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Alizarin Dye based ultrasensitive plasmonic SERS probe for trace level Cadmium detection in drinking water

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Abstract

Alizarin functionalized on plasmonic gold nanoparticle displays strong surface enhanced Raman scattering from the various Raman modes of Alizarin, which can be exploited in multiple ways for heavy metal sensing purposes. The present article reports a surface enhanced Raman spectroscopy (SERS) probe for trace level Cadmium in water samples. Alizarin, a highly Raman active dye was functionalized on plasmonic gold surface as a Raman reporter, and then 3-mercaptopropionic acid, 2,6-Pyridinedicarboxylic acid at pH 8.5 was immobilized on the surface of the nanoparticle for the selective coordination of the Cd (II). Upon addition of Cadmium, gold nanoparticle provide an excellent hotspot for Alizarin dye and Raman signal enhancement. This plasmonic SERS assay provided an excellent sensitivity for Cadmium detection from the drinking water samples. We achieved as low as 10 ppt sensitivity from various drinking water sources against other Alkali and heavy metal ions. The developed SERS probe is quite simple and rapid with excellent repeatability and has great potential for prototype scale up for field application.

Keywords

3-Mercaptopropionic acid; 2; 6-pyridinedicarboxylic acid; SERS; Gold nanoparticle

1. Introduction

Heavy metal contamination is a growing concern worldwide. Cadmium is one of the most toxic elements and widely found in fertilizers, fuel combustions, plastics toys and several other industrial products. According to the Centers for Disease Control and Prevention (CDC), cadmium ranks seventh out of 275 hazardous substances in the environment^{1,2}. Cadmium contamination is frequently found in jewelry and battery operated toys and paints. A recent investigation showed up to 90% of elemental Cadmium in more than 100 jewelry items for kids in various stores in Texas, New York, California and Ohio³. Number of

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methods have been developed to detect Cadmium such as Inductively Coupled Plasma Mass Spectrometry (ICP-MS), Atomic Absorption Spectrometry (AAS), electrochemical sensing, fluorescence turn on and off sensing techniques^{4–11}. Among many popular methods, fluorescence is a highly reliable and sensitive technique for metal detection. Kubo et al.¹² has reported a strategy based on the behavior of phenylboronic acids (PBAs) and Alizarin MeOH solution for the anion and cation sensing. There are many other reports based on fluorescence technique such as^{13–16}. However most fluorescence based Cd (II) sensors are quenched upon high Cd(II) coordination. They often rely on an irreversible Cd (II)-dependent chemical reactions to turn on the fluorescence^{10,17–19}. Also these systems are developed in organic solvent systems. As a result, these sensors display huge drawbacks for field applications due to interference with other heavy metal ion, hydrophobic nature, weak fluorescence enhancement and short emission wavelengths. Surface-enhanced Raman spectroscopy (SERS) is a powerful optical technique for sensitive and selective detection of toxic metals in aqueous solution. SERS based probes are very convenient and can be applied routinely at the single molecule level^{20–25}. The sensitivity of SERS in present work is reported to detect 2ppt divalent Cadmium ion selectively in aqueous solution. Recently, several groups including ours have reported that nanomaterial aided surface enhanced Raman spectroscopy is capable of measuring chemical and biological activity three times lower than other available methods^{26–41}. In this article we have developed a facile and practical Alizarin dye tagged SERS assay for the selective recognition of Cd(II) ions in aqueous solution. We have found that functionalized gold nanoparticles are aggregated in presence of Cd(II) by an ion template chelation process forming active traps of Alizarin molecule between gold nanoparticles which further enhances the SERS signal of about 30 times.

2. Experimental Section

2.1. Materials and Experiments

Hydrogen tetrachloroaurate(III) hydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), Sodiumborohydride (NaBH_4), Alizarin dye, 3-Mercaptopropionic acid (MPA), 2,6-Pyridinedicarboxylic acid (PDCA), HEPES buffer solution, Sodium chloride (NaCl) and Trisodium citrate dihydrate (TSC) were purchased from Sigma-Aldrich and used without further purification.

