Detection of Glutathione Conjugate Export from Yeast Cells

A Summary Report to The Electrochemical Society for the 2003 Edward G. Weston Summer Research Fellowship

by Janine Mauzeroll

To probe the transport activity of cells, we use Scanning Electrochemical Microscopy (SECM), a surface technique that allows one to detect electroactive ions or molecules that are transported from cells across membranes or ion channels into an outside solution.[1] As proof of concept, the rate of transport of thallium (I) ions across a lipid membrane into solution was quantified.[2] The experimental current with time relationships were fitted to numerical simulations and good agreement was obtained.[3]

Presently, we are extracting transport rates for the detection of a detoxification product that is released from yeast cells when the cells are put in contact with menadione, a quinone very similar to a drug used in chemotherapy treatment. Quinones are oxidants that are readily transported into cells and retain the ability to impose a chemical stress once inside cells. The quinone reaction in presence of intracellular oxygen leads to the formation reactive oxygen species that damage DNA and cause cell growth arrest. To cope with such stress, cells have enzymatic and non-enzymatic defenses, like antioxidants. Glutathione (GSH) is a non-enzymatic sulphydryl molecule that detoxifies cells by sacrificially reacting with quinones to form a conjugate and prevent the irreversible modification of important cellular macromolecules. The conjugates can then be degraded to other substances or actively removed from the intracellular media by a pump.[4] In the case of the menadione-glutathione conjugate, the conjugate remains intact[5] and is excreted into the extra cellular media where it can be detected using SECM.

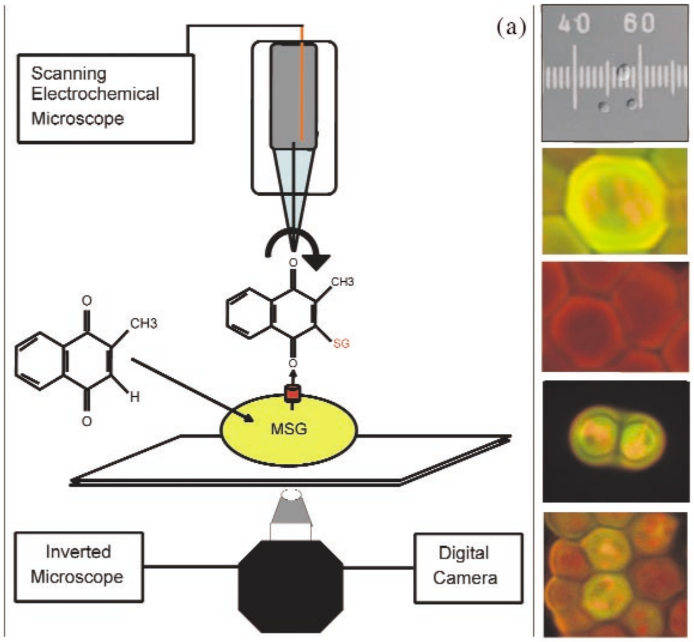

Fig. 1. (a) Experimental setup; (b) Transmission micrograph of DAY4 Saccharomyces Cerevisiae. Smallest division is 2μm; (c) fluorescence image of immobilized yeast cell used for positive control in viability experiments; (d) fluorescence image of immobilized yeast cell used for negative control in viability experiments. The cells were killed in the UV reactor; (e) fluorescence image of immobilized yeast cell following 1h incubation in 0.1 mM menadione; and (f) fluorescence image of immobilized yeast cell following a 30 minute exposition the microscope Hg lamp.

