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PII: S1386-1425(14)00719-7
DOI: http://dx.doi.org/10.1016/j.saa.2014.04.146
Reference: SAA 12110

To appear in: *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*

Received Date: 1 March 2014
Revised Date: 22 April 2014
Accepted Date: 23 April 2014

Please cite this article as: M.H. Mashhadizadeh, R.P. Talemi, A novel optical DNA biosensor for detection of trace amounts of mercuric ions using gold nanoparticles introduced onto modified glass surface, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* (2014), doi: http://dx.doi.org/10.1016/j.saa.2014.04.146

A novel optical DNA biosensor for detection of trace amounts of mercuric ions using gold nanoparticles introduced onto modified glass surface

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Abstract

In this work we report a DNA spectrophotometric biosensor for detection of Hg^{2+} ions in which a pair of oligonucleotides with four thymine–thymine (T–T) mismatched bases was immobilized onto modified glass surface. Firstly, glass surface modified with 3-(mercaptopropyl) trimethoxysilane (MSPT) and gold nano-particles respectively and then one oligonucleotide (P_1) modified with hexanthiol at 5-terminal was immobilized on gold nano-particles via self-assembly and inserted in methylene blue. Methylene blue can intercalate on single strand DNA (ss-DNA) and its absorption peak can measure spectrophotometrically. Then the other oligonucleotide was able to hybridize with P_1 by forming thymine–Hg^{2+}–thymine (T–Hg^{2+}–T) complexes in the presence of Hg^{2+}, and absorption signal of methylene blue reduced upon Hg^{2+} increasing concentration because inaccessibility of guanine base in DNA duplex. However, when Hg^{2+} was absent, the two oligonucleotides could not hybridize due to the T–T mismatched bases, and P_2 could not be fixed on the modified glass surface and any change in absorption peak of methylene blue takes place. The UV-Vis spectrum showed a linear correlation between the absorption peak of methylene blue and the concentration of Hg^{2+} over the range from 10

nM to 10 μM (R^2 = 0.9985) with a detection limit of 6 nM. This spectrophotometric biosensor could be widely used for selective detection of Hg^{2+}.

Keywords: DNA biosensor; Hg^{2+}; Modified glass surface; Gold nano-particles; Self-assembly; Methylene Blue

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1. Introduction

Mercury, an environmental toxin, can cause serious health problems such as kidney failure, brain damage, and DNA damage [1]. The mercury levels in drinking water must not exceed 10 nM, a limit set by the US Environmental Protection Agency. Therefore, it is critical to detect mercury at concentrations around this limit with high sensitivity and selectivity. There have been many studies on the detection of Hg^{2+} ions by utilizing electrochemistry or fluorescence [1,2]. It is necessary to develop methods for the highly selective and efficient determination of Hg^{2+} in environmental, food, and biomedical samples. The determination of Hg^{2+} in water samples is mainly carried out by instrumental methods, such as high performance liquid chromatography (HPLC) [3], inductively coupled plasma mass spectrometry (ICPMS) [4], electrochemical methods [5-8], and atomic absorption spectrometry (AAS) [9]. Although highly sensitive methods have been developed, the complication of the sample preparation and the high cost of the equipment are disadvantages for the determination of Hg^{2+} and an inexpensive, sensitive, and rapid analytical method is required.

Several types of sensing platforms based upon small organic molecules [10 – 13], conjugated polymers [14], oligonucleotides [15], DNAzymes [16], and proteins [17] have been examined for the detection of Hg^{2+}. Recently, DNA/gold nanoparticle (DNA/AuNP)-based colorimetric assay [18] offers a promising approach for simple and rapid tracking of mercuric ions. These assays rely on the size dependent Surface Plasmon Resonance (SPR) properties of AuNP and thymine – Hg^{2+} – thymine (T – Hg^{2+} – T) coordination chemistry [19]. Colorimetric sensor is one attractive approach for rapid and simple determination of Hg^{2+} [20–22], owing to its facile operation and simplicity. The color change can be easily caught by the naked eye and no special equipment is required. Unfortunately most of these methods are suffer from poor sensitivity because they are relying on absorption of gold nano-particles.

Indeed, in this work we developed a novel spectrophotometric DNA biosensor for highly selective and sensitive determination of Hg^{2+} ions. Firstly glass surface functionalized with MSPT and then gold nanoparticles has been attached to S-H group of MSPT. Thiolated probe DNA, immobilized onto gold nanoparticles and transferred in MB solution. MB can intercalate on single strand probe DNA and approximately a large absorption peak for MB can be observed. When no Hg^{2+} present immobilized probe DNA can't be hybridized with 4-mis matched DNA and no change in absorption peak of MB was observed. On the other hand, in the presence of Hg^{2+} due to T-Hg^{2+}-T complex formation and less affinity of MB accumulation onto ds-DNA, absorption peak of MB decreases. This decrease in MB absorption peak was linear with Hg^{2+} concentration at 10 μM to 10 nM range. The fabricated biosensor showed promising performance, e.g., good sensitivity, very high selectivity, simple in preparation, effective, and cheap. The

preparation methodology and main characteristic features were described and discussed in detail.

2. Experimental

2.1. Reagents and materials

Analytical reagent grade chemicals and double distilled water (DDW) were used for preparing all aqueous solutions. Hydrogen tetracholoroaurate ($HAuCl_4 \cdot 3H_2O$), potassium monohydrogen phosphate, potassium di-hydrogen phosphate, tri-sodium citrate and Methylene Blue (MB) were obtained from Merck (Germany. Mercaptohexanol (MCH) and 3-(mercaptopropyl)trimethoxysilane (MPTS), were purchased from Fluka (USA). Salts of metal nitrates (all from Merck) were of the highest purity available and used without any further purification.

