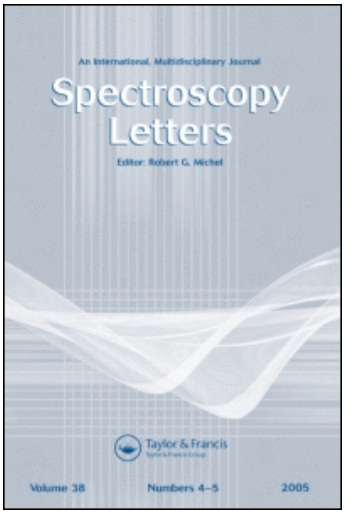

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Easily Constructed Microscale Spectroelectrochemical Cell

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ABSTRACT The design and performance of an easily constructed cell for microscale spectroelectrochemical analysis are described. A cation exchange polymer film, Nafion, was used as a salt bridge to provide ionic contact between a small sample well containing a coiled-wire working electrode and separate, larger wells housing reference and auxiliary electrodes. The cell was evaluated using aqueous ferri/ferrocyanide as a test system and shown to be capable of relatively sensitive visible absorption measurements (path lengths on the order of millimeters) and reasonably rapid bulk electrolysis (~5 min) of samples in the range of 1–5 μL volume. Minor alterations to the cell design are described that could allow for analysis of submicroliter volumes, rapid multi-sample analysis, and measurements in the ultraviolet spectral region.

KEYWORDS electrochemical cell, microscale analysis, spectroelectrochemistry

INTRODUCTION

Microchemical analysis techniques are beneficial to many applications in which the sample is either inherently small, of limited availability due to scarcity or expense, or hazardous enough to pose problems in personnel exposure and safe disposal. The development of spectral apparatus for microscale measurements has progressed significantly in the recent past, and several vendors presently offer sampling accessories and dedicated instruments for ultraviolet/visible/near-infrared analysis of sample volumes as low as ~1 μL. Although only a few commercially available electrochemical cells can accommodate microscale sample volumes (on the order of a few hundred microliters), there is considerable on going research directed toward the development and application of electrochemical cells and sensors, particularly biosensors, that accommodate very small sample volumes.[1,2]

There have been fewer reports of microscale apparatus permitting simultaneous electrochemical and spectral measurements, so-called "spectroelectrochemical" (SEC) techniques.[3] The most common approach to realizing small cell volumes is by entrapment of sample between two closely spaced, parallel planar surfaces, i.e., the "thin layer" electrochemical geometry. A variety of optical sampling modes is possible depending upon the identities of the planar substrates, i.e., solid electrodes, optically transparent electrodes (OTE), windows, and internal reflection crystals.

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Cells of this sort have been designed for batch analysis of sample volumes ranging from hundreds[4–6] to tens of microliters.[7–10]

Various applications of a versatile SEC sensor design employing film-coated internal reflection OTEs have been described by Heineman et al. in an extensive series of articles published from 1997 to 2007. In one application, the sensor was integrated with a commercially available electrochemical flow cell to permit visible fluorescence measurements on sample volumes as small as ~4 μL.[11] As those authors noted, however, this corresponds to the "sensing volume" of the flow cell, and all the results presented were for operation of the cell in flow mode. The calibration data reported were for fluorescence measurements made after 45 min of sample flow at 100 μL/min, corresponding to a total sample requirement of 4.5 mL. Although this cell could be used in static or "batch" mode with a much lesser volume requirement (but still greater than 4 μL due to the dead volume of the cell's sample introduction plumbing), such use would severely decrease sensitivity due to a greatly diminished preconcentration of analyte into the OTE's permselective film. Furthermore, if used for absorption measurements, the extremely short path length associated with attenuated total reflectance sampling would likewise result in a very low sensitivity.

Work in our laboratory is concerned with the development of SEC assays for species of biomedical and clinical interest. Toward this goal, we have sought to design a small-volume SEC cell that would permit the rapid analysis of large numbers of samples. Flow injection analysisis a useful approach to these objectives, and we previously developed an SEC flow cell for such purposes.[12] Despite the easy analysis of small volumes via the loop injectors common to flow methods, the unavoidable sample dispersion associated with these methods[13] serves to decrease sensitivity. In particular for SEC flow cells, the tradeoff between dispersion and electrolysis efficiency[12] makes batch analysis the more attractive option from the perspective of sensitivity.