2.2. Gold Nanoparticle Synthesis

Gold nanoparticles (AuNPs) of diameters 12nm were synthesized by controlling the ratio of HAuCl_4 , H_2O and TSC using reported method^{42–45}. AuNPs of 13 nm (absorption maximum 520 nm) were synthesized using our method published before <http://www.sciencedirect.com/science/article/pii/S0925400512013123-bib0075>⁴⁶(TNT paper). To obtain a concentrated AuNP solution, 50 mL of the AuNP solution was centrifuged for 90 min at 9000 rpm. After centrifugation, 45 mL of the supernatant was discarded, and the AuNPs were suspended in the remaining 5 mL of supernatant. The concentration of the AuNPs was determined with UV-Vis spectrophotometer. Further, transmission Electron Microscope (TEM) micrographs were used to characterize the size and topography of the nanoparticles. The particle concentration was measured by UV-visible spectroscopy using the molar extinction coefficients at the wavelength of the maximum absorption of each gold

colloid as reported recently [$\epsilon_{(15) 528\text{nm}} = 3.6 \times 10^8 \text{ cm}^{-1} \text{ M}^{-1}$, $\epsilon_{(30) 530\text{nm}} = 3.0 \times 10^9 \text{ cm}^{-1} \text{ M}^{-1}$, $\epsilon_{(40) 533\text{nm}} = 6.7 \times 10^9 \text{ cm}^{-1} \text{ M}^{-1}$, $\epsilon_{(50) 535\text{nm}} = 1.5 \times 10^{10} \text{ cm}^{-1} \text{ M}^{-1}$, $\epsilon_{(60) 540\text{nm}} = 2.9 \times 10^{10} \text{ cm}^{-1} \text{ M}^{-1}$, and $\epsilon_{(80) 550\text{nm}} = 6.9 \times 10^{10} \text{ cm}^{-1} \text{ M}^{-1}$] ⁴⁷.

2.3. Gold Nanoparticle Surface Modification

The pH of AuNPs was adjusted to pH 8.5 with HEPES buffer (5 mM) and to the final AuNP concentration of 15 nM. The color of the buffered AuNP solution was red. Gold nanoparticle surface was attached with MPA through Au-S bond using a similar method adopted before ⁴⁵. A 10 mM MPA (10 μL) aqueous solutions was added to gold nanoparticle solution (15 nM, 10 mL) while continuously stirring for two hours. These AuNP-MPA solutions at pH 6.0, 7.5, and 9.0 were stable for more than 10 days at room temperature. Notably, pH below 6.0 or above 9.0, precipitation of the AuNP occurred. To a 9 ml portion of the MPA functionalized gold nanoparticle solution, 1 mL of 500uM Alizarin solution was mixed and left for 4 hours at room temperature for complete adsorption of the dye on the gold nanoparticles surface. Water samples were collected from various sources around Mississippi area. They were further used for colorimetric and SERS experiments. We have used a continuous wavelength DPSS laser from laser glow technology (LUD-670) operating at 670 nm, as an excitation light source in combination with InPhotonics 670 nm Raman fiber optic probe for excitation and data collection. It is a combination of 90 μm excitation fiber and 200 μm collection fiber with filtering and steering micro-optics. Ocean Optics QE65000 spectrometers equipped with CCD were used to process SERS data and analysis.

