Iron telluride nanorods-based system for the detection of total mercury in blood

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**HIGHLIGHTS**

- Iron telluride nanorods (FeTe NRs) are prepared from tellurium nanowires (Te NWs).
- Mercury telluride nanorods (HgTe NRs) form by cation exchange reaction of FeTe NRs.
- Fe\textsuperscript{2+} ions released catalyze the oxidation of ABTS by H\textsubscript{2}O\textsubscript{2}.
- Mercury is effectively determined in blood with a LOD of 1.31 nM at S/N ratio 3.

**GRAPHICAL ABSTRACT**

Elucidation of the detection of mercury using iron telluride nanorods (FeTe NRs), and dose–response curve for varying concentrations of Hg\textsuperscript{2+}.

**ABSTRACT**

We have developed a simple, colorimetric iron telluride (FeTe) nanorods (NRs) based system for the detection of mercury, mainly based on the cation exchange reaction between FeTe NRs and Hg\textsuperscript{2+}. FeTe NRs (length, 105 ± 21 nm) react with Hg\textsuperscript{2+} to form HgTe NRs (length, 112 ± 26 nm) and consequently release Fe\textsuperscript{2+} ions that catalyzes the oxidation between a peroxidase substrate 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) and H\textsubscript{2}O\textsubscript{2}. The concentration of Fe\textsuperscript{2+} and thereby Hg\textsuperscript{2+} can be determined by measuring the absorbance of the ABTS oxidized product at 418 nm. This approach allows the detection of Hg\textsuperscript{2+}, with a limit of detection of 1.31 nM at a signal-to-noise ratio 3 and a linear range 5–100 nM (R\textsuperscript{2} = 0.99). The low-cost, simple, sensitive, and reproducible assay has been validated for the detection of Hg\textsuperscript{2+} in a blood sample (SRM 955c), with the result being in good agreement with that provided by National Institute of Standards and Technology.

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1. Introduction

Mercury is a severe pollutant that has a substantial bearing on the ecosystem because it is non-biodegradable, bio-accumulative and highly mobile [1]. Mercuric ion (Hg\textsuperscript{2+}) is one of the most stable inorganic forms of mercury, which can damage the brain, heart, kidney, stomach, and intestines even at very low concentrations [2]. When coming from industrial sources it gets converted into its highly toxic organic forms such as methyl mercury (CH\textsubscript{3}Hg\textsuperscript{+}) and ethyl mercury (C\textsubscript{2}H\textsubscript{5}Hg\textsuperscript{+}), which may end up in agricultural and food products. Dental amalgams have been another potential source of mercury accumulation into the blood stream, but the possible risk associated with vaccines such as thimerosal is a much newer concern [1]. The Environmental Protection Agency (EPA) in the United States sets allowable or safe daily intake of methyl mercury to be less than 0.1 \mu g of mercury per kilogram per day [2]. Normal concentrations of total whole blood Hg are typically less than 5.0 \mu g.L\textsuperscript{-1} (24.4nm) in adults [3], but recent data in the non-institutionalized US population show that the concentrations of whole blood Hg is 7.1 \mu g.L\textsuperscript{-1} for most adult females [4].

Atomic spectrometric techniques such as cold vapor atomic absorption spectroscopy have been primarily used for the...
detection of mercury in biological samples [3–5]. However, biological, organic residues may cause blockages within the microwave reaction coil and back pressure within the flow-injection manifold as a result of exothermic reactions occurring within the microwave cavity [6]. Inductively coupled plasma mass spectrometry (ICP-MS) has become more popular for the detection of mercury in biological samples, mainly because they relate to most atomic spectrometric techniques provide lower detection limits and wider dynamic ranges [7–9]. However for point-of-use applications, they are rather complicated and time-consuming for sample preparation as well as require expensive and sophisticated instrumentation and noble gas. Another method that has gained attraction is optical detection of Hg²⁺ ions using organic ionophores [10–15]. Nevertheless, instability of the probe, cross-sensitivity toward other metal ions, matrix interference, and photobleaching may occur. In addition, the probes usually allow detection of certain Hg species (not total amount). Gold as well as silver nanoparticles (Au, Ag NPs) and cadmium sulfide (CdS) quantum dots (QDs) have been used for the detection of mercury through optical and electrochemical techniques [16–19]. Although they are sensitive, use of expensive Au³⁺, Ag²⁺ ions, and potentially toxic Cd²⁺ ions are concerns, respectively. The aim of this study is to prepare low-cost FeTe NRs for the determination of total concentration of mercury species. The FeTe NRs interacted specifically with Hg²⁺ ions to release Fe²⁺ ions that catalyzed the oxidation of 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) with H₂O₂. We investigated the effects of pH, temperature, solvent effect and reaction time on the sensitivity of this approach toward Hg²⁺. Practicality of the present detection system was validated by the determination of the concentration of mercury in blood samples obtained from National Institute of Standards and Technology (NIST).

