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A sensitive atomic absorption spectrometric metalloimmunoassay with copper nanoparticles labeling

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ABSTRACT

A sensitive metalloimmunoassay based on copper nanoparticle labeling was demonstrated with atomic absorption spectrometry. Stable nanosized CuO particles of 5–10 nm diameter were synthesized by an alcothermal method, and conjugated to antibody under moderate conditions. The immunoassay conditions were optimized. After immunoreaction, the copper atoms were released with diluted nitric acid and measured by electro-thermal atomic absorption spectrometry. The atomic absorbance signal had a good linearity with logarithm of the C_{IgG} (concentration of IgG, g/mL). The detection limit was calculated to be 0.19 ng/mL and the assay was linear in the range of 10^{-9}–10^{-5} g/mL human IgG. The relative standard deviation (RSD) for 10 ng/mL human IgG was 3.0%. The human IgG results obtained by the proposed method correlated well with those obtained by inductively coupled plasma-based immunoassay.

1. Introduction

Immunoassay is based on the specific recognition of antibodies with the targeted antigens, which has been widely used to measure low amount of biomarkers in clinical samples, as well as trace level of drugs and chemicals in biological and environmental samples [1]. Immunoassay is often classified as radioimmunoassay, enzyme immunoassay, fluorescent immunoassay, chemiluminescent immunoassay, and so on, depending on the respective labels applied. In recent years, the development of metalloimmunoassay [2] (i.e., immunoassays applying metal-based labels) fostered the prosperous of atomic spectrometry including inductively coupled plasma mass spectrometry (ICPMS) [3–5], atomic fluorescence spectrometry (AFS) [6,7], atomic emission spectrometry (AES) [8], and atomic absorption spectrometry (AAS) [9]-based immunoassay. The atoms inside labels are directly detected, thus optical, electric, electrochemical, magnetic, or any other special properties are obviously unnecessary, which greatly alleviates the time and labor-consuming work of labels' design and synthesis.

The development of nanoscience and nanotechnology has brought great opportunities for metalloimmunoassays [10,11]. Zhang et al. [12] and Tanner et al. [13] reported the pioneer work about various kinds of ICPMS-based metalloimmunoassays by Au nanoparticles [14–22] labeling. CdSe quantum dots [23], PbS quantum dots [24] and silver nanoparticles [25] were employed by Hu et al. for disease biomarker quantification by ICPMS. HgS nanoparticles [26] were synthesized by Lv et al. for high sensitivity in AFS. TiO_2 [27] and CeO_2 [28] nanoparticles were obtained by Lim et al. for ICPMS determination of prostate-specific antigen and carcinoembryonic antigen. Sanz-Medel et al. [29] harvested CdSe/ZnS quantum dots for the comparison between ICPMS and molecular fluorescence. Metal nanoparticles are facile to bio-functionalize and possess large amount of detectable atoms (e.g., ca. 30,000 Au atoms in an Au nanoparticle of 10 nm diameter), making them promising in the fabrication of highly sensitive immunoassays.

Copper nanoparticles are currently under intensive investigation due to their special electronic and optical properties [30]. They have been widely applied in catalysis, optical limiting, photovoltaics and biosensors. Up to now, several highly sensitive immunoassays based on Cu nanoparticles have been reported. For example, Jiang et al. [31] reported a highly sensitive immunoassay based on CuO nanoparticles-labeled antibody and click chemistry. Chen et al. [32] developed a sensitive electrochemical immunoassay for H1N1 influenza virus based on the voltammetric signals of Cu^{2+} dissolved from CuO NPs. Recently, double strand DNA-templated formation of Cu nanoparticles also opens a new avenue for bioassays [33,34]. Cu nanoparticles featureeasy fabrication, cost-effectiveness, maturity and high sensitivity in the element detector.

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Inspired by the above-mentioned fact, Cu nanoparticles have an immediate feasibility and great potential in metalloimmunoassay. Therefore, in the present study, we used CuO NPs to label antibodies and designed a sensitive metalloimmunoassay. To the best of our knowledge, this is the first demonstration of copper nanoparticle-based atomic spectrometric immunoassay. The synthesis and bio-functionalization of stable CuO nanoparticles were investigated. The CuO NPs were dissolved by adding diluted nitric acid to produce copper ions, which in turn can be detected with high sensitivity and specificity by electrothermal AAS (ETAAS) detection. The results demonstrated that this immunoassay is applicable for human immunoglobulin G (IgG) detection in human serum. The proposed CuO nanoparticle-based metalloimmunoassay also offers an alternative strategy for the detection of other proteins and nucleic acids.

2. Experimental

2.1. Reagents and immunoreaction buffers

Bovine serum albumin (BSA), goat-anti-human IgG, human IgG, and 96-wells high binding ELISA plate (468667, NUNC, Denmark) were from Biodee Biotechnology Co. Ltd (Beijing, China). Copper acetate, acetic acid, dried ethanol, nitric acid, and sodium hydroxide were of analytical grade and obtained from Changzheng Chemical Reagent Co. Ltd. (Chengdu, China). Unless otherwise stated, all the reagents used in this study were at least of analytical grade.

Carbonate buffer (0.05 M, pH 9.6), dissolve 2.601 g of Na_2CO_3 and 3.437 g of $NaHCO_3$ in 1 L of deionized water; phosphate buffered saline (PBS, 0.01 M, pH 7.4), dissolve 2.204 g of $Na_2HPO_4 \cdot 12H_2O$, 0.600 g of $NaH_2PO_4 \cdot 2H_2O$, and 8.766 g of NaCl in 1 L deionized water; PBST, 0.01 M PBS including 0.1% Tween 20, pH 7.4.

