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Functionalized Fe_3O_4/graphene oxide nanocomposites with hairpin aptamers for the separation and preconcentration of trace Pb^{2+} from biological samples prior to determination by ICP MS

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ABSTRACT

Lead (Pb) as a topically poisonous metal represents a serious threat to the ecological environment and especially to human beings. Therefore, it is urgent to develop a rapid and reliable monitoring technique for this heavy metal in the environmental samples. In the present study, we have designed a selective and sensitive method for the determination of ultratrace contents of Pb^{2+} in biological samples, based on the guanine (G)-quadruplex formed by the aptamer with hairpin structure and Pb^{2+}. For this purpose, Pb^{2+} specific aptamer serving as affinity probe to capture and separate trace amounts of the analyte, was covalently linked to Fe_3O_4/graphene oxide (GO) surface by using a suitable cross-linking agent. Then, the G-quadruplex complex was formed by the opening of the "neck- ring" of the hairpin structure of aptamer in the presence of Pb^{2+}. Inductively coupled plasma mass spectrometry (ICP-MS) was used for determination of Pb^{2+} in biological matrices. The analysis conditions were optimized and the performance of the proposed method was investigated. Under optimum conditions, the calibration curve was linear over the range of 0.3–867.5 μg L^{-1} and an enrichment factor (EF) of 50 was obtained. The limit of detection (LOD) was 0.05 μg L^{-1} and the relative standard deviation (RSD) for single-sorbent repeatability and sorbent-to-sorbent reproducibility were <4.7% and 8.8% (n = 5), respectively. The accuracy of aptamer-based affinity purification method was confirmed by the analysis of quality control materials (QCMs, Seronorm™ Blood REF NO 201505 and Urine REF NO 2525).

1. Introduction

There is a worldwide concern about the high toxicity of environmental lead (Pb) for human and animal health. Environmental contamination with lead has been increased steadily since the industrial revolution, due to consumer demand and reliance on conventional energy resources such as coal and oil [1]. Exposure to high levels of this heavy metal may cause neurological disorders, high blood pressure and anemia. Lead (II) ions (Pb^{2+}) bind hemoglobin in red blood cells and slowly accumulate in the soft tissues (brain, spleen, liver, kidneys) and skeleton [2]. In view of the high toxicity of Pb^{2+} to public health, it is necessary to develop reliable, precise, and sensitive methods for the determination of trace amounts of this element in environmental and biological samples. The inductively coupled plasma optical mass spectrometry (ICP MS) technique is an excellent tool for the evaluation of ultratrace amounts of heavy metals in a variety of samples, but it cannot be directly applied to biological fluids. This is mainly due to the presence of high concentrations of interfering matrix components in most biological samples, which often necessitates the performance of a suitable pretreatment step before application of the ICP MS. Thereby, several methods for the separation and pre-concentration of Pb^{2+} have been previously reported in the literature. Cloud point extraction [3–5], co-precipitation [6–8], column extraction [9–11], liquid-liquid extraction [12–14] and solid phase extraction (SPE) [15–17] are among these methods. Although these methods are sensitive and selective, the magnetic solid phase extraction methods based on biosorbents have been well-established as effective, simple and environmentally friendly preconcentration techniques for this purpose.

In recent years, the application of biotechnology to controlling and removing of toxic elements has attracted much attention, and gradually becoming a hot topic in the field of metal pollution control. The biosorption process utilizes various certain natural materials of

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biological origin [18]. These biological probes are more effective alternatives for the removal of metallic elements from aqueous solution. Among a vast array of such biological probes used in this respect, in the past decade, the application of aptamers have attracted great attention for determination of metal pollutants, due to their high affinity, acceptable selectivity, low cost, thermal and chemical stability [19,20]. Aptamers are single-stranded oligonucleotides (ssDNA or RNA) with unique secondary structures that can bind specifically to the corresponding targets [21]. They are selected for a broad range of targets, including small molecules [22], tumor markers [23], ions [24], proteins [25], cells [26], tissues and organisms [27]. In the presence of certain metal ions, DNA or RNA can form G-quadruplex complexes, which is known to play a functional role in a variety of biological processes. The most effective ions for inducing quadruplex formation are K^+, Na^+ and Pb^{2+} [28]. The ability of cations to bind nucleic acids and induce quadruplex formation can be attributed to such cation properties as ionic radius, coordination behavior, and hydration effects [28].

Graphene oxide (GO), as a novel nanomaterial, has been attracting more and more attention in recent decades, due to its novel properties and wide range of potential applications including supercapacitors [29], fuel cells [30], catalysts [31], drug delivery [32], biosensors [25], etc. In some cases, it is necessary to regain graphene's desirable characteristics such as electrical conductivity, catalytic activity or magnetic properties. For these purposes, metallic nanoparticles are generally incorporated into GO or reduced GO nanosheets. Some of them cause an increase in electronic and catalytic effects of GO [31,33]. In addition, the combination of GO with iron oxide nanoparticles (Fe_3O_4) lead to magnetic nanocomposites with interesting properties for a variety of applications, especially, solid phase extraction (SPE). Some of the GO properties such as high surface area, ease of functionalization and good dispersibility due to the presence of the oxygen functionalities make it an ideal sorbent.