2. Experimental

2.1. Materials

Acetic acid (99.8%), hexadecyltrimethylammonium bromide (CTAB, 99%), methanol (99.5%), hexane (99%), chloroform (99%), iodine (99.5%), and iron (Ill) chloride hexahydrate (99%) were purchased from Acros (Geel, Belgium). Hydrogen peroxide (35%), hydrazine monohydrate (80%), and tellurium dioxide powder (99.8%) were purchased from SHOWA (Tokyo, Japan). Sodium acetate trihydrate (99%) was purchased from Merck (Milwaukee, WI, USA). ABTS (99.8%), fluorescein isothiocyanate (FITC, 90%), ethylenediaminetetraacetic acid (EDTA, 99.9%), mercury (II) acetate (99%), mercury (II) nitrate monohydrate (98%), and all metal chloride salts used in the experiment were purchased from Sigma Aldrich (St. Louis, MO, USA). Tris(hydroxymethyl)aminomethane (Tris) was purchased from ICN Biomedicals (Aurora, OH, USA). Nitric acid (70%) was bought from Panreac Quimica S.L.U (Barcelona, Spain). Monosodium phosphate monohydrate (99%) and disodium phosphate heptahydrate (99%) were purchased from J. T. Baker (Phillipsburg, New Jersey, USA) and Janssen Chimica (Geel, Belgium), respectively. Ethanol (99.5%) was purchased from Shimakyu's Chemicals (Osaka, Japan). Ultrapure water was obtained using a Milli-Q ultrapure (18.2 MΩ cm) system.

2.2. Synthesis of FeTe NPs

Hydrazine (10 mL) was added slowly to a beaker containing tellurium dioxide (0.016 g) at room temperature under constant magnetic stirring. The solution changed color from colorless to blue after 120 min, indicating the formation of FeTe NPs (average length 785 ± 170 nm; average diameter 16 ± 2 nm) from 100 counts [20]. To terminate the reaction and stabilize the Te NWs, the mixture was diluted 10-fold with CTAB (10 mM).

2.3. Preparation of FeTe NRs

The as-prepared Te NWs were subjected to centrifugation [relative centrifugation force (RCF): 25,000 × g for 10 min] and washed (10 mL of water) to remove most of the matrix (e.g., hydrazine). In a typical process for the synthesis of FeTe NPs, the Te NW pellet (ca. 30 mg) was re-dispersed in CTAB (9 mL, 10 mM). After 10 min, FeCl₃(aq) (1 mL, final concentration: 10 mM) was added to the mixture, which was then left at 60 °C for 1 h [21]. The solution changed color from blue to yellow, indicating the formation of FeTe NPs. Then the FeTe NPs were subjected to three centrifugation/wash cycles to remove most of the matrices. Centrifugation was conducted at RCF 12,000 × g for 10 min and ultrapure water (10 mL × 3) was used to wash the pellet. The pellets (FeTe NPs) were dried in air at ambient temperature (25 °C) prior to characterization and catalytic tests.

2.4. Characterization

A double-beam UV–vis spectrophotometer (Cintra 10e, GBC) was used to record the absorption spectra of the Te NWs and FeTe NPs. JEOL JSM-1230 and FEI Tecnai-G2-F20 transmission electron microscopes (TEM) were used to measure the sizes and shapes of the as-prepared Te NWs and FeTe NPs. The re-dispersed Te NWs and FeTe NPs were separately placed on formvar/carbon film Cu grids (200 mesh; Agar Scientific) and dried at room temperature. 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 (Dong Woo 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. ICP-MS for the determination of the concentrations of Fe²⁺ and Hg²⁺ ions in the HgTe and FeTe NPs was conducted using an Agilent Series 7700 ICP-MS system (Santa Clara, CA, USA).