P1 (5'-SH-TTATGGGGCAATGCA-3') and P2 (5'-TGCTTTGCCCCTTTT) oligonoleotides were purchased from the Aminsan Company (Tehran, Iran) and oligonucleotide stock solution was prepared in phosphate buffer solution (PBS, pH 7.0).

2.2. Apparatus and instrumentations

UV–Vis absorption spectra were recorded with a Lambda 25 Perkin Elmer UV-Vis spectrophotometer. The pH values were controlled with a pH/mV meter (Metrohm-827) supplied with a combined electrode. Scanning electron microscopy (SEM) was obtained by a TESCAN scanning electron microscope and TEM analysis was carried out with a Hitachi H-8100 transmission electron microscope.

2.3. Preparation of gold nano-particles

Approximately 30 nm diameter gold nanoparticles are prepared by the citrate reduction of $HAuCl_4$ [23]. All glassware is cleaned in aqua regia (3 parts HCl, 1 part HNO_3), rinsed with DDW, and then dried prior to use. An aqueous solution of $HAuCl_4$ (0.01% (w/v), 30 ml) was poured in a beaker, the temperature range is from 80 to 90°C, and then 1 mL of a 1% (w/v) tri-sodium citrate solution is added quickly, which results in a change in solution color from pale yellow to pale black. After another 10 minutes stirring, the solution color shifts to deep red.

After the appearance of red color, the solution is stirred for an additional 30 min, allowed to cool to room temperature. The prepared gold nano-particles was stored in dark glass bottles at 4 ◦C previously cleaned with freshly prepared 1:3 HNO_3–HCl solution and rinsed thoroughly with DDW.

2.4. Functionalization of glass surface

A commercially available glass slide (50× 24 mm, Citoglass) was used as the template for construction of Hg^{2+} DNA biosensor. The glass surfaces were cleaned using piranha solution (7:3 mixtures of concentrated sulfuric acid and 30% hydrogen peroxide) and a diluted alkaline detergent overnight; they were then washed extensively with DDW and dried before use.

The cleaned glass substrate was immersed in a solution of 290 mM solution of MPTS in toluene for 5 min, rinsed five times in ethanol with sonication for 5 min, and rinsed two times in water for 5 min. Both sides of the glass slide were coated with organosilane using this procedure and named Glass/MPTS.

2.5. Au nanoparticle deposition on silanizated glass surface

The glass substrates after functionalization were rinsed with DDW and immersed in a colloidal gold suspension for 3 h to fabricate gold nanoparticle layers on both sides of the substrate.

After incubation of the glass slide in the suspension, the glass slide was washed gently with DDW and named Glass/MPTS/GN.

2.6. Immobilization of S-H labeled probe DNA

Glass/MPTS/GN/S-H DNA was prepared by immersing the Glass/MPTS/GN in a 10 nM PBS solution of S-H DNA (pH 7.0) for 6 h. Afterward the glass surface was incubated in the MCH solution for 30 min. This process would produce a self-assembly well-aligned monolayer (SAM) of thiolated oligonucleotide. Then, the glass surface was rinsed thoroughly with DDW to remove physically adsorbed S-H DNA and obtain a modified glass surface denoted as Glass/MPTS/GN/S-H DNA.

2.7. DNA hybridization

The hybridization was carried out by immersing Glass/MPTS/GN/S-H DNA in 0.1 M PBS containing 10 nM of 4-miss ss-DNA for 30 min at 37 ◦C with stirring in presence different concentration of Hg^{2+}. Then the glass surface was washed with PBS for 3 times successively to remove the un-hybridized target ss-DNA, and this hybridized glass surface is denoted as Glass/MPTS/GN/ds DNA.

