Thin-Layer Differential Pulse Voltammetry

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The applicability of differential pulse anodic stripping voltammetry to thin-layer electrodes is demonstrated with Pb^{2+}, Cd^{2+}, Zn^{2+}, Cu^{2+}, and Tl^{+}. Typical calibration curves are linear over a range of 30 to 600 ng/mL with a detection limit of 10 ng/mL. Samples of blood, soybean, and oyster were analyzed for lead. The elimination of interferences in anodic stripping due to the Cu–Zn intermetallic compound is illustrated using a twin-electrode thin-layer cell in which Cu and Zn are deposited on separate electrodes. The utility of differential pulse voltammetry of drugs is shown with diazepam for which the calibration curve is linear over a range of 1 to 60 μg/mL with a detection limit of 0.1 μg/mL. Measurements were performed on 60 μL of solution.

The electrochemistry of thin solution layers has proved useful for a variety of studies such as adsorption at electrode surfaces; kinetics of homogeneous chemical reactions coupled to electrode processes; spectroelectrochemical measurements in the ultraviolet, visible, and infrared spectral regions; coulometric determination of n values; redox potential measurements; and diffusion coefficient measurements (*1–4*). For analytical purposes, thin-layer electrochemical cells possess the inherent advantage of small volume capability, experiments having been performed on as little as a few microliters of solution. They utilize the electroactive species very efficiently in the sense that the entire volume of solution adjacent to the electrode undergoes electrolysis. By contrast, the electroactive species in the diffusion layer surrounding the electrode is only a small fraction of the total solution serving to fill most electrochemical cells. In spite of this advantage, little use has been made of thin-layer cells for analytical purposes beyond the initial reports by Reilley and co-workers on the analysis of metal ions (Cu^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}) and halides (*5*). This is probably because analytical detection limits have been limited to about 10^{-5} M by thin-layer coulometry, the electroanalytical technique frequently used in conjunction with thin-layer cells (*1–3, 5*). Improvements in thin-layer analytical detection limits have been obtained with voltammetric techniques such as anodic stripping voltammetry (*6*) and second harmonic ac stripping voltammetry (*7*).

The feasibility of combining the excellent analytical sensitivity of differential pulse voltammetry (DPV) with the small volume capabilities of the thin-layer cell has recently been demonstrated as described in a preliminary communication (*8*). Thin-layer differential pulse anodic stripping voltammetry (DPASV) was performed on mixtures of Pb^{2+} and Cd^{2+}. Experiments on 70-μL samples gave a linear dynamic range of 25 ng/mL to 500 ng/mL with a detection limit of 10 ng/mL (5×10^{-8} M for Pb^{2+} and 9×10^{-8} M for Cd^{2+}). This detection level is two orders of magnitude lower than that obtained previously by thin-layer coulometry. The thin-layer aspect of the cell enabled complete electrolysis of the 6 μL of solution in the volume above the mercury film electrode to be achieved within 60 s with diffusion as the only mode of mass transport. Consequently, ca. 10% of the metal ions in the 70 μL required to fill the cell could be rapidly deposited into the mercury film without employing the controlled stirring conditions typically used for preconcentration in DPASV. Simultaneous with the deposition step, dissolved oxygen was removed by reduction to water, eliminating the need for nitrogen bubbling. This simplified the procedure and shortened the time required for analysis compared to conventional DPASV. These preliminary results clearly showed that differential pulse voltammetry is compatible with thin-layer cells.

The present paper further demonstrates the general applicability of thin-layer differential pulse voltammetry by (a) extending the anodic stripping technique to other metal ions and to the analysis of biological samples such as blood, soybeans, and oysters; (b) evaluating the potential of twin-electrode thin-layer cells for avoiding interferences from intermetallic compound formation in anodic stripping; and (c) demonstrating the use of thin-layer differential pulse voltammetry for organic compounds with the drug, diazepam.

EXPERIMENTAL

Apparatus. The small volume thin-layer electrochemical cell with a wax inpregnated graphite (WIG) electrode was constructed of Lucite and Teflon as shown and described in Figure 1 of reference (*8*). The total volume of the cell is 70 μL with approximately 50 μL of solution being required to fill the cell for analysis. The thin solution layer which undergoes electrolysis is sandwiched between the WIG and a Lucite top plate. This thin-layer electrolysis volume is calculated to be 5 μL for the WIG electrode radius of 0.225 cm and cell thickness of 0.030 cm. A cell volume of 5 μL was also obtained by thin-layer coulometry, the standard technique for calibrating thin-layer cell volumes (*1–3*), on a Hg^{2+} solution of known concentration. This cell was used for all DPASV and DPV with a single working electrode.