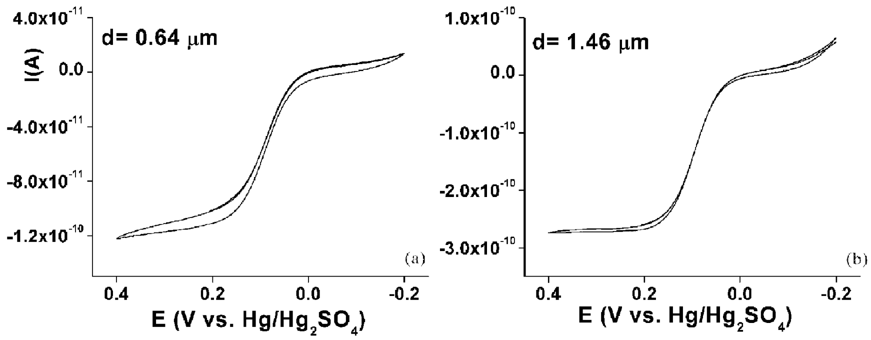

Fig. 2. Voltammograms in 1 mM ferrocene methanol and 0.1 M KCl solution with laser pulled Pt ultramicroelectrodes (a) Pt diameter = 640 nm; (b) Pt diameter = 1.46 μm) recorded at 20 mV/s.

Experimentally, cells are immobilized on a glass substrate and a small size electrode (microns to nanometer size) is then positioned a few microns above it using the piezoelectric positioning system of the SECM (Fig. 1a). An oxidative stressing agent, menadione, is then imposed such that the cells are forced to release the glutathione conjugate into solution. The released conjugate then diffuses to the nearby probe electrode and is detected as an oxidation current. Based on the reduction current of menadione and oxidation current of the glutathione conjugate, the rate of uptake and export respectively can be extracted.

The yeast cells adhere to poly-L-lysine or polyethylene imine treated glass (Fig. 1b). Under physiological conditions, Saccharomyces Cerevisiae cells have negatively charge cell surfaces[6] that stem from the presence of phosphodiesters, amino and carboxyl groups that regulate the charge of the cell to aid in adhesion processes.[7] The immobilized cells remained alive for several hours as confirmed by the fluorescence studies performed with fun1 viability dye (Fig. 1c). This dye also allowed us to perform cytotoxicity studies where the proper dose of mena-

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dione (0.1 mM) (Fig. 1e) and effect of the Hg lamp exposure (Fig. 1f) on the cells was evaluated based on comparative results obtained for positive (Fig. 1c) and negative (Fig. 1d) controls.

Considerable progress was also made in the development of small laser pulled Pt ultramicroelectrodes required for the collection experiments. These tips, first reported by Schumann *et al.*[8] can now regularly be made and characterized for Pt wire diameters of 200 nm to 10 µm. Figure 2 presents the voltammogram with two of these tips.

Now that the experimental setup and important ground work is passed, we are attempting to measure the rates of uptake and export of menadione and the menadione-glutathione conjugate respectively. ■

Acknowlegments

This work was funded by the 2003 Edward G. Weston Summer Research Fellowship of The Electrochemical Society and by the National Science Foundation (NSF) grant number CHE0109587 (UT). The yeast cells DAY4 and DAY4vac were graciously donated by Prof. D. R. Appling of the Chemistry & Biochemistry Department (UT). We appreciate the help and advice of Dr. S. Chang and the guidance of Dr. Allen J. Bard.

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About the Author

JANINE MAUZEROLL is completing her graduate work under the guidance of Prof. Allen J. Bard at the University of Texas at Austin.

Imaging Metal Ions with Stripping Voltammetry Scanning Electrochemical Miscropy

A Summary Report to The Electrochemical Society for the 2003 Colin Garfield Fink Summer Research Fellowship

by Mario A. Alpuche-Avilés

Scanning Electrochemical Microscopy (SECM) typically consists of an ultramicroelectrode (UME) probe that is used to scan a surface[1] while collecting one data point per position. Our group has developed voltammetric SECM: a full voltammogram is acquired at each point of the image, so different species can be detected based on their redox potentials.[2]

The present work uses a Hg-film UME probe to combine SECM with anodic stripping voltammetry (ASV). ASV is a highly sensitive technique that detects metal ions after preconcentrating them in the working electrode.[3] Coupling ASV with SECM provides both high sensitivity and simultaneous detection of different ions based on their oxidation potentials.

