Accelerated Articles

An Electrochemical Metalloimmunoassay Based on a Colloidal Gold Label

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A novel, sensitive electrochemical immunoassay has been developed using a colloidal gold label that, after oxidative gold metal dissolution in an acidic solution, was indirectly determined by anodic stripping voltammetry (ASV) at a single-use carbon-based screen-printed electrode (SPE). The use of disposable electrodes allows for simplified measurement in 35 μL of solution. The method was evaluated for a noncompetitive heterogeneous immunoassay of an immunoglobulin G (IgG) and a concentration as low as 3×10^{-12} M was determined, which is competitive with colorimetric ELISA or with immunoassays based on fluorescent europium chelate labels. The high performance of the method is related to the sensitive ASV determination of gold(III) at a SPE (detection limit of 5×10^{-9} M) and to the release of a large number of gold(III) ions from each gold particle anchored on the immunocomplex (e.g., 1.7×10^5 gold atoms are theoretically contained in a 18-nm spherical gold particle).

Methods that enable the rapid, selective, sensitive, accurate, and inexpensive detection of substances in a complex sample matrix are important tools in many analytical areas. Ligand-binding assays such as immunoassays exemplify such methods and are currently used to measure low concentrations of substances in clinical samples of biological fluids such as urine and blood and to detect trace amounts of drugs and chemicals such as pesticides in a sample. Immunoassays are based on the use of an antibody that reacts specifically with the substance (antigen) to be tested, and quantification is generally achieved by measuring the specific activity of a label, i.e., its radioactivity, enzyme activity, fluorescence, chemiluminescence, or bioluminescence. There is no ideal label, and each of them has its own advantages and disadvantages.

Metalloimmunoassays, i.e., immunoassays involving metal-based labels, were developed in the 1970s[1] in order to overcome problems associated with the common radioisotopic, fluorescent, or enzyme labels. Metal-based labels can be classified according to their chemical nature, i.e., colloidal metal particles,[2−9] metal ions,[10−14] organometallics,[15−18] coordination complexes,[19−21] or metalloproteins.[22] Many analytical methods are suitable for their quantitative determination, i.e., atomic[2,15] or colorimetric[2,5] absorption spectrophotometry, photothermal deflection spectroscopy,[3] acoustic plate mode sensor,[5] surface plasmon resonance,[4] scanning force microscopy,[7] infrared[16] or Raman[9] spectroscopy, time-resolved fluorescence,[19,20] and electrochemical techniques such as polarography[12] or voltammetry.[13,17,18] Metalloimmunoassays with

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electrochemical detection can offer several advantages; e.g., measurement can be performed in very low volumes of liquid (a few microliters), eventually in turbid media, with the possibility of having good sensitivity for a relatively inexpensive instrumentation (field-portable). Although the electrochemical techniques allow organometallic compounds[18,23] or metal ions[12,13] to be detected at nanomolar concentrations, their sensitivities remain insufficient compared with fluorescent europium chelate labels for which picomolar levels can be determined.[19]

In this work, it is shown that picomolar concentrations can be reached in a new electrochemical metalloimmunoassay involving the use of a colloidal gold label. The feasibility of the approach was investigated for a noncompetitive heterogeneous immunoassay of goat immunoglobulin G (IgG) as a model. The use of metallic colloidal particles as labels was not new, in particular the use of antibody-coated gold sols for cytochemistry studies,[24] in which the immunogold particles served as a contrasting agent in transmission electron microscopy for the visualization of the distribution of an antigen over a cell surface. The application of metallic colloidal particles as a label in ligand-binding assays is less common,[2–4,7,8] and it was first developed for an immunoassay with a colloidal gold or silver marker quantified by atomic or colorimetric absorption spectrophotometry.[2] However, no electrochemical technique has been involved so far for the determination of a colloidal gold label in a ligand-binding assay format. It is worth noting that the direct determination of colloidal gold-labeled IgG adsorbed on the surface of a carbon paste electrode was investigated in 1995 by Gonzalez-Garcia and Costa-Garcia using adsorptive stripping voltammetry.[25] The application to an immunoassay was envisaged by the authors; however, it has not yet been demonstrated.

The electrochemical approach developed in the present study is not based on the direct detection of the colloidal gold label but on its indirect detection, by determining the amount of ionic gold-(III) released in solution after oxidative treatment in acidic solution. For this purpose, we have employed disposable carbon-based screen-printed electrodes (SPEs) and the highly sensitive anodic stripping voltammetric technique (ASV). The use of disposable SPEs allows for simplified operation in microliter solutions and greatly reduced costs.

EXPERIMENTAL SECTION

Instrumentation and Electrodes. An Autolab potentiostat (EcoChemie) interfaced to a PC system with GPSE version 4.4 software was used for cyclic voltammetry (CV) and ASV. SPEs (eight per array; 9.6 mm² for the sensing disk area) were prepared with a manual screen printer (Circuit Imprimé Français, Bagneux, France) in association with a screen stencil of 77 threads cm^{-1} and using a homemade carbon-based ink composed of graphite particles (Ultra Carbon, UCP 1M, Johnson Matthey) and polystyrene.[26] A saturated calomel electrode (SCE) was employed as a reference in the preliminary results, and then a saturated Ag/AgBr reference electrode lengthened with a tube filled with a saturated NaBr solution was used. The counter electrode was a platinum wire.

