Detection of mercury ions using silver telluride nanoparticles as a substrate and recognition element through surface-enhanced Raman scattering

Chia-Wei Wang, Zong-Hong Lin, Prathik Roy and Huan-Tsung Chang*

Department of Chemistry, National Taiwan University, Taipei, Taiwan

Edited by:
Shusheng Zhang, Qingdao University of Science and Technology, Linyi University, China
Reviewed by:
Lingxin Chen, Chinese Academy of Sciences, China
Yingshu Guo, Linyi University, China

*Correspondence:
Huan-Tsung Chang, Department of Chemistry, National Taiwan University, 1, Section 4, Roosevelt Road, Taipei 106, Taiwan
e-mail: changht@ntu.edu.tw

INTRODUCTION
Monitoring the level of mercury ions in ecological systems is an extremely important issue, mainly because they are highly toxic, non-biodegradable, and bioaccumulated (Zahir et al., 2005; Clarkson et al., 2008). Several techniques such as atomic absorption/emission spectrometry, atomic fluorescence spectrometry, inductively coupled plasma spectrometry (ICP-MS) have been applied to detect Hg\(^{2+}\) in environmental and biological samples (Leermakers et al., 2005; Butler et al., 2006; Li et al., 2006). Among them, ICP-MS provides the highest sensitivity and a widest linear range, but the system is expensive because it requires expensive noble gas. In addition, it is not easy for the in-field analysis.

With advantages of simplicity, low cost, sensitivity, and selectivity, a number of optical and electrochemical sensors have been demonstrated for the detection of Hg\(^{2+}\) (Huang and Chang, 2007; Darbha et al., 2008; Jena and Raj, 2008; Zhu et al., 2009). Having extremely high stability, specificity, and ease in preparation, DNA-based optical sensors have become popular for the detection of Hg\(^{2+}\) (Chiang et al., 2008; Stewart et al., 2008; Dave et al., 2010). Polythymines (T\(_n\)) that are specific toward Hg\(^{2+}\) ions through T–Hg\(^{2+}\)–T coordination have been used for the selective and sensitive detection of Hg\(^{2+}\) ions through fluorescence detection based on the analyte induced changes in the DNA conformation, leading to enhanced efficiency in the fluorescence resonance energy transfer between the donor and the acceptor or increased quantum yield of the fluorophore. In addition, T\(_n\) conjugated with gold nanoparticles (NPs) have been used for the detection of Hg\(^{2+}\) ions through absorption or fluorescence modes (Huang et al., 2007; Lee et al., 2007; Wang et al., 2008; Xie et al., 2008). The detections are mainly based on the analyte induced changes in the DNA conformation and charge density on the surfaces of the Au NPs, leading to changes in absorbance (red shift upon aggregation) or fluorescence intensity (either decreases or increases). Surface-enhanced Raman scattering (SERS) using Ag NPs conjugated with T\(_n\) and organic dyes (reporter) are alternative for the sensitive detection of Hg\(^{2+}\) (Wang et al., 2009, 2011). Upon interaction with Hg\(^{2+}\), the DNA conformation changes, leading to changes in the distance of the reporter from the surfaces of Ag NPs and thus changes in the SERS signal (Grubisha et al., 2003; Doering et al., 2007). Although these sensing systems are sensitive and suitable for in-field analysis, the DNA is expensive and their sensitivity and selectivity are highly dependent on the ionic strength of the samples, limiting their wide practicality.

Relative to normal Raman scattering, SERS can provide enhancement factors (EFs) up to 10\(^{15}\) theoretically through a long-range electromagnetic (EM) effect such as “hot spots,” and/or chemical effect due to the charge-transfer excitation of chemisorbed molecules (Aravind et al., 1981; Kneipp et al., 1997; Nie and Emery, 1997). The EF values are dependent on the compositions, sizes, and shapes of the SERS substrates. Relative to spherical shaped silver NPs, silver plates and silver nanowires provide higher EF values; EF values up to 10\(^8\) have been demonstrated for the SERS signals of common reporters such as Rhodamine 6G (R6G), 4-mercaptobenzoic acid, and 5,5’-dithiobiis(2-nitrobenzoic acid) (Tao et al., 2003; Yang et al., 2007). Gold-tellurium nanodumbbells, gold-tellurium nanopeapods, and gold pearl-necklace nanomaterials (Au PNNs) providing EF values of R6G up to 5.6 \times 10^9 have been used for the selective detection of human serum albumin down to 70 pM using AB 580 as a reporter (Lin and Chang, 2008; Lin et al., 2011).
In this study, we developed a simple SERS approach using silver telluride (Ag$_2$Te) NPs as substrates for sensitive and selective detection of Hg$^{2+}$ in aqueous solutions. Ag$_2$Te NPs were prepared from tellurium dioxide and silver nitrate in the presence of hydrazine and sodium dodecyl sulfate (Samal and Pradeep, 2009). The Ag$_2$Te NPs provided SERS enhancement effect of R6G. Upon increasing the concentration of Hg$^{2+}$ ions, the SERS signal of R6G decreased. The novel SERS approach was further validated by the determination of the concentrations of Hg$^{2+}$ in pond water samples, showing advantages of sensitivity, selectivity, and simplicity.

