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COMMUNICATION

Visual test of subparts per billion-level copper(II) by Fe₃O₄ magnetic nanoparticle-based solid phase extraction coupled with a functionalized gold nanoparticle probe†

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By combining Fe₃O₄ magnetic nanoparticle-based solid phase extraction with a gold nanoparticle-based visual test, a novel method was developed for the field assay of Cu(II) in environmental water at subparts per billion-levels within 30 min. When a 200 mL water sample was treated with 12.5 mg L⁻¹ Fe₃O₄ nanoparticles by the proposed procedure, the detection limit with the naked eye was 0.2 µg L⁻¹ Cu(II). The proposed method is very specific to Cu(II), with tolerance against at least 100-fold amounts of other environmentally relevant metal ions except for Hg(II) (25-fold), and was successfully applied to the detection of trace Cu(II) in tap water, river water, and treated wastewater, and results agreed well with that determined by inductively coupled plasma-mass spectrometry (ICP-MS).

Copper is an essential trace element for living things, and functions in over thirty enzyme reactions in organisms, such as the metabolism of carbohydrate and lipid substances.¹ Insufficient and excess amounts of copper can both threaten human health.² Copper is not very biodegradable, and can accumulate in organisms along the food chain, resulting in significant side-effects for organisms if it accumulates to a certain concentration.³ To control the adverse environmental effects of copper ions [Cu(II)], sensitive methods to quantify trace or ultra-trace levels of Cu(II) in water are required.

Although traditional instrumental methods have high sensitivity and selectivity,⁴ most of them require strict operating conditions and complex or expensive instruments, which limit their application in field analysis. As an alternative, visual or photometric tests based on gold nanoparticles (AuNPs) have attracted great interest very recently. Jiang's group introduced a "click chemistry" method for the visual detection of 0.05 mM Cu(II) by the naked eye.⁵ Gooding *et al.*

discovered that L-cysteine modified AuNPs (Cys-AuNPs) respond to copper ions selectively, which was used for the visual detection of 0.01 mM Cu(II).⁶ Recently, Qu's group realized visual detection of 1 µM copper using magnetic silica nanoparticles clicked onto multi-walled carbon nanotubes,⁷ and AuNPs modified with glutathione (GSH) or 11-mercaptopundecanoic acid were applied as probes to determine Cu(II) based on fluorescence quenching.⁸

Although the probes described above overcome the requirement of strict control of detection conditions, special testing equipment such as a UV-vis or fluorescence spectrophotometer are needed to achieve low detection limits. The AuNP-based visual test requires no detection instruments, but the sensitivity of the currently available probes failed to detect Cu(II) in environmental water at µg L⁻¹ levels. Besides exploiting more sensitive probes, introducing an appropriate separation and preconcentration procedure prior to detection is a very efficient alternative to solve this problem.⁹ Recently, solid phase extraction with magnetic nanomaterials as adsorbents was demonstrated to have many marked advantages in both the removal and preconcentration of heavy metals.¹⁰ In addition to the reduced extraction equilibration time due to the nano-size of the extraction phase, the magnetic character makes it easy to implement the separation of the solid extraction phase from the liquid sample phases under a magnetic field, which greatly improved the efficiency of the entire analysis.

Herein, Fe₃O₄ NP-based magnetic-solid phase extraction (M-SPE) was coupled with a gold nanoparticle-based visual probe for the field testing of trace Cu(II) in water samples.

Scheme 1 Schematic representation of the proposed method for Cu(II) detection.

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As shown in Scheme 1, the entire process consists of two steps, the M-SPE and the chromogenic reaction. In the M-SPE, ammonium pyrrolidinedithiocarbamate (PDC) was added to the sample to complex with Cu(II), and the formed positively charged $[\text{Cu}(\text{PDC})]^+$ was anchored onto the surface of negatively charged Fe_3O_4 NPs by electrostatic interactions at an appropriate acidity. In the presence of a magnetic field, the Fe_3O_4 NPs loaded with Cu(II) were collected at the bottom of the container. In the chromogenic reaction step, the $[\text{Cu}(\text{PDC})]^+$ adsorbed on the Fe_3O_4 NPs acted as an adsorbent to specifically associate with Cys–AuNPs, to form the nanocomposite (Fe_3O_4 NPs– $[\text{Cu}(\text{PDC})]$ –Cys–AuNPs).⁶ Under the magnetic field, the nanocomposite collected at the bottom of the conical glass tube, which gave rise to the reduction of the red color of the upper aqueous Cys–AuNP solution. The Cu(II) concentration in the sample can be assayed by testing the reduction of the red color of the supernatant solution with the naked eye, or by measuring the absorbance change at 520 nm wavelength (ΔA_{520}).

