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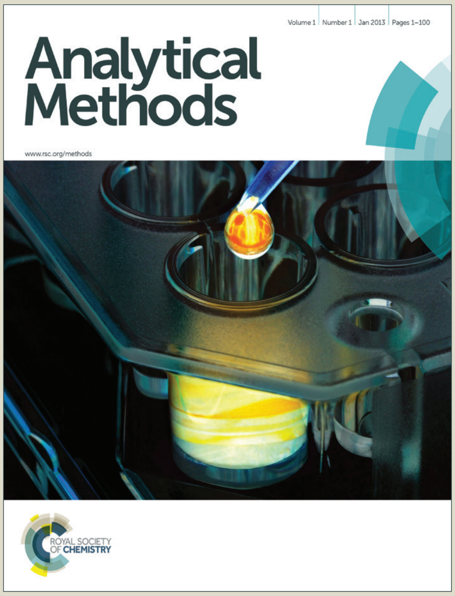

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Selective fluorescence turn-on sensing system for evaluation of Cu^{2+} polluted water based on ultra-fast formation of fluorescent copper nanoclusters

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We report on the rapid and selective fluorescence turn-on detection system for Cu^{2+} based on the formation of highly fluorescent copper nanoclusters (CuNCs). The facile detection of Cu^{2+} was achieved using a remarkable red colour emitting fluorescence upon addition of Cu^{2+}.

1. Introduction

Copper can be either healthy or unhealthy for humans based on the uptake amounts.[1-6] According to the US Environmental Protection Agency (EPA), the acceptable contaminant level for copper in drinking water should be lower than 1.3 ppm.[7] The excess level of Cu^{2+} intake will induce hypoglycemia, dyslexia, liver damage, or other diseases.[8] As a result, evaluation of the safety of Cu^{2+} is important for the environment. However, effective analytical methods such as ICP-MS, AAS and XRF for the detection of Cu^{2+} are expensive. For this reason, facile and cheap protocols are expected. One of the desired tools for the detection of Cu^{2+} is to utilise fluorescent sensors.[9-13] Organic sensors have been widely investigated for the detection of Cu^{2+},[14-16] although their synthesis methods usually involve complicated procedures, toxic starting materials and by-products.[17]

For the purpose of achieving low toxicity routes, fluorescent nanoparticles or nanoclusters have been employed for the recognition of Cu^{2+}, which provide environmentally friendly routes for analysis. Among these fluorescent sensors, the analysis mechanisms are mostly based on the quenching behaviour of Cu^{2+}, which are not favourable for the detection of pollutants because these probing systems demonstrate poor visible signal change.[18-20] Another problem exists for many reported fluorescent sensors. The signal change for other metal ions is less significant than the target metal ion but still comparable to it.[21-23] It would be worth investigating a sensor that describes better selectivity. Recently, due to their special molecular, fluorescent and low toxic properties, the application of nanoclusters has attracted more attentions.[24-36] Among the nanoclusters, CuNCs are the most cost effective, but their application is still limited because of the probes' natural instability. As a result, only a few examples CuNCs-products have been employed in sensing applications (see Table S1). However, none of these processes can be used for highly efficient turn-on detection of analytes. Stable CuNCs-products are difficult to obtain, which may prohibit the development of highly efficient probes. To the best of our knowledge, no study has been performed to make use of the formation process. If the synthesis process rather than the product of CuNCs is applied for sensing, the instability of CuNCs over time is no longer matters.

Herein, we employed the formation process of low toxic fluorescent CuNCs to develop selective Cu^{2+} turn-on sensor. The sensing mechanism is described in Scheme 1. After the combination of Cu^{2+} solution and the sensing system, highly bright red colour will be observed under a UV lamp due to the CuNCs formation induced fluorescence of CuNCs, which is similar to the fluorescence described on previous paper. [28] On the other hand, no fluorescence signal can be obtained by the introduction of other cations or anions. Thus, the signal change for Cu^{2+} will be extraordinary in this aspect. The sensing system was also developed for environmental pollution evaluation.

