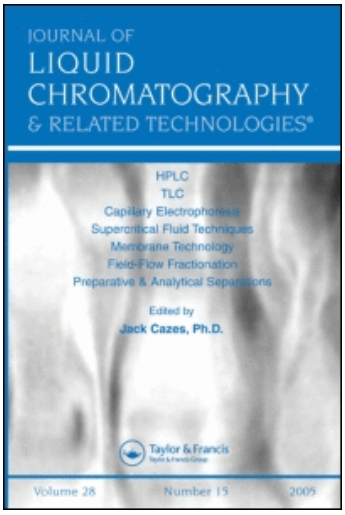

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John A. Roush[a]; Mark R. Anderson[a]
[a] Department of Chemistry, Virginia Polytechnic Institute and State University, Blacksburg, Virginia

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APPLICATION OF SQUARE WAVE VOLTAMMETRY FOR ELECTROCHEMICAL DETECTION IN GRADIENT ELUTION HPLC

JOHN A. ROUSH AND MARK R. ANDERSON*
Department of Chemistry
Virginia Polytechnic Institute and State University
Blacksburg, Virginia 24061-0212

ABSTRACT

The utility of square-wave voltammetry with a conventionally sized indicator electrode for use in the detection of gradient elution HPLC applications is demonstrated. A redesigned electrochemical detector cell, based upon a type of wall-jet design, provides more efficient potential control and allows the voltammetric detection. The minimum detectable quantity and linear dynamic range for the new detector design were found to be 13pg and 5 orders of magnitude, respectively, comparable to other electrochemical detectors. In the separation of a test mixture containing hydroquinone, bromohydroquinone, catechol, resorcinol, and phenylhydroquinone baseline resolution of all five components is obtained in 12 minutes using a nonlinear solvent gradient. Importantly, no change in the chromatographic baseline is observed during the course of the separation. Gradient separations of the extracts of fennel seeds and mainstream cigarette smoke are also demonstrated with no apparent change in the baseline.

INTRODUCTION

Electrochemical detection has been an important and sensitive method of detection for liquid chromatographic applications. Electrochemistry, however, has not been as widely utilized as other detection methods because of the difficulty generally encountered when used in conjunction with a mobile phase gradient (1,2). The problem with electrochemical detection and gradient elution lies in the different contributions to the measured current. Electrochemical

measurements are subject to three types of current: resistive, capacitive, and faradaic. The faradaic current is the analytical signal, while the others contribute to the background. During the course of a mobile phase gradient, the properties of the solvent continuously change, resulting in a continuous alteration of the background current contribution. The net result is that the baseline is continuously changing during the separation. Nevertheless, because of the sensitivity and simplicity of electrochemical detection, many reports have appeared since the early 1980's that describe techniques for making electrochemical detection compatible with a mobile phase gradient (3-13). In general, these methods require the application of some technique, unrelated to the chromatographic separation, which is designed to decrease the influence of the changing background. For example, Dorsey *et al.* altered the electrolyte concentration concurrent with the mobile phase change (5). This changes the conductivity of the mobile phase in a manner to offset the effect of the decreasing dielectric of the mobile phase during the solvent gradient. Likewise, Tjaden *et al.* utilized a dual electrode detection to provide for a continuous measurement and subtraction of the background current (8). In nearly all instances reported, the separation method must be developed that allows for the electrochemical detection, and is not necessarily optimized for maximum chromatographic efficiency. Such an approach may compromise the chromatographic efficiency, and cannot be generally applied.

Advances in the area of ultramicroelectrodes have increased the sensitivity of liquid chromatography with electrochemical detection (LCEC), making electrochemical detectors compatible with micro techniques such as capillary separations (3,14,15). In addition, microelectrodes have resulted in an increased compatibility of electrochemical detection with separations involving solvent gradients (12,13). The increased compatibility of microelectrodes with solvent gradients is due to the small currents (nanoamperes and lower) which are usually measured with microelectrodes. As the mobile phase composition changes in a gradient elution separation, changes in conductivity of the flowing stream will lead to only minor changes in the measured background current; hence, the chromatographic baseline does not change much.

