AgNPs Functionalized with Dithizone for the Detection of Hg$^{2+}$ Based on Surface-enhanced Raman Scattering Spectroscopy

Na Guo · Guangda Xu · Qijia Zhang · Peng Song · Lixin Xia

Received: 7 January 2022 / Accepted: 22 March 2022 / Published online: 7 May 2022
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2022

Abstract
Mercuric ion (Hg$^{2+}$), a poisonous metal ion that remained in water ecosystems, can severely damage the human central and peripheral nervous system and kidneys. Consequently, rapid and highly sensitive methods to determine trace Hg$^{2+}$ are meaningful to discuss. In recent years, methods for detecting heavy metals by complexation reactions have emerged one after another. We have proposed a novel approach of surface-enhanced Raman scattering (SERS) for the quantitative analysis of Hg$^{2+}$ in water samples using dithizone (DTZ) as a Raman reporter. DTZ-modified silver nanoparticles (AgNPs) produced a strong SERS signal. In the presence of Hg$^{2+}$, the DTZ can capture Hg$^{2+}$ composing a stable structure, resulting in DTZ leaving the surface of the AgNPs, with an accompanying decrease in the signal. The proposed SERS assay showed a linear range of $10^{-4}$–$10^{-8}$ M, with a limit of detection of $9.83 \times 10^{-9}$ M. The sensor has low detection cost, rapid detection speed, and uncomplicated sample pretreatment. Furthermore, this method can be successfully utilized to detect Hg$^{2+}$ rapidly in water samples, which sheds new light on the detection of Hg$^{2+}$ in the environment.

Keywords Hg$^{2+}$ · SERS · Dithizone · Sensor · Water analysis

Introduction
Heavy metals have a considerable impact on soil, plants, aquatic animals, and humans [1, 2]. Hg$^{2+}$ is one of the heavy metal ions, which is highly toxic in organisms and ecosystems even at a low concentration of 5 μg/L [3]. Mining activities, municipal waste, oil and coal combustion, cement production, consumer product emissions, waste–water emissions and so on are the major sources of mercury ion emissions into the environment, which has caused pollution due to improper treatment methods [4, 5]. Hg$^{2+}$ poisoning can cause neurological, renal, immune, cardiac, sports, reproductive, and genetic diseases in humans [6–8]. Therefore, it is of great significance to study the widespread distribution of Hg$^{2+}$ in the aquatic environment and bioaccumulation in the food chain [9–11].

At present, many analysis techniques have been utilized in the detection of Hg$^{2+}$ such as atomic absorption spectrometry, inductively coupled plasma–mass spectrometry (ICP–MS), high-performance liquid chromatography, electrochemistry, colorimetry, and fluorescence spectroscopy [12–19]. However, these methods have limitations [20, 21], such as complex sample preparation, expensive equipment, and time-consuming procedures. Therefore, a simple, highly selective, and sensitive method for measuring Hg$^{2+}$ must be developed to overcome these shortcomings. A non-destructive optical inspection is an attractive option, it can respond quickly in natural environments and organisms.

Surface-enhanced Raman scattering (SERS) is a non-destructive and rapid analysis technique that has a wide range of applications [21–24], especially the analysis of trace poisonous substances, such as heavy metal ions, dyes, and pesticides [25–28]. In recent years, using SERS to detect Hg$^{2+}$ has received widespread attention. The substrates which can enhance the signal of Raman, such as silver nanoparticles (AgNPs) and gold nanoparticles, are stable, easy to synthesize, and can be easily modified with different reagents, which improve the method of cognition Hg$^{2+}$ [29, 30]. Various methods have been put forward for detecting Hg$^{2+}$, based on differing SERS probes. Qi et al. studied a T–Hg$^{2+}$–T mismatch
base pair pattern for Hg\(^{2+}\) detection [31]. Hao et al. utilized acriflavine to detect Hg\(^{2+}\) quantitatively, since the interaction between acriflavine and AgNPs. Luo et al. introduced safranine T as a SERS probe, allowing trace analysis of Hg\(^{2+}\) with high selectivity [17]. Although these methods achieve the advantages of rapid and sensitive detection of Hg\(^{2+}\), they are susceptible to biological and environmental influences.

