Rapid Enrichment and Sensitive Detection of Multiple Metal Ions Enabled by Macroporous Graphene Foam

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ABSTRACT: Nanomaterials have shown great promise in advancing biomedical and environmental analysis because of the unique properties originated from their ultrafine dimensions. In general, nanomaterials are separately applied to either enhance detection by producing strong signals upon target recognition or to specifically extract analytes taking advantage of their high specific surface area. Herein, we report a dual-functional nanomaterial-based platform that can simultaneously enrich and enable sensitive detection of multiple metal ions. The macroporous graphene foam (GF) we prepared displays abundant phosphate groups on the surface and can extract divalent metal ions via metal-phosphate coordination. The enriched metal ions then activate the metal-responsive DNazymes and produce the fluorescently labeled single-stranded DNAs that are adsorbed and quenched by the GF. The resultant fluorescence reduction can be used for metal quantitation. The present work demonstrated duplexed detection of Pb²⁺ and Cu²⁺ using the Pb- and Cu-responsive DNazymes, achieving a low detection limit of 50 pM and 0.6 nM, respectively. Successful quantitation of Pb²⁺ and Cu²⁺ in human serum and river water were achieved with high metal recovery. Since the phosphate-decorated GF can enrich diverse types of divalent metal cations, this dual-functional GF-DNazyme platform can serve as a simple and cost-effective tool for rapid and accurate metal quantification in determination of human metal exposure and inspection of environmental contamination.

Heavy metals such as copper and lead are continuously released to our environment through industrial and human activities like gasoline processing, electronic waste disposal, fertilizer usage, et cetera. ¹—³ They are difficult to degrade and make their ways to plants and living organisms, imposing a persistent risk to our ecosystems. Metal pollution in the environment also represents a great threat to human beings, because it could cause severe health issues like memory loss, blindness and deafness, kidney damage, cancers, et cetera. ³—⁶ In particular, childhood exposure to lead can damage learning and recognition capabilities for the entire lifetime, and copper can induce the pathogenesis of hepatic disorder, neurodegenerative changes, and other disease conditions.⁷—⁹ Thus, it is of paramount importance to constantly survey heavy metal contents in environmental samples as well as in clinical specimens for pollution reduction and human exposure prevention.

With the acute toxicity of single heavy metals well documented, safety guidelines and regulations are established for individual metals in water, sediment, or other environmental subjects. However, little progress has been made to evaluate the impact of metal mixtures in the environment.¹⁰—¹² Metals in the mixture would compete or share binding sites to biological receptors, leading to different toxicity and uptake behaviors than single metals.¹³,¹⁴ The high complexity of the metal mixtures found in the environment and its potentially enhanced danger to the ecosystem and human health call for simple survey techniques that can detect multiple metals selectively and sensitively in a fast and high-throughput manner.

Detection of metal mixtures in complex biological or environmental samples demands higher sensitivity and selectivity compared to single metal detection. Electrochemical sensors have been developed for measurement of heavy metals, but with poor discrimination capability and low sensitivity.¹⁵—¹⁸ Mass spectrometric and optical spectroscopic methods are still the main approaches for assessment of metal mixtures in the environment, which include flame atomic absorption spectroscopy (FAAS), electrothermal atomic absorption spectroscopy (ETAAS), inductively coupled plasma optical emission spectroscopy (ICP-OES), and ICP-mass spectrometry (MS). While such instrumental analyses permit very sensitive and simultaneous detection of a large numbers of metals, they are...
expensive, take up a lot of space, and require well-trained scientists to operate, making it difficult for on-site and real-time detection. New methods for detection of metal mixtures are desired for a field survey of environmental contamination and point-of-care applications.

