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Rational Design of Magnetic Micro-nanoelectrodes for Recognition and Ultrasensitive Quantification of Cysteine Enantiomers

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Abstract

Driven by the urgent need for recognition and quantification of trace amino acids enantiomers in various biologic samples, we demonstrate for the first time an ultrasensitive electrochemical chiral biosensor for cysteine (Cys) based on magnetic nanoparticles ($\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$) as electrode units. D-Cys- Cu^{2+} -D-Cys formed in the presence of cysteine, exhibits strong stability and a shielding effect on the redox current of indicator Cu^{2+} , which can be used to quantify and recognize D-Cys by square wave voltammetry. Simultaneous detection of D-Cys and homocysteine (Hcy) is achieved in the presence of other amino acids, demonstrating an excellent selectivity of the sensor. Moreover, aided by the enrichment treatment effect of magnetic micro-nanoelectrodes, an ultra-high sensitivity up to $102 \mu\text{A} \mu\text{M}^{-1} \text{cm}^{-2}$ was achieved, the detection limit is reduced to picomolar level (83 pM) for D-Cys and can be used for the recognition of cysteine enantiomers. The proposed method has been verified by real sample analysis with satisfactory results. The results highlight the feasibility of our proposed strategy for magnetic micro-nanoelectrode sensor, electrochemical recognition and quantification of D-Cys, which can be more broadly applicable than that with traditional electrode structures and further advance the field of electrochemical sensors.

Introduction

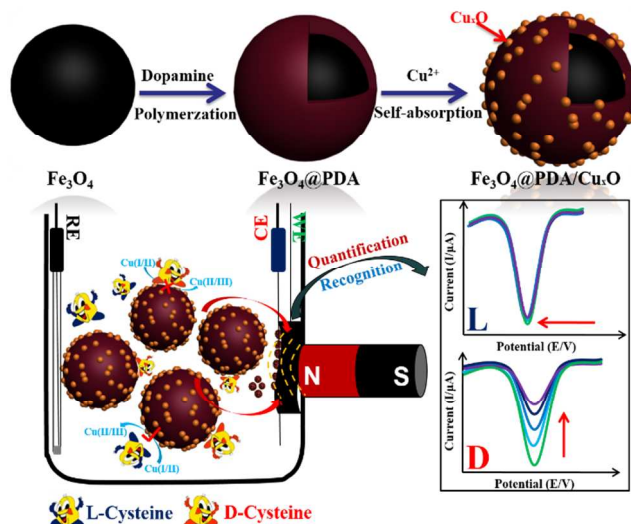
Amino acids, the most predominant chiral compounds in nature, are the basic building block of proteins. L-amino acids are almost exclusively present in higher animals, while the corresponding D enantiomers would only be utilized by microorganisms and bacteria. However, this view has recently been revisited, since substantial amounts of D-amino acids have been detected in mammals and humans.¹ Indeed, D- and L-amino acids often coexist in mammalian and human tissue, and are used to synthesize proteins or play an important role in the nervous system.²⁻⁴ Accordingly, this raises the important question concerning the analysis of amino acid enantiomers, as the role of the L- and D-amino acids enantiomers is not the same, one being beneficial to the organisms and another might be ineffective or even cause serious side effects.^{5,6} For example, the biological function of the D-amino acids are often closely related to a series of neurological diseases, such as schizophrenia, Alzheimer's, Parkinson's and other neurodegenerative or psychiatric disorders.^{4,7} Hence, the ability to quantify and recognize the enantiomers of amino acids with portable sensors is considered very promising for the diagnosis, and treatment of diseases.⁸ Cysteine (Cys) is a typical representative of this series of amino acid enantiomers. Efforts have recently been deployed for the quantification and recognition of cysteine to accurately report the total amount at a micromolar concentration level in the presence of other amino acids. Unfortunately, the techniques employed in these studies share some common drawbacks, such as lacking robustness, being time-consuming, requiring expensive equipment and tedious sample preparation.⁹⁻¹⁴ Electrochemical detection of L-Cys was greatly improved in recent years with simpler methods of high sensitivity.¹⁵⁻¹⁹ However, the rapid and portable identification and quantification of D-Cys is still limited.

Specifically, cysteine is a thiol-containing amino acid and can bind with many metal ions, such as Cu^{2+} , Ag^+ , Au^{3+} , Cd^{3+} and Pb^{2+} , via the strong interaction with its thiol group. More interestingly, the on-rate of D-type and L-type cysteine to the metal ions is evenly matched. However, the stability of the binding is widely different between the enantiomers, D-type being more stable (slower off-rate) than the L-type. This assumption has been confirmed experimentally and theoretically by Zhou *et al.*²⁰ Inspired by these reports, we strongly want to use this unique difference to develop a highly sensitive electrochemical chiral sensor. We report here that the binding of copper ions to cysteine enantiomers can significantly affect the

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3 electrochemical signal. Thereby, copper ions was used as an indicator with different response to
4 chirality of Cys.
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8 Magnetic composite materials have been intensively explored for various technological
9 applications.²¹⁻²³ These functional magnetic nanoparticles have the benefits of high dispersibility
10 and surface exposed active sites or functional groups, which are particularly important in
11 applications such as organic contaminant removal and colorimetric sensing among others.²⁴⁻²⁶
12 This simple mechanism is particularly useful, especially when a single, functionalized magnetic
13 nanoparticle electrode unit is used as an electrochemical sensor. The choice of surface functional
14 material is another crucial parameter. Dopamine (DA) can self-polymerize under alkaline
15 conditions to generate polydopamine (PDA), which is a mimic of the specialized adhesive foot
16 protein that can modify almost all material surfaces.²⁷ In addition, the functional groups (N-H, -
17 OH, C-O, and C-N) of PDA and its reduction ability endow PDA film with the ability to absorb
18 and reduce metal ions.²⁸ More importantly, cysteine can be covalently attached to the PDA-
19 coated sheet surface by thiol through Michael addition reaction.²⁹ Based on this, the PDA-coated
20 magnetic micro-nanoelectrodes can be dispersed in solution to enrich the cysteine enantiomers,
21 which then provides the conditions for the development of highly sensitive chiral sensors.
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33 Herein, an electrochemical chiral sensing strategy based on magnetic micro-nanoelectrodes
34 is proposed for highly sensitive and selective recognition and quantification of cysteine
35 enantiomers employing Cu^{2+} modified $\text{Fe}_3\text{O}_4@\text{PDA}$.^{30,31} In addition, the inhibition of oxidation
36 peak current of Cu^{2+} and D-Cys complex as well as the difference current changes between D-
37 and L-Cys are designed to quantify and recognize D-Cys. The proposed strategy was able to
38 measure D-Cys reaching a picomolar level and has satisfactory selectivity for typically
39 interferents, such as other amino acids and homocysteine (Hcy).
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Scheme 1. Schematic illustration of the $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ fabrication and the electrochemical responses of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ micro-nano-electrode.