3. Result and Discussion

The present approach for the selective and sensitive detection of Cd (II) ion in aqueous solution is based on the fact that, in the presence of divalent Cd^{2+} ion, multifunctional gold nanoparticle undergoes aggregation Figure 1(A) and Figure 1(B). As a result several hot spots formed providing a significant enhancement of the Raman signal intensity from Alizarin dye modified gold nanoparticle dye by several order of magnitude (3.8×10^9) through electromagnetic field enhancement. Alizarin is a Raman active molecule and is environmentally benign dye. For the chemical design, gold nanomaterial were first functionalized with Alizarin dye as shown in Figure 1(A) followed by MPA. Further a chelating ligand PDCA is added for the selective binding with Cadmium in alkaline medium (pH 8.5). For the SERS experiment a portable SERS probe is used as reported recently ⁴⁶. MPA functionalized gold nanoparticles were characterized using FTIR spectroscopy. In Figure 1(C), apart from other vibrational peak assignments, the band at 2564 cm^{-1} corresponds to the absorption of S-H group of MPA while in Au-MPA the band disappeared and strong S-Au band can be seen at 765 cm^{-1} , which is in excellent agreement with the several previous reports ⁴⁸⁻⁵¹. We recorded FTIR spectra of 10^{-4}M MPA solution only and later a freshly prepared $\sim 30 \text{ nM}$ gold nanoparticle colloid solution is added. After allowing gold nanoparticles to functionalize with MPA, the mixture was centrifuged and washed six times to remove all unbound ligands from the solution. From the IR spectra of Au-MPA, the disappearance of 2564 cm^{-1} (S-H band) and the appearance of a new band at 765 cm^{-1} band (Au-S) confirms the formation of gold-thiol bond at the nanoparticle surface.

The acquired Raman modes of Alizarin as we can see in Figure 2(A) are overwhelmed by an intense broad fluorescence background. We noticed that addition of aqueous Cd^{2+} in Alizarin functionalized gold nanoparticle solution enhances Raman modes of Alizarin and significantly quenches the fluorescence background. SERS peak assignment of Alizarin (as shown in Figure 3(A)) were found at 495.0, 562.0, 682.0, 758.0, 819.0, 865.0, 950, 1034.0, 1080.0, 1231.0, 1288.0, 1335.0, 1465.0, 1595.0 and 1627.0 cm^{-1} which are in excellent agreement to with the previous report⁵² and theoretical calculations⁵³. Both the high intensity of these bands and the quenched fluorescence of Alizarin suggest that a strong interaction exists between Alizarin Dye molecule and gold Nanoparticles.

The colloidal gold nanoparticle functionalized with MPA at alkaline pH 8.5 is highly water soluble and exhibit LSPR peak at 525 nm [Figure 1(B)]. We observed a red shifted to 6 nm in extinction spectrum of MPA functionalized AuNP relative to non-functionalized nanoparticle attributed due to change in size and refractive index after surface coating by functional group. LSPR peak remain unchanged in 5mM phosphate buffer solution confirming the excellent colloidal stability of the functionalized nanoparticle for sensor application. This is crucial step for colloidal nanoparticle based sensor because of signal incurred by nonspecific aggregation can be effectively eliminated. In contrast, nanoparticles with small molecular ligands typically exhibits low tolerance to the increased ionic strength of buffer solutions, and aggregation because of poor colloidal stability often produces false signals²⁷. This is crucial step for colloidal nanoparticle based sensor because of signal incurred by nonspecific aggregation can be effectively eliminated. In contrast, nanoparticles with small molecular ligands typically exhibits low tolerance to the increased ionic strength of buffer solutions, and aggregation because of poor colloidal stability often produces false signals²⁷.