In this paper, an SEC cell for batch analysis of microliter-scale samples is described. Unlike the more common thin-layer designs, this cell employs a small cylindrical cavity and a coaxial coiled-wire–working electrode to achieve microscale sample volumes, yielding improved sensitivity due to the much greater optical path length. Additional benefits include the ease of initial construction and assembly/disassembly and the possibility of modifying the design to accommodate sub-microliter volumes, multiple sample wells, and measurements in the ultraviolet spectral region.

MATERIALS AND METHODS

Reagents

Reagent grade potassium ferricyanide, $K_3Fe(CN)_6$(13746–66-2); and potassium nitrate, KNO_3 (7757–79-1); were used as received from Fisher Scientific (Pittsburgh, PA). Solutions were typically prepared using distilled or deionized water, stored under refrigeration in tightly capped polyethylene or polycarbonate bottles, and used within a few days of preparation.

SEC Cell Construction

Illustrations of the SEC cell are shown in Fig. 1. Base and well plates, approximately 3 cm × 7 cm and 3 cm × 5 cm, respectively, were cut from polymethylmethacrylate sheet (3 mm thick, United States Plastic Corp., Lima, OH) using a small hacksaw. Holes for the sample well (1.6 mm dia.), reference and auxiliary electrode wells (6.4 mm dia.), and connecting bolt feed-throughs (3.2 mm dia.) were drilled in the well plate using a bench top drill press and standard bits. Matching bolt feed-through holes were drilled in the base plate using the well plate holes as a guide. Ionic contact between the electrode wells was provided by a 1 cm × 5 cm sheet of 0.2 mm thick Nafion 117 perfluorinated membrane (31175-20-9, Sigma-Aldrich, St. Louis, MO). A 1 cm × 5 cm gasket was cut from Parafilm M film (Alcan Packaging, Neenah, WI) and hole-punched to match the sample well and electrode reservoirs drilled in the well plate. The volume of the empty sample well after cell assembly was estimated geometrically to be ~6 μL (after insertion of the Pt coil electrode, the well's void volume was ~5 μL; see below).

A coiled Pt-wire–working electrode (WE) was fashioned by tightly wrapping 0.25 mm dia. Pt wire (Alfa-Aesar, Ward Hill, MA) around a 1-mm dia. glass capillary tube to yield a coil length of ~3 mm. The capillary tube was then removed, leaving an adequately rigid Pt coil that could be handled without significant deformation. The volume of the Pt coil was estimated to be ~1 μL. Newly formed

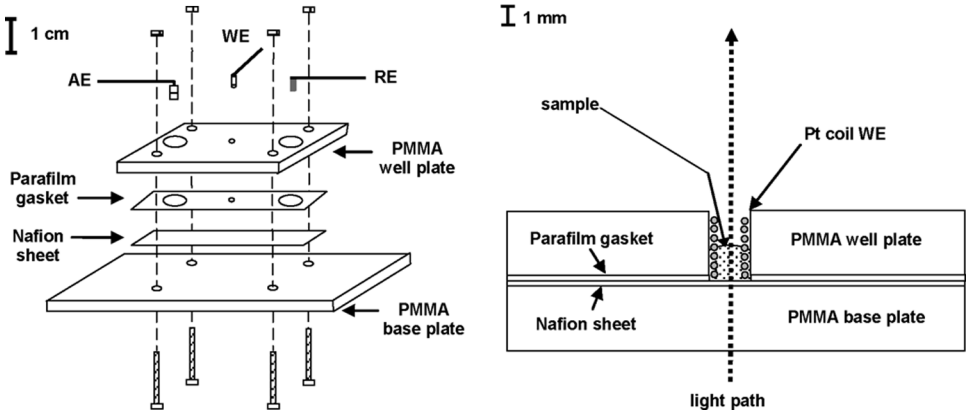

FIGURE 1 **Illustrations of the spectroelectrochemical cell showing an exploded view of the cell assembly (left) and a zoomed cross sectional side view of the sample well region (right).**

electrodes were typically cleaned by heating to incandescence in a butane lighter flame prior to use. A Pt or nichrome wire coil was used as the auxiliary electrode (AE), and a miniature AgCl/Ag electrode prepared from 0.5 mm dia. Ag wire (Alfa-Aesar, Ward Hill, MA, U.S.A.) according to the procedure reported by Nolan et al.[14] was used as the reference electrode (RE). All potentials are reported relative to this quasireference electrode.