Jorgenson and others have taken advantage of this attribute and have demonstrated the utility of ultramicroelectrodes as detectors for liquid chromatography both in isocratic and gradient elution modes (3,12-15).

An additional advantage of ultramicroelectrodes in chromatographic detection is that they may easily be utilized in a voltammetric mode to obtain, not only the chromatographic data, but also the electrochemical current-potential data. This additional degree of freedom of voltammetric detectors provides an opportunity to either (i) identify eluting components from their oxidation potential, or (ii) resolve in the electrochemical dimension multiple peaks that may coelute along the chromatographic time axis. Several different voltammetric modes have been utilized with microelectrodes. Jorgenson and coworkers have demonstrated the utility of a linear potential sweep in chromatographic detection (15). Kounaves *et al.* have similarly applied a square wave voltammetric detection method (16). With square wave voltammetry, a differential current is measured which results in a type of automatic correction of the background current. Because of this differential current measurement, square wave voltammetric detection provides an ideal opportunity for electrochemical detection coupled to gradient elution HPLC.

While ultramicroelectrodes provide better sensitivity than conventionally sized electrochemical detectors, they are also more difficult to prepare and utilize on a routine basis. Square wave voltammetric detection has previously been demonstrated with a macroscopic polarographic HPLC detector (17); however, the method has not been previously utilized when a separation involving solvent gradients and a conventional electrochemical detector is being studied. It is the intent of this research to demonstrate the applicability of square wave voltammetric detection to gradient elution HPLC using an indicator electrode of conventional size.

EXPERIMENTAL

Hydroquinone and resorcinol were obtained from Eastman Chemical Company (Rochester, NY). Phenylhydroquinone, bromohydroquinone, and catechol were obtained from

the Aldrich Chemical Company (Milwaukee, WI). Standard solutions were prepared for each of these compounds in 0.001M concentrations. Subsequent concentrations for the HPLC studies were prepared by dilution of the standard solutions. HPLC mobile phases were prepared from high purity UV grade acetonitrile (Burdick & Jackson, Muskegon, MI) and deionized water. The water was purified prior to usage with a Barnstead Nanopure II water purification system (Millipore, Milford, MA). Potassium Bromide and perchloric acid were utilized as supporting electrolyte and were purchased from Fischer Scientific Company (Fairlawn, NJ). All chemicals and solvents were used as received.

The HPLC equipment consisted of two Waters model 501 mobile phase pumps (Waters, Milford, MA), a Waters model 660 solvent programmer, a Rheodyne model 7010 high pressure injection valve (Rheodyne, Berkeley, CA), a 20μL sample loop, and a 4.6 X 250mm HPLC column packed with 5μm Econosil C18 stationary phase (Alltech, Deerfield, IL). Potential control of the electrochemical detector was by an EG&G Princeton Applied Research Model 273 potentiostat (Princeton, NJ). Data acquisition and application of the square wave potential wave form was accomplished with an in house written computer program running on a IBM model 50z microcomputer.

The electrochemical detector is a modified version of the wall-jet detector described by Fleet and Little (18). In the new detector, however, the electrode serves as the jet rather than the wall (figure 1). Eluents pass through the center of the platinum indicator electrode, encounter the wall, and are dispersed in a radial direction through the thin layer of solution that is formed between the wall and the disk shaped electrode. In this manner, the entire surface of the electrode is utilized in the voltammetric detection because there is no preferential flow path for the eluent. The radial flow path is confirmed visually by injection of a red dye solution. After clearing the detection zone, the eluent flows into a large volume cell that contains the reference and secondary electrodes. The platinum secondary electrode is concentric around the indicator electrode, and the tip of the saturated calomel reference electrode (SCE) is placed close to the