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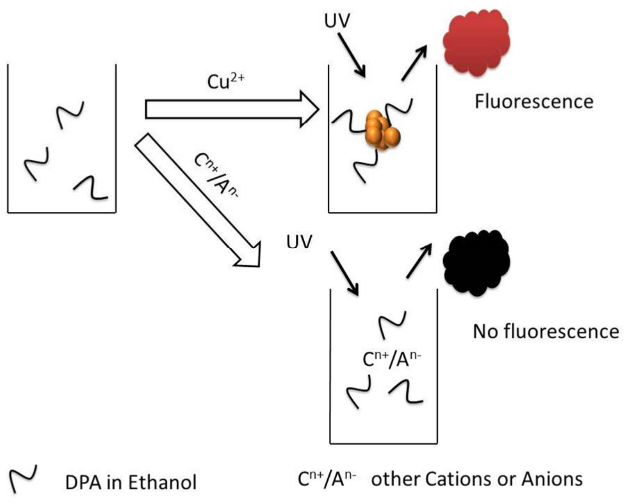

Scheme 1 Mechanism for sensing of Cu^{2+} based on ultra-fast formation of CuNCs by the assistance of DPA in ethanol.

2. Experiment

2.1. Chemicals and Apparatus

D-penicillamine (DPA) was obtained from Sigma-Aldrich (Korea). Ethanol and $Cu(NO_3)_2 \cdot 3H_2O$ were purchased from Samchun Reagent Company (Korea). All other reagents and metal salts were of analytical reagent grades. Deionized Water was used throughout all the experiments. UV-visible (UV-vis) absorption spectra were recorded using a Thermo Spectrophotometer (Hellios Zeta). Fluorescence spectra were collected on an FS-2 (Sinco). The X-ray photoelectron spectroscopy (XPS) measurements were carried out using a K-Alpha (Thermo Electron). Transmission electron microscopy (TEM) measurements were measured on a JEM-2100F high-resolution transmission electron microscope operated at 200 kV. The natural water samples were collected from the tap in our lab.

2.2. Fabrication of sensing system for standard samples

In a typical experiment, the sensor was prepared by dispersing 3.0 mg of DPA in 2 mL of ethanol (95%) solution. Then, 100 μL of testing samples were combined and shaken well. Immediately, the observation of bright red colour fluorescence under a 365 nm UV lamp indicated the formation of CuNCs. After the complete of the reaction after about one minute, the mixtures were shaken for 10 seconds to distribute the aggregates well before the fluorescence measurement.

2.3. Preparation of sensing system for safety evaluations

100 mL of Tap water was collected from our laboratory. The collected samples were filtered first. Then, 100 μL of HNO_3 was added. The water samples were carefully evaporated by heating to small volumes and diluted by deionized water. Assuming the original concentrations of all the samples for Cu^{2+} were 1.3 ppm, the final concentrations of Cu^{2+} were tuned to a same concentration to our detection limit by fixing the volumes. After that, the water samples were mixed with the designed sensing system to evaluate the pollution. The mixtures were measured by instrument and observed under a 365 nm UV lamp.

3. Results and Discussion

A previously reported paper[28] claims that it takes as long as 90 minutes for CuNCs to form when DPA is used in a pure water solution. In using a pure water system, it is difficult to design a sensor for Cu^{2+} due to the long reaction time. However, ethanol may greatly enhance the behaviour of 'formation induced fluorescence' based on the 'solvent induced formation' behaviour. According to a paper about the fabrication of fluorescent gold nanoclusters,[37] Au–thiolate was initially well dissolved in a water solution, and no fluorescence could be generated. However, in the presence of ethanol, the quantum yield of the fluorescence was greatly enhanced, from 0% to 15%, due to the mechanism of 'solvent induced formation'. This indicates that the Au–thiolate nanocluster formation much more easily in ethanol than in water. Similarly to gold nanoclusters, DPA protected CuNCs may be more easily formed in ethanol than in water due to the thiol-based structure of DPA. In this regard, we used ethanol to assist the reaction rate. Although we did not obtain fluorescence within minutes by using DPA systems in water, a bright red colour could be observed immediately with the assistance of ethanol, which indicats that fluorescent CuNCs were formed very quickly. The reaction time and fluorescence-time dependence are described in Table S1 and Fig. S1.

