Sensitive and Selective Detection of Mercury Ions in Aqueous Media Using an Oligonucleotide-functionalized Nanosensor and SERS Chip

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A surface-enhanced Raman scattering (SERS) platform for the selective trace analysis of Hg2+ ions was reported, based on poly-thymine (T) aptamer/2-naphthalenethiol (2-NT)-modified gold nanoparticles (AuNPs), which was an oligonucleotide-functionalized nanosensor and SERS chip. 2-NT was used as a Raman reporter, and T aptamer could form a T-Hg2+-T structure with Hg2+ ions making an SERS nanosensor absorbed to the SERS chip. The optimum concentrations of DNA and 2-NT were obtained. An average of 960 DNA molecules attached to each AuNP were measured. The limit of detection (LOD) was 1.0 ppt ($1.0 \times 10^{-12}$ g/mL), which is far below the limit of 10.0 ppb for drinking water, stipulated by the World Health Organization. The sensor has the advantages of low detection cost, a simple sample pretreatment, a green solution and reducing false positives. Furthermore, the nanosensor was used for the determination of trace Hg2+ in the water of a lake; a reliable result was obtained accurately.

Keywords  Mercury ions, nanosensor, SERS chip, aptamer-modified

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Introduction

Mercury and its compounds have a variety of hazards to human health, even at ultra-low concentrations. Mercury in water can enter the human body through food chain, especially aquatic products.1 Mercury accumulated in the human body is difficult to expelled through its own metabolism. Trace mercury can cause toxicity to the liver, kidney and other organs, resulting in oral inflammation, hand tremors, nervous system disorders and chronic mercury poisoning.2,4 With an increase of mercury accumulation, it shows severe irreversible cranial nerve damage, and can even lead to brain death.5–7 Therefore, the mercury content in natural water is a top priority in environmental monitoring and control. In order to render humans healthy, the maximum Hg2+ ions residue in drinking water stipulated by the American environmental protection association is 10 nM.8 Therefore, researchers urgently need to develop a fast, sensitive and selective way to detect Hg2+. Methods of detection and quantitative analysis of Hg2+ ions have been developed, including an enzyme-linked immunosorbert assay (ELISA),9 cold vapor atomic fluorescence spectrometry (CA-AFS),10 hydride generation atomic absorption spectroscopy (HGAAS),11 electrothermal atomic absorption spectrophotometry (ETAAS)12 and so on. These methods, however, are time-consuming and labor-intensive. They require expensive instruments and tedious preprocessing. A simple, cheap and sensitive detection method is therefore needed.

As a powerful detection method, SERS technology has been successfully applied to many aspects, such as environment,13,14 chemical engineering,15 life science,16–19 and food safety.20 At present, a widely accepted interpretation of SERS is a strong mechanism of the electromagnetic field of the electronic motion of the base surface and joint effect of the strong mechanism of the substrate and the electron transfer on the surface of the object to be measured.21–23 Up to now, the SERS substrate is one of the hotspots in the field of SERS technology that can be divided into a solid substrate and a metal colloidal substrate. The metal colloidal substrate can be uniformly dispersed in solution, which is suitable for SERS detection in liquid environments, such as molecular detection24 and cell detection.25 Meanwhile, the solid substrate can provide a stable platform for SERS detection. With the emergence of new materials, some novel SERS substrates, which are separated from the traditional ones, are gradually prepared, such as a flexible SERS solid substrate prepared by the electrodeposition of Au onto a carbon fiber cloth.26 In order to achieve specific detection, antibodies and SERS tags were attached to gold nanoparticles, and an immunoassay SERS nanoscale sensor was proposed based on different substrates.27 Previously, various antibody modified SERS nanoscale sensors have been successfully applied to the quantitative analysis of chemical and biological molecules,28–30 diseases and cells.31,32
In recent years, researchers have attached great importance to the research of aptamers. Aptamer is a single strand nucleic acid binding to a specific target, which has high affinity and specificity. Aptamers are more advantageous than antibodies because they are easy to be chemically synthesized, to preserve, and to modify. After being modified by aptamers, gold nanoparticles become a new type of biosensor for nanosensors. Hg\(^{2+}\) ions can selectively bind between two thymine bases, and promote these T-T mismatches to form stable T-Hg\(^{2+}\)-T base pairs. According to this principle, Hg\(^{2+}\) ions can be detected by fluorescence. The LOD is 12.9 nM and 10.2 pM. In addition, by this principle, Hg\(^{2+}\) ions can be detected by SERS. The LOD is 50 and 5 nM. Aptamer modified nanoscale sensors can also be applied to detect proteins, biotoxins, antibiotics and nucleocapsid proteins. In addition, there are other methods to detect mercury ions by other principles.

