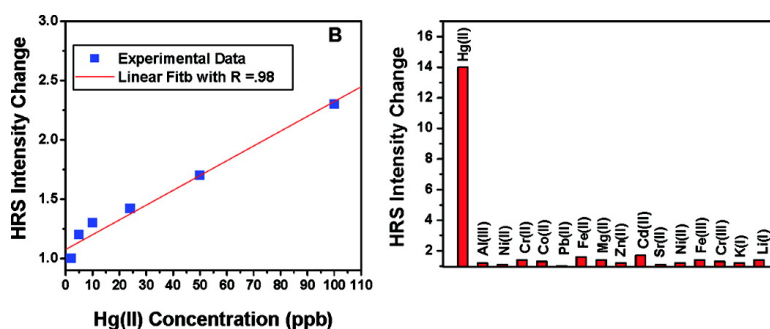


## Selective Detection of Mercury (II) Ion Using Nonlinear Optical Properties of Gold Nanoparticles

Gopala Krishna Darbha, Anant Kumar Singh, Uma Shanker Rai, Eugene Yu, Hongtao Yu, and Paresh Chandra Ray

*J. Am. Chem. Soc.*, **2008**, 130 (25), 8038-8043 • DOI: 10.1021/ja801412b • Publication Date (Web): 03 June 2008

Downloaded from <http://pubs.acs.org> on February 8, 2009



### More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 5 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

[View the Full Text HTML](#)

## Selective Detection of Mercury (II) Ion Using Nonlinear Optical Properties of Gold Nanoparticles

Gopala Krishna Darbha, Anant Kumar Singh, Uma Shanker Rai, Eugene Yu, Hongtao Yu, and Paresh Chandra Ray\*

Department of Chemistry, Jackson State University, Jackson, Mississippi 39217

Received February 25, 2008; E-mail: paresh.c.ray@jsums.edu

**Abstract:** Contamination of the environment with heavy metal ions has been an important concern throughout the world for decades. Driven by the need to detect trace amounts of mercury in environmental samples, this article demonstrates for the first time that nonlinear optical (NLO) properties of MPA–HCys–PDCA-modified gold nanoparticles can be used for rapid, easy and reliable screening of Hg(II) ions in aqueous solution, with high sensitivity (5 ppb) and selectivity over competing analytes. The hyper Rayleigh scattering (HRS) intensity increases 10 times after the addition of 20 ppm Hg<sup>2+</sup> ions to modified gold nanoparticle solution. The mechanism for HRS intensity change has been discussed in detail using particle size-dependent NLO properties as well as a two-state model. Our results show that the HRS assay for monitoring Hg(II) ions using MPA–HCys–PDCA-modified gold nanoparticles has excellent selectivity over alkali, alkaline earth (Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>), and transition heavy metal ions (Pb<sup>2+</sup>, Pb<sup>+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>).

### Introduction

Contamination of the environment with heavy metal ions has been an important concern throughout the world for decades. Mercury is one of the most toxic elements on the planet, probably second only to plutonium. Mercury is a known environmental pollutant routinely released from coal-burning power plants, oceanic and volcanic emissions, gold mining, and solid waste incineration.<sup>1</sup> The long atmospheric residence time of Hg<sup>0</sup> vapor and its oxidation to soluble inorganic Hg(II) provides a pathway for contaminating vast amounts of water and soil. Bacteria living in the sediments of aqueous environments transform inorganic Hg(II) into methylmercury, a potent neurotoxin that concentrates through the food chain in the tissues of fish and marine mammals. Since with sufficient exposure all mercury-based toxins damage the central nervous system and other organs or organ systems such as the liver or gastrointestinal tract, it is important to develop highly sensitive and selective Hg sensor that can provide real-time determination of Hg levels in the environment, water, and food. A number of fluorescence-based probes and sensors for detection of Hg(II) have been reported.<sup>2–10</sup> However, many fluorescent small-molecule-based Hg(II) sensors presented to date are quenched upon Hg(II) coordination, often rely on an irreversible Hg(II)-

dependent chemical reaction to give fluorescence turn-on, and/or require organic solvent systems. Most of these sensors display drawbacks in terms of actual applicability such as the lack of water solubility, cross-sensitivity toward other metal ions, weak fluorescence enhancement factor, and short emission wavelengths. Thus, the development of facile and practical assays for Hg(II) remains a challenge. In the last two years our group<sup>11</sup> and others<sup>12–15</sup> have directed their attention to gold nanoparticle-based colorimetric and nanomaterial based surface energy transfer (NSET) assay on recognition and detection of mercury ions in aqueous solution. But these assays identify mercury after the nanoparticle has been modified with a covalently linked DNA or labeled with a fluorescent tag. The necessity of tagging makes it costly to use those techniques as sensors for real life. Driven by the need, we demonstrate in this article, for the first time, that second-order nonlinear optical (NLO) properties of gold nanoparticles can be used for screening mercury from environmental samples without any DNA or fluorescent tag, with excellent sensitivity (5 ppb) and selectivity.

