

Electrochemistry in Nanovials Fabricated by Combining Screen Printing and Laser Micromachining

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The coupling of screen-printing and laser micromachining technology has been used to create a nanovial with “built-in” working and reference electrodes. The volume of the nanovial was calculated to be 7.2 nL using dimensions determined by SEM. The electrochemical nanovial was characterized using the ferri/ferrocyanide redox couple. Cyclic voltammetry and chronoamperometry experiments were performed with electrochemical nanovials utilizing 5% (v/v) glycerin in the solutions and a humidified headspace to control evaporation of the small-volume samples. Chronoamperometry experiments gave results consistent with a diffusion-limited process and revealed a working electrode surface area of $2.6 \times 10^4 \mu\text{m}^2$. The ultrasmall-volume cells represent a simple, reliable, low-cost approach for the fabrication of complete electrochemical nanovials.

The development of analytical techniques that can be used to analyze small-volume samples, especially those in the submicroliter range are of considerable interest. This interest is motivated by the need for analysis of samples of limited availability (micro-synthesis products, precious objects, high-throughput screening samples, etc.) and for monitoring single-cell events (e.g., release of cellular materials and neurotransmitter release by neurons). A number of techniques have been applied to small volumes (i.e., ≤ 10 nL) including acid–base^{1,2} and metal complexation titrations,³ binding assays,⁴ multireflective UV–visible spectroscopy,^{5,6}

fluorescence spectroscopy,^{7,8} graphite furnace atomic absorption microspectroscopy,⁹ Raman spectroscopy,¹⁰ ¹H NMR,¹¹ and mass spectrometry.¹² Nanoliter separations have been demonstrated using liquid column chromatography¹³ and are more routinely performed via capillary electrophoresis (for examples, see refs 14 and 15).

Different types of electroanalytical methods have been explored for ultrasmall-volume analyses. Physiology research groups have routinely used pH and ion-selective microelectrodes to measure intracellular ion activities since the late 1970s and early 1980s.^{16,17} The theory behind these electrophysiology studies was explored in 1982 by Simon and co-workers.¹⁸ A submicroliter analysis system based on electrochemically generated chemiluminescence at microelectrodes has been demonstrated.¹⁹

The bulk of electrochemical small-volume analysis has been performed using dc voltammetric techniques. One example of a small-volume voltammetric analysis used a droplet placed on a set of parallel band electrodes fixed in a matrix of heat-sealed Tefzel film.²⁰ More recent demonstrations used subnanoliter devices with electrodes at the bottom of the vials,^{21,22} microelectrodes in microfabricated picoliter vials,^{23,24} or microelectrodes inserted into nano- and picoliter aqueous droplets dispersed in

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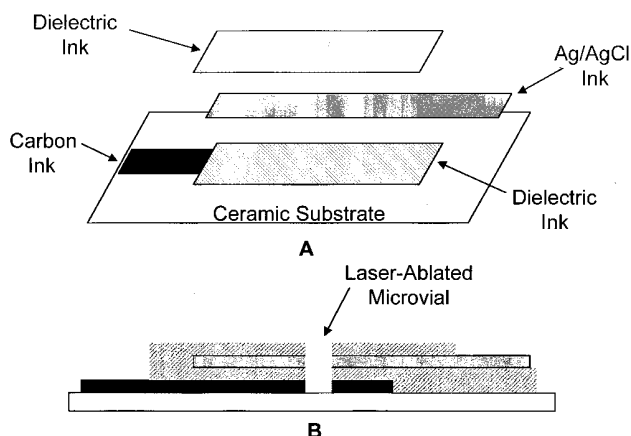


Figure 1. (A) Diagram showing the screen-printed layers of the electrochemical microvial. (B) Diagram of cross section of the finished electrochemical nanovial.

hexane.²⁵ However, each of these three approaches has disadvantages. The nano- and picoliter droplet analysis exposes the carbon fiber working electrode to heptane, which causes a change in the behavior of the electrode.²⁵ The disadvantage of the picoliter vial approach is that it requires manipulation of each of the two electrodes into position by separate micromanipulators, and a third micromanipulator is needed for the microinjection capillary.^{23,24}