A cell of similar design but modified to position a second working electrode (a rod of glassy carbon) directly above the WIG electrode was used for the twin-electrode studies. The inside diameter of this cell was just large enough to accommodate the WIG electrode, eliminating edge effects. The two parallel working electrodes were separated by ca. 0.4 mm so that 6 μL of solution was sandwiched between them.

A Princeton Applied Research Model 174 polarographic analyzer was used for differential pulse voltammetry and differential pulse anodic stripping voltammetry in the single-working-electrode cell. In all cases, the instrument was set for a scan rate of 5 mV s^{-1} with two 50-mV pulses per second. Signals were recorded on a Houston Instruments 2200-5-6 x–y recorder. Operational amplifier circuitry for the twin-electrode studies was based on the circuit reported in reference (*9*) with modifications for potential scan. Signals were recorded on a Hewlett-Packard 136A x–y–y' recorder.

Reagents. Solutions of Hg^{2+} for mercury deposition were prepared from either $Hg_2(NO_3)_2 \cdot H_2O$ (Baker Analyzed) or 1000 μg/mL Fisher atomic absorption standard (Fisher Scientific Co.). Zinc, cadmium, lead, and copper solutions were freshly prepared by the appropriate dilution of 1000 μg/mL Fisher atomic absorption standard. $TlNO_3$ (K&K Laboratories) was used to prepare thallium solutions. Sufficient diazepam hydrochloride (U.S.P.) was dissolved in 0.1 N HCl to make 1000 and 100 μg/mL stock solutions. The stock solutions were then diluted to the appropriate concentration with pH 7.0 phosphate buffer.

The following supporting electrolytes were used. A solution of 1 M in potassium acetate (Baker Analyzed) was buffered to pH 4.0 by the addition of glacial acetic acid. Buffer at pH 5.0 was prepared by adding glacial acetic acid to a solution containing

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1.5 M potassium acetate. A pH 8.5 solution was prepared by dissolving enough potassium acetate to make a 1.5 M potassium acetate solution. A solution of 1 N in NH_4Cl (Fisher Scientific Co.) was brought to pH 8.5 by adding concentrated NH_4OH (E.I. du Pont Co.). All of the above solutions were electrolyzed over a mercury pool at −1.4 V vs. SCE for 72 h to remove trace metal ions. Phosphate buffer, pH 7.0, 0.99 M was prepared from Na_2HPO_4 and $NaH_2PO_4 \cdot H_2O$. All added salts were reagent grade unless otherwise indicated. Concentrated nitric, perchloric, and sulfuric acids were certified low in heavy metals (G. F. Smith Co., redistilled acids). Doubly distilled acid and/or distilled, deionized water were used in all cases.

Procedures. *Glassware Cleaning.* To ensure that all glassware used in the trace metal determinations was free of metal contaminants, glassware was washed thoroughly with soap and water and rinsed in distilled, deionized water. Residual organic material was removed by a short rinse with chromic acid cleaning solution. Several rinses with 10% nitric acid followed. The glassware was then boiled in 10% nitric acid for 30 min and soaked in this solution for a minimum of 12 h. The glassware was rinsed thoroughly with distilled, deionized water, dried at 110 °C, and stored in a closed area.

Graphite Electrode Preparation. POCO FXI graphite was wax impregnated in molten paraffin under vacuum for 6 h. The electrode end was prepared by polishing on a flat surface with No. 600 grit paper followed by fine cerium oxide powder on rough filter paper, and then brought to a mirror-like finish by polishing with alumina (0.05 μm) on felt cloth (Fisher Scientific Co.). Cerium oxide and alumina polishing was repeated each day before experimentation was begun.

In Situ Formed Mercury Film. The solution to be analyzed was made 20 μg/mL in Hg^{2+} by the addition of the appropriate amount of Hg^{2+} (Fisher Scientific Co.) in the buffer being used. In this manner, the metal to be analyzed and the mercury film were deposited simultaneously on the electrode surface. At the end of each analysis, the potential was stepped to +0.4 V vs. SCE and held for 30 s to strip off the mercury film.

Procedure for Analysis of Standard Metal Ion Solutions. The WIG electrode was polished each day experimentation was begun and then press fitted into the bottom Teflon plate so that it was flush with the Teflon surface. The cell was rinsed with 5 to 10 mL of water between sample analyses. This was most effectively accomplished by placing the tip of a wash bottle at the inlet port and squeezing lightly while aspirating water through the cell, out the exit port, with a water aspirator. Enough analyte solution was then added to the cell (approximately 50 μL) to completely cover the working, reference, and auxiliary electrodes.

