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A competitive immunoassay for ultrasensitive detection of Hg^{2+} in water, human serum and urine samples using immunochromatographic test based on surface-enhanced Raman scattering

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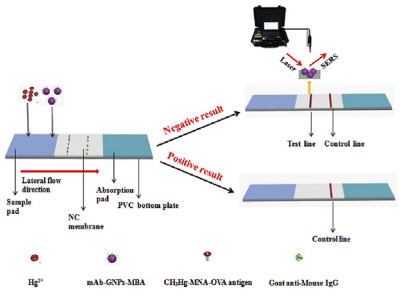
HIGHLIGHTS

- The proposed ICT was able to directly detect Hg^{2+} without formation of Hg^{2+}-ligand complex.
- The proposed ICT exhibited high sensitivity, specificity, stability, precision and accuracy for Hg^{2+} detection.
- The proposed ICT was applicable for the detection of trace amount of Hg^{2+} in water, human serum and urine samples.

GRAPHICAL ABSTRACT



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ABSTRACT

An immunochromatographic test (ICT) strip was developed for ultrasensitive competitive immunoassay of Hg^{2+}. This strategy was achieved by combining the easy-operation and rapidity of ICT with the high sensitivity of surface-enhanced Raman scattering (SERS). Monoclonal antibody (mAb) against Hg^{2+} and Raman active substance 4-mercaptobenzoic acid (MBA) dual labelled gold nanoparticles (GNPs) were prepared as an immunoprobe. The Raman scattering intensity of MBA on the test line of the ICT strip was measured for quantitative determination of Hg^{2+}. The ICT was able to directly detect Hg^{2+} without complexing due to the specific recognition of the mAb with Hg^{2+}. The IC_{50} and limit of detection (LOD) of the assay for Hg^{2+} detection were 0.12 ng mL^{-1} and 0.45 pg mL^{-1}, respectively. There was no cross-reactivity (CR) of the assay with other nineteen ions and the ICT strips could be kept for 5 weeks without loss of activity. The recoveries of the assay for water, human serum and urine samples spiked with Hg^{2+} were in range of 88.3–107.3% with the relative standard deviations (RSD) of 1.5–9.5% (n = 3). The proposed ICT was used for the detection of Hg^{2+} in urine samples collected from Occupational Disease Hospital and the results were confirmed by cold-vapor atomic fluorescence spectroscopy (CV-AFS). The assay exhibited high sensitivity, selectivity, stability, precision and accuracy, demonstrating a

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promising method for the detection of trace amount of Hg^{2+} in environmental water samples and biological serum and urine samples.

1. Introduction

Heavy metal pollution has increasingly attracted widespread attention for its human toxicity. The great efforts in the world, therefore, have been made for the examination and control of heavy metals in different matrices such as plant, soil, and water, etc. [1,2]. Among different heavy metals, mercury which displays bioaccumulative properties, high toxicity and severe adverse influence on human health is widely considered to be one of the most hazardous pollutants in the environment [3,4]. There is a variety of mercury species, such as mercuric ion (Hg^{2+}), mercurous ion (Hg^{+}), mercury sulfide (HgS), methylmercury (CH_3Hg^{+}), ethylmercury ($C_2H_5Hg^{+}$), and phenylmercury ($C_6H_5Hg^{+}$) in the environment. Owing to high affinity for thiol group in proteins and enzymes, mercury can cause cell dysfunction. Once it is absorbed into human body, serious damages to brain, kidney, central nervous system, mitosis and endocrine system will be produced [5]. Therefore, the development of simple and rapid methods for the detection of mercury species including mercuric ion (Hg^{2+}) in environmental and biological systems is of great urgency.

In the last decades, a variety of analytical methods for mercury detection such as atomic emission spectroscopy (AES) [6], inductively coupled plasma mass spectrometry (ICP-MS) [7], cold-vapor atomic absorption spectrometry (CV-AAS) [8], high performance liquid chromatography (HPLC) [9,10], and enzyme-linked immunosorbent assays (ELISA) [11] have been reported. In addition, various portable sensors including fluorescent sensors based on semiconductor nanocrystals [12] and polymeric materials [13], scanometric sensors [14], DNA sensors based on structure of the T-Hg^{2+}-T [15], electrochemiluminescence (ECL) sensors [16], etc. have been developed. These analytical techniques provide many advantages of quantitative nature, precision and low detection limit; however, they require sophisticated instrumentation, skilled analysts and time-consuming procedures, complicated sample preparation process, etc., which hinder their easy and fast applicability to real samples. Hence, it is necessary to develop sensitive and selective methods which are simple and low-cost.

