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Sensitive sandwich immunoassay based on single particle mode inductively coupled plasma mass spectrometry detection

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ABSTRACT

A sensitive sandwich type immunoassay has been proposed with the detection by inductively coupled plasma mass spectrometry (ICP-MS) in a single particle mode (time resolved analysis). The signal induced by the flash of ions ($^{197}Au^{+}$) due to the ionization of single Au-nanoparticle (Au-NP) label in the plasma torch can be measured by the mass spectrometer. The frequency of the transient signals is proportional to the concentration of Au-NPs labels. Characteristics of the signals obtained from Au-NPs of 20, 45 and 80 nm in diameters were discussed. The analytical figures for the determination of Au-labeled IgG using ICP-MS in conventional integral mode and single particle mode were compared in detail. Rabbit-anti-human IgG was used as a model analyte in the sandwich immunoassay. A detection limit (3σ) of 0.1 ng mL^{-1} was obtained for rabbit-anti-human IgG after immunoreactions, with a linear range of 0.3–10 ng mL^{-1} and a RSD of 8.1% (2.0 ng mL^{-1}). Finally, the proposed method was successfully applied to spiked rabbit-anti-human IgG samples and rabbit-anti-human serum samples. The method resulted to be a highly sensitive ICP-MS based sandwich type immunoassay.

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1. Introduction

Element-tagged immunoassay with inductively coupled plasma mass spectrometry (ICP-MS) detection has been extensively studied and well established in the past few years [1–5]. The advantages of using ICP-MS in immunoassays with element tagging include low detection limits, large dynamic range, multiplexing potential, good spectral resolution and free of radioactive isotope or toxic enzyme substrate reagents. In addition, this technique receives low biological matrix effects. Methods for the determination of biological analytes such as small biomolecule [6,7], protein [8–16], DNA [17], bacteria [18] and even cell [19–21] have been proved to be successful by several groups. The multiplexed detection ability has also been fully demonstrated by using this ICP-MS based immunoassay [8,22–24]. Besides, as an imaging tool, laser ablation (LA)-ICP-MS was proposed for the multi-analytes imaging in tissue section [25,26], protein microarray [24] and western blot membrane [27,28].

Previously, we described a single particle mode ICP-MS based immunoassay for α-fetoprotein [29], as enlightened from the spark of the single nanoparticle analysis with ICP-MS [30]. The strategy tends to be a sensitive readout method for nanoparticle tags, which is promising for applications in biological analysis. Nevertheless, the published work was mainly focused on the competitive type immunoassay, and had not fully illustrated the merits of the ICP-MS based single particle analysis. Sandwich type immunoassay, as a noncompetitive immunoassay, is arguably the most important immunoassay method and widely used to determine antigen concentrations in various endeavors including clinical diagnostics and biochemical studies [31–34]. Most sandwich immunoassays work in solid-phase where microwells or tubes are served as carriers of immunoreagent. Sandwich type immunoassay methods have several advantages over competitive assays. For instance, they are generally 5–20 times more precise and sensitive than their respective competitive assays and less affected by nonspecific or specific interferences [35]. Therefore, sandwich type immunoassay methods for biomolecules usually do not require preliminary extraction and purification of the sample and thus use a lower sample volume than competitive assays. Furthermore, sandwich type immunoassays generally have a larger working range [35]. These merits suggest that sandwich type immunoassays for biosamples may be more suitable for clinical routine than competitive assays.

In this work, a highly sensitive sandwich immunoassay based on ICP-MS detection in single particle mode (time resolved analysis), with Au-nanoparticles (Au-NPs) serving as model tags, was proposed. The characteristics of the signals obtained from different sizes of Au-NPs were studied. To illustrate the performance of this single particle mode, the analytical figures of merits for the

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Table 1
Working parameters of ICP-MS.

Parameters	Values
RF Power (W)	1200
Cool gas flow (L/min)	13
Auxiliary gas flow (L/min)	0.8
Nebulizer gas flow (L/min)	0.85
Sample uptake rate (mL/min)	0.5
Water stream flow rate (mL/min)	0.5
Torch	Shield torch
Cones	Nickel, HPI design
Data acquisition mode	Time resolved analysis (TRA)
Dwell time (ms)	10
TRA duration time (s)	60
Resolution	Standard
Analogue detector voltage (V)	2130
PC detector voltage (V)	3650

determination of Au-NPs labeled IgG using both modes (conventional integral mode and single particle mode) have been calculated for comparison. Characteristics of the signals obtained from Au-NPs labels, procedures to dissociate the antigen–antibody complex, effects of Au-NPs labels concentration and real sample analysis were discussed.

2. Experimental

2.1. Instrument

An X Series ICP-MS (Thermo Electron Co., Winsford, Cheshire, UK) was used throughout this work. The build-up of sample introduction manifold and the data acquisition parameters have been described previously [29], so only a brief account will be given here. The flow rates of the water stream and Au-NPs suspension were both 0.5 mL/min through a Y-shaped pipeline. Transient signals were recorded by ICP-MS in time resolved analysis (TRA) mode. With the dwell time of 10 ms, 6000 data points were provided during the time duration of 60 s. The working parameters of ICP-MS instrument were optimized and summarized in Table 1.

