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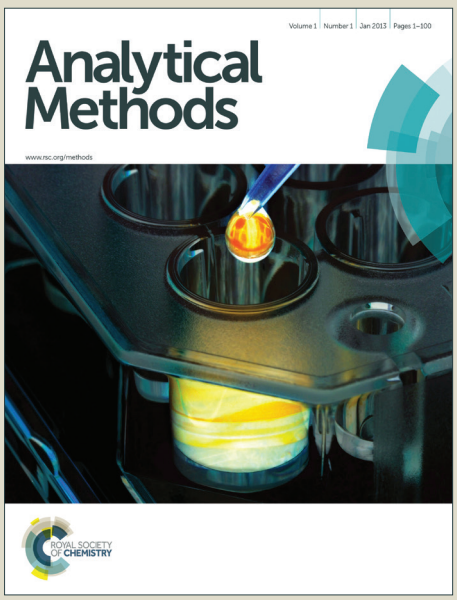

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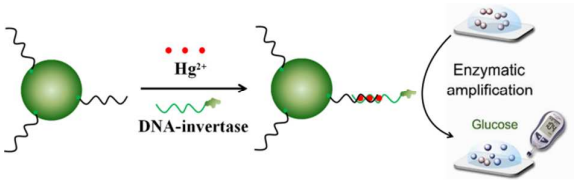

A highly sensitive and portable mercury (II) ions sensor based on personal glucose meter (PGM) recording

Highly Sensitive and Portable Mercury (II) Ions Sensor by Using Personal Glucose Meter

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Abstract

In this paper, a highly sensitive and portable mercury (II) ions sensor based on personal glucose meter (PGM) recording was proposed. Thymine-thymine (T-T) mismatches in the capture DNA and detection DNA were used to recognize target Hg^{2+}. The magnetic separation and hydrolysis of sucrose into glucose of DNA-invertase conjugation were employed to obtain the signal of PGM. There was a liner relationship between the signal of PGM and the concentration of Hg^{2+} in the range of 8.0 nM to 1 μM. A correlation coefficient of 0.995 was obtained and the relative standard deviation (RSD) was 3.6% for a concentration of 100 nM Hg^{2+} (n = 9). The selectivity and performance in lake water, tap water and river water of Hg^{2+} sensor were also studied, which suggested our method had a great positional to be used in real application.

1. Introduction

Mercuric ion (Hg^{2+}) is a widespread heavy metal which may cause deleterious effects on human health and the environment [1]. A very low concentration of Hg^{2+} can lead to adverse human health effects. Water-soluble Hg^{2+} is one of the most stable forms of mercury pollution [2, 3]. To prevent potential human exposure to Hg^{2+}, it is of great significant to selective and sensitive detection of Water-soluble Hg^{2+}. Great efforts have been made to develop sensitive and selective Hg^{2+} detection methods. Among those methods, some are constructed by using thymine (T) containing oligonucleotides as the sensing elements [4, 5]. As reported by previous papers, Hg^{2+} could bind to two T residues of DNA, with high selectivity and affinity, which provides a powerful tool to develop Hg^{2+} detection methods. Many Hg^{2+} detection methods based on traditional analytical techniques, such as atomic absorption spectrometry, atomic fluorescence spectrometry, mass spectrometry, inductively coupled plasmaeatomic emission spectrometry and cyclic voltammeter method, have been developed[6, 7]. Although high sensitivity and selectivity have been achieved, most of these analytical techniques are expensive, time-consuming and sophisticated instrumentation [8-12]. This has limited their wider applications, such as on-site applications. Therefore, it is interesting and significant to develop a highly sensitive,

inexpensive, simple and point-of-use method to detect Hg^{2+}.

Due to its low cost, simple operation and portability, personal glucose meter (PGM) has attracted worldwide attention [13, 14]. It is available in stores with low price (as low as $10 for a meter), which has been integrated into cell phones for point-of-use. PGM is mainly used to monitor the glucose concentration in diabetic patients. In 2011, Yi Lu linked PGM with functional DNA sensors to achieve portable, low-cost and quantitative detection of targets beyond glucose [15]. This has provided an excellent alternative to develop sensitive, inexpensive, simple and point-of-use Hg^{2+} sensors. However, it is an invasive DNA approach toward targets, which sacrificed the sensitivity of detection.

