A Colorimetric Aptamer Sensor Based on the Enhanced Peroxidase Activity of Functionalized Graphene/Fe₃O₄-AuNPs for Detection of Lead (II) Ions

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Abstract: Lead (II) is regarded as one of the most hazardous heavy metals, and lead contamination has a serious impact on food chains, human health, and the environment. Herein, a colorimetric aptasensor based on the graphene/Fe₃O₄-AuNPs composites with enhanced peroxidase-like activity has been developed to monitor lead ions (Pb²⁺). In short, graphene/Fe₃O₄-AuNPs were fabricated and acted as an enzyme mimetic, so the color change could be observed by chromogenic reaction. The aptamer of Pb²⁺ was decorated on the surface of the amine magnetic beads by streptavidin–biotin interaction, and the complementary strands of the aptamer and target Pb²⁺ competed for the binding Pb²⁺ aptamer. In the presence of Pb²⁺, aptamers bonded the metal ions and were removed from the system by magnetic separation; the free cDNA was adsorbed onto the surface of the graphene/Fe₃O₄-AuNPs composites, thus inhibiting the catalytic activity and the color reaction. The absorbance of the reaction solution at 652 nm had a clear linear correlation with the Pb²⁺ concentration in the range of 1–300 ng/mL, and the limit of detection was 0.63 ng/mL. This assay is simple and convenient in operation, has good selectivity, and has been used to test tap water samples, which proves that it is capable for the routine monitoring of Pb²⁺.

Keywords: peroxidase; aptamer; graphene; enzyme mimetic; colorimetric method; food safety; rapid detection

1. Introduction

Lead contamination is an increasingly important issue accompanied by the wide use of lead in industry sectors such as batteries [1], gasoline [2], metallurgies [3], and paints [4]. Lead poses a serious threat to food chains [5], human health [6], and the environment as a whole through chemical waste, soil, and water [7]. Due to its non-biodegradability, lead is hard to detoxify, and has different degrees of damage to many organs and tissues of the human body, especially the hematopoietic system, nervous system, and kidneys [8]. Even low levels of lead can pose serious threats for the central nervous system of infants and children, affecting their growth and development and leading to mental retardation [9,10]. For adults, long-term intake of lead-contaminated food or water may result in various health disorders such as anemia [11], hypertension [12], diabetes [13], and reproductive dysfunctions [14]. The maximum
contaminant level of Pb$^{2+}$ in drinking water is 15 ng/mL, as established by the U.S. Environmental Protection Agency (EPA) [15]. The U.S. Food and Drug Administration (FDA) sets the maximum level of Pb$^{2+}$ in food such as candy at 100 ng/g [16].

For food safety and public health, it is essential to develop advanced detection techniques for sensitive and selective monitoring of this heavy metal. The conventional methods for Pb$^{2+}$ determination are based on inductively coupled plasma mass spectrometry (ICP-MS) [17], high performance liquid chromatography (HPLC) [18], and graphite furnace atomic absorption spectrometry (GF-AAS) [19], which provide sensitivity and accuracy, but usually depend on sophisticated procedures, expensive equipment, and complicated sample preparation processes, limiting the practical sensing of Pb$^{2+}$, especially in developing and undeveloped countries or dealing with on-site emergencies. In this context, some alternative methods have been developed for Pb$^{2+}$ sensing including electrochemistry [20], fluorescence [21], colorimetry [22], and surface plasmon resonance [23]. These techniques have exhibited promising performance in the detection of Pb$^{2+}$. Xiong et al. proposed an electrochemiluminescence biosensor for sensing Pb$^{2+}$ using nitrogen doped carbon dots in a range of 1.0 ng/mL to 1375.0 ng/mL [24]. Chaiyo et al. developed a microfluidic paper-based analytical device using a boron-doped diamond electrode for the detection of Pb$^{2+}$, and the limit of detection was 0.1 ng/mL [25].

In recent years, aptamers have shown great advantages in establishing biosensors for the determination of heavy metals [26]. Aptamers are synthetic DNA or RNA single chain oligonucleotides selected by SELEX (Systematic Evolution of Ligands by exponential enrichment) procedures, can recognize targets ranging from ions and small molecules to cells with high binding affinity and specificity [27,28]. Compared with antibodies, aptamers possess the advantages of low cost, ease of synthesis and modification, great thermal stability, and without immunogenicity and toxicity [29]. Aptamer-based sensing methods coupled with colorimetry [30], electrochemistry [31], and fluorescence [32] have been regarded as competitive alternatives to overcome the above-mentioned limitations of conventional assays.

