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A novel phosphatidylserine-functionalized AuNP for the visual detection of free copper ions with high sensitivity and specificity

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In this paper, we have reported on novel phosphatidylserine-functionalized AuNPs (gold nanoparticles) for the visual detection of Cu^{2+} by employing phosphatidylserine for Cu^{2+} recognition and AuNPs for signal generation. The phosphatidylserine (1,2-dioleoyl-*sn*-glycero-3-phospho-L-serine, DOPS) was covalently assembled on the AuNP surface to obtain DOPS-functionalized AuNPs. It was demonstrated that DOPS-functionalized AuNPs could specifically bind Cu^{2+} and lead to the aggregation of AuNPs, which gave rise to a colour change from wine-red to blue and a new absorption band around 650 nm. This provides a sensing platform for the simple, rapid and field portable colorimetric detection of Cu^{2+}. By using the sensing platform, a selective and sensitive visual biosensor for the detection of Cu^{2+} was developed. The proposed biosensor has outstanding analytical advantages such as good stability, relatively high sensitivity, low cost and short analysis time. It can be used to detect concentrations of Cu^{2+} as low as 30 μM in river water by observation with the naked eye and of 1.55 μM Cu^{2+} in river water by UV-visible spectrophotometry, within 10 min and with a recovery of 98–103% and a relative standard deviation (RSD) < 4% (n = 6). The proposed biosensor is promising for on-site detection of trace Cu^{2+} in clinical diagnosis or environmental monitoring.

1 Introduction

Copper is one of the essential elements responsible for maintaining the homeostasis of living organisms in various biological processes, and it has potent redox activity and a propensity to engage in the catalytic activity of various enzymes.[1] However, uncomplexed Cu^{2+} is toxic to cells.[2,3] Indeed, the total copper ion concentration in healthy cells is generally around 100 μM, but most of this is present in complexed form in compounds. The free copper ion concentration in cells is on average less than one free ion per cell.[4] An excessive intake of copper is connected to a variety of neurodegenerative ailments, and can also cause damage to the liver and kidneys.[5,6] Accordingly, the US Environmental Protection Agency (EPA) has established a safe limit of 1.3 ppm (~20 μM) for copper in drinking water.[7] For the reasons mentioned above, it is thus crucial to monitor the concentrations of free copper ions (Cu^{2+}) in the human body and in the environment.

Traditional analytical techniques for Cu^{2+} include atomic absorption spectrometry, atomic emission spectrometry and inductively coupled plasma mass spectroscopy (ICP-MS), all of which require sophisticated and expensive equipment, which makes on-site and real time detection difficult.[8] The previously mentioned limitations of traditional analytical techniques have pushed researchers to develop novel methods for the rapid, sensitive and specific detection of Cu^{2+} *in situ*. In conjunction with this goal, various methods including optical and electrochemical sensors have been developed for the rapid or on-site detection of free copper ions.[7,9–25] The visual method in particular has received increasing attention because of its outstanding analytical advantages, including portability, low cost, short detection time, and lack of requirement for sophisticated equipment, which meet the needs of on-site detection. Currently, most colorimetric sensors previously reported have employed organic dyes for both Cu^{2+} recognition and for signal generation.[14,15,19–25] For colour signal generation, metallic nanoparticles such as gold nanoparticles (AuNPs) are particularly attractive because they possess much higher extinction coefficients in comparison with organic dyes, which allow more sensitive colorimetric detection of trace analytes. More importantly, metallic nanoparticles display distance-dependent optical properties.[26] For example, the dispersed AuNPs are red in colour, while the aggregated ones are purple or blue. Based on this optical property, many colorimetric sensing methods have been developed for the specific and visual detection of various toxic metallic ions including Cu^{2+}, Hg^{2+}, K^{+}, and Pb^{2+} by using AuNPs as a colour signal generator and metallic ion-

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specific oligonucleotides (DNAzyme or aptamer) or organic dyes as the recognition probe.[9–12,27–34] So far, some cleavage-based DNAzyme/AuNPs systems have been successfully developed for the visual detection of Cu^{2+};[9] however, the method based on DNAzyme has a relatively high background and poor stability because, in samples, the cleavage-based DNAzyme system is vulnerable to interference that can cause non-specific nucleic acid cleavage.

