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A test strip platform based on DNA-functionalized gold nanoparticles for on-site detection of mercury (II) ions

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ABSTRACT

A test strip, based on DNA-functionalized gold nanoparticles for Hg^{2+} detection, has been developed, optimized and validated. The developed colorimetric mercury sensor system exhibited a highly sensitive and selective response to mercury. The measurement principle is based on thymine—Hg^{2+}—thymine (T—Hg^{2+}—T) coordination chemistry and streptavidin–biotin interaction. A biotin-labeled and thiolated DNA was immobilized on the gold nanoparticles (AuNPs) surface through a self-assembling method. Another thymine-rich DNA, which was introduced to form DNA duplexes on the AuNPs surface with thymine—Hg^{2+}—thymine (T—Hg^{2+}—T) coordination in the presence of Hg^{2+}, was immobilized on the nitrocellulose membrane as the test zone. When Hg^{2+} ions were introduced into this system, they induced the two strands of DNA to intertwist by forming T—Hg^{2+}—T bonds resulting in a red line at the test zone. The biotin-labeled and thiolated DNA-functionalized AuNPs could be captured by streptavidin which was immobilized on the nitrocellulose membrane as the control zone. Under optimized conditions, the detection limit for Hg^{2+} was 3 nM, which is lower than the 10 nM, maximum contaminant limit defined by the US Environmental Protection Agency (EPA) for drinking water. A parallel analysis of Hg^{2+} in pool water samples using cold vapor atomic absorption spectrometry showed comparable results to those obtained from the strip test. Therefore, the results obtained in this study could be used as basic research for the development of Hg^{2+} detection, and the method developed could be a potential on-site screening tool for the rapid detection of Hg^{2+} in different water samples without special instrumentation. All experimental variables that influence the test strip response were optimized and reported.

1. Introduction

Mercury ion (Hg^{2+}) is one of the most toxic metallic pollutants that exerts harmful biological effects on the environmental and human health. Exposure to even very low levels of mercury can cause health damage to human. Mercury is accumulated in the vital organs and tissues, where it binds with sulfur-containing proteins and enzymes, causing organ dysfunction and a devastating effect on the whole central nervous system of human [1–3]. Mercury pollution received considerable public attention after the mercury poisoning incident referred as Minamata disease in Japan during 1950s. To protect public health, many countries and organizations have established the limits for Hg^{2+} in water samples. For example, the US Environmental Protection Agency (EPA) has set a maximum limit of 10 nM Hg^{2+} in drinking water [4]; whereas, the World Health Organization (WHO) permits a maximum level of 30 nM Hg^{2+} in drinking water [5]. With increasing pollution in the environmental waters, there is an urgent need for the rapid detection of Hg^{2+} in water samples.

Many analytical techniques are available to determine Hg^{2+}, for example, chromatography [6], inductively coupled plasma-mass spectrometry (ICP-MS) [7], atomic absorption/emission spectrometry [8,9], cold vapor atomic absorption spectrometry (CVAAS) [10,11], cold vapor atomic fluorescence spectrometry [12], electrochemiluminescence [13,14] and other electrochemical methods [15]. Despite the high sensitivity and specificity that can be achieved by these methods for the determination of Hg^{2+}, they require expensive instrumentation, time-consuming sample pretreatment and highly skilled personnel, and/or have cross-sensitivities toward other metal ions. Thus, a simple, direct and inexpensive method for detecting Hg^{2+} remains desirable.

One potential approach is the colorimetric method based on gold nanoparticles (AuNPs), which is extremely attractive, because it can be easily read out with the naked eye, in some cases at the point of use. DNA-functionalized AuNPs have become interesting nanomaterials for sensing Hg^{2+}, mainly because AuNPs are good indicators of binding and hybridization events, and Hg^{2+} can bind between thymines to form stable thymine—Hg^{2+}—thymine (T—Hg^{2+}—T) base pairs in DNA [16,17]. In recent years, a variety of

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colorimetric methods for detecting Hg^{2+} in aqueous media using DNA-functionalized nanoparticles have been reported [18–22]. However, two disadvantages have prevented their application for the on-site detection of Hg^{2+}. First, detection still requires professional instrumentation and highly skilled personnel, thus making it less useful for people who do not have a scientific background. Second, in aqueous media, the AuNPs give a strong red background. Although the sensitivity is high when the absorbance is recorded using a UV–vis spectrophotometer, it is often difficult to distinguish the blue color of aggregates against the red background from the dispersed nanoparticles by visual observation. At low Hg^{2+} concentrations, only a small fraction of nanoparticles aggregate and change to a blue color, which is likely to be masked by the strong red background.