Chemicals and Reagents. Goat IgG, donkey anti-goat IgG (Affinipure, H + L), donkey anti-goat IgG (H + L) conjugated colloidal gold (average diameter of 18 nm), streptavidin adsorbed on colloidal gold particles of 5 (S−Au$_5$) or 20 nm (S−Au$_{20}$), streptavidin conjugated with albumin labeled by 10-nm colloidal gold (S−A−Au$_{10}$), ovalbumin (OA), bovine serum albumin (BSA, fraction V), biotinylated BSA (B-BSA, 8−12 mol of biotin/mol of BSA), and Tween 20 were obtained from Sigma Chemical Co. Chloroauric acid (HAuCl$_4$), trisodium citrate, and concentrated bromine (CAUTION: bromine is a toxic and harmful reagent) were purchased from Aldrich Chemical Co. Sodium bromide, hydrochloric acid (30%), and hydrobromic acid (47%) were supplied by Merck as Suprapure grade reagents. Although commercialized as a Suprapure grade acid, the hydrobromic acid contained a significant amount of bromine (titration at a rotated glassy carbon disk electrode indicated a concentration of ∼5 mM bromine). To accurately control the bromine concentration in the solution used for gold dissolution, bromine was eliminated from the commercialized HBr by cathodic reduction of Br$_2$ to Br$^-$ at 0.0 V vs SCE using a graphite electrode (preparative scale). All of the solutions were prepared with Milli-Q 18-MΩ water (Millipore purification system).

Buffers. The following buffers were used in this study: (a) phosphate buffer (PB; 4.3 mM NaH$_2$PO$_4$, 15.1 mM Na$_2$HPO$_4$, and 50 mM NaCl, pH 7.4); (b) PB containing 0.5% ovalbumin and 0.1% Tween 20 (PBOAT); (c) PB containing 0.1% Tween 20 (PBT); (d) PB containing 0.1% BSA (PBBSA), (e) PBBSA containing 0.05% Tween 20 (PBBSAT).

Solutions. Goat IgG standard solutions were diluted from a stock solution (1.2 mg mL^{-1}) with PBT. Primary donkey anti-goat IgG (24 μg mL^{-1}) was prepared by dilution of a stock solution (1 mg mL^{-1}) with PB. The donkey anti-goat IgG colloidal gold conjugate solution was prepared as a 1:45 dilution of the stock solution with PBOAT. The solution of B-BSA (10 μg L^{-1}) was prepared in 15 mM Na$_2$CO$_3$ buffer (pH 9.6), and solutions of colloidal gold-labeled streptavidin were prepared by dilution of the stock solution in PBBSAT.

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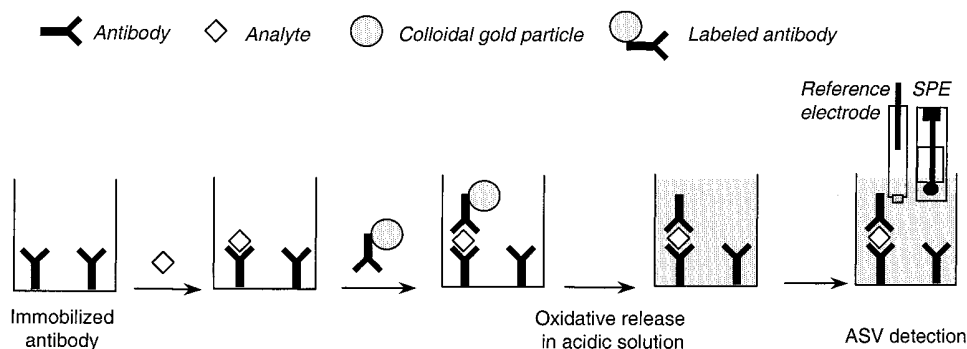(26) Bagel, O.; Limoges, B.; Schöllhorn, B.; Degrand, C. *Anal. Chem.* **1997**, *69*, 4688.

Figure 1. Schematic representation of the noncompetitive heterogeneous electrochemical immunoassay based on a colloidal gold label.

Au Colloid Preparation. Colloidal gold particles of average diameter 18 nm ± 5 nm were prepared according to Natan and Coll[27] with slight modifications. Briefly, a volume of 250 mL of 0.01% HAuCl$_4$ in pure water (Milli-Q water) was brought to a boil with vigorous stirring. To this solution was added 3.75 mL of 1% sodium citrate, which resulted in a change of color from pale yellow to deep purple within 1−2 min. Boiling was pursued for 10 min, and the solution was stirred for additional 15 min after the heating source was removed. The solution was allowed to cool to room temperature, and it was subsequently filtered through a Cyclopore membrane (0.4 μm polycarbonate filter, Whatman).

The complete reduction of chloroauric acid was previously assumed to take place during the reaction,[27b] which was verified as follows. A fraction of the resulting solution of colloidal particles was acidified with HCl (pH 4−5) and centrifuged (4000 rpm for 30 min), and the supernatant (10-fold diluted in a 0.1 M HCl−NaBr solution) was analyzed by ASV. The absence of anodic stripping current demonstrated that Au^{III} was entirely reduced in gold metal during the colloid synthesis.