Another promising material are enzyme mimetics. Enzyme mimetics have catalytic activity similar to natural enzymes to catalyze enzyme-like reactions. Compared with natural protein enzymes, enzyme mimetics offer various advantages such as simpler synthesis, higher stability, lower cost, easier modification, and are more efficient [33,34]. Over the past decade, a great number of nanoparticles have been found to have catalytic activity either alone or as hybrids in conjunction with other nanomaterials. These nanoscale enzyme mimetics are composed of carbon and its derivative nanomaterials and metallic nanozymes such as carbon nanodots, carbon nanohorns, graphene, Fe$_3$O$_4$ nanoparticles, Cu nanoclusters, and noble metals, most of which belong to peroxidase-like nanozymes that could induce the oxidation of peroxidase substrates in the presence of H$_2$O$_2$ [35,36]. These peroxidase nanozymes have been attractive for various applications due to the large surface-to-volume ratio as well as the fundamental and technological characteristics, and the development of enzyme mimetic-based biosensors has provided highly accurate and precise sensing of various targets from small molecules to proteins and cells including the Ebola virus, foodborne pathogens, and tumor cells [37–39].

Among which, graphene has a large specific surface area and is an excellent candidate for immobilizing a large number of functionalized metal oxide and noble metal nanoparticles such as Fe$_3$O$_4$ and AuNPs, which also have relative catalytic activity, thus, the synergy of these nanozymes could enhance the peroxidase-like activity through accelerating electron transfer [36,40–42]. Furthermore, Liu et al. reported that the catalytic activity of functionalized graphene materials may be inhibited by interaction with ssDNA nucleotides, which occupy the active catalytic sites of graphene [43].

Herein, for the first time, a colorimetric detection method using aptamer recognition and the synergetic effect of graphene/Fe$_3$O$_4$-AuNP composite triggered enzyme catalysis was established and used for the detection of lead ions. The aptamer of Pb$^{2+}$ was decorated on the surface of amine magnetic beads by streptavidin–biotin interaction, the complementary chains of aptamers
While in the absence of lead ions, cDNA was combined with the aptamer which anchored on beads, and then removed by magnetic separation. In this scenario, the graphene/Fe₃O₄-AuNPs composites could play the function of enhanced peroxidase-like activity to stimulate the color reaction, thereby visual detection of Pb²⁺ can be achieved with the addition of 3,3′,5,5′-Tetramethylbenzidine (TMB) chromogenic reagents.

2. Results and Discussion

2.1. The Principle of the Colorimetric Assay

The principle of the assay is shown in Figure 1. The biotin-labeled Pb²⁺ aptamer was connected to the surface of the streptavidin-coated amine magnetic beads to form a capture probe. In the absence of lead ions, the aptamer on the surface of the magnetic beads will combine with the cDNA, and then the magnetic beads are separated by magnetic adsorption. For the graphene/Fe₃O₄-AuNP composites, graphene plays a role as a nanozyme to catalyze the oxidation of TMB by H₂O₂ reduction, Fe₃O₄ nanoparticles, and AuNPs, which grow in situ on the surface of graphite, improving the efficiency of electron transfer from TMB, accelerating the catalytic reactions [44]. The color of the reaction solution changes from colorless to blue and can be observed by the naked eye. In addition, the absorbance value of the solution at 652 nm was measured by the microplate reader for quantitative analysis. In the presence of the lead ions, the aptamer preferentially binds to lead ions, leaving a large amount of cDNA in a free state. The magnetic beads and the aptamer bound metal ions were separated by magnetic adsorption, the free cDNA was adsorbed onto the surface of the graphene/Fe₃O₄-AuNPs composites by the strong π–π stacking interaction to occupy the active catalytic sites of the nanozyme, and the cDNA could also stick to the AuNPs due to the flexible structure [38]; all of these adsorptions prevent the catalytic ability of the nanocomposites to oxidize TMB to trigger the color reaction, so the color of the reaction solution turns lighter, enabling the quantitative analysis of lead ions by measuring the absorbance value of the solution.