The test strip platform based on DNA-functionalized AuNPs is well suited for one-step detection of Hg^{2+}, which has been used for DNA analysis [23], genotyping [24,25], protein analysis [26] and small molecule detection [27,28]. Torabi and Lu [29] recently reported a colorimetric sensor with functional DNA-linked AuNPs to achieve a detection limit of 5.4 nM for Hg^{2+} by determining the color change of the sensor on the dipstick test. Although this system has a high sensitivity for determination of Hg^{2+}, it has a disadvantage that AuNPs are vulnerable to aggregation, thus it is difficult to make AuNPs dissociate from the aggregates and change to a red color. Thereby, it can easily give false negative results. He et al. [30] also developed a colorimetric strip based on AuNPs and thymine-rich hairpin DNA probes. Nevertheless, the assay required three different sequences of DNA modified in three different groups for the detection. Instead, Zhou et al. [31] reported a competitive immunochromatographic assay for the detection of Hg^{2+}. In the absence of Hg^{2+}, two visible lines would appear; whereas, in the presence of Hg^{2+}, the density of the test line would be lighter than that of the control line. When the concentration of Hg^{2+} is high enough, the test line would disappear. This detection principle has a disadvantage that it is difficult to visually distinguish the slight differences between two lines when the concentration of Hg^{2+} approached the detection limit.

In this work, we designed a colorimetric mercury sensor system for the detection of Hg^{2+} using DNA-functionalized AuNPs. Based on the sensor system, a simple, highly selective and sensitive test strip for the on-site and colorimetric detection of Hg^{2+} at nM level was thus developed. The sensitivity achieved by this method is the highest among the reported methodologies. The mechanism of detection was also discussed.

2. Materials and methods

2.1. Chemicals and materials

Streptavidin from *Streptomyces avidinii*, bovine serum albumin (BSA) and guanidine hydrochloride (Gu-HCl) were purchased from Sigma–Aldrich (Steinhem, Germany). Succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC) was purchased from Pierce (Interchim, Montluçon, France). Two kinds of thymine-rich single-stranded DNA with different sequences, i.e. DNA_1 and DNA_2, as shown in Fig. 1, were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Hydrogen tetrachloroaurate(III) hydrate ($HAuCl_4{\cdot}4H_2O$) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). All other reagents were of analytical reagent grade and were used without further purification or treatment.

The AuNPs with a mean diameter of 13 nm were synthesized as described previously [32,33]. All metal ion solutions were prepared from nitrate salts. Working solutions of DNA_1, DNA_2 and streptavidin were prepared with 20 mM Tris buffer solution (pH 7.2), which contained 100 mM $NaNO_3$ and 8% sucrose. Milli-Q water (18 MΩ cm) was used throughout the experiment.

Hi-Flow Plus Assembly Kit was purchased from Millipore Corporation (Bedford, MA, USA), containing a Hi-Flow nitrocellulose (NC) membrane, cellulose fiber, glass fiber and plastic adhesive packing.

