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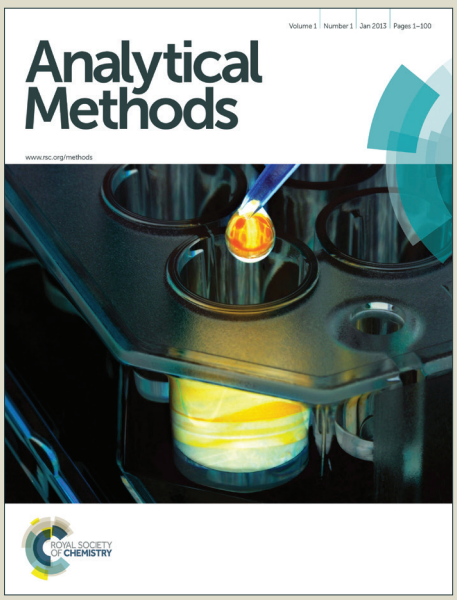

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One-step synthesis of water-soluble fluorescent copper nanoparticles for label-free detection of manganese ion

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This paper reports a one-step method for the synthesis of water soluble fluorescent copper nanoparticles stabilized by 3-mercaptopropoic acid and histidine. The resulting Cu nanoparticles exhibit intense blue fluorescence (λem = 449 nm), and proved highly effective in the selective detection of manganese ions. The intensity of Cu nanoparticles fluorescence was shown to decrease with an increase in manganese ion concentration in the range of 0.25–250 μM.

Noble metal nanoparticles with fluorescence have recently attracted considerable attention in the selective detection of a variety of metal ions.[1-8] Numerous methods have been developed for the synthesis of fluorescent nanoparticles in aqueous solutions, including the use of templates, such as amino acids,[9-12] peptides,[13] proteins,[1, 8] and DNA.[6, 14, 15] Copper is earth- abundant and low cost; however, Cu nanoparticles have attracted relatively little attention among researchers.[16, 17] Representatively, double-stranded DNA has been used as an efficient template for the formation of fluorescent Cu nanoparticles.[2, 18-20] In addition, Yang *et al* used L-cysteine as capping ligands to react in an aqueous solution of NaOH at 55°C in the synthesis of fluorescent Cu nanoparticles.[21]

Manganese (Mn) is a trace element essential to the growth of plant and animal, which under physiologic conditions, is generally found in the oxidation state Mn^{2+}. Manganese ions serve as a key co-factor in a wide range of enzymes, including superoxide dismutase, oxidases, dehydrogenases, DNA polymerases, decarboxylases, and sugar transferases.[22-24] A manganese deficiency in humans can result in skeletal deformation and impaired carbohydrate metabolism;[25] however, exposure to excessive oral or ambient air levels of Mn can have neurological effects, such as Parkinsonian-like disorder.[25-27] Despite the biological importance of manganese relatively few studies have focused on the development of specific fluorescent probes for Mn ions.[28, 29]

Herein, we report a facile, one-pot strategy for the preparation of fluorescent Cu nanoparticles by reducing Cu salt using 3-mercaptopropoic acid (MPA) and histidine as capping ligands and reducing agents in an aqueous solution. The resulting fluorescent Cu nanoparticles were then employed as fluorescent probes for the detection of Mn^{2+} ions. Our results demonstrate the excellent sensitivity and selectivity of Cu nanoparticles. This is the first study to report of a fluorescent Cu nanoparticle sensor capable of the selective detection of Mn^{2+} ions in an aqueous solution with linear dose-response curve over a wide range at concentrations of 0.25–250 μM.

Cu nanoparticles were prepared by mixing aqueous solutions of $CuSO_4$ (90 μL, 50mM), histidine (7.5 mL, 0.04 g/mL) and MPA (3 mL, 10mM). The mixture was first vortexed, and allowed to react without stirring for two days at 28°C. The color of resulting solution changed from light blue to light yellow (Fig. 1A). The absence of either histidine or MPA did not result in a noticeable change in color. Under UV light illumination, a blue emissive light indicated the formation of fluorescent Cu nanoparticles.