However, traditional Hg\(^{2+}\) ions SERS detection has some problems, such as difficult preparation, complex operation and false positive signals. Therefore, in this work a novel Hg\(^{2+}\) ions SERS detection method is proposed. In the general method, thymidine aptamers were electrostatically interacted, rather than covalently adsorbed on AuNPs. In this method, thymine-rich aptamers were assembled onto the surface of gold nanoparticles via a thiol gold bond. Thiolated aptamers and Raman tag were assembled on the surface of Au nanoparticles. Another thiolated aptamers were assembled on the surface of the solid substrate, and the T-Hg\(^{2+}\)-T structure was formed on the solid substrate (SERS chip), making the detection operation more simple and convenient. To our best knowledge, the presented nanosensor and SERS chip, for the first time, could be directly applied for Hg\(^{2+}\) detection in aqueous media with a simple detection process. The LOD is 1.0 ppt (1.0 × 10^{-12} g/mL), which is below the LOD of previous mercury ions detection methods. The proposed method was green because Hg\(^{2+}\) ions can be removed easily after being adsorbed onto the chip.

**Experimental**

**Materials and reagents**

Phosphate buffered saline (PBS; 10 mM, pH 7.0) was purchased from Molecular Research Center (USA). Bovine serum albumin (BSA) was purchased from Solarbio (Beijing, China). Chloroauric acid (HAuCl\(_4\)), trisodium citrate, 2-naphthalenemethiol, glutaraldehyde (GA) and NaCl were purchased from Kermel (Tianjin, China). A mercury single element standard solution (1 × 10^{-3} g/mL), 3-aminopropyl trimethoxysilane (APTMS), tris(2-carboxyethyl) phosphine (TCEP) and other chemicals were purchased from Aladdin China Ltd. (Shanghai, China). All other reagents were of analytical grade. All solutions were prepared in ultrapure water (≥18 MΩ, Elga water purification system, ELGA). Lake water samples were collected from QingNian Lake (Tianjin, China). The synthesis of DNA and the modification of SH, NH\(_2\) and FAM were done by Tataru (Dalian, China). Their sequences are as follows:

Sequence 1 (S1): SH-(CH\(_2\))\(_6\)-5’-TTTTTGTTCCTTGTTTCCT-3’;
Sequence 2 (S2): NH\(_2\)-(CH\(_2\))\(_6\)-5’-TTTTTGTTCCTTGTTTCCT-3’;
Sequence 3 (S3): SH-(CH\(_2\))\(_6\)-5’-TTTTTGTTCCTTGTTTCCT-3’-(CH\(_2\))\(_6\)-FAM

S1 and S2 are the Hg\(^{2+}\) recognition aptamers. S3 is fluorescent oligonucleotides used to determine the actual amount of S1 on per particle.

**Fabrication of DNA-AuNP-Raman tag nanosensor**

First, 50.0 μL of 0.01% chloroauric acid solution was heated and stirred vigorously. Under the stirring state, 1% of citric acid and three sodium aqueous solution of 1.5 mL were added quickly. Continued to heat and boil for 15 min. The pale-yellow solution gradually became a stable deep red. Finally, the liquid was cooled to room temperature, and a gold colloid with diameter of 30 nm was obtained.