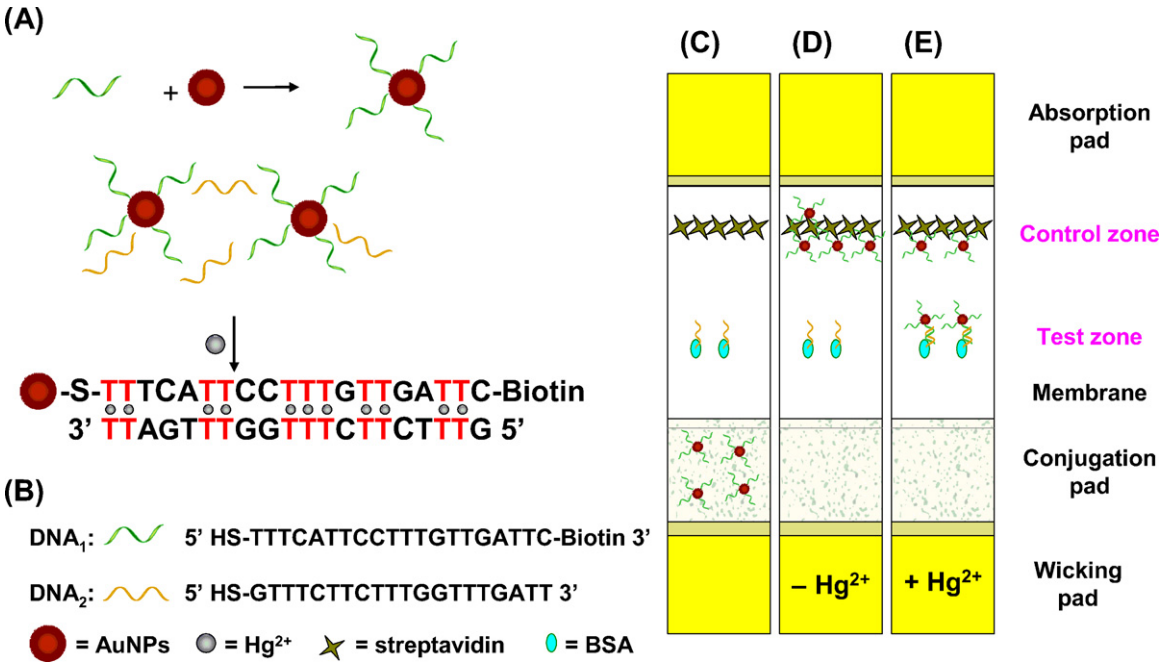

Fig. 1. Design of the test strip format. (A) Schematic illustration of sensors and theranostic agents for Hg^{2+}. (B) Description of DNA_1 sequence, DNA_2 sequence, AuNPs, Hg^{2+}, streptavidin and BSA. (C) Blank test strip loaded with the DNA-functionalized AuNPs (on the conjugation pad), DNA_2–BSA (test zone) and streptavidin (control zone). (D) Negative test: in the absence of Hg^{2+}, the DNA-functionalized AuNPs were captured at the control zone through streptavidin–biotin interaction, producing a single red line. (E) Positive test: in the presence of Hg^{2+}, the DNA-functionalized AuNPs were captured at the test zone by T—Hg^{2+}—T coordination to complementary DNA in addition to the control zone resulting in two red lines. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

2.2. Preparation of DNA_1-functionalized AuNPs

The DNA_1-functionalized AuNPs were prepared according to the method of Shim et al. [34]. Firstly, the pH of the AuNPs solution was adjusted to 7.2 with 0.1 M K_2CO_3. Secondly, 500 μL of AuNPs solution was added into a series of tubes, followed by 100 μM DNA_1 to each tube from 0 to 10 μL. All tubes were made up to 510 μL with 20 mM Tris buffer (pH 7.2), followed by mixing and incubating at room temperature for 10 min. Finally, 50 μL of 10% NaCl was added to all tubes and agitated for 1 min, and the absorbance of the solutions was measured with a UV/Vis spectra at 520 nm. If a tube contained a minimum amount of the DNA_1 for stabilization of AuNPs solution, the solution shows strong UV absorbability at 520 nm. A 3 μL of 100 μM DNA_1 was found to be the minimum amount to stabilize 500 μL of the AuNPs solution as prepared.

Thus, 6 μL of 100 μM DNA_1 and 1 mL of the AuNPs were mixed and incubated at room temperature for at least 12 h. Then, the mixture was centrifuged for 15 min at 14,000 rpm; the pellets containing DNA_1-functionalized AuNPs were re-dispersed in 50 μL of the Tris buffer solution as mentioned in Section 2.1, and were stored at 4 °C before use.

2.3. Preparation of DNA_2–BSA conjugates

The thiol group of DNA_2 was conjugated with the amino groups of BSA using SMCC as a coupling reagent. Briefly, BSA and SMCC solutions at the concentration of 5 mg mL^{-1} were obtained by dissolving them in 0.1 M PBS buffer. The BSA and SMCC solutions were mixed at the mass ratio of 20:3; incubated at room temperature for 1 h; and then dialyzed using 300 mL of 0.1 M PBS (pH 6.0) for 30 min. DNA_2 in 6 M Gu-HCl solution (pH 7.0) was added to the BSA–SMCC solution, with a mass ratio of BSA and DNA_2 being 20:7. The mixture was then incubated at room temperature with agitation for 2 h, followed by dialyzing with 20 mM Tris buffer solution (pH 7.2). Finally, DNA_2–BSA conjugate powder was obtained by freeze-drying.