The optical characterizations for the properties of the typical product are described in Fig. 1. As depicted in Fig. 1a, the absorption spectra did not demonstrate the characteristic absorption peak, which could be caused by the surface plasmon resonance of Cu nanocrystals. The results reveal the formation of CuNCs rather than copper nanoparticles. The typical formed CuNCs exhibit excitation at 391 nm and emission at 673 nm. The XPS and TEM properties of the CuNCs-product are described in Fig. S2. An XPS survey spectrum (inset, Fig.S2a) shows that the samples are composed of all the expected elements C, O, N, S, and Cu. No characteristic satellite peak (Fig. S2a) around 942 eV implies that all the Cu^{2+} is reduced to Cu^0 in CuNCs. The peaks at 952.2 eV and 932.4 eV are assigned to Cu2p3/2 and Cu2p1/2, which are attributed to Cu (0) and similarly with CuNCs as reported in previous papers.[38] Finally, the TEM

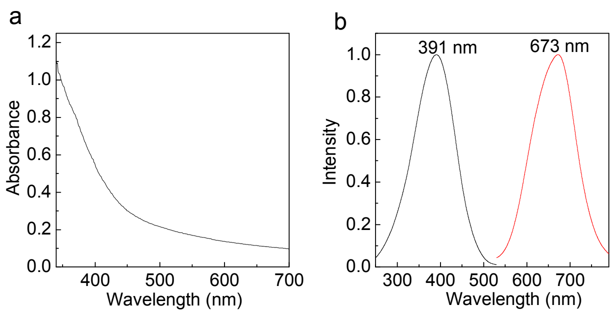

Fig. 1 The representative UV-vis absorption (a) and fluorescence excitation (left), emission (right) spectra (b) of the as-formed CuNCs.

image confirms the formation of small CuNCs. (Fig. S2b) The average size of CuNCs was determined to be 1.2 (±0.4) nm. On account of the formation process for CuNCs, the sensing systems of Cu^{2+} were evaluated by testing a variety of interferences including some cations and anions. Whether the introduction of other metal ions can induce the enhancement of fluorescence was studied first, see Fig. 2. Fig.2a describes the great enhancement of fluorescence by the combination of 10 ppm of Cu^{2+} with an excitation wavelength at 390 nm. Except for Cu^{2+}, no fluorescence could be noticed by the introduction of other metal ions including Ag^{+}, Au^{3+}, Hg^{2+}, Mn^{2+}, Fe^{3+}, Zn^{2+}, Ni^{2+}, Pb^{2+}, Na^{+}, the mixture of all other metal ions. In addition, the sensing system was also treated with a mixture of Cu^{2+} (10 ppm) and another metal ion such as Ag^{+}, Au^{3+} , Hg^{2+}, Mn^{2+}, Fe^{3+}, Zn^{2+}, Ni^{2+}, Pb^{2+}, Na^{+} or the mixture of other ions at 10 ppm. (Fig. 2b) There is little difference between the emission spectra in the presence of each mixture and single Cu^{2+}. The selectivity toward Cu^{2+} over other metal ions is also described in Fig. S3. It is noteworthy that the noise signal for other metal ions is not comparable to the high intensity of Cu^{2+} in our case. Sharp contasts between Cu^{2+} and other metal ions can be observed. This implies that the remarkable performance of Cu^{2+} is comparable to the results obtained by other papers.[39-41] It is possible that the present method can be used to detect Cu^{2+} with little interference by metal ions due to the great signal difference between Cu^{2+} and other metal ions. Additionally, the red colour emitting fluorescence can only be observed for Cu^{2+} under a UV lamp at 365 nm (Fig. 2c). The results are in good agreement with Fig. 2a and Fig. 2b. In addition, the influences for the sensing of Cu^{2+} by anions are demonstrated in Fig. S4. It can be seen that no anions induce significant fluorescence response for Cu^{2+}. Only a slight change can be observed in the presence of different anions due to the noise signal influence. Additionally, the fluorescence of the sensing system was observed in the presence of Cu^{2+} at different pH values (Fig. S5). It can be seen that fluorescence intensity shows an insignificant difference between pH 3 to 8. At pH 8, there is little changes in fluorescence possibly because, in our sensing system, OH^{-} is denatured in the presence of ethanol. On the other hand, most nanoclusters cannot be used in acid media because their fluorescence tends to be lost at low pH values.[42-44] This inhibits their application for solid sample analysis because there are always acid digestions involved. Notwithstanding, our sensing system would be promising for these applications due to an insignificant influence at low pH values. All these results indicate that Cu^{2+} sensing system based on the formation of CuNCs has potential for environmental applications.

