Analytica Chimica Acta 847 (2014) 10–15

Contents lists available at ScienceDirect

Analytica Chimica Acta

journal homepage: www.elsevier.com/locate/aca

Cerium oxide-deposited mesoporous silica nanoparticles for the determination of carcinoembryonic antigen in serum using inductively coupled plasma-mass spectrometry

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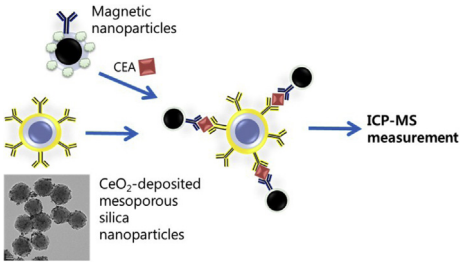
HIGHLIGHTS

- Sandwich-type immunoassay using ICP-MS and nanoparticles to determine biomarkers.
- CeO_2-deposited mesoporous silica nanoparticles were synthesized as a probe.
- Ratiometric measurement significantly improved the calibration linearity.
- Excellent detection limit was achieved by signal amplification.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Available online 21 August 2014

ABSTRACT

CeO_2-deposited mesoporous silica nanoparticles were synthesized as a probe to determine carcinoembryonic antigen (CEA) in serum by inductively coupled plasma-mass spectrometry (ICP-MS). The prepared mesoporous nanoparticles were modified and tagged to the target for sandwich-type immunoassay. Fe_3O_4 magnetic nanoparticles (MNPs) were also synthesized and immobilized with antibody to extract the target biomarker. The calibration curve of the synthesized CeO_2-deposited silica nanoparticles, which was plotted by the signal ratio of $^{140}Ce/^{57}Fe$ measured by ICP-MS vs. the concentration of CEA, showed excellent linearity and sensitivity owing to the signal amplification and low spectral interference. Under optimal conditions, the sandwich-type analytical method was applied to determine CEA in serum spiked in the range of 0.001–5 ng mL^{-1} and showed a limit of detection of 0.36 ng mL^{-1}. Since the deposited CeO_2 in the mesoporous silica layer can be substituted by other metal compounds, various kinds of metal-deposited nanoparticles can be prepared as probe materials for multiplex detection in bioanalysis.

1. Introduction

Recently, inductively coupled plasma-mass spectrometry (ICP-MS) using inorganic element tagging to determine the biomarkers for cancer diagnosis has been recommended for clinical analysis because of its high sensitivity and excellent linear dynamic range. However, since a biomarker can be expressed by various cancers, and alternatively, a cancer may contain various biomarkers, multiplex detection is inevitable for improving diagnostic accuracy and speed in clinical application. In this concept, element tagging demonstrated excellent analytical performance for multiplex detection of biomarkers using various

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http://dx.doi.org/10.1016/j.aca.2014.08.041
0003-2670/

rare earth elements [1–4]. Recently, nanoparticle tagging instead of element tagging demonstrated a significantly improved detection limit owing to signal amplification [5]. Despite this excellent analytical performance, there were still several limitations to be solved for nanoparticle tagging in sandwich-type immunoassay, such as improving measurement stability and developing various probe nanoparticles for multiplex detection. For the preparation of nanoparticles containing interference-free elements in ICP-MS measurement, two types of metal-embedded nanoparticles can be designed: doping at the core and embedding at the shell. Recently several metal-doped silica nanoparticles, such as Cs, Pb, and Cd were introduced for the potential use of multiplex detection, in which the metals were doped at the silica core and wrapped with silica shell [6]. The mesoporous inorganic nanoparticle structure can also be a candidate frame, in which the elements are deposited on the mesoporous silica shell instead of core. In this case, the deposited materials can be easily substituted with other elements because metal oxides can be inserted into the surface layer through the pores. Therefore, if possible, this type of structure can be a reference model for preparing various probe materials for a sandwich-type immunoassay using ICP-MS.

In order to achieve the study goal, we synthesized metal-deposited mesoporous silica nanoparticles for the first time, in which CeO_2 was deposited on the mesoporous shell of the silica core. Ce is an excellent element in ICP-MS measurement because it suffers almost no spectral interference from molecular ions and has a low background. Furthermore, the synthesized magnetic nanoparticles (MNPs) were also synthesized to extract and separate bio targets through magnetic extraction for high efficiency. The high sensitivity of the designed technique due to the signal amplification of tagged nanoparticle and long linear dynamic range by ICP-MS, was suggestive of excellent analytical performance in the determination of biomarkers.