Streptavidin Assay Procedure. All of the experiments were performed at room temperature. The solution of B-BSA (100 μL) was incubated in polystyrene microwells (Maxisorp, Nunc, France) for 2 h, and the microwells were washed with PB (110 μL) and incubated with PBBSA (100 μL) for 2 h. After the solution was removed, the wells were rinsed with pure water (3 × 110 μL), and colloidal gold-labeled-streptavidin standard solutions (35 μL, x μg mL^{-1} with $0.003 < x < 3$) were added to the wells and incubated for 3 h. Once drained, the microwells were washed with PBBSAT (3 × 110 μL) and with PB (2 × 110 μL). Then, 40 μL of acidic bromine−bromide solution (1 M HBr containing 10^{-4} M Br$_2$) was pipetted into the microwells, and after 5 min, 35 μL of the solution was transferred onto the SPE surface and mixed with 5 μL of a fresh solution of 3-phenoxypropionic acid solution (4 × 10^{-3} M in 1 M HBr). The released gold(III) ions were then quantified by ASV under the following instrumental conditions: 5-min deposition at −0.3 V vs saturated Ag/AgBr reference and positive potential scan at 50 mV s^{-1}. A new SPE was used for each measurement.

Immunoassay Procedure. All of the experiments were performed at room temperature. Primary donkey anti-goat IgG (60 μL) was incubated in polystyrene microwells (Maxisorp) for 1 h. After removing the solution, the wells were rinsed with PBOAT (100 μL) two times for 5 min each and an additional time for 1 h, and goat IgG standard solutions (35 μL) were added and incubated in the wells for 40 min. Next, the microwells were drained and rinsed with PBOAT (100 μL) two times for 5 min each and an additional time for 30 min. Following this step, 35 μL of colloidal gold-labeled donkey anti-goat IgG was added and the resultant mixture incubated for 3 h. A last washing cycle was then performed with PBOAT (3 × 200 μL for 5 min each), followed by PB (2 × 200 μL for 5 min each). After carefully removing the rinsing solution, 40 μL of acidic bromine−bromide solution (1 M HBr containing 10^{-4} M Br$_2$) was pipetted into the microwells, and after 5 min, 35 μL of the solution was transferred onto the SPE surface and mixed with 5 μL of a fresh solution of 3-phenoxypropionic acid solution (4 × 10^{-3} M in 1 M HBr). The released gold(III) ions were then quantified by ASV under the same instrumental conditions as in the case of the streptavidin assay procedure.

RESULTS AND DISCUSSION

The principle of the noncompetitive heterogeneous electrochemical immunoassay with a colloidal gold label is depicted in Figure 1, and it was applied to a goat IgG analyte. Primary antibodies specific for goat IgG are adsorbed passively on the walls of a polystyrene microwell. The goat IgG analyte is first captured by the primary antibody and then sandwiched by a secondary colloidal gold-labeled antibody. After removal of the unbound labeled antibody, the gold metal contained in the bound phase is dissolved in an acidic oxidative solution and the gold ions (Au^{III}) thus released in solution are quantitatively determined at a SPE by ASV. The electrochemical signal is directly proportional to the amount of analyte (goat IgG) in the standard or sample.

Determination of Gold(III) at a SPE. ASV has proved to be a very sensitive method for trace determination of metal ions.[28] In this analytical technique, the metal is cathodically electrodeposited onto the surface of an electrode during a preconcentration period (t_{acc}), and it is then stripped from the electrode by anodic oxidation. Gold(III) concentrations as low as 5 nM could be determined by ASV at a carbon paste electrode.[29] Nevertheless, the carbon paste electrode is mechanically unstable and it thus requires special care for resurfacing, which is often a source of irreproducibility. Clearly, its handling is not very convenient for routine application. Disposable SPEs are more attractive elec-

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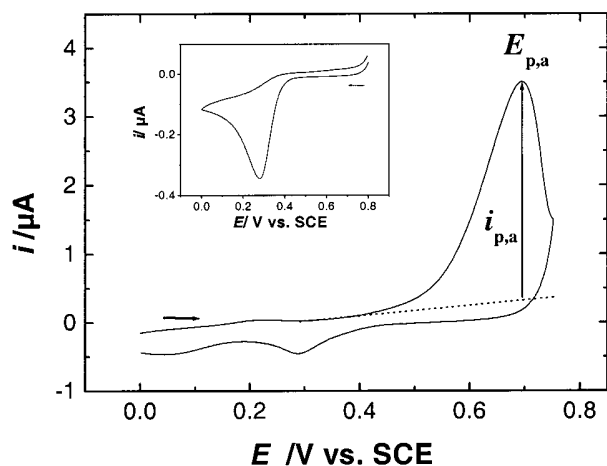(29) Jacobs, E. S. *Anal. Chem.* **1963**, *35*, 2112−5.

Figure 2. CV curve ($v = 50$ mV s^{-1}) recorded at a SPE immersed in 5 mL of 0.1 M HCl–NaBr containing 1 μM Au^{III} after electrodeposition at -0.8 V vs SCE during 2 min under magnetic stirring. Inset: CV curve (50 mV s^{-1}) at a SPE immersed in a solution of 10 μM Au^{III} in 0.1 M HCl–NaBr.