2. Experimental section

Materials and Chemicals Polyethylene glycol, glycol, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, NaAc, tetra-n-butyl titanate, dopamine hydrochloride, tris(hydroxymethyl)aminomethane (Tris), ethanol were purchased from Aladdin (Shanghai, China). Na_2HPO_4 , NaH_2PO_4 and $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ were purchased from Sinopharm Chemistry Reagent Co., Ltd (Shanghai, China). All of the chemicals were analytical grade and used directly without further purification. The deionized water was purified by a Millipore-Q system (Millipore Co., USA) and used for all aqueous solution preparation. As the supporting electrolyte, phosphate buffer (PB, 0.1 M phosphate, pH 5.0) solution was prepared with NaH_2PO_4 and Na_2HPO_4 . A glassy carbon electrode (3 mm diameter) with a removable magnetic core (MGCE), glassy carbon electrode (GCE) (3 mm diameter) and carbon cloth were purchased from Tianjin Incole Union Technology Co., Ltd. (Tianjin, China), and cylindrical magnets (3 mm diameter) were purchased locally. They were used for magnetic micro-nano-electrodes collection and redox reaction signal transmission.

Preparation of Fe_3O_4 , $\text{Fe}_3\text{O}_4@\text{PDA}$ and $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$

The Fe_3O_4 , $\text{Fe}_3\text{O}_4@\text{PDA}$ and $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ were fabricated according to our previously reported work³² and details were provided in supporting information (SI).

Electrochemical sensing

MGCE and GCE were cleaned before further use. Briefly, it was successively polished to a mirror finish with 1 μm , 0.3 μm , and 0.05 μm alumina slurry, then sonicated successively with anhydrous alcohol and double-distilled deionized water. Subsequently, it was dried with nitrogen and was ready for further use.

Standard D-Cys and L-Cys solutions with a series of concentrations were prepared in pure water. The target solutions were blended with $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ (1 mg/mL) by sonication for 30 s at room temperature. Subsequently, MGCE was immersed in these solutions for 2 min. After being rinsed with water, MGCE was electrochemically measured. To simplify the measurement process, we replaced the MGCE with a combination of carbon cloth and a cylindrical magnet.

Square wave voltammetry (SWV), and cyclic voltammetry (CV) experiments were performed with an Electrochemical Analyzer (Model: 120C, S/N: uEA120C10001, Homiangz LLC) at room temperature. The MGCE or carbon cloth that gathered by magnetic micro-nanoelectrodes ($\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$) in the effective area, platinum wire, and Ag/AgCl (KCl saturated) were served as working, counter and reference electrodes, respectively. The SWV and CV measurements were conducted in a PB solution (0.1 M, pH 5.0), and the voltage scanned from -1.0 to 1.0 V at a rate of 100 mV s^{-1} with a pulse width of 50 ms.

3. Results and discussion

Characterization of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$

The signal accumulation process of micro-nanoelectrodes ($\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$) for Cys chiral sensor system were provided in Scheme 1. The structure of the $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ sample was observed with SEM and TEM. As shown in Figure 1a-f, the Fe_3O_4 was coated with polydopamine (PDA) layer, approximately between 10 and 50 nm, which could be used as a precursor for a variety of multi-functional magnetic electrodes. This was mainly due to the large number of active groups on the PDA layer for functionalization, such as copper ions, that could be adsorbed on the surface and considered the active sites of the reaction. More importantly, functionalized Fe_3O_4 still maintained good dispersion, which provided favorable conditions for the electrode units of the sensor. At the same time, Figure 1g shows the TEM mapping images of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$. The results clearly demonstrated the magnetic core (Fe_3O_4) of the micro-nanoelectrode and further indicated that elements such as Cu, N, and C were uniformly

distributed outside the magnetic core. In addition, the content of the constituent elements was 62.13%, 0.82%, 2.54%, 14.2%, and 20.3% for Fe, Cu, N, C, and O, respectively (Figure S1). All these observations provided strong evidence to support that copper ions, which were used as an important active site for the recognition and quantitation of D-Cys, were successfully self-adsorbed on the PDA surface. In addition, the high electron transfer rate of PDA has also been confirmed, and has been widely used in the electrochemical field.³³⁻³⁶

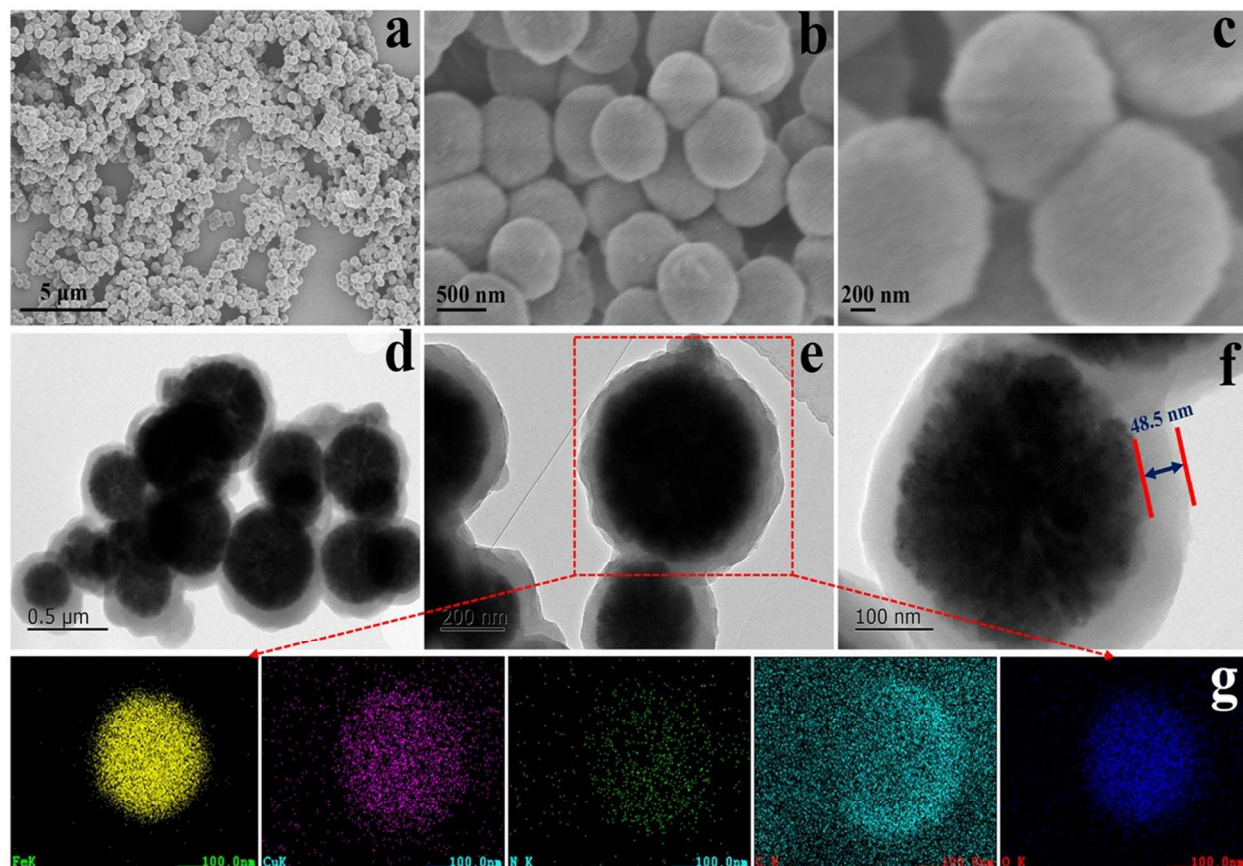


Figure 1. (a-c) SEM images of the Fe₃O₄@PDA/Cu_xO, (d-f) TEM images of the Fe₃O₄@PDA/Cu_xO, (g) TEM mapping of Fe, Cu, N, C and O for the Fe₃O₄@PDA/Cu_xO.

