Technical Notes

Measurement of Ultrasmall Volumes Using Anodic Stripping Voltammetry

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This paper discusses a new and effective method for small volume determination. The benefits of working with small volumes are numerous and include using less material, generating less waste, increased functionality in less space, portability, and high throughput. As sample size decreases, measuring its volume becomes more challenging. The most prevalent method for small volume determination is visual inspection. The method presented in this paper is precise, more quantitative, and adaptive to many solution geometries, because it is based on a coulometric approach. Demonstration of this method is performed on volumes of ∼1 nL containing silver and using a self-contained microcavity device that contains multiple electrodes. The silver is "exhaustively" deposited during a cathodic potential step, followed by anodic stripping voltammetry. It is expected that an even faster and more accurate analysis of volumes much smaller than those evaluated here is easily possible using this approach.

A popular direction in research today is to miniaturize chemical analysis methods.[1−7] Miniaturization involves reducing the overall size of the analysis device, using less material, generating less waste, providing for analysis of smaller samples (e.g., single cells) or multiple-sample analysis in a smaller space, and therefore, allowing higher throughput and the ability to analyze solutions having smaller volumes. Often, the actual volume of ultrasmall samples (≪1 μL) that are evaluated by such devices is not known.

The most prevalent method for measuring the volume of a small drop of solution is by visual inspection.[5,7−14] One visual inspection approach is based on the dimensions of a cavity or vial in which the solution resides and assumes that the top of the drop is flat and horizontal with the lip of the device with a lack of a lens effect.[5,7,8] Another method involves measuring the diameter of a spherical drop of solution and determining its volume based upon the volume of a sphere ($V_{sp} = 4/3\ \pi r^3$, where r is the radius) or hemisphere ($V_{hsp} = V_{sp}/2$).[10−14] Accurate measurement of the diameter of small drops requires the use of calibrated microrulers, microscopes, image-shearing monitors, and video monitors.

The method presented here is a relatively easier and more quantitative approach for determining small volumes of almost any shape, but it has two requirements: (1) the addition of a metal species that can be electrodeposited from solution and subsequently stripped off, and (2) either at least two electrodes within the solution or one electrode in the solution with a second electrode behind a fritted junction. This is especially convenient when electrochemical detection is to be used for the chemical analysis of small samples, in which case the electrodes are already available. The method involves cathodic deposition of all the metal species from the small volume of a known concentration and calculating the actual volume from the number of coulombs required to anodically strip the metal back off. This approach precludes the use of optics, and thus, a broader range of device designs, including those which are incompatible with optical measurements (e.g., enclosed or blocked spaces, opaque materials, etc.), may be considered. In addition, because the method is coulometric, calibration curves are not required.[15] A solution containing silver ions and a microcavity containing multiple electrodes were used to illustrate the concept.

EXPERIMENTAL SECTION

Reagents. All chemicals were reagent grade and used as received. Aqueous solutions were prepared with high-purity deionized water (Milli-Q, model RG). Potassium nitrate, hexaamineruthenium(III) chloride, and silver nitrate were obtained

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from Aldrich Chemical Co. (St. Louis, MO). Mineral oil was obtained from Sigma Chemical Company (St. Louis, MO).

Apparatus. Small volumes were formed using one or more of the following: a micropipet (Eppendorf Pipettor, 2100 series), microsyringe (Hamilton), and disposable pulled Pasteur pipets (VWR). The location of the small volume can be on or in any device, as long as there are enough electrodes to carry out the electrochemical experiment. A microcavity device was used here, the fabrication and characterization of which are described elsewhere in more detail.[16,17] Briefly, the device was constructed on a silicon wafer from alternating layers of conductor (Au with a Cr adhesion layer) and insulator (polyimide), which allows for multiple electrodes to be available in a small volume. These electrodes are individually addressable with a cavity depth of 8 μm and a diameter of $\sim$13 μm with a volume size of $\sim$1.0 pL. The microcavity has a recessed microdisk (RMD) electrode at its bottom and a tubular nanoband (TNB) electrode along the wall of the cavity. A third electrode, the Au top layer, resides in the plane of the upper rim of the cavity. This electrode configuration allows for self-contained electrochemistry, meaning that no external auxiliary or reference electrodes are needed to carry out an electrochemical experiment.