In this work, we developed a highly sensitive and portable mercury (II) ions sensor by using personal glucose meter. As shown in Figure 1, in the presence of Hg^{2+}, the Hg^{2+} and detection DNA were captured and concentrated onto the Streptavidin-MNBs through T-Hg-T linkage and magnetic separation, respectively. After the washing away of unbound Hg^{2+} and detection DNA, the bound DNA-invertase can be used to catalyze the hydrolysis of sucrose into glucose with millions of turnovers, which transformed the concentration of Hg^{2+} into the level of glucose for monitoring of PGM.

2. Experimental

2.1 Reagents and chemicals

Streptavidin-MNBs (350 nm in diameter, the aqueous suspension containing 0.05% Tween-20, 0.1% bovine serum albumin (BSA) and 10 μM EDTA at a concentration of 3.324×10^{11} beads mL^{-1}) were obtained from Bangs Laboratories Inc. (Fishers, IN), all oligonucleotides were synthesized and purified by Sangon (Shanghai, China), the sequences were as following:

Capture DNA:5'-Biotin-AAAAAAAAA*TTT*CCG*TTT*CGC*TTT*-3'

Detection DNA: 5'-HS-AAAAAAAAA*TTT*GCG*TTT*GCC*TTT*-3'

All DNA oligonucleotides were diluted by TE buffer (10 mM Tris-HCl and 1.0 mM Na_2EDTA, pH 8.0). They were denatured at 95 ℃ for 5 min and naturally cooled down to room temperature before use. Grade VII invertase was obtained from baker's

yeast (S. cerevisiae), Tween-20, sulfosuccinimidyl- 4- (N-maleimidomethyl) cyclohexane - 1 - carboxylate (sulfo-SMCC), Tris (2-carboxyethyl) phosphine hydrochloride (TCEP) and bovine serum albumin (BSA) were purchase from Sigma (St. Louis, MO). Other chemicals were in analytical grade obtained from standard reagent suppliers and used directly. All solutions were prepared with Milli-Q water (resistivity = 18 MΩ cm) from a Millipore system.

2.2 DNA-invertase conjugation

Firstly, a sum of 30 μL of detection DNA (1 mM), 2 μL of sodium phosphate buffer (1 M, pH 5.5) and 2 μL of TCEP solution (30 mM) were mixed, which was then incubated for 1 h at room temperature. The mixture was purified by Amicon-10K for 10 times using buffer A (0.1 M NaCl, 0.05% Tween-20, 0.1 M sodium phosphate buffer, pH 7.3). Then, in order to conduct invertase conjugation, 1 mg of sulfo-SMCC was added into 400 μL of invertase solution (dissolved in buffer A), which was incubated for 1 h on a roller. The obtained mixture was purified through centrifugation and Amicon-100K using Buffer A by 10 times. Finally, the obtained sulfo-SMCC-activated invertase was mixed with the detection DNA, which was incubated for 48 h at room temperature. The mixture was purified by Amicon-100K for 10 times using Buffer A.

2.3 Procedures for Hg^{2+} detection using PGM

Firstly, 5 μL of Streptavidin-MNBs was washed three times by using buffer A to remove the surfactants. Then, 50 μL of buffer A was added into the Streptavidin-MNBs suspension. One microliter above Streptavidin-MNBs suspension was added into a tube and 10 μL of capture DNA was added to bind on the Streptavidin-MNBs, which was placed on a roller for 30 min. It was washed using buffer A for 5 times and the Streptavidin-MNBs were separated by a magnet. Then, 100 μL of Hg^{2+} at different concentrations were mixed with obtained Streptavidin-MNBs and 100 μL of DNA-invertase conjugation (obtained in 2.2). It was allowed to incubate for 2 h on a roller. The mixture was further washed 5 times using buffer A containing BSA (2 mg/mL). It was washed 5 times using buffer A. Finally, 100 μL of sucrose in Buffer A (0.5 M) was added into the system and allowed

to incubate for 3 h. An amount of 5 μL obtained mixture was detected by a PGM.