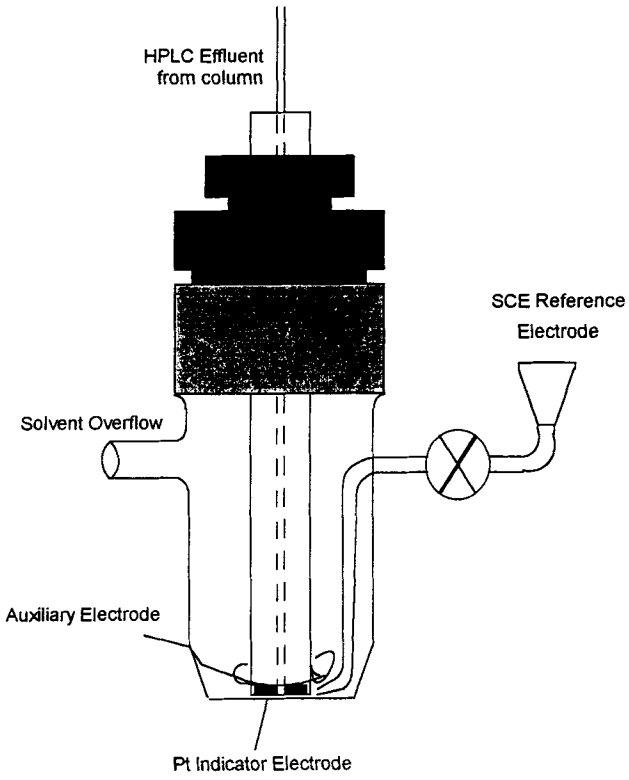

Figure 1. Schematic diagram of the Electrochemical Cell used for square wave voltammetric detection.

edge of the indicator electrode for better potential control. At the beginning of each day, the indicator electrode is polished with 0.05μm suspended alumina. No additional preparation was required, and the detector performance did not degrade during the day.

The extract of fennel seeds used in this study was prepared by extracting 1.728g of fennel seeds with 25mL of 20% acetonitrile-80%water for 10 minutes. The sample was filtered, and the resulting filtrate was separated without further sample preparation. Mainstream cigarette smoke was collected with a Phipps and Bird model 990-300 single port smoking machine

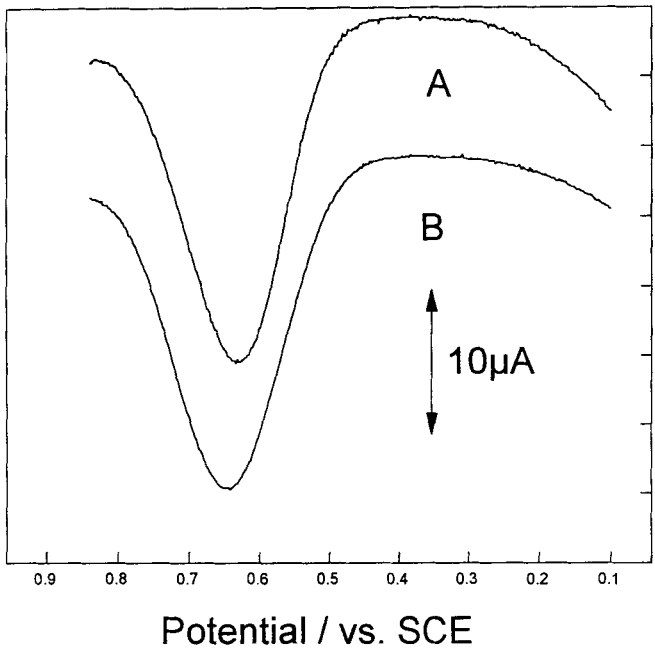

Figure 2. Square wave voltammograms for the oxidation of 0.001M Hydroquinone in the presence of aqueous 0.005M KBr and 0.001M $HClO_4$. (A) is the voltammogram when the electrode is pulled away from the wall, and (B) is the voltammogram when the electrode is pushed against the opposing wall forming the detector thin layer. For both voltammograms the potential was scanned at 15Hz with a pulse height of 25mV.

(Richmond, VA) using the FTC procedure (19). Five cigarettes were smoked per cambridge pad. The pad was then extracted with 20mL of 1% acetic acid at room temperature for 10 minutes. The extract was then filtered and used without further treatment. It was found that cigarette extract samples degraded rapidly with time, so chromatographic analyses were performed within two hours of extraction.

