Supplementary Information

Graphene oxide-based electrochemical sensor: A platform for ultrasensitive detection of heavy metal ions

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Experimental

Reagent

Graphite powder (99.5%) was purchased from Acros Organics. Potassium sulfuric 95-97%), permanganate $(KMnO_4),$ acid $(H_2SO_4,$ L-cysteine, 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), N-hydroxysuccinimide (NHS), ammonium acetate, potassium hexacyanoferrate (III), lead (II) acetate trihydrate, copper (II) chloride dehydrate, zinc perchlorate hexahydrate, cadmium perchlorate hydrate, iron (III) chloride anhydrous, potassium dichromate were purchased from Sigma-Aldrich. Mercuric acetate, nickel (II) sulphate hexahydrate, hydrogen peroxide (H₂O₂, 30%) were purchased from AccuChem Chemicals and nitric acid (HNO₃, 69%) from International Laboratory. All the materials were used as received without further purification.

All glassware was rinsed with 6 M HNO₃, and then thoroughly with Milli-Q water to remove contamination of metal ions.

Purification of graphite powder

Graphite powder was suspended in H_2SO_4/HNO_3 (3:1 by volume) for 24 h followed by dilution with Milli-Q water, filtration and washing with copious amount of Milli-Q water to remove impurities metals and volatile organic compunds. After drying at ambient, the graphite powder was calcined at 800 °C for 1 h to remove non-graphitic (which cannot be oxidized by Hummers' method) and low quality carbon content. The purified graphite is stored for later use.

Preparation of graphene oxide (GO)

Graphene oxide was prepared following modified Hummers' method. The purified

graphite powder (2 g) was suspended in a mixture of H_2SO_4 (46 mL) and HNO₃ (0.9 mL) in three-neck round bottom flask placed in ice bath. KMnO₄ (6 g) was slowly added into the flask with continuous stirring to keep the temperature below 10 °C. Afterwards, the ice bath was removed and the suspension was heated to 37 °C for 0.5 h. The mixture was diluted with Milli-Q water (92 mL) and heated to 98 °C and maintained for 15 min. The reaction was stopped by adding distilled water, and a further 30% H_2O_2 (45 mL) was added during which the color of suspension changed from dark brown to light yellow. The resulting GO suspension was centrifuged and washed thoroughly with warm water and dried in air. Colloidal suspension of GO (1 g L^{-1}) was prepared by dispersing dried GO flakes in Milli-Q water via sonication. The stock suspension can be stable for several months.

For the preparation of reduced graphene oxide (rGO), the colloidal GO colloidal suspension (1 g L^{-1} , 100mL) was adjusted to pH 9~10 using dropwise addition of 0.1 M NaOH. NaBH₄ (800 mg) was added directly into the vigorously stirred GO suspension, and held at 80 °C for 1 h. The final black rGO product was separated by filtration, washed with DI water, and dried in vacuum desiccator.

Stepwise modification of gold electrode

Gold electrodes (2 mm diameter, CH Instruments) were polished successively with 1.0, 0.3 and 0.05 um alumina slurries made from dry alumina and water on microcloth pads for 3 minutes each to obtain mirror-like surface. Trace alumina was removed from the surface by rinsing with Milli-Q water and brief sonication cleaning in ethanol and then water. The electrode was cleaned lectrochemically in 0.05 M H_2SO_4 by cyclic voltammetry (100 mV s⁻¹) between -0.3 and 1.5 V until a reproducible voltammogram was obtained. The cleaned electrode was rinsed with Milli-Q water and dried under N_2 stream.

The cleaned gold electrode was incubated in 0.1 M L-cysteine (in 75% ethanol, 25% water) for 4 h and followed by rinsing with absolute ethanol. As shown in Figure S1, the concentration of L-cysteine was optimized based on the sensing peak current of Pb²⁺ over the final GO-modified gold electrode. Activation of the carboxylic acid group of GO was carried out by addition of EDC (20 mM) and NHS (4 mM) into GO colloidal suspension (0.1 g/L, pH ~7), and stirred for 1 h. The L-cysteine modified gold electrode surface was covered by the activated GO suspension in an inverted Eppendorf® tube, sealed and incubated at 4 °C for 18 h. The resultant GO-modified gold electrode was rinsed with ammonium acetate buffer and dried under N₂ stream prior to use.



Figure S1. Optimization of the concentration of L-cysteine during the preparation of GO-modified gold electrode. The amount of surface functionalized L-cysteine determines the amount of GO that can be attached on the gold electrode, and further its sensing current, hereby shown with the SWV peak current of 25 ppb Pb^{2+} .

Physicochemical characterization

The morphology of the polished pristine gold surface and that after modification with

GO was observed with a scanning electron microscope (SEM, JEOL, JSM 6300). While the bare gold surface (Figure S2A) shows relatively smooth surface, high surface coverage of flaky GO can be evident (Figure S2B). X-ray photoelectron spectroscopy (XPS) was measured on Physical Electronics PHI-5802 using Al K α as the excitation source. The trace metal ions analyses for the construction of Langmuir adsorption isotherms and adsorption interference was obtained by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) carried out on Perkin-Elmer Optima 5300DV-ICP.