**MATERIALS AND METHODS**

**CHEMICALS**

Hydrazine monohydrate (80%) and tellurium dioxide powder (99.9%) were purchased from SHOWA (Tokyo, Japan). Sodium phosphate monobasic, dibasic, and tribasic, and sodium dodecyl sulfate were purchased from Acros (Geel, Belgium). Mercury chloride, R6G, silver nitrate, and other metal salts [Ca$^{2+}$, Co$^{2+}$, Cu$^{2+}$, Cd$^{2+}$, K$^+$, Mg$^{2+}$, Mn$^{2+}$, Ni$^{2+}$, Na$^+$, Pb$^{2+}$, Zn$^{2+}$, and Na$^+$ (chlorides)] used in this study were purchased from Sigma Aldrich (Missouri, USA). Ultrapure water was obtained using a Milli-Q ultrapure (18.2 MΩ·cm) system.

**SYNTHESIS OF Ag$_2$Te NPs**

Hydrazine (1 mL) was added slowly to a sample vial containing aqueous solution (9 mL) of tellurium dioxide (5 mM), silver nitrate (10 mM), and sodium dodecyl sulfate (30 mM). The mixture was then subjected to constant magnetic stirring at 90°C. The solution changed color from colorless to dark brown after 8 h, indicating the formation of Ag$_2$Te NPs. To terminate the reaction and to remove most of the matrix (e.g., hydrazine), the Ag$_2$Te NPs were subjected to three cycles of centrifugation [relative centrifugation force (RCF): 12000 g for 10 min] and wash (3 × 10 mL of water). For simplicity, the concentration of the as-prepared Ag$_2$Te NPs in 10 mL H$_2$O is represented as 1 X.

**CHARACTERIZATION**

JEOL JSM-1230 and FEI Tecnai-G2-F20 transmission electron microscopes (TEM) were used to measure the sizes and shapes of the as-prepared Ag$_2$Te NPs. The re-dispersed Ag$_2$Te NPs were separately placed on formvar/carbon film Cu grids (200 mesh; Agar Scientific) and dried at ambient temperature (25°C). An energy dispersive X-ray (EDAX) system (Inca Energy 200, Oxford) was used to determine the composition of the as-prepared NMs. Raman spectra were recorded using a Raman spectrometer (DongWoo 500i, Korea) equipped with a 50 × objective and a charge-coupled detector. The excitation wavelength was 532 nm and the spectral aperture was 50 μm. The signal collection time for each sample was 30 s.

**DETECTION OF Hg$^{2+}$ IONS USING Ag$_2$Te NPs**

Ag$_2$Te NPs (0.01 ×, 100 µL), phosphate buffer (PB) (1 mM, pH 4.0, 100 µL), and SDS (0.1 mM, 100 µL) were added to aqueous solutions (0.7 mL) containing various concentrations of HgCl$_2$ (final concentrations 10–150 nM). The mixtures were equilibrated under constant stirring at 37°C for 10 min. After centrifugation at 12000 g for 10 min, the supernatants were discarded and the pellets were dispersed in R6G solution (10 µM, 20 µL). Finally, drops (1 µL) of the R6G mixtures were added onto separate silica wafers and dried at ambient temperature (25°C) prior to SERS measurement.

**ANALYSIS OF REAL SAMPLE**

Pond water sample was collected from the National Taiwan University campus, and subsequently filtered through a 0.45 µm membrane. For comparison, aliquots (0.1 mL) of the pond water sample was mixed with HNO$_3$ (0.9 mL, final concentration 2%) prior to ICP-MS analysis. Aliquots of the pond water (100 µL) were spiked with standard solutions (100 µL) containing Hg$^{2+}$ at various concentrations (0.3–1.5 µM). Next, PB (1 mM, pH 4.0, 100 µL), SDS (0.1 mM, 100 µL), Ag$_2$Te NPs (0.01 ×, 100 µL), and water (500 µL) were added to the mixture to give final volumes of 1 mL. The mixtures were equilibrated under constant stirring at 37°C for 10 min. After centrifugation at 12000 g for 10 min, the supernatants were discarded and the pellets were dispersed in R6G solution (10 µM, 20 µL). Finally, drops of solutions (1 µL) were added onto separate silica wafers and dried at ambient temperature prior to SERS measurement.

