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A fluorimetric testing strip for the visual evaluation of mercury in blood using copper nanoclusters with DMSO-enhanced fluorescence and stability†

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A fluorimetric analytical method using test strips has been fabricated for detecting Hg^{2+} ions in blood by using copper nanoclusters (Cu NCs) prepared *via* a biomineralization route. Unexpectedly, the as-prepared Cu NCs displayed greatly amplified red fluorescence once dispersed in DMSO, the intensity of which decreased specifically in the presence of Hg^{2+}. Moreover, the resultant Cu NCs were deposited onto test strips to be further fast dried on superhydrophobic substrates in vacuum. The test strip-based fluorimetry can allow for the direct analysis of Hg^{2+} in blood in the linear concentration range of 0.10–1000 nM. Importantly, this solvent-enhanced fluorescence protocol for different metal probes such as Cu NCs promises extensive analysis applications for designing numerous fluorimetric platforms such as test strips.

Introduction

Mercury (Hg), one of the most prevalent toxic metals, can readily enter cells and gastrointestinal tissues by passing through biological membranes,[1–3] and can accumulate in the body to combine with proteins, making it hard to release.[4,5] The long-term exposure to high levels of mercury may bring the permanent deterioration of tissues such as brain and kidney tissues.[6,7] To date, numerous efforts have been focused on the preparation of numerous detection methods for monitoring Hg^{2+} ions in human body fluids such as blood,[8] typically including atomic absorption/emission spectroscopy, electrochemical analysis, inductively coupled plasma-atomic emission spectrometry (ICP-AES), and fluorimetry methods.[9–13] Among these classical methods, fluorimetric detection can facilitate the rapid and sensitive detection of numerous biomedical targets.[14–16] Nevertheless, they may hardly facilitate the direct and point-of-care analysis of targets due to the requirement of bulky and expensive instruments. Alternatively, studies have focused on the design of portable monitoring devices for field-applicable analysis, the most known being optical test strips.[4,17–20]

It is widely recognized that the detection capacities of fluorimetric analysis strategies can be mainly determined by the probe properties such as environmental stability and luminescence intensities.[21] Nowadays, nano-scaled noble metal materials such as Au and Ag nanoclusters (NCs) have numerous applications as fluorescent probes in numerous fluorimetric detection techniques because of their outstanding properties such as high emission intensities.[22,23] However, they may be trapped by some inherent disadvantages such as cost-ineffectiveness or storage instability. In particular, some attention has been drawn to the preparation of Cu NCs for the detection of a variety of targets.[22–24] For example, He *et al.* fabricated fluorescent Cu NCs as probes for the sensitive analysis of kojic acid.[22] Unfortunately, most Cu NCs are prepared show blue emission at the high-energy excitation, which may possibly risk the serious interference of biological backgrounds, resulting in practically limited applications. Recently, Cu NCs with red fluorescence (FL) have been successfully fabricated.[25] They may still be challenged by the limited fluorescence intensity and particularly poor environmental stability. Alternatively, some researches have demonstrated that the applications of organic solvents can alter the luminescence properties and/or environmental stability of fluorescent probes, such as DMSO,

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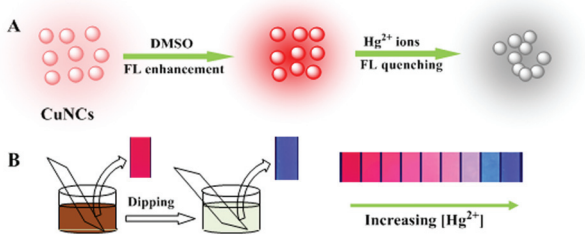
†Electronic supplementary information (ESI) available: TEM of Cu NCs, EDS analysis, the optimization of analysis conditions, anions effects, the environmental robustness, and storage stability of Hg^{2+} test strips and the table of fluorimetric analytical results of different methods for Hg^{2+}. See DOI: 10.1039/d0nr06896a



Scheme 1 (A) Schematic description of Cu NCs with the DMSO-triggered FL enhancement and the FL quenching induced by Hg^{2+} ions, and (B) the fabrication procedure of fluorimetric test strips for the detection of Hg^{2+}.

glycerin, and alcohol solvents. For example, the DMSO-glycerin mixture could serve as an optical clearing agent for enhancing the photonic transference towards the improved fluorescence imaging and analysis by increasing the translucent degrees of probe-containing solutions or tissues.[27,28]