The X-ray diffraction (XRD) pattern was then acquired for characterizing the Fe₃O₄@PDA/Cu_xO (Figure 2a). A series of diffraction peaks for CuO (002), (111), (202), (022), (220) and (004) were observed in the XRD pattern, as well as diffraction peaks ascribed to Cu₂O (111), (002) and (022) according to the PDF cards (JCPDS No. 01-1117 and JCPDS No. 96-901-2955, respectively). This result indicated that the copper ions on the surface of the magnetic micro-nanoelectrodes existed in the form of oxides, which was also in agreement with the results of

TEM mapping. The X-ray photoelectron spectroscopy (XPS) was conducted to further verify the reaction between $\text{Fe}_3\text{O}_4@\text{PDA}$ and CuCl_2 (Figure 2b-d and Figure S2 a-d). The Cu 2p XPS signal was commonly used for identifying the different states of copper ions. Figure 2b shows the deconvoluted XPS spectra of the Cu 2p peaks (main peak and satellite peak of Cu $2p_{3/2}$ and Cu $2p_{1/2}$) of the $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ samples. In agreement with XRD results, XPS analysis showed that surface copper ions were present in two states in each magnetic micro-nanoelectrode: Cu^{2+} in cupric oxide (CuO) and Cu^+ in cuprous oxide (Cu_2O). This result was similar to our previous report, indicating that copper ions were self-adsorbed to the surface of the PDA and formed CuO and Cu_2O .²⁸ Obviously, the band at a binding energy BE at 935-936 eV and the one at BE at 955-956 eV corresponded to Cu $2p_{3/2}$ and Cu $2p_{1/2}$ in CuO . Some derivative peaks at around 943-946 eV were assigned to Cu $2p_{3/2}$ satellite peaks of surface Cu^{2+} species. These core levels of Cu^{2+} were the evidence and diagnostic of 3d⁹ shell of Cu^{2+} . The positions and relative high intensities of the satellite peaks were indicative of the presence of a large amount of cupric ions in the surface.³⁷ Then, the bands at BE = 933.6 eV and BE = 953.6 eV were assigned to the Cu $2p_{3/2}$ and Cu $2p_{1/2}$ in Cu^+ .³⁸ However, Cu^0 and Cu^+ were not readily distinguishable from the XPS spectra (Cu 2p), since Cu^0 and Cu^+ have identical binding energies (± 0.1 eV) within the resolution of conventional XPS measurements.²⁸ In the X-ray excited Cu LMM Auger spectrum, a strong peak was seen at 570.8 eV, which could unambiguously be assigned to Cu^+ , confirming the valence state of copper in the sample as Cu^+ (Figure 2c). Meanwhile, no obvious Cu^0 signal expected around 568 eV was observed.³⁹ Overall, the concentration of Cu^{2+} on the surface of magnetic micro-nanoelectrode was much larger than that of Cu^+ . Figure 2d shows the high-resolution XPS spectra of C1s in $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$. Some species showed the presence of C=C/C-C (284.6 eV), C=N/C-O (286.5 eV), C=O (288.3 eV), and O=C-O (291.3 eV) groups. $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ showed increased peak intensities of O and N-containing groups, and the peaks associated with C=O (284.6 eV) and O=C-O (291.3 eV) become dominant. This was due to the functionalization of the PDA coating on the nanoparticle surface. In the N1s spectra (Figure S2c), $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ showed two major peaks of pyrrolic nitrogen (399.8 eV) and pyridinic nitrogen (398.5 eV), while only pyrrolic nitrogen was present in the parent PDA. We hypothesized that this was mainly due to the conversion of the part of pyrrole nitrogen to pyridine nitrogen when the prepared magnetic micro-nanoelectrodes were oven-dried at 60 °C. Interestingly, these pyridine nitrogen species could help improve the

conductivity of the sensor. The O1s spectra of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ (Figure S2d) was deconvoluted into three components of C=O (531.1 eV), O-C=O (532.3 eV), and C-O (533.3 eV). Obviously, these oxygen-containing groups were all from the PDA.⁴⁰

Raman spectra also provides some valuable information (Figure 2e). In the Raman spectra, $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ and $\text{Fe}_3\text{O}_4@\text{PDA}$ have a significant absorption band difference at $\sim 536\text{ cm}^{-1}$, and this peak could be attributed to Cu-N, from the chelation of Cu on polydopamine. This result further confirmed that the copper ions were successfully anchored on the surface of the micro-nanoelectrodes by the unique adsorption properties of PDA.⁴¹ The magnetic properties of Fe_3O_4 , $\text{Fe}_3\text{O}_4@\text{PDA}$ and $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ were quantitatively determined by vibrating sample magnetometer (VSM) (Figure 2f). The obtained magnetization curves of all three samples showed reversible, non-linear characteristics with no significant coercivity after removing the applied magnetic field, thereby depicting the superparamagnetic nature of the materials. The saturation magnetization (M_s) values of pure Fe_3O_4 , $\text{Fe}_3\text{O}_4@\text{PDA}$ and $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ were 46.4 emu g^{-1} , 28.2 emu g^{-1} and 25.2 emu g^{-1} , respectively. As compared to M_s of pure Fe_3O_4 , the M_s of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ was lower due to the coating of non-magnetic PDA. Nevertheless, the magnetism of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ was sufficient to realize the rapid recovery of the magnetic micro-nanoelectrode unit.⁴²