2.4. Preparation of test strips

The test strip consisted of a plastic adhesive backing, a NC membrane, a wicking pad, a conjugation pad and an absorption pad as shown in Fig. 1C. The wicking pad was cut from the Millipore cellulose fiber sample pad and saturated with a solution containing 1% polyvinylpyrrolidone (PVP) K-30 and 1% Tween 20 and was dried at 37 °C for 12 h. The conjugation pad was cut from the Millipore glass fiber conjugate pad, soaked in 20 mM Tris at pH 7.2 containing 0.5% sucrose and 0.25% Triton X-100, and was dried at 37 °C for 12 h. Afterwards, the treated conjugation pad was saturated with 2 μL of the DNA_1-functionalized AuNPs and dried at 37 °C for 2 h. DNA_2–BSA conjugates solution, 2 μL of 100 μM, was sprayed onto the NC membrane as the test zone, while 2 μL of 1 mg mL^{-1} streptavidin solution was sprayed at the control zone. The distance between the test zone and control zone was about 5 mm. The NC membrane was then dried at 37 °C for 2 h and cut into sections of about 25 mm in length. The pads and the NC membrane were pasted onto a plastic adhesive backing in a way as shown in Fig. 1C. The NC membrane was pasted at the center of the plastic adhesive backing, and the conjugation pad was attached by overlapping 2 mm with the NC membrane. The wicking pad was also pasted by overcrossing 4 mm with the conjugation pad, and the absorption pad was placed to the other side of the NC membrane with an overlap of 3 mm. The width of the strip was 4 mm. The loaded strips were stored overnight before use.

2.5. Detection procedures

For sample tests, various concentrations of the analyte Hg^{2+} and other interfering metal ions were dissolved in a buffer containing 20 mM Tris (pH 7.2) and 100 mM $NaNO_3$. The wicking pad of each strip was dipped into the sample solutions for approximately 3 min until the liquid had migrated to the NC membrane, in order to fully hydrate the conjugation pad. Then the strip was placed horizontally on a plastic surface for the flow to continue. After 6 min, the test results were evaluated visually. There was only one red line at the control zone in negative samples; whereas, the positive samples exhibited two red lines at the test and control zones. If no color development was observed at the control zone, the performance of the strip was considered invalid. A digital camera was used to document the images of the strips after ~10 min.

2.6. Validation of the assay

The strip was validated for the cross-reactivity with metal ions other than Hg^{2+} and matrix effect from samples by analyzing pool water samples spiked with various concentrations of Hg^{2+}. For the determination of cross-reactivity, other metal ions (Ca^{2+}, Co^{2+}, Zn^{2+}, Mg^{2+}, Cd^{2+}, Ni^{2+}, Mn^{2+}, Pb^{2+} and Cu^{2+}) at different concentrations were prepared in flow buffer, and they were applied to the strip. Pool water samples were collected and filtered through 0.22 μm pore-sized membrane filters immediately after sampling. Mercury-positive water samples were prepared by spiking Hg^{2+} to the final concentrations of 0, 1.0, 2.0, 3.0, 5.0, 10.0, 20.0, and 50.0 nM in pool water. River water, lake water and sea water samples were collected, filtered and spiked similiarly.

3. Results and discussion

3.1. Mechanism of detection

The colorimetric mercury (II) ion sensor system, as shown in Fig. 1A, consisted of AuNPs, two thymine(T)-rich single-stranded DNA and streptavidin as the capture protein. The sequence of DNA_1 was 5′ HS-TTTCATTCCTTTGTTGATTC-Biotin 3′ (Fig. 1B). The 5′ end of DNA_1 was functionalized with a thiol group, which could conjugate DNA_1 to 13 nm AuNPs to obtain DNA_1-functionalized AuNPs, and the 3′ end was modified with a biotin moiety. The sequence of DNA_2 was 5′ HS-GTTTCTTCTTTGGTTTGATT 3′ (Fig. 1B), which was designed to link DNA_1-functionalized AuNPs by T—Hg^{2+}—T coordination chemistry [27,28,35]. The 5′ end of DNA_2 was functionalized with a thiol group, which could conjugate DNA_2 to BSA. Streptavidin was used to capture DNA_1-functionalized AuNPs via streptavidin–biotin interaction.

In the absence of Hg^{2+}, the DNA_1-functionalized AuNPs would migrate on the NC membrane till they reach the control zone, where the biotin-containing DNA_1-functionalized AuNPs would be captured by streptavidin coated on membrane, thus producing a single red line at the control zone (Fig. 1D). In contrast, in the presence of Hg^{2+}, the DNA_1-functionalized AuNPs would be captured by both DNA_2–BSA conjugates at the test zone and streptavidin at the control zone. Therefore, two red lines would appear (Fig. 1E). Development of red color at the test zone was a qualitative confirmation of the Hg^{2+}; whereas, the visual observation of the optical density of the red band at the test zone was used to determine the concentration of Hg^{2+} quantitatively.