The morphologies of the Au-NPs were observed on a transmission electron microscope (TEM, Hitachi H-800) with an accelerating voltage of 100 kV. The enzyme-linked immunosorbent assay (ELISA) was performed with an ELISA reader (Tecan, Sunrise Remote/Touch Screen, Columbus plus, Austria).

2.2. Reagents and immunoreaction buffers

Deionized water with conductivity of 18.3 MΩ cm^{-1} from a Milli-Q water purification system (Millipore Milford, MA, USA) was used in this work. Polystyrene 96-well microtiter plates (Nikon, Sino American Biotechnology Co., Beijing, China) were used to perform the immunoreactions. Human IgG, rabbit-anti-human IgG antibody (RAH IgG), goat-anti-rabbit IgG antibody (GAR IgG) and bovine serum albumin (BSA) were purchased from Beijing Biosynthesis Biotechnology Co. (Beijing, China). Colloidal Au-Nanoparticles and GAR IgG-colloidal Au conjugate (Au-GAR IgG) were synthesized in our laboratory. The buffers used were as follows: (A) coating buffer, 0.05 M carbonate/bicarbonate buffer solution, pH 9.6 (dissolve 2.601 g Na_2CO_3 and 3.437 g $NaHCO_3$ in 1 L deionized water); (B) blocking buffer: 3% (w/v, g mL^{-1}) BSA in 0.01 M sodium phosphate-buffered saline (PBS, dissolve 2.204 g $Na_2HPO_4{\cdot}12H_2O$, 0.600 g $NaH_2PO_4{\cdot}2H_2O$ and 8.766 g NaCl in 1 L deionized water), pH 7.4. The blocking solution was stored at 4 °C and used within a week; (C) assay buffer, 0.01 M PBS containing 1% BSA (w/v), pH 7.4; and (D) washing buffer, 0.01 M PBS with 0.05% (v/v) Tween 20, pH 7.4.

2.3. Preparation of colloidal gold nanoparticles

Colloidal Au was prepared according to the literature with slight modification [33]. Briefly, after boiling 0.01% (m/v) $HAuCl_4{\cdot}4H_2O$ with an appropriate volume of 1% (m/v) trisodium citrate (namely, 50 mL 0.01% $HAuCl_4$ with 1.0, 0.50, 0.3 mL 1% trisodium citrate for 20, 45, and 80 nm average diameters of particles, respectively) in aqueous solution for 30 min, the resulted colloidal suspension was cooled and stored in 4 °C. The average diameters of particles were 20, 45, and 80 nm, respectively, as confirmed by TEM (Fig. 1). The Au-NPs suspensions used for detection were serially diluted from the stock suspensions with pure water after sonicating for 5 min.

2.4. Preparation of colloidal gold–antibody conjugates

Antibody–colloidal conjugates were prepared according to the modification in the literature [33]. The GAR antibody (10% more than the minimum amount, which was determined using a flocculation test) was added to 1 mL of pH-adjusted colloidal Au suspension followed by incubation at room temperature for 1 h. The conjugates were centrifuged at 10,000 rpm for 10 min, and the

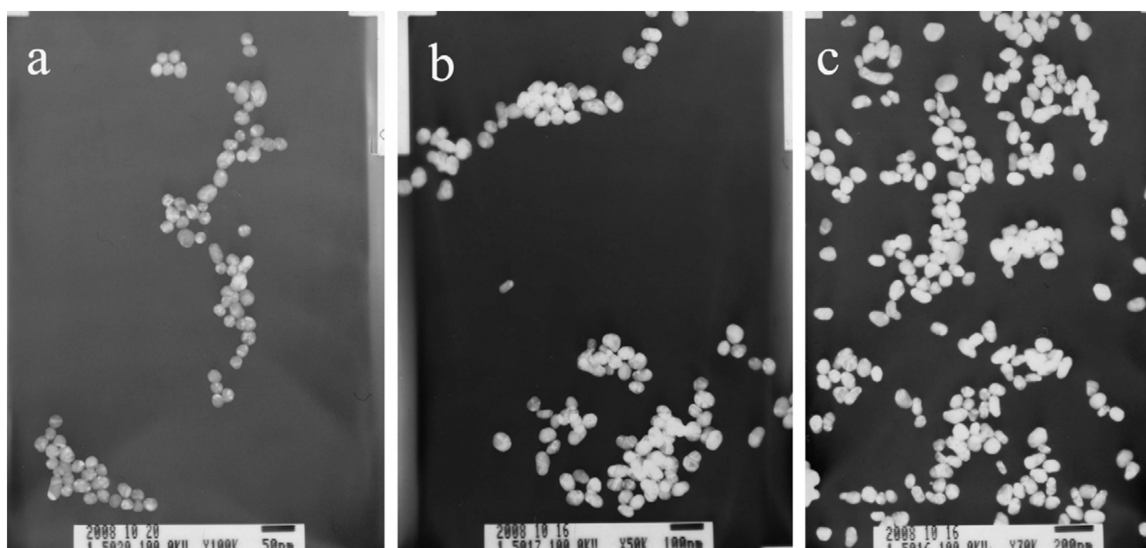

Fig. 1. TEM photograph of colloidal gold: (a) Au-NPs with 20 nm average diameter; (b) Au-NPs with 45 nm average diameter; and (c) Au-NPs with 80 nm average diameter.

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