2.8. Accumulation of MB onto ss-DNA and ds-DNA

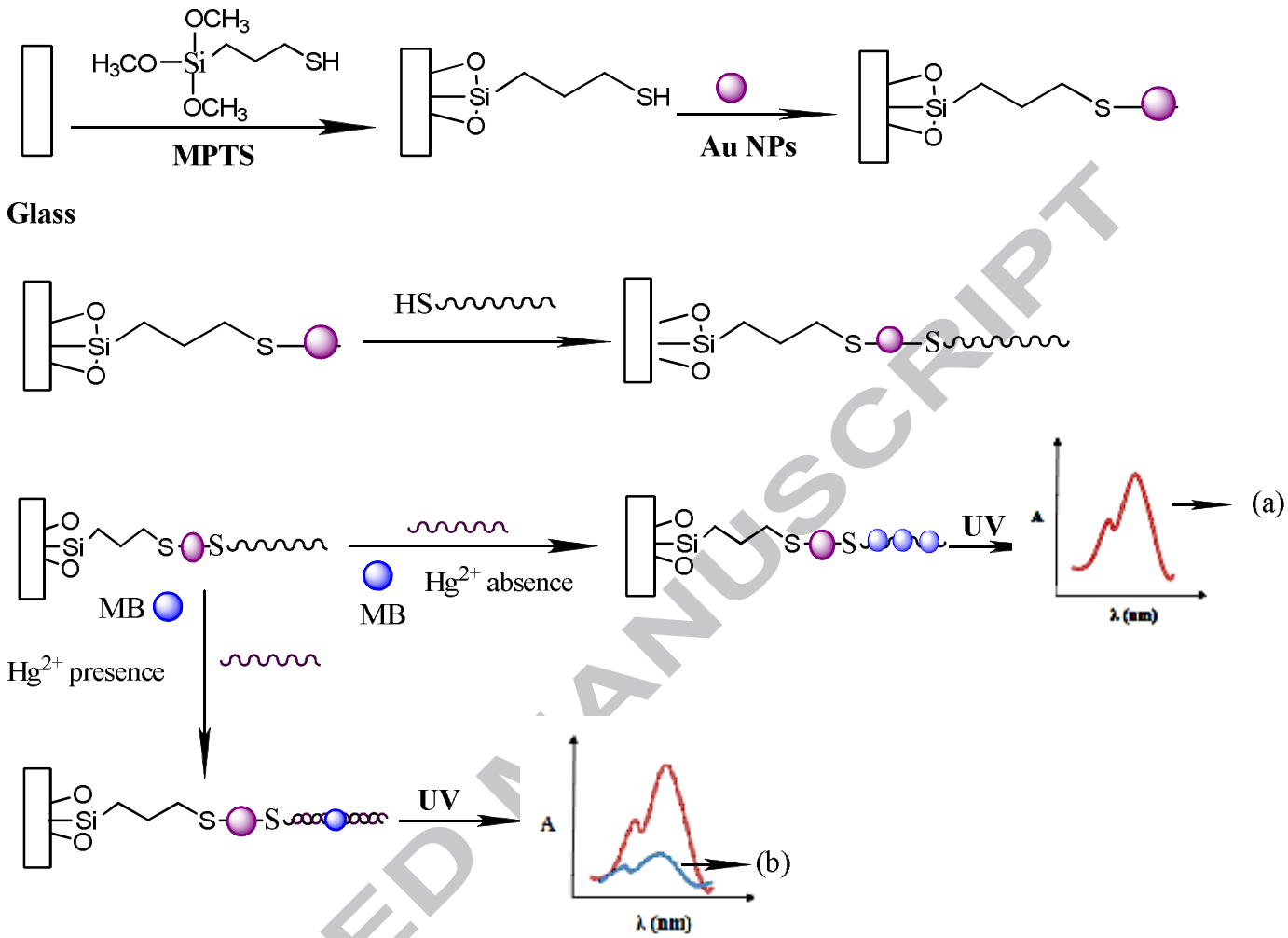
MB was firstly accumulated onto the S-H DNA modified glass surface by immersing the Glass/MPTS/GN/S-H DNA into the stirred solution containing 20 μM MB with 20 mM NaCl for 5 min. After accumulation of MB, the glass surface was rinsed with PBS buffer and water thoroughly. Accumulation of MB onto ds-DNA in Hg^{2+} presence was carried out by similar method to ss-DNA.

2.9. Detection of Hg^{2+}

All modified glass slides were stored in DDW until the SEM or UV-Vis measurement was carried out. To detect Hg^{2+} or other metal ions, the Glass/MPTS/GN/S-H DNA was immersed in a solution containing 10 nM 4-mis DNA, 0.1M NaCl, 10 μM MB and a series of different concentration of Hg^{2+} or 10 μM other metal ions in10 mM PBS (pH 7.4) for 30 min at 25^0C. The absorption of MB onto Glass/MPTS/GN/S-H DNA was recorded against Glass/MPTS/GN/S-H DNA without MB accumulation as reference and the value of the absorption peak of MB after the 4 mis-DNA hybridization with the probe DNA in Hg^{2+} presence obtained against Glass/MPTS/GN/ds DNA as reference.

3. Results and Discussion

Scheme 1 clearly illustrates the schematic of the spectrophotometric biosensor for Hg^{2+} determination based on Hg^{2+}-induced DNA hybridization. As mentioned above, the biosensor works on the basis of Hg^{2+} inducing two thymine-mismatched oligonucleotides hybridizing and the DNA hybridization process can be relatively easy to proceed with.



Scheme 1. Schematic illustration of the fabrication steps of the Hg^{2+} DNA biosensor.

3.1.Characterization of prepared gold nano-particles

A transmission electron microscope (TEM) and a UV spectrophotometer are performed to determine the size and characteristic absorption bands of the resulting nanoparticle solutions. Fig 1.A is the UV-Vis spectrum of gold nanoparticles that are produced using sodium citrate reducing agent. This figure demonstrates that the peak of the spectrum of the gold nanoparticles is at 523 nm. Fig. 1B is TEM image of the gold nanoparticles before. The size of the nanoparticles is estimated about 30 nm.

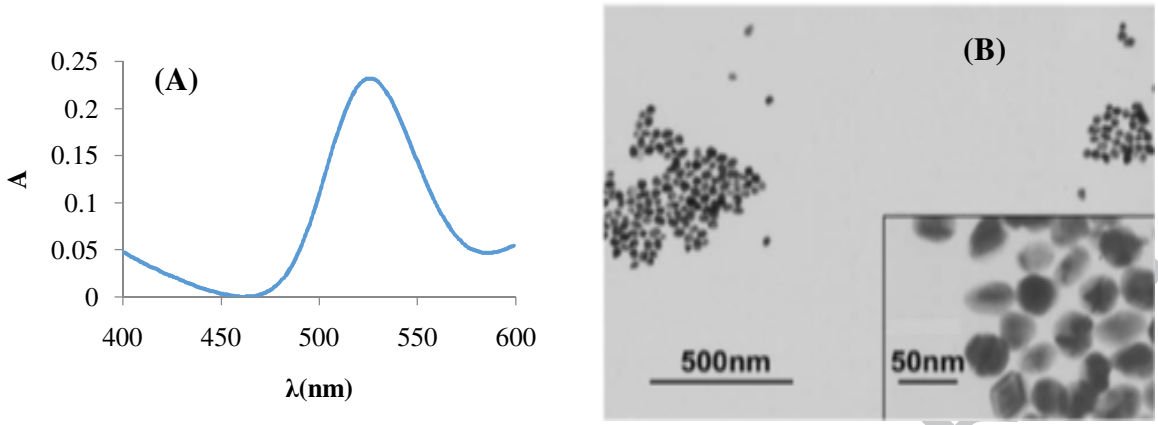


Figure 1. Absorption spectra (A) and TEM image (B) of the 30-nm gold nano-particle.