The SERS nanoscale sensor was based on a slightly improved preparation by Liu et al. Then, 10.0 μL of 2 μM 2-NT was added to 1.0 mL of 30 nm gold colloid. The mixture was gently stirred for 10 min. 2-NT labeled gold nanoparticles colloidal was centrifuged for 10 min at 8000 rpm and resuspended with 1.0 mL 10 mM PBS. The DNA (S1) with 10.0 μL concentration of 2 μM was mixed with TCEP with 1.5 μL concentration of 10 mM, and activated for 1 h at room temperature. The prepared DNA (S1) solution was added into colloidal gold to react for 12 h. After that, 20.0 μL 0.1 M NaCl was added to salt for 30 min. The mixture was then placed at 4°C to continue salting for 6 h. The mixture was centrifuged for 30 min at 8000 rpm and resuspended with 1.0 mL 10 mM PBS. The obtained nanosensor was stored at 4°C. The successful attachment was confirmed by a UV-Vis spectrometer.

**Fabrication of SERS chip**

We used APTMS and GA to modify the glass substrate. First, the glass slides were soaked in a Piranha solution (H\(_2\)SO\(_4\):H\(_2\)O\(_2\), 7:3 (v:v)) for 30 min to make its surface hydroxylated. After that, glass substrates were immersed in a methanol solution containing 5% APTMS and reacted at room temperature for 12 h. Then, glass substrates were immersed in a 2.5% GA solution for 4 h. GA reacted with the amino groups of APTMS modified on the substrate to form the APTMS-GA surface. Finally, the substrate was cleaned with ultrapure water and dried with nitrogen.

The 5’-labeled with-NH\(_2\) DNA (S2) strands were dropped onto the surface of the substrate and reacted at room temperature for 6 h. The substrate was washed with a 0.1 M PBS solution three times to remove nonspecifically bound strands. After that, 2.5% of the BSA solution was dripped onto the spot to block the active site. Finally, the substrate was cleaned with ultrapure water and dried with nitrogen.

**SERS detection of Hg\(^{2+}\)**

The 30.0 μL modified gold nanoparticles were mixed with Hg\(^{2+}\) ions with different concentrations of 20.0 μL, and then dripped onto the surface of the chip to react for 30 min at room temperature. Chips were washed for two times with PBS and dried with nitrogen. The detection process is shown in Fig. 1. First, 2-NT was marked on the 30 nm AuNPs as a Raman tag. The 5’-SH-DNA (S1) was modified by salinization to the Raman tag-AuNPs. Here, 12 mismatched T-T base pairs were designed for the capture of Hg\(^{2+}\) ions. Five intervening matched C-G base pairs were designed for enhancing the affinity between sequences S1 and S2, as well as for avoiding self folding. The 5’-NH\(_2\)-DNA (S2) strand was fixed on the surface of the glass substrate to form the SERS chip. Hg\(^{2+}\) sample solution and SERS nanoparticles were premixed. The mixed solution was then added to the capture substrate. After 30 min of incubation, glass slides were washed with PBS buffer two times to remove
unbonded nanoprobes, and then SERS signals were measured. The intensity of SERS signal increases with the increase of the Hg\(^{2+}\) concentration.

**Apparatus**
The SERS spectra were collected by using an inVia Raman microscope (British Renishaw Company) with a Renishaw CCD detector. SERS measurements were obtained with a 532-nm excitation laser and a power of 1 mW at the sample location. The magnification ratio of the objective lens was 50×. The accumulation times was 3 s. The exposure time was 10 s.