Prior to assembling the cell, the Nafion sheet was fully hydrated and cation-exchanged to the potassium form by soaking overnight in 1 M KNO_3 (the supporting electrolyte used in all measurements). The cell components were then stacked as shown in Fig. 1 and clamped together by tightening the four connecting bolts. Leak-free seals were typically easily achieved as confirmed by monitoring the volume of water added to the well and reservoirs (after capping with Parafilm) over a period of several hours. The assembled cell was stored, with all its wells filled with water, in a sealed container along with a small beaker of water to maintain the Nafion sheet in a fully hydrated state.

Instrumentation

The experimental arrangement is illustrated in Fig. 2. A BAS model CV-50 W voltammograph (Bioanalytical Systems, Inc., Layfayette, IN) was used for electrochemical control and measurement. Absorbance measurements were made with a model USB2000-FLG CCD spectrometer and model HL450 tungsten-halogen light source (Ocean Optics, Inc., Dunedin, FL). The output of the light source was directed to the cell via a 0.6 mm single-fiber patch cable terminated with a collimator assembly (Multimode Fiber Optics, Inc., Hackettstown, NJ) and affixed to the condenser mount of a compound, upright optical microscope (Micromaster II series, 12-561-4D, Fisher Scientific). After passing through the SEC cell's sample well as shown in Fig. 1, the light was collected by the microscope's objective piece and directed finally to the spectrometer via another single-fiber patch cable connected to the microscope's camera port via a model C-Mount-MIC adapter (Ocean Optics, Dunedin, FL).

Procedure

The SEC cell was placed on the microscope's X-Y translational stage and secured to its mounting clips

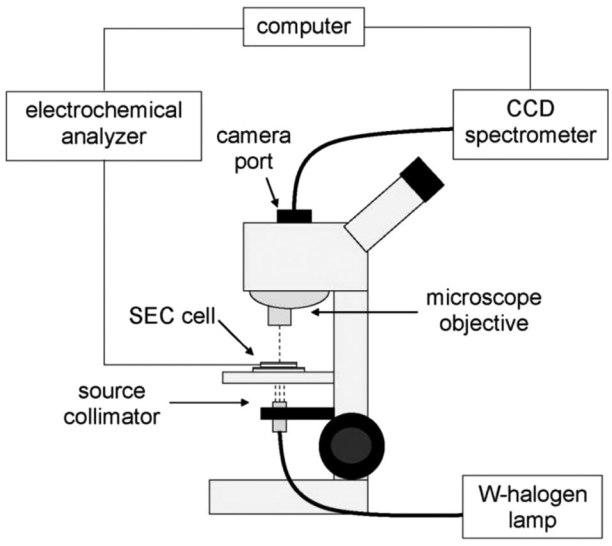

FIGURE 2 **Schematic diagram of the experimental setup depicting the electrical connections (light lines), fiber optic cables (heavy lines), and light path (dashed lines).**

with adhesive tape. The reference and auxiliary electrode wells were filled with 1 M KNO_3 using a disposable Pasteur pipette, and all three electrodes were then inserted into their respective wells and secured in place by taping their protruding ends to the top of the well plate. Small squares of Parafilm were used to cover the reference and auxiliary wells in order to minimize solvent evaporation during extended experimentation. A measured volume of sample solution (1–5 μL) was next added to the sample well using a Hamilton model 7105KH zero dead volume 5 μL syringe (Hamilton Company, Reno, NV, U.S.A.). Though any microliter syringe or digital pipette can be used to fill the sample well, a blunt, plunger-in-needle syringe is recommended as it most easily allows the introduction of the small sample volume to the bottom of the well with minimal risk of introducing air bubbles or damaging the surface of the Nafion film. During lengthy experiments, a small square of transparent polyethylene film wrap was used to cover the sample well to slow solvent evaporation and avoid dust contamination. The electrochemical analyzer leads were then connected to the SEC cell electrodes, and microscope stage translation controls were used to align the cell for optimal light throughput. This alignment procedure entailed an initial, rough visual positioning using the microscope's eyepiece to bring the sample well into the field of view with focus on the top of the Pt coil WE, followed by a fine adjustment of the cell's X-Y position (in the plane perpendicular to the light beam, via the stage translation controls) and its Z position (along the axis of the light beam, via the microscope focus controls) to maximize signal at the spectrometer. Unless otherwise specified, the microscope's 4X objective was used. The sample was then subjected to the desired electrochemical and/or spectral measurements as detailed in the "Results and Discussion" section. Samples were removed from the well via capillary action using a wick fashioned by tightly twisting a small piece of lint free tissue.