Figure 3. Calibration plots of Au^{III} recorded by ASV at a SPE. (A) Electrodeposition at -0.8 V vs SCE for 2 min under magnetic stirring in 5 mL of 0.1 M HCl–NaBr. (B) Electrodeposition at -0.3 V vs Ag/AgBr for 5 min in a 35-μL droplet of 0.1 M HCl–NaBr solution deposited on the SPE surface. Error bars represent the standard deviation of two measurements.

trodes, since they can be easily and reproducibly mass produced, and they are well adapted for the construction of a multichannel electrochemical microtiter plate.[30,31] Moreover, as in the case of carbon paste electrodes, SPEs have the advantage of displaying a very low double-layer charging current, thus leading to low detection limits in LSV or CV.[26] Since SPEs have never been the subject of gold determination by ASV, an exploratory work was necessary to examine the analytical performance of the SPE for the detection of Au^{III}. The study was carried out in a 0.1 M hydrochloric acid solution containing 0.1 M NaBr (0.1 M HCl–NaBr), since bromide is required for the efficient oxidative dissolution of gold in the final step of the metalloimmunoassay (vide infra). Under these conditions, the CV curve of a 10^{-5} M gold(III) solution (inset in Figure 2) shows that the cathodic reduction of Au^{III} takes place at 0.3 V vs SCE at a SPE. No oxidation peak was observed during the reversed anodic scan, reversal of the potential being set at 0.0 V, which suggests that a more negative potential is required for metal gold electrodeposition. Indeed, the ASV curve recorded at a SPE after cathodic polarization at -0.8 V for 2 min in a magnetically stirred solution containing 1 μM Au^{III} shows a well-defined anodic peak at 0.7 V (peak potential $E_{p,a}$) which is characteristic of the oxidation of electrodeposited gold. During the scan reversal, a small cathodic peak located at 0.3 V is visible and it probably corresponds to the reduction of Au^{III} ions anodically released and still present in the diffusion layer. A similar behavior was previously observed at a carbon fiber microelectrode, insofar as the electrode was electrochemically pretreated using a complex procedure.[32] Indeed, it was previously shown that electrochemical pretreatment of a glassy carbon electrode[33] or a carbon fiber microelectrode[32] was crucial for detecting trace amounts of gold. However, such a pretreatment is tedious and time-consuming and usually it increases the charging current. In contrast, the detection of gold at a SPE did not require any electrochemical pretreatment.

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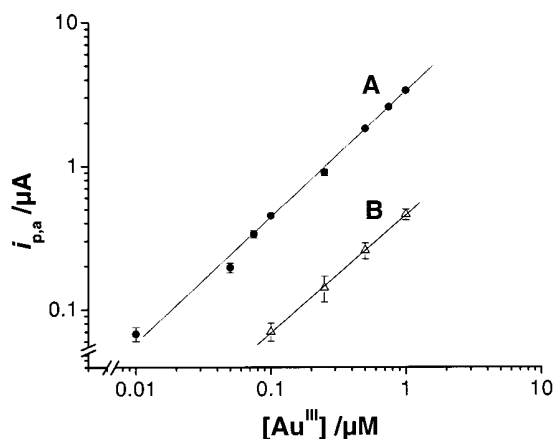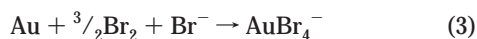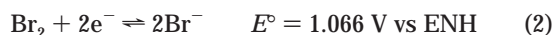
Several parameters were investigated in order to establish optimal conditions for the detection of Au^{III} in 5 mL of solution. The influence of the electrodeposition potential (E_d) was tested, and as E_d was decreased from -0.2 to -0.8 V, the anodic peak current $i_{p,a}$ was increased until it reached a constant value at more negative potentials (see Supporting Information). These results are consistent with those previously reported at a glassy carbon electrode[33] or a carbon fiber microelectrode.[32] A deposition potential of -0.8 V vs SCE was selected for the further studies, and a good linear dependence of $i_{p,a}$ with the gold(III) concentration over the 10^{-8}–10^{-6} M range was thus obtained (Figure 3A). It is to be noted that the same SPE was used to obtain all of the data plotted in Figure 3A, which could be achieved by preconditioning the electrode at 0.8 V for 60 s between each measurement. The error bars, which correspond to the standard deviation (SD) of two measurements, indicate the good reproducibility of the experiments (relative standard deviation, $2 < \text{RSD} < 16\%$). The detection limit calculated from 3 SD of the background noise was 5×10^{-9} M ($t_{acc} = 2$ min). Taking into account the short accumulation period, this value is competitive with the lowest detection limits previously obtained by ASV at a glassy carbon electrode (5×10^{-9} M, $t_{acc} = 5$ min),[34] an aza-crown ether-modified glassy carbon electrode (4.2×10^{-10} M, $t_{acc} = 20$ min),[33] a carbon paste electrode (5×10^{-9} M, $t_{acc} = 15$ min),[29] or a carbon fiber microelectrode (5.9×10^{-9} M, $t_{acc} = 10$ min).[32] Concerning the influence of the electrodeposition time, a linear relationship was observed between the $i_{p,a}$ value and the deposition time ranging from 2 to 20 min. An electrodeposition time of 5 min was chosen for all of the experiments, since longer accumulation time would increase the analysis time and consequently decrease sample throughput.
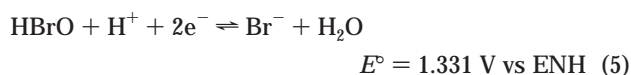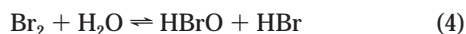
Gold Dissolution. To detect the colloidal gold label by ASV in the final step of the immunoassay, the gold metal must be released from the immobilized bound fraction by oxidation to the soluble ionic Au^{III} form. This was achieved with the use of an acidic