Differential pulse anodic stripping voltammograms were obtained by applying the appropriate negative potential to the working electrode for 60 s to reduce all the ions of interest, along with mercuric ions, to a metal amalgam on the electrode surface. The potential must be negative enough (at least −0.7 V vs. SCE) to reduce O_2 to H_2O. The voltammogram was recorded by scanning the potential positively to strip the metal ions of interest out of the amalgam.

Procedure for Lead Analysis in Blood. Human whole blood containing 5 mg/mL sodium citrate, used in the lead spiking experiments, was stored in a test tube at 4 °C. The blood was allowed to come to room temperature and shaken well before using. Then 100 μL of blood were pipetted into a clean 13 × 60 mm test tube using a 100-μL Eppendorf pipet. The pipet tip was rinsed twice with 100 μL of water. This rinse water was also injected into the test tube. If the sample was to be spiked, the appropriate volume of lead spiking solution was added at this time. This was followed by the addition of 0.30 mL of the acid digestion mix (24:24:1 concentrated HNO_3, $HClO_4$, and H_2SO_4). With each set of blood analyses, a blank was analyzed containing only 0.30 mL of acid mix and 200 μL of water.

Blood samples from the Cincinnati Health Department were prepared in a slightly different manner. The blood was supplied in 100-μL heparinized glass capillary tubes and stored at 4 °C until used. Two hundred μL of water were pipetted into the test tube. The blood sample was blown out of the capillary into the test tube. The water–blood mixture from the test tube was sucked up into the capillary and rinsed in and out several (ca. 10) times. The acid digestion mix (0.30 mL) was then added to the test tube.

After samples were prepared by either of the above two methods, the test tubes were placed in an aluminum heating block especially prepared to accept 13-mm diameter test tubes (Fisher Scientific Co.) and the temperature was increased slowly over a 2-h period to 330 °C on a hot plate. When dry, the tubes were cooled in air. While the test tubes were still slightly hot, 0.20 mL of 2% nitric acid was added to each tube. The warm test tube warmed the nitric acid and facilitated dissolution of the lead. The tubes were corked immediately to prevent loss of solution. It was necessary to swirl the nitric acid solution around the walls of the test tube to completely dissolve the lead. The tubes were then left to cool for 1 h, with occasional swirling. Immediately before analysis, the sample was diluted with 0.20 mL of pH 5.0 acetate solution containing 40 μg/mL Hg^{2+}. This results in an analyte solution at approximately pH 4.6 containing 20 μg/mL Hg^{2+}. The sample was analyzed immediately (before loss of lead due to adsorption on the glass) by the following method. Approximately 50 μL of solution was injected into the cell. Deposition for exactly 60 s at −0.80 V vs. SCE was followed by a positive scan to give the lead stripping peak.

The amount of lead in the sample was quantitated by the method of standard addition. A 10-μL aliquot of either 1.11 μg/mL or 0.384 μg/mL standard Pb^{2+} solution was added to 100 μL of the sample and a voltammogram recorded. The original concentration of Pb in the sample was calculated by the conventional equation for the method of standard addition.

Procedure for Lead Analysis in Soybean Samples. The soybean samples were acid digested at the Cincinnati FDA Laboratory and diluted with 2% nitric acid. To 100 μL of sample solution was added 100 μL of 1.5 M potassium acetate. This solution was then analyzed in the same manner as the blood samples.

Procedure for Diazepam. Stock solutions of diazepam hydrochloride in pH 7.0 phosphate buffer were injected with a 100-μL Eppendorf pipet into the thin-layer cell. A potential of −0.800 V was applied for 60 s to remove dissolved O_2 before recording the differential pulse voltammogram. Between samples, the cell was flushed with 0.1 M HCl, deionized water, and then phosphate buffer to minimize sample carryover.

RESULTS AND DISCUSSION

Thin-Layer Differential Pulse Anodic Stripping Voltammetry. Differential pulse anodic stripping voltammetry was performed in the thin-layer cell by a rather simple procedure. The sample to be analyzed was made ca. 20 μg/mL in Hg^{2+} and 50 μL of this analyte solution was injected into the cell. The metal ions and Hg^{2+} in the thin solution layer above the electrode were exhaustively deposited onto the WIG by maintaining the potential at −0.950 V vs. SCE for 60 s. This step also removed dissolved oxygen by reduction to water. Accurate deposition time was necessary because of edge concentration of the analyte into the mercury film. The metals were then stripped out of the thin mercury film by DPASV. Figure 1 shows a typical voltammogram for a 50 ng/mL Pb^{2+} solution. The oxidation peak is sharp and well-defined. No distortion due to the rather large solution resistance of thin-layer cells is evident. The voltammogram compares favorably with that reported in the literature for DPASV on mercury film electrodes in bulk solution (*11*). Voltammograms were obtained for solutions of Tl^+, Cd^{2+}, and Cu^{2+} at pH 4.0 and for Zn^{2+} at pH 8.5. In all cases the voltammograms were comparable to those reported in the literature at mercury film electrodes in conventional cells.