RESULTS AND DISCUSSION

In the detector design, better potentiostatic control of the electrode potential, relative to that of conventional electrochemical detector cells is obtained. This better potential control

allows the voltammetric detection using the thin layer cell. The importance of electrode placement is demonstrated in figure 2. Here, a square wave voltammogram for the oxidation of a static solution containing 0.001M hydroquinone is shown with and without the detector thin layer formed (figure 2A and B, respectively). As can be seen, the current response is virtually identical for the two experiments. This is indicative of adequate potential control, even with the increased resistance when the solution thin layer is formed. Attempts to obtain a square wave voltammogram under similar conditions using a commercial thin layer electrochemical detector cell were unsuccessful. The result in the conventional thin layer cell is likely due to the high resistance of the conventional design relative to that found in our new design.

The minimum detectable quantity and the linear dynamic range for the detector were determined in both an amperometric (constant applied potential) and in the square wave voltammetric modes of operation using isocratic elution conditions. In each case, hydroquinone was utilized as the test analyte. In the amperometric mode, the electrode potential was held at 0.6V *vs.* SCE, and the minimum detectable quantity (MDQ) was found to be 13pg (120 femtomoles) of hydroquinone at a signal to noise ratio of 3. For the determination of the MDQ in the square wave voltammetric mode, the square wave had a frequency of 35Hz and a pulse height of 100mV and the MDQ is found to be 710pg (6.5 picomoles) for hydroquinone. These MDQ values are comparable to those reported by Kounaves *et al.* for the square wave voltammetric detection of epinephrine using a carbon fiber microelectrode (16). Kounaves and coworkers also found an increase in the MDQ by approximately an order of magnitude when changing from amperometric to voltammetric detection. The MDQ values determined in the present research are comparable to values reported for microelectrode HPLC detectors, and are equivalent to or lower than values reported for other conventionally sized electrochemical detectors (15,16,20). The linear dynamic range was found to be approximately 5 orders of magnitude when operating in either the amperometric or voltammetric modes. This value is again comparable to the linear dynamic range reported for commercial amperometric detectors.

