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# Determination of Heavy Metals by Thin-Layer Chromatography-Square-Wave Anodic Stripping Voltammetry

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A square-wave anodic stripping voltammetric method is described for low parts per million determination of heavy metals separated by thin-layer chromatography (TLC). Heavy metal samples are separated on carboxymethyl cellulose TLC plates and detected by anodic stripping voltammetry (ASV) using a cellulose dialysis membrane-covered mercury film electrode (CM-MFE) placed directly on the TLC plate surface in a thin film of supporting electrolyte solution. The fast scan rates possible in square-wave voltammetry during the stripping step eliminate the need to deoxygenate the sample. Results are presented for a mixture of Pb(II), Cd(II), Cu(II), and Zn(II). Calibration curves for Pb(II) were linear over the range 10–500 ng, with a relative standard deviation of the peak current over a set of eight separate 100-ng Pb(II) samples of 16%.

## INTRODUCTION

Anodic stripping voltammetry (ASV) is a powerful technique for rapidly measuring trace levels of heavy metals, especially in conjunction with modern pulse voltammetric techniques that discriminate double-layer charging currents from Faradaic currents.<sup>1</sup> The application of ASV to analytes in complex sample matrices can be complicated by several factors, particularly the adsorption of interfering components to the electrode surface. A gradual loss of electrode activity ("poisoning") by the adsorption of proteins and surfactants or the accumulation of reaction products can be observed when ASV is used in determinations of heavy metals in complex mixtures. Mercury electrode surfaces in particular are poisoned readily by humic acids, which are usually present in samples of environmental origin.<sup>2,3</sup> In addition to these adsorptive interferences, the presence of overlapping peaks in mixtures containing several electroactive components can also hinder the successful application of ASV.

The coupling of electrochemical techniques to various column liquid chromatographic (LC-EC) techniques has proven to be an effective way of preventing adsorptive interferences and separating electroactive components.<sup>4</sup> In addition to LC-EC, chemically-modified electrodes have been used to improve ASV selectivity. Efforts have been reported with Nafion perfluorosulfonate resin films,<sup>5-7</sup> cellulose acetate films,<sup>8</sup> and cellulose dialysis membrane-covered electrodes.<sup>9,10</sup>

These membranes serve to control access to the mercury film. Furthermore, the presence of overlapping peaks in mixtures can be addressed by using a mercury film electrode (MFE), which has the highest resolving power of the electrodes used in ASV.<sup>1,9</sup>

Koval reported recently the novel application of in situ voltammetric detection for thin-layer (or planar) liquid chromatography (TLC).<sup>11</sup> Low-nanogram levels of *p*-anisidine and *p*-phenetidine were identified by square-wave voltammetry (SWV) using a Pt disk microelectrode after development on silica gel TLC plates, with linearity observed over several orders of magnitude. We demonstrate here the feasibility of using a cellulose acetate membrane-covered mercury film electrode (CM-MFE) to determine Pb(II), Cd(II), Cu(II), and Zn(II) by themselves and in mixtures by in situ ASV after separation on carboxymethyl cellulose (CMC) TLC plates.

## EXPERIMENTAL SECTION

**Apparatus.** Thin-layer chromatography (TLC) was performed in a 10- x 10-cm saturated (sandwich) vertical development chamber, fabricated in-house using Macor (a machinable ceramic material). The sandwich or "S-chamber" provides more reproducible chromatographic conditions.<sup>12</sup>

A glassy carbon disk working electrode (3-mm diameter) and Ag/AgCl (3 M NaCl) reference electrode (Model RE-1) were obtained from Bioanalytical Systems (West Lafayette, IN), while a Pt disk auxiliary electrode (0.25-mm diameter) was fabricated as described by Koval et al.<sup>13</sup> ASV was conducted on a Bioanalytical Systems Model 100A electrochemical analyzer (West Lafayette, IN) using Osteryoung square-wave anodic stripping voltammetry (SWASV), primarily because the fast scan rates attainable obviate the need to deoxygenate the sample. As Wojciechowski and Balcerzak point out,<sup>14</sup> SWV scan rates in excess of 40 mV/s allow stripping to be completed before a significant amount of dissolved oxygen can reach the electrode surface and oxidize the amalgam. Solution purging is not only time-consuming in any ASV measurement but also impractical for the in situ method developed here.