3 Results and Discussion

3.1 Design Principle of Hg^{2+} sensor by a PGM

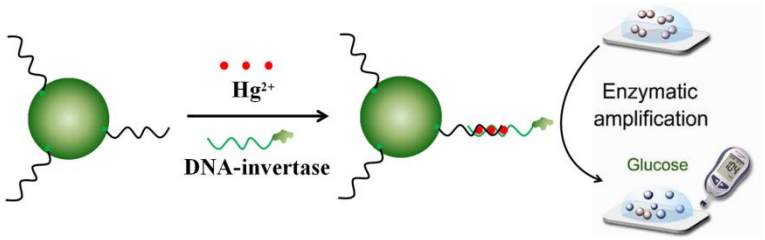

Figure 1 Schematic illustration of the design principle of Hg^{2+} sensor by a PGM.

The design principle of the Hg^{2+} sensor is schematically described in Figure 1. The capture DNA immobilized onto Streptavidin-MNBs through the reaction between Streptavidin and biotin. The detection DNA was modified with DNA-invertase conjugation. In this case, the detection DNA and capture DNA were separated, as the mismatch of T-T. In the presence of Hg^{2+}, the detection DNA and capture DNA stitched together after the forming of T-Hg-T complex. The Hg^{2+} and detection DNA were captured and concentrated onto the Streptavidin-MNBs through T-Hg-T linkage and magnetic separation, respectively. Therefore, only in the presence of Hg^{2+}, can the detection DNA modified with DNA-invertase conjugation be immobilized onto Streptavidin-MNBs. We assumed the concentration of Hg^{2+} was proportional to the amount of DNA-invertase bound to the Streptavidin-MNBs. After the washing away of unbound Hg^{2+} and detection DNA, the bound DNA-invertase can be used to catalyze the hydrolysis of sucrose into glucose with millions of turnovers, which transformed the concentration of Hg^{2+} into the level of glucose for monitoring of PGM.

3.2 Optimization of Experimental Parameters

Optimization the concentration of detection DNA. The concentration of detection DNA is essential to the performance of Hg^{2+} sensor, as shortage of detection DNA leads to the abundant of unbound Hg^{2+} and an excess of detection DNA leads to the increasing of the background signal. Therefore, different amount of DNA-invertase conjugation (5, 10, 50,100, 200 and 300 μL) were used for the detection of Hg^{2+}. The personal glucose meter signals for different amount of DNA-invertase conjugation

were showed in Figure 2. The personal glucose meter signal increased with the increasing of DNA-invertase conjugation amount until 100 μL and there was a peak at the amount of 100 μL. As the amount of DNA-invertase conjugation higher than 100 μL, there was a slight decline for the signal, which was caused by the increasing of the background signal. Therefore, an amount of 100 μL of DNA-invertase conjugation was selected for the following study.

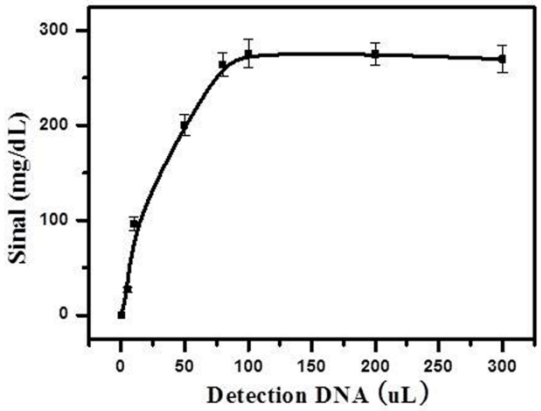

Figure 2 Relationship between the amount of detection DNA and signal. Condition: Each data point represents an average of 3 measurements (each error bar indicates the standard deviation).

Optimization of incubation time for Hg^{2+} and detection DNA. The performance of Hg^{2+} sensor was strongly affected by the incubation time of Hg^{2+} and detection DNA. As shown in Figure 3, the signal of personal glucose meter elevated gradually with the increasing of incubation time (in the presence of 100, 300 and 500 nM Hg^{2+}) at the early stage and a maximum was obtained at 100 min. In order to obtain the best signal-to-background level, 100 min was selected as the incubation time for sequent detection.