(1) <http://www.epa.gov>.

(2) Harano, K.; Hiraoka, S.; Shionoya, M. *J. Am. Chem. Soc.* **2007**, *129*, 5300.

(3) Butler, O. T.; Cook, J. M.; Harrington, C. F.; Hill, S. J.; Rieuwerts, J.; Miles, D. L. *J. Anal. Atom. Spectrom.* **2006**, *21*, 217.

(4) Leermakers, M.; Baeyens, W.; Quevauviller, P.; Horvat, M. *Trends Anal. Chem.* **2005**, *24*, 383.

(5) Valeur, B. Ed. *Molecular Fluorescence: Principles and Applications*; Wiley-VCH: Weinheim, 2002.

(6) Komatsu, H.; Miki, T.; Citterio, D.; Kubota, T.; Shindo, Y.; Kitamura, Y.; Oka, K.; Suzuki, K. *J. Am. Chem. Soc.* **2005**, *127*, 10798.

(7) Zhu, X.-J.; Fu, S.-T.; Wong, W.-K.; Guo, J.-P.; Wong, W.-Y. A. *Angew. Chem., Int. Ed.* **2005**, *45*, 3150.

(8) Mello, J. V.; Finney, N. S. A. *J. Am. Chem. Soc.* **2005**, *127*, 10124.

(9) Nolan, E. M.; Lippard, S. J. *J. Am. Chem. Soc.* **2003**, *125*, 14270.

(10) Caballero, A.; Martínez, R.; Lioveras, V.; Tatera, I.; Vidal-Gancedo, J.; Wurst, K.; Tarraga, A.; Molina, P.; Veciana, J. *J. Am. Chem. Soc.* **2005**, *127*, 15666.

(11) Darbha, G. K.; Ray, A.; Ray, P. C. *ACS Nano* **2007**, *1*, 208.

(12) Lee, J.-S.; Han, M. S.; Mirkin, C. A. *Angew. Chem., Int. Ed.* **2007**, *46*, 4093.

(13) Huang, C.-C.; Chang, H.-T. *Anal. Chem.* **2006**, *78*, 8332.

(14) Chih-Ching, H.; Zusing, Y.; Kun-Hong, L.; Huan-Tsung, C.; *Angew. Chem., Int. Ed.* **2007**, *47*, 5549.

(15) Xuejia, X.; Wang, F.; Xiaogang, L. *J. Am. Chem. Soc.* **2008**, *130*, 3244.

NLO properties have been monitored using a hyper-Rayleigh scattering (HRS) technique. The HRS technique<sup>16–26</sup> is based on light scattering. The HRS or nonlinear light scattering can be observed from fluctuations in symmetry, caused by rotational fluctuations. This is a second harmonic generation experiment in which the light is scattered in all directions rather than as a narrow coherent beam. The technique can be easily applied to study a very wide range of materials because electrostatic fields and phase matching are not required. Other advantages are that the polarization analysis gives information about the tensor properties, and spectral analysis of the scattered light gives information about the dynamics. Recently<sup>19,20</sup> we have shown that the HRS technique can be used to achieve detection of pathogenic DNA with excellent sensitivity (100 pM) and selectivity (single base-pair mismatch) through NLO properties of gold nanoparticle. Hupp and co-workers<sup>17</sup> have reported a concept on gold nanoparticle-based HRS and colorimetric techniques for the detection of small concentrations of aqueous heavy metal ions, including toxic metals such as lead, cadmium, and mercury. Their data (Figures 1, 2, and 3 in reference 17) show the colorimetric, TEM, and HRS intensity change due to the addition of Pb<sup>2+</sup> ion. Although they have not presented any data for Hg<sup>2+</sup> ion, they have indicated that similar responses were obtained with Hg<sup>2+</sup>- and Cd<sup>2+</sup>-containing solutions. Due to the lack of selectivity, the gold nanoparticle-based HRS assay reported by Kim et al.<sup>17</sup> cannot be applied to detect heavy metal ions from environmental samples. Here we report a highly selective and sensitive HRS assay for mercury (II) recognition at 5 ppb level in aqueous solution using gold nanoparticles modified with mercaptopropionic acid (MPA), homocystine (HCys), and 2,6-pyridinedicarboxylic acid (PDCA). Our results show that the HRS assay for monitoring Hg(II) ions using MPA–Cys–PDCA-modified gold nanoparticles have excellent selectivity over alkali, alkaline earth (Li<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Mg<sup>2+</sup>, Ca<sup>2+</sup>) and transition heavy metal ions (Pb<sup>2+</sup>, Pb<sup>+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>).