Procedure. Electrochemical measurements were obtained with a CH Instruments Electrochemical Workstation model 750A equipped with a picoamp booster and Faraday cage (CH Instruments, Inc, Austin, TX), controlled by a PC equipped with CHI-750A software. Initially, each electrode in the cavity device was characterized using cyclic voltammetry (CV) in a bulk solution of 5 mM $Ru(NH_3)_6^{3+}$ and 0.5 M KNO_3 with an external and internal electrode configuration, reported previously.[16,17] Small volume determination involved an internal electrode configuration that utilizes the TNB as the working electrode and the top Au layer as the reference and auxiliary electrode. The RMD electrode was not used in the studies reported here. The experiment was performed by pipetting 100 nL of 5 μM $AgNO_3$ in 0.5 M KNO_3 on the microcavity using a micropipet and then quickly placing it in mineral oil, which slowed the evaporation process. The smallest volumes evaluated here were formed by wicking the excess solution by touching the drop with a disposable pulled Pasteur pipet and allowing capillary action to remove the majority of the drop. We have demonstrated that there is no significant evaporation of these wicked drops for at least 60 min under mineral oil.[17] Drop manipulations were monitored using a stereoscope (40X, H-011, Olympus). This setup was then placed in an air-free environment in a glovebag (Instruments for Research and Industry, Cheltenham, PA) with continuous positive pressure (nitrogen, Air-gas, Radon, PA). Results were most reproducible when experiments were carried out in an air-free environment. Although we have not investigated the reason for this, we suspect that oxygen or its reduction products lead to unusually small anodic stripping waves of Ag° that are obtained when air is present.[18] Silver was deposited on the TNB electrode by stepping to a reduction potential (-0.7 V vs Au top layer pseudoreference) beyond the cathodic peak potential of Ag^+ for varying amounts

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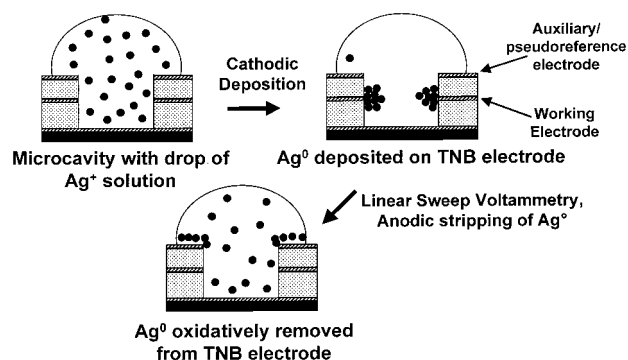
Figure 1. Cross-sectional diagram of the microcavity and procedure for measurement of small volumes by anodic stripping of cathodically deposited silver from the drop.

of time. The silver was anodically stripped off the TNB electrode using linear sweep voltammetry from -0.6 to 0.1 V vs the Au top layer, Figure 1. The volume was calculated from the measured area under the stripping wave. Any residual Ag° was removed from the electrode before the next experiment by sweeping the potential several times over anodic potentials. This was continued (usually five times) until the stripping peak disappeared. In general, devices were cleaned between uses and before storage by first rinsing thoroughly with deionized water, then sonicating (Branson 2210, Branson Ultrasonic Corporation) for 30 s in acetone to remove any residue, followed by drying with a jet of Ar for 15 s. Devices were stored in deionized water when not in use.

RESULTS AND DISCUSSION

Choice of Metal Species. Several factors should be considered when selecting the metal species for the volume determination method. Those that may be analyzed by stripping voltammetric analysis (e.g., Ag, Au, Pt, Hg, Cu, Tl, Bi, Cd, In, Sn, Sb, Zn, and Pb) are eligible candidates.[19−21] We chose silver for our demonstration, partly because it is low in toxicity and easily anodically stripped from gold electrodes.[20,22−25] In addition, its stripping potential does not interfere with oxidation of the electrode material or of the electrolyte solution.