Since the cell thickness was only 0.030 cm, complete electrolysis, during the deposition step, of the 5 μL adjacent to the WIG was rapid with diffusion as the only mode of mass transport as is typical for thin-layer electrochemistry (*1–3*). The time required for complete electrolysis was determined by depositing Tl^+ for varying lengths of time and measuring the height of the subsequent DPASV stripping peak. Figure 2 shows the height of the Tl stripping peak for deposition times varying from 10 to 300 s. The current increases rapidly for deposition times of up to about 60 s after which the rate

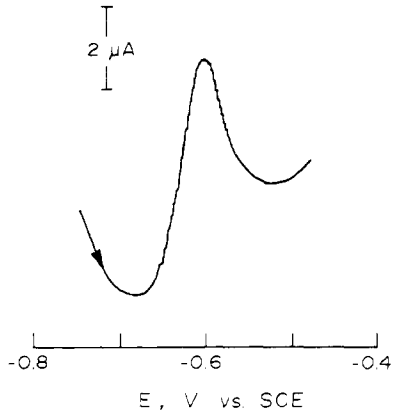

Figure 1. Thin-layer differential pulse anodic stripping voltammogram for Pb. Deposition at −0.950 V vs. SCE for 60 s. Scan rate, 5 mV s^{-1}; two 50-mV pulses per second. 50 ng/mL Pb^{2+}, 20 μg/mL Hg^{2+}, pH 4.0 acetate buffer

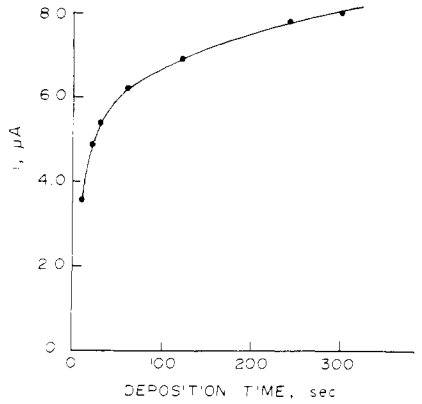

Figure 2. Height of Tl stripping peak as a function of deposition time. Deposition potential, −0.90 V vs. SCE. 405 ng/mL Tl^+, pH 4.0 acetate buffer

of current increase is much less. The inflection at about 60 s signifies that complete electrolysis of Tl during the deposition step has been achieved. The smaller increase in current for longer deposition times is attributable to diffusion into the cell along the edges of the electrode (*10*). Thin-layer coulograms of Tl^+ also showed an inflection at about 60 s, again signifying that complete electrolysis was achieved in this time. Because of the edge effect, the deposition time should be controlled.

In most voltammetric techniques, the magnitude of the peak current is used as the analytical parameter to measure concentration. Table I shows the peak currents obtained for standard solutions of Pb^{2+}, Cd^{2+}, Tl^+, and Cu^{2+} at pH 4 and of Zn^{2+} at pH 8.5. Each value of current represents the average of three analyses with relative precision better than 5%. For all five metals, the plot of peak current vs. concentration is linear over more than two orders of magnitude. The detection limit for Tl^+, Cd^{2+}, Pb^{2+}, and Zn^{2+} is 10 ng/mL while that of Cu^{2+} is somewhat higher (40 ng/mL) due to the fact that the copper stripping peak appeared as a shoulder on the mercury stripping peak.

To demonstrate the multielement capabilities of this method, voltammograms of mixtures of Zn^{2+}, Cd^{2+}, and Pb^{2+} at pH 8.5 were recorded. A typical voltammogram is shown in Figure 3. Sharp peaks were obtained for each metal with no overlap. (The small wave at −0.2 V is attributed to copper in the supporting electrolyte.) The corresponding peak currents for these metal ion concentrations are shown in Table II. The peak currents as a function of concentration were linear. Detection limits and precision data for the ion mixtures

Table I. Variation of Peak Current with Concentration of Pb, Cd, Tl, Cu, and Zn Standard Solutions

Pb[a]		Cd[a]		Tl[a]	
C, ng/mL	i, μA	C, ng/mL	i, μA	C, ng/mL	i, μA
25	3.0	25	1.5	20	0.2
50	4.8	50	2.2	87	1.1
100	8.3	100	5.0	170	2.7
200	16	200	10	405	6.7
500	38	500	24	742	13

Cu[a]		Zn[b]	
C, ng/mL	i, μA	C, ng/mL	i, μA
48	1.4	55	11
97	2.8	110	21
190	4.0	220	38
443	8.6	440	73

[a] pH 4.0 acetate buffer. [b] pH 8.5 ammonium buffer.