In this study, we fabricated a test strip-based fluorimetric method for detecting Hg^{2+} ions in blood using copper nanoclusters (Cu NCs). Herein, the Cu NCs were prepared by a biomineralization route using a protein template of bovine albumin serum (BSA). Unexpectedly, the resultant Cu NCs presented greatly enhanced red fluorescence when dispersed in DMSO, the intensities of which could be specifically quenched by Hg^{2+} ions, as described in Scheme 1A. Furthermore, as schematically illustrated in Scheme 1B, the fluorescent nanoprobes of Cu NCs in DMSO were deposited onto the test strips for probing Hg^{2+} ions *via* a vacuum-aided fast drying process using super-hydrophobic substrates to suppress the formidable "coffee stains". The developed test strips were further used in the fluorimetric analysis of Hg^{2+} ions in blood, showing dramatically improved environmental stability. To the best of our knowledge, this study reports for the first time the DMSO-enhanced fluorescence of Cu NCs for designing the fluorimetric platform with test strips for the analysis of Hg^{2+} ions in the blood. The improved analysis selectivity, sensitivity, and reproducibility are explored in detail.

Experimental section

Reagents

$CuCl_2$, bovine serum albumin (BSA), and hexadecyltrimethoxysilane (HDS) were bought from Sigma Aldrich (Beijing, China). $N_2H_4{\cdot}H_2O$ (80%), ethanol, isopropanol (IPA), ethyl acetate (EA), dimethyl sulphoxide (DMSO), dichloromethane (CH_2Cl_2), trimethylamine (TEA), polyethylene glycol (PEG), acetonitrile (ACN), *N*,*N*-dimethylformamide (DMF), dichloroethane (DCE), and butyl alcohol (NBA) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Whatman filter papers were purchased from Sigma-Aldrich (Beijing, China). Other chemicals were of analytical grade. Deionized water (>18 Mohm cm) was supplied from the Ultra-pure water system (Pall, USA).

Apparatus

The fluorescence measurements were performed on an FL spectrophotometer (Horiba, FluoroMax-4, Japan), which was operated at an excitation wavelength of 420 nm and an excitation slit of width 5.0 nm. The UV-vis spectra of functional materials were recorded on a UV-3600 spectrophotometer (Shimadzu, Japan). The morphological shapes and structures of Cu NCs in DMSO with and without Hg^{2+} ions were investigated *via* transmission electron microscopy (TEM, JEM-2100PLUS, Japan) operated at 100 kv and energy-dispersive X-ray spectroscopy (EDS). The photographs of reaction products were taken under UV light at an excitation wavelength of 365 nm of.

Preparation of fluorescent Cu NCs

In a typical experiment, $CuCl_2$ solution (250 μL, 0.10 M) was added to BSA solution (20 mL, 5.0 mg mL^{-1}). The above mixture was stirred at room temperature for 10 min, and a white hydrogel was formed due to the coordination between Cu^{2+} ions and the various functional groups of BSA such as –NH, –SH, and –OH. Then, $N_2H_4{\cdot}H_2O$ (1.0 mL, 80%) was slowly injected into the reactive mixture to be stirred for 5 h at room temperature. The light-yellow suspension of Cu NCs was formed and further stored in the refrigerator at 4 °C in dark.

A certain amount of Cu NCs was dispersed separately into numerous solvents including ethanol, IPA, EA, DMSO, CH_2Cl_2, TEA, PEG, CAN, DCE, NBA, and DMF. The fluorescence intensities were recorded for comparative evaluations of the solvent-enhanced fluorescence of Cu NCs.

Fabrication of test strips with Cu NCs in DMSO

The Hg^{2+} test strips were fabricated with the DMSO-containing Cu NCs *via* a modified fabrication method previously reported.[15] Typically, the Whatman filter papers were first cut into slices for designing test strips (10 mm × 10 mm), which were further immersed into Cu NCs (0.50 mM) containing DMSO (40%, v/v) for 10 min. After that, the Cu NC-coated test strips were dried onto super-hydrophobic substrates of HDS in vacuum for 20 min. Herein, the lotus surface-like HDS substrates with the hydrophobic constraining forces controlled the exterior transportation of dispersed nanoclusters on the strips, leading to the minimized "coffee stains" and improved the distribution of Cu NC probes on the strips, as described previously.[17] The resultant test strips for Hg^{2+} detection were stored in dark.

