-2; NRL, 7445-47-8; TMP, 77-99-6; Pt, 7440-06-4; acetonitrile, 75-05-8.

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Carbon Fiber Electrode Cell for Square Wave Voltammetric Detection of Biogenic Amines in High-Performance Liquid Chromatography

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INTRODUCTION

Application of voltammetric fast-scan techniques to high-performance liquid chromatography (HPLC) has been investigated by several groups (*1–4*). Samuelsson et al. (*1*) were the first to describe the use of square wave voltammetry (SWV) with HPLC. SWV is an electrochemical technique that allows the rapid scanning of a voltage range with a minimal contribution of charging current to the output signal (*5*). Using *N*-nitrosodiethanolamine and *N*-nitrosoproline, they showed the advantages and possibilities of the technique. Detection was limited by flow pulsation noise, the geometry of the cell, and the computer-controlled sampling interval for both the time and potential domains (ca. 200 s × 400 mV with sampling at 2-s and 10-mV intervals). More recently Goto and Shimada (*4*), using SWV in combination with a carbon fiber electrode (CFE) and HPLC, described the analysis of several catecholamines by three-dimensional HPLC–SWV. The results presented, even though of qualitative value, were of very low temporal resolution (4 scans/min or 3–4 peak data points/chromatographic peak) and thus of questionable quantitative value. Insufficient data was given to make a valid estimate of sensitivity or detection limits, and the authors did not provide any information on reproducibility. Even though both of the above studies suggested potential qualitative uses of three-dimensional HPLC–SWV, neither demonstrated its actual practical qualitative and quantitative use.

The use of single graphite fibers as working electrodes in HPLC has been reported by several groups. Recently Jorgenson et al. (*2, 6*) have used a micropositioner to insert and hold a carbon fiber electrode at the outlet of an HPLC column. Attempts to use a similar system in this laboratory found that, even though this system provides some versatility for research, it is rather bulky and highly susceptible to mechanical vibrations. Goto and Shimada (*4*) have fabricated an electrode by inserting and sealing the carbon fiber into a fused silica tube. We found it difficult, however, to duplicate the electrode since construction details were not adequately described.

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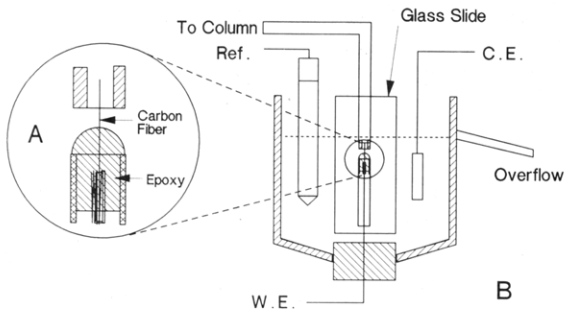

Figure 1. Construction details of (A) the carbon fiber electrode and (B) the flowthrough cell assembly.

Several electrodes made to their approximate specifications presented problems with mechanical instability and large background noise.

In attempting to use a fast-scan technique such as SWV for the detection scheme in HPLC, one must take into consideration several important factors when designing the electrochemical cell. Most carbon electrode flowthrough cells are designed for amperometric detection and, as such, have extremely poor transient response characteristics (i.e. RC > 5 ms). High-frequency SWV requires a cell configuration with an RC of about 0.1 ms or less. Another critical point to consider is the positioning of the electrode within the cell. The cell must permit the growth of the diffusion layer and the flux lines without any hindrance, otherwise the resistance will increase and so will the time constant.

In this paper we describe a chromatovoltammographic (HPLC–SWV) system using a commercially available potentiostat and microcomputer and a custom-fabricated working electrode consisting of a combination carbon fiber (permanently mounted at the HPLC column outlet on a glass slide) and flowthrough cell. The system is used to obtain qualitative and quantitative data from a solution containing a mixture of biogenic amines, with the results being presented in the form of contour and isochromic plots. We also look at the effect of electrode pretreatment on the position and intensity of the SW net-current peak for several biogenic amines. Combining SWV with HPLC provides a new "dimension" in the collection and analysis of chromatographic data in that both the chromatographic domain (time vs concentration signal) and the electrochemical domain (potential vs current) can be viewed simultaneously, allowing peaks that overlap in either technique to be resolved and detected separately.

