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Detection of Silver Nanoparticles by Electrochemically Activated Galvanic Exchange

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Abstract

Here we report on the seemingly simple process of galvanic exchange (GE) between electrogenerated AuCl_4^- and silver nanoparticles (AgNPs). The results were obtained in the specific context of using AgNPs as labels for bioassays in paper fluidic devices. Results obtained from a combined electrochemistry and microscopy study indicate that the GE process results in recovery of only ~5% of the total equivalents of Ag present in the system. This low value is a consequence of two factors. First, after an initial fraction of each AgNP undergoes GE, a Au shell forms around the remaining AgNP core preventing further exchange. Second, to simulate a true biological fluid, the experiments were carried out in a Cl^- -containing buffer. Consequently, some Ag^+ formed during GE precipitates as AgCl, and it also serves to block additional GE. Following optimization of the GE process, it was possible to detect AgNP label concentrations as low as 2.6 fM despite these limitations.

Graphical Abstract

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Supporting Information

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Description of the PLA electrochemical cell; TEM micrographs and size-distribution histograms; SEM micrographs; microscopy and EDX analyses; linear sweep voltammograms; experiment showing the relationship between the percentage of Au oxidized from the carbon/Au electrode as a function of chloride concentration; electrochemical data used to calculate the diffusion coefficient of AuCl_4^- ; morphology of AgNPs after one GE cycle; and EDX spectrum (PDF)

The authors declare no competing financial interest.

■ INTRODUCTION

We recently reported the use of silver nanoparticle (AgNP) labels for electrochemical detection of biomolecules¹⁻⁴ in paper microfluidic sensing devices.⁵⁻⁹ In this approach (Scheme 1), multiple AgNPs are linked to a single magnetic microbead (MμB) in the presence of a target. This MμB–AgNP conjugate is then drawn to an electrode surface by a magnetic force, and the number of AgNPs is analyzed electrochemically. Because there is a one-to-one correspondence between the number of target molecules and the number of AgNPs, the presence of the target is amplified in proportion to the charge stored in each AgNP.^{1,3,10}

We have used two closely related electrochemical methods to detect the presence of the AgNP labels. In both cases the AgNPs are chemically oxidized, the resulting Ag^+ is electrodeposited onto the surface of an electrode, and then the amount of deposited Ag is determined by anodic stripping voltammetry. The two methods vary only in the method of initial oxidation of the AgNPs. In one case they are chemically oxidized using a chemical reagent such as permanganate (MnO_4^-),^{4,10} bromine,^{11,12} or hypochlorite (bleach),³ and in the other the oxidation is accomplished by a process known as galvanic exchange (GE).^{1,2,13-16} Note that because the AgNPs are bound to MμBs and not in physical contact with the electrode surface, it is not possible to oxidize them directly.⁴

GE, which is sometimes called galvanic replacement, has been used for decades to efficiently replace one metal with another.^{14,15,17} GE occurs when a zerovalent metal is immersed in a solution containing the oxidized form of a more noble metal (e.g., a higher standard potential). The two metals undergo a spontaneous exchange of electrons, leading to oxidation of the less noble metal (e.g., Ag, $E^0 = 0.79$ V) and reduction of the more noble metal (e.g., Au, $E^0 = 1.52$ V).¹⁸ There have been a number of interesting fundamental and practical reports relating to GE. For instance, Adzic and coworkers studied the electrocatalytic oxygen reduction reaction using nanoparticles (NPs) having Pt shells deposited onto different types of metal cores by GE.^{19,20} In another report, Xia and co-

workers used AgNPs and Ag nanowires as templates to study the morphology of the materials resulting after addition of metal salts having higher reduction potentials.^{14,21-23}

Because AgNPs are available commercially in different sizes and shapes, and because Ag has a relatively low reduction potential, AgNPs are often used as templates for GE.^{14,15,24,25} For example, a number of groups have studied how the optical properties, composition, and morphology of AgNPs change upon addition of HAuCl₄.^{21,26-30} An important outcome of these studies is that, depending upon conditions, both AgCl and Ag⁺ can result, as shown in eqs 1 and 2.^{23,24,31-33}

Importantly, eq 1 can lead to deposition of both a Au shell and/or an insoluble AgCl shell on the surface of the AgNPs, thereby blocking penetration of AuCl₄⁻ to the underlying AgNP that remains and preventing complete GE.³³ As we will discuss later, this is an important consideration in our study.

