Effect of Nobel Metal Ions on the Synthesis of Metal Nanoclusters for Selective Detection of Various Heavy Metals

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Abstract

In this study, effect of noble metal ions (Au, Ag and Cu) on the synthesis of metal nanoclusters (MNCs) have been investigated. Through heating at 70°C, TSA/BSA–Au, –Ag and –Cu NCs were separately prepared from Au³⁺, Ag⁺ and Cu²⁺ respectively in the presence of bovine serum albumin (BSA) and thiosalicylic acid (TSA). They exhibit photoluminescence (PL) at 700, 624 and 430 nm, with an average life times of 1500, 100 and 11.71 ns, respectively, when excited at 350 nm. X-ray photoelectron spectroscopy (XPS) data support the presence of metal core (M⁰) and metal–thiolate shell (M_n–SR_m) in each of the TSA/BSA–Metal nanoclusters (MNCs). Spectroscopic measurements reveal the formation of Au₃₂–SR, Ag₉–SR and (Cu₄–Cu₁₃)–SR species in the TSA/BSA–Au, –Ag and –Cu NCs respectively. Through PL quenching of the TSA/BSA–Au, –Ag and –Cu NCs, they have been used separately for quantitation of Hg²⁺, As³⁺ and Cr⁶⁺, with linear ranges of 1–400, 4–18, and 40–400 nM and limits of detection (LODs) of 0.25, 2.34 and 3.54 nM, respectively. The PL quenching is mainly due to aggregation of the MNCs via metal–metal or metal–thiol interaction. The stable TSA/BSA–Au, –Ag and –Cu NCs have been employed separately for the determination of the concentrations of Hg²⁺, As³⁺ and Cr⁶⁺ ions in the spiked sea water samples, showing advantages of simplicity, rapidity, high selectivity, and sensitivity.

Keywords: Metal nanoclusters, Thiosalicylic acid, BSA, Fluorescence, Heavy metal, sensors

Introduction

Mercury (Hg²⁺), Arsenic (As³⁺) and Hexavalent chromium (Cr⁶⁺) are extremely toxic heavy metals and possess a devastating health risk to a living kind, hence causing the renal, respiratory and neural systems related disorders [1]. According to Environmental Protection Agency (EPA), the maximum permissible limit Hg²⁺, As³⁺ and Cr⁶⁺ in drinking water are 2, 10, and 100 ppb respectively. Conventional analytical methods used to detect such heavy metals in drinking or environmental samples includes HPLC, AAS/AES, GC–MS, etc., however these techniques are time-consuming, expensive, requires a skilled personals, sophisticated instruments, and lack of field deployability.

In the past decade, colorimetric methods using carbon (e.g., graphene oxide, carbon dots) and metal (e.g., Au, Ag, Cu, Fe) based nanomaterials (size ≥ 10 nm) functionalized with various labels (e.g., DNA, dye, protein, thiol ligands etc.) have been developed for detection of heavy metal ions [2]. Such visual inspection methods are high throughput, offered better sensitivity and selectivity, also enabled multichannel detection by modifying the labels. Nevertheless, label free methods with

improved features including simplicity, speed, compatibility, sensitivity, and selectivity are highly demanding. Recently, photoluminescent MNCs decorated with biomolecules (protein, DNA, etc.)/ligand (thiols, polymers etc.) have gained immense interest as sensing probes due of their fascinating core shell structure with extremely tiny size (~ 2 nm), and large stokes shift [3].

PL quenching based sensing strategy interest scientific community because it is sensitive, rapid, simple, highly selective and have on-site applicability. Various types of MNCs have been reported to exhibit PL properties via quantum confinement effect, charge transfer complex properties, and their interaction with analytes results in the change in their PL intensity which enable them for metal ion detection [4]. However, the application of MNCs for heavy metal ion detection is a challenge due to the poor selectivity, arising from the similar metal-metal interaction of different metal ions with the MNCs and also complexation with the ligands. Therefore, to achieve high selectivity of the MNCs-based systems, additional strategies, such as pretreatment of the samples and masking agents are required. In our previous study, we reported the effect of TSA isomers on the optical properties of copper nanoclusters aggregates prepared in different solvents [5].