![Figure 1](image_url). Principle of the detection of Pb²⁺ in this assay.

2.2. Characterization of Graphene/Fe₃O₄-AuNP Composites

The prepared graphene/Fe₃O₄-AuNPs composites were characterized by TEM and XRD. Figure 2a is the TEM of the composites, where the lamellar materials at the bottom of the figure are graphene, the light gray particles are Fe₃O₄ nanoparticles grown in situ, the dark particles are gold nanoparticles, and the TEM of the composites, where the lamellar materials at the bottom of the figure are graphene, the light gray particles are Fe₃O₄ nanoparticles grown in situ, the dark particles are gold nanoparticles, and the light red particles are AuNPs.
grown in situ, and the two kinds of nanoparticles are evenly distributed on the graphene. Figure 2b is the XRD spectrogram, from top to bottom are the XRD patterns of graphene/Fe$_3$O$_4$-AuNP composites (red pattern), AuNPs (green pattern), and Fe$_3$O$_4$ nanoparticles (blue pattern). As shown in the figure, characteristic peaks of Au crystal positioned in the 2θ scale at 38.6°, 44.7°, 64.9°, 77.9°, and 82.0° can be attributed to the Au(111), Au(200), Au(220), Au(311), and Au(222) planes, respectively, and the characteristic absorption peaks at 2θ = 30.5°, 36.0°, 43.6°, 57.9°, and 62.8° can be attributed to Fe(220), Fe(311), Fe(400), Fe(511), and Fe(440), respectively. These characteristic peaks can be observed in the XRD pattern of graphene/Fe$_3$O$_4$-AuNP composites, which proves that AuNPs and Fe$_3$O$_4$ nanoparticles have been successfully fabricated on the prepared composites.

![Figure 2](image-url)

**Figure 2.** Transmission electron microscope (TEM) image (a) and x-ray diffraction (XRD) patterns (b) of graphene/Fe$_3$O$_4$-AuNPs.

### 2.3. Characterization of Amine Magnetic Beads

The prepared amine magnetic beads were characterized by TEM, FTIR, and XRD. Figure 3a is the TEM image of the amine magnetic beads. It indicates that the prepared magnetic beads have a particle size of 50 to 70 nm and good dispersibility. Figure 3b is the Fourier infrared spectrum of the magnetic beads. The fabricated magnetic beads had characteristic absorption peaks of Fe–O at 575 cm$^{-1}$, and the characteristic absorption peaks of NH at 1630 cm$^{-1}$, 1049 cm$^{-1}$, and 832 cm$^{-1}$, which proved that the amino group had been successfully modified on the surface of magnetic beads. Figure 3c is the XRD pattern of amine magnetic beads, which represented six characteristic peaks at 2θ = 30.1°, 35.5°, 43.5°, 53.4°, 57.1°, and 62.5° corresponded to Fe(220), Fe(311), Fe(400), Fe(422), Fe(511), and Fe(440) crystallographic planes, respectively, which indicates the crystal structure of the amine magnetic beads.

### 2.4. Characterization of Streptavidin-Coated Amine Magnetic Beads

Streptavidin and amine magnetic beads are connected by using glutaraldehyde as a cross-linker, according to the principle that the aldehyde group reacts with the amino group to form a Schiff base. Ultraviolet spectrophotometry was used to verify the reaction. The supernatant of the solution was extracted by magnetic separation, and as shown in Figure 4, the characteristic absorption peak of streptavidin in the supernatant at 280 nm was about 0.15, and the absorption peak reduced to 0.05 after streptavidin connected with the magnetic beads, indicating that most of the streptavidin in the solution had connected to the magnetic beads.
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Figure 4. UV–Vis absorption spectra of the supernatants after the incubation of magnetic beads and streptavidin.