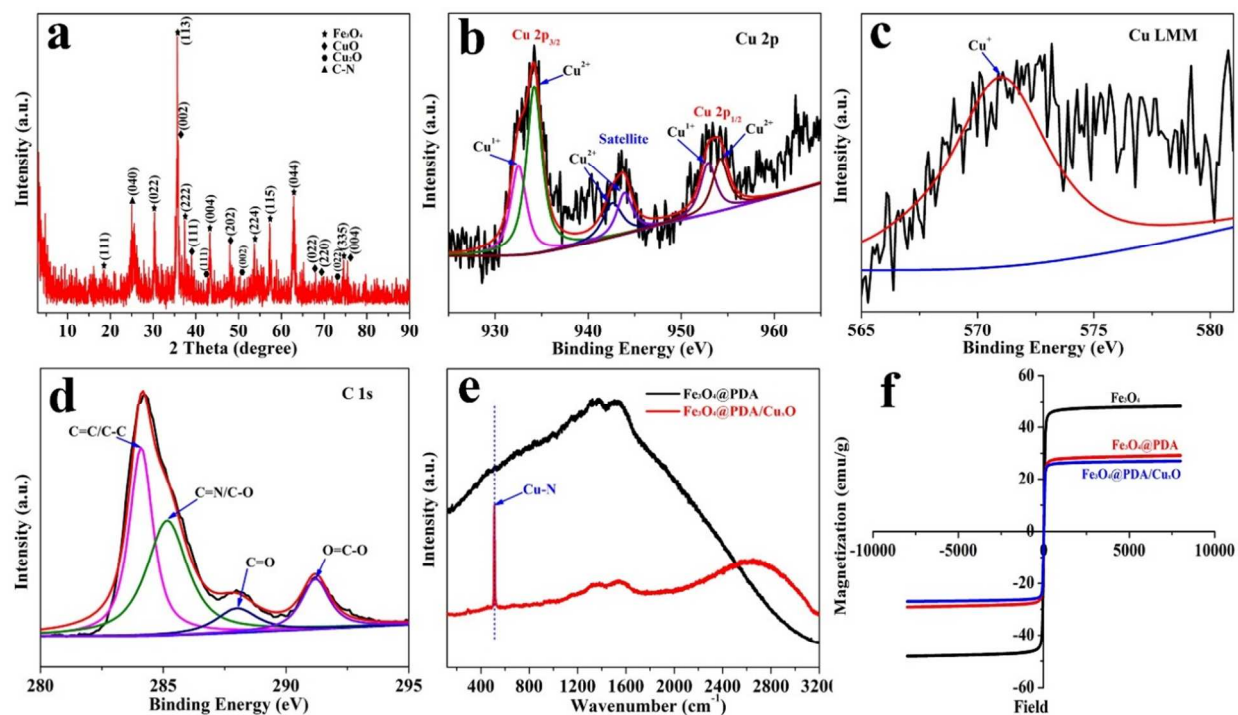


Figure 2. (a) XRD patterns for $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$, (b-d) XPS spectra in the Cu, Cu LMM and C regions of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$, (e) Raman spectra for $\text{Fe}_3\text{O}_4@\text{PDA}$ and $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$, (f) VSM measurement of Fe_3O_4 , $\text{Fe}_3\text{O}_4@\text{PDA}$ and $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$.

Sensing Principle

In this work, we have prepared $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$, which were employed as micro-nanoelectrodes in electrochemical system for the recognition and quantification of D-Cys. The characterization of the sensing parameters were provided in Figure 3. Generally, the electrochemistry of PB (0.1 M pH 5.0, see Figure S3 for details) was investigated at $\text{Fe}_3\text{O}_4@\text{PDA}/\text{MGCE}$ and $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}/\text{MGCE}$ by CV and SWV. As shown in Figure 3a, a pair of reversible redox peaks could be observed from the CV of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}/\text{MGCE}$ in the PB solution. The cathodic and anodic peaks were attributed to the reversible redox reaction of copper ions on the electrode surface. Specifically, it could be found that a small amount of Cu^+ in the electrode material was first rapidly oxidized to Cu^{2+} and then oxidized to a higher valence state (Cu^{3+}) together with the existing Cu^{2+} .^{43,44} On the contrary, the redox peaks of copper on the CV curve of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{MGCE}$ have not been observed. In addition, this conclusion was also confirmed by the SWV curves of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{MGCE}$ and $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}/\text{MGCE}$ (Figure 3b), which revealed that the redox reaction of copper ions was used as an indicator in the sensor. Furthermore, the D-Cys presented strong binding affinity with Cu^{2+} and had better stability compared to L-Cys, which masked the Cu^{2+} on the $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ surface and inhibited the redox reaction of Cu^{2+} . Clearly, the oxidation current change of the sensing system was related to the concentration of the added D-Cys (5 μM), as shown in Figure 3c. The CV and electrochemical impedance spectra (EIS) of the modified electrodes using redox probe $\text{Fe}(\text{CN})_6^{3-/4-}$ (5.0 mM, containing 0.1 M KCl) were hired to further observe the difference in the reaction mechanism of the cysteine enantiomer on the electrode surface (Figure 3d and Figure S4a). By fitting the data with Randles equivalent circuit (see the inset of Figure 3d), the electron transfer resistance of the probe were 265.2, 148.2, 115.1 and 62.6 Ω at the $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}/\text{D-Cys}$, $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}/\text{L-Cys}$, $\text{Fe}_3\text{O}_4@\text{PDA}$ and $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$, respectively. For the $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}/\text{D-Cys}$, the Nyquist plot had a large diameter, which was due to the fact that metal ions (Cu^{2+}) have a more stable binding to the D-type amino acids (D-Cys) than the L-type amino acids (L-Cys) because the growth at this stage

occurs primarily via Ostwald ripening and involved the detachment of a monomer from an existing Cu_xO .²⁰