Phosphatidylserine (PS) is a class of negatively charged phospholipid found in a moderate abundance of 5–10 mol% in eukaryotic membranes.[35] It directs the binding of proteins that bear C2 or gamma-carboxyglutamic domains and contributes to the electrostatic association of polycationic ligands with cellular membranes. The loss of PS asymmetry is an early indicator of apoptosis and serves as a signal to initiate blood clotting.[36] Not long ago, Monson *et al.* reported that one of the phosphatidylserine molecules, 1,2-dioleoyl-*sn-glycero*-3-phospho-L-serine (DOPS), could be used for specifically and reversibly binding Cu^{2+} with extremely high affinity through controlling the pH.[35] Their experimental results implied that DOPS is an excellent recognition molecule for detecting Cu^{2+}. Stimulated by their experimental results, in this research we developed a novel biosensor for the visual determination of Cu^{2+} with high sensitivity and specificity by using AuNPs as a signal generator and PS as the recognition probe, with the object of providing a convenient method for the rapid and on-site detection of free copper ions in living cells or in water.

2 Materials and methods

2.1 Reagent and materials

Cetyltrimethylammonium bromide (CTAB), sodium borohydride ($NaBH_4$) and ascorbic acid were purchased from Sinopharm Chemical Reagent Beijing Co., Ltd (China). Gold chloride acid ($HAuCl_4$) was purchased from Aladdin Industrial Corporation (Shanghai, China). The 99.999% copper sulfate pentahydrate ($CuSO_4 \cdot 5H_2O$) was purchased from Sigma-Aldrich Co., Ltd (China). The 1-palmitoyl-2-oleoyl-*sn-glycero*-3-phosphocholine (POPC) and DOPS were purchased from the Shanghai Advanced Vehicle Technology Company (China). The water used in this experiment was Milli-Q water (18.2 MΩ cm^{-1}). A TU-1950 UV-visible spectrophotometer (Persee, China) was used to obtain the UV-visible spectra, and a H-9000NAR transmission electron microscope (Hitachi, Japan) was used to obtain the transmission electron microscopy (TEM) image.

2.2 Preparation of CTAB-capped AuNPs

The CTAB-capped AuNPs were synthesized by using a seed mediated growth method. Firstly, CTAB stabilized gold seed particles were prepared by chemically reducing 140 μL of a mixture of 0.25 mM $HAuCl_4$ and 0.08 M CTAB with 9 μL of ice-cold 0.1 M $NaBH_4$ under full stirring for 2 min and then the mixture was kept at room temperature for 2 hours. At the same time, the "growth" solution was prepared by adding 5 mL of 2 mM $HAuCl_4$ to 40 mL of 0.02 M CTAB solution under full stirring. Then, 3 mL of 0.1 M ascorbic acid was added in to the growth solution, and 2.5 mL of 40-fold diluted as-prepared CTAB-protected gold seeds was added subsequently. The whole solution was then aged for 24 h in a 25 °C water bath to produce CTAB-capped AuNPs. The size of the as prepared AuNPs was about 15 nm and the solution was red in colour.

2.3 Preparation of DOPS-functionalized AuNPs

The DOPS-functionalized AuNPs used for the visual detection of Cu^{2+} were prepared using the lipid bilayer covalent method.[37] DOPS was covalently assembled on the surface of AuNPs by exchange or displacement of CTAB at the AuNPs surface with DOPS. Firstly, about 2.0 mL of 20 mM DOPS/POPC (DOPS : POPC = 12 : 88, mole ratio) chloroform solution was placed in a conical vial, and then the solution was evaporated to form a dried DOPS/POPC film on the inner wall of the vial by using a rotary evaporator. The dried film was further dried under high vacuum overnight to completely remove the chloroform. Then, about 2.0 mL water was added into the vial to dissolve the DOPS/POPC film for 1 hour at 40 °C with gentle agitation. Subsequently, the solution was treated under N_2 for 5 min to degas, followed by sonication for 5 min at a power of 10 W. The translucent solution obtained was centrifuged for 30 min at 12 000 rpm and the supernatant solution was further filtered through a 0.22 μm filter to obtain the DOPS/POPC liposome solution (filtrate).