RESULTS AND DISCUSSION

Electrochemistry

Cyclic voltammograms measured at several scan rates, ν, for a 3 μL sample of 1 mM potassium ferricyanide in 1 M potassium nitrate measured are shown in Fig. 3. The voltammetric peak currents display the expected linear dependence on $\nu^{1/2}$($r > 0.99$) over the examined scan rate range of 1–200 mV/s, and the increase in potential difference between the anodic and cathodic peaks with increasing scan rate is moderate, indicating that the cell functions essentially in bulk diffusion mode and that the Nafion salt bridge is adequately conductive. Repeating these measurements on the same sample more than 1 h after its initial loading showed no significant difference in the voltammograms, indicating that any leakage or other means of sample loss is negligible.

Spectrometry

The cell was used to measure visible spectra of various volumes of aqueous ferricyanide in order to assess its utility as an optical cuvette.In these measurements, a reference spectrum was first acquired with the cell's sample well containing a volume of electrolyte blank equal to the volume of sample to be used. The alignment procedure described in the "Materials and Methods" section was followed for each reference and sample spectrum measured. Spectra were obtained in this fashion for sample volumes of 1, 2, 3, 4, and 5 μL (the effective capacity of the sample well). A plot of peak absorbance for the ferricyanide ion at 420 nm versus sample volume was linear ($r > 0.99$) as expected from Beer's law and the geometric relation between volume and height (in this case, optical path length) for a cylinder:

$$A = \varepsilon bc = (\varepsilon c/\pi r^2)V \qquad (1)$$

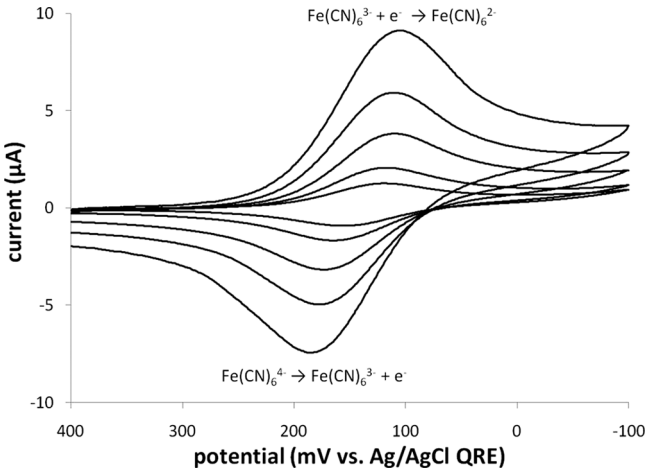

FIGURE 3 **Cyclic voltammograms for 1 mM potassium ferrocyanide in 1 M potassium nitrate measured at scan rates of 1, 2, 5, 10, and 20 mV/s.**

where A is absorbance, ϵ is molar absorptivity, b is pathlength, c is analyte molarity, r is sample well radius, and V is sample volume. Path lengths for sample volumes of a few microliters are in the range of 1–2 mm. In comparison to the typical transversely-sampled thin layer SEC cells that afford path lengths on the order of 0.01–0.1 mm, the optical sensitivity, ϵb, of the microscale SEC cell is thus one to two orders of magnitude greater.

Analyte detection limits, LOD, are determined by both the sensitivity and the noise level of the signal measurements. For absorbance measurements, LOD may be calculated as

$$LOD = 3\sigma/\varepsilon b \qquad (2)$$

where σ is the standard deviation of background measurements, a quantity that may be approximated as one-fifth of the peak-to-peak noise in the blank absorbance spectrum.[15] Meaningful comparison of the LOD possible with the microscale cell with that for other SEC cells would therefore require the use of equivalent spectrometer components (light sources, sampling optics, detectors) and signal processing (scan averaging, smoothing, etc.), since these experimental parameters are significant factors affecting noise level. Assuming that similar noise levels would result from the use of comparable instrumentation, it is reasonable to predict that the greater optical sensitivity provided by the microscale SEC cell will yield detection limits that are at least an order of magnitude lower than those for typical transversely sampled thin layer cells.