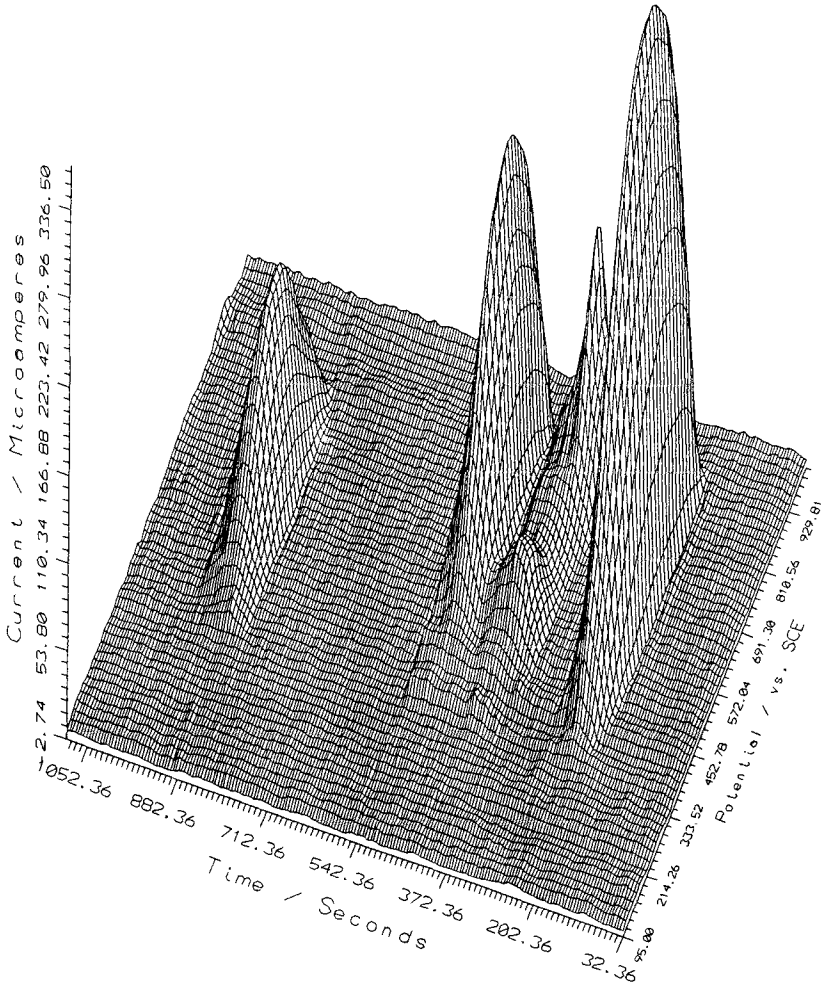

Figure 3. Three dimensional chromatovoltammogram of a simple test mixture obtained under isocratic conditions. The square wave potential program was scanned at 15Hz from 0.0V to 1.2V *vs.* SCE with a pulse height of 25mV. The solvent flow rate was 0.8mL/min., and the observed order of elution is hydroquinone, resorcinol, catechol, and bromohydroquinone.

Figure 3 shows a 3 dimensional chromatovoltammogram that is obtained for the isocratic separation of 15ppm hydroquinone, resorcinol, catechol, and bromohydroquinone using a mobile phase that contained 30% acetonitrile/water and was adjusted to pH 4 with perchloric acid. This chromatogram demonstrates the utility of voltammetric detection in that one obtains not only the chromatographic retention time data, but also the electrochemical oxidation potential data. If phenylhydroquinone is added to this test mixture, efficient separation of all five components under isocratic conditions is not obtained. The original four components of the mixture are separable in a few minutes using a weakly organic mobile phase; however, the phenylhydroquinone can only be eluted from the column with a strongly organic mobile phase. The best separation for this mixture was found to involve a nonlinear mobile phase gradient that changed from 30% to 80% acetonitrile over 15 minutes. A representative chromatogram is shown in figure 4. In this view, one can easily see that the baseline remains constant throughout the nonlinear mobile phase gradient. Importantly, the efficiency of the separation is good, as baseline resolution is obtained and all five peaks appear to be symmetrical.

A quantitative measure of the peak symmetry is obtained by measuring the tailing factor. Using the procedure described by McNair, the tailing factor was found to be 11.5% (21). Independent evaluation of the peak tailing due to sample injector and post column dead volume indicates a total contribution of 11.4% peak tailing coming from these sources. These results indicate that the electrochemical detector itself is contributing a negligible amount to the band tailing observed in the separation.

Following evaluation and optimization of the square wave voltammetric detector, the detector was utilized in the separation of complex, naturally occurring samples. Two samples were chosen because they were known to contain oxidizable components, although no attempt at identifying the constituents of the mixture was attempted in this study. Figure 5 shows the chromatogram for the separation of an extract of fennel seeds with a linear mobile phase gradient (20% to 80% acetonitrile over 15 minutes). Again, there is no drift in the baseline during the

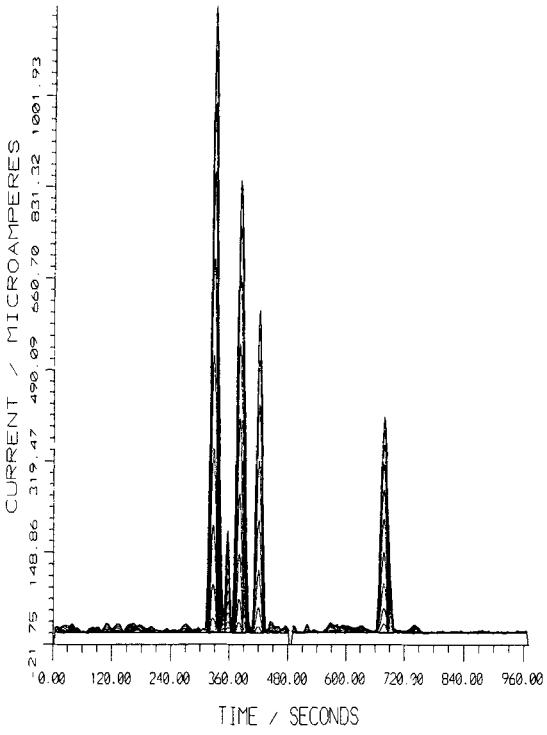

Figure 4. Chromatovoltammogram of a simple test mixture separated by gradient elution HPLC. The square wave potential program was scanned at 35Hz from 0.0V to 1.2V *vs.* SCE with pulse height of 20mV. The solvent flow rate was 0.8ml/min., and the observed order of elution is hydroquinone, resorcinol, catechol, bromohydroquinone, and phenylhydroquinone.