3.2. Characterization of modified glass surfaces

In this paper, we immobilized S-H labeled ss-DNA onto the glass surface by a new procedure through covalent interaction between gold nano-particles introduced onto modified glass surface and S-H labeled ss-DNA. To our knowledge, there are many methods for immobilization of DNA onto glass surfaces [25, 26], such as direct and non-covalent immobilization through adsorption or entrapment within polymeric films or immobilization of amino modified oligonucleotide using EDC and NHS or using an epoxide-opening reaction etc. Most of these methods suffer from some defect such as approximately weak interaction in direct and non-covalent and need to expensive material in amino modified oligonoleotides.

Gold nano-particles provide an appropriate template for successful immobilization of ss-DNA through strong covalent interaction by a well-known Au-S bond [27]. As a result using from gold nano-particles in our proposed method, thiolated ss-DNA can be tightly grafted on the gold nano-particles modified glass surface.

On the other hand, as we know, the most critical step while preparing a DNA biosensor is the immobilization of DNA probe on the surface of a sensing device. The amount of immobilized DNA probe will influence the accuracy, sensitivity, selectivity, and life of a DNA biosensor directly. Because of the high surface-to-volume ratio and excellent biological compatibility, gold nano-particles can enlarge the sensing surface area to increase the amount of immobilized DNA greatly, and the DNA mixed with nano-materials can keep its biological activity well [28].

Consequently, self-assembly of colloidal gold onto the modified glass surface enlarged the glass surface area and enhanced greatly the amount of immobilized ss-DNA and sensitivity of mercuric DNA biosensor.

The morphologies of the Glass/MPTS and Glass/MPTS/GN were investigated by scanning electron microscopy (SEM). Functionalization of glass surface with toluene solution of MPTS at room temperature led to occasional hemispherical features of about 200 nm diameter that were not self-associating (Fig. 2A). After addition of gold nanoparticles to MPTS modified glass surface, spherical gold nano particle can interact with S-H group of MPTS and accumulate onto MPTS surface (Figure 2B). Figure 2 also shows scanning electron micrographs of a Glass/MPTS/GN/S-H DNA without (Figure 2C) and after (Figure 2D) MB accumulation. When MB is accumulated at the modified electrode, more dense and homogeneous coverage is observed.

