Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 153 (2016) 709–713

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

Development of a novel method for determination of mercury based on its inhibitory effect on horseradish peroxidase activity followed by monitoring the surface plasmon resonance peak of gold nanoparticles

Javad Khodaveisi [a], Ali Mohammad Haji Shabani [a], Shayessteh Dadfarnia [a,*], Masoud Rohani Moghadam [b], Mohammad Reza Hormozi-Nezhad [c]

[a] Department of Chemistry, Faculty of Science, Yazd University, 89195-741 Yazd, Iran
[b] Department of Chemistry, Faculty of Science, Vali-e-Asr University, Rafsanjan, Iran
[c] Department of Chemistry, Sharif University of Technology, Tehran 11155-9516, Iran

ARTICLE INFO

Article history:
Received 4 June 2015
Received in revised form 24 August 2015
Accepted 4 October 2015
Available online 9 October 2015

Keywords:
Localized surface plasmon resonance band
Inorganic mercury (Hg^{2+})
Horseradish peroxidase
Gold nanoparticle

ABSTRACT

A highly sensitive and simple indirect spectrophotometric method has been developed for the determination of trace amounts of inorganic mercury (Hg^{2+}) in aqueous media. The method is based on the inhibitory effect of Hg^{2+} on the activity of horseradish peroxidase (HRP) in the oxidation of ascorbic acid by hydrogen peroxide followed by the reduction of Au^{3+} to Au–NPs by unreacted ascorbic acid and the measurement of the absorbance of localized surface plasmon resonance (LSPR) peak of gold nanoparticles (at 530 nm) which is directly proportional to the concentration of Hg^{2+}. Under the optimum conditions, the calibration curve was linear in the concentration range of 1–220 ng mL^{-1}. Limits of detection (LOD) and quantification (LOQ) were 0.2 and 0.7 ng mL^{-1}, respectively and the relative standard deviation at 100 ng mL^{-1} level of Hg^{2+} was 2.6%. The method was successfully applied to the determination of mercury in different water samples.

1. Introduction

Horseradish peroxidase (HRP) is a glycoprotein containing intrachain disulfide bonds [1]. HRP is isolated from horseradish roots (*Amoraciarusticana*) and belongs to the ferroprotoporphyrin group of peroxidases [2]. HRP has found enormous diagnostic, biosensing, and biotechnological applications [3–5] due to its high stability in aqueous solutions. HRP easily combines with hydrogen peroxide (H_2O_2) and the [HRP-H_2O_2] complex can oxidize a wide range of inorganic and organic compounds [6–10].

Inorganic mercury ions (Hg^{2+}) are highly toxic pollutants harmful to human health. Extreme accumulation of Hg^{2+} in human body has unfavorable effects on the nervous and immune system as well as the other organs, causing a number of diseases [11–14]. Thus, the development of a sensitive analytical method for the detection and determination of Hg^{2+} contamination has attracted considerable attention [15]. Various techniques including atomic fluorescence spectrometry (AFS) [16], inductively coupled plasma-optical emission spectrometry (ICP-OES) [17], gas chromatography-inductively coupled plasma-mass spectrometry (GC-ICP-MS) [18] as well as cold vapor atomic absorption spectrometry (CVAAS) [19,20] have been reported for the determination of low levels of mercury in aqueous samples. Although most of these methods are characterized by high sensitivity, they often suffer from the time consuming sample preparation and the preconcentration procedures besides the costly and complicated instrumentation. The test method, using enzymes characterized by high sensitivity, good selectivity and simplicity of operation, is a promising technique for the determination of micro quantities of different toxicants [21]. The test procedure and test devise for the determination of low amounts of mercury have been developed based on its inhibitory effect on the catalytic activity of HRP [22–25].

Colorimetric assays, based on nobel metal nanoparticles, have recently gained considerable interest due to their simplicity and great magnitude of molar absorption coefficients (~3×10^{11} mol^{-1} L cm^{-1}) [26,27]. The strong absorption band appears when the frequency of the incident photon is resonant with the collective excitation of the conduction electrons. This phenomenon is known as the localized surface plasmon resonance (LSPR). LSPR depends on the size, shape, and inter-particle spacing of the nobel metal nanoparticles as well as their dielectric properties and their local environment [28–31]. The spectrophotometric monitoring based on LSPR band of gold and silver nanoparticles was used for the determination of metal ions [32–38], anions [39], molecules [40,41], amino acids [42,43] and proteins [44,45].