**TLC-SWASV.** The apparatus used is shown in Figure 1. A Nylon "canopy" (15-mm height, 22-mm i.d., 24-mm o.d.) was machined with three holes symmetrically arranged for the electrode insertions, as well as a small (<0.5-mm i.d.) hole for venting (i.e., to relieve pressure during supporting electrolyte addition). Two small three-pronged laboratory clamps were used to secure the canopy over the TLC plate.

**Reagents and TLC Supplies.** All chemicals were of ACS reagent grade or better. All reagents were prepared in water than was de-ionized and then doubly-distilled. Heavy metal standards (nitrate salts) were prepared volumetrically from atomic absorption standards (Fisher Scientific, Pittsburgh, PA).

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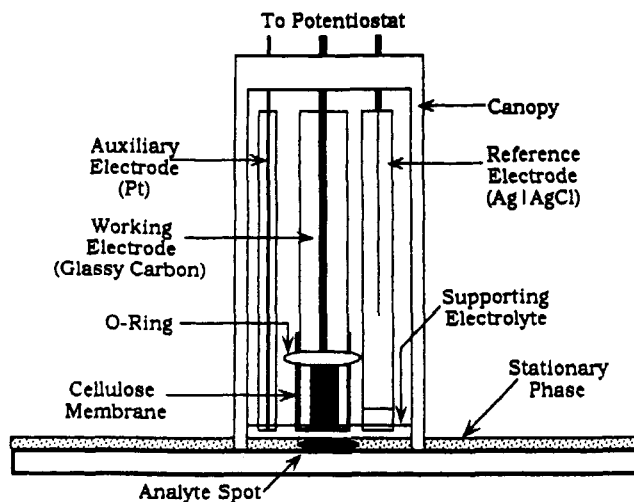


Figure 1. Schematic cross-sectional view of the TLC-ASV apparatus.

A pH between 2 and 3 was maintained in order to prevent the formation of metal hydroxides. Dithizone (Eastman Kodak, Rochester, NY) was repurified and then dissolved in chloroform as a 0.10% (w/v) solution.<sup>15</sup>

Nafion 117, a perfluorosulfonate cation-exchange resin, was obtained from Aldrich (Milwaukee, WI) as a 5% (w/v) solution in a mixture of lower aliphatic alcohols and 10% (v/v) water. Spectrum Spectra/Por 6 cellulose acetate dialysis membranes (28- $\mu\text{m}$  thickness) with a nominal molecular weight cutoff of 3500 were obtained from Fisher Scientific (Pittsburgh, PA).

Whatman polyester-backed carboxymethyl cellulose (CMC) plates (100- $\mu\text{m}$  thickness) were obtained from VWR Scientific (Cleveland, OH).

**Procedures. Container Cleaning.** Heavy metal reagent solutions were stored in polyethylene containers that had been soaked with 0.1 M  $\text{HNO}_3$  for at least 1 week.

**TLC Procedure.** The procedure of Cozzi et al. for the separation of metals on CMC was modified to use 0.60 M acetic acid/0.60 M sodium acetate as the mobile phase.<sup>16</sup> Four samples (0.5–1.0  $\mu\text{L}$ ) were applied to separate manually-scored lanes on the TLC plates using plastic Eppendorf pipets (Brinkmann, Westbury, NY) or glass Nanopipettes (Analtech, Newark, DE). Following a 25-min chromatographic development time, a standard lane was excised and analyte spot(s) were identified with 0.10% (w/v) dithizone in chloroform followed by 10% (v/v)  $\text{NH}_4\text{OH}$  using a glass atomizing sprayer. The location of samples in adjacent lanes was based on the retardation factors ( $R_f$ ) determined from dithizone identification of the standards, as depicted in Figure 2. The visual detection limit using dithizone was approximately 100 ng for each analyte.