The current work proposes a new method of fabricating a nanoliter-sized electrochemical system by coupling screen-printing techniques and laser micromachining. In comparison to the traditional thin-film microfabrication methods employed in refs 21–24, screen-printing (thick-film) fabrication is a relatively simple, inexpensive, and versatile technology that also allows a great range of flexibility in the electrode geometry. The use of screen-printed carbon inks as working electrodes in voltammetric analysis has been reviewed (ref 26 and references therein), and screen-printed electrodes have been the transducer of choice for most practical real-life applications (e.g., commercial glucose devices). Excimer laser ablation is a method that enables the production of high-resolution features in a wide variety of polymers.²⁷ The use of picovials fabricated in glass microscope cover slips by excimer laser micromachining has been demonstrated in bioluminescence binding assays.⁴ The combination of screen-printing carbon and insulating inks with excimer laser micromachining to expose the carbon ink working electrodes by ablation/removal of the insulator has been used to fabricate microelectrodes.²⁸ Recessed microdisk electrodes (ref 29 and references therein) have also been previously fabricated. Both the aforementioned electrode systems are used in conjunction with an external reference electrode to perform analyses in bulk solutions of large volume (i.e., greater than microliters). By layering screen-printed carbon, dielectric, and Ag/AgCl inks, a vertical assembly can be fabricated containing a working electrode and a reference electrode that are electrically insulated from each other (Figure 1A). Creation of a nanovial

through these layers with an excimer laser micromachining system results in a nanoliter vial with embedded electrodes (Figure 1B); this alleviates the need to manipulate the electrodes into position. To our knowledge, the present work represents the first demonstration of using the techniques of screen printing and laser micromachining in tandem to prepare nanoliter-sized electrochemical cells that contain both reference and working electrodes. This approach creates a reliable ultrasmall-volume stand-alone electrochemical cell using simple and effective techniques.

EXPERIMENTAL SECTION

Fabrication of the Screen-Printed Electrode Assembly. A semiautomated screen-printer (model TF-100, MPM Inc., Franklin, MA) was utilized for fabrication of the electrodes. Carbon ink (Ercon G-449(I)) was printed through a patterned stencil onto a 3.33×10 cm alumina ceramic substrate, resulting in a 1.5×30 mm carbon ink feature (working electrode). Subsequently, a layer of polyester-based dielectric insulating ink (Ercon R-488B(HV)-B2 Blue) was printed onto a portion of the ceramic substrate and carbon layer through another stencil of dimensions 5×20 mm, allowing a 1.5×5 mm section at one end of the carbon (working electrode) for the electrical contact. Then, two coatings of Ag/AgCl ink (Ercon R-421(DBE-68)) were printed through the same stencil used for the carbon ink, but offset, allowing a 1.5×5 mm section to be printed 180° from the carbon contact area. This ink was used as the reference/counter electrode. A final dielectric ink layer was placed on top of the reference strip to complete the electrode assembly. After each ink-printing step, the assembly was placed into an oven at 100°C for 30 min to cure the inks (all were low-temperature-curing inks). The overall geometry of the screen-printed electrodes is shown in Figure 1A. In this work, the screens were designed to create simultaneously five individual nanocell systems.

Excimer Laser Micromachining. Once the electrode assembly was fabricated, nanovials were ablated through the ink layers using a Resonetic Excimer Laser Micromachining System equipped with a model MPB X-100 excimer laser (Montreal, Canada). The micromachining system includes a computer-controlled X–Y translational stage, and therefore, each set of five printed electrode systems (see above) can easily be micromachined quickly. The laser medium was a Kr–F mixture, which produces UV radiation at 248 nm. The laser fluence used for the nanovials that were employed to study solutions *not* containing glycerin was 20 mJ cm^{-2} . The nanovials for all other studies were obtained with a laser fluence of 15 mJ cm^{-2} . The laser parameters common to all nanovials were a 2.5-ns pulse length and a repetition rate of 10 Hz. A total of 2000 pulses were used to fabricate each nanovial. The laser micromachining was performed in a vacuum of $\sim 1 \times 10^{-2}$ Torr. Once the laser machining was completed, the electrode assemblies were separated into individual strips, and the resulting nanovials were visually inspected under a stereomicroscope. The resistance between the working and reference electrode was also measured with a digital multimeter (Micronta model 22-166A, Radio Shack, Fort Worth, TX) to ensure that the laser ablation process did not short the electrodes.