Herein, for the first time, we propose a method for the detection of Hg\(^{2+}\) with DTZ. In this work, indirect SERS detection of Hg\(^{2+}\) is carried out based on dithizone (DTZ) as a Raman probe. The quantitative decrease of the SERS signal of the probe molecule of DTZ was observed when DTZ-functionalized AgNPs mixed with a certain amount of Hg\(^{2+}\), which suggests that the phenomenon of decreased SERS signal intensity should chiefly be attributed to the interaction between Hg\(^{2+}\) and DTZ [32, 33]. A linear correlation of Raman intensity with Hg\(^{2+}\) concentrations was from 10\(^{-4}\)–10\(^{-8}\) M with a limit of detection was 9.83 \times 10\(^{-9}\) M. Eventually, the SERS method with DTZ as Raman reporter is beneficial for the rapid and sensitive determination of Hg\(^{2+}\) in water. This quantitative detection strategy of the proposed method is shown in Fig. 1.

**Materials and Methods**

**Chemicals and Reagents**

All reagents and solvents were of analytical grade, and no further purification is required when used. DTZ, absolute ethanol, sodium citrate, AgNO\(_3\), HgCl\(_2\), KNO\(_3\), Al(NO\(_3\))\(_3\), Fe(NO\(_3\))\(_3\), Bi(NO\(_3\))\(_3\), Cu(NO\(_3\))\(_2\), NaNO\(_2\), Ni(NO\(_3\))\(_2\), and Pb(NO\(_3\))\(_2\) were all purchased from China Energy Chemical Co., Ltd. The solutions used in this experiment were prepared using deionized water.

**Instrumentation**

A JEM–2100 ultra-high-resolution transmission electron microscope (JEOL, Japan) was used to characterize AgNPs, and to determine the geometry and size of the AgNPs. A PerkinElmer Lambda 35 spectrophotometer (326 nm; Norwalk, CT, USA) was used to record the ultraviolet–visible (UV–Vis) absorption spectra. The SERS measurements were performed using a Renishaw InVia Reflex confocal microscope (Renishaw, UK). All measurements were performed using a He–Ne laser (532 nm, exposure time of 10 s). The laser spot diameter was 1 \(\mu\)m, and the laser power was 50 mW.

**Synthesis and Characterization of AgNPs**

AgNPs were prepared according to previous work [34]. In short, 36 mg AgNO\(_3\) was added to 200 mL of water and boiled under constant stirring. Then, trisodium citrate (4 mL, 1%) solution was added to the above solution quickly and boiling continued for another 30 min. Let the flask chill to room temperature, a green–gray colloid was acquired. Finally, the colloidal AgNPs were stored at 4 °C for later use.

![Fig. 1 Schematic illustration of Hg\(^{2+}\) sensor](image-url)
Preparation of Hg\(^{2+}\) Standard Solution and Samples

HgCl\(_2\) was dissolved in deionized water to make a 1 mM solution. A set of Hg\(^{2+}\) solutions with different concentrations was obtained by diluting 1 mM HgCl\(_2\) solution. Drain water from a tap was collected in Shenyang City, Liaoning Province, China. The samples passed a 0.22-μm filter membrane to remove impenetrable matter for the later experiments.

Detection for Hg\(^{2+}\)

DTZ solution (20 μL), AgNPs (60 μL), and Hg\(^{2+}\) (20 μL) or spiked water solution of different concentrations were transferred to a centrifuge tube in turn, and SERS detection was performed after fully sonicating and mixing at room temperature. Using a He–Ne laser (532 nm), laser power was 50 mW. The intensity of SERS change of DTZ at 1590 cm\(^{-1}\) was elected as a basis for quantification. Each experiment was performed three times in parallel.