**Electrode Polishing.** The working and auxiliary electrodes were polished on a felt pad daily before use with 0.5- $\mu\text{m}$   $\gamma\text{-Al}_2\text{O}_3$  (Buehler Ltd., Lake Bluff, IL).

**NC-MFE Preparation.** Nafion-coated mercury film electrodes (NC-MFE) were prepared as described by Hoyer and co-workers.<sup>5</sup> Nafion 117 was diluted with absolute ethanol to 0.24% (w/v), and 10  $\mu\text{L}$  was applied to the polished working electrode surface to cast the film. The electrode was covered for 1 h to allow dust-free evaporation and then dried in the warm air stream ( $\sim 50^\circ\text{C}$ ) of a heat gun for 1 min. NC-MFE's prepared in this manner correspond to the "thick" width used by Hoyer et al.,<sup>5</sup> with an approximate electrode coverage of 1.2  $\mu\text{g}/\text{mm}^2$ . Best results were obtained when the Nafion-coated electrode was equilibrated prior to MFE preparation for at least 12 h in a 10 mM  $\text{HNO}_3$ /10 mM  $\text{KNO}_3$  solution. This proved to be a critical step—the influence of Nafion film swelling on membrane transport efficiency is well-documented.<sup>17,18</sup>

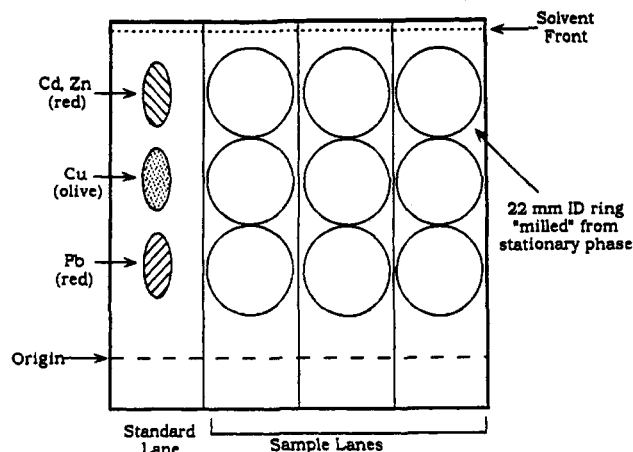


Figure 2. Schematic representation of a developed TLC plate (10  $\times$  10 cm). The color of the dithizone complex of the metal is indicated. Typical dimensions of the spots were 15  $\times$  5 mm.

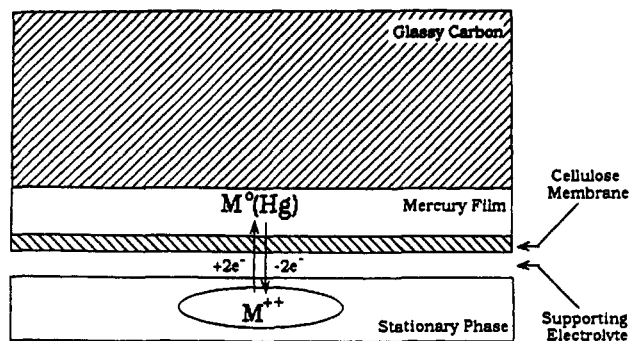


Figure 3. Apparent diffusion of metal cations from the TLC stationary phase to the MFE depicted schematically. Approximate thicknesses are 0.010, 28, and 100  $\mu\text{m}$  for the MFE, CM, and stationary phase, respectively.