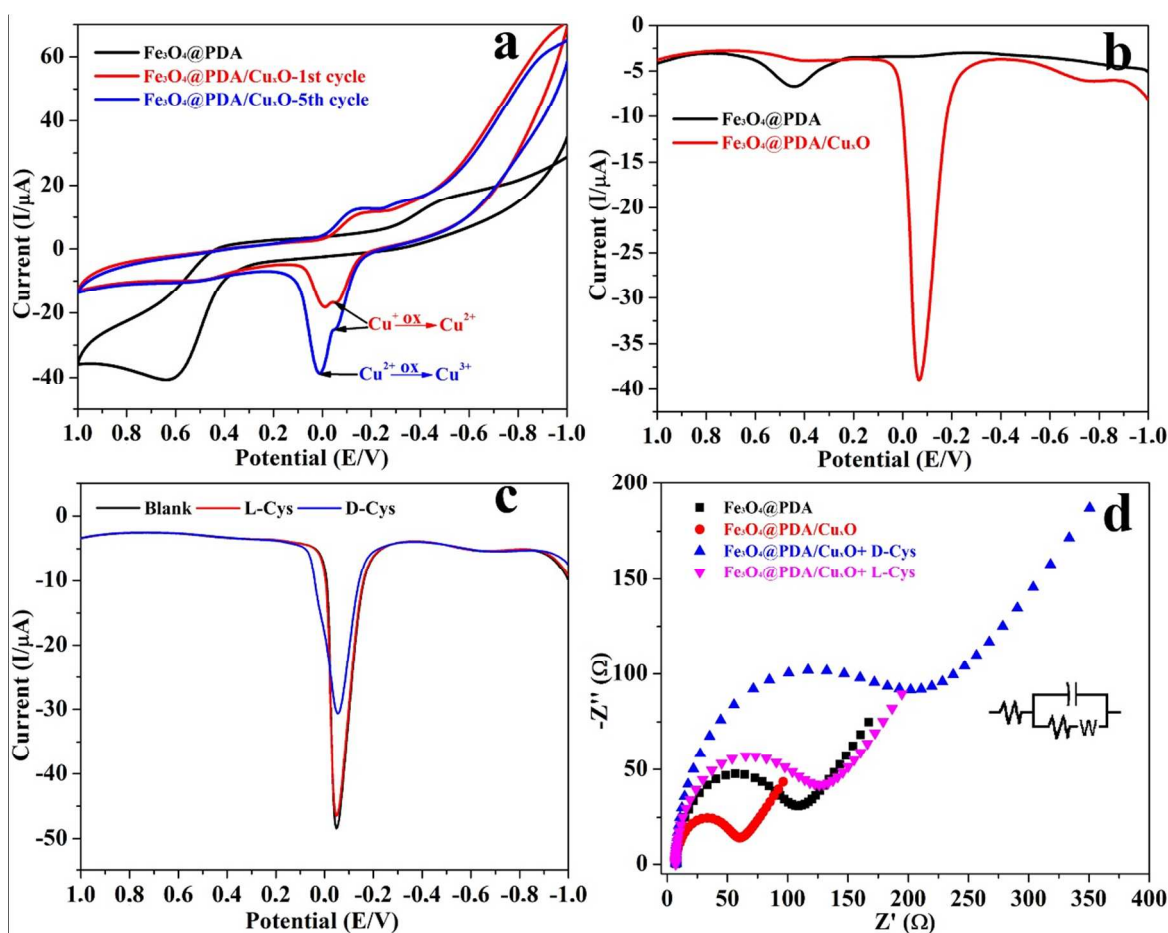


Figure 3. (a) CVs of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{MGCE}$ and $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}/\text{MGCE}$ in 0.1 M PB (pH 5.0). (b) SWV of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{MGCE}$ and $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}/\text{MGCE}$ in 0.1 M PB (pH 5.0). (c) SWV of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}/\text{MGCE}$ in 0.1 M PB (pH 5.0) and PB containing a mixture of 5 μM D/L-Cys. (d) EIS plots of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ (5.0 mM, containing 0.1 M KCl) that were recorded with different electrodes, Inset: Randle's equivalent circuit model for the impedance data.

Evaluation on the performance of micro/nanoelectrodes system

To prove the performance of the proposed micro-nanoelectrodes system, $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ was mixed with target solution (containing 5 μM D-Cys) to inhibit the redox reaction of copper ions. The detection rate was increased by sonication. The peak current intensity of the Cu^{2+} in the absence of sonication ($\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ was immobilized directly on the surface of MGCE)

was significantly increased after sonication ($\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ was first dispersed in the target solution and then magnetically recovered to the surface of MGCE) (Figure 4a). This result was attributed to the fact that sonicating significantly increased the rate of covalent attachment of PDA to cysteine, thereby capturing more target molecules (D-Cys) to mask the Cu^{2+} on the surface of the micro-nanoelectrodes and greatly improving the sensitivity of detection. By comparing the different sonication times (Figure S4b), the ultrasonic time of 30 seconds was an acceptable result, which will greatly increase the efficiency of detection compared with the two hours that is required from using the colorimetric method.^{45,46} Considering the detection sensitivity of D-Cys on the $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$, the concentration of Cu^{2+} loaded is very critical. The shielding effect of D-Cys produces current signals (Figure 4b) at different copper ion concentrations indicated that higher sensitivity was optimal at 5 mM. This conclusion was in agreement with experimental and theoretical data from Wu *et al.*⁴⁶ Hence, 5 mM was selected for the following detection experiments. Overall, these results demonstrated that we have established a novel strategy to improve the detection efficiency and sensitivity of micro-nanoelectrode system.

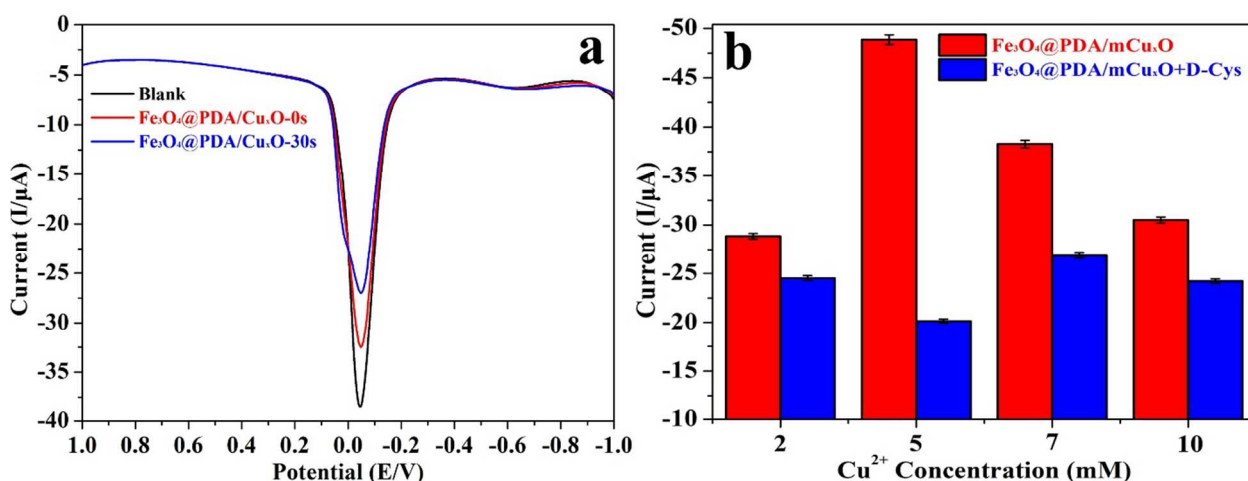


Figure 4. (a) SWV of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ in 0.1 M PB (pH 5.0) with and without sonication. (b) Effect of different Cu^{2+} concentrations on sensor performance in the presence of D-Cys (5 μM).

Quantification and recognition of cysteine enantiomers

SWV experiments were performed to probe the electrochemistry of the micro-nanoelectrodes (Figure 5), as described above. To improve the adsorption efficiency $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ was first mixed with target solution containing a certain concentration of D-Cys by sonicating for 30

s and then MGCE was immersed in the mixture for 2 min. Then, an electrochemical chiral sensing strategy for recognition and quantification of D-Cys was developed according to redox reaction of copper ions. Figure 5a shows the oxidation current of the sensing system with different concentration of D-Cys (from 10 nM to 500 μ M). With increasing concentration of D-Cys, the current signal decreased gradually. Especially, the proposed strategy showed an extremely high sensitivity ($102 \mu\text{A } \mu\text{M}^{-1} \text{ cm}^{-2}$) to D-Cys at low concentrations (from 10 nM to 5 μ M). Meanwhile, linear relationships were recorded over the range from 10 nM to 500 μ M, as shown in the inset of Figure 5b, and limit of detection (LOD) of 83 pM was obtained for the D-Cys detection through experimental measurements. To the best of our knowledge, there were still no electrochemical method for the quantitative detection of D-Cys, and our strategy has the advantages of ultra-sensitivity, low cost, and simple operation to fulfill this purpose.

Despite the previous reports of cysteine sensing mediated with copper ions, it has not been reported to identify cysteine enantiomers by electrochemical methods. As shown in Figure 5c and d, the oxidation current of Cu^{2+} was greatly decreased after incubation with D-Cys, comparing with that in the presence with L-Cys (10 nM-5 μ M). Meanwhile, a new oxidation peak appears in Figure 5c as the concentration of D-Cys increases, and this was absent in Figure 5d. The results were ascribed to the different binding affinity between Cu^{2+} and L-Cys and D-Cys, clearly, metal ions combined with D-Cys to maintain a higher stability than that of L-Cys. Fundamentally, the stable structure formed by the Cu^{2+} and D-Cys rapidly covers the surface of the electrode, acting as a barrier to the electron transfer and thus exhibiting the suppression of redox properties. In addition, the newly emerged oxidation peak also proves this view, which was due to the stable complex formed by D-Cys and copper ions on the surface of the micro-nanoelectrode. At this time, as the continuous electrochemical oxidation was performed, the composite was also oxidized and showed a new oxidation peak. However, the complex formed by L-Cys and copper ions was not stable, the oxidation peak could not be generated. This phenomenon was more pronounced at low concentrations (from 10 nM to 5 μ M), so it could be used to recognize D- and L-Cys.