Secondly, 1 mL of the previously prepared CTAB-capped AuNP solution was placed in a vial and was centrifuged at 12 000 rpm for 30 minutes, and then the supernatant solution was discarded. The residue (CTAB-capped AuNPs) was re-dispersed in 1 mL of deionized water and was centrifuged at 12 000 rpm for 30 minutes, and the supernatant solution was also discarded. Then, 100 μL of Milli-Q water and 2.5 μL of the previously prepared DOPS/POPC liposome solution were added to the residue in the vial (CTAB-capped AuNPs) and the mixture was incubated for 5 hours at 47 °C to modify the DOPS/POPC on the surface of AuNPs. The resulting solution was centrifuged at 8000 rpm for 30 min and the supernatant solution was discarded. The residue in the vial (the resulting DOPS-functionalized AuNPs) was re-dispersed in 400 μL of 2 mM 2-(*N*-morpholino)-ethanesulfonic acid (MES)–Tris (MES : Tris = 1 : 2, mole ratio) buffer solution (pH 8.0) to obtain a DOPS-functionalized AuNP solution. The resulting DOPS-functionalized AuNP solution was red in colour. This solution was stored at 4 °C and used for the detection of Cu^{2+} within four months.

2.4 Visual detection of Cu^{2+}

For the detection of Cu^{2+}, about 0.5 mL of Cu^{2+} standard or sample solution was taken and added into the same volume of DOPS-functionalized AuNP solution. The mixture was then incubated for 10 min at 40 °C, and then the colour change of the mixture was recorded with a digital camera and the absorption spectrum of the mixture was determined with a 1 cm path length cell using a spectrophotometer. The concentration of Cu^{2+} was quantified based on the absorption ratio (A_{650}/A_{535}) or naked eye observation.

3 Results and discussion

3.1 Detection principle of the DOPS-functionalized AuNPs

As mentioned previously, it was reported that DOPS can specifically bind Cu^{2+} with an extremely high affinity at basic pH (pH 8.0) (ref. 35) and that AuNPs are a particularly attractive colour signal generator. Thus, a novel colorimetric biosensor for the visual and specific detection of trace Cu^{2+} by using DOPS for Cu^{2+} recognition and AuNPs for signal generation was designed. As shown in Fig. 1, the DOPS/POPC liposome was covalently assembled on the surface of the AuNPs with the lipid bilayer covalent method.[37] The resulting DOPS-functionalized AuNPs can be stably dispersed in solution and display distance-dependent optical properties. Its solution is wine-red in colour and has a surface plasma resonance absorption peak at 535 nm (see Fig. 2A). In the presence of Cu^{2+}, DOPS assembled on the AuNPs surface can specifically bind Cu^{2+} to form PS–Cu^{2+}–PS complex at pH 8.0, which leads to the aggregation of AuNPs, as illustrated in Fig. 1. The aggregation of AuNPs resulted in a colour change from wine-red to blue and a new absorption band was seen at around 650 nm, which provided a sensing platform for the simple, rapid and field-portable colorimetric detection of Cu^{2+}.

In order to verify whether the biosensor worked as expected, the UV-visible spectra and TEM image of the DOPS-functionalized AuNPs was measured in the absence and presence of Cu^{2+}, respectively. The results are shown in Fig. 2A, and in the absence of Cu^{2+}, the DOPS-functionalized AuNP solution was wine-red in colour and only had an absorption peak at 535 nm in the UV-visible spectra. In contrast, in the presence of Cu^{2+}, the AuNP solution became blue-gray and the absorbance at 535 nm decreased and a new absorption band around 650 nm appeared, which indicated that DOPS-functionalized AuNPs aggregated in solution because of the formation of a Cu^{2+}–DOPS complex. The aggregation of the DOPS-functionalized AuNPs was also verified by the TEM image (see Fig. 2B and C).

For a rapid and field-portable colorimetric method, the rate of interaction between Cu^{2+} and DOPS-functionalized AuNPs is another key consideration. In the experiment, it was found that the rate of reaction between Cu^{2+} and DOPS-functionalized AuNPs was influenced by the reaction temperature. As the results in Fig. 3A show, the rate of reaction increased with an increase in the reaction temperature in the range of 25–40 °C, and then the rate of reaction tended to decrease when the temperature was higher than 40 °C. Therefore, 40 °C was selected as the optimum temperature in this experiment. The relationship between reaction time and net absorption ratio ($\Delta A_{650}/A_{535} = A_{650}/A_{535}$ of standard or sample $- A_{650}/A_{535}$ of reagent blank) was investigated in detail by using a 250 μM Cu^{2+} standard solution. The results shown in Fig. 3B clearly revealed that the aggregation of AuNPs was completed within 10 min.