**RESULTS AND DISCUSSION**

**SENSING STRATEGY**

Scheme 1 shows the detection of Hg$^{2+}$ based on differential SERS EFs of Ag$_2$Te and HgTe NPs. Ag$_2$Te relative to HgTe provides a higher EF value. The displacement reaction between Hg$^{2+}$ and Ag$_2$Te NPs leads to the formation of HgTe nanostructures and decomposition of Ag$_2$Te NPs. As a result, the SERS signal of R6G decreases upon increasing the concentration of Hg$^{2+}$, mainly because Ag$_2$Te relative to HgTe NPs provides a greater SERS EF value. Although the $K_{sp}$ values of Ag$_2$Te and HgTe are unavailable, the latter has a small one based on that of the metal selenides (Moon et al., 2010). For example, Ag$_2$Se relative to HgSe has a higher $K_{sp}$ value (1 × 10$^{-54}$ vs. 4 × 10$^{-59}$) (Wang et al., 2007). It
has been reported that the reaction of Ag$_2$Te nanostructures with Hg$^{2+}$ ions is spontaneous and fast (Samal and Pradeep, 2011).

To confirm the formation of HgTe NPs from the reaction of Ag$_2$Te NPs with Hg$^{2+}$, we conducted TEM, EDX, and XRD measurements. Figure 1A shows the TEM image of as-prepared Ag$_2$Te NPs with an average size of 26.8 ± 4.1 nm (100 counts). On the other hand, the TEM image displayed in Figure 1B shows HgTe NPs with an average size of 37.7 ± 6.8 nm. Different morphologies in the two TEM images reveal the changes in the Ag$_2$Te NPs. The EDX patterns (Figures 1C,D) confirm the displacement reaction between Hg$^{2+}$ and Ag$_2$Te NPs. The XRD patterns of Ag$_2$Te NPs (Figure 1E) and its reaction product (Figure 1F) with Hg$^{2+}$ agree with the literature data of Ag$_2$Te (JCPDS: 34-0142) and HgTe (JCPDS: 75-2084), respectively.

The EF value of Ag$_2$Te NPs (0.1 X) was investigated using R6G as a reporter. The limits of detection (LODs) at a signal-to-noise (S/N) 3 for R6G using silica wafers and Ag$_2$Te NPs as SERS substrates were 1.8 mM and 5 nM, respectively, which reveals that the Ag$_2$Te substrate provided a EF value of 3.6 × 10$^5$. The EF value of Ag$_2$Te NPs is comparable with that of Ag NPs (Wang et al., 1980; Kerker, 1987). The SERS signal of R6G on the Ag$_2$Te substrate was about 5-fold greater than that on an HgTe substrate that had been formed from the reaction of Ag$_2$Te NPs with 100 nM Hg$^{2+}$ ions. The decreased SERS signal of R6G at a constant concentration (10 µM) is related to the concentration of Hg$^{2+}$ ions (to be discussed later), revealing the potential use of this approach for the determination of the concentration of Hg$^{2+}$ ions. The decrease in SERS signal was due to the decreased amount of Ag$_2$Te NPs.

OPTIMIZATION OF DETECTION CONDITIONS

In order to optimize the sensing condition, we investigated several important parameters, including concentration of R6G, reaction time, and pH. The strongest SERS signal of R6G at 1361 cm$^{-1}$ (aromatic C-C stretching) was used to evaluate the effects of these parameters. Figure 2A shows that the SERS signal of R6G at 1361 cm$^{-1}$ increased upon increasing its concentration, with a saturated concentration of 10 µM. Figure 2B displays the reaction was completed within 10 min when using Hg$^{2+}$ at the concentration of 100 nM. Figure 2C displays that pH is not an important factor over the investigated pH range (4.0–10.0), mainly because the replacement reaction between Ag$_2$Te and Hg$^{2+}$ ions and the SERS signal of R6G are both not pH sensitive. Although larger Ag$_2$Te NPs provided greater SERS EF values, poor reproducibility of SERS signals due to their instability in aqueous solution is problematic.