Results and Discussion

SERS Measurement of DTZ and DTZ–Hg\(^{2+}\)

The TEM image showed that the size of uniform spherical AgNPs is about 30 nm (Fig. 2a, b) shows the UV–Vis absorption spectrum of AgNPs at 420 nm, which demonstrated the successful synthesis of AgNPs. The UV–Vis
peaks of AgNPs and DTZ shifted, indicating that DTZ was successfully adsorbed on the AgNPs surface. Figure 2c is X-ray photoelectron spectroscopy (XPS) for elemental and structural analysis. The image shows that Hg, O, N, and C elements exist in the system, which proves that AgNPs, DTZ, and Hg^{2+} exist in the detection system. The SERS signal of DTZ, DTZ with Hg^{2+} and ethanol are in Fig. 2d, in which the main SERS signals of DTZ were located at 510, 855, 994, 1156, 1219, 1310, 1377, and 1590 cm^{-1} [35]. It is found that the SERS peak at 1590 cm^{-1} attributed to the C=N vibration of DTZ, has conspicuous changes, so it is the most suitable for use as a basis for quantitative analysis. Comparing the blue and the red lines, after adding Hg^{2+}, the SERS intensity of the DTZ molecules reduced significantly. This is substantially due to the change in the number of DTZ molecules adsorbed on the SERS active sites. The SERS signal of DTZ was enhanced dramatically because the DTZ molecules drew closer to the SERS active sites through the Ag–N bond. However, after the addition of Hg^{2+}, the binding force between Hg^{2+} and DTZ is stronger than that with AgNPs, which leads to the desorption of DTZ molecules from SERS active sites. As a result, fewer DTZ molecules were left on the AgNPs surface and the SERS signal intensity was reduced.

Optimization of the Analytical Conditions

To increase the sensitivity of this method, we optimized the experimental parameters. The volume of AgNPs will affect the analytical performance. Figure 3a shows the intensity of the SERS signal of DTZ at 1590 cm^{-1}. Upon the increase in the volume of AgNPs, the corresponding SERS signal increased. However, when the volume exceeded 60 μL, the SERS signal achieved a steady value, which proved that DTZ is completely adsorbed on the AgNPs. Therefore, 60 μL AgNPs were chosen for the detection of Hg^{2+}. In this method, DTZ will connect AgNPs and Hg^{2+}, so we explored the influence of DTZ concentration. As shown in Fig. 3b, the SERS signal reached a maximum value when the concentration of DTZ is 10^{-4} M. Thus, we fixed 10^{-4} M as the optimum condition. Mixing time plays a critical part in this detection process. Figure 3c shows that after mixing for 8 min the signal tends to be stable, demonstrating that DTZ was entirely integrated with Hg^{2+}. So, we chose 8 min as the optimized mix time.

Selectivity for the AgNPs–DTZ–Hg^{2+} System

To study the selectivity of this method, various other environment-related metal ions were appraised (such as Fe^{3+}, Cu^{2+}, Al^{3+}, Na^{+}, K^{+}, Ni^{2+}, Bi^{3+}, and Pb^{2+}). Figure 4a shows the comparison to SERS spectra of the Hg^{2+} and other ions, based on the low binding affinity of DTZ to other ions, the SERS signal of DTZ did not reduce significantly in the presence of the other ions, which indicated the DTZ sensors for Hg^{2+} detection had an excellent selection.
To clearly show the SERS signal intensity corresponding to the 1590 cm⁻¹ peak of DTZ, Fig. 4b is presented in the form of a histogram. The Hg²⁺ and mixed groups showed significantly lower column heights, which indicates that the AgNPs–DTZ system can specifically detect Hg²⁺, and this method has an anti-interference ability.

Quantitative SERS Detection of Hg²⁺

To assess the sensitivity and potential quantitative analysis application of the proposed method, we measured different concentrations of Hg²⁺ standard samples. Under the optimum conditions, the present method showed a linear relationship for Hg²⁺ concentrations and the SERS peak in the range of 10⁻⁴ to 10⁻⁸ M with a limit of detection of 9.83 × 10⁻⁹ M, which conforms to the equation Y = 1096.3961X + 15738.17866 (R² = 0.999). The relevance between the SERS signal intensity and Hg²⁺ concentration is shown in Fig. 5. In the comparison, the experimental results of some other methods for measuring Hg²⁺ are listed in Table 1, which showed that our method is simpler and faster. More importantly, the detection result is inferior to the limit of 10 ppb (4.98 × 10⁻⁸ M) in drinking water recommended by the World Health Organization.