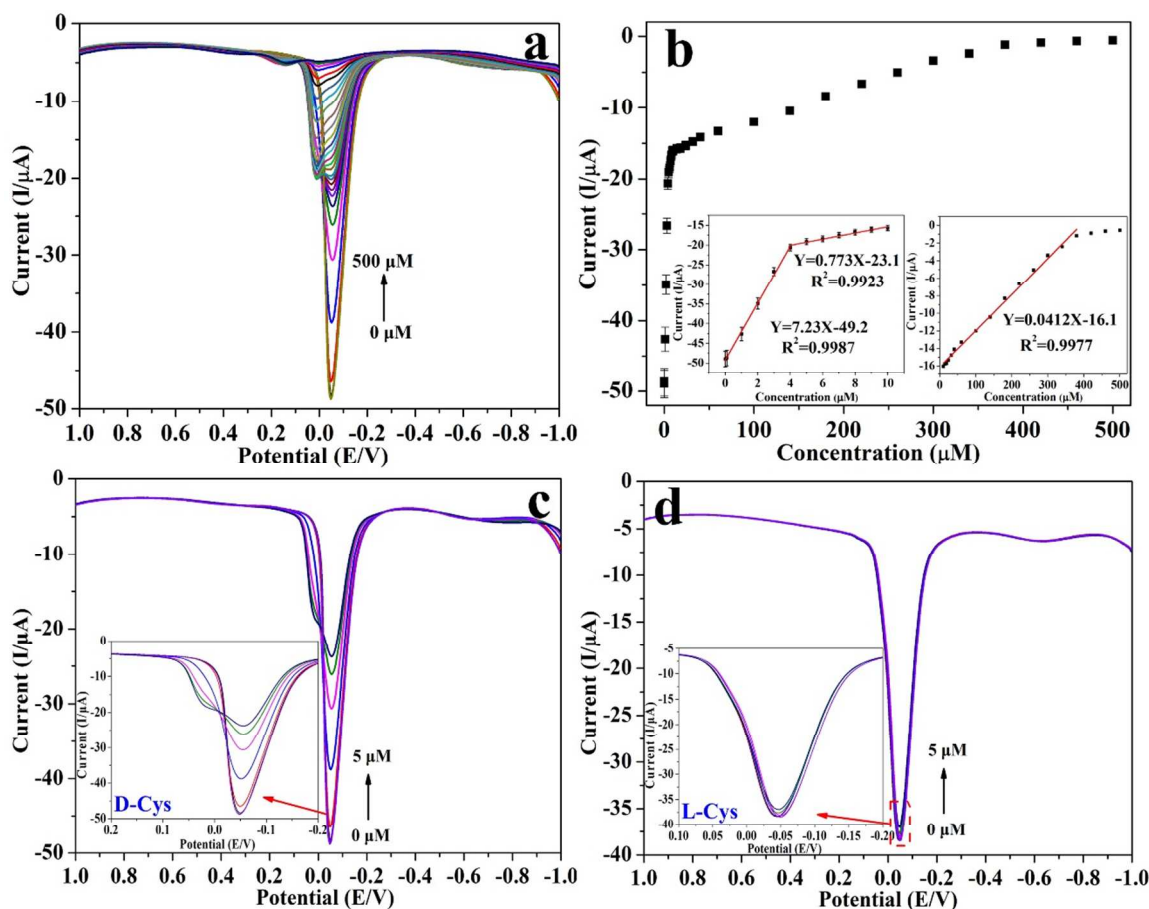


Figure 5. (a) SWV of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ in 0.1 M PB containing a mixture of 0.01-500 μM D-Cys; (b) the relationship of I_{pa} versus the concentrations of D-Cys; (c) (d) SWV of the $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ in 0.1 M PB containing a mixture of 0.01-5 μM D-Cys and L-Cys. Inset is the amplified SWV curve.

Evaluation of the stability and selectivity

To evaluate the performance of a novel analytical method, stability and selectivity are critical parameters. More importantly, the stability of copper ion oxidation peak is of great significance, which ensures that the reduction of oxidation peak was not caused by multiple cycles of itself. In the course of the experiment, a series of metal ions (Na^+ , K^+ , Pb^{2+} , Cd^{2+} , Zn^{2+} , Ca^{2+} , Fe^{3+} , Cr^{3+} , Cl^- , NO_3^- , and SO_4^{2-} in 100-fold excess) have been added into PB and detected by the proposed micro-nanoelectrode system. As shown in Figure 6a, the signal of Cu^{2+} (without the presence of D-Cys) still retained a good reproducibility with increasing number of cycles (signal change below 5%), which means that the proposed micro-nanoelectrode system has good stability. In

addition, some biological molecules (in 10-fold excess), such as glycine (Gly), L-aspartic acid (L-asp), L-isoleucine (L-iso), L-leucine (L-leu), L-lysine (L-lys), L-tyrosine (L-tyr), tryptophan (Trp), L-argine (L-arg), L-histidine (L-his), L-valine (L-val), L-threonine (L-thr), L-phenylalanine (L-phe), α -alanine (α -ala), proline (Pro), glutamic acid (Glu), L-serine (L-ser), homocysteine, thiourea, folic acid, glutathione (GSH), L-methionine (L-met) and D/L-Cys (5 μ M) also had negligible interference (Figure 6b). The results validated that the developed micro-nanoelectrode strategy presents excellent selectivity and could distinguish D-Cys from other amino acids. Thus, a highly sensitive and selective innovative electrochemical method for cysteine enantiomers recognition and quantification was realized in this work.