Our approach to overcome the aforementioned problems is to combine the high selectivity of the metal-responsive DNAzymes and the strong absorptivity of nanomaterials in designing sensors for ultrasensitive and multiplexed metal detection. Metal-responsive DNAzymes have been discovered by systematic evolution of ligands by exponential enrichment (SELEX), showing good catalytic ability and binding activity toward many specific metal ions.21–23 Nanomaterials, with judicious design, can provide large specific surface areas and tunable functional groups to facilitate metal ion absorption. They could also possess superior optical property or quenching capability to enable sensitivity and simple fluorescent or colorimetric detection. Herein, we constructed our sensor by combining the macroporous graphene foam (GF) with the Cu- and Pb-specific DNAzymes for simultaneous enrichment and detection of Cu2+ and Pb2+ from aqueous solutions. The GF acts as both an extractor for metal ions and a quencher for the fluorophores that label the DNAzymes (Scheme 1). The dual functionality comes from the phosphate groups on the GF surface that can coordinate with the metal cations for their extraction and the graphene backbone that can bind to single-stranded DNA (ssDNA) strongly and quench the fluorophores attached to the ssDNAs. Once Cu2+ and Pb2+ are enriched on the surface of GF, they can activate the corresponding DNAzymes for simultaneous enrichment and detection of Cu2+ and Pb2+ from aqueous solutions. The GF acts as both an extractor for metal ions and a quencher for the fluorophores that label the DNAzymes (Scheme 1).

Scheme 1. Detection of Metal Ions Based on GF and DNAzymes

Preparation of DNAzymes. Equimolar amounts of the enzyme strand and the Cy3 or FAM labeled substrate strand were added into the reaction buffer (50 mM MgCl2 and 5 mM Tris, pH ~ 8.0) and denatured at 98 °C for 2 min in a water bath. The obtained DNAzymes were stored at 4 °C after being cooled to room temperature. Detection of Metal Ions Based on GF. The procedure for extraction and detection of metal ions based on GF is shown in Scheme 1. For metal ion extraction, 20 μg of GF was added into 1 mL of the metal solution at different metal cation concentrations in 0.5 M of KCl-HCl (pH 1.5). The solution was stirred at room temperature for 30 min to reach maximum adsorption, and then the solution was centrifuged. After removal of the supernatant, the GF was resuspended in MgCl2-Tris buffer (50 mM MgCl2 and 50 mM Tris, pH ~ 8.0, 90 μL), followed by the addition of both DNAzymes, reaching the final concentration of 5 nM. Then, 5 μL of AA (5 mM) was supplied to reduce Cu2+ to Cu+ and incubated for 10 min at room temperature. Subsequently, solution fluorescence was measured as described in the Fluorescence Measurements section. Detection of metal contents in serum and environmental water samples was carried out in the same manner.

EXPERIMENTAL SECTION

Chemicals. The Pb2+-specific DNAzyme (Pb-Sub: 5′/-SCy3/ACT CAC TAT RAGG AGA AGA TG-3′ and Pb-Enz: 5′-CAT CTC TTC TCC GAG CCG GTC GAA ATA GTG AGT-3′) and the Cu2+-specific DNAzyme (Cu-Sub: 5′-TTT TTT TAG CCT TCT TCT AAT ACG GCT TAC C/36-FAM/-3′ and Cu-Enz: 5′-GGT AAG CCT GGG CCT TCT TTT TAA GAA AGA AC-3′) were synthesized and purified by Integrated DNA Technologies, Inc. (IDT; Coralville, IA). Graphene oxide (GO), phytic acid (PA), Tris base, and ascorbic acid (AA) were obtained from Sigma-Aldrich (St. Louis, MO). Cupric nitrate (Cu(NO3)2, 99%), lead acetate (Pb(CH2COOH)2 ≥ 98.0%), manganese nitrate (Mn(NO3)2, 99%), magnesium nitrate (Mg(NO3)2, 99%), cadmium nitrate (Cd(NO3)2, 99%), nickel nitrate (Ni(NO3)2, 99%), cobalt nitrate (Co(NO3)2, 99%), ferric nitrate (Fe(NO3)3, >98.0%), zinc nitrate (Zn(NO3)2, 99%), magnesium chloride (MgCl2, 99%), potassium chloride (KCl, 99%), and nitric acid (HNO3) were purchased from Fisher Scientific (Waltham, MA). All the reagents were used as received without further purification. All experiments and measurements were carried out at room temperature unless otherwise stated. Deionized water (18.4 MΩ) used for all experiments was obtained from a Milli-Q system (Millipore, Bedford, MA).