This paper deals with an indirect spectrophotometric method for the determination of Hg^{2+} based on the inhibitory effect of Hg^{2+} on the catalytic activity of HRP enzyme in the oxidation of ascorbic acid by hydrogen peroxide followed by the reduction of Au^{3+} to Au–NPs

* Corresponding author.
E-mail address: sdadfarnia@yazd.ac.ir (S. Dadfarnia).

http://dx.doi.org/10.1016/j.saa.2015.10.004
1386-1425/

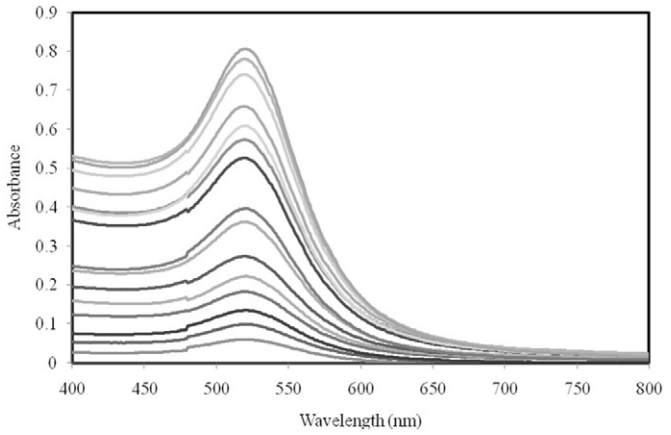


Fig. 1. Absorbance spectra of Au–NPs in the presence of different concentrations of Hg^{2+} (1.0–220.0 ng mL^{-1}). Conditions: Au^{3+} 0.2 mmol L^{-1}, H_2O_2 0.6 mmol L^{-1}, ascorbic acid 50.0 μmol L^{-1}, HRP 100.0 nmol L^{-1}, CTAC 1.0 mmol L^{-1}, pH 7.0.

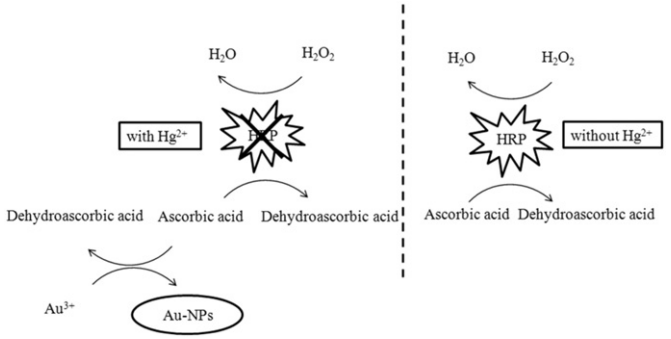
by unreacted ascorbic acid and the measurement of the absorbance of LSPR (at 530 nm) which is directly proportional to the concentration of Hg^{2+}. The developed method was finally applied to the determination of Hg^{2+} in water samples.

2. Experimental

2.1. Reagents and apparatus

Tetrachloroauric acid ($HAuCl_4.4H_2O$, 99.99%) was purchased from Sigma-Aldrich (Steinheim, Germany) and cetyltrimethylammonium chloride (CTAC, 99%) from Acros (Geel, Belgium). All other reagents were of analytical grade and were purchased from Merck (Darmstadt, Germany). Double distilled water was used for the preparation of the solutions. All the experiments were performed at the ambient temperature (25 ± 2 °C). Stock solution of $HAuCl_4$ (10.0 mmol L^{-1}) was prepared by dissolving 0.3398 g of $HAuCl_4$ in water and diluting it to 100 mL with distilled water. The solution of CTAC (0.1 mol L^{-1}) was prepared daily by dissolving 0.8 g of CTAC in water and diluting it to 25 mL with distilled water. Ascorbic acid (10 mmol L^{-1}) and hydrogen peroxide (48 mmol L^{-1}) solutions were prepared daily in distilled water. HRP solution (50 μmol L^{-1}) was prepared by dissolving an appropriate amount of the enzyme in distilled water. The enzyme concentration was determined by measuring its absorption at 404 nm ($\varepsilon = 1.33 \times 10^5$ mol^{-1} L cm^{-1}) [46]. The phosphate buffer (0.01 mol L^{-1}) was prepared by dissolving 0.3 g of $NaH_2PO_4.H_2O$ and 1.1 g of Na_2HPO_4 in distilled water, adjusting the pH to 7.0 and diluting to 1 l.