course of the gradient. In the electrochemical dimension, the complexity of the voltammograms indicates the possibility of coeluting peaks, a possibility that would have gone undetected with amperometric or another conventional detection scheme. Figure 6 shows a contour map for the separation of an extract of mainstream cigarette smoke using a linear mobile phase gradient (10% to 90% acetonitrile over 30minutes). The complexity of the sample was such that the three dimensional representation used previously provided no usable information because of the density of peaks. The contour map provides the same data as the three dimensional representation and it

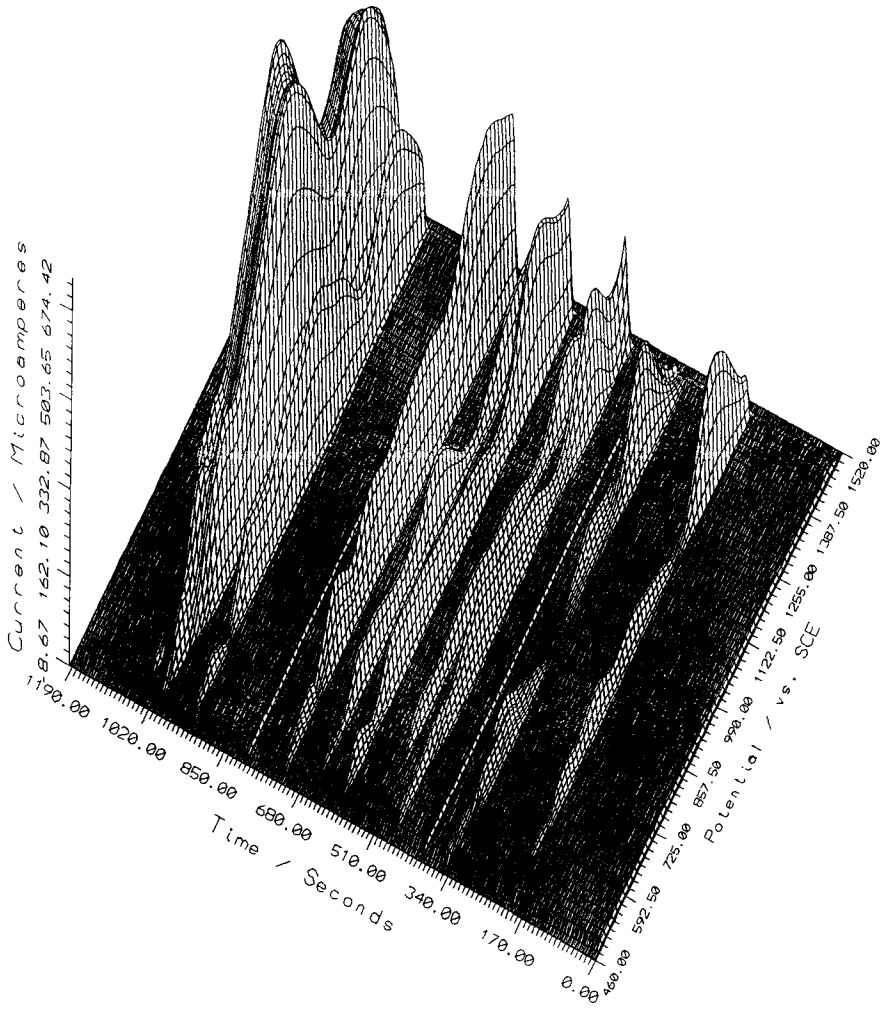

Figure 5. Chromatovoltammogram of the extract of Fennel seeds under gradient elution conditions. The electrochemical and chromatographic parameters utilized in this separation are the same as those given in the caption to Figure 4.