2.5. Detection of Hg²⁺ ions using FeTe NPs

Aliquots (100 μL) of HgCl₂ solutions (final concentrations 5–500 mM) in phosphate solution (10 mM, pH 6.4) were separately added to FeTe NPs (50 μL, 250 mM) in phosphate solution (10 mM, pH 6.4). The effects of pH, temperature and reaction time on the detection of Hg²⁺ were investigated over the pH range 2.0–12.0, temperature range 30–75 °C, and reaction period 15–120 min, respectively. Under constant stirring at 60 °C for 90 min, the color changed from yellow to dark brown, indicating the formation of HgTe NRs. After centrifugation at 15,000 × g for 10 min, the supernatants containing the released Fe²⁺ ions were collected and mixed with ABTS (24 μL, 60 mM) and H₂O₂ (24 μL, 0.1 μM). The mixtures were subsequently mixed with acetate buffer solution (185 μL, 0.2 M, pH 4.0), which were incubated at 30 °C for 10 min. Each of the mixtures was diluted by a factor of 10 with water prior to absorption measurement. To test the counter ion effect, Hg(NO₃)₂ and Hg(CH₃COO)₂ were used to replace HgCl₂. In order to evaluate the solvent effect, phosphate solution (10 mM, pH 6.4) containing 49% of methanol, ethanol, hexane or chloroform was used to prepare the mixture.

2.6. Determination of the concentrations of Hg²⁺ and Fe²⁺ ions using ICP-MS

ICP-MS was used to determine the concentrations of metal ions in the products obtained upon the reaction of FeTe NPs.
(50 μL, 0.32 μg mL⁻¹) with Hg²⁺ ions (10–100 nM). The mixtures were subjected to three centrifugation/wash cycles. Centrifugation was conducted at RCF 12,000 × g for 10 min and ultrapure water (10 mL × 3) was used to wash the pellets. The pellets were redispersed in HNO₃ solution (5 mL, 2%) for the ICP-MS measurement. Standard solutions of HgCl₂ (final concentrations of 10–100 nM) and FeCl₃ (10–100 nM) were prepared in HNO₃ solution (5 mL, 2%).

2.7. Detection of released Fe²⁺ ions upon formation of HgTe NRs

The supernatants of the mixtures of FeTe NRs (1.25 μg mL⁻¹) and Hg²⁺ (5–500 nM) ions were placed in vials, in which aliquots (600 μL) of phosphate buffers (10 mM, pH 6.4) and FITC in ethanol solution (final concentration: 1 μM, 100 μL) were added to. To each of the mixtures, iodine solution (90 μL, 0.3 mM) was added before dilution with ultrapure water (0.69 mL). The fluorescence intensity was then measured against a reagent blank at 515 nm with the excitation wavelength of 485 nm.

2.8. Detection of total mercury ions in blood

The blood sample (SRM 955c) was frozen at −20 °C prior to use. Aliquots of the 2-fold diluted blood sample (50 μL) were spiked with standard Hg²⁺ solutions (50 μL, 3–18 nM). Acidic digestion of whole blood samples was performed according to a routine sample-digestion method. Briefly, HNO₃ (300 μL, 1 M) was added to 200 μL of whole blood and then left for 30 min to rupture the red cell membranes and leach Hg species. The insoluble material was removed through centrifugation twice (RCF: 10,000 × g, 10 min). Acetonitrile (10 μL) was added subsequently to the supernatant and the mixture was centrifuged again (RCF: 10,000 × g; 10 min). The supernatant was then heated at 90 °C for 1 h to evaporate most of the acid and acetonitrile. After cooling to room temperature, the solution was passed through a filter (cutoff 3 kDa; nominal pore size ca. 0.3 nm) and diluted to 2 mL with Tris-acetate buffer (5 mM, pH 7.0). FeTe NRs were added to the dilute solutions, which were subjected to heating at 60 °C for 90 min under constant stirring. This was followed by centrifugation at 15,000 g for 10 min. The supernatants containing the released Fe²⁺ ions were collected and mixed with ABTS (24 μL, 60 mM) and H₂O₂ (0.1 μM). The mixtures were subsequently mixed with acetate buffer solution (185 μL, 0.2 M, pH 4.0) and incubated at 30 °C for 10 min, afterwards diluted by a factor of 10 with water prior to absorption measurement.