## Experimental Section

Hydrogen tetrachloroaurate (HAuCl<sub>4</sub>·3H<sub>2</sub>O), NaBH<sub>4</sub>, mercaptopropionic acid (MPA), homocystine (HCys), 2,6-pyridinedicarboxylic acid, buffer solution, sodium chloride, and sodium citrate were purchased from Sigma-Aldrich and used without further purification.

**Gold Nanoparticle Synthesis.** Gold nanoparticles of diameters of 15 nm or more were synthesized using the reported method.<sup>11,19,20</sup> Gold nanoparticles of different sizes and shapes were synthesized

by controlling the ratio of the HAuCl<sub>4</sub>·3H<sub>2</sub>O, and sodium citrate concentrations as we reported recently.<sup>11,19,20,27,28</sup> For smaller gold nanoparticles, we have used the sodium borohydride method as reported before. To 18 mL of deionized H<sub>2</sub>O were added 0.5 mL of 0.01 M HAuCl<sub>4</sub> trihydrate in water and 0.5 mL of 0.01 M sodium citrate in water and stirred. Next, 0.5 mL of freshly prepared 0.1 M NaBH<sub>4</sub> was added, and the solution color changed from colorless to orange. Stirring was stopped, and the solution was left undisturbed for 2 h. The resulting spherical gold nanoparticles were 4 nm in diameter. Transmission electron microscope (TEM) and UV–visible absorption spectrum were used to characterize 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<sup>11,25–28</sup> [ $\epsilon_{(15)} 528\text{nm} = 3.6 \times 10^8 \text{ cm}^{-1} \text{ M}^{-1}$ ,  $\epsilon_{(30)} 530\text{nm} = 3.0 \times 10^9 \text{ cm}^{-1} \text{ M}^{-1}$ ,  $\epsilon_{(40)} 533\text{nm} = 6.7 \times 10^9 \text{ cm}^{-1} \text{ M}^{-1}$ ,  $\epsilon_{(50)} 535\text{nm} = 1.5 \times 10^{10} \text{ cm}^{-1} \text{ M}^{-1}$ ,  $\epsilon_{(60)} 540\text{nm} = 2.9 \times 10^{10} \text{ cm}^{-1} \text{ M}^{-1}$ , and  $\epsilon_{(80)} 550\text{nm} = 6.9 \times 10^{10} \text{ cm}^{-1} \text{ M}^{-1}$ ].

**Gold Nanoparticle Surface Modification.** To detect Hg(II) ion selectively, we modified the surface of the gold nanoparticle with mercaptopropionic acid (MPA) and homocystine (bound to the gold nanoparticle surface through a Au–S bond), and we added a chelating ligand PDCA, to the solution, as we discussed before.<sup>11</sup> The gold nanoparticle surface was attached with MPA and HCys through a –SH bond using a method similar to one we have described before.<sup>11</sup> We have added 10 mM MPA (10  $\mu\text{L}$ ) and 2 mM HCys (10  $\mu\text{L}$ ) to the gold nanoparticle solution (15 nM, 10 mL) with stirring. After 2 h, (5–8) mM NaBH<sub>4</sub> was added, and the mixture was left for few hours without disturbance.

For the HRS experiments we have used a mode-locked Ti:sapphire laser delivering at fundamental wavelengths of 860 nm with a pulse duration of about 150 fs at a repetition rate of 80 MHz. After passing through a low-pass filter, a fundamental beam of about 100 mW was focused into a quartz cell containing the aqueous solutions of the metallic particles. The HRS light was separated from its linear counterpart by a high-pass filter and a monochromator and then was detected with a cooled photomultiplier tube; the pulses were then counted with a photon counter. The fundamental input beam was linearly polarized, and the input angle of polarization was selected with a rotating half-wave plate. In all experiments reported, the polarization state of the harmonic light was vertically polarized.