3.2. Optimization of experimental conditions

Coating the AuNPs with the optimal amount of DNA_1 can prevent degradation of the gold surface and keep the color of the AuNPs

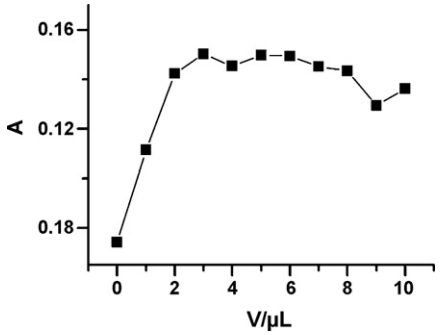

Fig. 2. Optimal amount of DNA_1 to stabilize the AuNPs solution determined by UV/vis spectra. The graph presents the UV absorption peak values of the AuNPs solution at 520 nm under different amounts of DNA_1.

solution, thus the amount of DNA_1 on the AuNPs surface would affect the response of the strip strongly. The optimal amount of DNA_1, i.e. the minimum amount of DNA_1 to stabilize the AuNPs solution, was determined as described in Section 2.2. According to the results obtained (Fig. 2), 3 μL of 100 μM DNA_1 was found to be the minimum amount to stabilize 500 μL of the AuNPs solution as prepared, because the UV absorption peak values of the AuNPs solution strongly increased at 520 nm.

Molecular weight of DNA_2 is very low, and it would be washed away easily by the sample flow if it was sprayed onto the NC membrane at the test zone directly. Therefore, a carrier protein was required to achieve the stable immobilization of DNA_2 on the NC membrane. BSA is a well-established carrier protein for the production of immunogen conjugates [36] due to its high molecular weight (66 kDa) and numerous functional groups. Therefore, the 5′ end of DNA_2 was functionalized with a thiol group, which could conjugate DNA_2 to BSA using SMCC as a coupling reagent, as described in Section 2.3.

The amount of DNA_1-functionalized AuNPs loaded on the conjugation pad and the amount of DNA_2–BSA conjugates loaded at the test zone on the membrane of the strip affected the sensitivity of the assays significantly. To develop a sensitive method, checkerboard assays were performed to determine the optimum amount of DNA_1-functionalized AuNPs and DNA_2–BSA conjugates. The test strip with 1 μL of DNA_1-functionalized AuNPs showed a faint red color at the test line, which was difficult to judge as a result. When the strip was loaded with 2 μL, the sensitivity increased; but there was no difference in sensitivity between 2 μL and 4 μL loaded strips. Thus, 2 μL of DNA_1-functionalized AuNPs was confirmed to be the minimum amount and coated onto the conjugation pad in further experiments. Similarly, 2 μL of 100 μM DNA_2–BSA conjugates solution was applied as the test zone.

Appropriate buffers would minimize the nonspecific adsorption and increase the sensitivity and reproducibility of the strip. The formation of narrow, intense and uniform bands of the reagents immobilized on the membrane strongly depends on both the concentration and the composition of the applied buffer solution. Therefore, the effect of the buffer solutions on the performance of the strip was investigated. Results revealed that flow buffer containing 20 mM Tris (pH 7.2) and 100 mM $NaNO_3$, working buffer solutions of DNA_1–AuNPs, DNA_2–BSA and streptavidin containing 20 mM Tris (pH 7.2), 100 mM $NaNO_3$ and 8% sucrose, resulted in sharper zones with higher intensity.

Sucrose, as a component of the buffer (20 mM Tris buffer, pH 7.2, 100 mM $NaNO_3$, 8% sucrose) that was used to prepare the working solutions of DNA_1-functionalized AuNPs, DNA_2–BSA conjugates and streptavidin, had the following special functions: (1) stabilize and facilitate the release of DNA_1-functionalized AuNPs from the conjugation pad; (2) reduce the nonspecific adsorption of the DNA_1-functionalized AuNPs on the NC membrane; (3) after rehydrating the conjugates, sucrose is dispersed in the running buffer, migrate along the strip, and block the NC membrane naturally without additional block steps [37]; (4) decrease the moving velocity of the DNA_1-functionalized AuNPs and increase the reaction time between the DNA_1-functionalized AuNPs and the test zone as well as the control zone, resulting in more sensitive detections [38]; and (5) helpful for the stability of the strips during storage. However, the intensity of the test zone and the control zone would decrease if the concentration of sucrose is too high. Therefore, various concentrations of sucrose were investigated, and 8% was found to be optimal.