Fluorimetric analysis for Hg^{2+} with Cu NC-coated test strips

The detection conditions of the developed fluorimetric test strips were first optimized in probing Hg^{2+} ions, including the different Cu NC concentrations of 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, and 0.70 mM, temperature (5.0, 20.0, 35.0, 50.0, 65.0, and 80.0 °C), pH values (1.0, 3.0, 5.0, 7.0, 9.0, 11.0, and 13.0), and ionic strengths of NaCl (0.0, 50, 100, 150, 200, 250, and 300 mM).

The fluorimetric assays for Hg^{2+} ions were conducted using test strips coated with Cu NCs in water containing DMSO by the following procedure. A certain amount of Hg^{2+} ions of different concentrations (0.0010, 0.0050, 0.010, 0.050, 0.10, 0.50, 1.0, 5.0, and 10 μM) were separately mixed in an aliquot of Cu NCs (0.50 mM) in a buffer containing 40% DMSO. The fluorimetric measurements were further carried out by recording the change in intensities and fluorescence spectra. The tests of solid fluorescence need to be carried out on a solid support. To perform the fluorescence detection on the solid sample, the as-prepared test strip sample needs to be fixed on the solid support in such a way that the excitation light and the sample are at an angle of 30°.

According to the above procedure, the as-fabricated fluorimetric test strips were employed to probe Hg^{2+} ions spiked in blood with different Hg^{2+} levels (0.0032, 0.0010, 0.032, 0.010, 0.10, 0.32, 1.0, 3.20, and 32.0 μM). Also, the control tests for 1.0 μM of different inorganic anions (Cu^{2+}, Zn^{2+}, Mg^{2+}, Pb^{2+}, Fe^{3+}, Fe^{2+}, Hg^{2+}, Co_2^{+}, Cd^{2+}, Cr^{3+}, Ni^{2+}, K^{+}, Ca^{2+}, and Na^{+} ions) were conducted accordingly. Herein, the quenching efficiencies are calculated according to the equation: quenching efficiencies = $(F_0 - F)/F_0$, where F_0 and F are the fluorescence intensities of the Cu NCs containing DMSO before and after adding Hg^{2+} ions, respectively. Moreover, F_1 refers to the fluorescence intensities of Cu NCs. The detection of Hg^{2+} ions in blood samples was performed with the approval of the Animal Ethics Committee at Qufu Normal University, P.R. China, and blood samples were obtained from the Hospital of Qufu Normal University.

Results and discussion

Main Hg^{2+}-sensing mechanism of Cu NCs in DMSO

Cu NCs were fabricated in water with red fluorescence simply by using a cheap protein of bovine serum albumin (BSA) and N_2H_4 as the stabilizer and reductant, respectively. It can be seen from Fig. 1A that 5% N_2H_4 can synthesize Cu NCs with maximum fluorescence intensity. Furthermore, several kinds of organic solvents were introduced separately into Cu NCs in water. To our surprise, DMSO enabled the dramatic enhancement of fluorescence emission of Cu NCs in water or on test strips. DMSO-increased environmental stability could also be expected for the resulting Cu NCs. More importantly, the fluorescence of Cu NCs containing DMSO could be specifically quenched after adding Hg^{2+} ions, which is described schematically in Scheme 1A. Moreover, Cu NCs in water containing DMSO were deposited onto the test strips, resulting in a solid-state fluorimetric strategy for the visual evaluation of Hg^{2+} (Scheme 1B). Herein, the DMSO-enhanced fluorescence and environmental stability of Cu NCs are thought to be based on two factors. On the one hand, DMSO might act as an optical clearing agent to increase the photonic transference and decrease the light scattering of fluorescent Cu NCs, as revealed previously for the optical clearing technique.[26,27] On the other hand, DMSO molecules might conduct the "solvent effect" by replacing water molecules towards the enhanced fluorescence of Cu NCs. Therefore, the introduction of DMSO should have a vital effect on the optical and sensing performances of Cu NCs. The DMSO-to-water ratios were examined, showing an optimal one at 40% (Fig. 1B). In addition, a fluorimetric analysis method was proposed for the analysis of Hg^{2+} in blood using Cu NC-coated test strips, in which Hg^{2+} ions might trigger the unstable aggregation of Cu NCs showing the fluorescence quenching.