Reproducibility of the Hg²⁺ Detection

To assess the reproducibility of the AgNPs–DTZ–Hg²⁺ system, the SERS intensity of Hg²⁺ at a concentration of 10⁻⁵ M under optimal experimental conditions is detected. Randomly collected 20 different points for testing with the result is shown in Fig. 6a. It is worth noting that the SERS intensity at 1590 cm⁻¹ is relatively uniform. From Fig. 6b, we find that the relative standard deviation of the 20 sets of data is less than 5%, which proves that the SERS sensor proposed here article has satisfactory reproducibility.

Analysis of Hg²⁺ in Tap Water Samples

To demonstrate the practicality of the developed SERS method, we detected the Hg²⁺ contents in tap water under the optimum conditions by the standard addition method. Different concentrations of Hg²⁺ were tested 3 times and the results are shown in Fig. 7. We further evaluated a comparison of the developed SERS method and the conventional ICP–MS method and the related statistics are shown.

| Method  | Linear range (μmol/L) | LOD (μmol/L) | Reference |
|---------|-----------------------|--------------|-----------|
| SERS    | -0.1–0.01             | 0.009        | This work |
| SERS    | -                     | 0.5          | [36]      |
| Electrochemistry | 0.1–2.0                | 0.1          | [37]      |
| Colorimetry      | -                     | 0.498        | [38]      |
| Colorimetry      | -                     | 0.05         | [39]      |
| Fluorescence     | 0.05–0.8              | 0.015        | [40]      |
| Fluorescence     | -                     | 0.011        | [41]      |
| ELISA      | 0.05–50.0             | 0.048        | [42]      |

Fig. 7 Detect the SERS spectra of different concentrations of Hg²⁺ in tap water.

Fig. 6 (a) The SERS intensity at 1590 cm⁻¹ was measured from 20 random positions. (b) A series of SERS spectra of 10⁻⁵ M Hg²⁺ solution from 20 random positions.
in Table 2, which indicates that the Hg$^{2+}$ concentrations detected by the SERS sensor are very consistent with those measured by the classic ICP–MS method. The recovery for Hg$^{2+}$ was 89–90%. The results showed that this method is reliable and applicable for the detection of Hg$^{2+}$ in tap water.

Table 2 Determination of Hg$^{2+}$ in tap water samples via the SERS method and ICP–MS

| Samples   | Spiked amount (μM) | SERS amount | SERS Recovery% | RSD% (n = 3) | Spiked amount (μM) | ICP–MS amount | ICP–MS Recovery% |
|-----------|-------------------|-------------|----------------|-------------|-------------------|---------------|------------------|
| Tap water | –                 | Not detected| –              | –           | 10                | 9.63          | 89.96            |
|           | 1                 | 1.12        | 89.90          | 1.31        | 1                 | 0.46          | 89.44            |
|           | 0.1               | 0.10        | 89.53          | 0.43        | 0.1               | 0.09          | 89.12            |

**Conclusion**

In short, we developed a time-saving, high-sensitivity SERS sensor for detecting Hg$^{2+}$. This sensor works with excellent sensitivity via the special recognition of Hg$^{2+}$ by DTZ, which belongs to the following: first, DTZ has a high SERS signal that can utilize for Hg$^{2+}$ quantitative detection. Moreover, DTZ captures Hg$^{2+}$ and forms a steady structure that can enhance the specificity of this method. We have merely fabricated the SERS sensor by mixing AgNPs and DTZ to achieve a rapid and ultrahigh sensitivity to detect Hg$^{2+}$, which can exhibit a limit of detection of 9.83×10$^{-9}$ M under the best conditions. More importantly, this sensor can detect Hg$^{2+}$ in a broad range of concentrations and the detection result is inferior to the limit of 10 ppb (4.98×10$^{-8}$ M) in drinking water recommended by the World Health Organization. Remarkably, we further demonstrate the SERS sensor is suitable for detecting Hg$^{2+}$ from tap water in reliable and quantitative manners. With these advantages, we anticipate this method will have fine potential in the detection of Hg$^{2+}$ in complex water environments.