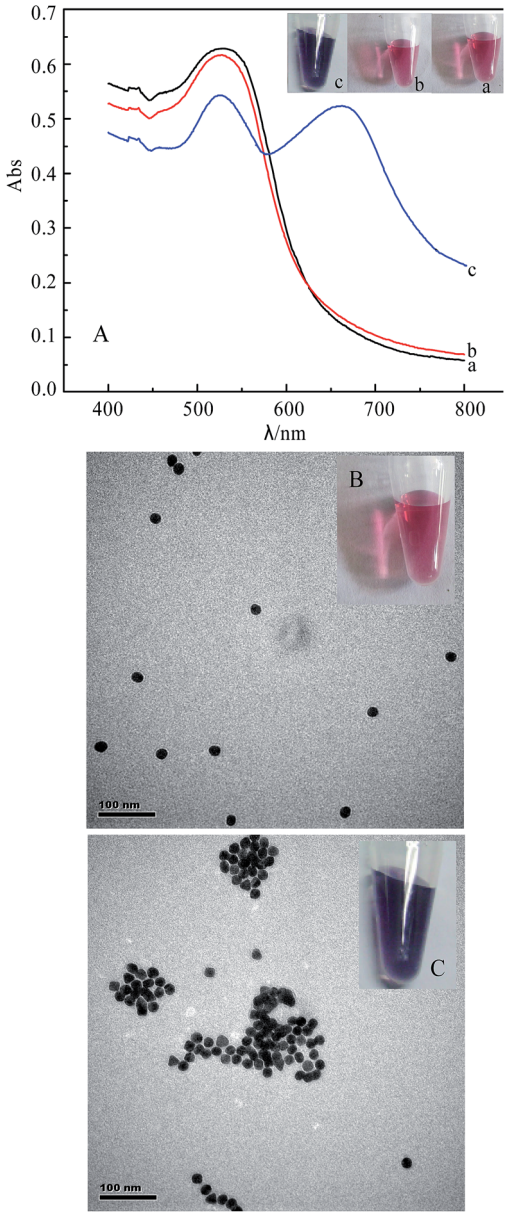

Fig. 2 (A) The UV-visible absorption spectra and photographs of AuNP solution. (a) CTAB-capped AuNP solution; (b) DOPS-functionalized AuNP solution; (c) DOPS-functionalized AuNP solution in the presence of 500 μM Cu^{2+}. (B) The TEM image of DOPS-functionalized AuNP solution. (C) The TEM image of DOPS-functionalized AuNP solution in the presence of 400 μM Cu^{2+}.

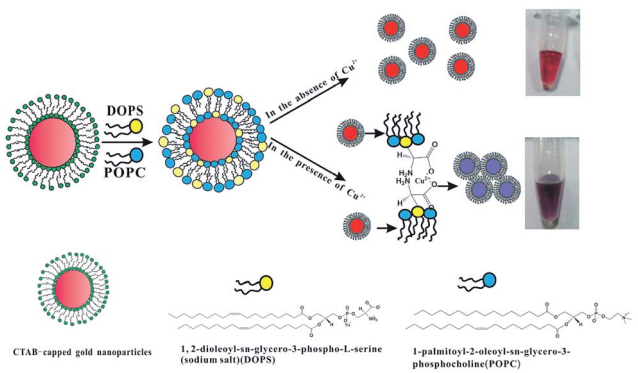

Fig. 1 Schematic illustration of the detection principle of the DOPS-functionalized AuNPs.

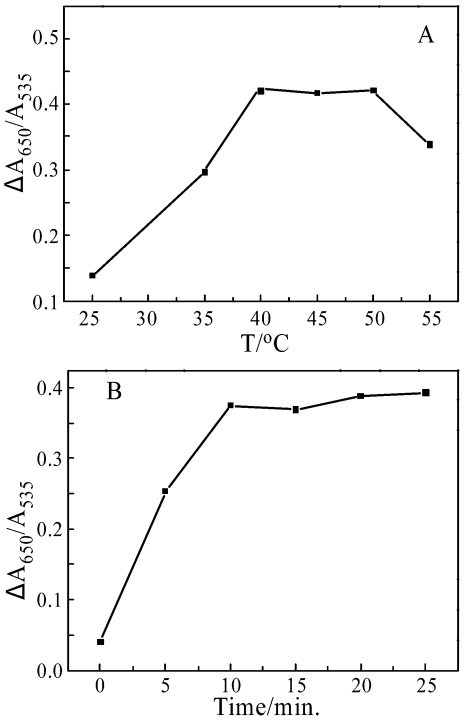

Fig. 3 (A) The temperature-dependent reaction rate between Cu^{2+} and the DOPS-functionalized AuNPs. The concentration of Cu^{2+} is 200 μM. (B) The relationship between Cu^{2+}-induced aggregation of DOPS-functionalized AuNPs and reaction time. The concentration of Cu^{2+} is 200 μM.