## Results and Discussion

The intensity  $I_{\text{HRS}}$  of the hyper-Rayleigh signal from an aqueous solution of gold nanoparticles can be expressed as<sup>16–25</sup>

$$I_{\text{HRS}} = G(N_w \beta_w^2 + N_{\text{nano}} \beta_{\text{nano}}^2) I_w^2 e^{-N_{\text{nano}} \epsilon_{2\omega} l} \quad (1)$$

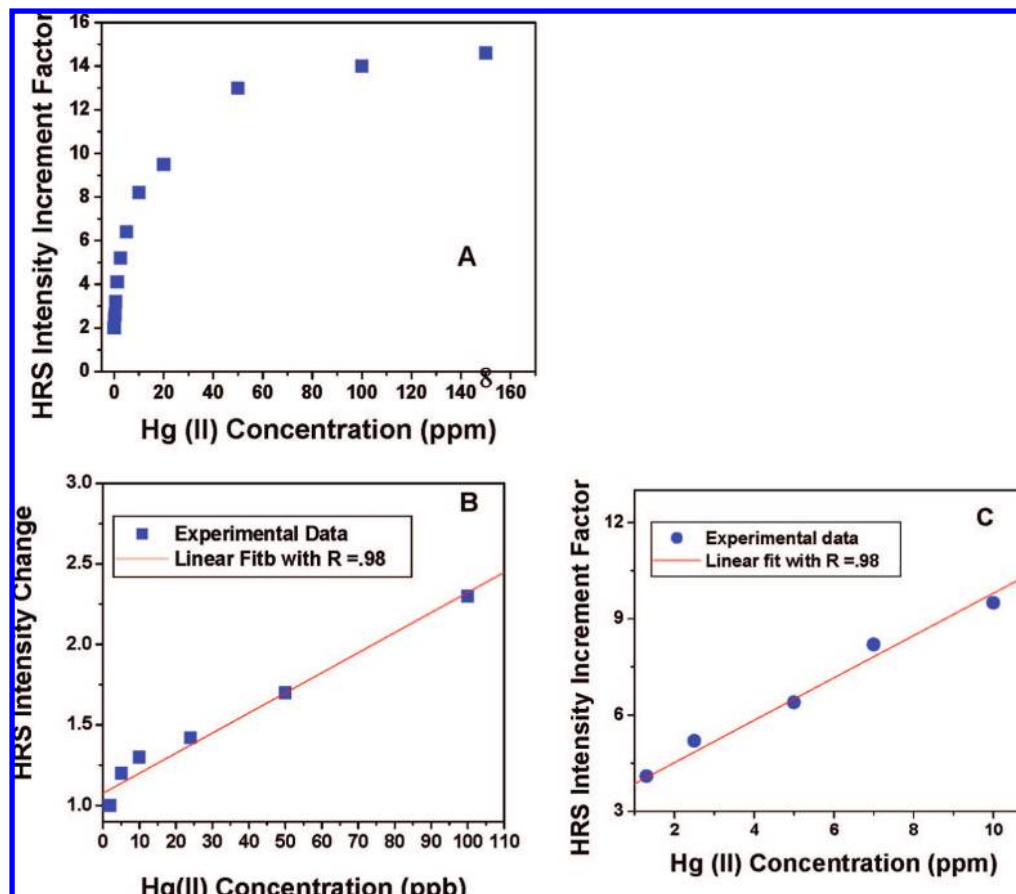
where  $G$  is a geometric factor,  $N_w$  and  $N_{\text{nano}}$  the number of water molecules and gold nanoparticles per unit volume,  $\beta_w$  and  $\beta_{\text{nano}}$  are the quadratic hyperpolarizabilities of a single water molecule and a single gold nanoparticle,  $\epsilon_{2\omega}$  is the molar extinction coefficient of the gold nanoparticle at  $2\omega$ ,  $l$  is the path length and  $I_w$  the fundamental intensity. The exponential factor accounts for the losses through absorption at the harmonic frequency. The HRS signal intensities were normalized to the square of the incident light intensity. To understand whether the two-photon scattering intensity at 430 nm light is due to second harmonic generation, we performed a power-dependent as well as a concentration-dependent study. Figure 1 shows the output signal intensities at 430 nm from MPA–HCys–PDCA-modified gold nanoparticles at different powers of 860 nm