Synthesis and Characterization of GF. Graphene foam (GF) was prepared by using phytic acid as the gelator and dopant, and graphene oxide (GO) was employed as the precursor, as reported by Chen et al.24 Briefly, 0.5 mL of PA was added into 15 mL of GO (2 mg/mL, aqueous solution) and sonicated for 40 min at room temperature. Then, the mixture was sealed in a 25 mL Teflon-lined autoclave tube and maintained at 180 °C for 12 h. Subsequently, the solid precipitate formed from the reaction was collected by tweezer after the autoclave tube was naturally cooled to room temperature. The product was washed by ethanol and water and then freeze-dried for 24 h to obtain the desired final product, GF.

Transmission electron microscopy (TEM) images were directly taken with a JEOl 2011 microscope operated at 200 kV (JEOl, Tokyo, Japan). Samples were suspended in ethanol and spotted on a carbon-coated copper grid. The infrared spectra were obtained by using a FTIR 360 manufactured by Nicolet (Thermofisher, USA). X-ray photoelectron spectroscopy (XPS) data were collected with an X-ray photoelectron spectrometer (PerkinElmer PHI 5000C ESCA System) equipped with Mg Kα radiation. Raman spectra were taken by a Labram-1B Raman spectrometer from Yobin Yvon with a laser (2 mW) excitation wavelength of 632.8 nm.

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ICP-AES Analysis. The Optima 200DV Inductively Coupled Plasma–Atomic Emission Spectrometer (ICP-AES; PerkinElmer, Norwalk, CT) was employed to verify the quantities of all metals recovered from the standard metal solutions and the unknown samples. The samples were acidified with 10% HNO3 before analysis. The instrument was run thoroughly with 10% HNO3 before injection to prevent memory effects. The argon source (>99%) was set at 90 psi. The data were acquired using the ICP Expert II software. A blank was run at the beginning of each measurement to establish the baseline level. Then, standard solutions and unknown samples were measured in triplicate. A standard curve was generated to determine the concentration of the unknown samples.

Fluorescence Measurements. Fluorescence measurements were conducted on a QM400 fluorometer (HORIBA, Japan). For detection of Pb2+ with the Cy3 labeled Pb-specific DNAzyme, the excitation and emission wavelengths (λEx and λEm) were set at 535 and 540–600 nm, respectively. Copper detection was performed with λEx/Ex at 496 nm/500–600 nm, which detected the FAM label on the Cu-specific DNAzyme. The slit width for both excitation and emission was set at 5 nm. A 100 μL sample was added to the cuvette, and the fluorescence spectra were scanned. The cuvette was washed with pure water three times and dried under N2 after each sample.

RESULTS AND DISCUSSION

Characterization of GF. GF was prepared from GO by a thermal annealing approach using phytic acid (PA) as the gelator and dopant.24 PA not only reduced the GO sheets and structural stability but also introduced many phosphate groups into the GF structure. The GO and GF exhibit the C=O bond stretch at 1615 and 1340 cm⁻¹ when examined by FT-IR. The spectrum for GF also contains the distinct transmittance peaks at 1161, 1057, and 886 cm⁻¹, which can be ascribed to the stretching vibrations of P=O, P−O−C (phosphate ester group), P−O, and P−O−H, respectively. The peak at 510 cm⁻¹ can be assigned to the deformation vibration of PO4. The Raman spectra of both GO and GF (Figure 2b) illustrate the typical G band at about 1580 cm⁻¹ and the D band at about 1340 cm⁻¹ for both GO and GF. The ratio of the intensities of the D and G bands (ID/IG) can be utilized to judge the degree of structural disorder and defects. The relatively large amounts of phosphate groups originating from PA reduce the relative number of the six-membered aromatic rings and thus increase the degree of structural disorder: the ratio of ID/IG was enlarged by 10% compared to that of GO.