3.2 The selectivity of the DOPS-functionalized AuNPs

As is well known, there are various metallic ions existing in living cells or found in water samples, and it is possible that the existence of these metallic ions will interfere with the detection of Cu^{2+}. In order to investigate whether other metallic ions would disturb the detection of Cu^{2+}, various metallic ions including Al^{3+}, Ba^{2+}, Ca^{2+}, Fe^{2+}, Fe^{3+}, K^{+}, Mg^{2+}, Mn^{2+}, Na^{+}, Ni^{2+}, Pb^{2+}, and Zn^{2+} were used to test the specificity of the DOPS-functionalized AuNPs. As the results in Fig. 4 show, only Cu^{2+} can bind with the DOPS-functionalized AuNPs and this results in the aggregation of the AuNPs, which gives rise to a change in solution colour from wine-red to blue and a high net absorption ratio ($\Delta A_{650}/A_{535}$). No colour change and only a negligible net absorption ratio ($\Delta A_{650}/A_{535}$) were observed for other metallic ions, even if their concentrations were higher than that of Cu^{2+}, indicating that other metallic ions do not induce the aggregation of the DOPS-functionalized AuNPs and thus interfere with the detection of Cu^{2+}. The experimental results above reveal that the proposed biosensor, which is based on the DOPS-functionalized AuNPs, has excellent specificity.

3.3 The linear range, sensitivity and reproducibility of the proposed biosensor

As mentioned in the principle of detection, the DOPS modified on the AuNP surface was employed for Cu^{2+} recognition by the formation of a PS–Cu^{2+}–PS complex. Therefore, the density of

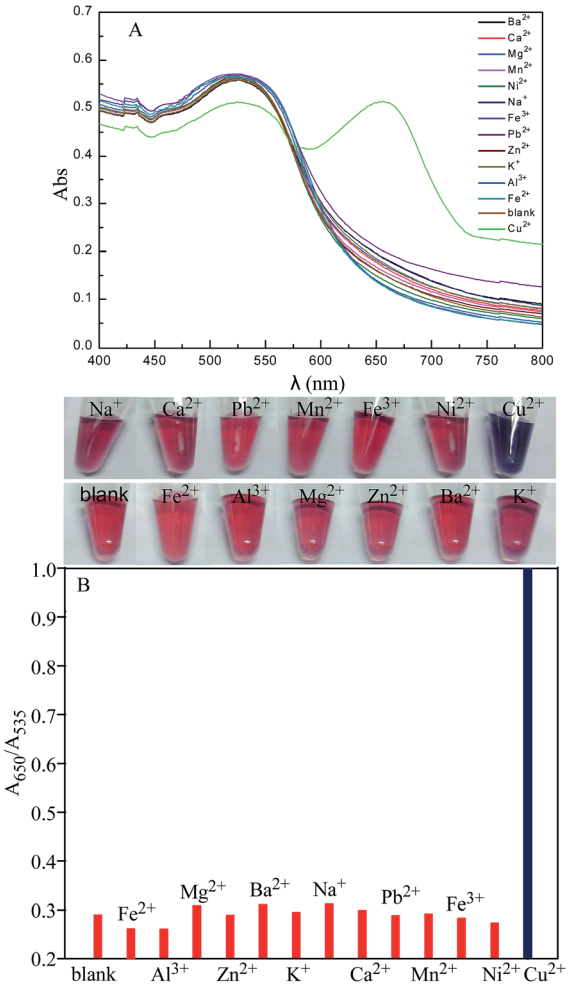

Fig. 4 The UV-visible absorption spectra (*A*), photograph and absorption ratio (A_{650}/A_{535}) (B) of the DOPS-functionalized AuNPs solution in the presence of 400 μM Cu^{2+} or 800 μM of other metal ions.