In the present article, we focus on the details of the GE processes represented by eqs 1 and 2. The experiments are carried out using the approach illustrated in Scheme 1, which, with one exception, is directly relevant to the detection scheme we have used in previous publications focused on chemical sensing.^{1,2} The one exception is that here we have used 110 nm AgNPs, and in our prior studies the size was 20 nm. We found it necessary to use the larger particles so that they could be easily imaged by electron microscopy (vide infra).

As shown in Scheme 1a, GE is implemented as follows. First, a small amount of Au is electrodeposited onto a carbon paste working electrode. Next, the M₄B-AgNP conjugates are drawn to the electrode surface by a magnetic force. At this point, a fraction of the Au is electrochemically oxidized to initiate GE with the AgNPs. During GE, eqs 1 and 2 proceed from left to right. This results in formation of Ag⁺ (Scheme 1b), which is subsequently electrodeposited onto the electrode surface as zerovalent Ag (Scheme 1c). A final anodic stripping voltammetry step makes it possible to determine the total Ag charge recovered from the GE reaction. Note, however, that Scheme 1 is a highly simplified representation of the GE process. For example, it ignores the possibility of AgCl formation, incomplete GE, and mass transfer considerations. The objective of the present study is to better define the complex nature of this seemingly simple process so that it can be used for electrochemical sensing applications.

■ EXPERIMENTAL SECTION

Chemicals and Materials.

All solutions were made using deionized (DI) water ($>18.0\text{ M}\Omega\text{ cm}$, Milli-Q Gradient System, Millipore, Bedford, MA). NaCl, NaOH, HCl, HAuCl_4 , KNO_3 , citric acid monohydrate, 4-(2-hydroxymethyl)-1-piperazineethanesulfonic acid (HEPES), Whatman grade 1 chromatography paper ($180\text{ }\mu\text{m}$ thick, $20\text{ cm} \times 20\text{ cm}$ sheets, linear flow rate of water = 0.43 cm/min), and siliconized low-retention microcentrifuge tubes were purchased from Fisher Scientific (Pittsburgh, PA). Boric acid was purchased from EM Science (Gibbstown, NJ). Citrate-capped AgNPs (nominal 110 nm diameter) were purchased from Ted Pella (Redding, CA). A solution containing 0.10 M borate and 0.10 M NaCl (referred to henceforth as BCl) was prepared by dissolving appropriate amounts of boric acid and NaCl in DI water, and then adjusting the pH to 7.5 with NaOH.²

Conductive carbon paste (CI-2042) was purchased from Engineered Conductive Materials (Delaware, OH). Cylindrical neodymium magnets ($1/16\text{ in} \times 1/2\text{ in}$, N48) were purchased from Apex Magnets (Petersburg, WV). Streptavidin-coated $\text{M}\mu\text{Bs}$ (Dynabeads, M-270, $2.8\text{ }\mu\text{m}$ diameter) were obtained from Invitrogen (Grand Island, NY). Lyophilized thiol-DNA-biotin ($5'\text{ d thiol C6 SS-ACATTAAATTC-biotin }3'$) was purchased from Biosearch Technologies (Petaluma, CA). Before use, the DNA-biotin was hydrated with the appropriate amount of DI water to make a final concentration of 1.0 mM .

Electrochemistry.

All electrochemical measurements were performed using a CH Instruments model 760B electrochemical workstation (Austin, TX). For all electrochemical procedures, the working electrode was stencil-printed carbon paste partially covered with electrodeposited Au (vide infra). The counter and reference electrodes were a Pt wire and a saturated $\text{Hg/Hg}_2\text{SO}_4$ electrode, respectively, both purchased from CH Instruments.