**CM-MFE Preparation.** The CM-MFE was prepared daily and stored as described by Stewart and Smart.<sup>9</sup> Spectra/Por 6 cellulose dialysis membranes were heated for 20 min at  $70^\circ\text{C}$  in reagent water, followed by 48 h in reagent water to remove traces of the sodium azide preservative. To prevent degradation by cellulytic microbes, the tubing was transferred to freshly boiled water daily. Stock dialysis membranes (i.e., unwashed) were refrigerated per the manufacturer's instructions. A silicone rubber O-ring was used to hold the membrane ( $\sim 1\text{ cm}^2$ ) in place on the end of the working electrode. Care must be exercised such that the membrane is neither stretched nor allowed to dry out.<sup>19</sup>

**MFE Preparation.** MFE's were prepared in solution ex situ from 1.0 mM  $\text{Hg}(\text{NO}_3)_2$  in 10 mM  $\text{KNO}_3$ /10 mM  $\text{HNO}_3$  for 10 min at  $-1.000\text{ V}$  (all potentials are vs a  $\text{Ag}|\text{AgCl}$  (3 M  $\text{NaCl}$ ) reference electrode). The mercury film was prepared under a  $\text{N}_2(\text{g})$  blanket to improve its uniformity.<sup>14</sup> The supporting electrolyte (10 mM  $\text{KNO}_3$ /10 mM  $\text{HNO}_3$ ) also included 0.1 mM  $\text{Hg}(\text{NO}_3)_2$  to help maintain the mercury film during the measurement. Best results were obtained by electrically preconditioning the CM-MFE in a deaerated 10 mM  $\text{KNO}_3$ /10 mM  $\text{HNO}_3$  solution by cycling the potential between  $+100$  and  $-1000\text{ mV}$  for eight cycles (each of 2-min duration) immediately following MFE preparation. The estimated MFE thickness is 100  $\text{\AA}$ .

**SWASV.** The following conditions were typically used for on-plate measurements: initial (deposition) potential of  $-1.100\text{ V}$ ; final potential of  $+0.100\text{ V}$ ; deposition time of 30–60 s; quiet time of 0 s; square-wave amplitude of 25 mV; frequency of 25 Hz; potential step of 4 mV—thus the scan rate was 100 mV/s.

**TLC-SWASV.** A schematic of the apparent transport process is depicted in Figure 3. The TLC-SWASV experimental procedure is as follows: (1) after chromatography, the plate is

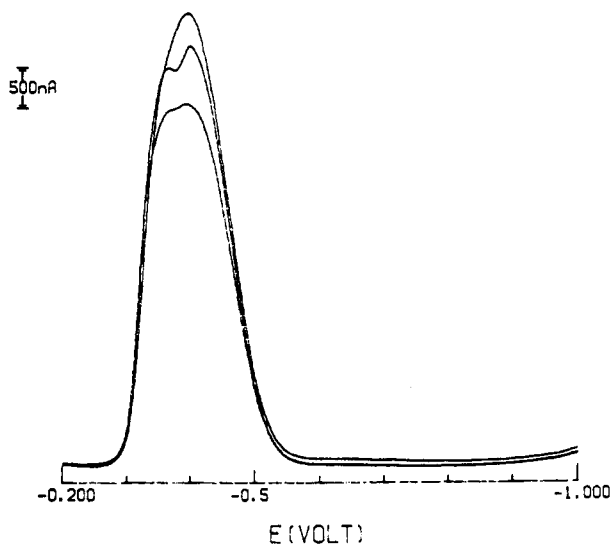
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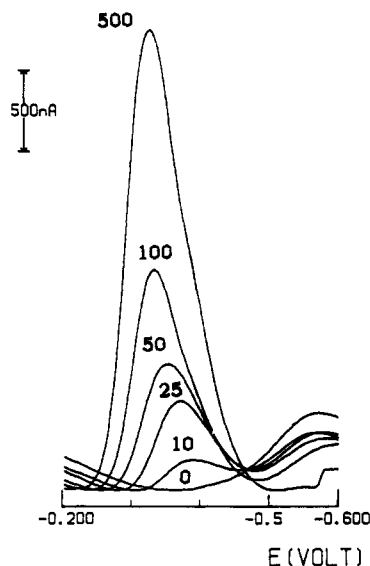