Spectroelectrochemistry

The SEC performance of the cell was evaluated by monitoring sample absorbance while performing a double-potential step chronoabsorptometric experiment. This measurement entails abruptly changing the working electrode potential from a value where one member of a redox couple is predominant to a value where the other member is predominant, the electrode potentials being sufficiently beyond the couple's standard potential, E^o, to result in diffusion-limited electrolysis.[16] In this case, the electrode potential was held constant at each value until the sample was exhaustively electrolyzed as evidenced by the attainment of a constant absorbance and a near-zero current. A plot of absorbance at

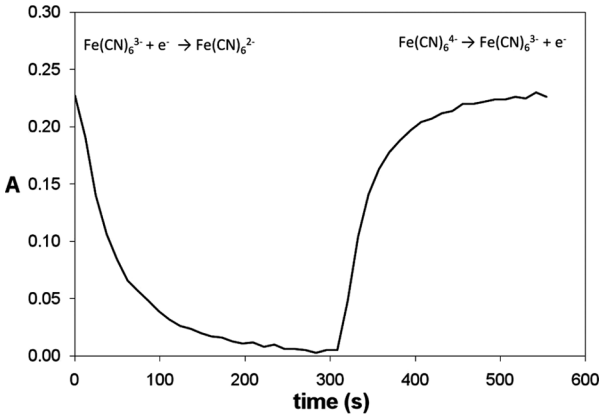

FIGURE 4 **Plot of absorbance at 420 nm versus time for the double-potential step electrolysis of a 3 μL sample of 1 mM potassium ferrocyanide in 1 M potassium nitrate.WE potential was stepped from 400 mV to −100 mV at t = 0 s and from −100 mV to 400 mV at t = ~300 s.Reference spectrum was measured after exhaustive electrolysis at −100 mV (only ferrocyanide was present).Spectra were acquired using 47 ms integration, 256 scan average, and 19-point smoothing (acquisition time = ~12 s/spectrum).**

420 nm versus time for such a measurement using a 3 μL sample of 1 mM potassium ferricyanide is presented in Fig. 4 (see figure caption for experimental details). These data show that electrolysis of the sample is essentially complete in approximately 5 min, a reasonable result considering the diffusion times estimated by the Einstein equation, $t = x^2/2D$, where x is distance, and D is diffusion coefficient. Using a value of $7.26 \times 10^{-6}\,cm^2s^{-1}$ for ferricyanide's diffusion coefficient[17] and defining d as the diameter of cylindrical volume within the Pt-coil electrode (~0.11 cm), times of ~200 and ~400 s are estimated for species diffusing to the Pt coil's inner wall from the center ($x = d/2$) and the opposite side ($x = d$) of the sample solution.

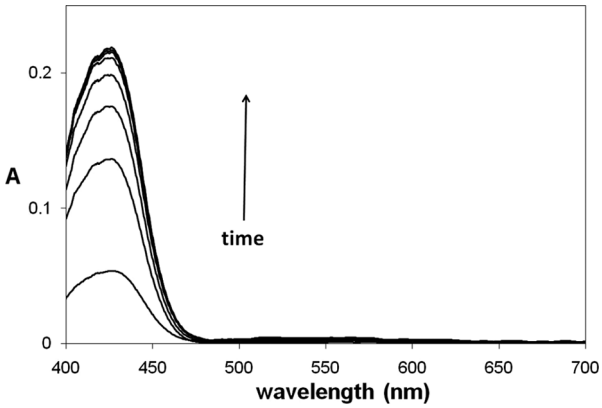

FIGURE 5 **Absorbance spectra measured at various times after the second step (−100 mV to 400 mV) of a double-potential step experiment (conditions as described in Fig. 4).**

Full-range visible spectra measured at selected times during the second step of a double-potential step experiment like the one described above are shown in Fig. 5. The quality of these spectra is very good, particularly considering the small volume of sample, 3 μL, and the modest signal acquisition time, 12 s.

CONCLUSIONS

The cell described in this manuscript has been shown to be useful for electrochemical, spectral, and SEC measurements on sample volumes in the range of 1–5 μL. Several advantages relative to most of the comparable designs previously published have been demonstrated, including

- simple construction, requiring no specialized machine or glass shop services;
- easy assembly/disassembly and sample loading;
- greater sensitivity due to longer optical paths (one or twoorders of magnitude greater than transversely sampled thin layer cells).

Additionally, some relatively minor alterations to the cell design are envisioned that could expand the scope of its application, for example,

- use of an anion exchange polymer film as the salt bridge to permit the analysis of cationic species;
- incorporation of multiple sample wells in a single device (similar to standard multi-well plates) to increase sample throughput;
- use of smaller diameter sample wells to accommodate sub-microliter sample volumes (with accompanying decreases in bulk electrolysis times);
- addition of a matched hole in the base plate beneath the sample well to avoid ultraviolet absorption by the PMMA and thus allow measurements in this spectral region (or, alternatively, use of an ultraviolet-transparent base plate material).

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