An immunoassay, based on the specific reaction between an antibody and its antigen, is immunochromatographic test (ICT) which has received great attention from researchers recently [17]. As a popular method for point-of-care applications, the great advantages of ICT are its user-friendly operation, fast performing time, on-site detection, low cost, and fairly good shelf life, etc. [18]. Nowadays, most test kits in the market (e.g., pregnancy testing) based on ICT are qualitative and they aren't applicable when the quantitative analysis of a chemical is needed and important [19]. In the past years, some ICT methods using fluorescence or quantum dots for quantitative analysis have been reported [20,21]. Nevertheless, a significant limitation of these modified ICT methods is that the results may suffer from optical interference (e.g., photobleaching). Other quantitative ICTs such as chemiluminescent and electrochemical ICT have been published [22,23]. The drawbacks of them is their insufficient sensitivity; moreover, the test line on the ICT strip should be cut down and put into another solution for chemiluminescent or electrochemical reaction, leading to a complicate signal measurement.

Since surface-enhanced Raman scattering (SERS) phenomenon was first reported, it has received continuous attention by researchers for its high specificity and sensitivity in sensing chemical and biological molecules in trace amounts [24]. Applications employing SERS are observed in research fields such as surface science, biology, material science, art and analytical chemistry [25]. On account of its ultra-sensitivity, SERS has been applied in immunoassays. Many SERS-based immunoassays in sandwich method for detecting macromolecule antigen [26,27] or in competitive format for determination of small molecules [28,29] have been reported. In spite of SERS-based immunoassays providing an ultrasensitive detection, they have a few shortcomings including long incubation time in each immunoreaction, complication of washing steps and hardness to manufacture surfaces for highly reproducible enhancements.

With the widely application of the colloidal gold nanoparticles (GNPs), there are some clear preponderances when GNPs are used as the substrate for the preparation of probe due to their controllable-size distribution, long time stability, friendly biocompatibility with large biological protein (e.g antibody and antigen), and good enhancement of Raman intensity. In this work, GNPs were used as a bridge to combine ICT and SERS. On the basis of the successful preparation of the monoclonal antibody (mAb) against Hg^{2+} in our lab [30], the aim of this work was to establish an ultrasensitive competitive ICT based on SERS for rapid determination of Hg^{2+} in water, human serum and urine samples. MAb against Hg^{2+} and Raman active substance 4-mercaptobenzoic acid (MBA) dual labelled gold nanoparticles (GNPs) were prepared as an immunoprobe. Hg^{2+} in water, human serum and urine samples competes with the coating antigen immobilized on the test line for the limited mAb on the immunoprobe. After ICT procedures, the Raman intensity of the MBA on the strip was measured for quantitative detection of Hg^{2+}.

2. Experimental

2.1. Chemicals and materials

Bovine serum albumin (BSA), chicken egg ovalbumin (OVA), mercuric chloride ($HgCl_2$), 6-mercaptonicotinic acid (MNA) and tween-20 were purchased from Sigma (St. Louis, MO, USA). 4-mercaptobenzoic acid (MBA) was obtained from Aladdin China Ltd. (Shanghai, China). Trisodium citrate, chloroauric acid ($HAuCl_4$), potassium carbonate (K_2CO_3), sodium carbonate (Na_2CO_3) and sodium bicarbonate ($NaHCO_3$) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Saturated ammonium sulfate [$(NH_4)_2SO_4$] and polyethylene ketone of alkanes (PVP) were obtained from Kelon chemical reagent factory (Chengdu, China). Goat anti-mouse IgG was obtained from Golden-Bridge Biotechnology Co., Ltd. (Beijing, China).

2.2. Apparatus

Nitrocellulose (NC) membranes were purchased from Whatman (Shanghai, China). PVC sheets, adhesive tape and filter paper were purchased from Jieyi Biotechnology Co. Ltd (Shanghai, China). Ultraviolet visible spectrophotometer (UV-2300) was purchased from Techcom Com. (Shanghai, China). Deionized-RO water machine