In the ASV-SECM experiment, the potential is swept at 100 V/s to collect an image in a short time. As the tip moves from one position to the next, the tip potential is kept at a negative potential to reduce and preconcentrate the metal ions. The Hg-film is produced by electrodeposition of Hg onto a 10-µm diam Pt electrode by cycling the potential of the Pt electrode continuously for 300 times at 50 V/s from 0 to -1.6 V vs. MSE in a solution of 5.7 mM $HgNO_3$, 1 M KCl and 0.5% concentrated HNO_3. A preconcentration time of 300 ms was set by adjusting the raster scan rate of the tip.

A calibration curve for the peak current of Pb oxidation is shown in Fig. 1. Calibrating solutions contained equal concentrations of Pb^{2+} and Cd^{2+}, but only the peak current at the potential region of Pb oxidation is graphed. Accurate calibration required that voltammograms be continuously run after a 300 ms delay to emulate the conditions of ASV-SECM imaging. The graph is linear in the concentration range from 1 to 100 µM of metal ion. Linear calibration curves were found for Cd, although with different slopes. Thus, under imaging conditions Cd^{2+} and Pb^{2+} can be simultaneously detected.

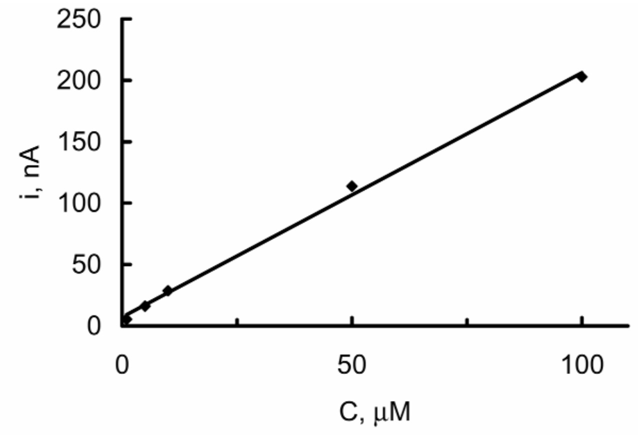

Fig. 1. Calibration curve of a Hg-covered Pt disk electrode 10 µm in diameter. ASVs were run from -1,300 to -450 mV vs. MSE at 100 V/s, the peak for Pb oxidation was observed at ca. -700 mV. The currents correspond to 1, 5, 10, 50 and 100 µmol/l. The slope obtained was 1.989 nA/µM, y-intercept value of 7.282 nA, and R^2 of 0.997.

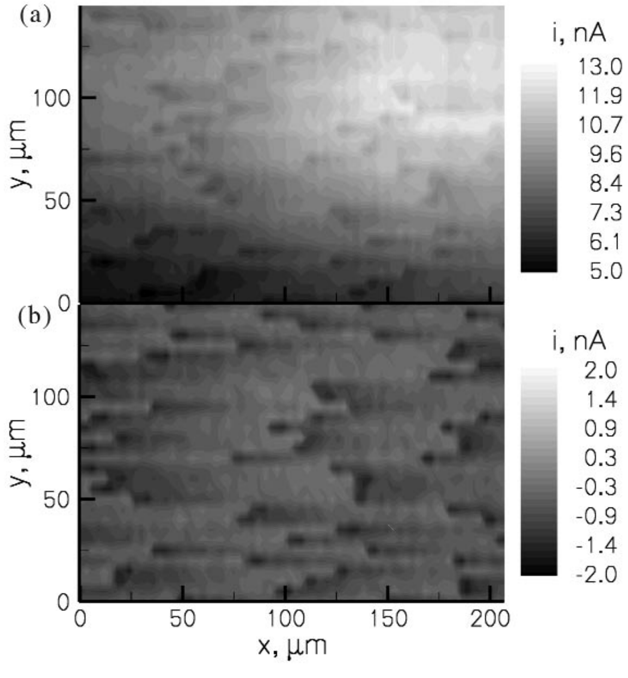

Fig. 2. ASV-SECM images extracted from the same experiment: (a) current at a potential value of -695 mV vs. MSE, which is the peak of Pb oxidation from the Hg-film electrode (10-µm diam.). The surface consists of two 25-µm diam. Pt disks electrodeposited with Pb, (higher current) and a second electrode covered with Cd does not appear in the image, but its center is located at ~(100, 50) µm; and (b) current at -1,298 mV, the initial potential of the ASVs.