2.5. Catalytic Performance Verification of Graphene/Fe₃O₄−AuNPs

To verify that the synergy of graphene/Fe₃O₄/Au enhanced the catalytic specific activity, graphene/Fe₃O₄/AuNPs, graphene/AuNPs, graphene/Fe₃O₄, and graphene were simultaneously tested for specific activity. Figure 5A shows the time–absorbance curves of the reaction. The absorbance increased within the whole reaction time, and was linear in the initial 60 s, so 60 s was selected as the initial rate period (Figure 5B). According to the above-mentioned equation, the specific activities of graphene/Fe₃O₄/AuNPs, graphene/AuNPs, graphene/Fe₃O₄, and graphene were calculated as 25.55 U/mg, 11.90 U/mg, 6.37 U/mg, and 4.37 U/mg, respectively. Among these, graphene/Fe₃O₄/AuNPs
had the highest specific activity, which proved that the synergetic coupling effect of graphene, Fe₃O₄, and AuNPs enhanced the catalytic activity of the nanozyme.

![Figure 5](image-url)

**Figure 5.** The time–absorbance curve of the 3,3',5,5'-Tetramethylbenzidine (TMB) chromogenic reaction catalyzed by graphene/Fe₃O₄/AuNPs, graphene/AuNPs, graphene/Fe₃O₄, and graphene in 5 min (A). The calibration curve of the first 60 s of the reaction, the specific activities (SA) of graphene/Fe₃O₄/AuNPs, graphene/AuNPs, graphene/Fe₃O₄, and graphene were calculated (B).

2.6. Optimization of the Concentration of Complementary Strands

Complementary strands of the aptamer can be adsorbed on the surface of graphene/Fe₃O₄-AuNP composites and inhibit the catalytic activity of the nanozyme. In the optimization process, eight different concentrations (0, 10, 20, 30, 40, 50, 60, and 70 nmol/L) of cDNA were studied. As shown in Figure 6, the A652 value decreased as the concentration of the cDNA increased, indicating that the catalytic activity of the graphene/Fe₃O₄-AuNP composite was suppressed due to the adsorption of the cDNA onto the surface. When the concentration of cDNA reached 50 nmol/L, the A652 value tended to be stable, which proves that the catalytic activity of graphene/Fe₃O₄-AuNPs had reduced to the lowest level. Thus, 50 nmol/L was employed as the concentration of the cDNA.

![Figure 6](image-url)

**Figure 6.** Optimization of the concentration of complementary strands. Measurements were performed in triplicate.

2.7. Optimization of the Concentration of Magnetic Beads

The concentration of the aptamer decorated magnetic beads was optimized, and seven different concentrations (0.2, 0.4, 0.6, 0.8, 1, 1.2, and 1.4 mg/mL) were selected. As shown in Figure 7,
as the concentration of the complex increased from 0 to 1 mg/mL, the value of A652 gradually increased, indicating that the cDNA had been combined with the aptamer decorated magnetic beads and subsequently removed from the system by magnetic separation. When the concentration of the complex reached 1 mg/mL, the A652 value tended to no longer increase, which proved that almost all of the cDNA in the system had been removed by magnetic separation, and the optimal concentration was 1 mg/mL.

2.8. Detection Performances

Under the optimal conditions, the proposed method was used to detect lead ion standard samples of different concentrations. As shown in Figure 8, the absorbance value at 652 nm decreased when the concentration of Pb$^{2+}$ increased, and the color of the solution gradually changed from dark blue to light blue, which could be observed by the naked eye, exhibiting good linear relationship within the working range from 1 to 300 ng/mL ($R^2 = 0.9931$). The limit of detection was 0.63 ng/mL, which was determined by a 3 Sb/slope. The relative standard deviation of the ten test results was 2.3%, which proves that this assay has good repeatability. The proposed method and other recently developed methods for the detection of Pb$^{2+}$ are summarized and compared in Table 1, and indicate that the linear range and the LOD of this assay are competitive among the listed methods.

![Figure 7](image7.png)

**Figure 7.** Optimization of the concentration of magnetic beads. Measurements were performed in triplicate.

![Figure 8](image8.png)

**Figure 8.** The linear relationship between the concentration of Pb$^{2+}$ and A652 value. Measurements were performed in triplicate.

