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A rapid and portable sensor based on protein-modified gold nanoparticle probes and lateral flow assay for naked eye detection of mercury ion

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ABSTRACT

Contamination of the environment with heavy metal ions has been an important worldwide concern for decades. Among them, mercury ion (Hg^{2+}) is one of the heavy metal ion maintained in the detection because of its toxicity. In this study, we have designed the gold nanoparticle (AuNP) probes of carboxylic modified protein and employed the lateral flow assay to detect the Hg^{2+} without complicated instrumentations and technical expertise. The captured AuNP probes on the test zone and control zone of the sensor produced the characteristic red bands, enabling visual discrimination of Hg^{2+} concentrations. The demand of Hg^{2+} analysis in environmental applications requires parallel analytical strategies; such a method with simplicity and rapidity has enormous potential for the application of Hg^{2+} monitoring in environment, water, and food samples.

1. Introduction

The sensing of biological agents, diseases, and toxic materials is an important goal for biomedical diagnosis, forensic analysis, and environmental monitoring [1–3]. Designing easy-to-use sensors for trace metal ions in the environment is of considerable importance as these metal ions are large in number, small in quantity, and high in toxicity. Mercury, which can accumulate in vital organs and tissues, such as the liver, brain, and heart muscle, is highly toxic and can have lethal effects on living systems. Mercury originates mainly from coal-burning power plants, oceanic and volcanic emissions, gold mining, and waste combustion [4]. Furthermore, microbial biomethylation of mercury ion (Hg^{2+}) yields methyl mercury, a potent neurotoxin that passes through the food chain to the tissues of fish and marine mammals [5]. The conveniently routine detection of Hg^{2+} is central to the environmental monitoring of rivers and larger bodies of water and for evaluating the safety of aquatically derived food supplies. Therefore, it is highly desirable to develop a sensitive mercury detection method that can provide simple, practical, and high-throughput routine determination of levels of Hg^{2+} for both environmental and water samples.

Recent progress in the laboratory has been a result of improvements in rapid analytical techniques and detection limits for sensing Hg^{2+} by using the unique physicochemical properties of gold nanoparticles (AuNPs) [6–8]. While these AuNPs-based colorimetric sensors have taken an important step towards real-time sensing as the signal is detectable by the naked eye, they still require laboratory type operations, such as precise transfer and mixing of multiple solutions. Additionally, those experiment procedures for the analysis of metal ion require expensive equipment and skilled analysts by the assist of UV–Vis or fluorescence spectrophotometer and are limited in laboratory scientific research. Therefore, it still remains a challenge to develop simple, rapid, and specific colorimetric methods to probe the metal ion.

Immunochromatographic assays also known as lateral flow assays (LFA) are an ideal platform to further improve the performance of the colorimetric sensors [9,10]. Most commonly these tests are used for medical diagnostics either for home testing, point of care testing, or laboratory use. They are easy to develop and inexpensive to manufacture in high volumes. Their popularity with users stems from their low cost and simplicity that allows non-specialized users to perform complicated tests at the point of need without additional equipment. Therefore, with the combination of the AuNP-based visual test with LFA, a rapid, cost-effective, highly sensitive, and portable technique was developed for the field detection of Hg^{2+} in this study. As illustrated schematically in Fig. 1, we have designed the AuNP probes of carboxylic modified protein (COOH-BSA) and employed the LFA to detect the Hg^{2+} without complicated instrumentations and technical expertise.

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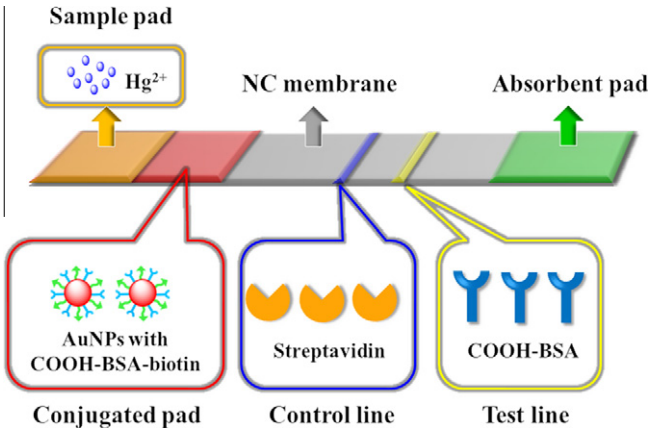

Fig. 1. The manufacture of lateral flow assay, which indicated all the reagents for necessary on the device.