Figure 2 shows two different images of a substrate from the same experimental data set. In ASV-SECM the current is a function of position (x, y) and potential (E(t)), so different images can be obtained by extracting the tip currents at a particular potential for all of the x-y positions. Figure 2a shows the tip currents at a potential corresponding to the oxidation of Pb from the Hg electrode. The high current region (10 - 11 nA) is above a 25-µm diam Pt disk covered with Pb. Compare this to Fig. 2b, which shows tip current at the initial tip potential for the ASVs (E_t = -1,298 mV). The currents in Fig. 2b are much smaller and serve as background for Fig. 2a; they are cathodic as the result of the metal ion reduction. By looking at different potentials of the same experiment we can simultaneously observe different processes on the tip. The solution is 0.5 M KNO_3 and the

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Pb detected (Fig. 2a) is from the substrate. The concentrations are in the low micro-molar region, determined from a separate calibration curve made at the same electrode used for this image. The surface also contains a second 25-μm diam electrode covered with Cd that does not show in the image since at this potential the current corresponds only to Pb^{2+} concentration. The substrate was kept at -1.3 V vs. MSE, which is in the noble region of the metals, so the presence of Pb^{2+} in solution is interesting and may be due to chemical oxidation by dissolved oxygen.

In conclusion we have expanded the SECM experiment to simultaneously detect different metal ions in the same ASV-SECM experiment. It is then possible to selectively image different metal ions based on their oxidation potentials. Extension of voltammetry SECM to the cathodic stripping imaging of halides and to lower the detection limit are goals of our laboratory. ■

Acknowledgments

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About the Author

Mario A. Alpuche-Avilés *is a PhD candidate in Chemistry at Mississippi State University under the direction of Professor David O. Wipf.*

New Theory for Aging Mechanisms of Li-ion Batteries

A Summary Report to The Electrochemical Society for the 2003 Joseph W. Richards Summer Research Fellowship

by P. Ramadass, H. J. Ploehn, and R. E. White

There has been tremendous interest in using Li-ion batteries to power many devices in consumer electronics. One of the problems associated with the performance of Li-ion batteries is the capacity fade. Various mechanisms for the capacity loss in Li-ion cells, including electrode passivation, electrolyte decomposition, active material dissolution, phase change, overcharge, self-discharge, and several other phenomena, have been reviewed in the literature.[1] The prime objective of our research work is to analyze, quantify, and to model the capacity fade of commercial Li-ion batteries for the prediction of cycle life and calendar life under several operating conditions.

This report summarizes the results of a rigorous continuum mechanics model of solvent diffusion developed to describe the growth of solid-electrolyte interfaces (SEI) in Li-ion cells incorporating carbon anodes. The one-dimensional solvent diffusion model developed was able to explain the capacity loss of Li-ion cells during storage under float potentials at various temperatures. The model was developed on the assumption that a reactive solvent component diffuses through the SEI and undergoes two-electron reduction at the carbon-SEI interface.

Based on several morphological studies of SEI,[2-4] we believe that the porosity of SEI plays a major role in Li-ion capacity fade, for both charge-discharge cycling and self-discharge under storage conditions. We thus believe that the SEI has a significant level of porosity (or permeability) that permits the ingress of electrolyte components (solvents and/or solvated ions). This hypothesis thus provides a common physical basis (in terms of SEI composition and morphology) for understanding both the initial quality of the as-formed SEI, and its long-term role in capacity fading and cell cycle life.

