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# An electrochemiluminescence biosensor for mercury ion detection based on gamma-polyglutamic acid-graphene-luminol composite and oligonucleotides

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ABSTRACT

An ultrasensitive electrochemiluminescence (ECL) biosensor based on gamma-polyglutamic acid-graphene-luminol (γ-PGA-G-luminol) composite and oligonucleotides for the detection of mercury ion ($Hg^{2+}$) was developed. γ-PGA-G-luminol composite coated on the glassy carbon electrode could produce a strong and stable ECL signal by luminol, and provide carboxyl groups to cross-link with amino-modified DNA1 enriching in thymine (T) by γ-PGA. Upon addition of $Hg^{2+}$ and biotin-modified DNA2 enriching in T, T–$Hg^{2+}$–T interaction occurred and mediated the coordination between DNA1 and DNA2. Afterwards, streptavidin was connected to the end of DNA2 through specific binding of streptavidin to biotin, which obviously reduced the ECL intensity due to the inert protein layer with poor electron transfer ability. Based on the specific T–$Hg^{2+}$–T coordination chemistry and the specific binding of streptavidin-biotin system, ultrahigh sensitivity and selectivity for $Hg^{2+}$ detection were obtained. Results revealed that the ECL intensity was logarithmically linear with the concentration of $Hg^{2+}$ in a wide range from 0.01 to 100 nmol/L (about 0.002–20 ppb). Moreover, the biosensor also exhibited excellent selectivity for $Hg^{2+}$ ions without significant interference from commonly co-existing metal ions in the sample matrix. Excellent sensitivity and selectivity make the developed biosensor a potential and simple tool for the detection of $Hg^{2+}$.

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## 1. Introduction

Mercury is considered as one of the most toxic metallic pollutants in the environment [1]. Because of its high affinity for thiol group in proteins and enzymes, low level exposure to mercury can lead to serious bioaccumulation in organisms and cause damage to the nervous system, immune system, reproductive system, and cardiovascular system [2–4]. With an estimated annual release of 4400–7500 metric tons of mercury into the environment [5], mercury pollution has been considered as a common health concern. Considering its serious health hazard, different regulatory agencies have set maximum limits for mercury. The World Health Organization and the United States Environmental Protection Agency imposed a maximum mercury level of 6 ppb and 2 ppb, respectively, for drinking water [6,7], while the European Commission set the maximum limit of 1 ppb as early as 1998 [8]. These low maximum limits of mercury in drinking water necessitate sensitive analytical methods.

The most commonly used techniques for mercury detection mainly include inductively coupled plasma atomic emission spectrometry [9], inductively coupled plasma mass spectrometry [10], cold vapor generation-atomic absorption spectrometry [11], and cold vapor generation-atomic fluorescence spectrometry [12]. These methods are sensitive, selective, and have a low detection limit, but they are instrument-intensive, expensive, and require trained technicians. Furthermore, sample preparation may be tedious, and require a long time in some cases; thus hampering the on-site application. Currently, other assays such as electrochemical techniques [13], fluorescence methods [14], colorimetric methods [15], and test strips [16], have also been developed to allow miniaturization and field applications. However, these methods usually have practical limitations due to the poor aqueous solubility, sophistication needed to synthesize probe materials, and matrix interference or cross-sensitivity toward other metal ions. Thus, a

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sensitive, selective and inexpensive method that requires minimal sample handing and permits rapid detection remains highly desirable.

Electrochemiluminescence (ECL) is a well-known detection method with acknowledged advantages such as simplified optical setup, strong signal response, low background values, wide dynamic range, and inherent sensitivity [17,18]. In the fields of biomedical and chemical analysis, ECL has been widely used as an important analytical method for the determination of extremely low concentrations of nucleic acids [19,20], proteins [21–23], cells [24,25], and small molecules [26–28]. It has been reported that $Hg^{2+}$ could specifically bind two DNA thymine (T) bases to form strong and stable T–$Hg^{2+}$–T complexes [29], which was directly confirmed using NMR spectroscopy [30]. According to this principle, biosensors that combine the advantages of ECL and the specific binding of T–$Hg^{2+}$–T have been recently employed for the sensitive and selective detection of $Hg^{2+}$ [31–38].

Graphene (G), as a one-atom-thick sheet of $sp^2$-bonded carbon atoms, has gained much attention in recent years due to its distinctive properties of electrical conductivity, large surface area, high mechanical strength, and good biocompatibility [39,40]. Owing to these properties, G has been applied extensively in the fabrication of ECL biosensors [41]. The most commonly used ECL reagent, luminol, is an excellent luminophore with good electrochemical stability and high ECL efficiency [42]. Gamma-polyglutamic acid (γ-PGA) has been reported to be the major component of the viscous sticky mucilage in natto produced by *Bacillus subtilis* and has been used in a wide variety of application due to its nontoxic nature [43].