**Results and Discussion**

**Characterization of DNA-AuNP-Raman tag nanosensor**

Figure S1 (Supporting Information) shows TEM images of the nanoparticles. Figure S2 (Supporting Information) shows the dynamic light scattering of the nanoparticles. As shown, the gold nanoparticles with an average diameter of 30 nm display a well-defined spherical structure. Figure 2 shows the TEM images of a single one nanoparticle. We can find the indication of the modification surround the AuNPs. It is a hydrophobic group containing thiol (2-NT, SH-DNA). This shell shows the successful combination of thiol and gold nanoparticles. Furthermore, the existence of DNA was explored. Figure S4 (Supporting Information) shows AFM images of the DNA-AuNP-2-NT. Moreover, the attachment of probe DNA (S1) to AuNPs was shown by ultraviolet visible light spectroscopy. From Fig. 3, we can see a red shift of the peak, which indicates that the combination of DNA (S1) and AuNPs was successful. In addition, the absorbance spectra intensities of AuNP and

![Graph](image)

**Fig. 3** UV-vis spectra result of nanoprobe.

![Diagram](image)

**Fig. 1** (A) and (B) The 5'-SH-DNA (S1) was modified to the AuNPs after 2-NT was marked on the 30 nm AuNPs as a Raman tag. (C) DNA (S2) was assembled onto the substrate surface. The modified AuNPs were mixed with Hg\(^{2+}\) ions with different concentrations, and then dripped on the surface of the chip to react 30 min at room temperature. (D) Different concentrations of Hg\(^{2+}\) formed different quantities of the T-Hg\(^{2+}\)-T structure, resulting in different intensities of the Raman signals.

![Diagram](image)

**Fig. 2** TEM images of 30 nm AuNP (A) and DNA-AuNP-2-NT (B).
DNA-AuNP-2-NT were different. This is probably because AuNPs with no molecular modification had strong localized surface plasmon resonance (LSPR). This will lead to an increase in the absorbance.\textsuperscript{53} No matter whether DNA (S1) was attached to AuNPs, SERS spectrum shows that only the characteristic peak of 2-NT was observed, as shown in Fig. S3 (Supporting Information).

Furthermore, through experiments, the optimum concentration of DNA and 2-NT was obtained. As shown in Figs. S5 and S6 (Supporting Information), when the DNA concentration and the 2-NT concentration were both 2 μM, the experimental results were the best. In addition, the number of DNA molecules attached to each gold nanoparticle was estimated to be 1122, which was going to be about the actual measured number, 950.\textsuperscript{54}

Sensitivity of the sensor

Based on the T-Hg\textsuperscript{2+}-T structure of Hg\textsuperscript{2+} ions and thymine, a Hg\textsuperscript{2+} ions detection sensor was proposed. When more Hg\textsuperscript{2+} ions were added into the sample solution, more T-Hg\textsuperscript{2+}-T structures would be formed, so that more Raman labeled nanoparticles was incorporated into the substrate. The increase in the number of AuNPs was apparent from the SEM images (Fig. S10, Supporting Information). Because of the surface plasmon resonance (SPR), more gold nanoparticles were adsorbed onto the substrate, resulting in enhanced SERS signal. The results show that the intensity of SERS signal increased with the increase of the Hg\textsuperscript{2+} concentration. The relationship between the intensity of SERS signal and the concentration of Hg\textsuperscript{2+} ions is shown in Fig. 4. Obviously, the intensity of SERS signal increases with the increase of Hg\textsuperscript{2+} ions concentration. The Raman spectrum shows that the strongest Raman characteristic peak was at 1380 cm\textsuperscript{–1}, so the 1380 cm\textsuperscript{–1} peak was selected to quantitatively determine the concentration of Hg\textsuperscript{2+}.

The standard curve is shown in Fig. 5. Obviously, the results show that the standard curve had good correlation, and the correlation coefficient was 0.9727. The standard deviation is shown in Table 1. The sensitivity was estimated to be 1864.3 cps/log\textsubscript{10} (pg mL\textsuperscript{–1}) based on the intensity-to-quantity ratio (I/Q). The standard deviation of 11 blank samples was 6.245 (N), and the LOD was estimated to be 1.0 ppt (1.0 × 10\textsuperscript{–12} g/mL) based on a signal-to-noise ratio of 3 (S/N = 3). The test results are much better than that form traditional Hg\textsuperscript{2+} ions detection methods, such as 8 × 10\textsuperscript{–11} g/mL by ELISA\textsuperscript{9}, 9 × 10\textsuperscript{–10} g/mL by CA-AFS\textsuperscript{10}, 1.6 × 10\textsuperscript{–9} g/mL by HGAAS\textsuperscript{11}, 4 × 10\textsuperscript{–10} g/mL by ETAAS\textsuperscript{12}. Compared with other methods, our results have excellent sensitivity to Hg\textsuperscript{2+} detection. More importantly, the detection result is lower than the limit of 10 ppb detected in the drinking water of the World Health Organization.