Figure S2. SEM images of (A) polished bare gold surface and that of (B) after attachment of GO flakes through the EDC/NHS coupling strategy.

Electrochemical measurement

All electrochemical measurements were performed in a conventional three-electrode consisting of the modified gold surface working electrode, platinum auxiliary electrode and Ag/AgCl (3.0 M KCl, CH Instruments) reference electrode. Cyclic voltammetry (CV), square wave voltammetry (SWV) and electrochemical impedence spectroscopy (EIS) were measured on CHI660D (CH Instruments). CV was conducted at scan rate of 100 mV s⁻¹. In SWV the pulse amplitude is 25 mV with

a step of 4 mV and frequency of 25 Hz. All measurements were performed in deaerated 0.05 M ammonium acetate buffer (pH 7.0) containing 50 mM KCl.



Figure S3. CV of bare, L-cysteine and GO-modified gold electrodes towards ferricyanide in 0.05 M KCl and 0.05 M NH₄Ac buffer solution (pH 7.0). Note the reduced intensity and separated redox peaks of GO-modified gold electrodes relative to bare gold electrode due to the hindrance of direct access of ferricyanide to the electrode surface.



Figure S4. Nyquist plots of bare, L-cysteine and GO-modified gold electrodes towards ferricyanide in 0.05 M KCl and 0.05 M NH₄Ac buffer solution (pH 7.0). Also

shown is the magnified image with fitting at high frequency (upper inset), modeled using the equivalent circuit (bottom inset) consisting of solution phase resistance (R_s), charge-transfer resistance (R_{ct}), double-layer capacitance (C_d), and Warburg impedance (Z_w).

Electrochemical impedence spectroscopy (EIS) characterization of the bare, L-cysteine and GO-modified gold electrodes by are shown in Figure S4, using ferricyanide as the redox probe dissolved in 0.05 M KCl and 0.05 M NH₄Ac buffer solution (pH 7.0). The equivalent circuit is shown in the bottom inset of Figure S4. Minimum electron transfer resistance (R_{ct}) was measured for the bare gold electrode given the high substrate conductivity and the direct interfacial charge transfer between ferricynanide probe and the gold surface. A small high frequency semicircle is evident upon modification with L-cysteine, which can be described by with R_{ct} value of 950 Ω . The R_{ct} increases to 1360 Ω after GO attachment due to the enhanced resistance for electron transfer from GO to gold substrate through L-cysteine. The results are consistent with the CV responses.



Figure S5. CV (left) and SWV (right) of GO-modified gold electrode before (black line) and after 10 min accumulation (red line) in 50 ppb Pb^{2+} , 25 ppb Cu^{2+} and 10 ppb Hg^{2+} in 0.05 M NH₄Ac buffer solution (pH 7.0) and 0.05 M KCl.



Figure S6. Optimization of the accumulation time of analyte ions $(Pb^{2+}, Cu^{2+} \text{ and } Hg^{2+})$ on the GO-modified gold electrodes, and their effects on the SWV currents measured in 0.05 M NH₄Ac buffer solution (pH 7.0) and 0.05 M KCl. Optimum accumulation time of 10 min was measured for all analytes.



Figure S7. Calibration plot of SWV peak current against metal ion concentrations over L-cysteine modified gold electrode for (a) Pb^{2+} , (b) Cu^{2+} and (c) Hg^{2+} . Fitted red line shows the region of linear response.



Figure S8. Stripping peak current of (a) the minimum detection limits of Pb^{2+} (0.4 ppb), Cu^{2+} (1.2 ppb) and Hg^{2+} (0.8 ppb), and (b) the maximum limit of linear range detection of Pb^{2+} (51.2 ppb), Cu^{2+} (200 ppb) and Hg^{2+} (12.8 ppb) using GO-modified gold electrodes fabricated independently on five different occasions. The relative standard deviations for minimum detection limits are 4.1% for Pb^{2+} , 2.2% for Cu^{2+} and 2.6% for Hg^{2+} ; and for the maximum linear range detection limits are 4.6% for Pb^{2+} , 5.3% for Cu^{2+} and 8.5% for Hg^{2+}



Figure S9. SWV of Pb²⁺ in the absence (zero concentration, black line), 100 ppb (low concentration, red line) and 1000 ppm (high concentration, blue line) of interfering ions: (a) Zn^{2+} , (b) Fe^{3+} , (c) Ni^{2+} , (d) Cu^{2+} , (e) Hg^{2+} , (f) Cd^{2+} and (g) $Cr_2O_7^{2-}$.



Figure S10. Stripping peak current of 50 ppb Pb^{2+} , 25 ppb Cu^{2+} and 10 ppb Hg^{2+} using electrodes fabricated independently on five different occasions. Note the high repeatability of the measurements, with relative standard deviations of 2.1% for Pb^{2+} , 3.8% for Cu^{2+} and 3.3% for Hg^{2+}