The working electrodes were fabricated by patterning sheets of chromatography paper with wax using a Xerox ColorQube 8570DN printer. After printing, the wax was melted through the thickness of the paper by placing it in an oven at $120\text{ }^\circ\text{C}$ for 25.0 s . Next, the paper was cut into 12 rectangles ($5.0\text{ cm} \times 6.5\text{ cm}$). A stencil for defining the 2.0 mm diameter, disk-shaped electrodes was created using CorelDRAW (Ottawa, ON), and then it was cut into a thin plastic sheet of transparency film using an Epilog laser engraving system (Zing 16). Finally, the stencil was placed over the paper (wax side up); the electrodes were printed through the stencil using conductive carbon paste, and then the carbon paste was left to dry in air for 14 h .

Au was electrochemically deposited onto the carbon paste electrodes using a polytetrafluoroethylene electrochemical cell.^{1,2} Au electrodeposition was carried out using a solution containing $300.0\text{ }\mu\text{L}$ of 6.0 mM HAuCl_4 and 0.10 M KNO_3 .^{1,2,34} Au electrodeposition onto the carbon paste working electrode was initiated by stepping its potential from 0 to -0.20 V vs $\text{Hg/Hg}_2\text{SO}_4$ for 2.0 s . This potential was chosen based on the AuCl_4^- reduction wave.^{1,2} During reduction, Au nucleated on the carbon paste electrode resulting in dispersed gold nanoparticles.^{2,35} This modified electrode will be referred to

hereafter as the “carbon/Au working electrode”. After Au electrodeposition, the electrode was rinsed twice with DI water and dried with a Kimwipe.²

The GE reactions and Ag anodic stripping voltammetry were carried out using an electrochemical cell printed with polylactic acid (PLA) using a Modified MakerBot Replicator 2s 3D printer. This cell is referred to hereafter as the “PLA cell” (Figure S1). After the PLA cell was printed, a single coat of clear nail polish, obtained from Electron Microscopy Sciences (Hartfield, PA), was applied to the interior of the cell to seal it and prevent leakage of BCl electrolyte solution. The charge corresponding to AgNP concentration was calculated by integrating the anodic stripping voltammetry peaks using Origin Pro8 SR4 v. 8.0951 software (Northampton, MA).

Preparation of the M μ B–AgNP Conjugate.

Biotinylated DNA was immobilized on 110 nm AgNPs using a previously reported fast, pH-assisted method.^{2,36,37} The biotinylated DNA immobilized on the AgNPs will henceforth be referred to as “AgNP-biotin”. Next, 400 μ L of the AgNP-biotin (2.4×10^9 AgNPs/mL) was mixed with 16 μ L of streptavidin-coated M μ Bs ($(6-7) \times 10^8$ M μ Bs/mL), and the solution was shaken (1400 rpm) for 30 min at 23 °C.^{2,4,38} Following conjugation, the M μ B–AgNP conjugates were washed three times by holding the microcentrifuge tube up to a magnet, removing the supernatant, and resuspending in 16 μ L of BCl.^{2,4}

Transmission Electron Microscopy (TEM).

Size analysis of the AgNPs before and after biotin modification was carried out using a JEOL 2010F TEM instrument having a point-to-point resolution of 0.19 nm. Samples were prepared by pipetting 2.0 μ L of unmodified or modified AgNPs onto a carbon-coated Cu grid obtained from Electron Microscopy Sciences (Hartfield, PA). The size distributions were determined using ImageJ software and 200 randomly selected particles (Figure S2).

Energy-dispersive X-ray (EDX) analysis of the M μ B–AgNP conjugates was performed using a JEOL 2010F TEM instrument. The samples for this analysis were prepared by removing the M μ B–AgNP conjugates from the carbon/Au working electrode (after electrochemical analysis) using a micropipette. The M μ B–AgNP conjugates were then added to 100.0 μ L of isopropylalcohol in a microcentrifuge tube, and sonicated for 10.0 min. Next, the sample was pipetted onto a Si₃N₄ TEM grid (Ted Pella, Redding, CA) and dried for 15.0 min under a heat lamp. Finally, a 200 mesh carbon-coated Cu grid obtained from Electron Microscopy Sciences (Hartfield, PA) was laid on top of the sample to prevent the M μ Bs from adhering to the magnetic portions of the TEM instrument.

Scanning Electron Microscopy (SEM).