SENSITIVITY AND SELECTIVITY OF Hg$^{2+}$ DETECTION

Figure 3A shows that the SERS signal of R6G decreased upon increasing the Hg$^{2+}$ concentration, with a linear relationship between the SERS ratios ($I_{R6G}-I_{Hg} / I_{R6G}$) at 1361 cm$^{-1}$ and the Hg$^{2+}$ concentration ranging from 10 to 150 nM ($R^2$ = 0.98). This approach provided an LOD at an S/N = 3 of 3 nM for Hg$^{2+}$ ions. The sensitivity is better than that provided by SERS approaches using different reporters (Zamaria et al., 2008; Han et al., 2010; Senapati et al., 2011; Luo et al., 2012). Control experiments were carried out to test the specificity of the developed approach for Hg$^{2+}$ ions (100 nM) under optimal conditions in the presence of
various metal ions (each at a concentration of 1 µM). The results displayed in Figure 3B reveal that the sensing approach is selective to Hg²⁺ ions. The potential interferences could not replace Ag⁺ ions from the Ag₂Te NPs, resulting in negligible changes in the SERS signal of R6G at 1361 cm⁻¹.

REFERENCES
Aravind, P. K., Nitzan, A., and Metiu, H. (1981). The interaction between electromagnetic resonances and its role in spectroscopic studies of molecules adsorbed on colloidal particles or metal spheres. Surf. Sci. 110, 189–204. doi: 10.1016/0039-6028(81)90595-1
Butler, O. T., Cook, J. M., Harrington, C. F., Hills, S. I., Rieuwerts, J., and Miles, D. L. (2006). Atomic spectrometry update. Environmental analysis. J. Anal. At. Spectrom. 21, 217–243. doi: 10.1039/BS16025C
Chiang, C.-K., Huang, C.-C., Liu, C.-W., and Chang, H.-T. (2008). Oligonucleotide-based fluorescence probe for sensitive and selective detection of mercury(II) in aqueous solution. Anal. Chem. 80, 3716–3721. doi: 10.1021/ac080142k
Clarkson, T. W., Magos, L., and Myers, G. J. (2008). The toxicity of mercury-current exposures and clinical manifestations. N. Engl. J. Med. 349, 1731–1737. doi: 10.1056/NEJMra022471
Darbha, G. K., Singh, A. K., Rai, U. S., Yu, E., Yu, H., and Ray, P. C. (2008). Selective detection of mercury (II) ion using nonlinear optical properties of gold nanoparticles. J. Am. Chem. Soc. 130, 8038–8043. doi: 10.1021/ja801412b
Dave, N., Chen, M. Y., Huang, P.-J. J., Smith, B. D., and Liu, J. (2010). Regenerable DNA-functionalized hydrogels for ultrasensitive, instrument-free mercury(II) detection and removal in water. J. Am. Chem. Soc. 132, 12668–12673. doi: 10.1021/ja106098j
Doering, W. E., Piotti, M. E., Natan, M. J., and Freeman, R. G. (2007). SERS as a foundation for nanoscale, optically detected biological labels. Adv. Mater. 19, 3100–3108. doi: 10.1002/adma.200701984
Grubisha, D. S., Lipert, R. J., Park, H.-Y., Driskell, J., and Porter, M. D. (2003). Femtomolar detection of prostate-specific antigen: an immunoassay based on surface-enhanced Raman scattering and immunogold labels. Anal. Chem. 75, 5936–5943. doi: 10.1021/ac034536f
Han, D., Lim, S. Y., Kim, B. J., Piao, L., and Chung, T. D. (2010). Mercury(II) detection by SERS based on a single gold microshell. Chem. Commun. 46, 5587–5589. doi: 10.1039/CDC000895H
Huang, C.-C., and Chang, H. T. (2007). Parameters for selective colorimetric sensing of mercury(II) in aqueous solutions using mercaptopropionic acid-modified gold nanoparticles. Chem. Commun. 1215–1217. doi: 10.1039/B615385F
Huang, C.-C., Yang, Z., Lee, K.-H., and Chang, H.-T. (2007). Synthesis of highly fluorescent gold nanoparticles for sensing mercury(II). Angew. Chem. Int. Ed. 46, 6824–6828. doi: 10.1002/anie.200700803
Jena, B. K., and Raj, C. R. (2008). Gold nanoelectrode ensembles for the simultaneous electrochemical detection of ultratrace arsenic, mercury, and copper. Anal. Chem. 80, 4836–4844. doi: 10.1021/ac071064w
Kerker, M. (1987). Estimation of magnetic intensities. J. Opt. Soc. Am. A 4, 1400–1407. doi: 10.1364/JOSAA.4.001400
C.-W., and Chang, H. T. (2008). Selective detection of various metal ions (each at a concentration of 1 µM). The results displayed in Figure 3B reveal that the sensing approach is selective to Hg²⁺ ions. The potential interferences could not replace Ag⁺ ions from the Ag₂Te NPs, resulting in negligible changes in the SERS signal of R6G at 1361 cm⁻¹.