For the experimental demonstration, carcinoembryonic antigen (CEA) was selected because it is a widely used tumor marker in clinical diagnosis. It is a member of cell surface glycoproteins that appear at high levels in the blood of patients for colon, breast, pancreas, and lung carcinomas [7]. Since it has been expressed only in such cancer cells for adults, the measured level can provide important information in cancer diagnosis. For example, a serum CEA level in the range of 2.5–5.0 ng mL^{-1} is regarded as a reference for healthy individuals, whereas a level higher than 10 ng mL^{-1} is a marker for colorectal carcinoma. Due to this narrow diagnostic range, highly reliable and sensitive detection methods are required for clinical application. Various analytical methods have been developed so far, such as amperometry, chemiluminescence, surface plasmon resonance, enzyme-linked immunosorbent assay, as shown in Table 1. However, they showed limitations in detection limit and linearity of calibration. Although the electrochemiluminescence recently showed the best detection limit of 0.001–10 ng mL^{-1} as listed in the table, the complicated and long reaction of various amplifiers induced some difficulties in analytical measurement, and led to poor analytical efficiency for practical application.

2. Experimental

As a sandwich-type platform, two nanoparticles were used in this work, i.e., Fe_3O_4 MNPs for magnetic separation and CeO_2-deposited mesoporous silica nanoparticles as a probe. First, the CEA targets were collected by the antibody-immobilized MNPs and then tagged by the mesoporous silica nanoparticles through antigen–antibody reaction. The deposited Ce atoms of the tagged nanoparticles were determined by ICP-MS for quantification of CEA.

2.1. Synthesis and amine-functionalization of mesoporous CeO_2-deposited silica nanoparticles and Fe_3O_4 MNPs

The mesoporous CeO_2-deposited silica nanoparticles were prepared by incorporating CeO_2 components into the mesoporous shell of the silica using a method similar to that reported previously [17]. The silica spheres (0.25 g) were completely dispersed in a 20 mL of ethanol (99.99%, Sigma–Aldrich Chem. Co., LLC, MO, USA) by sonication. Ethanol (20 mL), $Ce(NO_3)_3{\cdot}6H_2O$ (0.5 g, 99.99%, Sigma–Aldrich Chem. Co., LLC, MO, USA) and IGEPAL CO-520 (1 mL, Sigma–Aldrich Chem. Co., LLC, MO, USA) were added to the solution. After stirring for 1 h, ethanol (20 mL) and NH_4OH (0.05 mL, 28%, Sigma–Aldrich Chem. Co., LLC, MO, USA) were added to the mixed solution. The resultant colloidal particles were separated by centrifugation, followed by drying in an oven. The solid particles were annealed at 500 °C for 5 h, yielding CeO_2-deposited silica nanoparticles. For functionalization of the amine group, 10 mg of the prepared nanoparticles stored in anhydrous ethanol was dispersed in 2 mL ethanol and sonicated for 1 h. Then, 100 μL of 3-aminopropyltrimethoxysilane (APTMS, 99%, Sigma–Aldrich Chem. Co., LLC, MO, USA) was added dropwise, followed by vigorous stirring for 3 h. The amine-functionalized silica nanoparticles were purified by centrifugation and washing four times with ethanol.

Fe_3O_4 NMPs were synthesized by co-precipitation according to a previously reported method [18]. After 0.5 g $FeCl_2{\cdot}H_2O$ (97% reagent grade, Sigma–Aldrich Chem. Co., LLC, MO, USA) and 1.35 g $FeCl_3{\cdot}H_2O$ (97% reagent grade, Sigma–Aldrich Chem. Co., LLC, MO, USA) were dissolved in 25 mL de-ionized water, the solution was heated to 80 °C under Ar environment while stirring. After the precipitates were formed, 12.5 mL NH_4OH was added slowly and reacted for 20 min. The MNP cores were washed with ethanol three times using a permanent magnet. In the Stöber method, used for silica shell coating, 350 mg of the prepared MNPs was dispersed in 20 mL ethanol and sonicated. Then, 520 μL NH_4OH, 520 μL de-ionized water, and 3.3 mL tetraethoxysilane (TEOS, 99.999%, Sigma–Aldrich Chem. Co., LLC, MO, USA) were added and stirred

Table 1
Typical analytical methods and performance for the determination of CEA.

Immunoassay method	Linear range (ng mL^{-1})	Detection limit (ng mL^{-1})
Amperometric immunoassay [8]	0.01–160	0.005
Chemiluminescence enzyme immunoassay [9]	2–162	0.69
Potentiometric immunoassay [10]	1.5–200	0.5
Surface plasmon resonance immunosensor [11]	0.1–40	0.1
Magnetic immunoanalysis with ICP-MS [12]	0.1–50	0.041
Chemiluminescent and colorimetric methods immunoassay chip [13]	0.5–80	0.41
Resonance light scattering spectral immunoassay [14]	0.1–60	0.03
Electrochemiluminescent (ECL) immunosensor [15]	0.001–50	0.00038
Electrochemical immunoassay based on three-dimensional macroporous gold nanoparticles/graphene composite platform [16]	0.001–10	0.00035
CEA ELISA kit (Abcam, UK)	0.343–250	0.2