In our experiments, Fe_3O_4 NPs and Cys–AuNPs were prepared through a co-precipitation¹¹ and a reduction method¹² respectively, and their characterisation (transmission electron microscopy images and the magnetic hysteresis curve) is shown in Fig. S1.† Factors that influence the M-SPE were optimized to enhance the extraction efficiency and thus the method sensitivity. The extraction efficiency was evaluated based on the variation of absorbance at 520 nm (ΔA_{520}) in the colorimetric test. A previous study showed that in M-SPE the target analytes associate onto the extractant surface mainly through electrostatic interaction.¹³ Since the charges of the analytes and nanomaterials are usually dependent on the solution acidity, the solution pH can affect the extraction efficiencies significantly. Herein, the sample pH influenced both the surface charge of the Fe_3O_4 NPs, and the complex formed between the Cu(II) ions and PDC. As shown in Fig. 1a, the value of ΔA_{520} reached a maximum at pH 8.0. In strongly acidic solutions, the weak organic acid PDC could not easily dissociate to form a complex with Cu(II), and the charge of the Fe_3O_4 NPs surface was positive (Fig. 2). Thus the positively charged Cu(II)

Fig. 2 Zeta potentials of Fe_3O_4 NPs and Cys–AuNPs with varying pH.

ions were barely adsorbed onto the Fe_3O_4 NPs. In strongly basic conditions, however, the Fe_3O_4 NPs surface was negatively charged and Cu(II) was present mainly in the neutral form of $\text{Cu}(\text{OH})_2$, which is unfavourable for the adsorption of Cu(II) onto the Fe_3O_4 NPs surface.

Commonly used complex reagents for Cu(II) such as PDC and sodium diethyldithiocarbamate (DDTC) were tested, and it was found that PDC is more favourable for complexing with Cu(II) to form a water-soluble and colorless complex, which in weakly alkaline conditions is positively charged and can be adsorbed onto the negatively charged Fe_3O_4 NPs surface (Fig. 2). The PDC concentration was optimized in the range of 0–25 μM , and the result depicted in Fig. 1b indicates that the highest ΔA_{520} , and thus extraction efficiency, was obtained at 2.5 μM PDC. The reduction of the ΔA_{520} value at higher concentrations of PDC might be attributed to the formation of the neutral $[\text{Cu}(\text{PDC})_2]$ complex, which prohibited their adsorption onto the negatively charged Fe_3O_4 NPs, and also prohibited complexation with the cysteine coated on the AuNPs, which is essential in the following chromogenic reaction step. The Fe_3O_4 NPs concentration was optimized in the range of 2.5–50 mg L^{-1} , and results shown in Fig. 1c indicate that the ΔA_{520} value, representing extraction efficiency, increased with the increase of Fe_3O_4 NPs concentration from 2.5 mg L^{-1} to 12.5 mg L^{-1} . Higher concentrations of Fe_3O_4 NPs caused a slight decrease in the sensitivity of the method, which might be ascribed to the enhanced sorption of Cys–AuNPs in the following chromogenic test step.

The addition of inert inorganic salts such as NaCl, NaNO_3 , and NaClO_4 had little impact on the extraction efficiency, but could reduce equilibration time as a result of the salt effect.¹⁴ As illustrated in Fig. 1d, the ΔA_{520} value increased gradually with NaCl concentration up to 1.0% (w/v), and then levelled off. The effect of sample volume was studied in the range of 50–1000 mL, and results showed that the ΔA_{520} value increased with sample volume up to 750 mL (Fig. S2†). As a large sample volume causes inconvenience in extraction operation, a 200 mL sample was selected in this study. Additionally, the shaking rate and time were taken into account to pursue a higher sensitivity (Fig. S3†).

To sum up, the optimized M-SPE procedure was as follows: into 200 mL water samples, adjusted to pH 8.0, were added 12.5 mg L^{-1} Fe_3O_4 NPs, 1% NaCl (w/v), and 2.5 μM PDC. Then the mixture was shaken at 200 rpm for 10 min, and the Fe_3O_4 NPs loaded with Cu(II) were magnetically separated and transferred into a 10 mL centrifugal tube.

Apart from the M-SPE conditions, the chromogenic procedure was also optimized. The results (Fig. S4†) demonstrated that the

Fig. 1 Effect of sample pH (a), PDC concentration (b), Fe_3O_4 NPs concentration (c) and NaCl concentration (d) on the extraction efficiency of Cu(II), represented by ΔA_{520} . Sample: 100 mL of Cu(II) at a concentration of 2.0 $\mu\text{g L}^{-1}$ (a) or 1.0 $\mu\text{g L}^{-1}$ (b, c and d); extraction time and shaking rate: 30 min at 250 rpm; separation: 3 min by a common permanent magnet; chromogenic reagent: 7.5 nM Cys–AuNPs; desorption: ultrasonic (10 min).