**Figure 4.** Example of SWASV using the NC-MFE on a CMC TLC plate. Pb(II) measured in triplicate (200-ng sample) reveals a gradually increasing current response and distortion of the peak shape.

dried at room temperature; (2) on the basis of the standard's  $R_f$ , analyte spots are isolated using a milling device (22-mm i.d., 24-mm o.d.) to remove the stationary phase around the spot as depicted in Figure 2; (3) a cylindrical Nylon canopy (15-mm height, 22-mm i.d., 24-mm o.d.) containing the three electrodes (arranged symmetrically within it) is clamped over the spot, such that the edges of the canopy fit securely within the milled ring; (4) 600  $\mu$ L of supporting electrolyte is immediately added through a port in the top of the canopy to form a shallow ( $\sim 0.4$ -mm) film across the plate surface that is confined by the canopy; (5) the ASV measurement is made in quadruplicate (3–6 min/sample). In this way, the measurement is made directly on the TLC plate. Approximately 20-fold analyte losses were observed as a result of chromatographic development, in basic agreement with Koval's estimate for their voltammetric method.<sup>11</sup> Between samples the working electrode is rinsed in deoxygenated 10 mM  $\text{KNO}_3$  and polarized in solution under a  $\text{N}_2(\text{g})$  blanket to +100 mV (by cycling the BAS 100A "step" function between +99 and +100 mV indefinitely).

## RESULTS AND DISCUSSION

**Nafion-Modified Electrodes.** Initial characterization of Nafion-coated (NC) polymer-modified MFE's was performed using Pb(II) standards applied to CMC TLC plates without chromatographic development. Heavy metal standards (10  $\mu$ L) were applied to CMC and allowed to air dry. Figure 4 shows a typical voltammogram using a NC-MFE. Three disadvantages in using the NC-MFE on-plate were observed. First, Nafion's ability to preconcentrate cations proved to be impractical. A stable peak current signal could only be obtained after at least six measurements. Supporting electrolyte (blank) samples revealed a high background signal which increased with coating thickness. Furthermore, when samples over a range of concentrations were analyzed in random order, the peak current signal for a given sample was proportional to that of the previous spiked sample (i.e., hysteresis). Second, the variability of measurements on successive days was very high, as the reproducibility in casting a fresh Nafion coating each day by the evaporative procedure was poor. In fact, roughly half of the NC-MFE's yielded erratic responses following MFE preparation and were not used further. Finally and most importantly, although NC-MFE's have been successfully used for solution-phase ASV measurements,<sup>5–7</sup> the Nafion films used in this work were too thin to protect the MFE from abrasive contact with the TLC stationary phase surface. The prospect of having to prepare a fresh MFE after each sample was unattractive from

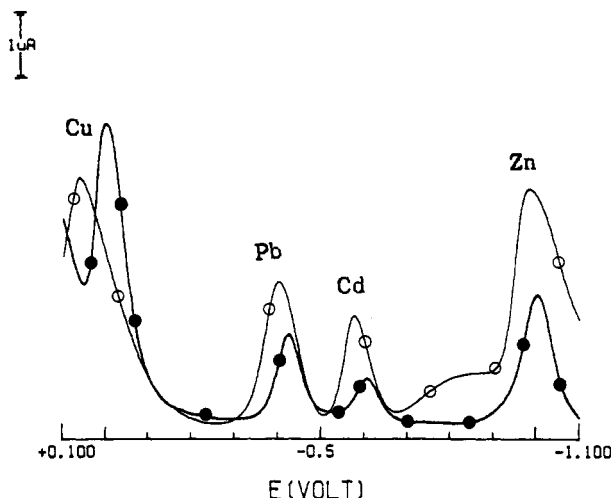


**Figure 5.** Calibration standards for on-plate SWASV of Pb(II) using the CM-MFE. Pb(II) was applied as 0-, 10-, 25-, 50-, 100-, and 500-ng samples. Each sample was measured in triplicate.

the standpoint of sample throughput. Attempts to design an electrode housing for a bare MFE that would prevent abrasive damage (e.g., use of spacers) were unsuccessful, apparently because the distance from the working electrode to the TLC stationary phase was much greater than the diffusion layer.