EXPERIMENTAL SECTION

Apparatus. Square wave voltammetry was carried out with an EG&G potentiostat/galvanostat Model 273 (EG&G Princeton Applied Research, Princeton, NJ) controlled by a Vectra PC (Hewlett-Packard, Sunnyvale, CA) with 640K memory, 40-MB hard disk, 10-MHz clock, and a GPIB-488 interface card. Generation of square waveforms through the EG&G Model 273, data acquisition from the EG&G Model 273, and display of the resulting three-dimensional chromatovoltammographic data were accomplished by software written and compiled with QuickBASIC V.4 (Microsoft, Redmond, WA).

The liquid chromatography system consisted of a Model 196 minipump (Milton Roy, Riviera Beach, FL), a 50-μL loop injection valve (Model 7125, Rheodyne, Berkeley, CA), a pulse dampener, and a 150 mm × 4 mm Bio-Sil ODS.5S reversed-phase column (Bio-Rad, Richmond, CA). The mobile phase contained 0.1 M sodium acetate (Fisher), 0.04 M citric acid (Mallinckrodt), 0.8 mM sodium octyl sulfate (Kodak), 0.1 mM ethylenediaminetetraacetic acid (EDTA) (Fisher), and 5% methanol (Fisher) and was adjusted to a pH of 5.2 with sodium hydroxide. The flow rate was 1 mL/min.

Electrodes. The carbon fiber electrode and cell assembly are shown in Figure 1. The carbon fiber electrodes were constructed by threading a bundle of the 7-μm carbon fibers (ca. 70–80 mm long, Avco/Textron, Lowell, MA) through a 0.8 mm × 30 mm capillary tube (Kimax-51, Kimble, Vineland, NJ). The bundle was pulled through in such a way as to allow one fiber to protrude at least 4–6 mm from one end. This end was then sealed with a bead of fast-drying epoxy (2R:1H, DURO-Master Mend, Loctite Corp., Cleveland, OH) with some of the epoxy being drawn 2–3 mm into the tube (Figure 1A). After about a half-hour was allowed for hardening, the bundle of fibers at the other end was tightly wound around a copper stranded wire (ca. 20 mm striped wire 20 cm long) and twisted into the capillary. Any excess fibers were cut off, and this end was also sealed with epoxy and allowed to dry; then ca. 1 cm of heat-shrinkable tubing was placed over the capillary/wire joint. The electrode was then mounted with epoxy onto a slide (7.5 mm × 25 mm × 1 mm) on which Teflon capillary tubing (0.3 mm i.d. × ca. 20 cm long) had been previously mounted, by inserting the fiber into the end of the Teflon tubing to within ca. 0.5 mm of the seal (Figure 1A), and held in place till dry (ca. 4 min). The entire assembly was cured at 70 °C for 3 h. The complete electrochemical detector, shown in Figure 1B, consists of the above electrode assembly mounted in a modified high-density polyethylene (HDPE) bottle along with a Pt-wire counter electrode and saturated NaCl calomel reference electrode. The cell is filled with mobile-phase solution and kept at constant volume by suction. Unless otherwise indicated, the carbon fiber electrode was pretreated only once at the very beginning before being used. The pretreatment consisted of applying a 1-Hz triangular waveform potential varying between 0 and +1500 mV for 60 s with a mobile-phase flow rate of 1 mL/min.