Table II. Variation of Peak Current with Concentration of Pb, Cd, and Zn Mixtures[a]

	i, μA		
[M], ng/mL	Pb	Cd	Zn
72	8.5	9.0	17
117	15	30	49
350	22	47	71
580	33	84	133

[a] pH 8.5 ammonium buffer.

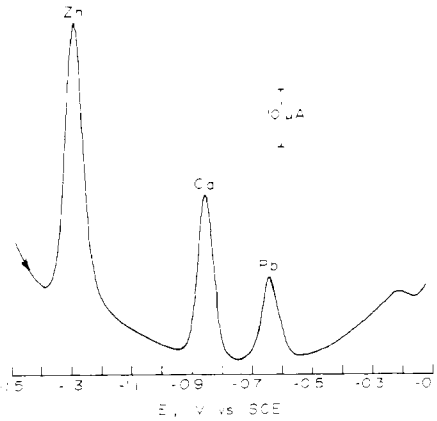

Figure 3. Thin-layer differential pulse anodic stripping voltammogram for 218 ppb Zn^{2+}, Cd^{2+}, and Pb^{2+} in 20 ppm Hg^{2+}, pH 8.5 ammonia buffer. Deposition at −1.5 V vs. SCE for 60 s

were the same as those reported above for the single ion analyses.

Analysis of Lead in Blood. The detection of lead in blood is one of the most frequent analyses of a metal in a biological sample. Its importance stems from the opportunities for lead poisoning by ingestion of paint in old houses (by children) and by the general exposure to lead in the environment. Consequently, the analysis of lead in blood was selected to test the utility of thin-layer DPASV for analysis of biological fluids. Since more than 90% of the lead in blood is bound in the red blood cells, samples were acid digested before analysis.

Prior to the actual analysis of lead in blood, lead solutions of known concentrations were analyzed by the method of standard addition. This served two purposes: (a) the amenability of the standard addition method to thin-layer DPASV was checked, and (b) the retention of Pb through the acid digestion procedure was evaluated. Known volumes of a standard lead solution were added to test tubes and acid digested, as described in the Experimental section. The digested material was diluted, buffered, and analyzed for lead

Table III. Determination of Pb by Thin-Layer DPASV: Recovery Studies

A. Analysis of Standard Lead Solutions Carried through Digestion Procedure

Pb added, ng	Pb found, ng	Deviation, ng
6	6	0
11	12	1
22	24	2
28	28	0

B. Analysis of Blood Samples Spiked with Pb

Pb added to 10.9 ng	Total Pb, ng	Pb found, ng	Deviation, ng
0	10.9	10.9	...
5.5	16.4	16	0.4
16.7	27.6	23	-4.6
27.8	38.7	44	+5.3

Table IV. Determination of Lead in Blood Samples[a]

ESA procedure, ng/mL[b]	Thin-layer DPASV, ng/mL	Deviation, ng/mL
190	200	10
200	170	-30
230	240	10
170	150	-20
240	250	10
290	260	-30
340	290	-50
210	260	50
290	350	60

[a] Blood samples provided by the Cincinnati Lead Program, Cincinnati Health Department. [b] Results reported by Cincinnati Lead Program using Environmental Science Associates method (*12*).

in the thin-layer cell. Results are given in Table IIIA. Each sample was run in duplicate with a relative precision of better than 4%. The percent deviation of the amount of lead found from the amount of lead added was better than 9% in all cases. The precision and accuracy of these data are comparable to other electrochemical trace methods at these low lead levels. Spiked blood samples were then analyzed by the same procedure mentioned above. First, two separate blood samples were analyzed for lead, and the average value was taken to be 10.9 ng absolute (100-μL blood sample). Varying amounts of lead were then added to 100-μL blood samples and the total amount of lead was determined. The results tabulated in Table IIIB verify recovery of the lead added to the blood samples.

Table IV shows the results for the analysis of lead in blood samples obtained from the Cincinnati Lead Program. The data obtained by thin-layer DPASV agree quite well with the values reported by the Cincinnati Lead Program. Data obtained by the authors were based on the acid digestion of the blood samples. (The data obtained by the Cincinnati Lead Program Laboratory were based on the method developed by Environmental Science Associates (*12*). In this method, 100 μL of blood are added to a specially prepared reagent mix which releases the lead from the red blood cells. The resulting solution is then analyzed by anodic stripping voltammetry at a mercury-film carbon electrode in bulk solution.)