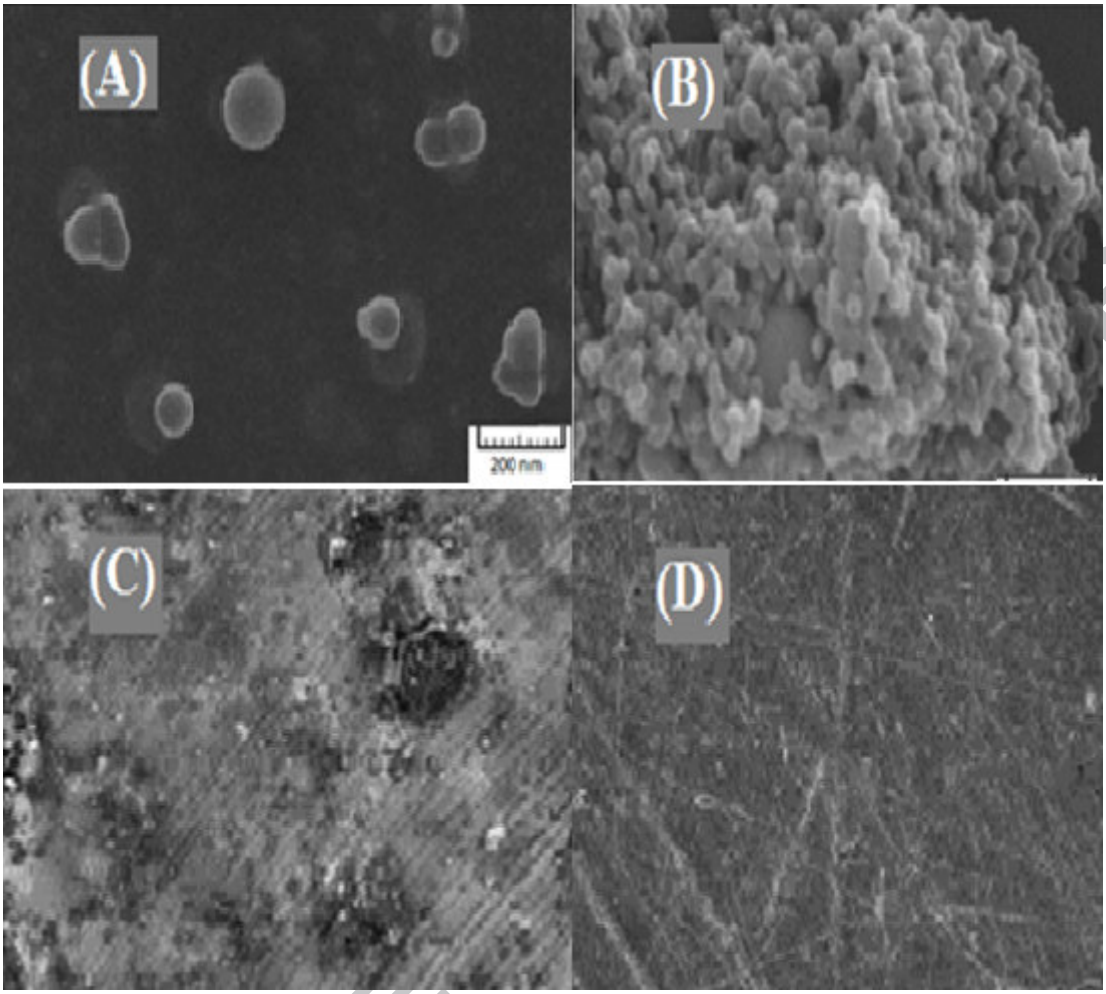


Figure 2. SEM images of Glass/MPTS (A), Glass/MPTS/GN (B), Glass/MPTS/GN/S-H DNA (C), and Glass/MPTS/GN/S-H DNA-MB.

3.3. Optimization of experimental conditions

The effect of reaction temperature on the response of the sensor was investigated. The Glass/MPTS/GN/S-H DNA hybridized with 4-mis DNA with the help of 0.1 μM Hg^{2+} at 15 to 35 °C, respectively. The difference in the value of the peak absorption of MB before and after hybridization was 0.168, 170, 0.174, 0.173, and 0.173 respectively. It is obvious that the difference in the value of the peak currents of MB before and after hybridization at 25 °C was bigger than that at all other temperatures, thus 25 °C was

selected as experimental temperature to realize the sensitive determination of Hg^{2+} in our study.

The concentration of probe DNA immobilized on modified glass surface was optimized to let the maximum P_2 loading on the glass surface through T - Hg^{2+} - T mediated hybridization. This accomplished with a fixed immobilization time of 2 h and varying probe concentration from 10 μM to 1 nM in the immobilization buffer during the self-assembly process. As shown in Fig. 3A, the largest difference in absorption peak of MB before and after hybridization in presence of 0.1 μM Hg^{2+}, was observed at the modified glass surface prepared with 10 nM probe P_1 in the immobilization buffer. This concentration of probe DNA was optimal to the surface hybridization between P_1 and P_2 and thus maximum difference in MB absorption peak before and after hybridization was observed.

The appropriate ionic strength is important for the stability of DNA complexation with Hg^{2+}. The dependence of the difference in absorption peak of MB before and after hybridization in presence of Hg^{2+} on the ionic strength was investigated. The difference in absorption peak of MB before and after hybridization increases with the increase of NaCl concentration from 0.020 M to 0.10 M (Fig. 3B). This is probably due to the fact that the DNA is a negatively charged biopolymer, its folding strongly depends on the presence of cations such as Na^+ to neutralize the negatively charged backbone. With the further increase of the concentration of NaCl from 0.10 M to 1.0 M, however, the difference in absorption peak of MB before and after hybridization decreased. In this case, the decreased in Hg^{2+} activity due to the increase in ionic strength may have played

a dominated role. As a result, 0.10 M NaCl was chosen in the present work to maximize the difference in absorption peak of MB before and after hybridization.