Extinction spectra in Figure 2(B) shows the inter-particle plasmonic coupling is immediately turned on upon the addition of Cd(II) ions at very low concentration (~50ppt) and very clear visual color change in the solution mixture Figure 3(A). Enhancement of Alizarin Raman modes Figure 4(A) by 20 times also confirms this inference. In the extinction spectra, a pronounced shoulder at 650 nm and gradual shift toward higher energy end of electromagnetic spectrum with increasing Cd(II) ion concentration is observed and attributed due to the formation of gold nanoparticle aggregate which was further confirmed by TEM image Figure 2(D). At the same time, the intensity of the original LSPR peak at 525 nm progressively decrease, which is accompanied by a colorimetric red to blue transition of sample mixture. Transmission electron microscopy observation Figure 2(C) and Figure 2(D) reveals that the functionalized nanoparticle remained as dispersed single particles in the absence of Cd(II) , while addition of Cd(II) induces clustering of the nanoparticle to form aggregates. This is obvious due to the coordination of Cd(II) chelating ligands, yielding both a substantial shift in the plasmon band energy to longer wavelength and a red to blue color change. The colorimetric trend in presence of various alkaline and transition metal ions, along with absorption spectra measurement in figure 1 clearly show that Alizarin- MPA-modified gold nanoparticle probe at pH 8.5 is highly selective for Cd(II) ions in aqueous media.

The high selectivity towards of MPA Cd(II) ion is mainly due to the fact that at pH 8.5, metals like Fe^{2+} , Cu^{2+} and Zn^{2+} has weaker binding affinity. On the other hand cadmium ion has ability to coordinate up to six oxygen (instead of eight in case of Pb^{2+}) atom or four acetate molecule and as a result, it is able to form smaller aggregates, which is highly favorable condition for SERS enhancement from Alizarin dye sitting in close proximity of plasmonic gold nanoparticle^{52,53}. To avoid the interference from other metal ions, we have performed our experiment at pH 8.5, and it is well documented that $-\text{COO}^-$ is good binding site for to binding Cd(II) at alkaline pH,^{8,17,27,54,55}. To understand the effect of pH on the selectivity of this assay, we have also performed the same experiment at two different pHs 5.5 and 9.0. As shown in Figure 2(B), 2(C), at pH 8.5 and pH 9.0, MPA modified gold nanoparticle is highly stable, but interference from other metal like ions are very high. Ethylene Diamine Tetraacetic acid (EDTA) is strong metal ion chelator approved by FDA to remove Cadmium and Lead. In similar lines we have used EDTA to understand the reversibility of aggregation process. We have added 700 μM EDTA after the aggregation in presence of Cd(II) ion and interestingly we have noted that the color change is completely reversed Figure 3(B). Similar observation has also been reported by several authors including us⁵⁶. Therefore our experimental result shows that the color change due to addition of Cd(II) ion is mainly due to the formation of chelating complex between Cd(II) and MPA-PDCA modified gold nanoparticle, which helps gold nanoparticle to form aggregate results in Alizarin Dye-AuNP interfacial hotspot formation and enhancement in Raman modes of dye.

The large Raman Scattering enhancement, even single molecule SERS have been described for molecules residing in the fractal space between aggregated colloidal nanoparticles²⁴. This attribute to Plasmonic coupling between nanoparticles in close proximity, which result in huge local electromagnetic enhancement in these, confined junctions or SERS hot spots²². Our data clearly show, Cadmium ion helps to bring nanoparticle together through coordinate covalent bond to generate hot spots via aggregation of functionalized gold nanoparticle. As result, we obtained about 17 order of magnitude in the enhancement of Raman signal. As shown in Figure 4(A), at higher concentration of Cadmium ion, we note a gradual enhancement of SERS signal. This may be attribute to the fact that presence of Cd(II) helps in trapping the Raman active Alizarin in to the aggregate, which further results in Raman mode enhancement due to the interaction of the dye with the surface electromagnetic field of plasmonic nanoparticle. As seen in Figure 4(B) a saturation in SERS signal and decrement after a critical concentration attributed due to the formation of bigger aggregate and the loss of SERS properties.