The absorbance spectra were recorded by a UV–vis spectrophotometer (HACH DR 3900, Loveland, Colorado, USA) using a 1.0 cm glass cell. The pH was measured with an EcoMet Model P25 pH meter (Guro-gu, Seoul, Korea) equipped with a combined glass calomel electrode. The



Scheme 1. Inhibitory effect of Hg^{2+} on HRP activity.

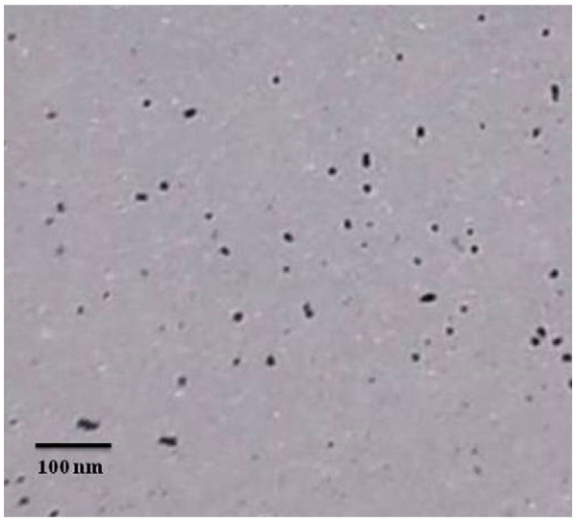


Fig. 2. TEM image of Au–NPs formed by ascorbic acid.

size of the Au–NPs was characterized by transmission electron microscopy (TEM) using a Zeiss transmission electron microscope (Jena, Germany) operating at an accelerating voltage of 80 kV.

2.2. General procedure

1 mL phosphate buffer, 20 μL ascorbic acid (10.0 mmol L^{-1}), 10 μL HRP (50.0 μmol L^{-1}) and 50 μL of hydrogen peroxide were added to 3 mL of sample or standard solution containing 5.0–1100.0 ng of Hg^{2+} in a 5 mL volumetric flask at 25 ± 2 °C. After the addition of hydrogen peroxide the mixture was left for 2 min to guarantee the irreversible inactivation of HRP [19]. Then, 0.1 mL $HAuCl_4$ (10 mmol L^{-1}) and 0.05 mL CTAC solution (0.1 mol L^{-1}) were added and the mixture was diluted to the mark with distilled water. The solution was then mixed, transferred into a 1 cm glass cell and after 2 minutes when the formation of NPs was completed, the absorbance of the intense LSPR band of Au–NPs was measured against the reagent blank at 530 nm.

3. Results and discussion

It has been verified that several compounds inhibit the catalytic activity of HRP through interaction with SH group of cysteine of the active site of the enzyme. This effect has been used for the spectrophotomtric or electrochemical determination of Hg^{2+} or the organomercury in the oxidation of o-dianisidine, o-phenylenediamine, 3,3′,5,5′-tetramethyl

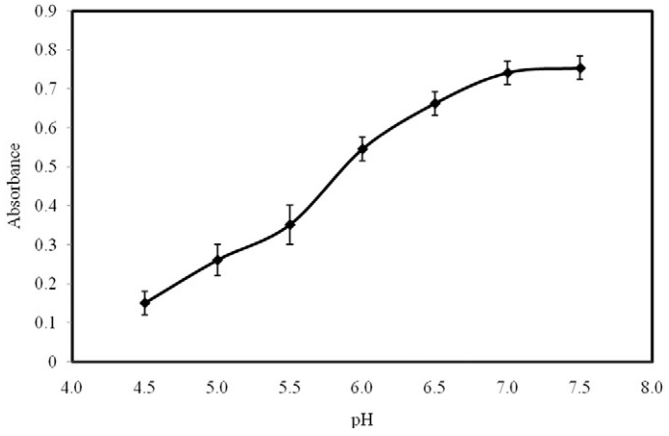


Fig. 3. Effect of pH on the synthesis of Au–NPs. Conditions: Au^{3+} 0.2 mmol L^{-1}, ascorbic acid 50.0 μmol L^{-1}, CTAC 1.0 mmol L^{-1}.