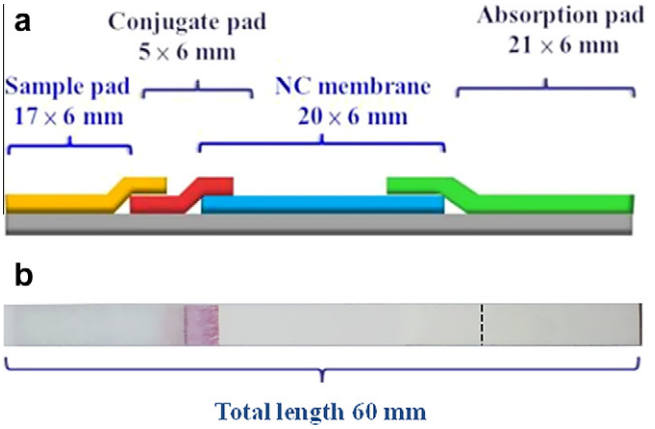

Fig. 2. The (a) component and (b) photographic image of the lateral flow assay test strip.

2. Experimental section

2.1. Synthesis of AuNPs

Citrate-stabilized AuNPs were synthesized using the classical Turkevich/Frens procedure [11,12]. In this method, aqueous solutions of 1 mM $HAuCl_4 \cdot 3H_2O$ (Sigma–Aldrich) 10 mL and 38.8 mM trisodium citrate dihydrate (SHOWA, Chemical Co., Japan) 1 mL were mixed. Heating of the solution under reflux was continued until the color of the boiling solution changed from dark purple to purplish-red. After cooling to room temperature, colloidal AuNPs were formed in the solution, and filtered before use through a 0.45 μm syringe filter (Millipore). Absorption spectra (HITACHI, U-3310) and scanning electron microscopy (SEM) (JEOL, JSM-6700F) images confirmed the sizes and shapes of these AuNPs.

2.2. Modification of carboxylic acid group on BSA

COOH-BSA is modified carboxylic acid group on BSA for the recognition of heavy-metal ions on the test line [6]. The amino group of BSA can be replaced the amino group to carboxylic acid group by succinylation. In this experiment, 5 mg BSA (Sigma) was dissolved in sodium borate buffer (pH 8.5, 5 mL). A pH electrode was placed within the solution to monitor the pH value. As the solution was continuously stirred using magnetic bars, succinic anhydride (Acros) was added in 0.5 mg gradually. During the process, NaOH solution (Tokyo Chemical Industry) was used to maintain the pH value at 7. After the addition of succinic anhydride, the reaction was still proceed and stirred at least 30 min. The modified BSA called COOH-BSA was purified by ultra-filtration through centrifugation (14,000 rpm, 15 min) and re-dispersion in buffer at the concentration of 1 mg/mL. BSA-biotin (Sigma) was also succinylated and purified by the same procedures. After the carboxylic modification of BSA-biotin, 1 mg/mL COOH-BSA-biotin (5 mL) was mixed with 7.5 nM AuNPs (5 mL) for the preparation of AuNPs with COOH-BSA-biotin.

2.3. Fabrication of lateral flow strip

The LFA strip (Millipore) is comprised of a sample pad (17 mm × 6 mm), conjugate pad (5 mm × 6 mm), nitrocellulose (NC) membrane (20 mm × 6 mm), and an absorbent pad (21 mm × 6 mm) as shown in Fig. 2.

NC membrane and conjugate pad were prepared by immersing in 5% sucrose (Sigma–Aldrich) solution ahead, and then dried at 37 °C for 2hr in dry box. Next, AuNP probes (AuNPs with COOH-BSA-biotin; 30 μL) were dropped on the conjugated pad, and then dried at 37 °C for 2 h and stored at 4 °C in a dry state. 50 μg/mL streptavidn (Acros) was sprayed on the control line (CL); 125 μg/mL COOH-BSA was sprayed on the test line (TL) by the Dimatix Materials Printer (FUJIFILM, DMP-2800). Both the width of the CL and TL was 2 mm. Finally, the sample pad, conjugate pad, NC membrane, and absorption pad were assembled on a plastic adhesive backing using the clamshell laminator. Each part overlapped 1 mm to ensure that the sample solution can migrate through the strip during the assay.

3. Results and discussion

At the first step in our analysis, we have to find an accurate method to characterize the quality and quantity of AuNPs. We used a standard chemical reduction method to prepare spherical AuNPs having mean diameter of 13 ± 1 nm, under conditions that ensured

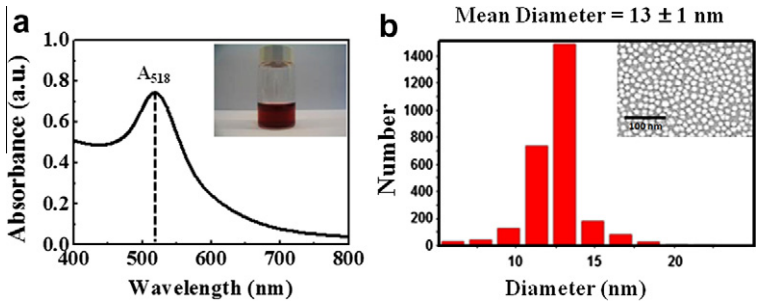

Fig. 3. (a) UV–Vis absorption spectrum and color of aqueous AuNPs. (b) SEM image and particle size distribution of AuNPs with mean diameter of 13 ± 1 nm. Scale bar: 100 nm.