| Detection Method                  | LOD (ng/mL) | Linear Range (ng/mL) | Reference |
|----------------------------------|-------------|----------------------|-----------|
| Surface plasmon resonance based fiber probe | 1.2         | 1–20                 | [23]      |
| Carbon dots based electrochemical sensor | 0.33        | 1–1375               | [24]      |
| Nanoporous bismuth electrode sensor | 1.5         | 5–40                 | [45]      |
| ZnO@graphene modified electrode | 0.8         | 10–200               | [47]      |
| Aptamer based colorimetric assay | 0.63        | 1–300                | this work |
| Microfluidic paper-based sensor | 0.1         | 0.5–70               | [25]      |
| Paper-based analytical device    | 10          | 10–100               | [46]      |
1.031 ± 0.024 & 1.06 ± 0.032 & 106.0 \\
2 10 & 10.41 ± 0.33 & 10.58 ± 0.25 & 105.8 \\
3 50 & 48.76 ± 1.01 & 47.63 ± 1.17 & 95.3 \\
4 200 & 208.5 ± 5.57 & 206.6 ± 4.08 & 103.3

### 2.9. Evaluation of Specificity

To explore the effect of the potentially foreign ions on this assay, ten metal ions including Hg²⁺, Cd²⁺, Fe³⁺, Mg²⁺, K⁺, Ag⁺, Cu²⁺, Zn²⁺, Ca²⁺, and Na⁺ were studied under the optimized conditions, the concentration of Pb²⁺ was 50 ng/mL, each concentration of the competitive metal ions was 10-fold higher than Pb²⁺, and the results are shown in Figure 9. Compared to the A652 value of the blank sample, no noticeable changes were detected in the presence of interference metal ions, while an obvious variation in the absorbance value was observed after the addition of Pb²⁺, indicating that the developed method possesses great selectivity for Pb²⁺ detection.

### 2.10. Analysis of Pb²⁺ in Real Samples

To further determine whether the assay was applicable to the real samples, the prepared method was applied to detect Pb²⁺ in tap water. The water samples were filtered with a 0.22 µm membrane (Millipore) and then diluted with 10 mmol/L Tris-HCl buffer (pH 7.4). The lead ions contained in the spiked water sample were measured by this proposed method and atomic absorption spectrometry. The measurement results are exhibited in Table 2, where there were no noticeable differences between the two methods, and the recoveries of water samples tested by this assay ranged from 95.3% to 106.0%, which demonstrates that the proposed method is capable of the accurate detection of Pb²⁺ in water samples.

### Table 2. Detection and recovery results of lead in tap water samples by the developed method (n = 5).

| Spiked Samples | Added (ng/mL) | Found (ng/mL) (Graphite Furnace Method) | Found (ng/mL) (This Method) | Spike Recovery (%) |
|----------------|---------------|----------------------------------------|-----------------------------|-------------------|
| 1              | 1             | 1.031 ± 0.024                          | 1.06 ± 0.032                | 106.0             |
| 2              | 10            | 10.41 ± 0.33                           | 10.58 ± 0.25                | 105.8             |
| 3              | 50            | 48.76 ± 1.01                           | 47.63 ± 1.17                | 95.3              |
| 4              | 200           | 208.5 ± 5.57                           | 206.6 ± 4.08                | 103.3             |
3. Materials and Methods

3.1. Chemicals and Apparatus

Graphene, graphene/Fe$_3$O$_4$, and graphene/AuNPs were bought from Jiangsu XFNANO Materials Co. Ltd. (Nanjing, China). Standard solution of Pb$^{2+}$ (1000 µg/mL in 1.0 mol/L nitric acid) was obtained from Aladdin Reagent Company (Shanghai, China). Glutamic acid, sodium acetate, and TMB chromogen reagent were obtained from Sigma-Aldrich (Shanghai, China). CH$_3$OH, CH$_3$CH$_2$OH, C$_2$H$_6$O$_2$, C$_5$H$_8$O$_2$, FeCl$_3$·6H$_2$O, HAuCl$_4$, and other chemicals used without further purification were obtained from Sinopharm Chemical Reagent Ltd. (Shanghai, China). Ultrapure water used throughout this work was purified by a Milli-Q Integral water system. The aptamer and complementary strand were synthesized according to Li et al.’s report [48] by Sangon Biotech Co. Ltd. (Shanghai, China) and the sequences were as follows:

Biotin labeled Pb$^{2+}$ aptamer sequence: 5′-biotin-GGGTGGGTGGGTGGGT-3′;
Pb$^{2+}$ aptamer complementary strand sequence (cDNA): 3′-CCACCCCTCCACC-5′.