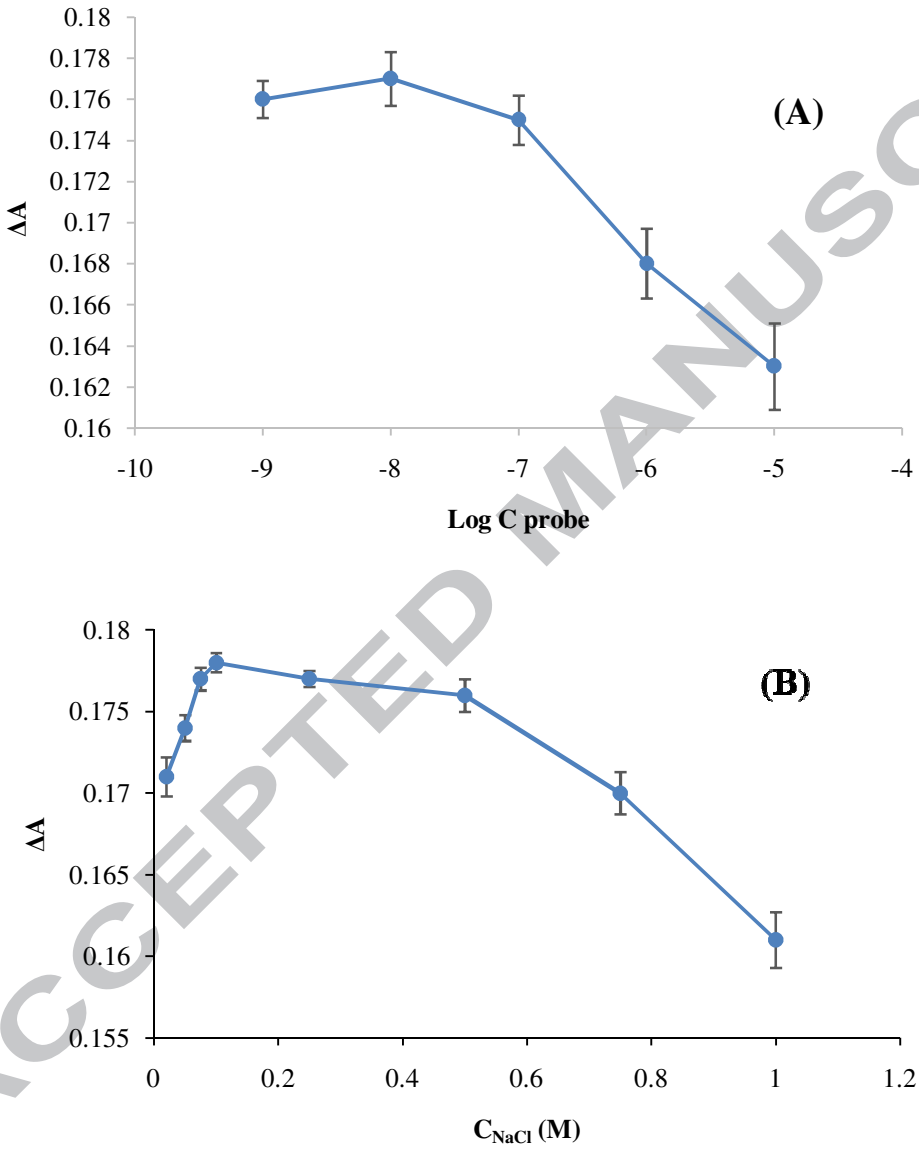


Figure 3. Absorption peak at λ_{max} of MB in presence of 0.1 µM Hg^{2+}dependence on the probe (A) and NaCl concentration (B).

Also some important parameter such as concentration of MPTS and gold nanoparticles, immersion time of deposition of gold nano-particles and MPTS optimized as mentioned in literature [30] for optimum analytical performance. Best result gained with this conditions: concentration of MPTS and gold nanoparticles was 290 mM and 0.58 nM respectively and optimum immersion time of MPTS and gold nanoparticles was 5 min and 3 h respectively. These results are approximately in agreement with [30] and hence next experiments were done with these concentrations and immersion times.

3.4. Detection of Hg^{2+}

The performance of the T-Hg^{2+}-T based biosensor for different concentrations of Hg^{2+} was intensively investigated. When no Hg^{2+} is added into the hybridization solution, P_1 and P_2 do not hybridize due to the mismatched bases, and P_2 cannot be fixed on the glass surface, so no notable corresponding change in signals of the MB respect to S-H DNA modified glass surface occurs. On the other hands, When a certain amount of Hg^{2+} exists in the hybridization solution, P_2 is able to hybridize with P_1 by forming T-Hg^{2+}-T base pairs and is modified on the glass surface and change in MB absorption can be observe. The spectrophotometric biosensor was applied to measure a wide range concentration of Hg^{2+} from 100 µM to 1 nM. The difference in absorption peak of MB as showed in Fig. 4 B exhibit a good linear relationship with the logarithm of Hg^{2+} concentration in the range of 10 µM-10 nM (R^2 = 0.9985). The limit of detection is evaluated to be 6 nM based on the signal-to-noise protocol (S/N = 3).

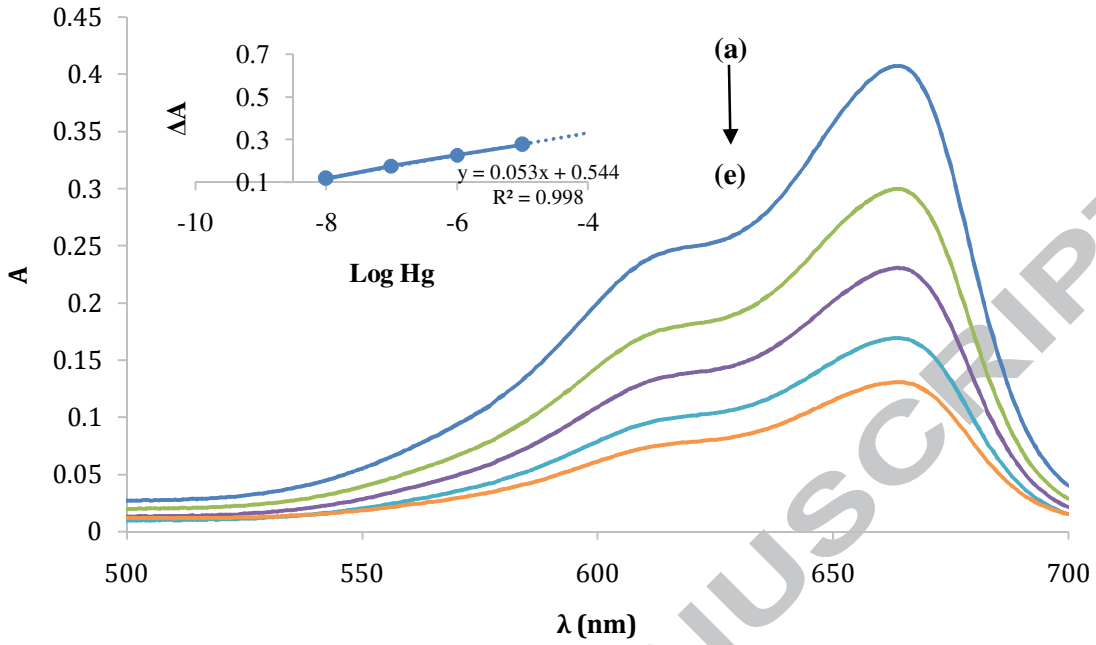


Figure 4. Absorption peak obtained at DNA-modified glass surface in a 0.1 M PBS buffer solution containing 10 nM of P_2. Prior to the spectrophotometric measurements, these glass surface were incubated in the hybridization solution containing 0 (a), 10 nM (b), 100 nM (c), 1 μM (d) and 10 μM (e) Hg^{2+}, respectively. Inset: Linear relationship between the absorption of MB at its λ_{max} and the logarithm of Hg^{2+} concentration.