SEM micrographs were obtained using a Hitachi S5500 SEM instrument having an accelerating voltage of 30 kV, and a point-to-point resolution of 0.4 nm. Samples of the M μ B–AgNP conjugates were prepared by drop casting 2.0 μ L of the M μ B–AgNP conjugates on a carbon-coated Ni grid (Electron Microscopy Sciences, Hartfield, PA) and then drying in air for ~12 h. The size distribution of the AgNPs on the M μ Bs was also analyzed by randomly selecting 200 particles in ImageJ (Figure S2). The AgNP coverage on the M μ Bs

was determined by counting the number of AgNPs present on the surface of the M μ Bs using 10 different SEM micrographs (Figure S3).

SEM micrographs of the carbon/Au working electrode before and after immobilization of the M μ B–AgNP conjugates were obtained by removing the electrode from the PLA cell, cutting out a section of the electrode (3.0×5.0 mm), drying in air for 12.0 h, and then attaching the electrode section to the stage of a Hitachi SEM standard holder with conductive carbon tape. The AgNP size distribution (Figure S2) and coverage of the AgNPs on the M μ Bs (Figure S3) were both quantified as previously described. EDX was carried out using Bruker ESPRIT imaging software (Billerica, MA). The EDX detector was a Bruker XFlash 4010 with a detector area of 10 mm² and an energy resolution of 129 eV at the Mn K α peak.

■ RESULTS AND DISCUSSION

Electron Microscopy of the M μ B–AgNP Conjugates.

The primary goal of this project is development of a better understanding of the complex nature of the seemingly simple GE process between AuCl₄[−] and AgNPs.^{14,15,24,39} As mentioned earlier, Scheme 1 neglects numerous potential subtleties that are important when using this labeling approach for biosensing applications. Accordingly, to better understand this process, we first examined the M μ B–AgNP conjugates by electron microscopy.

Figure 1a is a representative SEM micrograph of a M μ B–AgNP conjugate dried onto a carbon-coated Ni grid prior to GE. EDX data (Figure S4) confirm that the NPs present on the M μ Bs are composed of Ag. Qualitatively, it is clear that AgNPs sparsely cover the M μ Bs, and quantitative analysis of 20 M μ Bs (Figure S3) indicates the presence of 106 ± 100 AgNPs/M μ B. On the basis of the AgNP:M μ B ratio used to prepare the conjugates, the maximum coverage should be 94 AgNPs per M μ B. We conclude, therefore, that essentially 100% of the AgNPs introduced to the M μ Bs are immobilized thereon. Due to the variability in the number of M μ B–AgNP conjugates measured experimentally, we adopt the calculated average number of 94 AgNPs/M μ B for subsequent analysis.

The size of the M μ B-bound AgNPs is 131 ± 10 nm, which is larger than the size of AgNP conjugates prior to immobilization on the M μ Bs (106 ± 10 nm, Figure S2). It is unlikely that this discrepancy is real, but rather it probably arises from the difficulty of accurately measuring the AgNP size after immobilization on the M μ Bs.

Figure 1b is a representative SEM micrograph of the M μ B–AgNP conjugates dried on the carbon/Au working electrode. The shape (spherical) and size of the AgNPs before and after immobilization of the M μ Bs on the electrode (131 ± 10 nm vs 128 ± 8 nm, respectively) are the same. Additional SEM micrographs of the M μ B–AgNP conjugates following immobilization onto the working electrode are provided in Figure S3b. The main point of this initial assessment is that immobilization of the M μ B–AgNP conjugates onto the carbon/Au working electrode does not result in any significant alteration of their physical properties.

As one final point, the micrographs shown in Figures S2–S4 suggest that a small number of AgNPs may be in direct contact with the electrode surface. However, control experiments (Figure S5) indicate that if this is the case they are not electrochemically detectable.

Galvanic Exchange.

After ensuring the fidelity of the $M\mu B$ –AgNP conjugates after adsorption onto the working electrode, we sought to understand how they change following GE. These experiments were carried out as shown in Scheme 1. First, 0.50 μL of the $M\mu B$ –AgNP conjugates was pipetted onto the carbon/Au working electrode, and then the electrode was dried in air for 20.0 min. Given the number of $M\mu B$ s in this volume of solution, the surface area of the electrode, and assuming the $M\mu B$ s are in a hexagonal close-packed lattice, this would correspond to a coverage of about 1.5 monolayers of $M\mu B$ s on the electrode. Because the $M\mu B$ s are not close-packed, however, this represents a lower limit on the thickness of the $M\mu B$ layer. An optical micrograph of the $M\mu B$ s on the electrode is provided in Figure S6.

