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Chemically modified cellulose strips with pyridoxal conjugated red fluorescent gold nanoclusters for nanomolar detection of mercuric ions

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ABSTRACT

One-pot approach was adopted for the synthesis of highly luminescent near-infrared (NIR)-emitting gold nanoclusters (AuNCs) using bovine serum albumin (BSA) as a protecting agent. The vitamin B_6 cofactor pyridoxal was conjugated with the luminescent BSA-AuNCs through the free amines of BSA and then employed for the nanomolar detection of Hg^{2+} in aqueous medium *via* selective fluorescence quenching of AuNCs. This nano-assembly was successfully applied for the real sample analysis of Hg^{2+} in fish, tap water and river water. The study also presents chemically-modified cellulosic paper strips with the pyridoxal conjugated BSA-AuNCs for detecting Hg^{2+} ion up to 1 nM.

1. Introduction

Mercuric ion (Hg^{2+}) is an extremely hazardous pollutant and highly carcinogenic transition metal ion due to its high toxicity and bio-accumulative nature (Aschner and Aschner, 1990; de Vries et al., 2007; Vasimalai and John, 2011). Various forms of mercury exist in soils (*i.e.* elemental mercury, organic and inorganic mercury) and their potential toxicity depends on their concentration and types present in the soil solution (Revis et al., 1989). Mercuric ions tend to have strong affinity for the ligands containing sulphur group and causes the blocking of sulfhydryl groups of proteins and enzymes that can damage the central nervous system, DNA mitosis and the endocrine system, and results in different severe disorders and diseases such as cognitive and motor disorders, Minamata disease, coronary heart disease and cardiovascular diseases (Gopal, 2003). Therefore, mercury loading into the ecosystems is inexorable as the compounds of mercury are highly reactive, extremely volatile and highly soluble in water. These detrimental results greatly demands for the development of analytical procedures for the determination of trace amounts of mercury. Various methods have been adopted for the detection are atomic absorption spectrometry (Krata et al., 2007), stripping voltammetry (Ashrafi and Vytřas, 2011) and inductively coupled plasma atomic emission spectrometry (Zhu and Alexandratos, 2007). However, these techniques are expensive and required sophisticated instrumentation and complicated sample pre-treatment methods. Thus, chemosensors based on colorimetric and fluorescence responses have significantly gained importance due to their ability for simple, rapid and sensitive on-site monitoring of target analytes in various biological and environmental samples (Hu et al., 2010; Sahoo and Baral, 2009).

The applications of fluorescent gold nanoclusters (AuNCs) consisting of only several to hundreds of gold atoms into the sensing and imaging fields have become the growing field of research because of their simple and one-pot green synthesis, high fluorescence quantum yield, superior catalytic activity, low toxicity, more biocompatibility and readily bio-conjugation as compared with semiconductor and carbon QDs (Shang et al., 2011; Shiang et al., 2012; J. Zhang et al., 2013; P. Zhang et al., 2013; Zhang et al., 2014). Controllable synthesis of gold nanoclusters with a specific number of gold atoms and tuneable optical properties can be achieved by controlling the reaction conditions such as pH, temperature etc. (Wang et al., 2015). Thus, fluorescence emission of AuNCs could be readily adjusted from the visible to near-infrared region leading to wide range of applications, including chemical and biological sensing (Shang et al., 2011; J. Zhang et al., 2013; P. Zhang et al., 2013), cellular and animal imaging (Zhang et al., 2014), as well as cancer therapy (Wang et al., 2011). Nowadays, Protein scaffolded AuNCs especially bovine serum albumin (BSA)-capped red-emitting gold nanoclusters (BSA-AuNCs) are becoming increasingly attractive for biological labeling and imaging, diagnostic and therapeutic applications due to their good biocompatibility, water solubility and high photostability (Tao et al., 2015; Xie et al., 2009). Recently, the BSA-AuNCs system has been successfully applied for the detection of metal ions (Durgadas et al., 2011; Hu et al., 2010), anions (Liu et al., 2010; J. Zhang et al., 2013; P. Zhang et al., 2013), biological thiols (Park et al., 2013) and proteins (Hu et al., 2012).