In this work, an ultrasensitive ECL biosensor using gamma-polyglutamic acid-graphene-luminol (γ-PGA-G-luminol) composite and T-rich oligonucleotides for the determination of $Hg^{2+}$ was developed. In the fabrication process, γ-PGA was used as the biopolymer to immobilize G-luminol on the glassy carbon electrode surface and to provide an aqueous microenvironment. Moreover, amino-modified DNA1 enriching in T can be cross-linked to γ-PGA through carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS). To the best of our knowledge, use of γ-PGA in building a stable composite film to develop an ECL biosensor has not been reported. Upon addition of $Hg^{2+}$ and biotin-modified DNA2 enriching in T, T–$Hg^{2+}$–T interaction occurred directing a coordination between DNA1 and DNA2. Streptavidin, a 66 kDa protein purified from the bacterium *Streptomyces avidinii*, has been widely used as an intermediate link in immunoassay due to its high specificity and strong affinity for biotin [44,45]. Based on the highly selective recognition of streptavidin to biotin, streptavidin can be efficiently immobilized at the end of DNA2. Herein, streptavidin was first used as an inert protein to inhibit the electron transfer on the electrode interface, resulting in an obvious reduction on ECL intensity. The reduction in ECL intensity in presence of $Hg^{2+}$ enabled realizing sensitive and selective determination of $Hg^{2+}$.

Compared with other analytical methods based on ECL and T–$Hg^{2+}$–T coordination chemistry [31–35], this proposed method using γ-PGA-G-luminol composite film as supporting matrix has a higher sensitivity. Because luminol was directly doped into G and γ-PGA without labeling procedures, a large number of luminophore can take part in the ECL reaction that will greatly enhance the sensitivity. Benefited from the specific binding of streptavidin to biotin, streptavidin-biotin system was first used to inhibit the ECL reaction and block the ECL signal more effectively, which also improved the sensitivity significantly. Furthermore, it also possesses other advantages such as good stability, high specificity, simple instrumentation and short assay time. Therefore, this novel biosensor demonstrated a balance between ultra-high sensitivity, high selectivity and easy operation.

## 2. Experimental

### 2.1. Apparatus

ECL measurements were performed with a custom-built ECL detection system which mainly contains a BPCL ultra-weak chemiluminescence analyzer (Institute of Biophysics, Chinese Academy of Sciences, Beijing, China) and a CHI 1100A electrochemical workstation (Chenhua Instrument Company, Shanghai, China). A conventional three-electrode system including a bare or modified glassy carbon electrode (GCE, $\Phi = 3$ mm) as the working electrode, a platinum wire electrode as the counter electrode, and a Ag/AgCl/3 mol/L KCl electrode as the reference electrode was used throughout the experiment. Electrochemical impedance spectroscopy (EIS) analysis was performed with a CHI 660E electrochemical workstation (Chenhua Instrument Company, Shanghai, China). The concentrations of $Hg^{2+}$ in spiked water samples were validated using Agilent 7500cx ICP-MS (Agilent Technologies Inc., Santa Clara, USA).

### 2.2. Reagents

Two types of oligonucleotides were synthesized and HPLC-purified by TaKaRa Biotechnology Co. Ltd. (Dalian, China), and the sequences are: 5′-$NH_2$-CTGTGTCTTGCTCGGTATC-3′ (DNA1) and 5′-biotin-GATTCCGTGCATGACTCAG-3′ (DNA2). Tris(Hydroxymethyl)aminomethane (Tris) was purchased from Acros Organics (Geel, Belgium). Gamma-polyglutamic acid (γ-PGA), bovine serum albumin (BSA, 98–99%), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), 3-aminophthalhydrazide (luminol) and streptavidin were purchased from Sigma–Aldrich (St. Louis, MO, USA). Graphite powder (8000 mesh, 99.95%) and hydrazine monohydrate were purchased from Aladdin (Shanghai, China). Mercury nitrate and other metal nitrate salts were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Other chemicals were of analytical reagent grade or higher, and used without further purification or treatment. Tris–HCl buffer solution (pH 8.6) containing 0.05 mol/L Tris was used as the working solution for the ECL measurement. Ultrapure water (18 MΩ cm) obtained from a Heal Force PW ultrapure water system (Hong Kong, China) was used throughout the experiment.

### 2.3. Preparation of gamma-polyglutamic acid-graphene-luminol (γ-PGA-G-luminol) composite

Graphene (G) was synthesized according to reported routes involving the steps of graphite oxidation, exfoliation and chemical reduction. Firstly, graphene oxide (GO) was synthesized from graphite powder using a method as presented by Kovtyukhova et al. [46]. Then, exfoliation process was carried out by dissolving GO in deionized water using sonication, and the small amount of unexfoliated GO was removed by centrifugation. Finally, G was prepared from GO as described by Li et al. [47].

The γ-PGA-G-luminol composite was prepared as follows: 30 μL of 2 mg/mL G solution and 30 μL of 1 mmol/L luminol solution were mixed and vortexed for 5 min to obtain a homogeneous solution. Then, the mixed solution was left for 1 h for G and luminol to bond with each other adequately due to the π–π interaction between them. Next, 300 μL of 2 wt% γ-PGA was added and vortexed for 5 min to obtain the γ-PGA-G-luminol composite, which could produce a stable and strong ECL intensity after coated on GCE surface.

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