In this study, we investigated the effect of metal core on PL properties and interaction with heavy metal ions, when stabilized by bovine serum albumin (BSA) and TSA in the aqueous solution. We find ourselves inclined towards building a sensor array for the detection of toxic heavy metal ions using different metal core such as Au, Ag and Cu with a same surface ligand, by simple procedure. We used a simple hydrothermal method to prepare Au, Ag and Cu NCs from respective ions in a separate solution containing TSA and BSA to form TSA/BSA-Au, TSA/BSA-Ag and TSA/BSA-Cu NCs with sub nanometer size and possess a strong interaction with Hg^{2+} , As^{3+} and Cr^{6+} , respectively. A facile, rapid, inexpensive, highly selective and sensitive technique for the detection of Hg^{2+} , As^{3+} and Cr^{6+} in aqueous solution, based on metallophilic and metal-thiol complexation or inner filter effect induced PL quenching of MNCs have been developed. A schematic representation of the synthesis of BSA and TSA capped MNCs and the method of detection of heavy metal ions is presented in Figure 1.

Figure 1 Schematic representation of noble metal ion effect on the synthesis of TSA/BSA stabilized MNCs. Equivalent concentration (5 mM) of Au^{3+} , Ag^+ and Cu^{2+} ions when incubated with BSA (100 μM) and TSA (10 mM) at 70°C results in the formation of tiny sized photoluminescent TSA/BSA-Au NCs (red), TSA/BSA-Ag NCs (orange), and TSA/BSA-Cu NCs (blue) and further demonstration of the selective interaction with Hg^{2+} , As^{3+} and Cr^{6+} respectively. Digital photographs showing the real time solutions of MNCs under day light (left side) and UV-light (right side).

Material and Methods

Synthesis of TSA/BSA-Au, Ag and Cu NCs

In a 1 mL vial HAuCl_4 , AgNO_3 and $\text{Cu}(\text{NO}_3)_2$ (100 mM, 50 μL) was added separately to the solution containing BSA (6.64 mg/mL), TSA (10 mM) which were then subjected to heat at 70°C . As prepared Au, Ag and Cu NCs were cooled down to room temperature, purified using a dialysis and stored at 4°C dark.

Characterization

UV-Vis absorption and PL spectra of TSA/BSA-Au, Ag and Cu NCs were recorded using a double beam UV-Vis spectrophotometer (Cintra 10e, GBC, Victoria, Australia) and Cary Eclipse Fluorescence spectrophotometer (Varian, Palo Alto, CA, USA), respectively.

Detection of heavy metals

Sensing probes such as TSA/BSA–Au NCs ([Au]=1000 nM) and TSA/BSA–Ag NCs ([Ag]=100 nM) were mixed separately with the aliquots containing mixture of EDTA (10 mM) and Hg^{2+} (0 – 400 nM) and EDTA (10 mM), TSA (100 μM) and As^{3+} (0 – 20 nM) respectively dispersed in sodium phosphate buffer (10 mM, pH 7.0) at room temperature for 10 min. However, as prepared TSA/BSA–Cu NCs ([Cu]=80 nM), were spiked into the solution containing Cr^{6+} (0 – 400 nM) in sodium phosphate buffer (10 mM, pH 9.0) without any further treatment. Each of the solutions were then transferred individually into 96–well microtiter plates and their PL spectra were measured using a Synergy 4 microplate reader (BioTek, Winooski, VT, USA) at a fixed excitation wavelength of 350 nm. Limit of detections (LOD) were determined using the following formulae: $\text{LOD} = c \cdot \text{SD}/m$, where c is the signal to noise ratio, SD is the standard deviation of the blank and m is the slope of regression line.

Results and Discussion

Characterization

Figure 1 briefly describes the one–step synthesis approach used for the preparation of highly fluorescent, ultra–stable metal NCs. BSA actuates the nucleation and growth of metal NCs which are facilitated by the amino acids such as cysteine, histidine (coordination with metal ions) and tyrosine (assist in cluster formation) [5]. Figure 2 shows the absorption (2A), and emission (2B) spectra of TSA/BSA–Au, –Ag, and –Cu NCs respectively. UV–Vis absorption spectra of TSA/BSA–Au, –Ag and –Cu NCs shows a small hump around 300 nm due to the presence of TSA/BSA–MNCs, whereas absence of characteristic peak around 500 to 600 nm confirms that solution is devoid of larger particles. TSA/BSA–Au, –Ag and –Cu NCs emitted maximum PL intensity at 700 (bright red), 624 (orange) and 430 (blue) nm respectively upon excitation with 350 nm. Such large stock shifts attributed to the coordination of Au^+ and Ag^+ with the surface groups of TSA and BSA inducing ligand–to–metal–metal charge transfer (LMMCT) and/or ligand–to–metal charge transfer (LMCT) [6].

Figure 2 (A) UV–Vis absorption, and (B) PL emission spectra of TSA/BSA–Au, TSA/BSA–Ag and TSA/BSA–Cu NCs. Each solution was separately diluted 1000–fold with sodium phosphate buffer (10 mM, pH 7.4) for the absorption and PL measurements.