**Cellulose-Modified Electrodes.** Voltammograms obtained using a CM-MFE on a CMC TLC plate are shown in Figure 5. Several improvements compared to the NC-MFE results became evident. First, preparation of the CM-MFE is simple. Working electrodes can be prepared quickly on the day of use since membrane equilibration is not required for each electrode. Second, the background signal is low, as cellulose does not preconcentrate cations as significantly as Nafion. Cellulose is less specific than Nafion, which is an important consideration analytically as rapid diffusion across the membrane minimizes sample carryover as well as shortens the analysis time. Although a delay in reaching the maximum current response was observed, the signal stabilizes quickly (usually by the third replicate of a given sample). Furthermore, a useful diagnostic was that peak currents above  $\sim 4$   $\mu$ A were indicative of an overloaded MFE, as distorted peaks (very broad with "jagged" crests) were consequently observed. A slight positive drift in the peak potential was observed for NC- and CM-MFE's, though this usually stabilized by the second replicate of a given sample for the CM-MFE's. Third, the peaks are narrower than those obtained with the NC-MFE which again suggests less restricted ion transport through the membrane. Fourth, the membranes protect the MFE from abrasive contact with the TLC surface during the course of the day.

The relative standard deviation (RSD) for eight separate 100-ng samples (10  $\mu$ L of 10 ppm) of Pb(II) spotted on CMC was 15.9%—by comparison, a bare glassy carbon electrode in solution (250 ppb) exhibited a RSD of 4.00%. Calibration curves for Pb(II) applied to the surface were linear from approximately 10 to 400 ng. For a series of calibration standards (e.g., Figure 5), each sample was measured in triplicate and the third measurement was plotted. For example, four separate calibration curves over a period of several weeks yielded an overall least-squares linear fit of  $y$  ( $\mu$ A) =  $0.00719 \pm 0.00412$  ( $\mu$ A/ng) +  $0.211 \pm 0.253$  ( $\mu$ A); correlation coefficient,  $r = 0.976 \pm 0.0258$ . Thus in qualitative terms, the variability in the slope and intercept values illustrates the high day-to-day variability of the CM-MFE calibration curves, while the low variability in the correlation



**Figure 6.** On-plate (O) and solution (●) SWASV of a four-component mixture using the CM-MFE. The solution concentrations were 3.00 ppm Zn(II), 0.250 ppm Cd(II), 0.250 ppm Pb(II), and 0.500 ppm Cu(II), while a 10- $\mu$ L sample containing 100 ppm Zn(II), 10 ppm Cd(II), 10 ppm Pb(II), and 20 ppm Cu(II) was applied to the TLC plate.

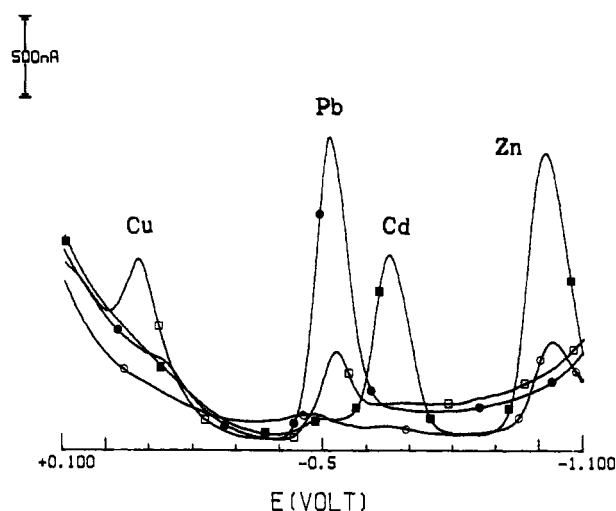
coefficient shows that CM-MFE's prepared for a given day possessed good linearity.