2.2. Instrumentation

An AAnalyst™ 800 atomic absorption spectrometer (PerkinElmer, Inc., Shelton, CT, USA) was used throughout this work. The instrument is equipped with a graphite furnace atomizer and an AS 800 autosampler to facilitate sample introduction and free atom generation. A hollow cathode lamp (HCL, PerkinElmer Lumina™, Part No. N305-0121) of copper was used as the radiation source. The signal measurements were performed using the peak area (integrated absorbance) method.

2.3. Procedure

2.3.1. Preparation of colloidal copper oxide

Colloidal CuO was prepared according to the literature with some modifications [35]. In a typical experiment, 1 mmol of $Cu(CH_3COO)_2$ and 2 mmol of acetic acid were dissolved in 50 mL of dried ethanol, respectively. The solution was heated to the boiling temperature of ethanol (78 °C). 4 mmol of the NaOH powder was then added to the solution under vigorous stirring. The colloidal copper oxide was obtained after 1 hour reaction.

2.3.2. Preparation of CuO NPs antibody conjugates

Briefly, 0.5 mL pure water was added into 1 mL colloid copper oxide. After 2 min vortex, the solution was centrifuged for 2 min at 1000 rpm, to get rid of CuO nanoparticles of larger diameter. The solution in the upper layer was centrifuged for 5 min at 5000 rpm, then the precipitate was washed three times with pure water. After washing, the precipitate was re-dispersed into 1 mL of pure water. 100 μL goat-anti-human IgG (1 mg/mL) was added in the CuO nanoparticle solution. The mixture was gently shaken for 3 h. Then 200 μL 10% *m*/*v* BSA in PBS was added to stabilize the solution. After 30 min, the solution was centrifuged for 5 min at 5000 rpm. The precipitate was re-dispersed with 1 mL 1% *m*/*v* BSA. The CuO nanoparticles labeled antibody was obtained and stored at 4 °C.

2.3.3. Immunoassay protocol

The schematic diagram of the proposed sandwich immunoassay is shown in Fig. 1. The assay was performed in a polystyrene 96-well high binding ELISA plate. Briefly, 200 μL of 10 μg/mL goat-anti-human IgG in carbonate buffer (0.05M, pH 9.6) was added in the 96-well plate and incubated at 4 °C overnight. Goat-anti-human IgG was then steadily attached on the solid substrate via physical adsorption between hydrophobic groups of antibody molecule and polystyrene. The glass slide was washed off three times with 200 μL of washing buffer (PBST, PBS including 0.1% Tween 20) to remove unbound antibodies, and the uncoated active sites of polystyrene substrate were saturated with 200 μL of blocking buffer, in which 3% BSA in PBS was used as a blocking agent to prevent nonspecific adsorption. 200 μL of diluted human IgG standard solutions or human serum sample (in 1% BSA, *m*/*v*) was then incubated for 1 h at 37 °C. The wells were washed three times with PBST to remove the unbound antigens. 100 μL of CuO NPs labeled

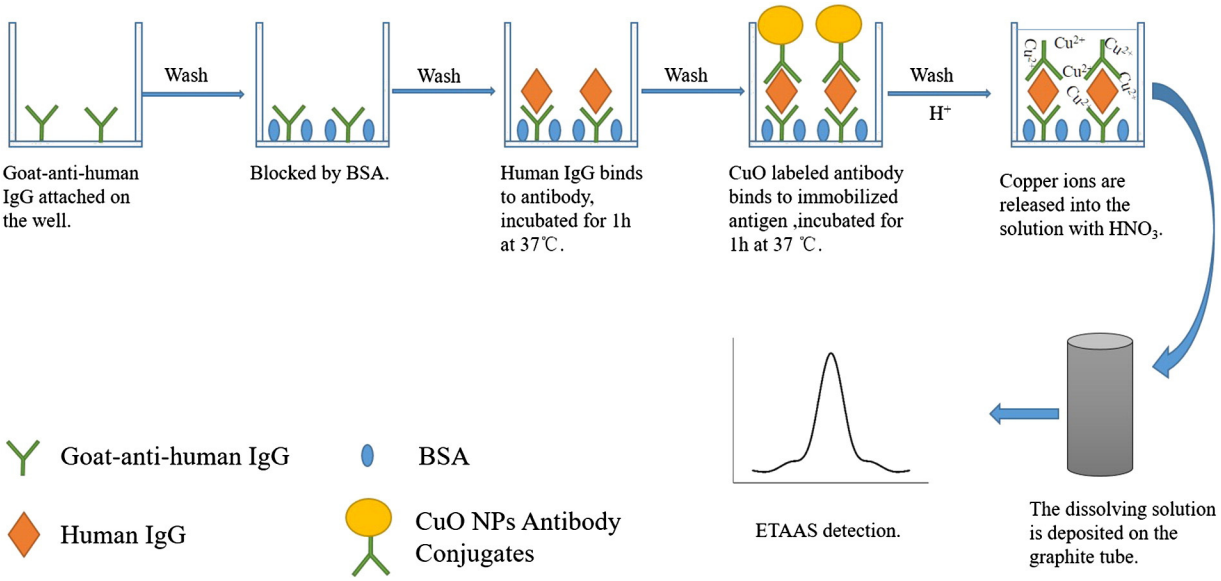

Fig. 1. Sandwich-type immunoassay based on CuO-labeled antibody and ETAAS analysis.