The hybridization time, which depends on the migration time of sample flow in the NC membrane, also influences the sensitivity of the test. Three NC membranes with the nominal capillary flow time of 140 s/4 cm, 180 s/4 cm and 240 s/4 cm were investigated to prepare the strips. The intensity of the test line with the 240 s/4 cm NC membrane was significantly higher than that of the other two, perhaps due to that there was enough reaction time between DNA_1-functionalized AuNPs and the capture zone DNA_2–BSA conjugates. Therefore, the 240 s/4 cm NC membrane was used to prepare the strips.

3.3. Selectivity and sensitivity

To study the selectivity of the sensor system, solutions containing non-specific metal ions (Ca^{2+}, Co^{2+}, Zn^{2+}, Mg^{2+}, Cd^{2+}, Ni^{2+}, Mn^{2+}, Pb^{2+} and Cu^{2+}) were tested under the same conditions as in the case of Hg^{2+}. As shown in Fig. 3A, only one red band (control zone) is visible in the presence of those unspecific metal ions, implying that no cross-reactivities occurred with these metal ions up to 1 mM. The result indicated that the sensor has an excellent selectivity for Hg^{2+} against other metal ions, due to the high specificity of the interaction between T–T mismatches and Hg^{2+} which had been previously demonstrated [16]. In addition, interference of some common anionic species and organic compounds in environmental water was also tested, such as 10 mM of Cl^- and SO_4^{2-}, 100 μM of F^-, ClO_2^-, ClO_3^- and formaldehyde, 1 μM of trichloroacetic acid, dichloroacetic acid, trichloromethane, phenol, di(2-ethylhexyl) phthalate and dimethoate. As expected, these species would not interfere with the determination of Hg^{2+} (Fig. 3B). The results further confirmed the selective binding between thymine base and Hg^{2+}. Therefore the test strip could be used for the selective detection of Hg^{2+}.

To determine the sensitivity of the system, the test strip was dipped in a flow buffer containing various concentrations (0, 1.0, 2.0, 3.0, 5.0, 10.0, 20.0, 50.0 and 100.0 nM) of Hg^{2+} ($n = 5$ for each concentration). As expected, the color development in the control zone alone on the NC membrane indicates that the test is negative, and Hg^{2+} concentration was considered to be below the detection limit. Whereas the existence of control and test zones means that the Hg^{2+} concentration was above the detection limit, and the result was considered as positive. As indicated in Fig. 4, the detection limit of the assay is about 3 nM. In addition, the intensity of the test zone band increased with increasing Hg^{2+} concentration, indicating that this test can be semi-quantitative.

3.4. Stability

The stability of the strip was evaluated by performing the assay after storage in sealed aluminum pouches for a month at room temperature. After preparation of test strips as described above, the strips were stored without any stabilizers for a month at room temperature under dark and dryness. During the storage, the strips were tested without and with mercury solutions ($n = 20$

