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Anodic stripping voltammetry of gold nanoparticles at boron-doped diamond electrodes and its application in immunochromatographic strip tests

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ABSTRACT

Anodic stripping voltammetry (ASV) of colloidal gold-nanoparticles (AuNPs) was investigated at boron-doped diamond (BDD) electrodes in 50 mM $HClO_4$. A deposition time of 300 s at −0.2 V (vs. Ag/AgCl) was fixed as the condition for the ASV. The voltammograms showed oxidation peaks that could be attributed to the oxidation of gold. These oxidation peaks were then investigated for potential application in immunochromatographic strip tests for the selective and quantitative detection of melamine, in which AuNPs were used as the label for the antibody of melamine. Linear regression of the oxidation peak currents appeared in the concentration range from 0.05–0.6 μg/mL melamine standard, with an estimated LOD of 0.069 μg/mL and an average relative standard deviation of 8.0%. This indicated that the method could be considered as an alternative method for selective and quantitative immunochromatographic applications. The validity was examined by the measurements of melamine injected into milk samples, which showed good recovery percentages during the measurements.

1. Introduction

In recent years, metal nanoparticles have gained considerable attention as labels in analytical methods based on affinity reactions, such as immunosensors and immunoassays [1–5]. In particular, immunochromatographic strip tests, which combine chromatographic techniques with immunoreactions, offer wide applications in protein analysis and clinical diagnosis [5–11]. Among many types of metal nanoparticles, gold nanoparticles (AuNPs) have been the most extensively studied due to their unique optical, chemical, electrical, and catalytic properties[12]. In addition, AuNPs can provide good affinity for covalent bonds with proteins[12] . Based on these properties, the application of AuNPs for immunochromatographic strip tests has been reported [7–11]. Furthermore, although qualitative detections using immunochromatographic techniques offer selectivity, simplicity, and versatility [5–10], quantitative determination is also required [10–12]. Optical methods were generally incorporated to quantify the AuNP labels in immunochromatography [5–9]. However, the optical methods have limited applicability due to optical interference as well as the liquids that are used as solvents.

On the other hand, boron-doped diamond (BDD) electrodes are superior to other conventional solid electrodes due to their wide potential window, very low charging current, chemical inertness, mechanical durability, and good biocompatibility [13–15]. Electrochemical applications using BDD electrodes have been reported for many types of chemicals and biochemical sensors [13–20], including metals and heavy metals sensors [14,16–20]. Accordingly, anodic stripping voltammetry (ASV) is generally considered as the most suitable method for trace-level metal detections since the method offers several advantages for metal analysis, including high selectivity and sensitivity, low detection limits, simple operation, and economical cost [16–20]. Since ASV involves deposition and stripping processes at the working electrodes, the inert surfaces of BDD electrodes make a significant contribution in that they provide better stability in comparison to other solid electrodes [16–20]. Furthermore, ASV of AuNPs in immunochromatographic strip test applications has already been reported using carbon paste electrodes [10,11]. However, because the presence of proteins causes fouling of the electrodes, a disposable system was suggested not only for the strip tests but also for the working electrodes [10,11].

In this work, ASV of AuNPs at BDD electrodes was studied. $HClO_4$ was found to be the most suitable supporting electrolyte. It was also found that after an initial coating of gold had been

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deposited at the BDD surface, a new peak at around −0.1 V that is attributable to the formation of Au_2O_3 could be observed. As a model for further applications, the method was combined with immunochromatographic strip tests for the quantitative detection of melamine. Melamine is a synthetic compound that is used as an industrial chemical for the production of plastics, amino resins, and flame retardant materials [21–23]. However, it was recently found that melamine has been deliberately added to milk and to cheese [23,24]. Since the conventional detection method for proteins (Kjehldahl method) only determines the nitrogen content, the presence of melamine can create a false indication regarding the protein content. Various analytical methods have been developed for the detection of melamine, such as chromatography [8,23–25], capillary electrophoresis [11], and enzyme-linked immunosorbent assay (ELISA) [12], which require expensive instruments as well as highly skilled operators. Therefore, a rapid, sensitive, and inexpensive detection method for melamine is definitely necessary [7]. The melamine strip test was prepared with reference to the earlier reports, based on the complex reaction between melamine and the antibody of melamine (anti-melamine) [10,11,23,26]. AuNP was used for the label for anti-melamine and the detection of melamine was performed based on the assumption that the quantity of AuNPs involved in the system was equivalent to the concentrations of the antibody and of melamine [10,11]. The utilization of BDD for ASV measurements of AuNPs was demonstrated to provide a quantitative detection of melamine using immunochromatographic strip tests. The stability of BDD electrodes enabled a low limit of detection (LOD) and a good stability of the current responses to be achieved. Moreover, good percentage recovery of the measurements in milk sample matrices was shown, indicating that the method is promising for applications in immunochromatographic strip tests with metal nanoparticle labels.