Analysis of Lead in Soybeans and Oysters. In order to further explore the utility of the thin-layer DPASV technique for the analysis of plant and animal tissues, soybeans and oysters were analyzed for lead. Digested soybean and oyster samples were provided by the Cincinnati Food and Drug Administration (FDA) Laboratory. The soybeans had been

Table V. Determination of Lead in Soybeans and Oyster[a]

DPASV, ng/mL[b]	Thin-layer DPASV, ng/mL	Deviation, ng/mL
Soybean 22	25	3
Soybean 22	24	2
Oyster 28	26	-2

[a] Samples provided by Cincinnati District Laboratory of the Food and Drug Administration. [b] Results reported by FDA using DPASV in conventional cell.

grown on waste sludge and were suspected of containing high levels of heavy metals. The lead content of these samples was analyzed at the FDA laboratory by DPASV at a hanging mercury drop electrode. Table V presents the data for duplicate analyses of the soybean digest, and a single analysis of the oyster sample. Good agreement was obtained at a lead level very near the detection limit of the thin-layer DPASV method.

Twin-Electrode Thin-Layer Voltammetry: Elimination of Intermetallic Compound Interferences. A difficulty with DPASV involves interactions between materials which have been preconcentrated into or onto the electrode. One such interaction is the formation of intermetallic compounds by metals deposited into mercury. A variety of intermetallic compounds have been reported: Ag–Cd, Ag–Zn, Au–Cd, Au–Mn, Au–Sn, Au–Zn, Cu–Cd, Cu–Mn, Cu–Ni, Cu–Zn, Fe–Mn, Mn–Ni, Ni–Sb, Ni–Sn, Ni–Zn, Pt–Sb, Pt–Sn, Pt–Zn (*13*); Cu–Ga (*14*); Pt–Hg (*15*); and Ag–Cu (*16*). Because of the high demand for the determination of copper and zinc, the Cu–Zn intermetallic is one of the most frequently encountered difficulties. The formation of this compound precludes the simultaneous determination of Cu^{2+} and Zn^{2+}, since the Cu–Zn intermetallic strips out at a potential very close to that of free Cu, causing enhancement of the Cu peak and diminution of the Zn peak. Both Cu and Zn can be determined by a more complex procedure in which Cu is first determined without depositing Zn; Zn is subsequently determined after addition of Ga^{3+} which preferentially forms a Ga–Cu intermetallic, leaving the Zn free (*14*).

An electroanalytical method which circumvents the intermetallic problem associated with the simultaneous determination of Cu and Zn has been explored. The approach involves the twin-electrode thin-layer cell [developed by Reilley and co-workers (*3*, *10*, *17*)] in which a thin layer of solution is sandwiched betwen two parallel working electrodes. The essence of the method is to selectively deposit the Cu from the thin solution layer onto one electrode and the Zn onto the other electrode, thereby avoiding formation of the Cu–Zn intermetallic compound by physical separation of the interferents. This can be implemented by independently controlling the potential of the two working electrodes.

A typical twin-electrode thin-layer stripping voltammogram for Cu and Zn is shown in Figure 4. The voltammogram was obtained by first introducing 10 μL of solution containing 20 μg/mL Cu^{2+}, 60 μg/mL Zn^{2+}, and 100 μg/mL Hg^{2+} into the cell. The two electrodes were then simultanecusly potentiostated to −0.97 V (WIG, electrode 1) and −0.20 V (glassy carbon, electrode 2) for 3 min. This caused codeposition of Hg and Cu on the WIG electrode and formation of a Hg film on the glassy carbon electrode. The potential of the glassy carbon electrode was then switched to −1.25 V for 3 min, causing exhaustive deposition of Zn into the mercury film on this electrode and, also, the removal of dissolved O_2 by reduction to H_2O. The anodic stripping voltammograms were then recorded by first scanning the potential of the glassy carbon electrode from −1.25 V to −0.02 V, giving the stripping peak for Zn. The potential of the WIG electrode was then