Second, the $M\mu B$ –AgNP conjugate-covered electrode was placed into the PLA cell, and then 150.0 μL of BCl electrolyte solution was added. The BCl electrolyte solution contained 0.10 M NaCl, which is sufficient to oxidize Au from the carbon/Au working electrode (Figure S7). Next, the working electrode potential was stepped from 0 to 0.60 V for 25.0 s. This potential results in oxidation of a fraction of the previously electrodeposited Au thereby initiating GE.^{1,2} Moreover, on the basis of the diffusion coefficient calculated for AuCl_4^- (Figure S8), 25.0 s is sufficient time for AuCl_4^- to reach all of AgNPs present in the vicinity of the electrode.¹⁸ Following GE, the electrode potential was immediately stepped from 0 to -0.70 V for 100.0 s to electrodeposit Ag. The combination of electrochemical oxidation of Au and subsequent deposition of Ag is referred to hereafter as “one GE cycle”. Finally, the electrode was removed from the PLA cell at the open-circuit potential, dried, and analyzed by SEM (Figure 2).

The micrograph in Figure 2a shows the $M\mu B$ –AgNP conjugates dried on the carbon/Au working electrode immediately following GE. At the resolution of this micrograph, there are no significant changes in morphology of the AgNPs compared to the images obtained prior to GE shown in Figure 1. Higher-resolution images of the AgNPs (Figure 2b,c) are somewhat more illuminating, however. For example, Figure 2b shows that some of the NPs maintain their size, smooth texture, and generally spherical shape even after GE (Figure 1). In contrast, the AgNPs shown in Figure 2c exhibit a morphology change, probably resulting from GE. Note that when the same electrochemical program is applied, but in the absence of Au (Figure S9), the spherical shape and smooth texture of the imaged AgNPs are conserved. This control experiment further implicates the GE process as being responsible for the morphological changes observed in Figure 2c. Indeed, it has been shown previously that GE between AgNPs and AuCl_4^- leads to changes in the shape of the parent AgNPs. Specifically, AuAg alloys form with shapes that include nanocages^{21,32,40,41} and core@shell structures.^{28,42,43}

To further confirm that GE is responsible for the changes shown in Figure 2, the $M\mu B$ –AgNP conjugates were analyzed by TEM and EDX following GE (Figure 3). For this experiment, the $M\mu B$ –AgNP conjugates were dried onto a freshly prepared carbon/Au

working electrode. Following one GE cycle, the $M\mu B$ -AgNP conjugates were removed from the electrode and prepared for TEM analysis as discussed in the Experimental Section. Figure 3a is a TEM micrograph of a portion of a $M\mu B$ -AgNP conjugate after GE. The edge of the $M\mu B$ is outlined by the white arc, and three NPs are also shown. One of these is highlighted with a red circle, and a pair of overlapping NPs is highlighted with a white circle. The rough surface morphology of all three NPs resembles those shown in Figure 2c, and therefore we conclude that they have undergone GE.

EDX analysis of the two NPs highlighted by the white circle is shown in Figure 3b. Here, the green shell corresponds to Au, and the red core corresponds to Ag. The width of the Au shell is ~ 10 nm, and the two Ag cores are 25 and 50 nm in diameter. Figure S10 provides quantitative EDX data for the two NPs circled in white, and it shows that the Au shell comprises ~ 21 atom % of the NPs. Two conclusions can be drawn from Figure 3b. First, electrochemically initiated GE results in only partial exchange of Au for Ag for these ~ 110 nm diameter AgNPs. Second, the Au shell that encapsulates the Ag core is probably responsible for the observed incomplete GE.

Clearly, it is only possible to obtain TEM and EDX data for a few NPs following GE, but the foregoing results confirm that there is a correlation between the morphology of the NPs and whether they have undergone GE: rough morphologies correlate to GE, while smooth morphologies correlate to no GE. This finding has allowed us to image many NPs using SEM, which has sufficient resolution to distinguish morphology, and hence determine a statistically meaningful value for the percentage of AgNPs that undergo GE. A detailed explanation for how this analysis was carried out is provided in Figure S11. Briefly, we examined 547 NPs on 25 different $M\mu B$ s, and of these 52% exhibited a morphological change correlated to at least partial GE. We now turn our attention to understanding why the GE reaction does not go to completion.