REAL SAMPLE ANALYSIS
To examine the practicality of our approach, the concentration of Hg²⁺ in a pond water sample was determined. Our ICP-MS result showed that no Hg²⁺ was detected. By applying a standard addition method, the Raman ratios against the concentration of Hg²⁺ was found to be linear (R² = 0.98) over 30–150 nM (Figure 4). The recovery percentage values of Hg²⁺ over the concentration range (30–150 nM) were 96–103%, showing low matrix interference. Our results reveal that this approach holds great potential for the determination of the concentrations of Hg²⁺ in environmental samples.

CONCLUSIONS
We have demonstrated a SERS-based approach for the detection of Hg²⁺ ions using Ag₂Te NPs as a substrate and recognition element and R6G as a reporter. To the best of our knowledge, this is the first example using a single material (Ag₂Te) as the substrate and recognition element in SERS technology. Relative to Ag₂Te, HgT e is a less SERS active substrate, thus the SERS signals of R6G decreased upon increasing Hg²⁺ concentration when using Ag₂Te NPs as a substrate. This novel approach is sensitive (LOD 3 nM) and selective for the detection of Hg²⁺ ions over a wide pH range. With its high sensitivity, selectivity, and simplicity, the SERS-based approach holds great potential for the determination of the concentrations of Hg²⁺ in environmental samples.

AUTHOR CONTRIBUTIONS
Chia-Wei Wang: He did the characterization of Ag₂Te nanoparticles and the detection of Hg²⁺. Zong-Hong Lin: He developed the method to synthesize Ag₂Te nanoparticles and designed the sensing strategy. Prathik Roy: He gave some advice about synthesis of Ag₂Te nanoparticles and sensing strategy of Hg²⁺. Huan-Tsun Chang: He is the advisor of this group.

ACKNOWLEDGMENTS
This study was supported by the National Science Council of Taiwan under contracts NSC 101-2113-M-002-002-MY3.
Detection of Hg$^{2+}$ through SERS

Wang et al.

Lin, Z.-H., Chen, I.-C., and Chang, H.-T. (2008). Colorimetric detection of mercuric ion (Hg$^{2+}$) in aqueous media using DNA-functionalized gold nanoparticles. Angew. Chem. Int. Ed. 46, 4093–4096. doi: 10.1002/anie.200700269

Lee, J.-S., Han, M. S., and Mirkin, C. A. (2007). Colorimetric detection of mercury using single nanoparticles as substrates. J. Phys. Chem. C 113, 13539–13544. doi: 10.1021/jp076716f

Samal, A. K., and Pradeep, T. (2009). Assembly of dandelion-like Au/PANI nanocomposites and their application as SERS nanosensors. Biosens. Bioelectron. 26, 3063–3067. doi: 10.1016/j.bios.2010.11.044

Xue, X., Wang, F., and Liu, X. (2008). One-step, room temperature, colorimetric detection of mercury (Hg$^{2+}$) using DNA/nanoparticle conjugates. J. Am. Chem. Soc. 130, 3244–3245. doi: 10.1021/ja076716c

Yang, Y., Shi, J., Tanaka, T., and Nogami, M. (2007). Self-assembled silver nanochains for surface-enhanced Raman scattering. Langmuir 23, 12042–12047. doi: 10.1021/la0701610s

Zahir, F., Rizwi, S. I., Haq, S. K., and Khan, R. H. (2005). Low dose mercury toxicity and human health. Environ. Toxicol. Pharmacol. 20, 351–360. doi: 10.1016/j.etap.2005.03.007

Zamrion, V. M., Timm, R. A., Araki, K., and Toma, H. E. (2008). Ultrasensitive SERS nanoprobes for hazardous metal ions based on trimercaptotriazine-modified gold nanoparticles. Inorg. Chem. 47, 2934–2936. doi: 10.1021/ic80122v

Zhao, Z., Su, Y., Li, J., Li, D., Zhang, J., Song, S., et al. (2009). Highly sensitive electrochemical sensor for mercury(II) ions using a mercury-specific oligonucleotide probe and gold nanoparticle-based amplification. Anal. Chem. 81, 7660–7666. doi: 10.1021/acid9010809