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bromine−bromide solution, a mixture that was proved to be more efficient than the traditional cyanide reagent for the extraction of gold from ores.[35] The bromine reagent transforms Au into Au^{III}, whereas the bromide anion assists the gold dissolution in coordinating the generated Au^{III} with formation of a strong complex. The gold dissolution in a bromine solution is an electrochemical process described by reactions 1−3:[35]

$$Au + 4Br^- \rightleftharpoons AuBr_4^- + 3e^- \qquad E° = 0.854 \text{ V vs ENH} \quad (1)$$

$$Br_2 + 2e^- \rightleftharpoons 2Br^- \qquad E° = 1.066 \text{ V vs ENH} \quad (2)$$

$$Au + {}^3/_2Br_2 + Br^- \rightarrow AuBr_4^- \qquad (3)$$

Moreover, Br$_2$ can be hydrolyzed in dilute solution into bromhydric and hypobromic acids (eq 4), and consequently hypobromic acid can also be involved as a strong oxidative agent (eq 5).

$$Br_2 + H_2O \rightleftharpoons HBrO + HBr \qquad (4)$$

$$HBrO + H^+ + 2e^- \rightleftharpoons Br^- + H_2O$$
$$E° = 1.331 \text{ V vs ENH} \quad (5)$$

Clearly, the dissolution of gold in bromine solution can be affected by several factors including pH and bromine and gold concentrations. On the basis of the Pourbaix diagrams given in ref 35, a bromide concentration of >0.1 M was required to favor the formation of the stable AuBr$_4^-$ complex and to facilitate thermodynamically the redox reaction 3.

Colloidal gold particles of average diameter 18 ± 5 nm were prepared from a 0.28 mM HAuCl$_4$ solution and sodium citrate.[27] A 0.1 M HCl−NaBr solution containing 0.25 mM Br$_2$ was initially adopted to investigate whether the bromine−bromide solution was efficient to dissolve the colloidal gold particles. Addition of 1 mL of the purple colloidal gold solution into 4 mL of the bromine−bromide solution resulted in an instantaneous change of color from purple to pale yellow, confirming that the gold dissolution was effective and fast under these conditions. After waiting for 20 min to make sure that the gold dissolution was complete, the mixture was centrifuged, and then the supernatant was 60-fold diluted with the 0.1 M HCl−NaBr solution and analyzed by ASV. The resulting anodic peak current in Figure 4A shows that the colloid was dissolved and a gold(III) concentration of 0.7 μM could be evaluated from the calibration curve of Figure 3A. Taking into account the initial concentration of Au^{III} in the mother colloidal gold solution and the two successive dilutions (5-fold, and then 60-fold dilution), this corresponds to a 75% recovery. A 100% recovery was not achieved because the gold sol solution was filtered after colloid synthesis, and so a nonnegligible amount of large gold particles and aggregates were retained on the filter. The same experiment repeated in the absence of bromine did not provide any anodic stripping peak current (Figure 4B), thus confirming that Br$_2$ was required for gold dissolution in the 0.1 M HCl−NaBr medium. A centrifugation step was required to differentiate residual gold particles in suspension from the soluble
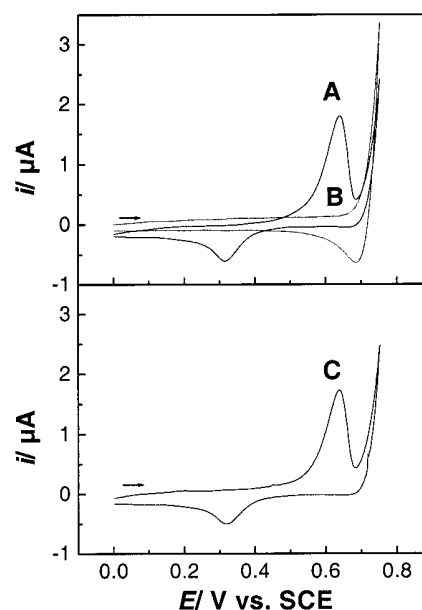
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Figure 4. CV curves (v = 50 mV s^{-1}) at a SPE immersed in the supernatant of solutions obtained from 1 mL of the mother colloidal gold solution (0.28 mM Au) pretreated with 4 mL of 0.1 M HCl−NaBr solution containing (A) 0.25 mM Br$_2$ or (B, C) no Br$_2$, followed by centrifugation (30 min at 4000 rpm) and 60-fold dilution in 0.1 M HCl−NaBr. The centrifugation step was avoided for curve C. t_{acc} = 2 min at −0.8 V vs SCE.

Au^{III} complex, because colloidal gold particles can be adsorbed on the electrode and directly detected by anodic oxidation (Figure 4C), in agreement with a previous work.[25] The cathodic signal appearing at the beginning of the reversed scan (∼0.7 V) in Figure 4B is probably due to the reduction of Br$_2$ anodically generated from Br$^-$ at the end of the forward scan (discharge part of the curve).