Reagents. Potassium ferricyanide was obtained from Mallinckrodt (St. Louis, MO). Potassium chloride was purchased from Fisher Scientific (Fair Lawn, NJ). Glycerin was used as received from Harshaw (Cleveland, OH). All solutions were prepared with

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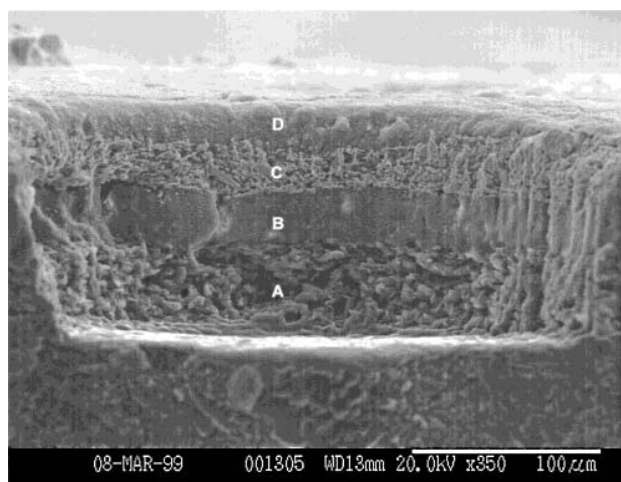


Figure 2. Scanning electron micrograph of the electrochemical nanovial cross section showing carbon layer (A), dielectric layer (B, D), and Ag/AgCl layer (C). Layers are printed onto a ceramic substrate. The length of the white bar at the lower right of the figure represents the scale of 100 μm .

distilled, deionized (Milli-Q water purification system, Millipore, Bedford, MA) water.

Apparatus and Procedure. Electrochemical experiments were performed using a PAR 273 potentiostat and model 270 software (EG&G Princeton Applied Research, Princeton, NJ). The electrochemical nanovials were filled using a micropipetor (Transjector 5246, Eppendorf, Hamburg, Germany) and a micromanipulator (Narishige, Sea Cliff, NY). The capillaries used for injections were prepared from borosilicate glass capillaries (Catalog No. BF100-78-15, Sutter Instrument, Navato, CA) using a horizontal capillary puller (model P-97, Sutter Instrument). Cyclic voltammetry experiments were performed by cycling the potential between +0.500 and -0.050 V. Chronoamperometry experiments were performed by stepping the potential from +0.500 to 0.00 V. All sample solutions contained 0.10 M KCl and, unless otherwise stated, 5% (v/v) glycerin. Before each series of experiments, a laser-machined electrode assembly was mounted on a custom-built microscope stage. The potentiostat leads were connected to the electrode contacts, and sample solution was injected into the nanovial. All experiments were performed within an in-house-built humidity chamber equipped with a cool-vapor humidifier (Duracraft model DH-830, Southborough, MA).

Scanning electron microscopy (SEM) was performed using a Hitachi model S-3200N (San Jose, CA) on a cross section of an electrochemical nanovial to characterize the overall shape of the nanovial. The SEM data were also used to determine the representative diameter and overall depth of the vials, as well as the thickness of the carbon layer.