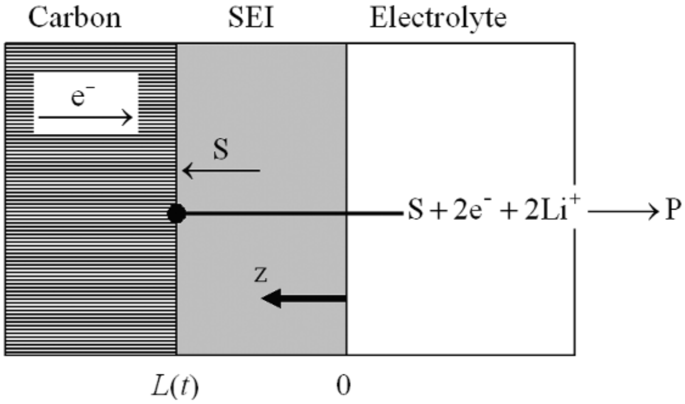

Fig. 1. Schematic diagram of SEI growth via solvent diffusion through the SEI.

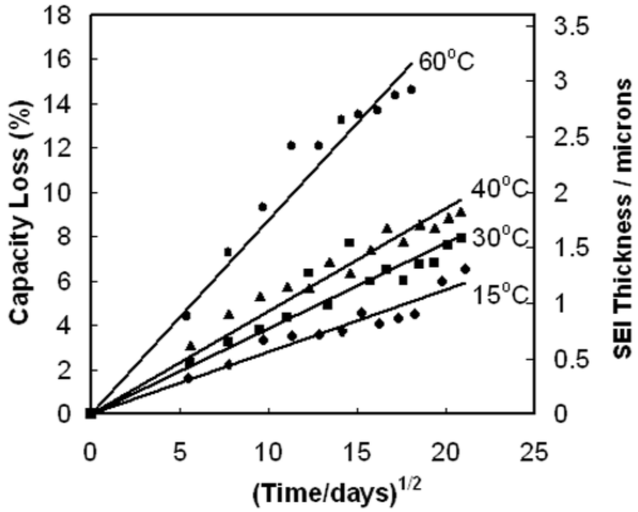

Fig. 2. Measured capacity loss[6] and estimated SEI thickness as functions of time and temperature for MP prototype cells stored at a float potential of 3.8 V.

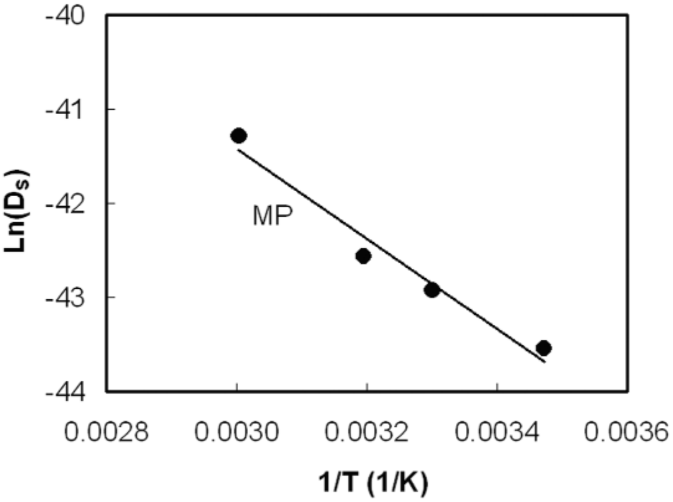

Fig. 3. Arrhenius plot of solvent diffusivity versus temperature for MP prototype cells. Diffusivity values estimated from data in Fig. 2.

Figure 1 presents a schematic of the Li-ion half-cell illustrating the transport processes and reactions occurring near the surface of a carbon anode under float charge conditions. We assume planar symmetry of the anode and consider only transport in one spatial Cartesian coordinate, valid away from the edges of the anode. Frame indifference[5] enables us to work in reference frame in which SEI-electrolyte interface is stationary, located for convenience at z = 0. For our model, we assume a two-electron reduction of the solvent, ethylene carbonate (denoted as S) at the carbon-SEI interface , producing an insoluble product (P) with constant molar density.