During the course of this work, it was observed that the $E_{p,a}$ value of the anodic stripping peak current of gold was dependent upon the Cl$^-$ content. Indeed, it was shifted positively with increasing amounts of Cl$^-$, owing to the difference of stability between the AuBr$_4^-$ and AuCl$_4^-$ complexes. To avoid irreproducible results due to the continuous leaching of chloride anion from the SCE, the reference electrode was replaced with a NaBr-saturated Ag/AgBr reference electrode and the experiments were performed in a 1 M HBr solution instead of the initial 0.1 M HCl−NaBr solution. With this new reference electrode, the anodic peak potential of gold was located at 1.0 V and the deposition potential was fixed at −0.3 V.

The stoichiometry of eq 3 indicates that 1.5 equiv of bromine is required to oxidize the gold metal. However, a large excess of Br$_2$ has to be employed in the context of an immunoassay, because the amount of colloidal gold label to be dissolved is unknown, and moreover, some bromine can also be consumed by oxidizing substances contained in proteins (e.g., tyrosine). A concentration of 10^{-4} M Br$_2$ in 1 M HBr was selected in this study, which unfortunately led to a cathodic baseline current (∼−0.6 μA at 50 mV s^{-1}) owing to the reduction of Br$_2$, and consequently interfered in the detection of trace amounts of gold (see Supporting Information). Furthermore, the presence of bromine should decrease the efficiency of the electrodeposition of gold through its reoxidation. Therefore, the excess of bromine was suppressed
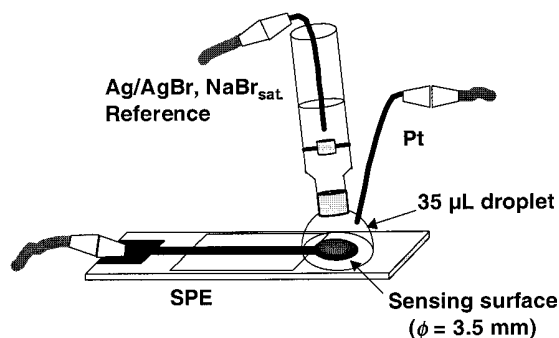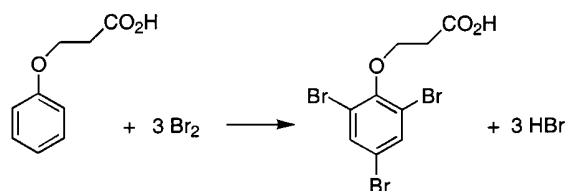
Figure 5. Schematic representation of the electrode assembly for electrochemical measurement in a 35-μL droplet.

after oxidative treatment and before ASV recording by addition of a soluble bromine-trapping reagent, i.e., 3-phenoxypropionic acid, which, as many phenol derivatives, has the property to react spontaneously with bromine.

The interfering reduction of bromine was completely suppressed by the addition of 5×10^{-4} M 3-phenoxypropionic acid (see Supporting Information).

Reduction of the Assay Volume. All of the experiments previously described were carried out in 5 mL of solution. However, immunoassays require working with much smaller volumes of liquid, because expensive reagents are used (antibodies, labels, etc.) and small quantities of samples are assayed (serum, blood, etc.). The assay volume was thus reduced to a 35-μL droplet directly deposited onto the SPE surface, as depicted in Figure 5. To avoid the nonnegligible contamination of the assay solution by silver ions leaking from the reference electrode, a small-sized saturated Ag/AgBr reference with a double glass-frit separation was built. Moreover, the SPEs were single-use to avoid fouling or cross-contamination between each experiment. Under these conditions, a new calibration curve of Au^{III} was plotted for an electrodeposition time of 5 min (Figure 3B). Although the deposition time was 2.5-fold longer than previously, the sensitivity was 8-fold lower (compare curves A and B in Figure 3). This drop of sensitivity was mainly ascribed to the decrease of the mass transport efficiency in the 35-μL quiescent solution, since it was evaluated that depletion of Au^{III} was not significant (less than 10%). A detection limit of 5×10^{-8} M Au^{III} was achieved and the reproducibility of the single-use SPEs remained good. The conditions corresponding to curve B in Figure 3 were adopted for the development of the immunoassay.

Detection of Colloidal Gold-Labeled Streptavidin in a Polystyrene Microwell Coated with Biotinylated Bovine Serum Albumin. The concept of the indirect electrochemical detection of a colloidal gold label was first explored with the high-affinity binding of streptavidin to biotin. A semisandwich assay

Table 1. Characteristics of the Three Commercially Available Streptavidin Colloidal Gold Conjugates Used in This Work

conjugate	av diam of gold particle, nm	no. of particles/mL	streptavidin concn, μg mL^{-1}	N^a
S$-$Au$_5$	5.2	2.2×10^{14}	27.5	~ 1
S$-$Au$_{20}$	19.2	3.1×10^{12}	100	~ 300
S$-$A$-$Au$_{10}$	8.5	4.0×10^{13}	47	~ 10

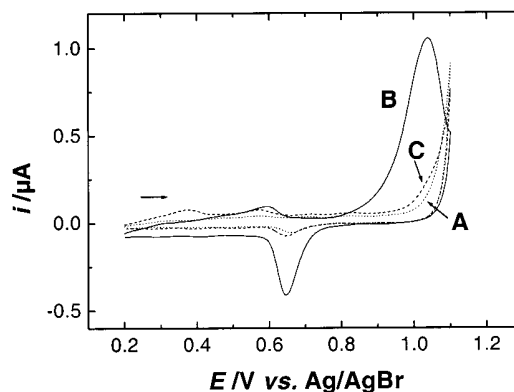^{a} Number of streptavidin units per gold particle.