- (16) Clays, K.; Persoons, A. *Phys. Rev. Lett.* **1991**, *66*, 2980.  
 (17) Kim, Y.; Johnson, R. C.; Hupp, J. T. *Nano Lett.* **2001**, *1*, 165.  
 (18) Viau, L.; Bidault, S.; Maury, O.; Brasselet, S.; Ledoux, I.; Zyss, J.; Ishow, E.; Nakatani, K.; Le Bozec, H. *J. Am. Chem. Soc.* **2004**, *126*, 8386.  
 (19) Ray, P. C. *Angew. Chem., Int. Ed.* **2006**, *45*, 1151.  
 (20) Darbha, G. K.; Rai, U. S.; Singh, A. K.; Ray, P. C. *Chem. Eur. J.* **2008**, *14*, 3896.  
 (21) Kang, H.; Evmenenko, G.; Dutta, P.; Clays, K.; Song, K.; Marks, T. J. *J. Am. Chem. Soc.* **2006**, *128*, 6194.  
 (22) Coe, B. J.; Harries, J. L.; Helliwell, M.; Jones, L. A.; Asselberghs, I.; Clays, K.; Brunshwig, B. S.; Harris, J. A.; Garin, J.; Orduna, J. *J. Am. Chem. Soc.* **2006**, *128*, 12192.  
 (23) Ghosh, S.; Krishnan, A.; Das, P. K.; Ramakrishnan, S. *J. Am. Chem. Soc.* **2003**, *125*, 1602.  
 (24) Chandra, M.; Indi, S. S.; Das, P. K. *J. Phys. Chem. C* **2007**, *111*, 10652.  
 (25) Russier-Antoine, I.; Benichou, E.; Bachelier, G.; Jonin, C.; Brevet, P. F. *J. Phys. Chem. C* **2007**, *111*, 9044.  
 (26) Nappa, J. I.; Russier-Antoine, E.; Benichou, G.; Bachelier, C.; Brevet, P. F. *J. Chem. Phys.* **2006**, *125*, 184712.

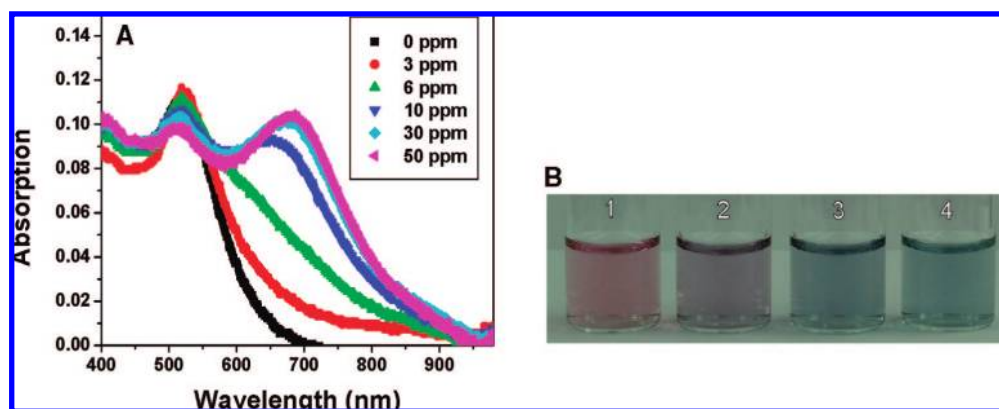
- (27) Ray, P. C.; Fortner, A.; Darbha, G. K. *J. Phys. Chem. B* **2006**, *110*, 20745.  
 (28) Kim, C. K.; Kalluru, R. R.; Singh, J. P.; Fortner, A.; Griffin, J.; Darbha, G. K.; Ray, P. C. *Nanotechnology* **2006**, *17*, 3085.







**Figure 2.** (a) Plot of HRS intensity increment factor (ratio of the HRS intensity after the addition of Hg(II) to the HRS intensity before the addition of Hg(II)) vs Hg(II) concentration in ppm. (b) Plot of HRS intensity change vs Hg(II) concentration in ppb. Linear correlation exists over the range of 5–100 ppb with  $R = 0.988$ . (c) Plot of HRS intensity increment factor vs Hg(II) concentration in ppm. Linear correlation exists over the range of 1–10 ppm with  $R = 0.988$ .