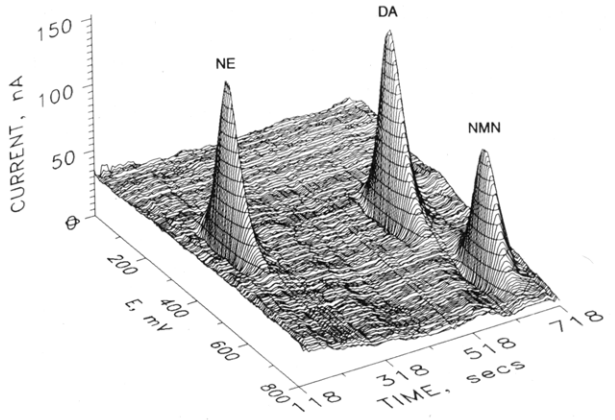

Figure 2. Square wave chromatovoltammogram of NE, DA, and NM, all at 1 × 10^{-5} M, represented as a three-dimensional *XYZ* plot. Chromatographic and voltammetric conditions are given in text.

Reagents. All solutions were prepared with 18-MΩ water from an ion-exchange system (Millipore) fed from a singly distilled source. Epinephrine (E), norepinephrine (NE), dopamine (DA), 5-hydroxytryptophan (5-HTP), 3,4-dihydroxybenzylamine (DHBA), and normetanephrine (NM) were obtained from Sigma Chemical Co., St. Louis, MO. Standard solutions were made up containing 1.0 mM concentrations of each amine in 0.1 M perchloric acid and stored at 4 °C.

RESULTS AND DISCUSSION

A three-dimensional chromatovoltammograph of a mixture of biogenic amines is shown in Figure 2. The sample contained 1 × 10^{-5} M NE, DA, and NM in 0.1 M $HClO_4$, 50 μL of which was injected onto the column with a mobile-phase flow rate of 1 mL/min. The SWV potential was scanned every 2 s from 0 to 800 mV with a step height (ΔE_S) of 5 mV, square wave amplitude (E_S) of 25 mV, and square wave frequency (f) of 100 Hz. The raw SWV data collected consisted of forward (i_f) and reverse (i_r) current as a function of potential and elution time. The data shown in Figure 2 is the resulting net current ($i_n = i_f - i_r$) after background subtraction and smoothing. For this sample, with relatively few peaks, we can clearly identify each component in the three-dimensional plot. A contour plot is displayed immediately after the analysis as an isochromic plot (current signal displayed in 15 different