$M\mu B$ -AgNP Conjugates after Oxidation by $AuCl_4^-$.

As discussed earlier, the time allotted for GE (i.e., the duration of the Au oxidation step shown in Scheme 1a) is sufficient for $AuCl_4^-$ to diffuse well beyond the layer of $M\mu B$ s present on the electrode surface. Hence, it is surprising that a higher percentage of the AgNPs do not undergo GE. In this part of the study we sought to address this issue by identifying phenomena that could limit GE. This was accomplished using EDX analysis of the $M\mu B$ -AgNP conjugates following just the first half of the GE cycle (described next).

This experiment was carried out exactly as described in the previous section, except that it was halted immediately after oxidation of Au (e.g., only the steps shown in frames a and b of Scheme 1 were carried out: no redeposition of Ag as in frame c). Specifically, the working electrode potential was stepped from 0 to 0.60 V for 25.0 s to initiate GE. The electrode was then removed from the PLA cell at open-circuit potential, dried, and analyzed by SEM (Figure 4).

Figure 4a is an SEM micrograph of the $M\mu B$ -AgNP conjugates present on the electrode following oxidation of Au. The blue arrow points to a $M\mu B$, and the green arrow points to a NP structure present on the electrode surface. The area outlined by the orange box contains

an unknown material that overlays multiple $M\mu$ Bs. EDX analysis was performed to identify the composition of this material and to determine the identity of the NP indicated by the green arrow.

Figure 4b is an EDX color map corresponding to the micrograph in Figure 4a. The element map is focused on Fe, Au, Ag, and Cl because of the roles they play during GE. Specifically, Fe is present in the core of the $M\mu$ Bs (blue arrow in Figure 4a), and Cl^- is present in the electrolyte. The object highlighted by the green arrow in Figure 4a is now clearly identified as a nodule of Au that was electrodeposited during the fabrication of the electrode (green arrow in Figure 4b). The presence of this feature indicates that only a fraction of the Au originally deposited onto the carbon electrode is converted to soluble $AuCl_4^-$ during the positive potential step (Figure S7). It also indicates that Au electrodeposited onto the carbon electrode during device fabrication is randomly distributed as NPs on the carbon surface rather than being present as a conformal coating.^{2,35} Finally, the structure outlined with an orange box in Figure 4a is composed of both Ag and Cl, suggesting that it is primarily AgCl. Figure S12 indicates that a small fraction of NaCl is present as well. These findings indicate that AgCl ($K_{sp} = 1.8 \times 10^{-10}$)⁴⁴ is a product of GE (eq 1) under the conditions used in our experiment.^{23,24,31,33,45} Indeed, AgCl is a known byproduct of this GE reaction, and can lead to inefficient GE.^{24,31-33,41}

The results shown in Figure 4 suggest that AgCl encapsulates a significant fraction of the $M\mu$ B–AgNP conjugates. This most likely reduces access of $AuCl_4^-$ to the underlying AgNPs and hence reduces the GE efficiency.^{23,24,32,33,41,45} Importantly, and as shown in Figure 2, the mass of AgCl present in Figure 4 (orange box in Figure 4a) is not visible after a complete GE cycle (all three steps shown in Scheme 1). Apparently, some or all of the AgCl is in direct contact with the electrode surface and is reduced to Ag^0 during the second half of the GE cycle (Ag electrodeposition).^{1,2}

To summarize, there are two factors that prevent complete oxidation of the AgNPs: first, the presence of a Au shell that prevents access of $AuCl_4^-$ to underlying Ag (Figure 3); second, precipitated AgCl, which also blocks access of $AuCl_4^-$ to some AgNPs.

GE of Remaining AgNPs.