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Figure 1. (a) SEM image, (b) TEM image, (c) XPS pattern, and (d) elemental analysis of GF.

Figure 2. (a) FT-IR and (b) Raman spectrum of GF.
Cu²⁺, on the GF in the optimal buffer. As shown in Figure 3a, more than 90% of the added metal ions were enriched by GF within 30 min. In particular, Pb²⁺ and Cu²⁺ exhibited the fastest adsorption rates, reaching adsorption maxima of 92% and 96% within 120 min. The adsorption capacity was also examined at the extended incubation period of 120 min. Most of the metals, including Cu²⁺, can reach a maximum capacity, q_max, of 15–50 mg of metal per gram of GF (46.3 ± 0.9, 35.7 ± 1.8, 24.1 ± 3.3, 21.5 ± 0.5, 13.1 ± 2.5, 29.1 ± 4.3, and 27.8 ± 2.6 mg/g GF for Cd²⁺, Zn²⁺, Mn²⁺, Pb²⁺, Co²⁺, Mg²⁺, Ni²⁺, and Cu²⁺, respectively), but the q_max of Pb²⁺ was 97.4 ± 0.6 mg/g GF, more than 3 times higher than the others. For both Pb²⁺ and Cu²⁺, we tested the recoveries at various metal concentrations. We found that even with lower than 1 mg/L of the metal ion, at which concentration the adsorption efficiency would be limited by the concentration-driven diffusion to the surface of GF, the recovery was more than 80% (Figure S2, Supporting Information). All of the above results confirm that GF can rapidly capture and concentrate trace metal ions. The enrichment should benefit sensitive detection of trace metals in samples.

**Construction of the GF Sensor for the Detection of Metal Ions.** Besides its large specific area functionalized with groups beneficial for metal enrichment, GF contains the graphene structure that can help with in situ detection of the enriched ions. It has been well studied that single-stranded DNA (ssDNA) can bind strongly to graphene via π−π stacking between the bases on nucleotides and the sp²-hybridized carbon atoms in the extended π conjugation on graphene. In particular, guanine (G) shows enhanced binding via the NH−π interaction, supported by both computational simulation and experimental measurement.²³,²⁶ As shown in Figure S3 (Supporting Information), DNA can be adsorbed by GF within 10 min, with the maximum capacity reaching 11.6 mg of DNA (g GF)⁻¹. In addition, the planar carbon π system on the graphitic domain can establish long-range resonance energy transfer with the adsorbed dye molecules, quenching a wide range of fluorophores with high efficiency.²⁷,²⁸ On the basis of these features, we designed our metal sensor by coupling the porous GF with the fluorescently labeled, metal-responsive DNAzymes: the enriched metals on the GF surface can specifically cleave the substrate of the DNAzyme, and the cleaved product would be subsequently adsorbed and quenched by the GF.

Diverse DNAzymes have been reported for metal sensing.²⁹−₃₁ Thus, the two DNAzymes specific for Pb²⁺ and Cu²⁺ were chosen, and the substrate strands were labeled with Cy3 and FAM, respectively. The fluorophores were not quenched by GF when the DNAzymes were intact, owing to the double-stranded regions formed between the substrate and enzyme strands. Once the DNAzymes were mixed with the Pb²⁺ or Cu²⁺ enriched by GF, the substrate strand was cleaved, the released ssDNA was adsorbed, and the fluorophore was quenched by GF. As shown in Figure S4 (Supporting Information), the 100 nM Cy3-labeled ssDNA product resulting from the Pb²⁺-induced substrate cleavage could be quenched completely by 20 μg/mL of GF, while the fluorescence of the intact DNAzyme was not affected by the presence of GF. TEM was used to examine the GF before and after metal enrichment and DNAzyme cleavage and revealed no difference on the GF (Figure S5, Supporting Information). The presence of GF did not affect the cleavage efficiency, as proved by using gel electrophoresis to monitor product generation with or without GF (Figure S5c).