Figure 6. ASV curves recorded at SPEs immersed in a microwell coated with (A, B) B-BSA or (C) BSA followed by incubation with the S$-$Au$_{20}$ conjugate and then by an acidic treatment in 1 M HBr containing (A) no Br$_2$ and (B, C) 10^{-4} M Br$_2$. $t_{acc} = 5$ min at -0.3 V vs Ag/AgBr.

based on the reaction of a colloidal gold-labeled streptavidin with a monolayer of biotinylated BSA adsorbed on the bottom of a polystyrene microwell was investigated for three different streptavidin colloidal gold conjugates (Table 1), i.e., streptavidin adsorbed on colloidal gold particles of two different diameters (S$-$Au$_5$ and S$-$Au$_{20}$) and streptavidin conjugated with albumin labeled by colloidal gold (S$-$A$-$Au$_{10}$). The streptavidin contained in the S$-$A$-$Au$_{10}$ complex is coupled through a spacer to albumin-coated colloidal gold for enhanced detection of biotinylated compounds.[36] Assuming spherical gold particles, it could be estimated that a particle of diameter 5, 10, and 20 nm contained 4×10^3, 3×10^4, and 2.5×10^5 gold atoms, respectively. A first set of experiments was performed with S$-$Au$_{20}$, and the results are shown in Figure 6 where voltammograms A and B were recorded in the absence and in the presence of Br$_2$, respectively. The absence of anodic stripping current for curve A indicates that the acidic medium is not sufficiently harsh to release the bound colloidal gold particles from the solid phase and to allow their direct detection at a SPE, as was shown in Figure 4C. In contrast, a well-defined anodic peak was recorded in the presence of 10^{-4} M Br$_2$ (curve B), which demonstrates that immobilized gold could be efficiently released by oxidation from the wall of the microwell and dissolved as Au^{III} ions. In addition, when biotinylated BSA was replaced with BSA, the low residual signal observed in Figure 6C indicates a low level of nonspecific adsorption of streptavidin colloidal gold conjugate.

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Table 2. Comparison of Immunoassay Methods Developed for IgG

label[a]	immunoassay format	analytical technique	analysis time	detcn limit, ng mL^{-1}	ref
Au	immunosensor/sandwich	surface-enhanced Raman scattering	4 h	30	9
Au	immunosensor/sandwich	surface plasmon resonance	na[b]	1	4
Au	microwells/sandwich	ASV at a SPE	5 h	0.5	c
Au	polystyrene beads/semisandwich	photothermal deflection spectroscopy	overnight	0.2	3a
AP	microwells/sandwich	voltammetry at interdigitated array electrodes	1 h 20 min	10	39
AP	immunosensor/competitive	amperometry at a sol−gel SPE	2 h 45 min	5	40
AP	microwells/sandwich	liquid chromatography with amperometric detection	3 h	0.05	41
AP	microwells/sandwich	time-resolved fluorescence	3 h	0.03	42
AP	microwells/sandwich	colorimetry	2 h 45 min	1	42
AP	immunosensor/competitive	alternating current voltammetry at a carbon paste electrode	75 min	165	43
GOx	immunosensor/sandwich	amperometry at a rotating disk electrode (electrocatalysis)	2 h 45	16	44
HRP	microwells/competitive	amperometric flow injection analysis	1 h 15	100	45
Urease	immunomagnetic beads/competitive	potentiometric flow injection analysis	30 min	1200	46
Bi−DTPA	beads/competitive	differential pulse stripping voltammetry at mercury electrode	2 h 25	600	11b
Eu−BCPDA	microwells/sandwich	time-resolved fluorescence	2 h 45	0.1	42

[a] Au, colloidal gold; AP, alkaline phosphatase; GOx, glucose oxidase; Bi−DTPA, bismuth chelate of diethylenetriaminepentaacetic acid; Eu−BCPDA, europium chelate of 4,7-bis(chlorosulfophenyl)-1,10-phenanthroline-2,9-dicarboxylic acid. [b] Not available. [c] This work.
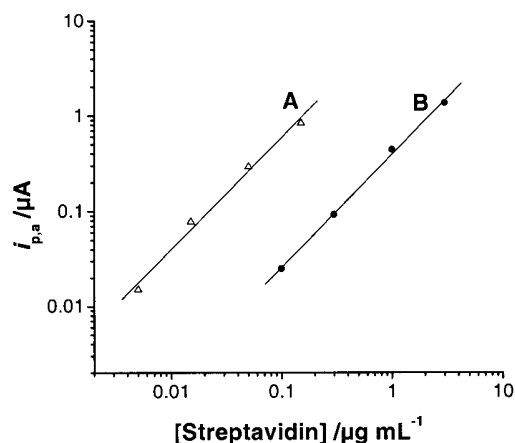
Figure 7. Log−log calibration plots for streptavidin contained in (A) S−A−Au$_{10}$ and (B) S−Au$_{20}$.
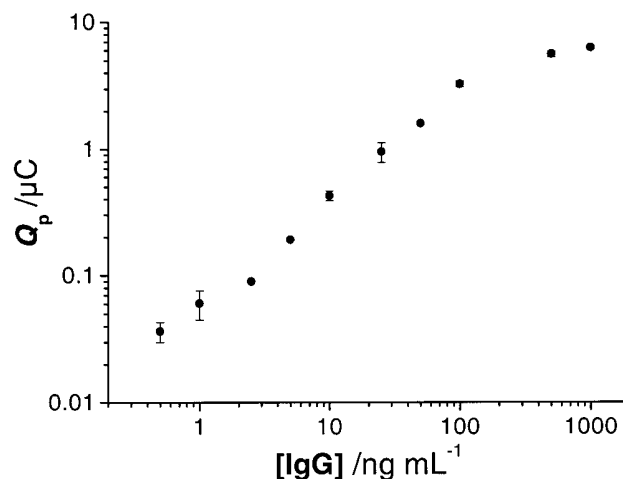