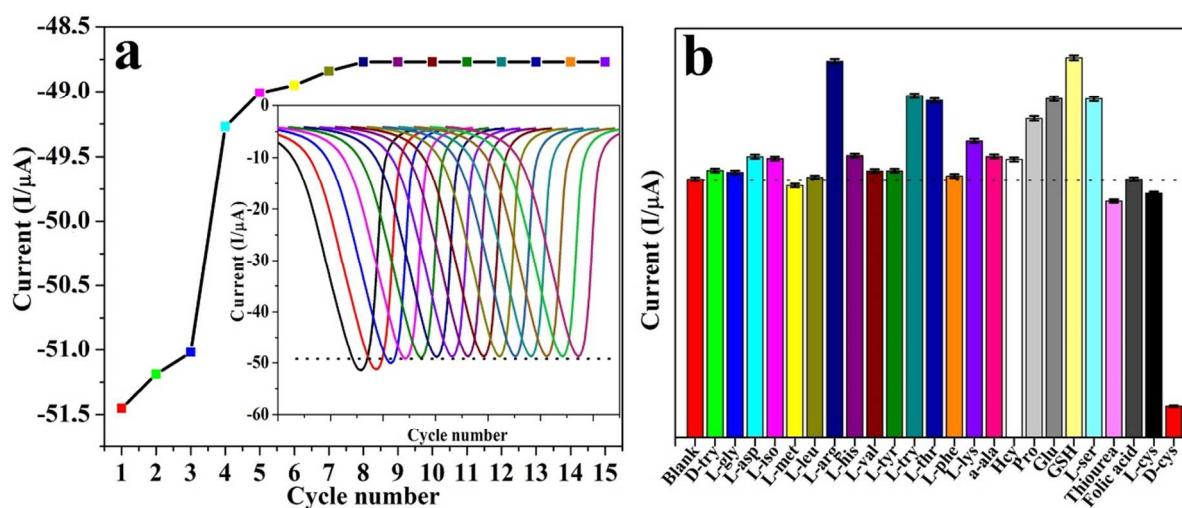


Figure 6. (a) Stability of the voltammetric current for sequential scans of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ in the presence of interfering inorganic ions (Na^+ , K^+ , Pb^{2+} , Cd^{2+} , Zn^{2+} , Ca^{2+} , Fe^{3+} , Cr^{3+} , Cl^- , NO_3^- , and SO_4^{2-} in 100-fold excess), different cycles are represented with different colors; (b) selectivity of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ to cysteine enantiomers (5 μM) after consecutive addition of 50 μM other amino acids, homocysteine, folic acid and thiourea into PB (pH 5.0).

Real sample analysis

The performance of the proposed sensing strategy was verified with the detection of D-Cys in clinical samples. In order to make the practical application more convenient, a flexible combination of magnet and carbon cloth replaces the bulky MGCE as the carrier of the working electrode, as shown in the inset of Figure 7a. Meanwhile, $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ was further utilized to measure D-Cys in human serum spiked with D-Cys. In brief, the human serum spiked with a

standard solution of D-Cys (200 μL) was added to the PB (5 mL, 0.1 M pH 5.0) mixed with magnetic micro-nanoelectrodes, and then the magnetic nanoelectrodes were collected on the carbon cloth. Subsequently, the signals of Cu^{2+} were recorded for quantitative analysis (Figure 7 and Table S1). The calibration curve of the oxidation peak currents versus concentrations of D-Cys exhibited good linearity (Figure 7b). In addition, the recoveries were in range of 99.0–100.4% (measures at 1 and 5 μM), suggesting good feasibility and reliability of the proposed micro-nanoelectrodes sensor for detection of D-Cys in human serum.

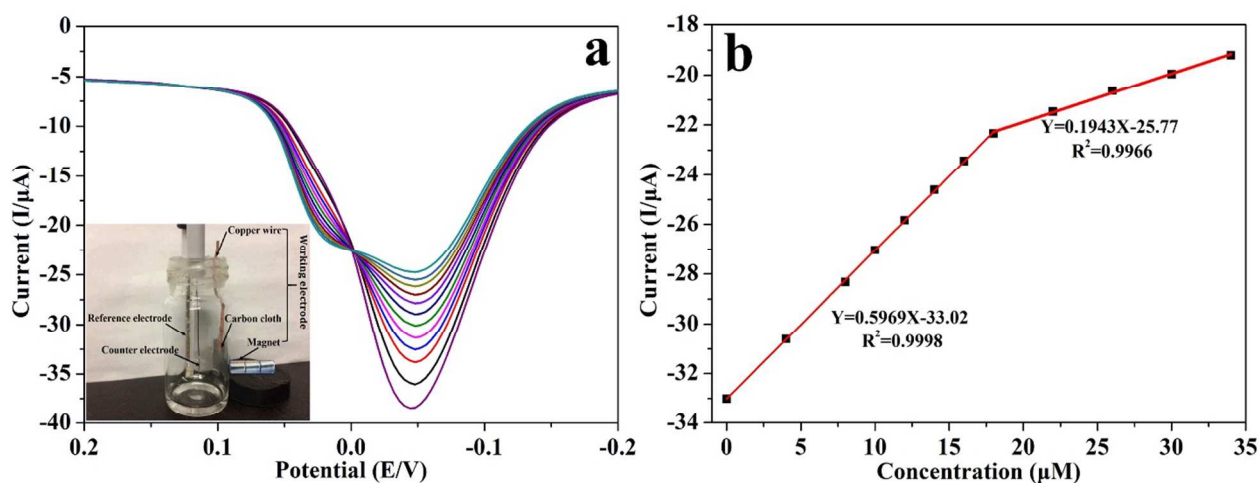


Figure 7. (a) SWV of $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$ in 0.1 M PB containing a mixture of 3–34 μM D-Cys.

The inset shows a simple measuring device designed for the detection of D-Cys; (b) the calibration curve in human serum for D-Cys

4. Conclusion

In conclusion, an ultrasensitive sensing strategy for recognition and quantification of cysteine enantiomers has been developed using magnetic micro-nanoelectrodes ($\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$). The PDA-coated micro-nanoelectrodes were shown to play a role in enrichment and exhibited high sensitivity on the shielding of the redox reaction of Cu^{2+} in the presence of D-Cys. Based on the difference in the structural stability of Cu^{2+} and selectivity of the sensor for D-Cys and L-Cys, we showed the ability to recognize and quantify selectively the D-Cys enantiomer. The detection limit for D-Cys was measured at 83 pM. Clinical samples were analyzed to confirm the potential of this electrode to sense D-Cys in serum. To the best of our knowledge, our method fills the gap of electrochemical methods in identifying and detecting D-Cys in clinical samples.

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Notes

The authors declare no competing financial interest.

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Supporting Information

The supporting Information is available free of charge on the ACS publications web site.

Synthesis and characterization of materials (Fe_3O_4 , $\text{Fe}_3\text{O}_4@\text{PDA}$ and $\text{Fe}_3\text{O}_4@\text{PDA}/\text{Cu}_x\text{O}$); survey XPS analysis; evaluation of electrolyte pH; sonication time dependence of the sensor response; and the practical application for detection of D-Cys in human serum. (PDF)