Furthermore, our developed probe is stable for over 3 months when stored at 4° C dark (Figure 3A) and withstand high salt concentration i.e., upto 800 mM (Figure 3B) and UV (330 nm) exposure upto 12 h (Figure 3C).

Figure 3 The stability of TSA/BSA–Au, TSA/BSA–Ag and TSA/BSA–Cu NCs. The PL intensity at 700, 624 and 430 nm of as prepared photoluminescent TSA/BSA–Au, TSA/BSA–Ag and TSA/BSA–Cu NCs, respectively, (A) storage at 4 °C, (B) NaCl (0–800 mM), (C) photo–irradiation (UV–lamp, 365 nm).

Sensor performance

PL responses of TSA/BSA–Au, –Ag and –Cu NCs at 700, 624 and 430 nm, respectively, upon separate incubation with various metal ions (Cd^{2+} , Ni^{2+} , Mg^{2+} , Pb^{2+} , Mn^{2+} , Zn^{2+} , Co^{2+} , As^{3+} , Cr^{6+} , Cu^{2+} , Ag^+ , Fe^{3+} and Hg^{2+} ion, 100 μM) in sodium phosphate buffer (10 mM, pH 7.0) at room temperature for 10 min were studied. To circumvent the interference from the undesired metal ions and enable specific detection, EDTA (10 mM) and TSA (100 μM) & EDTA (10 mM) were mixed with the TSA/BSA–Au, and –Ag NCs respectively prior to addition of Hg^{2+} , and As^{3+} , however TSA/BSA–Cu NCs were directly employed for Cr^{6+} detection (Figure 4(A–C)–i). The analytically significant PL quenching of TSA/BSA–Au, –Ag and –Cu NCs by Hg^{2+} , As^{3+} and Cr^{6+} ions, respectively, were determined by measuring the PL intensity ratio $(I_{F0}-I_F)/I_{F0}$, where I_{F0} and I_F are the PL intensities (a. u) in the absence and presence of Hg^{2+} , As^{3+} or Cr^{6+} . PL intensity of TSA/BSA–Au, –Ag and –Cu NCs were gradually decreased with an increase in Hg^{2+} , As^{3+} and Cr^{6+} ions concentration respectively, resulting in a good linear relationship with the concentration of respective ions ranging from 1 to 400 nM ($R^2=0.9913$), 4 to 18 nM ($R^2=0.9897$), and 40 to 400 nM ($R^2=0.9905$), respectively and Limit of detection (LOD) of Hg^{2+} , As^{3+} and Cr^{6+} ions were found to be 0.25 nM (0.068 ppb), 2.34 nM (0.463 ppb) and 3.54 nM (1.04 ppb), respectively (Figure 4(A–C)–ii).

Figure 4 A–C (i) PL ratios $[(I_{F0}-I_F)/I_{F0}]$ at 700, 624 and 430 nm for TSA/BSA–Au, –Ag and –Cu NCs, respectively, measured in the absence and presence of TSA (100 μ M) and EDTA (1 mM) after the addition of 100 μ M metal ions in 5 mM sodium phosphate buffer solution. Figure 4A–C (ii) shows the linear range of TSA/BSA–Au, –Ag and –Cu NCs upon addition of Hg^{2+} , As^{3+} and Cr^{6+} respectively.

Conclusions

The effect of noble metal ions (Au, Ag and Cu) on the synthesis and optical properties of metal nanoclusters (MNCs) was studied. In conclusion a new, facile, rapid, and highly selective and sensitive technique for detection of Hg^{2+} , As^{3+} and Cr^{6+} ions using TSA/BSA–Au, –Ag, and –Cu NCs, respectively, in aqueous media based on metallophilic and metal–thiol interaction, and inner filter effect induced PL quenching have been developed. The TSA/BSA–Au and TSA/BSA–Ag NCs demonstrated remarkably high selectivity for Hg^{2+} and As^{3+} ions over other metal ions, in presence of masking agent EDTA and TSA–EDTA, respectively. However, TSA/BSA–Ag NCs exhibited intrinsic specificity toward Cr^{6+} without any pretreatment of the analytes or masking agent. The MNCs could detect as low as 0.25, 2.34 and 3.54 nM of Hg^{2+} , As^{3+} and Cr^{6+} ions, respectively. This strategy primarily involved green chemistry approach and due to great aqueous solubility (>100 days), photostability, salt tolerance (against NaCl up to 0.8 M) and therefore successfully employed for Hg^{2+} , As^{3+} and Cr^{6+} ions detection in the sea water.

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