Further, we quantitatively describe the fluorescence behaviour as a function of Cu^{2+} and the linearity of the current sensing system (Fig. 3). As indicated in Fig. 3a, when concentrations of Cu^{2+} are increased, the fluorescence emission intensity at 673 nm increases gradually. There is an excellent linear relationship between fluorescence intensity and concentration of Cu^{2+} within the range of ca. 0.95 – 6.35 ppm (Fig. 3b). However, the range can be tuned for higher concentrations of Cu^{2+} by using greater amounts of DPA because up to a certain extent, there is no maximum limitation for formation. The fitted linear data can be expressed as $Y = 3902X - 2556$ ($R^2 = 0.991$), where Y is the fluorescence intensity at 673 nm X is the concentration of Cu^{2+}. Under the current experimental conditions, the detection limit of Cu^{2+}, at the smallest response, is estimated to be 0.3 ppm, which is smaller than the EPA acceptable value, 1.3 ppm. The fluorescent photograph for different concentrations of Cu^{2+} is described in Fig. 3c. Though the exact concentration of Cu^{2+} is not obvious from our photograph, it can be expected that the pollution value, 1.3 ppm, may be easily monitored by a suitable design. Based on the current analysis method, the spike samples in the presence of various concentrations of Cu^{2+} were evaluated in the presence of 10 ppm of other metal ions (Table S2). The results suggest that the present sensing system shows promise for use to evaluate the safety of Cu^{2+} in aqueous water.

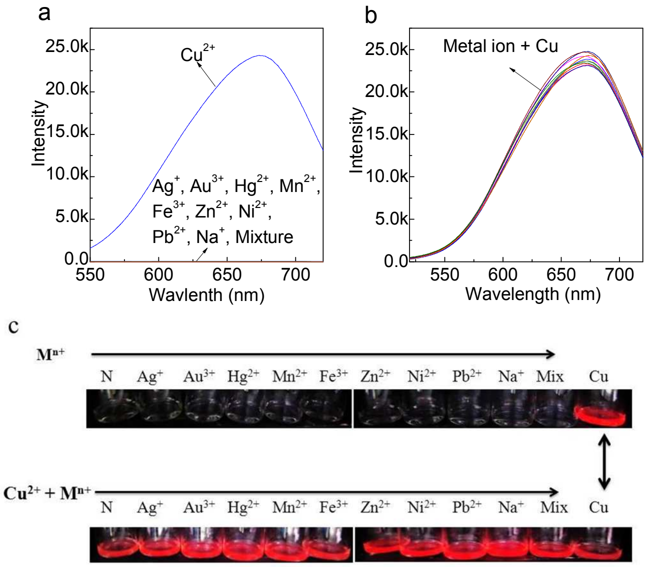

Fig. 2 Fluorescence emission spectra in the presence of various single metal ion at 10 ppm, metal ion mixture (a) and the addition of Cu^{2+} to each single metal ion, metal ion mixture (b) at 10 ppm; The corresponding photograph of the sensing system after the combination of different metal ions at 10 ppm under a 365 nm UV lamp (c).

After that, the sensing system for safety evaluation (< 1.3 ppm) was studied. The original concentrations of the samples are assumed as 1.3 ppm. Then, the final concentrations are fixed as the same concentration to our detection limit as described in 2.3. The purpose is that no obvious fluorescence could be observed under a 365 nm UV lamp in the presence of Cu^{2+} within safe value range. On the other hand, when the concentration of Cu^{2+} is beyond the acceptable value, the red colour fluorescent warning signal will be detected. To evaluate the performance of the method for pollution, we studied using spiked tap water samples. The fluorescence spectra and intensities were measured and compared (Fig. 4). Tap water samples containing different standard concentrations of Cu^{2+} were measured by instrument (Fig. 4a and Fig. 4b) and observed under a 365 nm UV lamp (Fig. 4c). For tap water, no fluorescence signal could be detected by instrument measurement or visual observation under the UV lamp. Therefore, we concluded that the concentration of Cu^{2+} in tap water is within the acceptable safety level. This is in agreement with the ICP-OES measurement (< 2 ppb) of enrichment of water samples we studied. On the other hand, unpolluted and polluted concentrations of Cu^{2+} were added to tap water to fabricate spiked natural water samples. Next, these samples were treated using the procedure in 2.3. Then, the spiked samples were evaluated using instrument and visiblilty tests. When the concentration of Cu^{2+} is lower than 1.3 ppm (Tap, Tap+0.6 ppm), no obvious signal is demonstrated. On the other hand, a red colour is observed under a UV lamp at 365 nm when the concentration of Cu^{2+} is equal or higher than 1.3 ppm. The method is fast, convenient and cost effective. These results indicate that the proposed sensor is promising for safety evaluation of Cu^{2+} in environmental samples.