DOPS on the surface of AuNPs will substantially affect the aggregation of the DOPS-functionalized AuNPs, and thus the sensitivity of the biosensor, by influencing the formation of the PS–Cu^{2+}–PS complex. A high density of DOPS will lead to the formation of a PS–Cu^{2+}–PS complex at the same AuNP concentration and eventually decrease the sensitivity of the method. However, a low density of DOPS will weaken the ability to form the PS–Cu^{2+}–PS complex among the different AuNPs, and eventually decrease the sensitivity of the method as well. In this experiment, the density of DOPS on the surface of the AuNPs was controlled by adjusting the mole ratio of DOPS/POPC, and the results shown in Fig. 5 clearly indicate that the mole ratio of DOPS/POPC = 12/88 is the best selection. Monson *et al.* reported that DOPS can specifically bind Cu^{2+} to form a PS–Cu^{2+}–PS complex only at basic pH,[35] and our results verified this. As shown in Fig. 6, Cu^{2+} cannot induce the aggregation of the DOPS-functionalized AuNPs when the pH is lower than 6. When the pH is higher than 8, Cu^{2+} can quickly induce the aggregation of DOPS-functionalized AuNPs and lead to a colour change from red to blue within 10 min.

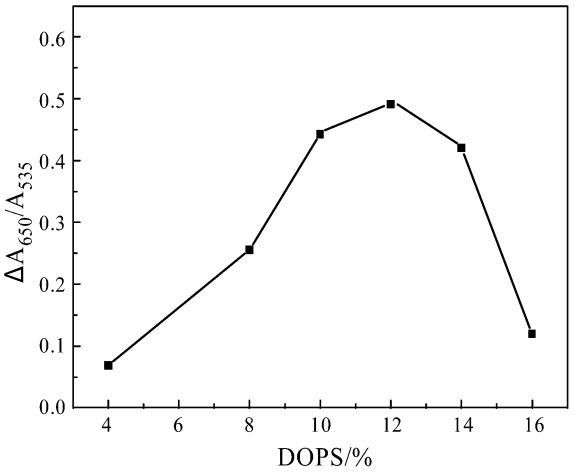

Fig. 5 The relationship between the surface density of DOPS on the AuNPs and the sensitivity of the proposed method. The concentration of Cu^{2+} is 250 μM.

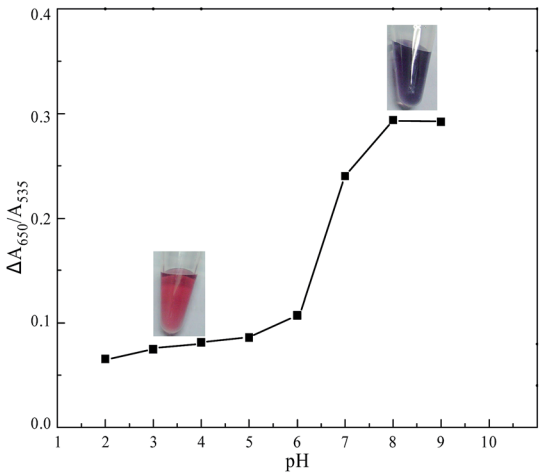

Fig. 6 The pH-dependent absorption ratio (A_{650}/A_{535}) and colour of the DOPS-functionalized AuNP solution in the presence of 200 μM Cu^{2+}.

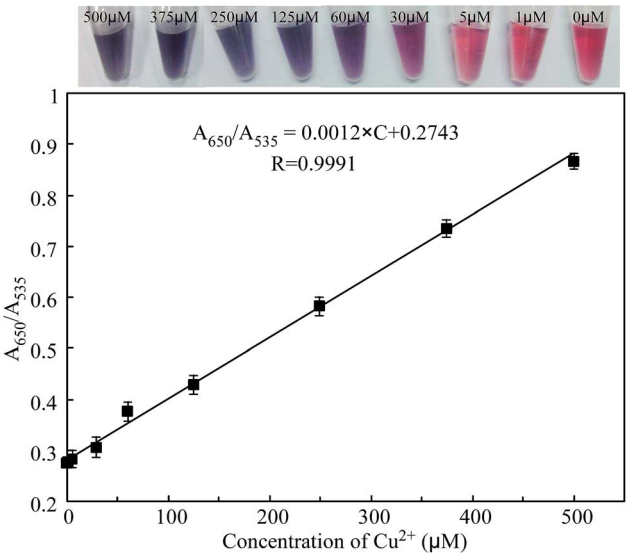

Fig. 7 Photographs and calibration curve for detecting Cu^{2+}. The concentrations of Cu^{2+} are 0.0, 1.0, 5.0, 30.0, 60.0, 125.0, 250.0, 375.0 and 500.0 μM.