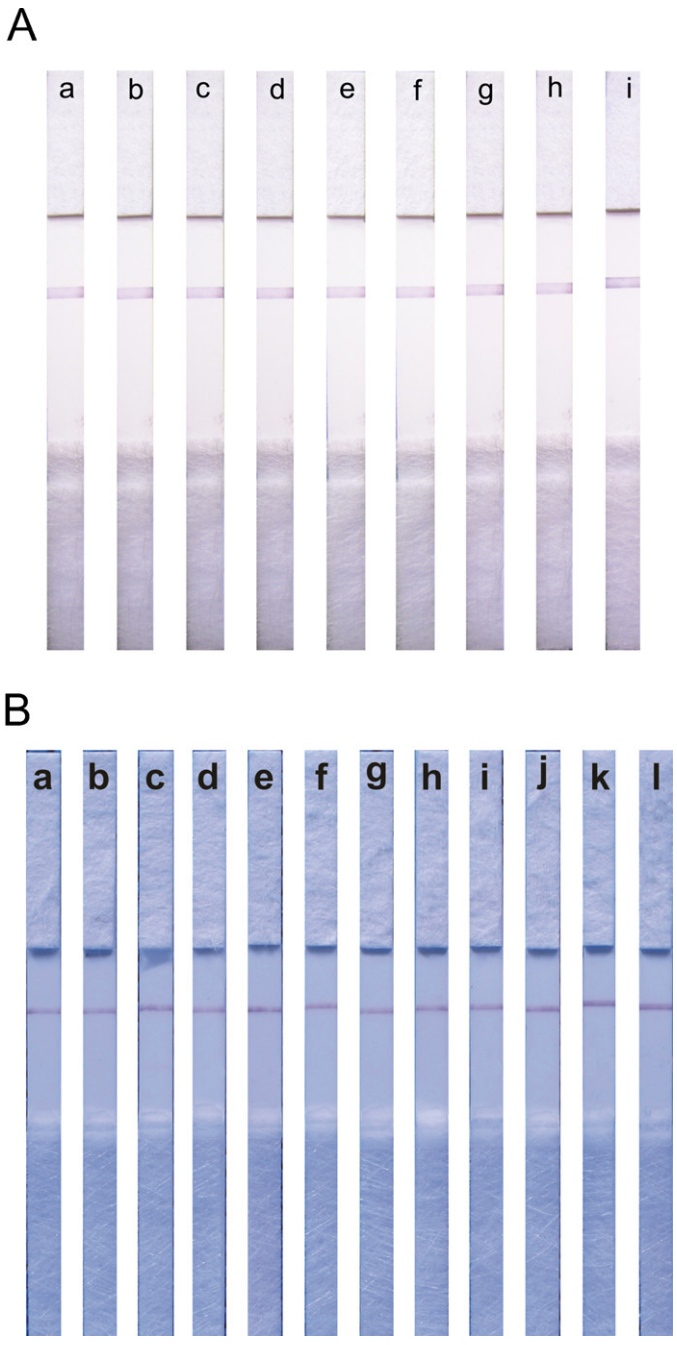

Fig. 3. (A) Cross-reactivity of other metal ions by the test strip. (a) Ca^{2+}, (b) Co^{2+}, (c) Zn^{2+}, (d) Mg^{2+}, (e) Cd^{2+}, (f) Ni^{2+}, (g) Mn^{2+}, (h) Pb^{2+}, and (i) Cu^{2+}. (B) Cross-reactivity of some anionic species and organic compounds by the test strip. (a) 10 mM Cl^-, (b) 10 mM SO_4^{2-}, (c) 100 μM F^-, (d) 100 μM ClO_2^-, (e) 100 μM ClO_3^-, (f) 100 μM formaldehyde, (g) 1 μM trichloroacetic acid, (h) 1 μM dichloroacetic acid, (i) 1 μM trichloromethane, (j) 1 μM phenol, (k) 1 μM di(2-ethylhexyl) phthalate, and (l) 1 μM dimethoate.

each). Compared with those strips prepared newly by paired *t*-test, the conjugates did not show any significant loss of its activity and color intensity even after a month. This was attributed to strong dative bonds between the gold and the thiol-modified DNA molecules. Additionally, the presence of sucrose in the dilution buffer improved the stability of the strip and retained the activity of the DNA_1–AuNPs conjugates. To make the strip suitable as a commercial kit, the stability for a month is not enough, so additional research may be required to confirm the shelf life of the test strip and will be the subject of future investigations.

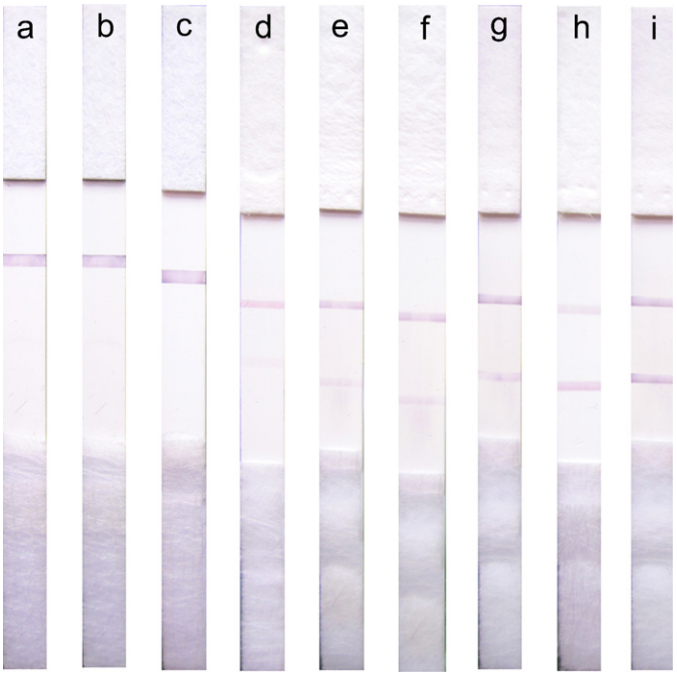

Fig. 4. Sensitivity analysis of the test strip with varying concentrations of Hg^{2+}. (a) 0 nM, (b) 1 nM, (c) 2 nM, (d) 3 nM, (e) 5 nM, (f) 10 nM, (g) 20 nM, (h) 50 nM, and (i) 100 nM.