Figure 8. Log−log calibration plot of the integrated anodic stripping peak current vs goat IgG concentration. Error bars represent the standard deviation of two measurements obtained at two distinct SPEs.

Similar results were obtained for the S−A−Au$_{10}$ conjugate. In contrast, an important nonspecific adsorption was observed in the case of S−Au$_5$.

Figure 7 compares the calibration plots for streptavidin contained in the S−A−Au$_{10}$ (curve A) and S−Au$_{20}$ (curve B) conjugates. A better sensitivity was achieved with S−A−Au$_{10}$ than with S−Au$_{20}$, although the average diameter of the gold particle label was lower for the former. This can be tentatively explained by considering in Table 1 the data related to the number of streptavidin units available per gold particle (N). In the case of S−A−Au$_{10}$ for which it is assumed that each streptavidin contained in the conjugate is coupled to one adsorbed albumin, the value of $N = 10$ corresponds approximately to a packed monolayer of ellipsoidal albumin (4 nm × 4 nm × 14 nm)[37] adsorbed on each gold particle surface of spherical diameter 8.5 nm (area of 230 nm^2). In the case of S−Au$_{20}$, a packed monolayer of labeled

streptavidin involves 50 cubic units (5 nm × 5 nm × 5 nm)[38] on a 19.1-nm gold particle (area of 1150 nm^2). Since $N = 300$, a 5-fold excess of nonlabeled steptavidin is present in the gold particle solution, which competes with the labeled streptavidin for the biotin/streptavidin interaction.

Noncompetitive Heterogeneous Immunoassay of IgG. The metalloimmunoassay of goat IgG was performed as depicted in Figure 1 using donkey anti-goat IgG labeled by colloidal gold of average diameter of 18 nm, and the corresponding standard calibration plot is shown in Figure 8. The analytical response resulting from the integration of the stripping peak current (Q_p) was chosen because it was observed to be more reproducible than the $i_{p,a}$ response. The dynamic range for the assay extended between 0.5 and 100 ng mL^{-1}. The signal saturated above 100 ng

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mL^{-1} goat IgG, owing to the limited amount of antibody available on the surface of microwells. The detection limit was estimated to be 0.5 ng mL^{-1}, i.e., 3×10^{-12} M goat IgG (0.1 fmol in the 35-μL droplet), and it is competitive with or better than other immunoassay formats (see Table 2).

Metal labels were previously detected by anodic stripping voltammetry,[12,13] but in these systems, only one metal atom (or a number <10) was present in every tracer. In the case of colloidal gold label, each sol particle contains thousands of atoms (e.g., 1.7×10^5 gold atoms for a 18-nm gold particle), and consequently, picomolar detection limits can be attained considering the detection limit of Au^{III} in a 35-μL droplet (5×10^{-8} M). This explains the high sensitivity of the present electrochemical immunoassay compared with the previous electrochemical metalloimmunoassay of IgG with a bismuth chelate label (detection limit of 600 ng mL^{-1}).[12b]

CONCLUSION

It has been demonstrated that colloidal gold label when involved in an immunoassay can be sensitively detected by ASV at a disposable SPE after oxidative release of Au^{III} ions. In the case of IgG, the concentration range and the detection limit of the proposed method compare favorably with classical colorimetric ELISA or immunoassays based on fluorescent europium chelate label (Table 2). The approach can be readily extended to a large variety of bioaffinity assays of analytes of environmental or clinical significance.

Colloidal gold label possesses the advantage over radioisotopic or enzyme labels to be stable, and the gold sol labeling procedure is very simple and does not affect generally the biochemical activity of the labeled compound. Arrays of single-use SPEs can be easily built at low cost and they can be used with a simple and compact multichannel potentiostat for on-site multimeasurements. Several analytes may be detected simultaneously insofar as the determination of different colloidal metal labels with distinct anodic stripping potentials (e.g., gold and silver) is feasible. We now envisage simplifying the method by performing the entire protocol on the SPE surface (i.e., with the primary antibody directly immobilized on the electrode surface instead of on the wall of a polystyrene microwell) and improving the sensitivity by increasing the diameter of the colloidal particle and/or by using microelectrodes to favor the mass transfer during the deposition period.

SUPPORTING INFORMATION AVAILABLE

Plot of the influence of the electrodeposition potential on the anodic peak current and the ASV curves showing the interference of bromine on the gold detection and its elimination. This material is available free of charge via the Internet at http://pubs.acs.org.

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