2. Experimental

2.1. Chemicals and materials

Melamine, trisodium citrate, hydrogen tetracholoroaurate (III) tetrahydrate, perchloric acid, trimethoxyborane, and other chemicals were supplied from Wako. Bovine serum albumin (BSA), millipore glass-fiber filters AP2002500 (borosilicate with an acrylic binder suitable to protect a 0.8–8 μm membrane), and a nitrocellulose membrane with a pore size of 5 μm was purchased from Sigma Aldrich. Melamine antibody (polyclonal antibody of melamine, anti-melamine, 500 μg/L) was obtained from Beacon. Silicon wafers were purchased from Mitsubishi Metal Corp. Plastic backing and a liquid blocker super pap pen mini were supplied by Cosmo Bio (Japan).

2.2. Electrode preparation.

The BDD electrode was prepared using microwave plasma-assisted chemical-vapor deposition (MPACVD) (ASTeX Corp.), and a mixture of 50 mL of acetone and 4 mL of trimethoxyborane was used to provide sources of carbon and boron, respectively. Details of the preparation procedure are described elsewhere [13]. Scanning electron microscopy (SEM) showed that the grain sizes of the polycrystalline film were ~2–5 μm with 5 μm thickness. Characterization with Raman spectroscopy (Renishaw System 2000) provided a typical spectrum with a peak at 1333 cm^{-1} related to sp^3 carbon bonds and a couple of peaks at ~500 and 1200 cm^{-1} that confirmed the existence of boron doping in the diamond structure [13,27,28]. The absence of a peak at ~1600 cm^{-1}, which is generally attributed to 'non-diamond' carbon, suggested that the BDD thin films were of fine quality [13,27,28].

2.3. Preparation of AuNPs and AuNPs-modified anti-melamine (AuNP-antimel)

AuNP was synthesized by boiling 100 mL of 0.01% $HAuCl_4$ solution with constant stirring. Then, 2.0 mL of 1% trisodium citrate was added into the solution and the boiling process was continued for 15 min. After cooling to room temperature, the volume of the colloidal AuNPs was readjusted using deionized water. In order to prepare AuNP-antimel, 10 mL of colloidal AuNPs was adjusted to pH 7 using 0.1 M K_2CO_3 before carefully adding 0.5 mL of antimel (0.1 mg/mL). After incubation was completed, 3 mL of 5% BSA was added followed by further incubation. The incubation time of AuNP-antimel was monitored using the adsorption peak of AuNPs at ~520 nm by UV-visible spectroscopy. The solution was then centrifuged at 180,000 rpm for 30 min. The red precipitate was rinsed as a re-suspension in 2 mL 0.01 mL of phosphate buffer solution (PBS) containing 5% sucrose, 5% BSA, and 0.4% Tween-20 pH 7.4 and kept in storage at 4 °C when not in use. TEM, UV–vis and FT-IR spectroscopy were used to characterize the AuNPs and the AuNP-antimel.

2.4. Fabrication of immunochromatographic strip test [23,26]

The immunochromatographic strip test was assembled from several components, including a sample pad, a conjugate pad, a nitrocellulose membrane, a capturing antibody pad/test zone, a control line pad, and an absorbent pad. All of the components were arranged on a plastic backing sheet as shown in Fig. 1. The sample, the conjugate, and the absorbent pads were made of

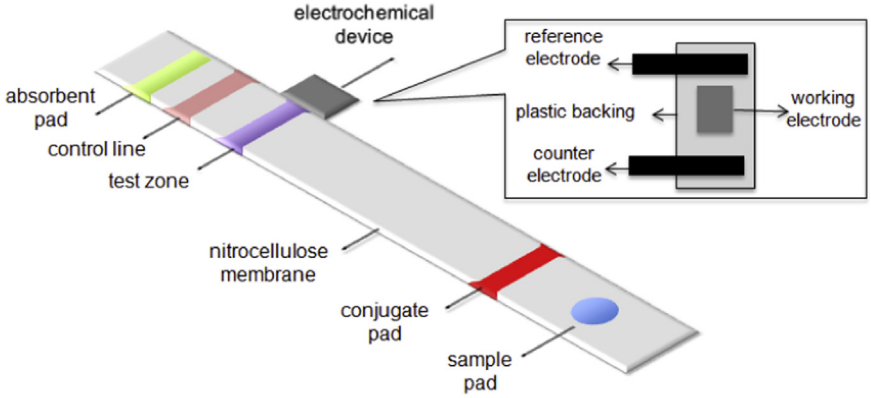

Fig. 1. Scheme of the immunochromatographic strip test. Inset shows the scheme of the electrochemical device.