A comparison of FL intensities was carried out between Cu NCs in the presence and absence of DMSO for detecting Hg^{2+} ions (Fig. 2). Cu NCs containing DMSO (curve a) could exhibit two-fold stronger fluorescence than the ones without DMSO (curve b). More importantly, in the presence of Hg^{2+} ions, a three-fold larger change in the fluorescence intensities could be achieved for the Cu NCs containing DMSO. Fig. 2B manifests the UV-vis spectra of Cu NCs containing DMSO before and after the addition of Hg^{2+} ions, in comparison with those without DMSO. It was found that a UV-vis absorption peak at 280 nm was observed for all solutions of Cu NCs, which is assigned to the protein used during the fabrication procedure. Accordingly, compared with Cu NCs in water (curve a), a decreased absorption of proteins could be observed for Cu NCs containing DMSO (curve b), demonstrating that DMSO might increase the photonic transference of Cu NC suspensions. Moreover, the addition of Hg^{2+} could trigger the aggregation of the protein-encapsulated Cu NCs (curve c), the products of which might have precipitated resulting in lower absorbance of supernatants. Interestingly, a slight change in the protein absorption was witnessed for Cu NCs containing

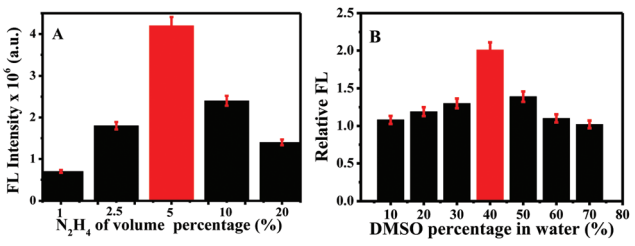


Fig. 1 (A) FL intensities of Cu NCs synthesized using different volume percentages of N_2H_4. (B) The FL intensities of Cu NCs depending on the volume percentages of DMSO in water.

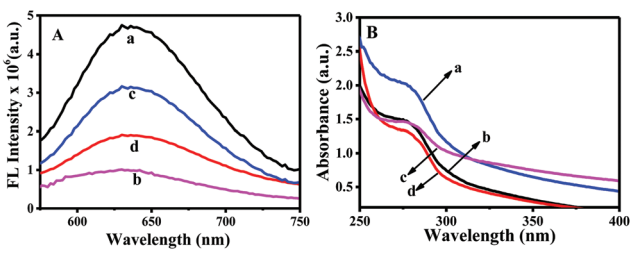


Fig. 2 (A) FL spectra and (B) UV-vis spectra of Cu NCs (a) without and (b) with DMSO, with (c) and (d) corresponding responses to Hg^{2+}.

DMSO without (curve b) and with Hg^{2+} ions (curve d), presumably due to the optical clearing capacity of DMSO.

The changes in the morphological structures of Cu NC containing DMSO in the absence and presence of Hg^{2+} ions were comparatively explored using transmission electron microscopy (TEM) imaging. As shown in Fig. 3A, Cu NC containing DMSO could exhibit uniform mono-dispersion showing spherical shapes with an average size of about 3.0 nm in diameter and no significant change in shape and size as compared with Cu NCs in water (Fig. S1†). Yet, greatly enhanced red fluorescence could be expected as well as environmental stability as demonstrated afterwards. Herein, the DMSO-enhanced fluorescence and environmental stability of Cu NCs could be attributed to DMSO acting as an optical clearing agent to increase the photonic transference and decrease the light scattering of fluorescent Cu NCs, as confirmed elsewhere.[26,27] DMSO molecules might also conduct the "solvent effect", so that water molecules were replaced resulting in the enhanced fluorescence of Cu NCs. However, it should be pointed out that the detailed mechanism should be investigated in future studies. More importantly, the addition of Hg^{2+} ions could trigger the instability and aggregation of Cu NCs (Fig. 3B), presumably due to the occurrence of a replacement reaction between Cu NCs and Hg^{2+}, thus changing the size, morphology, and composition of Cu NC probes, thereby inducing the unstable aggregation of Cu NCs. In contrast, such a replacement reaction might not take place between Cu NCs in DMSO and other cations mentioned (*i.e.*, Zn^{2+} and Fe^{3+}). In addition to the TEM imaging for their morphological structures above, the EDS analysis was further performed for Cu NCs in DMSO with and without Hg^{2+} ions, confirming the change in chemical compositions as expected (Fig. S2A†). Therefore, DMSO evidently has a vital effect on the Hg^{2+} sensing performances of Cu NCs in addition to their optical properties.