It is also important to consider whether the dispensing and device materials are compatible with the metal-containing solution. Because the method is coulometric, any uncontrolled addition or removal of the metal species in the sample will cause inaccurate volume determinations. For example, loss of 33% (for an 8 mM solution) to 52% (for a 5 mM solution) of silver ion occurs, as determined by cyclic voltammetry, when a 10-nL drop is dispensed from a 0.5-μL Hamilton syringe with a stainless steel needle. In

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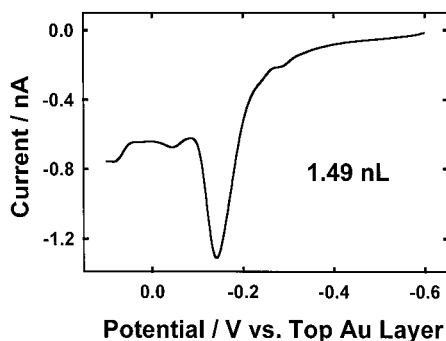
Figure 2. Representative silver stripping peak at a TNB working electrode in a wicked drop of 5 μM $AgNO_3$ in 0.5 M KNO_3 under mineral oil. The top layer of Au served as the auxiliary/pseudoreference electrode. The Ag^+ was cathodically deposited for 30 min before anodic stripping at 0.1 V/s in an air-free environment.

Figure 3. Volumes calculated from anodic stripping voltammetry under conditions similar to those in Figure 2. Cathodic deposition times were for 5, 15, 30, and 45 min. Two data sets are shown (circles and squares), which correspond to two different wicked drop experiments with the same microcavity device.

contrast, loss of silver does not occur when a plastic micropipet tip is used instead. This can be explained by removal of Ag^+ ($E^{\circ}_{Ag} = +0.7791$ V vs NHE) through reduction by the stainless steel needle ($E^{\circ}_{Fe} = -0.44$ V vs NHE).[19]

Choice of Metal Concentration. Another concern before performing the volume determination is choosing a concentration of metal ion that is high enough to provide a measurable stripping wave, but low enough to prevent electrical shorting between working and auxiliary or reference electrodes during the deposition time. The appropriate concentration depends on the size of the volume (which defines the total number of metal species available for reduction) and the distance between electrodes. In our device, enough Ag^0 buildup onto the working electrode causes electrical shorting with the reference/auxiliary electrode, which is 4 μm away, when a cathodic deposition time of 220 s is used with a solution of 8 mM $AgNO_3$ in 0.5 M KNO_3. A concentration that avoids electrical shorting at any time of deposition (i.e., exhaustive removal of metal from solution) with ~1 nL drops was 5 μM $AgNO_3$ in 0.5 M KNO_3.

Use of Anodic Stripping Voltammetry to Determine Volume. A representative anodic stripping peak of exhaustively electrodeposited silver in a wicked drop under mineral oil is shown in Figure 2. Wicking is a process that has been used by others to decrease the volume of a drop of solution.[4,7,8] The shape and size of the resulting drop can depend on the wettability of the surface of the substrate and the surrounding environment. Thus, this approach for forming ultrasmall volumes is a good application for a volume-determination method.

The volume of a drop of sample solution, V_s, is calculated from eq 1.

$$V_s = \frac{Q}{nFC^*} \quad (1)$$

Q is the number of coulombs obtained by integrating the anodic stripping wave, n is the moles of electrons transferred per mole of metal ions ($n = 1$ for Ag^+), F is the Faraday constant (96 485 C/mol of electrons), and C^* is the known starting concentration of the metal ion. The volume determined from the stripping peak shown in Figure 2, using eq 1, is 1.49 nL.

Volumes were calculated from stripping waves after deposition times of 5, 15, 30, and 45 min in two different sets of experiments,
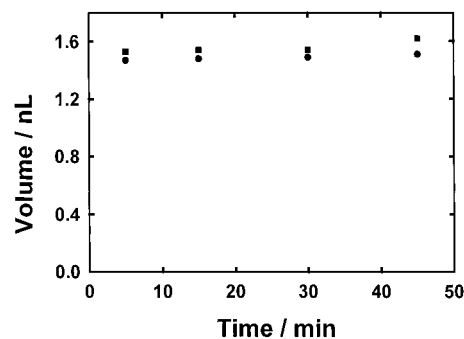
each set involving a different wicked drop, but using the same microcavity device. The results are shown in Figure 3. After each deposition and stripping sequence, the TNB electrode was electrochemically cleaned to ensure that all residual Ag^0 was removed from the electrode surface before the next sequence. The calculated volumes were almost identical within each set of experiments: 1.49 ± 0.02 nL and 1.56 ± 0.04 nL.