As just discussed, AgCl is a byproduct of the GE reaction between $AuCl_4^-$ and Ag^0 , when it is carried out in Cl^- -containing media, that prevents oxidation of about half the AgNPs immobilized on the $M\mu$ Bs. For sensing purposes, we wish to oxidize all the AgNP labels, and in this section, we show that a second GE cycle results in at least partial GE of all the AgNPs present. For these experiments, the potential of the working electrode was stepped from 0 to 0.60 V for 25.0 s and then to -0.70 V for 100.0 s. This program was then repeated. At this point the electrode was removed from the PLA cell at open-circuit potential, dried, and analyzed by SEM (Figure 5).

The micrograph in Figure 5a shows four $M\mu$ B–AgNP conjugates present on a carbon/Au working electrode after two GE cycles. The expanded views in Figure 5b,c correspond to the red and blue boxes in Figure 5a, respectively. As discussed previously, the distorted surface morphology of these NPs indicates that they have undergone GE. A total of 547 NPs were

examined following two GE cycles, and all of them resembled those in Figure 5b,c. Accordingly, we conclude that two potential cycles result in GE (or, more precisely, at least partial GE) of essentially all the AgNPs present on the electrode surface.

Electrochemical Detection of AgNPs after GE Cycles.

Thus far we have shown that 52% and ~100% of AgNPs exhibit morphological changes after one and two GE cycles, respectively. We correlate these changes to full or partial GE between AuCl_4^- and Ag^0 . In this section, we compare these findings to quantitative electrochemical results obtained by Ag anodic stripping voltammetry.

These experiments were carried out exactly as described in the previous sections for one, two, and three GE cycles, except rather than halting the experiment after Ag electrodeposition the potential was swept twice from -0.70 to 0.20 V at 50 mV/s to oxidize Ag after the final GE cycle. The charge under the second voltammogram for each experiment was then determined by integration.^{1-4,10}

Figure 6 presents histograms of the charge recovered for 12 independently prepared electrodes for the indicated number of GE cycles. The average Ag charge after one GE cycle was 0.5 ± 0.6 μC . After two and three GE cycles, the charge more than doubled to 1.3 ± 0.3 μC and 1.3 ± 0.4 μC . On the basis of these results, we conclude that only two GE cycles are required to capture all of the accessible Ag charge.

It is now possible to correlate the results obtained by microscopy to the electrochemical data in Figure 6. Recall that the microscopy data indicated that the number of AgNPs exhibiting morphological changes doubled after a second GE cycle. Now we find that the electrochemical data in Figure 6 are in near-quantitative accord with this finding.

On the basis of the AgNP concentration used in these experiments, detection of 100% of the Ag present in the AgNPs would correspond to 26.7 μC . However, the experimental results indicate that just 5% of this charge is recovered after two GE cycles. This low collection efficiency must, therefore, be attributed to the Au shell (Figure 3) that forms during the initial stages of GE. A rough calculation based on data like those shown in Figure 3 suggests that ~85% of the total Ag present in the system is within the core of the Ag@Au NPs that form during GE. The remainder of uncollected Ag (~10%) is likely present as AgCl that is not in direct contact with the electrode surface.

Electrochemical Detection of Different AgNP Concentrations.

Up to this point, we have only reported results for a single, average number density of AgNPs on the M μ B–AgNP conjugates. Because we wish to use AgNPs as labels in bioassays, we now turn our attention to correlating the electrochemical charge recovery to the concentration of AgNPs used to prepare the conjugates. This provides a good model for understanding the dynamic range of this detection method. These experiments were carried out as described for the two-cycle histogram shown in Figure 6, except for one minor change: Ag oxidation voltammograms were obtained only after both GE cycles were complete, whereas voltammograms were obtained after each of the two cycles in Figure 6.

The voltammograms in Figure 7a indicate that the Ag stripping current increases as a function of the concentration of the AgNPs. The concentrations listed in the legend of Figure 7a represent the moles of AgNPs dried at the working electrode and rehydrated in 150 μ L of BCl. The voltammograms for the two lowest concentrations are expanded in Figure 7b. Note that concentrations below 2.6 fM could not be differentiated from the baseline. The charges under voltammograms like that shown in Figure 7a were obtained by integration, and the results for five independent experiments per concentration are plotted in Figure 7c. These data show that the charge increases linearly from 2.6 to 337.0 fM AgNPs, and then it begins to saturate. The lower end of the linear range is shown on an expanded scale in Figure 7d. The point is that, by understanding and optimizing this detection system, it has been possible to obtain a very low detection limit and a respectable linear range (2 orders of magnitude).