The sensing performances of the Cu NC-based test strip for Hg^{2+}

Comparative studies were carried out on the changes in the fluorescence properties of the test strips fabricated using Cu NCs in water containing different common solvents (Fig. 4A). Again, it was found that the fluorescence intensities of Cu NCs dramatically enhanced in the presence of DMSO. In contrast, they could be weakened and even quenched by adding other kinds of solvents. Furthermore, the analytical selectivity of the

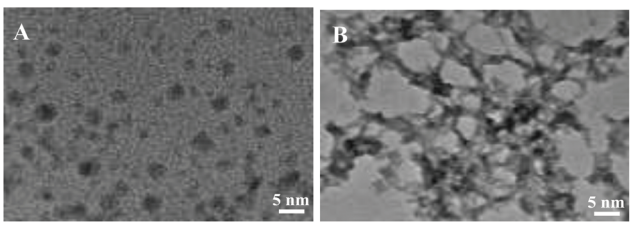


Fig. 3 TEM images of Cu NCs containing DMSO in (A) the absence and (B) presence of Hg^{2+}.

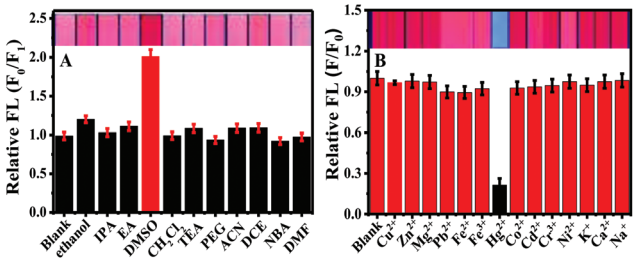


Fig. 4 (A) Relative FL intensities of fluorimetric test strips with Cu NCs in water containing different types of solvents: ethanol, IPA, EA, DMSO, CH_2Cl_2, TEA, PEG, ACN, DCE, NBA, and DMF at the Cu NC-to-DMSO ratios of 40%. (B) Relative fluorescence intensities of test strips coated with Cu NCs containing DMSO with different cations alone (top: the photographs of testing solutions under UV light, from the left to right: Cu^{2+}, Pb^{2+}, Mg^{2+}, Zn^{2+}, Fe^{3+}, Hg^{2+}, Co_2^+, Cd^{2+}, Cr^{3+}, Ni^{2+}, K^+, Ca^{2+}, and Na^+ ions).

fabricated test strips was examined by using different inorganic cations (Cu^{2+}, Zn^{2+}, Mg^{2+}, Pb^{2+}, Fe^{3+}, Fe^{2+}, Hg^{2+}, Co_2^+, Cd^{2+}, Cr^{3+}, Ni^{2+}, K^+, Ca^{2+}, and Na^+ ions) (Fig. 4B) and inorganic anions (NO_3^-, SO_4^{2-}, MnO_4^-, I^-, Cl^-, S^{2-}, and CN^- ions) (Fig. S3†). Accordingly, only Hg^{2+} ions could significantly cause the fluorescence quenching of Cu NC containing DMSO in contrast to other cations inducing no apparent change in the fluorescence intensity of the test strips.

Environmental stability of fluorescent Cu NCs probes

It is well recognized that Cu NCs may be unstable in water or on test strips. Herein, the environmental stability of the fluorometric test strips coated with Cu NCs containing DMSO was explored under different detection conditions of storage and strong light exposure by comparing with the ones coated with Cu NCs in water (Fig. S4A†). To our surprise, the test strips coated with Cu NCs containing DMSO could achieve greatly improved stability, which could be stored in dark for up to 12 months (red), showing no apparent change in the FL intensities. However, a great decay was observed in the intensities for test strips coated with Cu NCs in water (black). Moreover, Fig. S4B† manifests the comparison of optical robustness for the Cu NC-based test strips by exposure directly to the xenon lamp over different intervals of time. It was discovered that the test strips with DMSO-containing Cu NCs could interestingly present no obvious change of FL intensities even when they were exposed up to 5.0 h (red), whereas the test strips with Cu NCs in water showed a significant decrease (black). Therefore, the developed test strips exhibited greatly improved environmental stability, so that the light bleaching and self-quenching might be largely depressed in storage.