3.5. Selectivity

The selectivity of proposed Hg^{2+} biosensor was also evaluated by substituting Hg^{2+} in the hybridization buffer with various metal ions such as Co^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Ag^{+}, Cd^{2+}, pb^{2+}, Mn^{2+}, Fe^{3+}, Mg^{2+} at 10 μM concentration, respectively. As shown in Fig. 5, the biosensor exhibits remarkable response to 10 nM Hg^{2+}, but hardly responds to other metal ions, which indicates a good selectivity for Hg^{2+} detection. What should be stressed is the possible interference of Ag^{+}, because Ag^{+} is able to form C-Ag^{+}-C structure according to reports [30, 31]. We also tested the effect of 10 μM Ag^{+} on the

determination of Hg^{2+} using the T-Hg^{2+}-T based biosensor, and found that no notable interference response was observed. This reveals that the introduced electrochemical biosensor offers good selectivity for Hg^{2+} determination. Actually, to our best knowledge, only Hg^{2+} has been demonstrated that can induce the formation of T-T complexes.

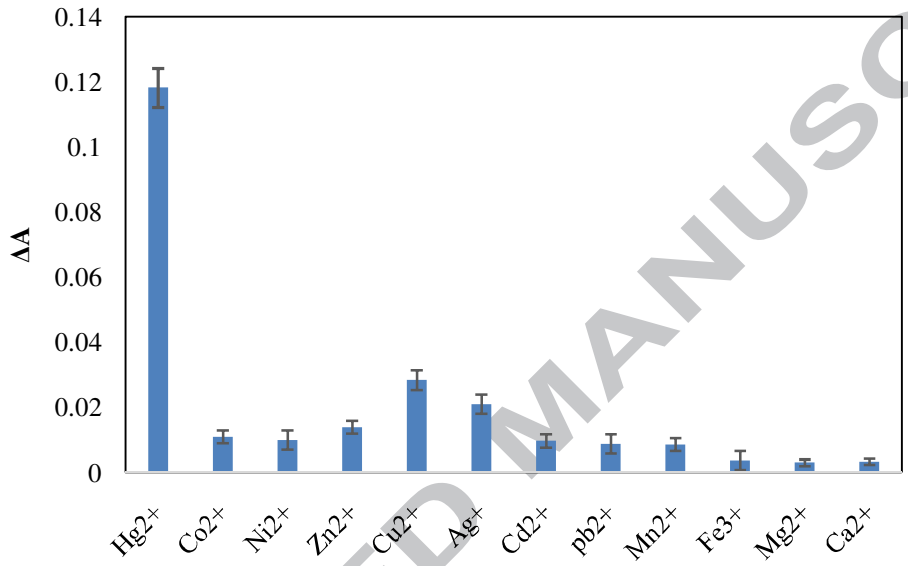


Figure 5. Selectivity of the Hg^{2+} ion measurement over other metal ions. The concentration of the Hg^{2+} is 10 nM and concentration of other metal ions is 10 µM. Error bars show the standard deviations of measurements taken from three independent experiments.

3.6. Comparative study

For comparative purposes, Table 1 lists the linear range and detection limit of recently published Hg^{2+} colorimetric sensors [31-35] against the proposed sensor. It is noteworthy that the limit of detection and linear range of the proposed sensor are also considerably

improved with respect to those of the recently reported Hg^{2+} sensors. Furthermore most of the interfering ions such as Co^{2+}, Ni^{2+}, Zn^{2+}, Cu^{2+}, Ag^{+}, and Cd^{2+} do not have any effect on the response of our sensor.

Table 1. Comparison of the proposed Hg^{2+} DNA biosensor with the previously reported sensor.

Ref.	Method	Linear range	Detection limit
31	An optode constructed by modified immobilization of indigo carmine on a triacetylcellulose membrane	24.0-468.0 µM	7.2 µM
32	Redox reaction between stannous ion and mercury ion enhanced by gold nanoparticles	0.1-30 µM	0.051 µM
33	Potentiometric sensor based on N,N'-bis(salicylaldehyde) phenylenediamine	3.2×10^{-7}-3.2×10^{-4} M	1.5×10^{-7} M
34	DNA electrochemical biosensor	0-2 µM	50 nM
35	Colorimetric detection based on nitrophenyl-aminothiourea	-	1.0×10^{-7} M
36	Structure-switching DNA optical biosensor	-	1.2 nM
37	An envanescent wave DNA-biosensor based on a direct structure-competitive detection mode	-	2.1 nM
This work	Spectrophotometric determination based on functionalizing of glass surface	10 µM-10 nM	6 nM

High sensitivity and low detection limit of proposed biosensor can be assigned to the effect of gold nano-particles in successful immobilization of probe DNA, high absorption of MB in visible region and its great differentiation ability between ss and ds DNA before and after Hg^{2+} addition.

3.7. Analytical application

The practical application of the designed sensor was evaluated by determination of the recovery of spiked Hg^{2+} in tap and spring water samples. The analytical results are shown in Table 2. The analytical results show the acceptable relative standard deviation and quantitative recoveries, implying that the proposed sensor was applicable for practical Hg^{2+} detection.

Table 2. The concentration of Hg^{2+} in water samples detected using the proposed spectrophotometric DNA-based sensor.