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The solvent diffusion model has only one adjustable parameter, namely the solvent diffusivity (D_S). The primary result of the model is the prediction that capacity loss increases with the square root of time (t), in accord with experimental data.[6] Additional reasonable assumptions about the composition of the SEI lead to plausible estimates of SEI thickness, which also grows in proportion to the square root of time.

Figure 2 shows data (symbols from Ref. 6, Fig. 1) for capacity loss versus for MP prototype cells[6] stored at various temperatures and at a float potential of 3.9 V. Despite the scatter in the data, linear regression provides a satisfactory fit.

The solvent diffusivity was obtained from linear regression of capacity loss plotted versus square root of time. An Arrhenius plot (Fig. 3) of diffusivities extracted from experimental data is linear, consistent with the behavior expected for temperature (T)-dependent diffusivity of solvent through a solid, perhaps microporous SEI. The solvent diffusion model also provides a starting point for understanding and predicting the effect of solvent composition on SEI growth and capacity fade. For example, we may use the model to explore the effect of varying EC concentration on long-term capacity loss and SEI layer growth for cells under float potential conditions. The model development and other features of this solvent diffusion model are explained in detail elsewhere.[7]

To extend this study further, a similar mechanism of solvent diffusion was assumed to explain the capacity fade with continuous cycling and a first principles capacity fade model was also developed for commercial Li-ion batteries.[8] Although the solvent diffusion model invokes many simplifying assumptions, it points toward the possibility of a new, realistic, tractable picture of the SEI on carbon anodes in Li-ion cells. ■

Acknowledgments

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About the Author

Premanand Ramadass *is a doctoral candidate in chemical engineering at the University of South Carolina, working under the direction of Professor Ralph E. White and Branko N. Popov. He may be reached by e-mail at ramadass@engr.sc.edu.*

Anodic Stripping Voltammetry Enhancement by Redox Magnetohydrodynamics

A Summary Report to The Electrochemical Society for the 2003 F. M. Becket Summer Research Fellowship

by Emily A. Clark

Anodic stripping voltammetry (ASV) is commonly used to analyze metals (*e.g.* lead, silver, cadmium, copper, zinc, indium, etc.) in streams and lakes,[1,2] in soil samples,[3] in biological fluids,[4,5] and for many other applications, such as paint chip analysis.[6] ASV involves a preconcentration step via reduction of the metal at the electrode, followed by sweeping the voltage through the oxidizing potential, which results in a current for oxidation of the deposited material. The peak current or peak area is proportional to the concentration of the metal species in solution. Stirring analyte solutions during the electrodeposition step allows for shorter deposition times and improved limits of detection. However, mechanical stirring of small samples (and stirring in portable devices) is a challenge. In this work, redox magnetohydrodynamics (MHD) has been used to induce convection in analyte solutions during the deposition step without mechanical stirring.

A Lorentz force on charge-carrying ions, $\mathbf{F}_L$ (N/m^3), arises from the interaction of an electric field, expressed as current density, $\mathbf{J}$ (A/m^2), and a magnetic field, $\mathbf{B}$ (T). The force is given by the following equation:[7,8]

$$F_L = J \times B$$

The direction of $\mathbf{F}_L$ is governed by the right-hand rule. In this work, the Lorentz force results from the interaction of a large magnetic field from an electromagnet and a large cathodic current during the deposition step of ASV analysis. To produce a large cathodic current, a high concentration of mercury was added to an analyte solution of lead for co-deposition. The Lorentz force induces solution convection, which delivers species in solution to the electrode at a faster rate. Thus, a higher concentration of lead is present in the mercury thin film, resulting in a larger oxidative peak in the stripping voltammogram.