A JEM-2100HR transmission electron microscope (TEM, JEOL Ltd., Japan) was used to observed the size and morphology of the nanocomposites. X-ray diffraction (XRD) was performed on a D8-Avance (Bruker AXS Ltd., Germany) to detect the crystal phase of the composites. The amine magnetic beads were verified by a Nicolet Nexus 470 FTIR ESP spectrometer (Thermo Nicolet Co., USA). The UV–Visible absorption was measured by a UV-1800 spectrophotometer (Shimadzu Co., Japan). The background of Pb$^{2+}$ in real samples was measured by an AA240 atomic adsorption spectrometer (Varian Co., USA). Absorbance values were measured using a Synergy H1 hybrid multi-mode microplate reader (BioTek Instruments Inc., USA).

3.2. Preparation of Graphene/Fe$_3$O$_4$-AuNPs Composites

Based on Yuan et al.’s method [44], 4 mg of graphene oxide, 37.6 mg of glutamic acid, 69 mg of FeCl$_3$·6H$_2$O, and 8.3 mL of HAuCl$_4$ (1%) were added into 7.5 mL ethylene glycol and mixed thoroughly. The pH of the mixture was adjusted to 10 with the addition of NaOH (1 mol/L). After sonication for 2 h at room temperature, the solution was transferred to a Teflon lined autoclave in the next 12 h at 180 °C for the solvothermal reaction. After cooling to room temperature, the fabricated composite was adsorbed to the bottom by magnetic adsorption, the supernatant of the solution was discarded, and then ultrapure water was added and the solution sonicated for 5 min. The product was again adsorbed to the bottom by magnetic adsorption, the supernatant was discarded, and finally followed by repeated washing three times with ultrapure water and ethanol, respectively. Finally, the fabricated graphene/Fe$_3$O$_4$-AuNPs was kept in an oven at 60 °C, then the dried composite was ground in an agate mortar into powders, and characterized by transmission electron microscopy (TEM) and X-ray diffraction (XRD).

3.3. Preparation of Amine Magnetic Beads

Amine magnetic beads were prepared by the one-step solvothermal reaction. Two grams of FeCl$_3$·6H$_2$O and 4 g of sodium acetate were mixed with 60 mL of ethylene glycol, and then 13 g of 1,6-ethylenediamine was added. The mixture was heated and stirred at 50 °C until the color of the solution turned yellow-brown, subsequently, the solution was transferred to a Teflon lined autoclave for 12 h at 198 °C. After cooling to room temperature, the magnetic beads were adsorbed to the bottom of the autoclave by magnetic adsorption, the supernatant was discarded, and then ultrapure water was added and the solution was sonicated for 5 min. The composite was adsorbed to the bottom by magnetic adsorption again, the supernatant was discarded, and followed by repeated washing three times with ultrapure water and ethanol, respectively. The fabricated product was kept in an oven at 50 °C; the dried amine magnetic beads were ground in an agate mortar into powders and characterized by TEM, FTIR, and XRD.
3.4. Streptavidin Coated Amine Magnetic Beads

A total of 5 mg of the fabricated amine magnetic beads were added into 5 mL of Tris-HCl buffer solution (10 mmol/L, pH 7.4) and sonicated for 30 min, and then 200 µL of 25% (v/v) glutaraldehyde was added in the solution and incubated in a shaker in the dark at 37 °C for 3 h. The solution was washed three times by phosphate buffer saline (PBS buffer) and dispersed in 4.5 mL of Tris-HCl buffer. A sample of 0.5 mL of 1 mg/mL streptavidin was added thereto and the solution was placed in a shaker in the dark at 4 °C for 12 h. After the reaction, the magnetic beads were adsorbed to the bottom by magnetic adsorption, the supernatant was discarded, and then Tris-HCl buffer was added and the solution was sonicated for 1 min before the supernatant was discarded again after magnetic adsorption of the product, followed by repeated washing three times with PBS buffer. The streptavidin coated amine magnetic beads were dissolved in 5 mL of Tris-HCl buffer (10 mmol/L, pH 7.4), kept in a refrigerator at 4 °C, and the coating process was verified by ultraviolet spectrophotometry.