To further evaluate the sensitivity of our SERS probe, different concentration of Cd(II) ion were added to the dye-MPA conjugated nanoparticle. As shown in Figure 4(C) and Figure 4(D) SERS intensities are highly sensitive to the concentration of cadmium ion. The experimental result clearly demonstrates that the sensitivity of our SERS Cadmium probe is as low as 10ppt in aqueous solution. Further the experimental result also demonstrate that addition of other environmentally toxic heavy metal ion like Hg(II), Pb(II), Cr(III), Co(III), As(III), Ni(II), Zn(II), Fe(II), Cu(II) and Zn(II), SERS intensity remains almost unchanged. This is due to inclusive coordination of cadmium ion at favorable alkaline pH 8.5, and

exclusive non coordination of other heavy metal ion, which favors plasmonic gold nanoparticle to achieve aggregation for the hot spots formation. It is also well known that small clusters are better for hot spots ^{42,47,57}. We have observed similar trends as we have noted drastic decrease in the SERS intensity, after reaching certain concentration of Cadmium ion the solution Figure 4(B). In order to understand the change in SERS intensity that occurs due the nonspecific aggregations in presence and absence of Cd(II) at particular pH, we have calculated SERS enhancement factor in presence of Cd(II) and other metal ions. The Raman enhancement factor G , is measured experimentally using our previous report by direct comparison (1) ⁵⁸,

$$G = \frac{I_{SERS}}{I_{Raman}} \times \frac{M_{bulk}}{M_{ads}} \quad (1)$$

In which I_{SERS} is the Raman intensity at 1335 cm^{-1} ν (CC) vibrational mode in the surface enhanced Raman spectrum in presence of Cd(II), Pb(II), Hg(II), As(III) ions, and I_{Raman} is the Raman intensity of the same mode in the bulk Raman Spectrum from only respective ions. M_{bulk} is the number of molecule used in the bulk, M_{ads} is the number of molecule adsorbed and sampled on the SERS active substrate, all spectra are normalized on the same integration time. The enhancement factor estimated from SERS signal is approximately 3.2×10^7 . As shown in Figure 4(A), we have observed that, at a Cd (II) ion concentration of 30ppt, the SERS intensity started to decline and continued to further drop as Cd (II) ion addition is continued.

This may be reasoned due to the fact that at lower concentration of Cd (II) ion functionalized plasmonic nanoparticles form smaller aggregate than at higher ppb level. As a result, decrement in the SERS signal at higher level of cadmium is observed. Transmission electron microscope image as shown in Figure 5 also confirms our inference. It is a well known that smaller clusters are better for hot spots formation. Increasing the cluster size due to the excess addition of cadmium ion, might decrease the number of hot spots, and as a result, the intensity decrease after a certain concentration of cadmium ion. As shown in TEM image Figure 5(B, C, D), at the lower concentration range (ppt level), the nanoparticles are well dispersed. However, as we increase the amount of cadmium ion concentration, nanoparticles are drawn closer and eventually result in high level of agglomeration. This clearly demonstrates that Cadmium ion form a coordination complex with MPA-PDCA functionalized nanoparticles.

To further demonstrate the practical application of the Cadmium SERS assay in environmental samples, spiking experiment were performed and recovery values were determined. For this purpose, we collected water sample from Mississippi river, Bayou Pierre, Little Bayou Pierre, Big Black River and Alcorn State University tap water. Subsequently, different concentrations of interfering heavy metal ions including Cadmium ions were spiked and SERS assay was performed to determine the sensitivity of the probe and tabulated Table 1. The spiked heavy metal ion concentration and SERS enhancement determined by our probe in different environmental samples were compared. Our data shows the recovery values are quite satisfactory within the experimental error. This experimental

data confirms the capability of Alizarin based SERS assay to detect trace level concentration of cadmium ion as low as 70 ppt from environmental samples and with excellent discrimination against other common heavy metal ions.

Further, our experiment demonstrates that, the sensitivity of this SERS assay is rapid, takes less than 30 minute from nanoparticle functionalization to detection and analysis. The assay is highly sensitive towards cadmium level in water samples and is about three order of magnitude higher than the EPA standered limit and about two order of magnitude higher than any other gold nanoparticle based reported assay.