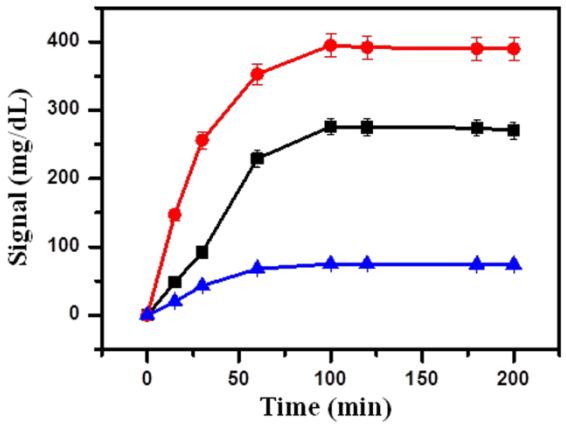

Figure 3 Relationship between the incubation time and signal. The concentration of Hg^{2+} from up to below is 500, 300 and 10 nM.

3.3 Sensing Performance for Hg^{2+} Detection

In order to evaluate the performance of Hg^{2+} sensor, we challenged the Hg^{2+} sensor with a series of concentrations of Hg^{2+}, covering a range of nearly 3 orders of magnitude (8.0 nM to 1 μM). Improved signal of personal glucose meter was observed with the increase of Hg^{2+} concentration and intensity of signal increased monotonically (nearly linearly) with the concentration of Hg^{2+} (As shown in Figure 4). There was a liner relationship between the signal of personal glucose meter and the concentration of Hg^{2+} in the range of 8.0 nM to 1 μM. A correlation coefficient of 0.995 was obtained and the relative standard deviation (RSD) was 3.6% for a concentration of 100 nM Hg^{2+} (n = 9), which was comparable or even better than some of other reported methods. Such an attractive detection limit of our sensing strategy can be primarily attributed to the enrichment effect of Streptavidin-MNBs and the million turnovers of sucrose hydrolysis into glucose. Substantial Hg^{2+} and detection DNA in solution were collected onto the surface of PGM and one sucrose on the detection DNA turns into millions of glucose for monitoring of PGM. According to the maximum contamination level of Hg^{2+} in drinking water issued by the United States Environmental Protection Agency (EPA), our Hg^{2+} sensor have great application prospects for the point-of-use and routine monitoring of Hg^{2+} with the wide dynamic range and superior detection sensitivity. The comparison of the detection

performance with traditional method using different equipment for determination of Hg^{2+} is listed in Table 1.

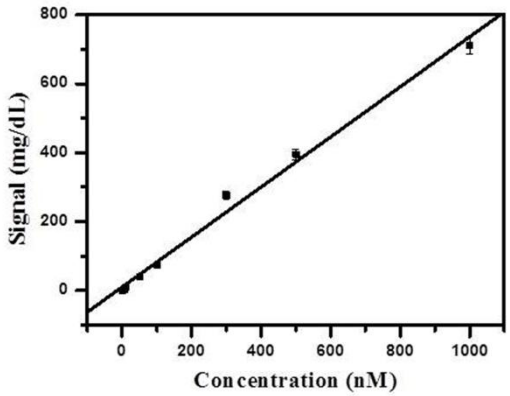

Figure 4 Relationship between the concentration of Hg^{2+} and signal. Condition: Each data point represents an average of 3 measurements (each error bar indicates the standard deviation).

Table 1 Comparison of the detection performance with traditional method using different equipment for determination of Hg^{2+}

Equipment	Method	Liner range (10^{-7} mol L^{-1})	reference
spectrofluorimeter	QDs/DNA/Au NPs based nanosensor	0.02–0.6	Analytical Chemistry , 83,7061–7065.
Test paper detection	Variation of color upon binding with reagent	100-4000	RSC Advances, 2012, 2, 3714 – 3721.
UV-Visible spectrophotometer	coordination of Hg^{2+} to the gold nanoparticle	0.1-5	Analyst, 2011, 136, 1690 – 1696
Electrochemical workstation	Modification of glassy carbon electrode	0.01-1	Anal. Methods, 2014, 6, 4988 – 4990
Renishaw 2000	interaction between silver nanoparticles and Hg^{2+}	90.9 pM detected	Nanoscale, 2012, 4, 5902 – 5905.
Personal Glucose Meter	T-Tmismatches recognize	0.08-10	This work