3. Results and discussion

3.1. Sensing mechanism

Scheme 1 shows the detection of Hg²⁺, mainly through the cation exchange reaction between the Fe²⁺ ions in FeTe NRs and Hg²⁺ ions as shown in Eq. (1), leading to the formation of HgTe nanostructures and consequently the release of Fe²⁺ ions.

\[
\text{FeTe} + \text{Hg}^{2+} \rightarrow \text{HgTe} + \text{Fe}^{2+}
\]  

We note that the FeTe NRs were stable at 4 °C for at least 30 days. Although the solubility product of FeTe is unavailable from literature, the solubility product of FeTe is not much smaller than that of FeS (10⁻¹⁹) [22,23], which is much larger than that (10⁻⁷⁰) of HgTe. The released Fe²⁺ ions present in the supernatant catalyze the reaction of ABTS with H₂O₂ as shown in Equation (2).

\[
\text{H}_2\text{O}_2 + \text{ABTS}^{*} \rightarrow 2\text{H}_2\text{O} + \text{oxidized ABTS}
\]  

The amount of ABTS product formed is proportional to the concentration of Fe²⁺ ions and thus to that of the Hg²⁺ ions. We note that the oxidized product of ABTS has an absorption wavelength maximum at 418 nm. Thus, by using a calibration curve of the absorbance values at λ_max = 418 nm against the concentration of the Fe²⁺ ions in the standard solutions, the concentration of Fe²⁺ displaced and thus that of Hg²⁺ ions in the sample solution can be determined.

To support the formation of HgTe nanostructures from the reaction of FeTe NRs and Hg²⁺ ions, we conducted TEM, EDAX, and Raman measurements. Fig. 1A displays the TEM image of the as-prepared FeTe NRs, with average length and width (the widest part) of 105 ± 21 and 19 ± 2 nm (100 counts), respectively. On the other hand, the TEM image of HgTe NRs depicted in Fig. 1B displays their average length and width were 112 ± 26 and 23 ± 8 nm, respectively. The HgTe NRs had a marginally larger lattice constant (6.458 Å) in comparison to that (6.26 Å) of the FeTe NRs [24,25]. Moreover, the HgTe NRs relative to the FeTe NRs had a comparatively smoother surface. The Raman scattering spectrum of the HgTe NRs (Fig. 1C) reveals characteristic transverse optical (TO)
and longitudinal optical (LO) peaks at 119 and 139 cm\(^{-1}\), which are close to those (118 and 140 cm\(^{-1}\), respectively) for the bulk HgTe. In comparison, the Raman peaks of the Te NWs are at 46, 62 and 182 cm\(^{-1}\) [26], while those for the FeTe NRs are at 148 (\(A_{1g}\)) and 168 cm\(^{-1}\) (\(B_{1g}\)) [21]. The EDAX pattern clearly shows the Hg and Te signals (Fig. 1D), further confirming the complete replacement of Fe\(^{2+}\) ions by Hg\(^{2+}\) and the formation of FeTe NRs. The Fe peak was still apparent in the FeTe NRs in intermediate steps (e.g. after the reaction for 30 min as depicted in Fig. S1). We also detected the concentration of Fe\(^{2+}\) ions released by using FITC as a fluorophore. The fluorescence of FITC increased upon increasing the concentration of Fe\(^{2+}\) ions (Fig. S2), supporting the formation of Fe\(^{2+}\) ions between the reaction of FeTe NRs and Hg\(^{2+}\) ions. In addition, the Fe\(^{2+}\) ions released were stable in the acidic solution during the analysis.

3.2. Effect of reaction time, temperature, and pH

It has been known that Fe\(^{2+}\) ions have the highest catalytic activity toward the reaction of ABTS with \(\text{H}_2\text{O}_2\) at pH 4.0 (0.2 M acetate buffer) [21]. Fig. S3 displays the response curve against reaction time, showing that 90 min was optimal. The absorbance decreased slightly upon further increase in reaction time, mainly because of the decomposition of ABTS product. When the reaction was conducted at pH 4.0 and at the temperature range over 30–75 °C for 90 min, the optimal temperature was 60 °C (Fig. S4A). The kinetic energy increased upon increasing reaction temperature, accelerating the reaction. At a temperature higher than 60 °C, the oxidation of Fe\(^{2+}\) by oxygen and decomposition of ABTS took place more rapidly. The solvent effect is negligible since our sensing mechanism is mainly through the reaction between FeTe and Hg\(^{2+}\) ions, with support of no effect from methanol, ethanol, hexane and chloroform.