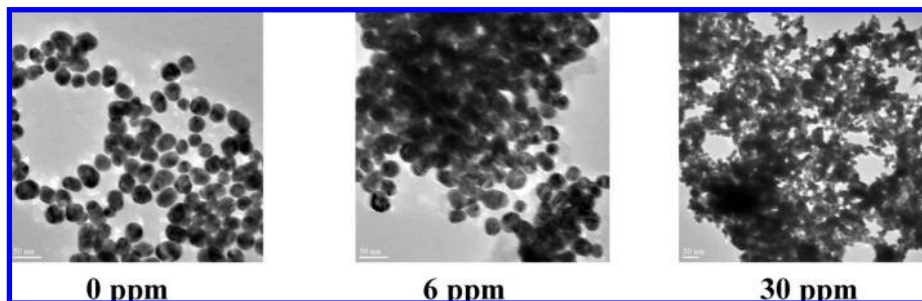


**Figure 3.** (a) Absorption profile of modified gold nanoparticles before and after addition of different concentrations of Hg(II) ions. (b) Photographic images of color of MPA-PDCA-modified gold nanoparticles (13 nM) in presence of different concentration of Hg(II) ion, (1) 3 ppm, (2) 6 ppm, (3) 10 ppm, (4) 50 ppm.

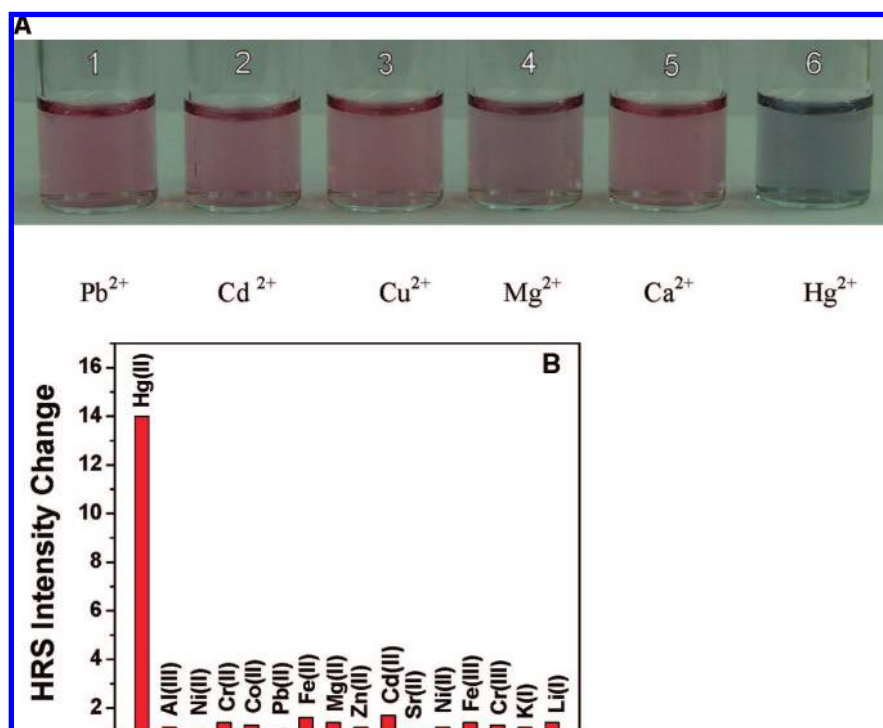
chelating ligand PDCA to the solution as we discussed before. When we modified the surface with only MPA or HCys (bound to the gold nanoparticle surface through the Au-S bond), our assay shows negligible responses toward Fe(II), Mn(II), Zn(II), Ni(II), K(II), Cr(III), and Sr(II), but we have noted substantial shift in the plasmon band energy to longer wavelengths and a red-to-blue color change, in the presence of Hg(II), Pb(II) as well as Cd(II). It has been reported<sup>30</sup> in the literature that the stability constants between heavy metal ions and chelating ligand like MPA are  $\log K(\text{Pb}) = 4.1$ ,  $\log K(\text{Hg}) = 10.1$ ,  $\log K(\text{Cd}) =$

3.2, and  $\log K(\text{Zn}) = 1.8$  respectively. So stability constant of mercury-MPA complex is about 6 orders of magnitudes higher than with other interfering metal ions. But when we modified the surface with both MPA and HCys, (5:1) our assays shows negligible responses toward Cd(II), but we noted red-to-blue color change, in the presence of Hg(II) or Pb(II). Further higher selectivity of our probe toward Hg(II) ions was achieved by

(30) Morel, F. M. M. *Principles of Aquatic Chemistry*; Wiley-Interscience: New York, 1983; pp 237.