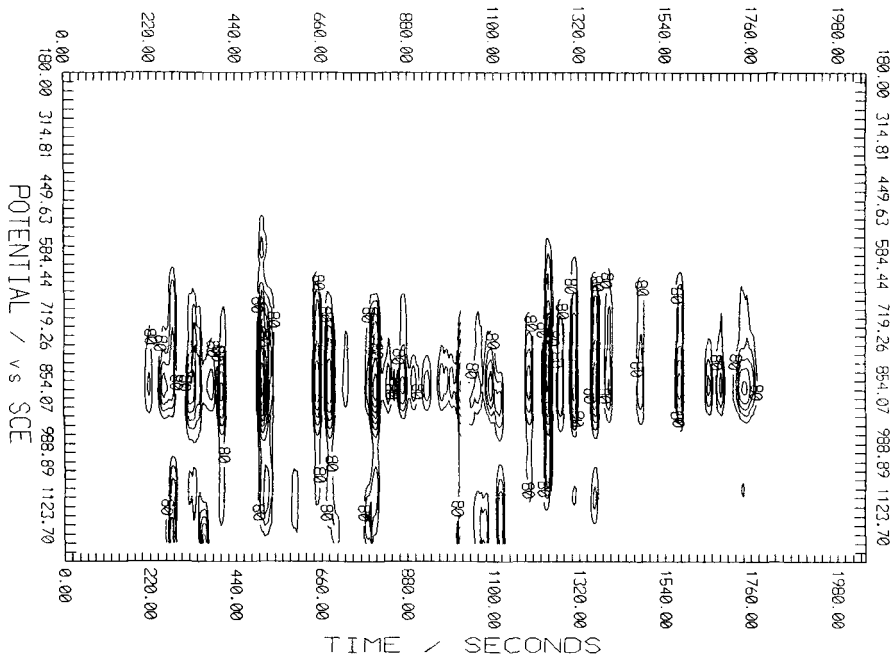

Figure 6. Topographic map representative of a chromatovoltammogram obtained for the separation of an extract of mainstream cigarette smoke under gradient elution conditions. The electrochemical and chromatographic parameters utilized in this separation are the same as those given in the caption to Figure 4.

also indicates the possibility of coeluting peaks in the chromatogram without the clutter of the 3 dimensional representation. By taking one dimension slices of the chromatovoltammogram at various potentials (figure 7), it can be seen that the baseline does not drift during the course of the separation, and that peaks are symmetric.

SUMMARY

A square wave voltammetric detector for use in HPLC applications has been developed using an indicator electrode of conventional dimensions. The design is similar to more common

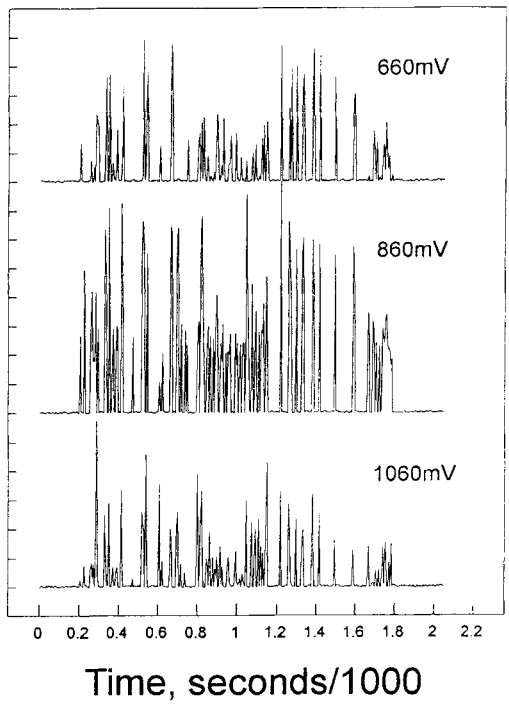

Figure 7. Two dimensional slices of the data shown in Figure 6. The slices were taken at 660mV, 860mV, and 1080mV and are illustrative of the resolution that is possible using the square wave detection.

wall jet detector designs; however, in the new design the electrode serves as the jet. Efficient control of the electrode potential, even when the detector thin layer is formed, allows application of the complex waveform required of square wave voltammetry. The linear dynamic range and the minimum detectable quantities are comparable to those reported for other electrochemical detectors. Importantly, this detector has been shown not to degrade in efficiency when mobile phase gradients are utilized in conjunction with the HPLC separation. Conventional mobile phase gradients, both linear and nonlinear, have been applied with no noticeable affect upon the

baseline. Improvement in the design to minimize post-column dead volume should increase the efficiency of future separations that make use of this detector.

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