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Peroxidase activity of biogenic platinum nanoparticles: A colorimetric probe towards selective detection of mercuric ions in water samples

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ABSTRACT

A biogenic green method for the synthesis of platinum nanoparticles (Pt NP) was developed using non-toxic, renewable, biodegradable plant exudate gum, gum olibanum (*Boswellia serrata*). The effect of parameters such as concentrations of gum (0.1–0.5%) and chloroplatinic acid (0.125–1.0 mM) on nanoparticle synthesis was studied. Techniques such as UV–vis spectroscopy (UV-vis), X-ray diffraction (XRD), Fourier transform infrared spectroscopy (FTIR), transmission electron microscopy (TEM), dynamic light scattering (DLS) and zeta potential measurements were used to characterize the generated nanoparticles. The Pt NP formation was evidenced from the broad continuous absorption in the UV–vis spectra and appearance of black colour. The XRD pattern established the face centered cubic crystal structure of the fabricated nanoparticles. The produced NP were quasi-spherical in shape with a particle size of 4.4 ± 0.5 nm. The resulting nanoparticles exhibited excellent peroxidase activity which catalyzes the oxidation of the chromogen 3, 3′, 5, 5′-tetramethylbenzidine (TMB) to a blue colour product, in the presence of hydrogen peroxide. The peroxidase activity of the nanoparticles was selectively inhibited by mercuric ions (Hg^{2+}) due to the formation of amalgam by Hg-Pt specific interaction. Notably, the inhibition was not affected by other metal ions even at a concentration of 5 μM. The decrease in oxidized TMB intensity at 652 nm (blue colour), upon addition of mercuric ions was linear in the range of 50–500 nM for MilliQ, tap and ground waters and the respective limit of quantification values for Hg^{2+} using the developed method were 16.9, 26 and 47.3 nM. The proposed method was effectively applied for the determination of Hg^{2+} in various ground water samples and verified with CVAAS. We envisage that the biogenic Pt NP based colorimetric probe can have promising applications in the screening and field detection of mercuric ions in various water bodies and public drinking water distribution systems.

1. Introduction

Mercury, a toxic heavy metal with extensive distribution in air, water and soil and occurs in different chemical forms including metallic (Hg^0), inorganic (mercurous (Hg_2^{2+}), mercuric (Hg^{2+}) and organic mercury (methyl, ethyl, phenyl mercury) forms. Mercury contamination in environment is mainly due to anthropogenic activities such as emissions and effluents resulted from mining and combustion of coal by thermal power plants, incineration of solid wastes, industrial applications, and volcanic activity. It mainly affects various parts of the body such as gastrointestinal, cardiovascular, nervous, renal, reproductive, endocrine, respiratory and immune systems. The toxicity results from its high affinity towards sulfhydryl groups and bonding to sulfur-containing amino acids [1–3]. Among the existing forms, mercuric ion (Hg^{2+}) is the most stable state of inorganic mercury, and mostly exists in surface waters accounted for its higher water solubility [4]. Thus, the presence of divalent mercuric ions in drinking waters cause considerable public health hazards [5]. Regulatory bodies such Environmental Protection Agency (EPA) and World Health organization (WHO) have stipulated the maximum permitted limits for mercury in drinking water as 10 nM and 30 nM, respectively [6].

In literature, a variety of excellent standard methods have been used for mercury detection, which includes cold vapour atomic absorption spectrometry (CVAAS), atomic absorption/emission spectrometry (AAS/AES), inductively coupled plasma mass spectrometry (ICP-MS), atomic fluorescence spectrometry (AFS), high-performance liquid chromatography (HPLC), ion selective electrode (ISE), flame photometry and stripping voltammetry. However, these selective and sensitive techniques are limited for routine detection due to the expensive and complex instrumentation, time-consuming sample preparation and preconcentration

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procedures and non-portable nature [4,7]. While, the noble metal nanoparticles of silver, gold have been utilized as colorimetric and fluorimetric probes for visual detection of mercury based on the associated colour change with surface plasmon resonance (SPR) and fluorescence phenomenon [4,5,7–14]. Again, the selectivity and sensitivity of these systems are restrained by the selection surface label molecules making them as complex and less practical options for regular mercury monitoring [15].

Various nanoparticles such as magnetite, gold, copper oxide, copper sulphide etc are known to exhibit intrinsic peroxidase activity and the phenomenon has been extensively utilized for the colorimetric detection of hydrogen peroxide, glucose, melamine etc [16–19]. Currently, the nanoparticles are gaining importance as enzyme mimics/artificial enzymes due to the absence of inherent limitations of natural enzymes such as the availability of natural resources, tedious, time consuming and expensive purification process; rigorous storage conditions and sensitivity towards high temperatures, string acid and alkaline pH conditions, proteases leading to low stability and shelf life [16,18]. In addition, the inorganic catalytic nanoparticles are bestowed with qualities including resistant to high concentration of substrate, high stability, low cost and ease of synthesis and storage [20,21]. Platinum nanoparticles are widely used in different industries as catalyst [22–24] and the shape [23–27] and size [28] controlled synthesis of them can be easily achieved by various methods. It is reported that the platinum nanoparticles can exhibit various enzymatic activities such as catalase, oxidase, superoxide dismutase and peroxidase. But, the studies on application of the peroxidase activity of platinum nanoparticles towards colorimetric detection of mercury are limited [15,29]. This has inspired us to the development of real-time, peroxidase based colorimetric method towards the detection of mercury, which is rapid, simple, feasible, selective and sensitive.