**Figure 4.** TEM images of MPA–PDCA-modified gold nanoparticle solution in the presence and in the absence of Hg(II) ions.



**Figure 5.** (a) Photographic images of color of MPA–HCys–PDCA-modified gold nanoparticles in presence of different metal ions with 40 ppm concentration. (b) HRS intensity change upon the addition of 40 ppm different metal ions on gold nanoparticle–MPA–HCys–PDCA solution (5 nM)

adding another chelating ligand, PDCA. Stability constants of heavy metal ions with PDCA are  $\log K(\text{Pb}) = 8.2$ ,  $\log K(\text{Hg}) = 20.2$ ,  $\log K(\text{Cd}) = 10.0$ , and  $\log K(\text{Mn}) = 8.5$ .<sup>31</sup> So PDCA will be able to form much more stable complex with Hg(II) than with other metal ions. Therefore, when we modified the surface of the gold nanoparticle with MPA and HCys and we added a chelating ligand PDCA, to the solution, excellent selectivity was achieved over alkali, alkaline earth, and transition heavy metal ions. To achieve better selectivity, we added PDCA to each MPA–HCys–gold nanoparticle solution at a concentration about 7–10 times greater than that of Hg(II) ions. Figure 5a shows the colorimetric response, and Figure 5b shows the HRS response in the presence of various environmentally relevant metal ions. Our result shows excellent selectivity over alkali, alkaline earth ( $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{2+}$ ,  $\text{Ca}^{2+}$ ) and transition heavy metal ions ( $\text{Pb}^{2+}$ ,  $\text{Pb}^+$ ,  $\text{Mn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ). We also tested the selectivity in the presence of only PDCA, our results show red-to-blue color change, in the presence of Hg(II), Fe(II) and Cr(III). From all the results we

conclude that modification of the gold nanoparticle surface with MPA and HCys is very important for increasing the selectivity toward Hg(II). We believe that PDCA ligands bound to the MPA–HCys–AuNP species through Au–N bonds improved the selectivity toward Hg(II) ions through a cooperative effect, while the PDCA ligands in the bulk solutions formed complexes with other metal ions, suppressing their interference with the probes.

To understand the response rate of the HRS signal upon addition of Hg(II) we have measured the HRS intensity at different time intervals, and our HRS experimental data indicate that the reaction is complete within 400 s.

## Conclusions

In conclusion, in this article, we have demonstrated for the first time a label-free, selective, and highly sensitive HRS assay for mercury (II) recognition in 5 ppb level in aqueous solution. Our HRS assay will have several advantages, and these are: (i) one does not need to use DNA or fluorescent dyes to probe mercury (II) ion in solution by the HRS technique; (ii) it can be 2–3 orders of magnitude more sensitive than the usual colorimetric technique; (iii) it is highly selective; and (iv) it

(31) Norkus, E.; Stalnioniene, I.; Crans, D. C. *Heteroat. Chem.* **2003**, *14*, 625.

takes only 6–7 min to find out the concentration of mercury in aqueous solution. Our experimental results reported here open up a new possibility of rapid, easy, and reliable diagnosis of toxic metal ions from environmental samples by measuring the HRS intensity of MPA–HCys–PDCA-modified gold nanoparticles. For developing a practical assay, more research needs to be done on improvement of the HRS experimental system, and the HRS intensity variation with the shape and size of metal nanoparticles should be investigated. It may be possible to improve the HRS intensity by several orders of magnitudes by choosing proper materials and detection systems. We believe

that the HRS method has enormous potential for application of toxic metal detection from environmental samples.

**Acknowledgment.** P.C.R. thanks NSF-PREM Grant DMR-0611539 and NSF-MRI Grant 0421406 for their generous funding. We thank Sara H. Bayley, Instrumentation Facilities Coordinator, University of Southern Mississippi for helping to acquire TEM data. We also thank reviewers whose valuable suggestions improved the quality of the manuscript.

JA801412B