**ASV Determination in Mixtures by Carboxymethyl Cellulose TLC.** A mixture containing Cu(II), Pb(II), Cd(II), and Zn(II) in reagent water was applied (1  $\mu$ L) to carboxymethyl cellulose TLC plates (250 ng/analyte) and developed. The  $R_f$  zones were lead (0.30–0.48), copper (0.51–0.70), and zinc/cadmium (0.73–0.86). The TLC spots "tailed" slightly more in a multicomponent mixture, and the  $R_f$  values increased by  $\sim 10\%$  compared to the values observed for single component determinations. It is important to note that the origin is well-separated from the heavy metals—that is, where most potentially interfering organic substances would remain under these chromatographic conditions.

Figure 6 shows voltammograms comparing the four-component mixture on-plate (i.e., undeveloped) to a qualitatively identical mixture in solution, using the same CM-MFE. The peak resolution does not degrade noticeably, and only minor drift in peak potentials was observed. Voltammograms from each of the chromatographic zones given above (origin, Pb, Cu, and Zn/Cd) are superimposed on the same potential axis in Figure 7, using a CM-MFE after development of the four-component mixture. Only minor carryover is apparent: some Pb(II) was observed in the Cu(II) zone, while Zn(II) was found at the origin. Intermetallic compounds of Cu(II), particularly of Cu(II)–Zn(II) and Cu(II)–Cd(II), can be significant problems in ASV.<sup>1</sup> The separation of Cu(II) from Zn(II) and Cd(II) in the method reported here reduces this phenomenon to a negligible level. Although Cd(II) and Zn(II) coelute, they are resolved by the inherent electrochemical selectivity of SWASV.

## CONCLUSIONS

There are few quantitative detection methods in TLC and even fewer that are electrochemical in nature. Work using amperometric detection<sup>20,21</sup> and voltammetric detection<sup>11,22</sup> are the only other reports of electrochemical detection in TLC that could be identified in the literature, although the



**Figure 7.** On-plate SWASV of a four-component mixture after chromatographic development using the CM-MFE. Voltammograms from the origin (O), Cu (□), Pb (●), and Cd/Zn (■) region of the TLC plate (as shown in Figure 2) are overlaid on the same potential axis.

isolation of specific regions of various surfaces to perform diverse electrochemical measurements has been the subject of recent efforts in several laboratories.<sup>23–25</sup>

The accurate measurement of free metal ion concentration is important in environmental, forensic, and clinical applications. This technique is simpler than atomic spectroscopic methods and potentially more powerful than the basic ASV technique; thus it may hold promise for field laboratory use, e.g., trace metals in hazardous waste samples. While the MFE is known for its stability and ease of preparation and usage, the cellulose dialysis membranes used in this study also possess many attractive features. These membranes are chemically inert, nonelectroactive, hydrophilic, and insoluble in water—ideal properties for an electrode membrane material. A highly variable film casting procedure is not needed to prepare the working electrode—the cellulose membranes are in fact quite easy to handle. Furthermore, the cellulose membranes are nontoxic, inexpensive, and widely available.

Work is in progress to modify the experimental configuration by using an array of ultramicroelectrodes, as the accuracy of working electrode placement is a major source of variability in the present system (variability of electrode placement was revealed by staining samples with dithizone after the ASV measurement). The methodology will also be optimized, especially the sensitivity (deposition time and volume of supporting electrolyte within the canopy), and the apparatus will be miniaturized to increase the sample throughput. Finally, potentiometric stripping analysis is being explored as an alternative to SWASV because it not only also allows one to forego sample deoxygenation but also may possess selectivity and sensitivity advantages.

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