In the present communication, we have developed a facile, green method for the biogenic synthesis of platinum nanoparticles from chloroplatinic acid using gum olibanum (*Boswellia serrata*), as a reducing and stabilizing agent. In an earlier studies carried out by us, this renewable, exudate biopolymer was successfully employed for the biosynthesis of silver and palladium nanoparticles and applied as antibacterial and catalytic agents, respectively [30,31]. The produced nanoparticles were characterized for UV–vis absorption, crystal structure, particle morphology, size, hydrodynamic diameter and zeta potential. The fabricated nanoparticles produced by autoclaving were sterile and exhibited smaller particle size compared to many green synthetic methods. They also showed excellent tolerance/stability towards higher temperatures, pH change and storage conditions. The platinum nanoparticles were evaluated for peroxidase activity by catalyzing the oxidation of peroxidase substrate 3, 3′, 5, 5′-tetramethylbenzidine (TMB) by hydrogen peroxide, producing blue coloured product, which can be detected by naked eye or spectroscopy. Further, a sensitive and selective colorimetric method for mercuric ion detection was developed, based on the down regulating activity of mercuric ions towards the peroxidase activity of platinum nanoparticles. A linear relationship in the concentration range of 50–500 nM of Hg^{2+} was found for MilliQ water, tap water and ground waters, with respective limit of quantification (LOQ) of 16.9, 26 and 47.3 nM. The developed method provides sufficiently good sensitivity for screening and monitoring of mercuric ions in various water matrices. While, the other colorimetric methods employ unstable citrate capped gold nanoparticles and demand complex ligand functionalization process for selectivity. Also, the used ligands are costly and require lower temperatures for improved shelf life. In this study, we exploit highly stable, biogenic Pt NP for selective detection and quantification of mercuric ion; which require no ligand conjugation for direct application.

2. Materials and methods

2.1. Materials

Chloroplatinic acid hexahydrate (Sigma, Bengaluru, India) and Grade-1 gum olibanum (Girijan Co-operative Corporation Ltd., Hyderabad, India) was used for nanoparticle synthesis. All the solutions were prepared in MilliQ water with resistivity of 18 MΩ cm. The gum was powdered in a Prestige Deluxe-Vs high speed mechanical blender (Bengaluru, India) and sieved to obtain a particle size of 38 μm. A 0.5% (w/v) of homogenous gum stock solution was made in ultrapure water by stirring overnight at room temperature. Then, the solution was centrifuged (5500*g*, 10 min) to remove the insoluble materials and the supernatant was used for synthesis. 3, 3′, 5, 5′-tetramethylbenzidine (TMB), metal salts and hydrogen peroxide (H_2O_2) of AR grade was used (E. Merck, Mumbai, India). Mercuric chloride standard stock solution (1000 mg/L) in 5% HNO_3 traceable to NIST 3133 (SD Fine Chemicals Ltd, Mumbai, India) was used. Other standard metal stock solutions of were prepared by dissolving an appropriate amount of their corresponding nitrate and chloride salts in ultrapure water.

2.2. Synthesis of platinum nanoparticles

The platinum nanoparticles were synthesized by autoclaving the gum solutions containing chloroplatinic acid (H_2PtCl_6) at 121 °C and 103 kPa for 30 min. The concentration effect of gum (0.1–0.5%) and H_2PtCl_6 (0.125–1.0 mM) on nanoparticle synthesis was investigated.

2.3. Characterization of synthesized platinum nanoparticles

The UV–vis absorption spectra of the prepared platinum nanoparticle solutions were recorded against autoclaved gum blanks using Analytik Jena AG, Specord 200 Plus UV–vis spectrophotometer (Jena, Germany), in a wavelength range of 200–500 nm. The X-ray diffraction analysis was conducted with a Rigaku, Ultima IV diffractometer (Tokyo, Japan) using monochromatic Cu Kα radiation (λ = 1.5406 Å) running at 40 kV and 30 mA. The intensity data for the nanoparticle suspension deposited on a glass slide was collected over a 2θ range of 35–85° with a scan rate of 1°/min. The size and shape of the nanoparticles were obtained with FEI Tecnai 20 G2 S-Twin (Eindhoven, Netherlands) transmission electron microscope (TEM), operating at 200 kV. The samples for TEM were prepared by depositing a drop of colloidal suspension on a carbon coated copper grid and drying at room temperature. The z-average particle size and zeta potential of the produced platinum nanoparticles were recorded with a Malvern Zetasizer Nano ZS90 (Malvern, UK). The nanoparticle solution was made into powder using an FTS Systems, Dura-Dry™ MP freeze dryer (New York, USA). The IR spectra of the lyophilized samples were recorded using Bruker Optics, TENSOR 27 FTIR spectrometer (Ettlingen, Germany); over a spectral range of 1000–4000 cm^{-1}.

2.4. Peroxidase activity of synthesized platinum nanoparticles (Pt NP)

The peroxidase activity of Pt NP was studied in a microtiter plate at room temperature by probing the catalytic oxidation of TMB in 0.1 M acetate buffer (pH 4) in the presence of H_2O_2 to generate a blue coloured reaction product, which can be detected spectrophotometrically at 652 nm. The mechanism of catalytic activity was monitored with (a) TMB, (b) TMB + Pt NP, (c) TMB + H_2O_2 and (d) TMB + H_2O_2 + Pt NP. The effect of variation in pH (2–6), temperature (20–60 °C) and concentration of Pt NP (1–15 μM), H_2O_2 (0.25–10 mM) and TMB (62.5–500 μM) on peroxidase activity was