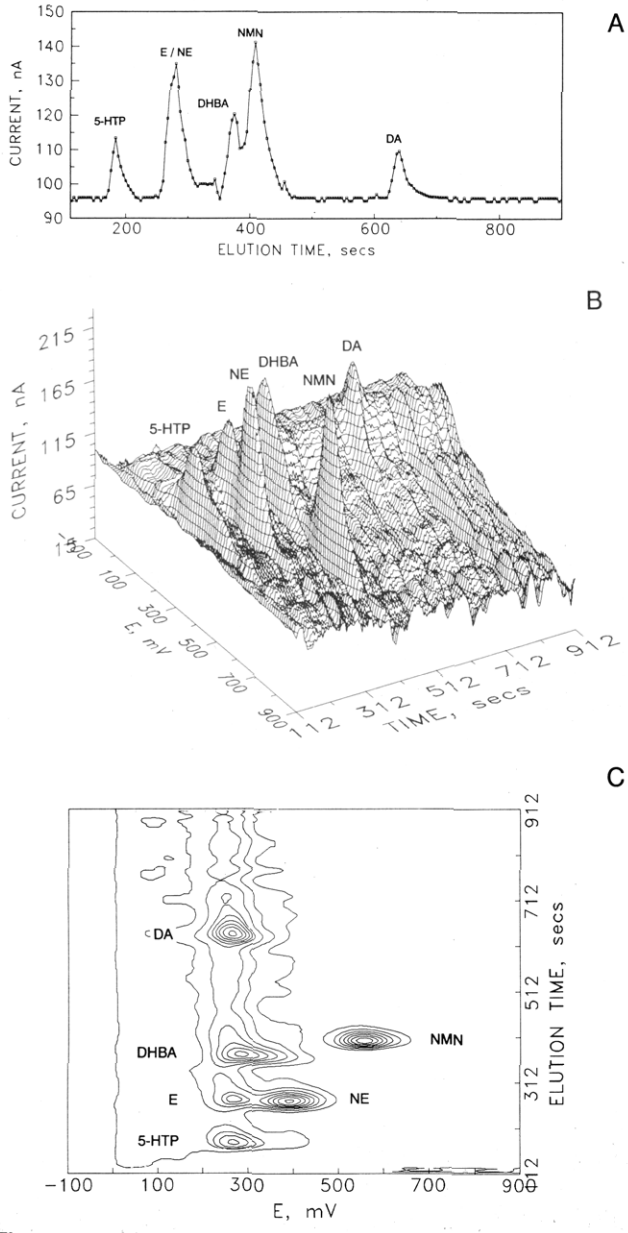

Figure 3. (A) Standard chromatogram of sample containing 5-HTP, E, NE, DHBA, NM, and DA, all at 1×10^{-6} M, with the CFE detector potentiostated at 650 mV. (B) Same sample analyzed with the chromatovoltammographic technique as a three-dimensional plot and (C) as a isogalvanic contour plot.

colors) on the computer monitor. The program allows the user to select (via hairline cursors) and read the current/concentration signal at any time–potential coordinates. The utility of this system becomes more evident during analysis of samples containing a large number of components at lower concentrations.

Figure 3A shows a standard chromatogram of a 50-μL injection of a sample containing 5-HTP, E, NE, DHBA, NM, and DA, all at 1×10^{-6} M, with the chromatographic conditions the same as before, except that the potential was fixed at 650 mV for the entire run. As can be seen, the peaks for E and NE are completely overlapping and those for DHBA and NM are partially overlapping. A 50-μL injection of the same sample run under the same conditions, except with a SWV potential scan range of −100 to 900 mV, scan interval = 2 s, ΔE_S = 5 mV, E_{SW} = 25 mV, and f = 600 Hz, shows the dramatic improvement in both the qualitative and quantitative information obtained (Figure 3B,C). With a larger number of components and lower concentrations, the three-dimensional chromatovoltammograph (Figure 3B) is useful only for a "qualitative feeling" of the results, with all of the biogenic amines in the sample being clearly resolved and quantified in the contour plot (Figure 3C).

The detection limit for these amines is between 6 and 4 pmol with a 2:1 signal/noise ratio. This limit was obtained without using a Faraday cage or a nonpulsating pump. The detection limit for a "slice" at 650 mV was ca. 5–2 pmol. These limits are an order of magnitude higher than obtained with the same detector in the amperometric mode but are several orders of magnitude lower than previously obtained with other scan voltammetric techniques (*2–4*).

The detector's linearity was assessed by injecting 50-μL samples containing 10^{-7} to 10^{-4} M epinephrine. The log–log plot of concentrations vs i_{np} gave a slope of 0.983, relative standard deviation of 0.020%, and correlation coefficient of 0.998.

Effect of Electrode Pretreatment on SWV Current Response. Previous research by several groups (*7–9*) has shown that the voltammetric sensitivity of carbon fiber electrodes to catechol- and indole-based compounds is significantly increased after "modification" of their surface by electrochemical pretreatments. Our studies, using SWV, have shown a similar behavior with these electrodes. However, care must be taken in making comparisons since SWV operates under a different set of assumptions and conditions (especially in terms of scan rate). As expected, pretreatment caused an increase in the SWV peak current response. However, an optimum time was reached for all the amines after approximately 50–60 s (except DA, 125 s). Unlike previous results (*7–9*), which showed a 10-fold increase in sensitivity, the difference in this case between treated and untreated electrodes was about 2-fold and the same for all the amines. However, it is interesting to note that the background current between the untreated and treated electrodes decreased by about 50%. No significant difference from the above results was seen with the use of a higher pretreatment frequency (up to 100 Hz) or potential excursion (up to 3.0 V). Electrochemical pretreatments have also been shown to cause a shift in the oxidation potentials of catecholamines (*8*). We found only a slight shift in the peak potentials between the treated and untreated electrodes of about 10 mV and no significant shift in peak net currents after any of the pretreatment electrolysis times (0–300 s).