**Author Contribution** All authors contributed to the study conception and design. Material preparation, data collection, and analysis were performed by Na Guo, Guangda Xu, Qijia Zhang, Peng Song, and Lixin Xia. The first draft of the manuscript was written by Na Guo and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

**Funding** This work was supported by the Special Project of Guiding Local Scientific and Technological Development by the Central Government of Liaoning Province (Grant No. 2022010010-JH6/1001), the National Natural Science Foundation of China (Grant No. 11974152 and 21671089), the Liaoning Revitalization Talents Program (Grant No. XLYC1807162), the Shenyang Highlevel Innovative Talents Program (RC200565), the Scientific Research Projects of Liaoning Provincial Department of Education (L2020002), the Liaoning Provincial Natural Science Foundation Joint Fund Project (2020–YKLH–22), the Science program of Liaoning Provincial Department of Education (LJKZ0097), and the Intercollegiate cooperation project of colleges and universities of Liaoning Provincial Department of Education.

**Data Availability** All data generated or analyzed during this study are included in this published article.

**References**

1. Amit P, Ajay K, Zhong H (2018) Adverse effect of heavy metals (As, Pb, Hg, and Cr) on health and their bioremediation strategies: a review. Int Microbiol 21:97–106. https://doi.org/10.1007/s10123-018-0012-3
2. Abdu N, Abdullahi A, Abdulkadir A (2017) Heavy metals and soil microbes. Environ Chem Lett 15:65–84. https://doi.org/10.1007/s10311-016-0587-x
3. Kothalam R, Perumal P, Ayyanu R (2019) Magnetic core–shell fibrous silica functionalized with pyrene derivative for highly sensitive and selective detection of Hg (II) ion. J Disper Sci Technol 40:1368–1377. https://doi.org/10.1080/01932691.2018.1468265
4. Hao BQ, Bu XF, Wu JW, Ding YR, Zhang LX, Zhao B, Tian Y (2020) Determination of Hg$^{2+}$ in water based on acriline functionalized AgNPs by SERS. Microchem J 155:104736. https://doi.org/10.1016/j.microc.2020.104736
5. Wang J, Deng BL, Chen H, Wang XR, Zheng JZ (2009) Removal of aqueous Hg(II) by polyaniline: sorption characteristics and mechanisms. Environ Sci Technol 43:5223–5228. https://doi.org/10.1021/es803710k
6. Rajeshwari A, Karthiga D, Natarajan C, Amitava M (2016) Anti–aggregation–based spectrometric detection of Hg(II) at physiological pH using gold nanorods. Mat Sci Eng C-Mater 65:711–716. https://doi.org/10.1016/j.msec.2016.05.066
7. GianPaolo G, Caterina AM, La P (2008) Molecular mechanisms triggered by mercury. Toxicology 244:1–12. https://doi.org/10.1016/j.tox.2007.11.002
8. Juma O, Christopher RF, Christopher C (2021) Concentration–dependent health effects of air pollution in controlled human exposures. Environ Int 150:106424. https://doi.org/10.1016/j.envint.2021.106424
9. Yu LY, Zhang LY, Ren G, Li JS, Zhu BY, Chai F, Qu FY, Wang CG, Su ZM (2018) Multicolorful fluorescent–nanoprobe composed of Au nanocluster and carbon dots for colorimetric and fluorescent sensing Hg$^{2+}$ and Cr$^{6+}$. Sensor Actuat B-Chem 262:678–686. https://doi.org/10.1016/j.snb.2018.01.192
22. Sehan J, Wang QX, Lei M, Liu YL, W. Daisa BH, Alex K, Qiu T, Ma LB, Chu PK, Oliver SG (2020) Flexible surface–enhanced Raman scattering chip: a universal platform for real–time interfacial molecular analysis with femtomolar sensitivity. Anal Chem 92:4164–4219. https://doi.org/10.1021/acs.analchem.0c00069

23. Wang Y, Zou C, Wang W, Xu DD, Zeng FY, Zhan C, Gu JH, Li MY, Zhao WW, Zhang JH, Guo JH, Feng HH, Ma X (2018) Photocatalytically powered matchlike nanomotor for light–guided active SERS sensing. Angew Chem Int Ed 40:13110–13113. https://doi.org/10.1002/anie.201807033

24. Surabhi K, Thomas C, Kuang Y (2022) Silver enriched silver phosphate micromes as an efficient recyclable SERS substrate for the detection of heavy metal ions. J Colloid Interf Sci 605:173–181. https://doi.org/10.1016/j.jcis.2021.07.084