We further investigated the pH effect on the reaction of the FeTe NRs with Hg\(^{2+}\) ions over pH values 2.0–12.0, as it might also play a critical role in the reaction of the FeTe NRs with Hg\(^{2+}\) ions. Taking the account of pH having a significant effect on the formation of iron oxide (at high pH values) and the dissolution of FeTe NRs (at low pH values) [27], Fig. S4B shows prominent differences in the absorbance values occurred at pH 3.0 and 4.0. At the pH values > 4.0, the formation of iron oxide and mercury hydroxide is a concern. The solubility product values of iron oxide and mercury hydroxide are 2.6 × 10\(^{-38}\) and 3.6 × 10\(^{-36}\), respectively [28]. At the pH values < 3.0, dissolution of FeTe and HgTe NRs occurred.

3.3. Detection of Hg\(^{2+}\) ions

Fig. 2A shows that the absorbance of ABTS product increases upon increasing the Hg\(^{2+}\) concentration, displaying the dose response curve for the detection of Hg\(^{2+}\) ions, with a linear relationship between the absorbance and the HgCl\(_2\) concentration ranging
from 5 to 500 nM ($R^2 = 0.99$) and a linear range from 5 to 100 nM (Fig. 2A inset). This approach provided a limit of detection at a signal-to-noise ratio of 3 of 1.31 nM for Hg$^{2+}$ ions. We also conducted ICP-MS measurements to determine the amounts of the Fe and Hg in the as-formed HgTe NRs (100 nM). The amount of Fe$^{2+}$ ions decreased upon increasing the amount of Hg$^{2+}$ ion detected in the as-formed HgTe NRs (Fig. S5), further supporting the displacement of Fe$^{2+}$ ions in the FeTe NRs by Hg$^{2+}$ ions.

3.4. Sensitivity and selectivity of FeTe NRs toward Hg$^{2+}$ ions

Control experiments were carried out to test the specificity of the developed system for Hg$^{2+}$ ions (50 nM) under optimal conditions in the presence of various metal ions and anions such as acetate and nitrate ions (each at a concentration of 1 μM). The results displayed in Fig. 2B reveal that the sensing system is specific to Hg$^{2+}$ ions. Relative to the solubility product of HgTe (10$^{-17}$), those for the other metal complexes with Te$^{2-}$ are much higher. In other words, the other metal ions relative to Hg$^{2+}$ ions displaced Fe$^{2+}$ ions from the FeTe NRs with much lower degree [29,30].

3.5. Analysis of real sample

To examine the practicality of our developed approach, the concentration of mercury in blood (SRM 955c) was determined. We determined the concentration of mercury in the spiked blood samples, with a linear plot ($R^2 = 0.99$) of the absorbance (Y) against the mercury concentration (X) as displayed in Fig. 3. We calculated the mercury concentration using the above equation to be $8.70 \pm 0.37$ nM ($n = 5$). The certified concentration of mercury in the blood sample is $8.95 \pm 0.80$ nM. The t-value of 0.78 at a 95% confidence level was smaller than the t-value of 2.015 ($n = 5$), showing the two approaches provided results without significant difference. Our results reveal that our approach using FeTe NRs holds great potential for the determination of the concentration of mercury in biological samples.

4. Conclusion

We have developed a simple, sensitive, and selective sensing system using FeTe NRs for the detection of mercury through the release of Fe$^{2+}$ ions that catalyzes the reaction of ABTS with H$_2$O$_2$. Because the solubility product of HgTe is relatively lower than those of other tested MTes (M = Ca$^{2+}$, Co$^{2+}$, Cu$^{2+}$, Cd$^{2+}$, Ag$^+$, K$^+$, Mg$^{2+}$, Mn$^{2+}$, Ni$^{2+}$, Cr$^{3+}$, Fe$^{3+}$, Pd$^{2+}$, Zn$^{2+}$, Pb$^{2+}$, Sr$^{2+}$, Na$^+$), the sensing approach is selective toward Hg$^{2+}$ ions over other tested metal ions. The sensing approach was validated by the determination of the concentration of total Hg in a blood sample (SRM 955c), with a result showing no significant difference from the certified value. Relative to other optical approaches for detecting Hg$^{2+}$ ions [15–17], this approach provides comparable sensitivity, with an advantage of no need for masking agents.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jhazmat.2012.10.033.
References