This lack of improved sensitivity and shift in the oxidation potentials is in contrast to that obtained in several previous studies (*7–9*). There are several possible reasons for these discrepancies, such as the following: the lower current densities used during our electrolysis pretreatments; the composition of the buffer solution; the source and/or fabrication process of the carbon fibers; or factors intrinsic to the SWV technique, which uses comparatively high scan rates such that the usual adsorption equilibrium (*7*) may not reached. Since the exact nature of the pretreatment effects on the carbon fiber surface is not well understood (*9*), it is difficult to venture a guess as to the specific reason for these discrepancies at this point. We are currently looking at the SWV response of carbon fibers in greater detail, the results of which will be published in the near future.

The treated electrodes retained their properties for several weeks and did not seem to be affected by the number of times they were used for three-dimensional HPLC–SWV analysis.

Electrochemistry of Biogenic Amines with SWV. Two interesting aspects of using SWV for the electroanalysis of catecholamines are that no electrode-fouling products appear to be generated during the oxidation steps and only single-peak SW voltammograms are obtained at pHs >3. It has been previously shown (*10*) that for cyclic voltammetry (CV) at pHs >3 the unprotonated oxidation product of epinephrine (ep-

inephrine–quinone, EQ) is available in sufficient concentration to allow a rapid cyclization reaction to take place (k_{cyc} = 0.6 s^{-1}, t = 1.2 s), converting it to the indole (5,6-dihydroxyindoline). The indole however is more easily oxidized than epinephrine so that it is oxidized by the EQ to the respective aminochrome, which then proceeds to polymerize. This complex ECC mechanism (electrochemical reaction followed by two chemical reactions) is responsible for the multiple peaks normally seen in linear scan voltammograms of catecholamines and for the fouling of electrode surfaces under amperometric conditions. With SWV, using a frequency of 600 Hz and scanning from 0 to 1000 mV (0.2 s), the rate of reduction of the quinone back to the catecholamine is an order of magnitude greater than the rate of cyclization reaction. Thus, the cyclization reaction occurs to an insignificant degree so that the SW voltammogram shows only one peak and there is no chance for polymerization (fouling) to occur on the electrode surface. In addition, by changing the frequency of the SW, one could use the above situation to advantage to determine the heterogeneous electron-transfer rates of catecholamines and indoleamines.

In conclusion, it seems evident that many of the factors involved in using SWV with CFEs are only partially understood at this time. The pretreatment and configuration of the CFE is crucial to the signal response when one is using SWV. We feel that with proper cell configurations and conditions, SWV will prove to have much greater sensitivity and resolution than the other voltammetric techniques currently used.

ACKNOWLEDGMENT

The expert technical assistance of S. Gunn is gratefully acknowledged. This work was supported in part by the National Institutes of Health, Grant No. HL-37871.

Registry No. E, 51-43-4; NE, 51-41-2; DA, 51-61-6; 5-HTP, 56-69-9; DHBA, 37491-68-2; NM, 97-31-4.

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CORRECTIONS

Bienzymatic Electrode for the Determination of Aspartame in Dietary Products

Orlando Fatibello-Filho, Ahmad A. Suleiman, George G. Guilbault, and Glenn J. Lubrano (*Anal. Chem.* 1988, *60*, 2397–2399).

Equation 1 on page 2397 should read

$$\text{aspartame} \xrightarrow{\text{carboxypeptidase A}} \text{L-aspartic acid} + \text{L-phenylalanine}$$

Multifrequency Phase Fluorescence Study of Hapten-Antibody Complexation

Frank V. Bright (*Anal. Chem.* 1989, *61*, 309–313).

Equation 12 on page 310 should read

$$\frac{r_0}{r} = \frac{1}{F_f}\{1 + (\tau_F/\phi_f)\} + \frac{1}{F_b F_L}\{1 + (\tau_L/\phi_L)\} + \frac{1}{F_b F_G}\{1 + (\tau_G/\phi_G)\}$$

As a result, Figure 1 should appear as