In this work, we aim to achieve two goals. First, we report a convenient route to synthesize functionalized Fe_3O_4/GO nanocomposite with affinity probe (aptamer) as the new magnetic biosorbent. As aforementioned, weak interactions including hydrophobic, electrostatic, π-π stacking interaction between phenyl groups of the oligonucleotides and plane of the graphene can drive DNA immobilization on the GO [24,34,35]. Here, despite previous strategies, the aminated aptamer was covalently immobilized on Fe_3O_4/GO surface using an activating system based on 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and N-Hydroxysuccinimide (NHS). Second, we applied the designed biosorbent for separation and preconcentration of Pb^{2+} based on hairpin oligonucleotides forming G-quadruplex structure in presence of target ions. An important advantage of proposed approach is immobilization of DNA probes through covalent bonds in a controlled orientation resulting in a functional active affinity matrix. To our best knowledge, the biosorbent based on the lead specific aptamer functionalized Fe_3O_4/GO was designed for the first time in our study and applied for determination of ultratrace amounts of Pb^{2+} (as a model analyte) with ICP MS in real samples including blood and urine matrices.

2. Experimental

2.1. Reagents

All reagents were of analytical grade and were used as received without further purification. The lead specific oligonucleotide (5′-H_2N-$(CH_2)_6$-TTTTT ACCCA GGGTGGGTGGG TGGGT-3′) was prepared from Invitrogen (USA). Stock metal solutions of 1000 mg L^{-1} used for the experiments as well as for the interferences studies were purchased from Merck (Germany). Working solutions were prepared by appropriate dilution of the stock solutions with doubly distilled water. The natural graphite powder, sulfuric acid (H_2SO_4, 98%), nitric acid (HNO_3, 70%), acetic acid (CH_3COOH, ≥99.85%), ethanol (C_2H_5OH, ≥99.8%), methanol (CH_3OH, 99.8%), ethylenediaminetetraacetic acid (EDTA, ≥99.0%), potassium permanganate ($KMnO_4$, 99.9%), hydrogen peroxide (H_2O_2, 30%), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$, 97%), ferrous chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, 98%), and ammonium hydroxide (NH_4OH, 25%) were also purchased from Merck. *N*-Hydroxysuccinimide (NHS, 98%), *N*-(3-Dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDC HCl, ≥98%), sodium hydroxide (NaOH, ≥97.0%) and sodium nitrate ($NaNO_3$, ≥99.0%) were prepared from Sigma-Aldrich (USA).

All laboratory wares, including pipette tips and autosampler cups, were cleaned thoroughly with detergent and tap water, rinsed with distilled water, soaked in dilute nitric acid, and then rinsed thoroughly with deionized distilled water.

2.2. Instruments

Lead (II) ion was analyzed by a Perkin Elmer ICP MS, model Elan6000 DRC (USA) with RF power of 250 W and sampling depth of 7.0 mm. Scanning electron microscopy (SEM) was performed with a EM3200 KYKY instrument (Madell Technology Corporation, USA). Energy dispersive X-ray spectroscopy (EDX) analysis of the nanocomposite was obtained using a XL30 scanning electron microscope (Philips, Netherlands) equipped with an energy dispersive X-ray analyzer. Powder X-ray diffraction (XRD) was performed using a Philips X'pert diffractrometer with a Cu-Kα source (Netherlands). The products were also characterized by a dispersive Raman microscope (DRM) system (Bruker, Germany) with a confocal depth resolution of 2 μm. Fourier transform infrared (FTIR) spectral studies were carried out by using a Mattson ATI Genesis FTIR Spectrometer in the range of 400–4000 cm^{-1} (USA). A sonoreactor UTR200 (Hielscher, Germany) with maximum output power of 200 W, operating frequency of 24 kHz and ultrasonic intensity of 80 W cm^{-2} was employed for ultrasound-assisted exfoliation of GO. A Metrohm 780 pH Meter (Switzerland) was used for measuring pH at 25 °C.

2.3. Preparation of the aptamer functionalized Fe_3O_4/GO nanocomposites

The Fe_3O_4/GO nanocomposite was synthesized by an inverse chemical co-precipitation method according to the reported procedure by our group [36]. For functionalization of Fe_3O_4/GO with aptamer probe, 50 mg of magnetic nanocomposite dispersed into 0.5 mL MES buffer (pH = 5) and then, 300 μL of EDC (150 mM) and NHS (300 mM) solutions were added to GO to activate its carboxyl-terminated surface. The EDC molecules are considered zero-length carboxyl-to amine crosslinkers. EDC reacts with the carboxylic acid groups to form an active O-acylisourea intermediate, allowing it to be coupled to the primary amino groups in the reaction mixture [37]. The suspension was sonicated for 30 min at room temperature. 100 μL of the aminated aptamer (10 μM) was then added to the above suspension. This immobilization mixture was incubated at room temperature for 1 h with gentle rotation using an overhead shaker. The prepared aptamer-functionalized Fe_3O_4/GO nanocomposites were isolated by a magnet and washed with deionized water and alcohol three times. In this step, unbound aptamers were separated from bound ones. After drying in vacuum oven (50 °C), they were ready for use in separation and preconcentration process.

2.4. Magnetic solid phase extraction procedure

To incubation of molecular recognition element and target, 50 mg of aptamer modified Fe_3O_4/GO nanocomposite was added into 25 mL of analyte solution (pH ~7), and shaken for 20 min at room temperature. The "stem-loop" structure of hairpin aptamer was opened via adding Pb^{2+} and, then a G-quadruplex structure was formed [38,39]. After 20 min incubation at room temperature on a shaking platform, Pb^{2+} attached magnetic biosorbent was isolated by placing a strong magnet and the supernatant was discarded. Then, 500 μL of EDTA (0.4 M, pH = 10) was used to elute the bounded analyte onto the biosorbent by