4. Conclusion

In this article, we have reported the development of naturally abundant Alizarin functionalized plasmonic gold SERS nano probe for the selective detection of Cd (II) at ultrasensitive level. Plasmonic gold nanoparticle conjugated with 3-mercaptopropionic acid, 2,6-Pyridinedicarboxylic acid and Alizarin dye, which has capability of enhancing Raman modes of Alizarin dye through aggregation and hotspot formation after the addition of Cd (II) ion. Our results also demonstrate that MPA and PDCA bound plasmonic gold can detect trace level of Cd (II) ion upto 10 ppt in aqueous solution which is 2 orders of magnitude higher than any reported method. Our experimental result shows that Cd (II) ion can be detected quickly and accurately with excellent discrimination against other Alkali and heavy metal ions and takes less than 30 minutes from sample preparation and diagnosis. We have also demonstrated that our SERS assay is capable measuring amount of Cd (II) in water sample from various sources as well as from extensively used samples like drinking water. The experimental result reported here open up a new possibility of an assay, reliable and rapid method for of Cadmium contamination in drinking water. Since Alizarin is environmentally friendly dye and given the sensitivity and speed of detection, the proposed assay may easily be extended to public domain and can become a new method of choice for safe drinking water supply.

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Figure 1.

(A) Schematic representation of Alizarin adsorption, and MPA-PDCA conjugation on gold nano particle surface. (B) Mechanism of Cadmium ion complex formation and SERS from Alizarin. (C) FTIR Spectrum of freshly prepared gold nanoparticle mixed with 3-mercaptopropionic acid showing the existence of 765cm⁻¹ (S-Au) indicates the formation of gold-thiol linkage.

Figure 2. (A) SERS spectra and Raman modes assignment from Alizarin trapped in MPA-PDCA functionalized plasmonic gold Nanoparticle. (B) Extinction spectrum of functionalized plasmonic gold at various concentration of Cd (II) addition. (C) Transmission Electron Microscope Image [TEM] of PDCA-MPA functionalized Plasmonic gold nanoparticle. (D) Transmission Electron Microscope Image [TEM] of functionalized sample mixture after addition of 100ppt Cd (II) ion.

Figure 3.

(A) Photograph showing colorimetric image of MPA-PDCA conjugated gold nanoparticle in presence of different heavy metal and transition metal ions (B) Photograph showing reversal of aggregation process with EDTA addition.

Figure 4.

(A). Change in SERS signal at various concentrations of Cd (II) ions added in Alizarin functionalized Gold nanoparticle. (B) Critical monitoring the Effect of Cd (II) addition on the intensity of C-C 1335 cm^{-1} vibrational Raman mode of Alizarin Dye. (C) Demonstrating the SERS spectra of Alizarin conjugated on functionalized Gold nanoparticle in presence of Cd(II) and other Alkali and transition metal ions. (D) Bar graph demonstrate the selectivity of functional gold nanoparticle for Cd (II) ion over other Alkali and transition metal ions.

Figure 5.

Comparison of TEM image of (A) MPA-PDCA and Alizarin dye conjugated gold nanoparticle (B) 30 ppt Cd(II) (C) 60 ppt Cd(II) and (D) 100 ppt Cd(II) MPA-PDCA and Alizarin dye functionalized gold nanoparticle.

Table 1

Comparison of SERS signal recovered from the Cadmium SERS assay and various environmental drinking water sources.

	SERS Enhancement (80 ppt each ion spiked in each water sample)				
	Mississippi river	Bayou Pierre	Little Bayou Pierre	Big Black River	Tap water
Cd(II)	3.1×10^7	3.1×10^7	3.2×10^7	3.1×10^7	2.9×10^7
Hg(II)	1.3×10^2	1.4×10^2	1.4×10^2	1.3×10^2	1.1×10^2
Pb(II)	1.1×10^2	1.1×10^3	1.6×10^3	1.3×10^2	1.1×10^2
As(III)	No Change	No change	No Change	No Change	No Change
Zn(II)	No Change	No change	No Change	No Change	No Change