Main fluorimetric conditions for Hg^{2+}

The main analytical conditions of the developed fluorimetric method with test strips were optimized by analyzing Hg^{2+} ions (Fig. S5†). It is noted that the Cu NC amounts can have large influences on the FL quenching efficiencies of the test strips for sensing Hg^{2+} ions, with the maximum at 0.50 mM Cu NCs

(Fig. S5A†). Also, the sensing temperature was optimized to be at about 37 °C (Fig. S5B†). Herein, a low temperature might induce DMSO to freeze and slow down the interaction between Cu NCs and Hg^{2+}. Moreover, a high temperature might bring about the instability of Cu NCs towards aggregation and oxidation. Furthermore, the fluorescence responses of Cu NCs containing DMSO to Hg^{2+} ions were explored under the changing pH values (Fig. S5C†). Obviously, the optimal pH value for Hg^{2+} sensing should be at pH 7.0, where too high or low pH values might challenge the stability of Cu NCs. Moreover, the effects of ionic strengths on Hg^{2+} responses were evaluated for Cu NC-containing DMSO (Fig. S5D†). Obviously, no significant influence on the responses to Hg^{2+} occurs in the testing solutions with the NaCl concentrations up to 300 mM, suggesting that the electrostatic interactions hardly exert any influence on the chelating reaction of Hg^{2+} ions with Cu NCs containing DMSO.

The Cu NC-based test strips for probing Hg^{2+} in samples

Under the optimal analytical conditions, the Cu NC-based test strips were applied for the detection of Hg^{2+} ions at various concentrations in the buffer (Fig. 5). One can see from Fig. 5A that an increase in the Hg^{2+} concentration causes a rational decrease in the FL intensities of the test strips. Furthermore, Fig. 5B displays the calibration curve of FL quenching efficiencies *versus* the logarithms of Hg^{2+} concentrations, which linearly range from 0.10 to 1000 nM (R^2 = 0.9886), with the limit of detection of about 0.030 nM, as estimated by the 3σ rule. Furthermore, the performance of the developed fluorescence analysis strategy was compared with other recorded analysis methods, and the result shown in Table S1.† It was found that the developed detection method can show better performance in terms of detection range and LOD. Therefore, the developed DMSO-containing Cu NC-based method can sensitively and selectively detect Hg^{2+} ions in complex samples such as blood.

The application feasibility of the developed fluorimetric analysis method with DMSO-containing Cu NCs was explored for sensing Hg^{2+} ions in human blood samples by comparing with traditional method such as inductively coupled plasma emission spectrometer (ICP) (Table 1). It should be noted that the measured Hg^{2+} concentrations are close to those obtained

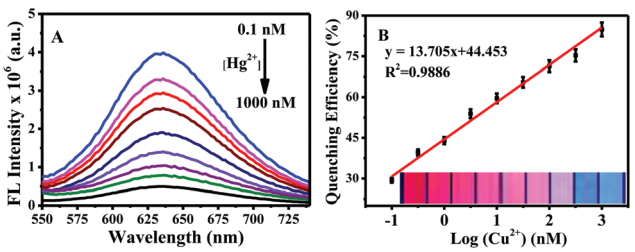


Fig. 5 (A) FL spectra of test strips with DMSO-containing Cu NCs in sensing Hg^{2+} ions of different concentrations, and (B) the calibration curve of the FL quenching efficiencies *versus* the logarithmic Hg^{2+} concentrations in the buffer (inset: the photographs of testing solutions under UV light).

Table 1 Practical analysis of Hg^{2+} ions in blood samples

Samples	ICP (nM)	Detected (nM)	RSD (%) (n = 5)
1	0.79	0.74 ± 0.019	2.56
2	0.63	0.67 ± 0.014	2.07
3	0.76	0.83 ± 0.016	1.89

by the classic ICP method, showing the relative standard deviations ranging from 1.89% to 2.56%. Therefore, the DMSO-containing Cu NC-based detection method is reproducible and accurate and shows great potential for applications in the analysis of Hg^{2+} in practical samples.

Conclusions

In summary, fluorescent Cu NCs dispersed in water containing DMSO exhibited enhanced red fluorescence and improved environmental stability unexpectedly. A solid-state fluorimetric analytical method with test strips was further fabricated using Cu NCs for detecting Hg^{2+} ions in blood. In particular, the advantageous introduction of DMSO could enable Cu NCs coated on test strips with stable red fluorescence for probing Hg^{2+} ions in the complicated blood samples, with the Hg^{2+} level down to about 0.030 nM. The developed fluorimetric method with test strips promises wide practical applications for monitoring Hg^{2+} ions with high selectivity and sensitivity in the clinical diagnosis, food safety, and environmental monitoring fields. More importantly, this discovery may open new avenues towards extensive applications for the design of different noble metal probes such as Cu NCs with solvent-improved fluorescence and environmental stability. However, the detailed mechanism should be investigated in future studies.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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