■ SUMMARY AND CONCLUSIONS

To summarize, we have investigated the seemingly simple process of GE between AuCl_4^- and AgNPs conjugated to M μ Bs in Cl^- -containing buffer. The study was carried out to develop a better understanding of (and hence to improve) an electrochemical detection modality that we recently reported.^{1,2} The simplistic view of the detection system is summarized in Scheme 1, but after analysis of both SEM and TEM micrographs, we found that the reality of the situation is quite a bit more complicated. For example, each AgNP label undergoes only partial GE due to formation of a $\sim$ 10 nm Au shell that shields the remaining Ag that ends up in the core of the NP. The formation of this core@shell structure is a consequence of the rather large AgNPs (110 nm) that were used in this study (to facilitate structure determination). Because the Au shell is 10 nm thick, however, it is likely that 20 nm AgNPs will undergo complete GE. Therefore, in forthcoming biosensing applications, we plan to use AgNP labels in the 20 nm size range.

We also discovered that only about half the AgNPs undergo any detectable level of GE after one cycle, but that two GE cycles results in partial GE of essentially all AgNPs originally present. This, we believe, is a consequence of precipitation of AgCl, which prevents cross-reaction between AuCl_4^- and AgNPs.

Finally, we established that this basic GE-based detection of AgNPs is both quantitative and sensitive. The relevant metrics for biosensing detections are a 2.6 fM AgNP detection limit, and a linear range that spans 2 orders of magnitude.

We are presently working on a metalloimmunoassay using this detection strategy and a paper fluidic platform.^{5,7,8} The results of those experiments will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. SEM micrograph of the $M\mu B$ -AgNP conjugate (a) on a carbon-coated Ni grid and (b) dried onto a carbon/Au working electrode prior to electrochemical activation.

Figure 2. SEM micrographs of MμB-AgNP conjugates imaged on the carbon/Au working electrode after one GE cycle. (a) Low-resolution view. (b, c) Expanded views of the regions highlighted by the red and blue boxes, respectively, in part a.

Figure 3.

(a) TEM micrograph of a portion of a MμB-AgNP conjugate after one GE cycle. The white arc approximates the edge of the MμB; the red circle highlights one associated NP, and the white circle highlights a pair of closely spaced NPs. (b) EDX element map of the NPs in the white circle in part a. It reveals a Ag core (red) surrounded by a Au shell (green).

Figure 4.

(a) SEM micrograph of $M\mu B$ -AgNP conjugates on the carbon/Au working electrode after oxidation of Au. The orange rectangle highlights salt around the $M\mu B$. (b) Element map of the $M\mu B$ -AgNP conjugates in part a. The blue and green arrows point to the same $M\mu B$ and Au nodule, respectively, in part a.

Figure 5. SEM micrographs of M μ B-AgNP conjugates after two GE cycles imaged on the carbon/Au working electrode. (a) Low-resolution view. (b, c) Expanded views of the regions highlighted by the red and blue boxes, respectively, in part a.

Figure 6.

Histogram of the average Ag charge measured at 12 different working electrodes after one, two, and three GE cycles. The $M\mu B$ -AgNP conjugates were dried on the carbon/Au working electrode and rehydrated in 150 μL of BCl to give a final AgNP concentration of 45 fM.

Figure 7.

(a) Anodic stripping voltammograms (ASVs) obtained by drying the $\text{M}\mu\text{B}$ -AgNP conjugates onto the carbon/Au working electrode, rehydrating in 150 μL of BCl , and then carrying out the ASV protocol described in the text. The concentrations listed in the legend represent the total concentration of AgNPs in the 150 μL volume. The scan rate was 50 mV/s. (b) Expanded view of the ASVs for the lowest two AgNP concentrations in part a. (c) Plot of charge, determined by integrating the ASVs in part a, as a function of AgNP concentration. Each data point represents five replicate measurements obtained using independently prepared electrodes. Outliers were eliminated using the Grubb's test with a 95% confidence level.⁴⁴ (d) Expanded view of the linear range in part c. The lowest detectable concentration is 2.6 fM AgNPs. The data were obtained using two GE cycles.

Scheme 1.