RESULTS AND DISCUSSION

A representative cross-sectional view of a nanovial by SEM (Figure 2) shows the profile of the nanovial and that of the working carbon ink electrode. The nanovial is formed by laser ablation through all four layers of cured ink. The shape of the laser micromachined vials is very close to being a right cylinder, with a diameter of 270 μm and a depth of 125 μm . Using these dimensions and assuming the nanovial to be a right cylinder, the

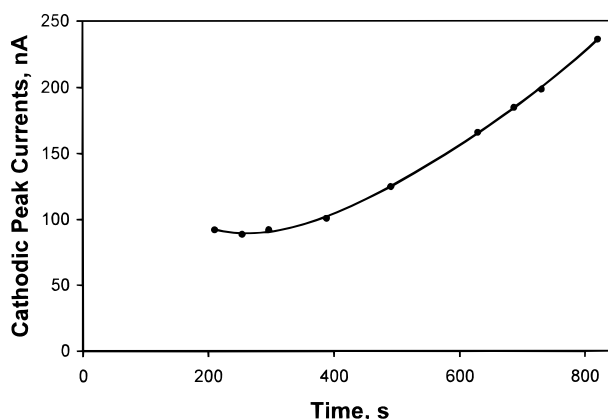


Figure 3. Cathodic peak currents of 10 mM $\text{K}_3\text{Fe}(\text{CN})_6$ in 0.1 M KCl at a scan rate of 100 mV/s obtained with the electrochemical nanovial.

volume of the nanovial is 7.2 nL. The scanning electron micrograph in Figure 2 also shows that the carbon working electrode (labeled A) is a tubular band on the wall at the bottom of the nanovial. The carbon layer has a rough surface and is approximately 50 μm thick.

Work in small volumes requires special considerations due to the problem of evaporation. Evaporation in small volumes leads to significant changes in volume, concentration, and density. Two major methods of controlling evaporation effects in small samples are based on (a) working with aqueous droplets in or under organic liquids (e.g., heptane, mineral oil, etc.)^{1,3,9,21,22,25} and (b) adding a water-soluble organic compound, glycerin, that retards evaporation.^{23,24} It has also been suggested that an increase in the headspace humidity of small-volume systems helps to decrease the effect of evaporation.^{20,23}

Cyclic voltammetry was repetitively performed on a $\text{K}_3\text{Fe}(\text{CN})_6$ sample solution that contained no glycerin in an electrochemical nanovial. Figure 3 shows that the peak cathodic current remains relatively constant (<5% change from original value) for up to approximately 5.5 min. After this period, the effect of evaporation causes dramatic increases in the apparent concentration of $\text{K}_3\text{Fe}(\text{CN})_6$. At 13 min, the peak current is approximately 160% higher than the original peak current. While the response for short-term experiments is relatively unaffected by evaporation, longer term electrochemical experiments (> 5 min) would greatly be affected by evaporation of the sample solution.

Cyclic voltammetry was repeated in the electrochemical nanovials using a 0.5 mM $\text{K}_3\text{Fe}(\text{CN})_6$ solution with 5% (v/v) glycerin. The cathodic peak currents obtained over a 9-min period were recorded. The average value for the collected peak currents was 22.1 nA with a relative standard deviation of 1.0% ($n = 11$). Thus, it is demonstrated that the addition of small amounts of glycerin to an aqueous sample, along with a humidified headspace, greatly reduces the evaporation of the sample in an electrochemical nanovial.

Cyclic voltammograms of a $\text{K}_3\text{Fe}(\text{CN})_6$ solution with 5% (v/v) glycerin in the electrochemical nanovial were recorded at various scan rates. The scan rate was varied from 20 to 250 mV/s. The resulting voltammograms from a single sample injection are shown in Figure 4. Cyclic voltammograms were acquired at 100 mV/s at various times throughout the experiment to verify the stability