3.5. Applications

To test the practicality of our developed approach for the determination of mercury, pool water samples, spiked with 0, 1.0, 2.0, 3.0, 5.0, 10.0, 20.0, and 50.0 nM of Hg^{2+}, were analyzed by the test strip. In parallel, cold vapor atomic absorption spectrometry (CVAAS) analysis was performed as a confirmatory method for the quantitation of Hg^{2+} in pool water samples, experimental conditions of which were approximately same as reported [39]. As presented in Table 1, no red lines on the test zone appeared for water spiked with 0, 1.0, and 2.0 nM Hg^{2+}, but clear red lines on the test zone were obtained for the water samples spiked with 3.0, 5.0, 10, 20, and 50 nM Hg^{2+}. The analytical results of the strip test were in good agreement with the results obtained from CVAAS, indicating that the strip test we developed had good reliability. Besides, the strip test gave neither false positive nor false negative results, indicating that the matrices of the samples, where Hg^{2+} may be found do not interfere with the assay. Some more river water, lake water and sea water samples without and with spiking were tested, results were also satisfactory (Table 2). Thus, this proposed

Table 1
Analysis of artificially spiked water samples by the test strip and cold vapor atomic absorption spectrometry (CVAAS) method ($\bar{x} \pm s$, $n = 5$).

Hg^{2+} spiked (nM)	CVAAS		Test strip	
	Found (nM)	Recovery (%)	Test line	Control line
0	ND[a]	NC[b]	−, −, −, −, −	+, +, +, +, +
1.0	1.1 ± 0.3	110.0	−, −, −, −, −	+, +, +, +, +
2.0	1.9 ± 0.2	95.0	−, −, −, −, −	+, +, +, +, +
3.0	3.2 ± 0.4	106.7	+, +, +, +, +	+, +, +, +, +
5.0	5.4 ± 0.6	108.0	+, +, +, +, +	+, +, +, +, +
10.0	9.7 ± 0.8	97.0	+, +, +, +, +	+, +, +, +, +
20.0	21.5 ± 1.2	107.5	+, +, +, +, +	+, +, +, +, +
50.0	47.1 ± 1.9	94.2	+, +, +, +, +	+, +, +, +, +

(−) No line was observed. (+) A red line was observed.
[a] Not detected.
[b] Not calculated.

Table 2
Results of real samples and spiked ones by the test strip ($n = 5$).

Real sample	Code	Original[a]	Spiked[b]
River	1	–, –, –, –, –	+, +, +, +, +
water	2	–, –, –, –, –	+, +, +, +, +
	3	–, –, –, –, –	+, +, +, +, +
	4	–, –, –, –, –	+, +, +, +, +
	5	–, –, –, –, –	+, +, +, +, +
Lake	1	–, –, –, –, –	+, +, +, +, +
water	2	–, –, –, –, –	+, +, +, +, +
	3	–, –, –, –, –	+, +, +, +, +
	4	–, –, –, –, –	+, +, +, +, +
	5	–, –, –, –, –	+, +, +, +, +
Sea	1	–, –, –, –, –	+, +, +, +, +
water	2	–, –, –, –, –	+, +, +, +, +
	3	–, –, –, –, –	+, +, +, +, +
	4	–, –, –, –, –	+, +, +, +, +
	5	–, –, –, –, –	+, +, +, +, +

(–) Only control line was observed. (+) Both test line and control line were observed.
[a] Real samples without any spiking.
[b] Real samples spiked by 10 nM Hg^{2+}.

approach shows potential application for the determination of Hg^{2+} in water samples.

4. Conclusion

A simple, easy to perform, sensitive and accurate test strip method for the determination of Hg^{2+} in water samples was developed. Results can be obtained within 10 min without the need of expensive handling, equipment or scientific expertise. Under the optimized conditions reported in this work, the detection limit for Hg^{2+} was 3 nM, which is the lowest detection limit based on the test strip methods. Besides, the developed assay format offers potential as a reliable on-site screening tool.

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