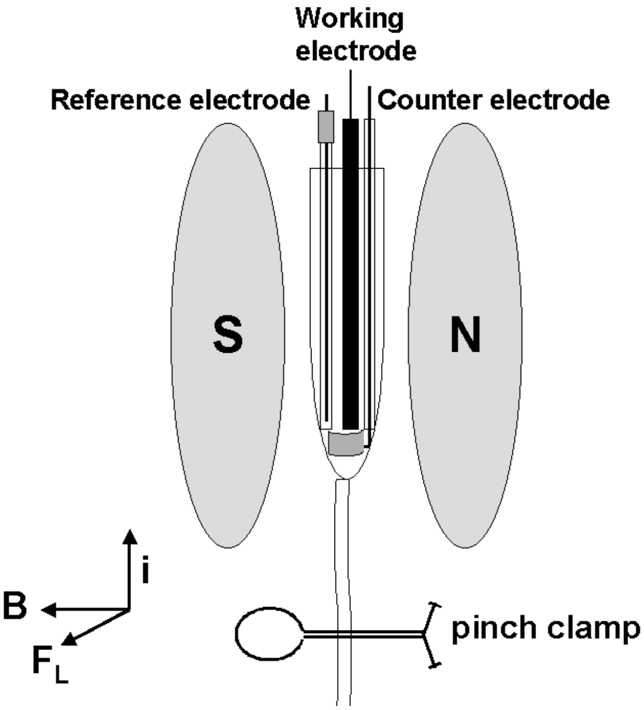

FIG. 1. Experimental set-up. The electrochemical cell is positioned between the poles of an electromagnet with the working electrode (3 mm glassy carbon) facing down. The perpendicular electric and magnetic fields result in a Lorentz force.

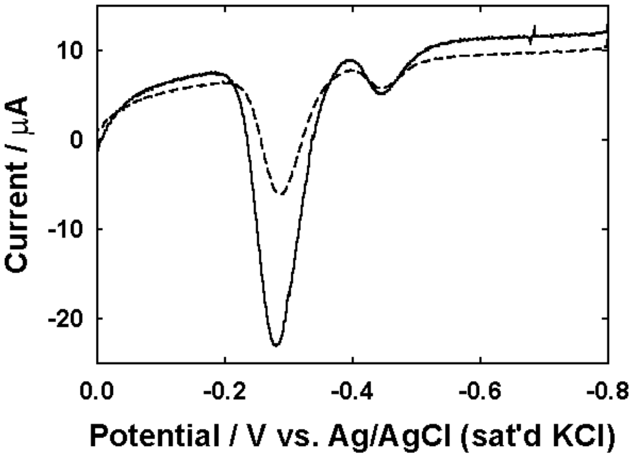

FIG. 2. ASV responses of 0.82 µM $Pb(NO_3)_2$/ 40 mM $Hg(NO_3)_2$/ 30 mM HNO_3/ 0.1 M KNO_3 at 0 T (dashed curve) and 1.77 T (solid curve). Scans were performed at 2 V/s after a 60 s deposition at -0.8 V and a 30 s rinsing time.

A disadvantage of this technique is the requirement of a high concentration of mercury to produce a high background cathodic current in the stripping voltammogram. Using a flow-through cell to wash away the mercury solution, and performing the stripping step in pure electrolyte avoids the high background. The experimental set-up is shown in Fig. 1.

Figure 2 shows stripping voltammograms after a preconcentration step in a solution of 0.82 µM $Pb(NO_3)_2$ and 40 mM $Hg(NO_3)_2$ in the presence and absence of a magnetic field. The peak current of the lead signal increased by 160% with the 1.77 T magnet. The increase in peak current indicates that the Lorentz force was able to induce convection in solution without any moving parts. This method of convec-

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tion has potential applications in ASV of small volumes and in portable ASV devices. Other experiments (not shown here) suggest stirring is possible in volumes as low as 1 nL. In the future, the use of strong permanent magnets would eliminate the need for the electromagnet. ■

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About the Author

__Emily Clark__ is a PhD candidate at the University of Arkansas. She is performing research under the direction of Dr. Ingrid Fritsch.