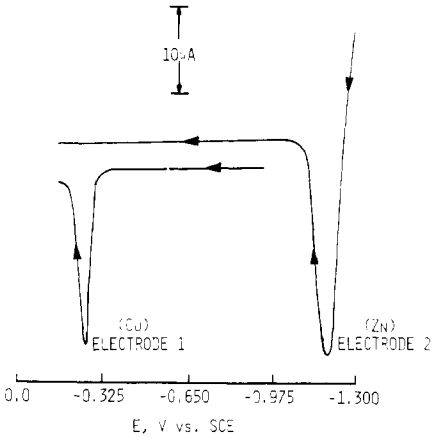

Figure 4. Anodic stripping voltammograms for twin-electrode thin-layer cell. Electrode 1: Potential scan from −0.97 V (deposition potential) to −0.02 V. WIG electrode. Electrode 2: Potential scan from −1.25 V (deposition potential) to −0.02 V. Glassy carbon electrode. Scan rate, 13 mV s^{-1}. 60 μg/mL Zn^{2+}, 20 μg/mL Cu^{2+}, pH 8.5 ammonia buffer. Cell volume, 6 μL

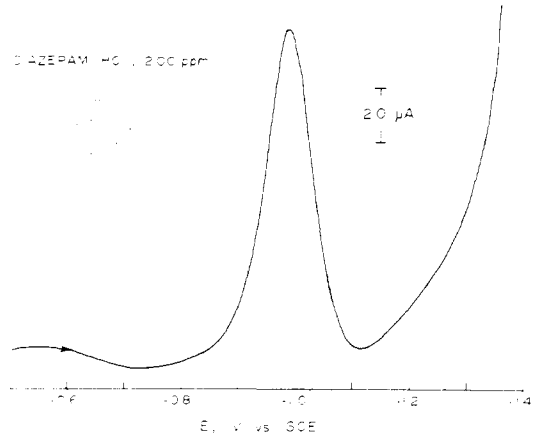

Figure 5. Thin-layer differential pulse voltammogram for diazepam. Potential scan initiated at 0.00 V vs. SCE. 2.00 μg/mL diazepam hydrochloride, pH 7.0 phosphate buffer

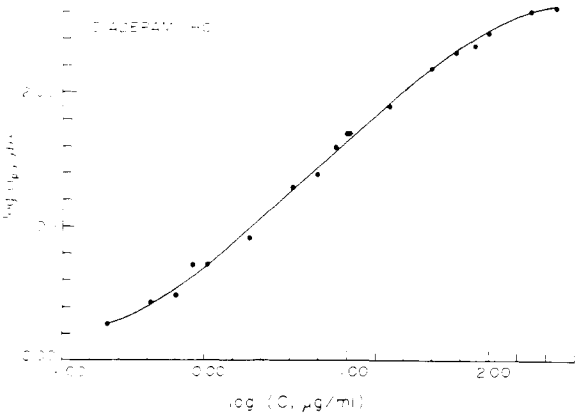

Figure 6. Peak height vs. concentration for thin-layer DPV of diazepam hydrochloride. Conditions given in Figure 5. Electrode potentiostated at −0.800 V for 1 min for O_2 removal prior to recording voltammogram

scanned from −0.97 V to −0.02 V giving the stripping peak for Cu. It can be seen that complete separation of the two metals by deposition onto the two different electrodes has been achieved.

Preliminary studies show a linear response for Cu and Zn in the micrograms per milliliter range with linear sweep voltammetry as the stripping technique. Circuitry is presently being developed to enable twin-electrode differential pulse voltammetry to be performed with independent control of the deposition and stripping at the two working electrodes. DPASV should lower the detection limit into the lower parts per billion range as has been demonstrated above for the single electrode thin-layer cell.

Thin-Layer Differential Pulse Voltammetry of Diazepam. Differential pulse voltammetry (DPV) can be used in conjunction with the thin-layer cell for the analysis of electroactive species which are not amenable to the preconcentration aspect of DPASV. An example of this is diazepam hydrochloride, which is the active drug in the tranquilizer dispensed under the trademark of Valium. Diazepam has been shown to exhibit a reduction wave with differential pulse polarography (E_P = −0.645 V vs. SCE in 0.1 M HCl) (*18*).

A well-defined reduction wave is obtained by DPV in the thin-layer cell as shown by the voltammogram in Figure 5 for 2.00 μg/mL of diazepam hydrochloride. This voltammogram was obtained with the WIG electrode which gave a sharper peak than the mercury coated WIG. The peak potential is −0.965 V vs. SCE in the pH 7.00 phosphate buffer. A plot of peak current as a function of diazepam concentration gives a linear relationship between 1 and 60 μg/mL as shown in Figure 6. The detection limit of 0.1 μg/mL is lower than the value of 0.4 μg/mL reported for differential pulse polarography (*18*). Some memory effects were observed when samples of very high concentration were followed by samples of low concentration. This emphasizes the necessity of thorough rinsing of the thin-layer cell between samples, since the cell has a very large surface area-to-volume ratio.