Selectivity of the sensor

To develop a specific analysis method that can detect Hg\textsuperscript{2+} ions, we must minimize the SERS response to other potential pollutants. In order to determine its specificity, we have studied the SERS response of other metal ions to this method, and the...
results are shown in Fig. 6. As expected, even the typical metal ions of the 2 × 10⁻⁵ g/mL concentration, including Zn²⁺, Ca²⁺, Cd²⁺, Cr³⁺, Cu²⁺, Fe³⁺, K⁺, Mg²⁺ and Mn²⁺, produced a weaker signal than that of Hg²⁺ of 1 × 10⁻⁶ g/mL concentration.

Practical application
In order to prove the accuracy and reliability of the method in actual samples, a certain amount of Hg²⁺ ions (5 × 10⁻¹², 5 × 10⁻¹¹, 1 × 10⁻¹⁰, 5 × 10⁻⁹ g/mL) was added into the lake water which from Tianjin Qingnian Lake and tap water. Compared with ultra pure water or tap water, lake water contains more complex chemical components. Before the formal test, the sample of lake water was filtered three times, and the solid impurities were removed. In addition, there were no other purification or purification steps. In the actual sample detection, the composition of the lake water is more complex, resulting in more noise. However, the SERS peak (1380 cm⁻¹) for specific detection of Hg²⁺ is still very obvious (Fig. 7). The results show that the sensor has considerable application prospects in the field of environmental safety. In addition, these are preliminary experiments with the aim of giving only a proof-of-concept of the used principle, so a reference method was not given. Furthermore, this method is expected to expand the detection direction of other single ions or multiple ions.

Table 2 Determination of Hg²⁺ spiked into lake water and tap-water samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Spiked Hg²⁺ concentration/ng mL⁻¹</th>
<th>Detected Hg²⁺ concentration average ± standard deviation/ng mL⁻¹</th>
<th>Recovery, % (n = 3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lake water</td>
<td>0.005</td>
<td>0.00501 ± 0.00011</td>
<td>100.2</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.0512 ± 0.0021</td>
<td>102.4</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.104 ± 0.0029</td>
<td>104.0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>0.972 ± 0.025</td>
<td>97.2</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>5.17 ± 0.085</td>
<td>103.4</td>
</tr>
<tr>
<td>Tap water</td>
<td>0.005</td>
<td>0.00092 ± 0.00015</td>
<td>98.4</td>
</tr>
<tr>
<td></td>
<td>0.05</td>
<td>0.0494 ± 0.0025</td>
<td>98.8</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.101 ± 0.0018</td>
<td>101.0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.037 ± 0.020</td>
<td>103.7</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>5.11 ± 0.072</td>
<td>102.2</td>
</tr>
</tbody>
</table>

The RSD of each sample comes from three measurements.

Conclusion
In this study, a portable Hg²⁺ ions SERS sensing chip with a simple preparation method, green solution, high sensitivity and specificity was proposed by using the T-Hg²⁺-T mismatch principle. The limit of detection is 1.0 ppt. The sensor chip could detect Hg²⁺ ions in a broad range of concentrations. The proposed method depends on the absorb-wash mode, which reduces the probability of a false positive signal. The qualitative and quantitative analysis of Hg²⁺ ions in environmental water samples has been realized. The results show that the sensor has considerable application prospects in the field of environmental safety. In addition, these are preliminary experiments with the aim of giving only a proof-of-concept of the used principle, so a reference method was not given. Furthermore, this method is expected to expand the detection direction of other single ions or multiple ions.

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Supporting Information
This material is available free of charge on the Web at http://www.jsac.or.jp/analsci/

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