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As a part of our ongoing research on vitamin B_6 cofactors (Bothra et al., 2015; Sharma et al., 2015), the luminescent gold nanoclusters was first synthesized using BSA as a template and then conjugated with the vitamin B_6 cofactor pyridoxal (Py). It was expected that the free amines present in the coated BSA react with the aldehyde group of pyridoxal. Vitamin B_6 cofactor plays crucial roles in enzymatically catalysed transaminations to form an α-keto acid and pyridoxamine 5-phosphate as well as in many other biosynthetic processes (Hayashi et al., 2003; Liu et al., 2005). Pyridoxal containing enzymes are central to numerous metabolic pathways such as decarboxylations of amino acids (Fogle et al., 2005), racemization of amino acids (Sun and Toney, 1999) and aldol type addition of the pyridoxal stabilized glycine carbanion to formaldehyde or acetaldehyde (Mukherjee et al., 2011). The developed pyridoxal conjugated BSA-AuNCs system was successfully applied for the selective fluorescent detection of Hg^{2+} in aqueous medium with low limit of detection in comparison with the reported papers (Hu et al., 2010). For real on-site detection, we have also designed a simple and robust paper-based analytical technology for the sensing of Hg^{2+} in water *via* covalent anchoring of the nanosensor onto the cellulose paper. The paper-based sensors offer portability and operational simplicity by physisorption of molecular sensors and can be applied in many applications such as bio-diagnosis, water and seafood control as well as environmental markers (Martinez et al., 2007, 2010).

2. Experimental section

2.1. Reagents and instruments

Bovine serum albumin (BSA) was obtained from SRL Pvt. Ltd. Gold(III) chloride trihydrate ($HAuCl_4{\cdot}3H_2O$), pyridoxal hydrochloride, pyridoxamine hydrochloride and pyridoxine were purchased from Sigma Aldrich, India. All the metal salts and sodium hydroxide used for the experiments were procured from Rankem Pvt. Ltd., India. All the anions used in the form of tetra-n-butyl ammonium (TBA) salts were purchased from Spectrochem Pvt. Ltd., India. Stock solutions of the metal ions (1 mM), vitamin B_6 cofactors (1 mM) and anions (1 mM) were prepared freshly in double distilled water. These solutions were used for all spectroscopic studies after appropriate dilution. Hydrochloric acid (0.1 N) and sodium hydroxide (0.1 N) solutions were used to adjust the pH. Whatman® grade 1 filter paper (3.0 cm) was used as cellulose source.

The UV–Vis absorption spectra were recorded on a Cary 50 Varian UV–Vis spectrophotometer at room temperature using quartz cells of 1.0 cm optical path length in the range of 200–800 nm. Fluorescence measurements were carried out at an excitation wavelength of 360 nm, and the emission range of 380–800 nm by using a Cary Eclipse Fluorescent spectrophotometer. The excitations and emission slits were 5 nm and 10 nm, respectively. The observed pH was measured as $-\log(H^+)$ using a HANNA HI 2223 pH meter equipped with a calibrated combined glass electrode with standard buffer solutions. The FT-IR spectra of the bio-conjugated NCs before and after addition of analytes were recorded by using the ATR-FTIR method from 4000 to 600 cm^{-1} using Shimadzu IR Affinity1 FTIR. High resolution transmission electron microscopy (HRTEM) was recorded on a Tecnai T 20 S-Twin. For the TEM analysis, the samples were prepared by drop-coating NCs dispersions onto copper TEM grids of 3 mm diameter, which were subsequently air-dried. Field emission scanning electron microscopy (FE-SEM) was done using a NovaNanoSEM 450 instrument. Energy-dispersive X-ray (EDX) was performed on a JEOL JSM-7600F FEG-SEM (field emission gun, accelerating voltage: 0.1–30 kV, in lens detector of secondary electrons). A Multimode-8-AM AFM instrument was used for height, amplitude, and phase imaging. The X-ray photoelectron spectroscopy (XPS) analysis was carried out on an Omicron ESCA (Electron Spectroscope for Chemical Analysis), Oxford Instrument Germany. Circular dichroism (CD) spectroscopic measurements were performed using a Jasco Corp, J 715 Dichroism Spectrometer.