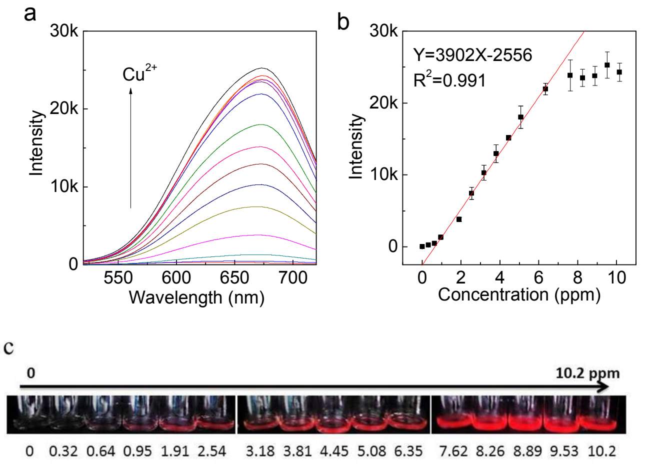

Fig.3 Fluorescence emission spectra (a) of the sensing system and the corresponding fluorescence intensities at 673 nm (b) in the presence of various concentrations of Cu^{2+}; The fluorescence photograph of the corresponding sensing products with different concentrations of Cu^{2+} from 0 to 10.2 ppm (No. 1 – No. 15) (c).

Furthermore, we have studied whether the Cu^{2+} turn-on sensor can be designed based on the formation of CuNCs by other synthesis methods. In the environment, red colour fluorescence is more favourable for the application. Thus, the synthesis system for red colour fluorescent BSA-CuNCs according to a previously reported method was investigated.[43] Different concentrations of Cu^{2+} were used for the formation of CuNCs, see Fig. S6. Firstly, it takes 6 -8 hours for the sensing results. Secondly, it can be seen that the concentration of Cu^{2+} will not be evaluated until the concentrations are beyond 127 ppm. This means that the detection limit and reaction time are not favourable for real applications. As well as this, most other protocols based on the formation of CuNCs will not show promise for development as facile Cu^{2+} sensors due to the long reaction time and the less favourable fluorescence emitting colour, see Table S1. Though there are several methods for the synthesis of CuNCs, our system based on the formation process of CuNCs describes the advantages for the development of a facile Cu^{2+} turn-on sensor.

4. Conclusion

To summarize, a Cu^{2+} turn-on sensing system based on the fast formation of highly fluorescent CuNCs through the assistance of ethanol was developed. The sensing system shows good selectivity and Cu^{2+} has been successfully detected over 1.3 ppm under optimal conditions, This strategy may provide a new approach for developing a low cost, simple sensor for safety evaluation of Cu^{2+} in water.

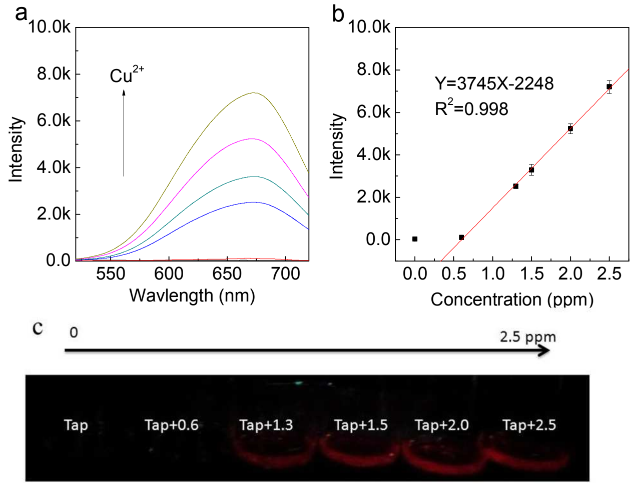

Fig.4 Fluorescence emission spectra (a) and intensity at 673 nm (b) of the sensing system for Tap water in the absence and presence of various concentration of Cu^{2+}; Fluorescence photograph for the evaluation of unpolluted and polluted water samples under a 365 nm UV lamp (c).

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