Fig. 3 The photographic image (above) and the values of ΔA_{520} (below) of the final test mixture for various metal ions by the proposed method. The concentrations were $1.0 \mu\text{g L}^{-1}$ for Cu(II) , $25 \mu\text{g L}^{-1}$ for Hg(II) , and $100 \mu\text{g L}^{-1}$ for other ions.

highest detection sensitivity was reached using 7.5 nM AuNPs and 0.1 M tris-borate acid buffer ($\text{pH } 8.5$), with an ultrasonic mixing time of 10 min and a magnetic separation time of 2 min .

Under the above optimized conditions, the method selectivity, sensitivity, and reproducibility were evaluated using standard solutions. Fig. 3 indicates that the environmentally relevant metal ions, including $100 \mu\text{g L}^{-1}$ each of K(I) , Ca(II) , Mg(II) , Al(III) , Co(II) , Zn(II) , Mn(II) , Cd(II) , Pb(II) , and $25 \mu\text{g L}^{-1}$ of Hg(II) did not interfere with the detection of $1.0 \mu\text{g L}^{-1}$ Cu(II) , demonstrating that the proposed method was quite specific to Cu(II) .

Fig. 4 indicates that ΔA_{520} showed a good linear relationship with Cu(II) concentration in the range of $0.2\text{--}1.0 \mu\text{g L}^{-1}$, with a linear regression equation of $\Delta A_{520} = 0.2876 C_{\text{Cu(II)}} + 0.2379$, and a correlation coefficient (R^2) of 0.993 . The upper solution was nearly colorless when the Cu(II) concentration was over $2.0 \mu\text{g L}^{-1}$. The detection limit with the naked eye was $0.2 \mu\text{g L}^{-1}$ Cu(II) , which was reduced to $0.06 \mu\text{g L}^{-1}$ (3σ , σ = standard deviation of eleven successive measurements of blank solution) by measuring with a multimode microplate spectrophotometer (Varioscan Flash, Thermo). To the best of our knowledge, this is the lowest concentration reported for Cu(II) detection with the naked eye. The precision was evaluated by testing five standard solutions with $0.5 \mu\text{g L}^{-1}$ Cu(II) . A similar color-change was observed with the naked eye, and the relative standard deviation of ΔA_{520} by a spectrophotometer

Fig. 4 The absorbance change (ΔA_{520}) and photographic images obtained at different concentrations of Cu(II) under the optimized assay conditions.

Table 1 Determination of Cu(II) concentration ($\mu\text{g L}^{-1}$, mean $\pm$ SD, $n = 3$) in real samples

Samples	This method	ICPMS
Tap water	1.04 ± 0.19	0.97 ± 0.08
River water	1.09 ± 0.17	1.05 ± 0.03
Treated wastewater	0.83 ± 0.05	0.79 ± 0.11

(Varioscan Flash, Thermo) was 5% , demonstrating the satisfactory repeatability.

The applicability of the proposed method was evaluated by testing Cu(II) in three real samples including tap water, river water and treated wastewater. A visual test using the proposed method indicates that the Cu(II) content in all the three water samples was above $0.8 \mu\text{g L}^{-1}$, and thus the samples were diluted 3 times with reagent water to determine the Cu(II) concentration using a spectrophotometer. The results (Table 1) obtained using the proposed method agreed well with those by inductively coupled plasma mass spectrometry (ICPMS, Agilent 7500ce), demonstrating that the proposed method is applicable for the accurate determination of ultra-trace Cu(II) in real environmental water samples.

Conclusions

In summary, by coupling Fe_3O_4 NP-based M-SPE with Cys-AuNP-based visual detection, a rapid method was developed for detecting ultra-trace amounts of Cu(II) with the naked eye. As the Fe_3O_4 NP extractant was quickly and effectively isolated from the sample solution by a permanent magnet, it took less than 30 min to finish the whole analytical process, including extraction and chromogenic reaction. The proposed method exhibited high sensitivity and selectivity toward Cu(II) , and was successfully applied to detecting Cu(II) in real water samples. This work further demonstrated that coupling with a sample pretreatment procedure is a very efficient approach to enhancing the selectivity and sensitivity of the AuNP-based visual test, which is essential for its application in real sample analysis. Furthermore, this proposed method is very suitable for field analysis as the equipment required is very simple. It is expected that by introduction of a proper complex reagent and modified AuNPs, this approach can be extended to test other metal ions.

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