2.2. Synthesis of BSA-AuNCs

BSA-AuNCs were synthesized using BSA as “soft templates” to grow highly luminescent NCs following the procedure of Xie et al. with some modifications (Xie et al., 2009). In a typical experiment, all glasswares used in the experiments were cleaned in a bath of freshly prepared aqua regia ($HCl:HNO_3$ volume ratio=3:1), and rinsed thoroughly in double distilled water prior to use. Under vigorous stirring at 37 °C, 5 mL aqueous $HAuCl_4$ solution (10 mM) was added to the BSA solution (5 mL, 50 mg/mL) followed by the addition of 0.5 mL 1.0 N NaOH solution. The mixture was incubated at 37 °C for 12 h. The color of the solution changed from light yellow to deep brown. The product was dialyzed against double distilled water for 48 h to remove unreacted $HAuCl_4$ or NaOH using dialysis membrane and was stored at 4 °C in refrigerator. The 1.5 mL of prepared NCs was diluted to 25 mL with water prior to further use. The concentration of BSA-AuNCs was 17 μM calculated by spectrophotometry using a molar absorptivity of 44,000 M^{-1} cm^{-1} at 278 nm (Shang et al., 2007).

2.3. Interaction of pyridoxal with BSA-AuNCs

Interaction of various vitamin B_6 cofactors (pyridoxal, pyridoxamine and pyridoxine) with BSA-AuNCs was studied using UV–Visible and fluorescent measurements. The titration studies were carried out by adding different concentrations of pyridoxal solution (1 mM) to BSA-AuNCs solution, and then allowed to interact for 15–20 min. From the titration data, the calibration curve (fluorescent intensity/Abs *vs* [Py]) was plotted, and then the obtained slope was used to calculate the limit of detection (LOD). The LOD based on the IUPAC definition can be estimated using the formula, LOD=K (α/s) (where K=3, α=standard deviation of blank signal and s=slope) (Sharma et al., 2013). To calculate the relative standard deviation, the fluorescence measurements of ten blank samples were taken.

The optimized concentration of 0.32 mM pyridoxal was added into 1.0 mL diluted BSA-AuNCs (17 μM) and maintained for 10 min. Selectivity studies were performed by recording the fluorescence spectra of the developed sensor solution after 10 μM addition of each representative metal ions (Cu^{2+}, Co^{2+}, Mg^{2+}, Hg^{2+}, Ni^{2+}, Mn^{2+}, Zn^{2+}, Cd^{2+}, Fe^{2+}, Pb^{2+}, Fe^{3+}, Cr^{3+} and Al^{3+}) and anions (Cl^-, Br^-, I^-, F^-, HSO_4^-, CN^-, AcO^- and $H_2PO_4^-$). Interference study was carried out by addition of equimolar amount of other metal ions under a competitive environment of Hg^{2+} to the developed fluorescent system. A continuous titration study was carried out by successive addition of 10 μM Hg^{2+} solution to the developed sensor system under optimized conditions.

2.4. Fluorescent detection of Hg^{2+} in Fish

The developed fluorescent system was applied for the determination of Hg^{2+} in the fish samples obtained from river (roho labeo i.e. *labeo rohita*) and sea (Indian Mackerel i.e. *Rastrelliger Kanagurta*) water. Nitric acid (50%) and sulphuric acid (25%) were used for the digestion of the fish. The small parts from the head, tail and viscera of river fish (4.4 g) and sea fish (4.2 g) was kept in a round bottom flask. Subsequently, 4.3 mL nitric acid and 2.8 mL sulphuric acid were added. The round bottom flask was placed in an oil bath at a temperature of 120 °C for 2 h. After filtration of the digestion solution, the pH of supernatant was adjusted to ~7.0 using 1 M NaOH solution. Then, the solution was diluted appropriately and added in to the sensor solution for fluorescent measurement.