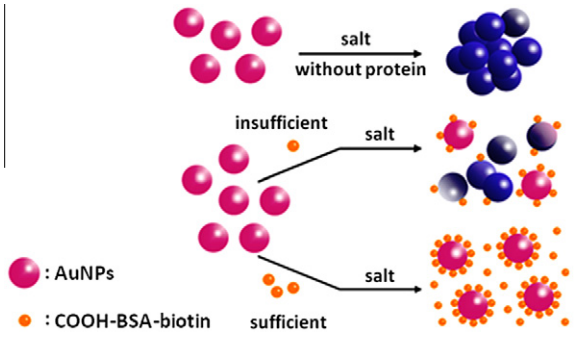

Fig. 4. The diagram of determining the optimum COOH-BSA-biotin concentration to cover on the AuNPs surface by monitoring the absorbance of AuNP solutions.

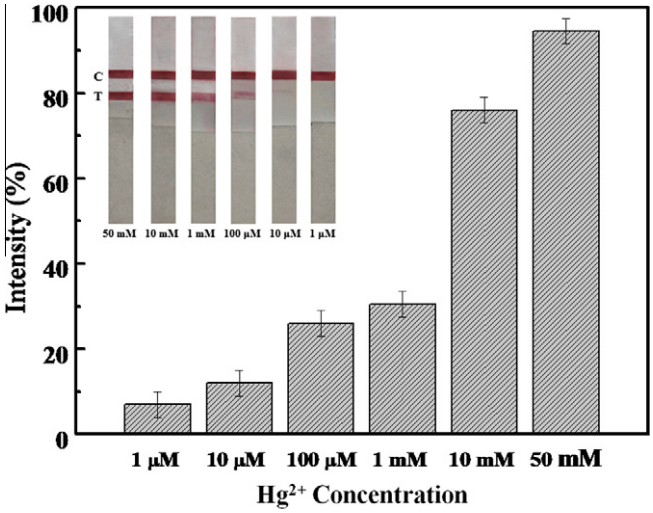

Fig. 5. Determination of the Hg^{2+} concentrations from 1 μM to 50 mM by the related optical intensities and the color of test line (Inset). C = control line and T = test line.

their stability and lack of aggregation. The color of the colloid AuNPs was purplish-red, and the max absorbance was 518 nm in UV–Vis spectrum as shown in Fig. 3(a). The particle size distribution of AuNPs was determined by analyzing the SEM image in Fig. 3(b).

As shown in Fig. 4, by exploiting interactions between the AuNPs and protein, colorimetric changes of the AuNPs could sensitively differentiate the COOH-BSA-biotin concentrations after titrating with the salt solution (10% NaCl; Tokyo Chemical Industry); thus, the formation of protein–AuNPs complex can be optimized with regard to its colloidal stability [13,14]. We employed the appropriate conditions for the preparation of AuNP probes (AuNPs with COOH-BSA-biotin), determined by optimizing the ratio of protein molecules and AuNPs to ensure the conjugate stability.

Combining the optical property of AuNPs and the high efficiency of recognition between Hg^{2+} and carboxylic acid group was realized on the LFA system for the detection of Hg^{2+}. When a contaminated sample was dropped on the sample pad, liquid migrated along the strip releasing the AuNP probes from the conjugate pad. As the AuNP probes migrated through the device, the test line and control line of the sensor produced the characteristic red bands, enabling visual discrimination of Hg^{2+} in the concentration range of 1 μM to 50 mM. In this range, the test zone displayed the deep red at a higher Hg^{2+} concentration. Quantitative detection was performed by recording the peak areas of the red bands in the test zone with the aid of photo images (Fig. 5).

4. Conclusions

We have successfully developed a simple, rapid, inexpensive, high-throughput, and quantitative sensor for the detection of Hg^{2+} based on LFA system. The AuNP probes performed excellent resistance from aggregating in the salinity solutions due to the protection of protein and exhibited the detection limit of Hg^{2+} concentration as low as 1 μM. The method for the detection of Hg^{2+} with naked eye was realized on this system, which eliminated multiple operation steps and expensive instrumentations compared to the reported technologies. The lateral flow device is the most commonly used sensor that contained powerful potential for commercial products because of the low-price and the easy-expertise. New generations of analytical devices have to be accurate, speed, simple, and cost-effective. This technology could provide a promising approach for this purpose.

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