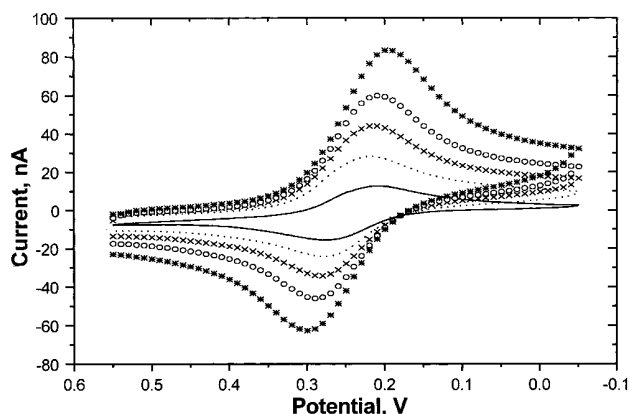


Figure 4. Cyclic voltammograms of 1 mM $\text{K}_3\text{Fe}(\text{CN})_6$ in 0.1 M KCl with 5% (v/v) glycerin obtained with the electrochemical nanovial at scan rates of 20 (solid line), 50 (dashed line), 100 (X's), 150 (circles), and 250 mV/s (*).

of the current. The geometry of the working electrode is similar to tubular band electrodes. Studies at tubular band electrodes have determined that they give rise to hemicylindrical diffusion.^{30,31} When the diameter-to-width ratio of these electrodes is between 2 and 100, significant edge effects to diffusion also occur according to ref 31. The value of the diameter-to-width ratio of the electrochemical nanovial working electrode is 5.4, suggesting there is a contribution to diffusion from edge effects in the described system.

The response of the electrochemical nanovial was also studied by injecting solutions of varying $\text{K}_3\text{Fe}(\text{CN})_6$ concentrations into the same nanovial used for the work described above. To replace a solution, the nanovial was flushed with electrolyte/glycerin solution followed by removal of the solution from the area above the nanovial with a syringe fitted with a blunt-tip 25-gauge needle. This process was repeated until a cyclic voltammogram that was nearly identical to the background was obtained. This was necessary since the sample solutions are not easily drawn out of the nanovial by simple suction. Three to five repetitions of the flushing procedure were necessary to clean the nanovials. Concentrations of $\text{K}_3\text{Fe}(\text{CN})_6$ from 0.1 to 5 mM were used to construct the calibration plot shown in Figure 5.

When a large enough potential step is made at a planar electrode, reduction of the electroactive species occurs at a diffusion-controlled rate and the resulting current is described by the Cottrell equation. A representative chronoamperogram of $\text{K}_3\text{Fe}(\text{CN})_6$ in an electrochemical nanovial is shown in Figure 6A. While the screen-printed carbon electrode is not planar, it has been suggested that the planar diffusion approximation applies in experimentally short time periods (<1 s).³² Our experiments showed that, at these short experimental times, the electrochemical nanovial system produces a linear dependence of current on the square root of time. The slopes of the Cottrell plots obtained from the chronoamperometric data (example shown in Figure 6B)

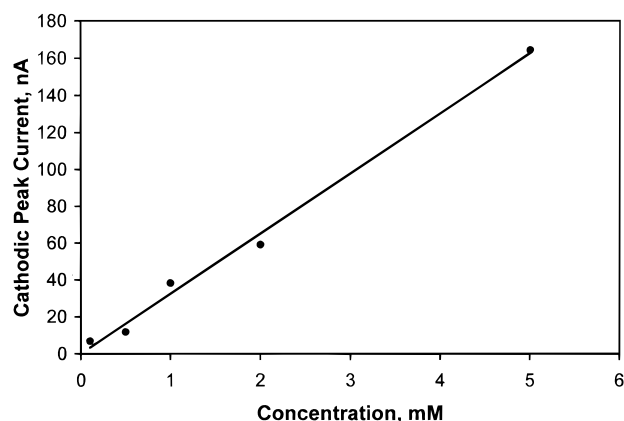


Figure 5. Calibration curve for $\text{K}_3\text{Fe}(\text{CN})_6$ in 0.1 M KCl with 5% (v/v) glycerin using cathodic peak currents of cyclic voltammograms obtained with the electrochemical nanovial at a scan rate of 50 mV/s. The slope of the calculated line was 32.5 nA/mM with an associated standard deviation (S_m) of 1.50 nA/mM.