Samples	Added Hg^{2+} concentration (M)	Concentration found (M)	Recovery (%)	RSD (%, n=3)
Tap water	1.0×10^{-8}	9.78×10^{-7}	97.8	3.6
	1.0×10^{-7}	9.88×10^{-6}	98.8	2.9
Spring water	1.0×10^{-8}	1.06×10^{-8}	106	4.7
	1.0×10^{-7}	1.03×10^{-7}	103	3.8

4. Conclusion

We have pioneered to introduce the GNPs onto modified glass surface to construct a platform for immobilization of S-H labeled so-DNA. We have also fabricated spectrophotometric biosensors for mercury detection based on T–Hg^{2+}–T complexation by UV-Vis spectroscopy. The hybridization of the two oligonucleotides through T–Hg^{2+}–T coordination chemistry leads to the decrease in the peak currents of MB. The difference in the value of the peak absorption of MB before and after DNA hybridization was linear with the concentration of Hg^{2+} in the range from 10 μM to 10 nM with a linear coefficient of 0.9985. The detection limit was 6 nM. The proposed method is rapid, convenient and low-cost for effective sensing of Hg^{2+}. Particularly, the proposed method was applied successfully to the determination of Hg^{2+} in tap and spring water samples.

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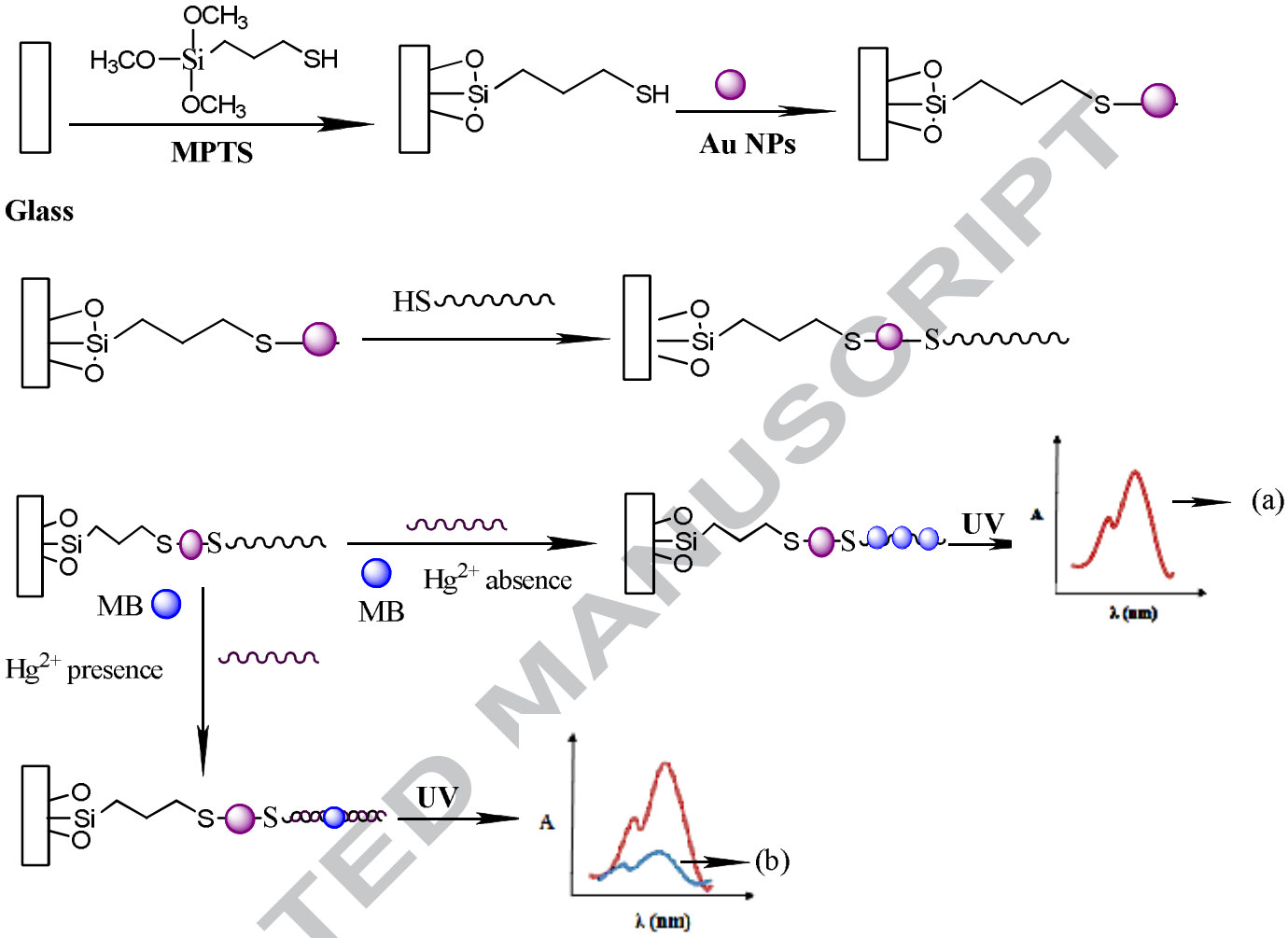

The fabrication steps of an Hg^{2+}-DNA biosensor.

Highlights:

1. A novel optical DNA biosensor for sensitive determination of Hg^{2+} was reported.
2. The biosensor showed good sensitivity, very high selectivity, simple, and cheap.
3. The absorption peak was linear to Hg^{2+} concentration from 10 nM to 10 μM.