Reference

- (1) Thanzeel, F. Y.; Wolf, C. *Angew. Chem., Int. Ed.* **2017**.
- (2) Walsh, C. T. *ACS Chem. Biol.* **2014**, *9*, 1653-1661.
- (3) Fuchs, S. A.; Berger, R.; de Koning, T. J. *Brain Res.* **2011**, *1401*, 104-117.
- (4) Billard, J.-M. *Amino Acids* **2012**, *43*, 1851-1860.
- (5) Gu, J.; Dai, H.; Kong, Y.; Tao, Y.; Chu, H.; Tong, Z. *Synth. Met.* **2016**, *222*, 137-143.
- (6) Yan, T. Q.; Orihuela, C. J. *Chromatogr. A* **2007**, *1156*, 220-227.
- (7) Fuchs, S. A.; Berger, R.; Klomp, L. W.; de Koning, T. J. *Mol. Genet. Metab.* **2005**, *85*, 168-180.
- (8) Turner, A. P. *Chem. Soc. Rev.* **2013**, *42*, 3184-3196.
- (9) Lee, M. H.; Kim, J. S.; Sessler, J. L. *Chem. Soc. Rev.* **2015**, *44*, 4185-4191.
- (10) Li, H.; Fan, J.; Wang, J.; Tian, M.; Du, J.; Sun, S.; Sun, P.; Peng, X. *Chem. Commun.* **2009**, 5904-5906.
- (11) Han, M. S.; Kim, D. H. *Tetrahedron* **2004**, *60*, 11251-11257.
- (12) Dai, X.; Wang, Z.-Y.; Du, Z.-F.; Cui, J.; Miao, J.-Y.; Zhao, B.-X. *Analy. Chim. Acta* **2015**, *900*, 103-110.
- (13) Shen, J.-S.; Li, D.-H.; Zhang, M.-B.; Zhou, J.; Zhang, H.; Jiang, Y.-B. *Langmuir* **2010**, *27*, 481-486.
- (14) Kuang, X.; Ye, S.; Li, X.; Ma, Y.; Zhang, C.; Tang, B. *Chem. Commun.* **2016**, *52*, 5432-5435.
- (15) Luo, Y.; Zhang, L.; Liu, W.; Yu, Y.; Tian, Y. *Angew. Chem., Int. Ed.* **2015**, *127*, 14259-14262.
- (16) Pazalja, M.; Kahrović, E.; Zahirović, A.; Turkušić, E. *Int. J. Electrochem. Sci* **2016**, *11*, 10939-10952.
- (17) Hassan, S. S.; El-Baz, A. F.; Abd-Rabboh, H. S. *Analy. Chim. Acta* **2007**, *602*, 108-113.
- (18) Wang, L.; Tricard, S.; Yue, P.; Zhao, J.; Fang, J.; Shen, W. *Biosens. Bioelectron.* **2016**, *77*, 1112-1118.
- (19) Wang, X.; Luo, C.; Li, L.; Duan, H. *J. Electroanal. Chem.* **2015**, *757*, 100-106.
- (20) Zhou, Y.; Yang, M.; Sun, K.; Tang, Z.; Kotov, N. A. *J. Am. Chem. Soc.* **2010**, *132*, 6006-6013.
- (21) Yu, H.; Chen, M.; Rice, P. M.; Wang, S. X.; White, R.; Sun, S. *Nano Lett.* **2005**, *5*, 379-382.
- (22) Frey, N. A.; Peng, S.; Cheng, K.; Sun, S. *Chem. Soc. Rev.* **2009**, *38*, 2532-2542.
- (23) Yang, C.-H.; Huang, K.-S.; Lin, Y.-S.; Lu, K.; Tzeng, C.-C.; Wang, E.-C.; Lin, C.-H.; Hsu, W.-Y.; Chang, J.-Y. *Lab Chip* **2009**, *9*, 961-965.
- (24) Lei, Y.; Chen, C.-S.; Tu, Y.-J.; Huang, Y.-H.; Zhang, H. *Environ. Sci. Technol.* **2015**, *49*, 6838-6845.
- (25) Yu, C.-J.; Lin, C.-Y.; Liu, C.-H.; Cheng, T.-L.; Tseng, W.-L. *Biosens. Bioelectron.* **2010**, *26*, 913-917.
- (26) Liang, M.; Fan, K.; Pan, Y.; Jiang, H.; Wang, F.; Yang, D.; Lu, D.; Feng, J.; Zhao, J.; Yang, L. *Anal. Chem.* **2012**, *85*, 308-312.
- (27) Guo, L.; Liu, Q.; Li, G.; Shi, J.; Liu, J.; Wang, T.; Jiang, G. *Nanoscale* **2012**, *4*, 5864-5867.
- (28) Zhou, H.; Huang, T.; Chen, D.; Li, S.; Yu, H.; Li, Y.; Song, Q. *Sens. Actuat. B-Chem.* **2017**, *249*, 405-413.
- (29) Li, P.; Cai, X.; Wang, D.; Chen, S.; Yuan, J.; Li, L.; Shen, J. *Colloids Surf. B: Biointerfaces* **2013**, *110*, 327-332.
- (30) Wu, D.; Wang, Y.; Zhang, Y.; Ma, H.; Pang, X.; Hu, L.; Du, B.; Wei, Q. *Biosens.*

Bioelectron. **2016**, 82, 9-13.

(31) Miao, P.; Tang, Y.; Wang, L. *ACS appl. matr. interfaces* **2017**, 9, 3940-3947.

(32) Li, S.; Liang, W.; Zheng, F.; Zhou, H.; Lin, X.; Cai, J. *Sens. Actuat. B-Chem.* **2016**, 224, 48-54.

(33) Han, L.; Lu, X.; Wang, M.; Gan, D.; Deng, W.; Wang, K.; Fang, L.; Liu, K.; Chan, C. W.; Tang, Y. *Small* **2017**, 13.

(34) He, F.; Chen, G.; Yu, Y.; Zhou, Y.; Zheng, Y.; Hao, S. *Chem. Commun.* **2015**, 51, 6824-6827.

(35) Yameen, B.; Rodriguez-Emmenegger, C.; Preuss, C. M.; Pop-Georgievski, O.; Verveniotes, E.; Trouillet, V.; Rezek, B.; Barner-Kowollik, C. *Chem. Commun.* **2013**, 49, 8623-8625.

(36) Li, R.; Parvez, K.; Hinkel, F.; Feng, X.; Müllen, K. *Angew. Chem., Int. Ed.* **2013**, 125, 5645-5648.

(37) Chusuei, C. C.; Brookshier, M.; Goodman, D. *Langmuir* **1999**, 15, 2806-2808.

(38) Iijima, Y.; Niimura, N.; Hiraoka, K. *Surf. interface anal.* **1996**, 24, 193-197.

(39) Peng, Y.; Shang, L.; Cao, Y.; Waterhouse, G. I.; Zhou, C.; Bian, T.; Wu, L.-Z.; Tung, C.-H.; Zhang, T. *Chem. Commun.* **2015**, 51, 12556-12559.

(40) Xian, S.; Xu, F.; Zhao, Z.; Li, Y.; Li, Z.; Xia, Q.; Xiao, J.; Wang, H. *AIChE J.* **2016**, 62, 3730-3738.

(41) Liu, T.; Luo, Y.; Wang, W.; Kong, L.; Zhu, J.; Tan, L. *Electrochim. Acta* **2015**, 182, 742-750.

(42) Kumar, A.; Khan, M.; Fang, L.; Lo, I. M. *J. Hazard. Mater.* **2017**.

(43) Casella, I. G.; Gatta, M.; Guascito, M. R.; Cataldi, T. R. *Anal. Chim. Acta* **1997**, 357, 63-71.

(44) Luo, S.; Su, F.; Liu, C.; Li, J.; Liu, R.; Xiao, Y.; Li, Y.; Liu, X.; Cai, Q. *Talanta* **2011**, 86, 157-163.

(45) Ghasemi, F.; Hormozi-Nezhad, M. R.; Mahmoudi, M. *Sci. Rep.* **2017**, 7.

(46) Wu, C.; Fan, D.; Zhou, C.; Liu, Y.; Wang, E. *Anal. Chem.* **2016**, 88, 2899-2903.

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