25. Gao Y, Li LF, Zhang X, Wang XN, Ji W, Zhao JZ, Yukihiro O (2019) CTAB–triggered Ag aggregates for reproducible SERS analysis of urinary polycyclic aromatic hydrocarbon metabolites. Chem Commun 55:2146–2149. https://doi.org/10.1039/c8cc09008d

26. Tehseen Y, Hongbin P, Sun DW (2019) Fabrication of silver–coated gold nanoparticles to simultaneously detect multi–class insecticide residues in peach with SERS technique. Talanta 196:537–545. https://doi.org/10.1016/j.talanta.2018.12.030

27. Jeongan C, Jiwon L, Jae JH (2020) Fully integrated optofluorometric SERS platform for real–time and continuous characterization of airborne microorganisms. Biosens Bioelectron 169:12611. https://doi.org/10.1016/j.bios.2020.112611

28. Li Q, Xiao MS, Wang F, Wang LH, Ji W et al (2017) Poly–cytosine–mediated nanotags for SERS detection of Hg2+. Nanoscale 9:14184–14191. https://doi.org/10.1039/c7nr05165d

29. Cui JB, An MY, Wang LY (2013) Nanocomposite–based rapid, visual, and selective luminescence turn–on assay for Hg2+ sensing in aqueous media. Talanta 115:512–517. https://doi.org/10.1016/j.talanta.2013.05.069

30. Kiwan MA, Hassan MF, Hamdan W (1989) Studies on tetracycline–dihydrzone isomers and their reactions with metal ions. B Chem Soc Jpn 62:325–329. https://doi.org/10.1246/bcsj.62.325

31. Gu X, Jon CP (2015) Surface–enhanced Raman spectroscopy–based approach for ultrasensitive and selective detection of hydrazine. Anal Chem 87:6640–6646. https://doi.org/10.1021/acs.analchem.5b01566

32. Sujitrita S, Nootcharin W, Mayuko K, Siriripapa S, Narisara T (2021) Raman enhanced scattering and DFT studies on the adsorption behaviour of dihydrozine on silver nanoparticle. Inorg Chem Commun 126:108480. https://doi.org/10.1016/j.ijinche.2021.108480

33. Donghoon H, Sung LY, Beom KJ, Piao LL, Taek CD (2010) Mercury(ii) detection by SERS based on a single gold microshell. Chem Commun 46:5587–5589. https://doi.org/10.1039/c000895h

34. Donghoon H, Yang KR, Jeong OW, Tae KH, Rakesh MK, Jong HS, Hasuck K (2009) A regenerative electrochemical sensor based on oligonucleotide for the selective determination of mercury(II). Analyst 134:1857–1862. https://doi.org/10.1039/b908457f

35. Ridhima C, Abhishek D, Anil DK, Sudhir K, Nandita M (2021) 2–thiazoline–2–thiol functionalized gold nanoparticles for detection of heavy metals, Hg(II) and Pb(II) and probing their competitive surface reactivity: a colorimetric, surface enhanced Raman scattering (SERS) and x–ray photoelectron spectroscopic (XPS) study. Colloid Surf A 615:126279. https://doi.org/10.1016/j.colsurfa.2021.126279

36. Li T, Dong SJ, Wang EK (2009) Label–free colorimetric detection of aqueous mercury ion (Hg2+) using Hg2+-modulated G–quadruplex–based DNAzymes. Anal Chem 81:2144–2149. https://doi.org/10.1021/ac900188y
40. Xie WY, Huang WT, Luo HQ, Li NB (2012) CTAB–capped Mn–doped ZnS quantum dots and label–free aptamer for room–temperature phosphorescence detection of mercury ions. Analyst 137:4651–4653. https://doi.org/10.1039/c2an35777a

41. Luo L, Song T, Wang HQ, Yuan QH, Zhou SH (2018) A highly selective fluorescence sensing platform for nanomolar Hg(II) detection based on cytosine derived quantum dot. Spectrochim Acta A 193:95–101. https://doi.org/10.1016/j.saa.2017.11.044

42. Zhan XJ, Xi T, Zhou P (2013) Indirect competitive immunoassay for mercury ion determination using polyclonal antibody against the Hg–GSH complex. Environ Forensics 14:103–108. https://doi.org/10.1080/15275922.2012.760175

Publisher’s Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.