3.5. Biotin-Labeled Aptamers Decorated on the Surface of Magnetic Beads

A total of 2.8 mL of the solution of the streptavidin-coated amine magnetic beads and 20 µL of the 10 µmol/L biotin-labeled aptamer were mixed in a 5 mL centrifuge tube and the mixture was incubated in a shaker at 37 °C for 30 min. After the reaction, the product was adsorbed to the bottom by magnetic adsorption, the supernatant was discarded, Tris-HCl buffer was added, the solution was sonicated for 1 min, and the supernatant was discarded after magnetic adsorption of the product, followed by repeated washing three times with PBS buffer. The aptamer modified magnetic beads were dissolved in 5 mL of Tris-HCl buffer (10 mmol/L, pH 7.4) and kept in a refrigerator in the dark at 4 °C.

3.6. Evaluation of the Specific Activity

The specific activity (SA) of graphene/Fe₃O₄/AuNPs, graphene/AuNPs, graphene/Fe₃O₄, and graphene were evaluated according to the protocol by Jiang et al. [49]. The specific activity of the enzyme mimics (units) was evaluated using the Equation as follows:

\[
\alpha = \frac{\Delta A}{\Delta t} \times \frac{V}{m} \times \frac{1}{l \varepsilon}
\]

where \(\alpha\) is the specific activity of the enzyme mimics (U/mg); \(\Delta A/\Delta t\) is the initial rate of change in absorbance (min⁻¹); \(m\) is the amount of enzyme mimics (mg); \(V\) is the volume of reaction solution (µL); \(l\) is the optical path length through the reaction solution (cm); and \(\varepsilon\) is the molar absorption coefficient of TMB (39,000 M⁻¹ cm⁻¹ at 652 nm).

The experiments were performed at 37 °C, where 20 µL of 0.01 mg/mL solution of the four materials were added into 200 µL TMB solution respectively and mixed, and the absorbance value at 652 nm was measured at a 10 s interval within 5 min.

3.7. Detection Procedure of Lead Ion Standards Solution

The lead standard solution was diluted by gradient (1, 5,10, 50, 100, and 300 ng/mL), 500 µL of the diluted lead standard solution, 50 µL of 50 mmol/L complementary strand, and 50 µL of 1 mg/mL aptamer-decorated magnetic beads were mixed and heated at 95 °C for 10 min, and then reacted in a shaker at 37 °C. After the reaction, the magnetic beads were adsorbed to the bottom by magnetic adsorption, and then 100 µL of the supernatant was extracted and 100 µL of 1 mg/mL graphene/Fe₃O₄-AuNPs was added into the system before the solution was reacted in a shaker at 37 °C for 30 min. A total of 100 µL of color reaction solution (containing 1.25 mmol/L of TMB and 2.21 mmol/L of hydrogen peroxide) was added, reacted at room temperature for 5 min, and the absorbance of the solution at 652 nm was measured. All of the measurements were performed in triplicate.
3.8. Detection of Lead Ions in Real Samples

The proposed assay was used to test tap water for verification. The water samples were filtered with a 0.22 µm membrane. One, 10, 50, and 100 ng/mL of Pb²⁺ standard solution were added to the samples and detected by this assay under the optimal sensing conditions and graphite furnace atomic absorption spectrometry. For the atomic absorption spectrometry, microwave digestion was performed in sample pre-treatment, the calibration standards were prepared by serial dilution of lead reference solution, the pyrolysis temperature of 750 °C and atomization temperature of 2300 °C were employed, the absorption wavelength of 283.3 nm was selected, and the sampling volume of 20 µL was used for determination.

4. Conclusions

In summary, a colorimetric aptamer based method for the sensitive and selective detection of Pb²⁺ was developed and validated. In this system, Fe₃O₄NPs and AuNPs were grown on the graphene to form graphene/Fe₃O₄-AuNP composites, which served as an enzyme mimetic of peroxidase to catalyze a color reaction. The aptamer of Pb²⁺ was anchored on the amine magnetic beads. In the presence of lead ions, the complementary strands of the Pb²⁺ aptamer were adsorbed onto the surface of the nanozyme composites, inhibiting the catalytic activity and the color reaction. The concentration of target lead ions was inversely proportional to the color of the solution and A₆₅₂ value. Pb²⁺ was detected in the range of 1–300 ng/mL with a low detection limit of 0.63 ng/mL. The results show the potential of this method for practical application in the detection of Pb²⁺, especially in on-site analysis.

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