Under the previously discussed optimum conditions, the aggregation of the DOPS-functionalized AuNPs induced by Cu^{2+} was monitored by UV-visible spectroscopy to obtain a calibration curve. As results in Fig. 7 show, upon the addition of Cu^{2+} from 0 to 500 μM, the absorbance of the AuNP solution at 535 nm decreased gradually and the absorbance around 650 nm obviously increased. At the same time, the colour of the AuNP solution changed from wine-red to purple and finally progressively to blue. As the photographs in Fig. 7 show, the colour change could be clearly observed with the naked eye when the concentration of Cu^{2+} was 30 μM, *i.e.*, the visual detection limit of Cu^{2+} was about 30 μM. The absorption ratio (A_{650}/A_{535}) showed a good linear relationship with the concentrations of Cu^{2+} in the range of 0 to 500 μM. The equation was: $A_{650}/A_{535} = 0.0012 \times C + 0.2743$ ($R = 0.9991$), where C is the concentration of Cu^{2+} (μM). The detection limit ($3\sigma/S$) was calculated to be 1.55 μM for Cu^{2+} and the RSD ($n = 5$) was calculated to be less than 2% for the detection of 10 μM Cu^{2+}.

As mentioned previously, so far several visual biosensors have been developed for the rapid and on-site detection of Cu^{2+}. However, most colorimetric sensors previously reported employed organic dyes for both Cu^{2+} recognition and for signal generation.[14,15,19–25] These sensors have relatively low sensitivity because the organic dyes have lower extinction coefficients in comparison with AuNPs. In comparison with the previous sensors, the proposed biosensor has outstanding analytical advantages, including good stability and specificity, relative high sensitivity, low cost and short analysis time, which makes this biosensor meet the requirements of on-site Cu^{2+} detection.

3.4 Detection of Cu^{2+} in a river water sample

The applicability and reliability of the biosensor developed was demonstrated by analysis of river water samples, which were collected from the Tingjiang river of Fujian, China. The river water was firstly filtered through a 0.22 μM membrane filter to remove any sediment, and then the amount of Cu^{2+} in the water was directly determined with our method by using UV-visible spectrometry. The results obtained were compared with the results obtained by ICP-MS, a standard method for Cu^{2+} determination. From the results shown in Table 1, the recovery was in the range of 97–102% and the RSD ($n = 6$) was smaller than 4%. The results obtained with our method were consistent with those of ICP-MS (see Table 1), which indicated that the proposed biosensor is reliable. The success of this study provides a promising approach for the rapid and on-site detection of free copper ions in living cells or water.

Table 1 The analytical results for Cu^{2+} in water samples collected from the Tingjiang river, Fujian, China

Sample	Added con. (μM)	Detected con. (μM)	Recovery (%)	RSD ($n = 6$)	ICP-MS[a] (μM)
1	0.0	—[b]	—	—	0.088
2	5.0	5.1	102	3%	5.11
3	20.0	20.1	101	4%	20.05
4	40.0	38.6	97	2%	40.12

[a] Data obtained with ICP-MS method. [b] "—" indicates that the concentration is lower than the detection limit.

4 Conclusions

In summary, DOPS-functionalized AuNPs were first synthesized. It was then demonstrated that the DOPS-functionalized AuNPs could specifically bind Cu^{2+} to induce the aggregation of AuNPs at basic pH. The aggregation of AuNPs resulted in a colour change from wine-red to blue and a new absorption band around 650 nm was found, which provided a sensing platform for the simple, rapid and field-portable colorimetric detection of Cu^{2+}. By using this sensing platform, a selective and sensitive visual biosensor for the detection of Cu^{2+} was developed. The proposed biosensor can be used to detect as little as 30 μM of Cu^{2+} in river water using naked-eye observation and 1.55 μM of Cu^{2+} in river water using UV-visible spectrophotometry, within 10 min and with a recovery of 98–103% and a RSD < 4% ($n = 6$). The proposed biosensor is promising for the rapid and on-site detection of trace Cu^{2+} in clinical diagnosis or in environmental monitoring.

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