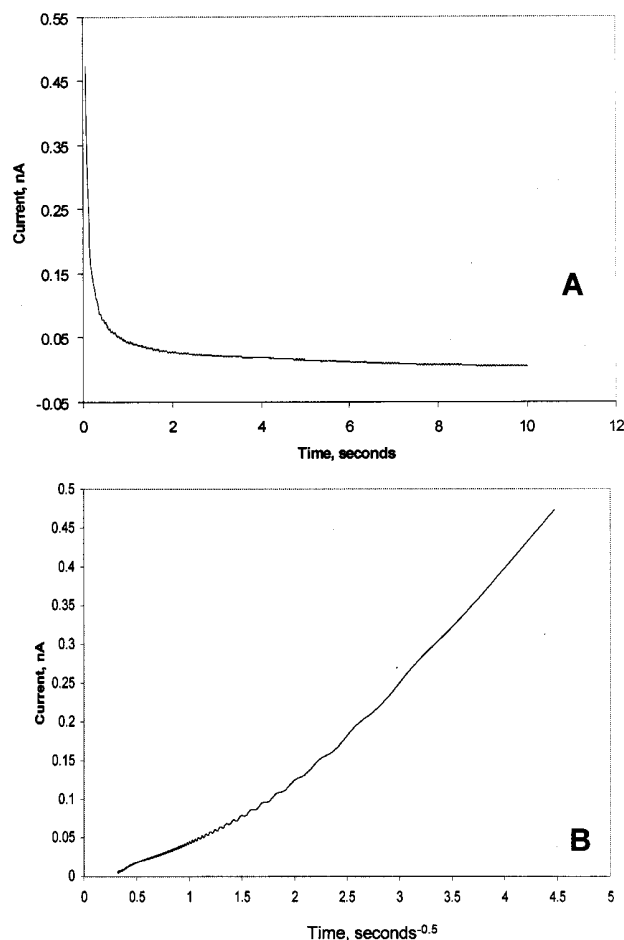


Figure 6. (A) Chronoamperogram of 0.5 mM $\text{K}_3\text{Fe}(\text{CN})_6$ in 0.1 M KCl with 5% (v/v) glycerin obtained with the electrochemical nanovial. (B) Cottrell plot of the data from (A).

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for three trials with 0.5 mM $\text{K}_3\text{Fe}(\text{CN})_6$ and three trials with 1.0 mM $\text{K}_3\text{Fe}(\text{CN})_6$ were used to calculate the surface area of the carbon electrode, taking the diffusion coefficient for the ferricyanide to be $6.9 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$. This was obtained by correcting the value of $7.8 \times 10^{-6} \text{ cm}^2 \text{ s}^{-1}$ in aqueous solution³³ for the

increased viscosity of the 5% (v/v) glycerin solution.³⁴ The average area for all six trials was $2.6 \times 10^4 \mu\text{m}^2$ with a RSD of 5.4%.

CONCLUSION

A nanoliter cell that simplifies manipulations necessary for small-volume experiments has been fabricated via the coupling of screen-printing and laser micromachining technologies. The data obtained with the electrochemical nanovials gave results that are consistent with a diffusion-controlled process. The effect of evaporation on this small-volume technique is shown to be greatly reduced by the addition of glycerin to the sample solution while experiments are conducted in a humidified headspace. It should be noted that the cost-effectiveness of thick-film electrodes allows

the fabrication of disposable, single-use electrochemical systems. Finally, the ease and flexibility of both screen-printing and laser micromachining techniques open an array of possible future applications of electrochemistry in ultrasmall environments.

ACKNOWLEDGMENT

This work was supported by the National Aeronautics and Space Administration and by a National Science Foundation Integrated Graduate Educational Research and Training (NSF-IGERT) Grant. The authors thank B. Tian (NMSU) for assistance with thick-film microfabrication. J.W. acknowledges NIH support.

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Received for review October 6, 1999. Accepted November 4, 1999.

AC991163C