The data reported for diazepam typify results obtained in this laboratory for other electroactive pharmaceuticals (*19*). Materials of this sort are quite amenable to analysis by thin-layer DPV.

CONCLUSIONS

The data presented in this paper clearly demonstrate the compatibility of the thin-layer cell with pulsed voltammetric techniques for electrochemical analysis. Linear calibration curves for DPASV of standard solutions of Zn^{2+}, Cd^{2+}, Tl^{+}, Pb^{2+}, and Cu^{2+} were obtained over a wide dynamic range. Blood, soybean, and oyster samples were analyzed for lead, and the data obtained compared favorably to the lead concentrations reported by other laboratories. Precision and accuracy in the small volume TLE agree quite well with other electrochemical methods for trace analysis. The absence of stirring and oxygen removal by nitrogen bubbling greatly simplifies the procedure compared to conventional anodic stripping.

Twin-electrode thin-layer electrochemistry shows potential as a means of eliminating interferences due to the formation of intermetallic compounds such as Cu–Zn. The true capability for trace analysis will be realized when circuitry is developed for application of the differential pulse waveform with independent control of the two electrodes.

The data reported for diazepam illustrate the potential utility of thin-layer DPV for the analysis of drugs and other organic compounds at low levels of concentration. It is in this area that the small volume aspect of the thin-layer cell may be most useful, especially for small biological samples. Since species of this type are usually not amenable to stripping voltammetry, the decrease in analytical detection limit caused by dilution to fill a conventional cell cannot be recovered by preconcentration at an electrode.

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High Speed Synchronous Data Generation and Sampler System: Application to On-Line Fast Fourier Transform Faradaic Admittance Measurements

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A *sy*nchronous *da*ta *ge*neration and *s*ampler (SYDAGES) system constructed to assist broadband FFT electrochemical relaxation measurements is described and evaluated. SYDAGES consists of a digital-to-analog converter, two analog-to-digital converters, three 1024-word shift register memories, and control circuitry. It functions as a programmable signal generator and two signal averaging data acquisition channels which are synchronized up to data rates of 500 kHz. SYDAGES is run directly by a minicomputer without manual control. Its performance characteristics are demonstrated here using dummy cell and electrochemical cell admittance data. In the latter instance, good quality cell admittance data are obtained to 125 kHz, enabling dynamic nonfaradaic measurement and compensation to reveal the faradaic admittance up to 40 kHz.

Previous work in this laboratory (*1–5*) has demonstrated the benefits of on-line multiple frequency Fast Fourier Transform (FFT) faradaic admittance measurements using a particular applied pseudo-random waveform. The benefits include the ability to acquire kinetic and thermodynamic parameters with unprecedented speed and precision and to characterize reactions with very high rates. The particular waveform suggested has the following important properties.

(a) It is periodic.

(b) It contains a limited number of frequency components of approximately equal amplitude which are selected specifically to cover the frequency range of interest without being overly redundant.

(c) All frequency components are *odd* harmonics of the lowest.

(d) The phases of the various frequency components are randomized as a function of frequency and measurement pass.

(e) The signal is generated by a digital-to-analog converter (DAC) from a data array of 2^n points (n is an integer).

(f) The applied waveform generation is synchronized to the analog-to-digital converter (ADC) operations which acquire the reference-working electrode potential and the cell current signals.

In reported work the signal generation and sampling was controlled directly by a minicomputer, which could repeat a complete conversion cycle (two sampling and ADC steps, one DAC operation, and data storage) on a period no shorter than 100 μs. The effects of solution ohmic resistance (R_s) and double-layer capacitance (C_{dl}) were removed by analog techniques restricted to liquid-metal electrodes under strictly controlled conditions, including accurate potentiostat operation. One overall result of this strategy was an effective measurement bandpass limitation of a few kiloHertz.

A far more general method of acquiring the faradaic admittance is the frequency domain analysis of the total cell admittance, as recommended originally by Sluyters (*6*), but modified to include correlation of measured input and response waveforms as invoked by deLevie (*7*) and Pilla (*8*). This approach has the advantage of providing, in the presence or absence of a faradaic component, rapid, dynamic measurement of R_s and C_{dl} which will detect changes in these variables with time. This can be very important in many situations, such as when monitoring reaction kinetics as a mercury electrode grows (*9*), or with solid electrodes, especially outside the clean laboratory environment. Of course, the acquisition of the nonfaradaic component magnitude enables convenient vectorial subtraction of these contributions from the total cell admittance to reveal the faradaic response. However, for many chemical systems this total cell admittance analysis requires that the data be acquired at significantly higher frequencies than a 100-μs conversion cycle will allow. Another stimulus toward performing higher-frequency

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