The results in Figure 3 illustrate several points. First, "exhaustive" reduction of Ag^+ in the wicked drop occurs in <5 min. This is consistent with back of the envelope calculations using Einstein's equation,[26] which predict that species from the periphery of a 1-nL drop should reach the TNB electrode after ~2 s, assuming a diffusion coefficient of 1.6×10^{-5} cm^2/s[27] and that the drop is perfectly hemispherical and centered over the microcavity. However, the actual time to maximize depletion of the silver ions must be longer than this and depends on electrode dimensions and geometry. For example, the time spent at reducing potentials during a cyclic voltammogram at 0.1 V/s (estimated to be 6 s) and in 5 μM solution with our microcavities is *not* long enough to produce a visible stripping wave at the TNB electrode.

The fairly constant volumes obtained after a 5 min deposition time are not an artifact of the method. Experiments performed under similar conditions on a much larger volume of 500 nL (dispensed from a plastic micropipet without wicking under mineral oil) did *not* achieve a constant area under the stripping waves with deposition times up to 105 min. The volumes calculated at different deposition times on the 500 nL drop were 7.84 nL after 15 min, 36.7 nL after 45 min, 68.3 nL after 75 min, and 119 nL after 105 min. Using the Einstein equation to calculate the time in which a species can reach the electrode from a radius length equal to that of a hemisphere of 500 nL is only about 2 min, further demonstrating the importance of electrochemical efficiency of the electrode in defining the required deposition time for exhaustive reduction of the metal species.

We are confident that the stripping wave for silver is not due to contaminant metal in the electrolyte. This is because lower

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concentrations than 5 μM Ag$^+$ provide proportionally smaller stripping peaks.

Because the volume determination method is coulometric, the shape of the sample solution is unimportant, as compared to optical methods. However, if the *distance* between the metal species and the working electrode is very large, then the time required for exhaustive reduction may be unreasonably long. For example, a hemispherical drop of 1 μL should require less time for exhaustive deposition than a 1 μL drop that is spread thin under a glass slide that covers the microcavity,[17] where diffusion to the electrode from the periphery of the flattened drop would involve distances of centimeters.

Another point that Figure 3 illustrates is the high reproducibility of the volume determination procedure. As long as the deposition time is long enough for exhaustive reduction, the volume calculated from the stripping wave for the same drop is the same. In addition, multiple analyses of the same sample do not cause loss of Ag$^+$ because of irreversible deposition elsewhere on the device (e.g., auxiliary electrode) or due to homogeneous reactions. The typical lifetime of a microcavity device under these conditions lasted more than 60 experiments.

Finally, comparison of the two sets of experiments in Figure 3 provides an example of how volume variations from drop to drop can be quantitatively distinguished. Although the volumes of the two wicked drops are within 5% of each other, they are outside of one standard deviation of each other. Slightly different volumes will be produced each time, because it is difficult to control precisely the amount of solution that is wicked away. Reproducibility of volumes produced on different devices is much poorer, presumably because of slight variations in wetting properties of the top Au layer. For example, a volume of 3.5 nL was obtained using the wicking procedure on two devices that were different from the one whose results are shown in Figure 3.

CONCLUSIONS

A new electrochemical method for measuring small volumes is reported, which has several advantages over optical methods. Appropriate metal species, concentrations, and deposition times depend on the application, electrode and volume geometries, and conditions. The volume determination method is expected to perform better on volumes much smaller than ~1 nL, where exhaustive reductive depletion occurs at shorter times.

Our studies have not addressed whether volume determination can be performed on a sample that also contains an analyte of interest. If volume formation is reproducible, it would be unnecessary to perform simultaneous volume and analyte analysis on the same sample. However, the method should work when a chemically compatible metal species is added to the sample, which is especially important when volume formation is irreproducible. If the analyte is electroactive, a metal species must be chosen with a redox potential that does not interfere with that of the analyte.