Since the salt content, concentration, and pH value of the reaction buffer could influence the structure stability of the DNAzymes and interaction between DNA and GF, we compared the quenching efficiency of GF in three kinds of common buffers (50 mM NaCl in 50 mM phosphate buffer at pH 7.4; 50 mM MgCl₂ in 50 mM Tris buffer at pH 8.0; and 50 mM NaN₃ in 50 mM Tris-acetate at pH 7.8). The quenching efficiency was defined as (F₀ − F)/F₀, where F and F₀ are the fluorescence intensities of the DNA solutions with and without the presence of the nanomaterial, respectively. As shown in Figure S6 (Supporting Information), these buffers showed similar quenching efficiency with GF. We chose the MgCl₂-Tris buffer because it exhibited better quenching stability in repeated measurements. We also compared the quenching capability of GF with other common graphene materials: graphene (G), graphene oxide (GO), and the hydrophobic macroporous graphene foam (MGF), in this buffer (Figure S7, Supporting Information). The signals were measured at 30 min after the metal ion, nanomaterials, and the DNAzyme were mixed. While fixing the concentrations of the DNAzyme and the metal ions in the mixture, the quenching efficiency increased linearly with the increase of GF concentration until reaching a plateau (larger than 90%) at around 40 μg/mL. On the contrary, the other materials showed similar trends but with much slower rates of increase, and no plateau was attained even with 200 μg/mL of the material used. The higher quenching efficiency exhibited by GF compared to the other graphene-based materials could be attributed: (I) the inherent aromatic structure and amphiphilic property of GF and (II) the increased structural disorder and defects of GF. The former feature facilitates highly efficient adsorption of ssDNA, and the latter benefits long-range energy transfer and results in an enhanced quench of fluorescence.³²−³⁴ Moreover, the adsorption event occurred very rapidly: within 5 min, the quenching efficiency of GF for ssDNA reached a maximum value of 98% (Figure S8, Supporting Information). The high quenching efficiency can help improve the signal-to-noise ratio of our sensing method, and prompt adsorption of the cleaved product can ensure fast detection upon metal enrichment, allowing us to perform a sensitive and quick survey of these two toxic metals in samples of interest.

**Performance of the GF Sensor in the Detection of Metal Ions.** The performance of our GF-based metal sensor was examined. Figure 4a,c shows the fluorescence spectra of the sensing system upon enriching Pb²⁺ and Cu²⁺ from the 1 mL solution at various concentrations using the GF, followed with detection in the 100-μL DNAzyme solution. The fluorescence...
intensity decreased dramatically as the concentrations of Pb\(^{2+}\) increased. The limit of detection (LOD) was calculated to be 50 pM and 0.6 nM for Pb\(^{2+}\) and Cu\(^{2+}\), respectively, using the 3\(\sigma\) method. These LODs are much lower than most of the previously reported approaches for Pb\(^{2+}\) and Cu\(^{2+}\) detection, as shown in Table 1, which compares the LODs of various techniques for Pb\(^{2+}\) and Cu\(^{2+}\) detection. The high sensitivity of our sensing system can be attributed to both the excellent metal enrichment capability of GF and its high quenching efficiency over the fluorescently labeled ssDNA. Furthermore, we evaluated the impact from Cu\(^{2+}\) to detection of Pb\(^{2+}\), and vice versa. The fluorescence intensity change of the Pb-specific DNAzyme caused by incubation with the GF enriching 50 nM Pb\(^{2+}\) did not vary with the presence of Cu\(^{2+}\) ranging from 1 \(\times\) 10\(^{-12}\) M to 1 \(\times\) 10\(^{-4}\) M. Similarly, the coexistence of 1 \(\times\) 10\(^{-12}\) M to 1 \(\times\) 10\(^{-4}\) M Pb\(^{2+}\) did not affect the signal from 0.6 nM Cu\(^{2+}\) (Figure S9, Supporting Information).