target Hg^{2+}

3.4 Selectivity

In order to evaluate the selectivity of the Hg^{2+} sensor for Hg^{2+}, we challenged the Hg^{2+} sensor with a series of environmentally relevant metal ions, including Cd^{2+}, Pb^{2+}, Mn^{2+}, Fe^{3+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Al^{3+}, Ca^{2+}, Cr^{3+}, Sn^{2+}, Zn^{2+}, and Ag^{+}, using the same experimental procedures as those for Hg^{2+}. Above metal ions were selected as they may coexisted with Hg^{2+} in the drinking water. The response of Hg^{2+} sensor to above metal ions is showed in Figure 5. We found that the presence of those metal ions at 1 μ M exhibits negligible responses compared with that of 300 nM of Hg^{2+} and their mixture. The signal of personal glucose meter was almost equal to the background signal. These observations suggested that binding of Hg^{2+} relies on specific recognition and that capture DNA and detection DNA was highly specific for the capturing of Hg^{2+}. What is more, the presence of abundant metal ions in the solution did not affect the detection for Hg^{2+}, which suggests that our sensor holds great promise as a powerful tool to be applied for detection of Hg^{2+} in real samples.

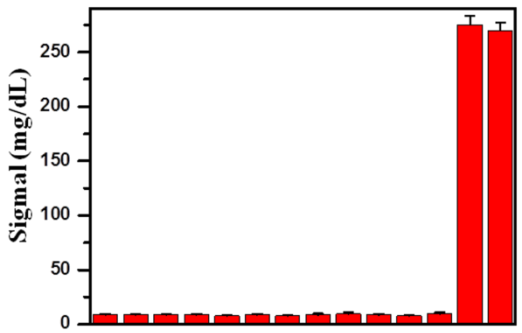

Figure 5 The response of Hg^{2+} sensor to different metal irons. The metal iron from left to right is Cd^{2+}, Pb^{2+}, Mn^{2+}, Fe^{3+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Al^{3+}, Ca^{2+}, Cr^{3+}, Sn^{2+}, Zn^{2+}, Hg^{2+} and mixture of above metal irons.

3.5 Real Sample Analysis

In order to evaluate the practical application of proposed Hg^{2+} sensor, we

challenged the Hg^{2+} sensor with a series of environmental water samples, including lake water, tap water and river water, using the same experimental procedures as those for Hg^{2+} detection in buffer solution. The results of Hg^{2+} detection in different water sample suggested that the signal of PGM in lake water, tap water and river water were similar to that of in buffer solution. Furthermore, the Hg^{2+} sensor was challenged with different amount of Hg^{2+} in different water samples for recovery tests. The results were summarized in Table 1. Satisfactory values between 94 and 106% were obtained for the recovery experiments, which indicated that the possible interference from the different background composition of water samples on the Hg^{2+} sensor was negligible. The above results demonstrate that our introduced Hg^{2+} sensor can be successfully applied to Hg^{2+} analysis in real environmental samples.

Table 1 Determination of Hg^{2+} (nM) in Environmental Water Samples

sample	added	found	Recovery (%)
Tap water	20	18.9±4.6	94.5
	100	103.2±7.9	103.2
	300	285.0±9.4	95.0
Like water	20	19.1±4.9	95.5
	100	106.1±8.1	106.1
	300	282.3±9.2	94.1
River water	20	19.2±5.1	96.0
	100	98.5	98.5
	300	287.9	96.0

Note: Each sample was analyzed using our proposed sensor, and all values were obtained as an average of three repetitive determinations±standard deviation (mean ±SD).

4. Conclusion

In summary, a highly sensitive and portable Hg^{2+} sensor was developed based on thymine (T) containing oligonucleotides recognizing and personal glucose meter

recording. The Hg^{2+} was captured and concentrated on the Streptavidin-MNBs and the DNA-invertase conjugation on the detection DNA catalyzes the hydrolysis of sucrose into glucose with millions of turnovers, which transformed the concentration of Hg^{2+} into the level of glucose for monitoring of PGM. There was a liner relationship between the signal of personal glucose meter and the concentration of Hg^{2+} in the range of 8.0 nM to 1 μM. A correlation coefficient of 0.995 was obtained and the relative standard deviation (RSD) was 3.6% for a concentration of 100 nM Hg^{2+} (n = 9). What is more, the Hg^{2+} sensor has a high selectivity both in buffer solution and an comparable performance in lake water, tap water and river water, which suggests that our proposed Hg^{2+} sensor has a great positional to be used in real application.

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