We further tested whether other divalent metals could affect selective detection of Pb\(^{2+}\) and Cu\(^{2+}\). The fluorescence response was monitored when the sensing system was challenged by the presence of other metal ions, including Mn\(^{2+}\), Mg\(^{2+}\), Cd\(^{2+}\), Ni\(^{2+}\), Co\(^{2+}\), Fe\(^{2+}\), and Zn\(^{2+}\). As shown in Figure 5, the GF sensor yielded much more quenching with 10 nM Pb\(^{2+}\) or Cu\(^{2+}\), compared to that obtained with other metal ions at 100-fold higher concentrations. The excellent selectivity is originated from the high specificity of each DNAzyme to its target cation, as well as from the good capability of GF in differentiating ss- and ds-DNA.

**Application of the GF Sensor.** The above results indicate the potential of our GF-DNAzyme sensing platform in the extraction and detection of Pb\(^{2+}\) and Cu\(^{2+}\) present in complex biological and environmental systems. Most of the metal ion exposure can be measured by testing ion concentration in serum.\(^4^4\),\(^4^5\) To demonstrate this, we spiked Pb\(^{2+}\) and Cu\(^{2+}\) into the human serum obtained from Sigma and detected their contents using our sensor. Each metal was spiked at two concentrations: 0.1 and 1.0 nM for Pb\(^{2+}\) and 1.0 and 10.0 nM for Cu\(^{2+}\). The metal concentration obtained with our sensing method was divided by the actual spiked concentration to achieve the recovery, which was presented in Table 2. The recoveries for both metal ions at the two concentrations tested were more than 95 \(\pm\) 3%. Switching serum with plasma, low concentrations of Pb\(^{2+}\) and Cu\(^{2+}\) were still determined successfully with excellent recoveries using the developed approach (Table S2, Supporting Information).

Environmental water is another type of sample that could be subjected to a survey of heavy metal contamination (Table S1, Supporting Information). We collected some water samples from the Santa Ana River at \(\sim\)100 m downstream from the wastewater treatment plant for the City of Riverside. The river sample did not contain a detectable level of Pb\(^{2+}\) or Cu\(^{2+}\), indicating no heavy metal contamination in the discharged water from the treatment plant. If spiked with these two metal ions, spiked metal ions were added to the river sample. The resulting recoveries are presented in Table S2. The spiked metal ions were not detected in the river samples, indicating no heavy metal contamination in the discharged water from the treatment plant.
In summary, we present a simple method for quick, sensitive, and selective detection of metal ions using the macroporous GF. Taking advantage of the abundant phosphate groups on its surface, its high specificity, and the unique DNA adsorption and fluorophore quenching properties, the macroporous GF enables both metal ion extraction and fluorescence-based detection. The enrichment and detection can be completed within 1 h, and multiple metals can be enriched simultaneously, with detection limits for specific metals such as Pb²⁺ and Cu²⁺ reaching the low nanomolar or even picomolar range. The sensor is also tolerant to complex sample matrices, as demonstrated by metal quantification in serum, plasma, and environmental water, eliminating the need of sample pretreatment. With the availability of numerous DNAzymes specifically targeting different metals and fluorophores detectable at various wavelengths, our method can be expanded for multiplexed detection of several metal ions for quick and easy assessment of metal contents in environmental samples and medical specimens. It will be valuable for an on-site survey of heavy metal contamination and for the diagnosis of metal exposure in patients.

**ASSOCIATED CONTENT**

* Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.7b03336.

Adsorbed amount of metal ions and DNA by GF under different conditions, recoveries of Pb²⁺ and Cu²⁺ at low concentrations after being enriched by GF, comparison of fluorescence spectra of Cy3-ssDNA and Cy3-dsDNA with and without GF, influence of different buffers on the quenching efficiency of GF, fluorescence quenching efficiency by different material, and determination of Cu²⁺ and Pb²⁺ in environmental water and plasma sample (PDF)

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**Notes**

The authors declare no competing financial interest.

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