We examined the absorption and fluorescence spectra to investigate the optical properties of the fluorescent Cu nanoparticles, and conducted a comparison of the absorption spectra of $CuSO_4$, histidine, MPA, and Cu nanoparticles (Fig. 1B). The Cu nanoparticles displayed a weak absorption peak at 335 nm, with no absorption peak in this range detected for the $CuSO_4$, histidine or MPA solutions. As shown in Fig. 1C, the Cu nanoparticles exhibited blue emissions at approximately 449 nm upon excitation at 350 nm. In contrast, pure $CuSO_4$, histidine and MPA present no fluorescent emissions, which is a clear indication that the observed fluorescence change cannot be attributed to the reactant molecules. We investigated the effect of reaction time and reaction temperature on the intensity of fluorescence in order to establish optimal synthesis conditions (Fig. S1).

The fluorescence quantum yield of Cu nanoparticles is 2.1% (relative to quinine sulfate in 0.1 M of H_2SO_4), and the

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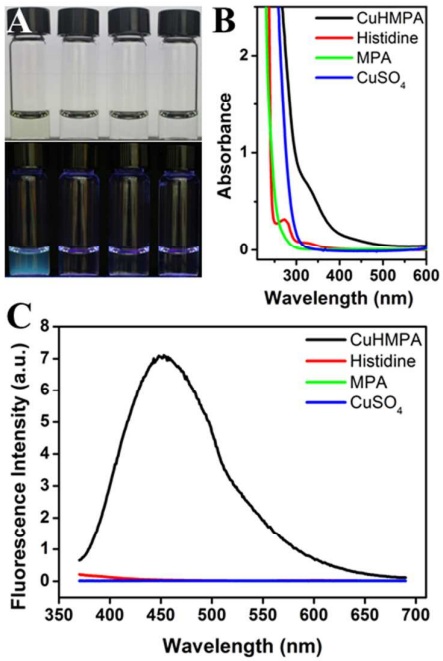

Fig. 1 (A) The photograph of (from left to right) copper nanoparticles (CuHMPA), $CuSO_4$, histidine and MPA solutions under the visible (top) or UV light (bottom) illumination. (B) UV-vis spectra (C) Fluorescence spectra of CuHMPA, $CuSO_4$, histidine and MPA solutions.

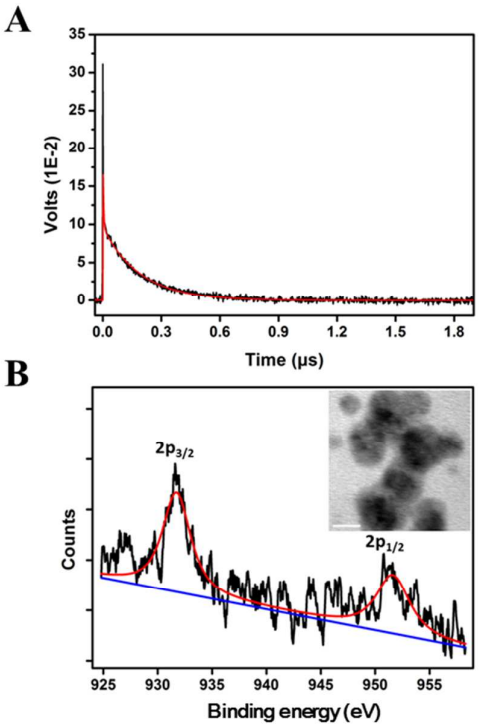

Fig. 2 (A) Fluorescence decay of the aqueous Cu nanoparticles (black line). The red line is the best-fit curve (B) XPS spectra of Cu 2p region for the resulting Cu nanoparticles. The inset shows the photo of HRTEM image of Cu nanoparticles. Scale bar: 5nm.

fluorescence lifetime was measured (Fig. 2A). The fluorescence lifetime collected at 460 nm was 10.07 ns. Similar fluorescence lifetime was observed for lyophilized powder (Fig. S2A). Analysis using high resolution transmission electron microscopy (HRTEM) revealed that Cu nanoparticles were 11 nm (Fig. 2B, inset). The analysis of energy dispersive X-ray spectroscopy (EDS) was used to confirm the presence of copper (Fig. S2B). X-ray photoelectron spectroscopy spectrum was measured to determine the oxidation state of copper. As shown in Fig. 2B, two peaks are located at 932.3 eV and 952.2 eV, which are attributed to binding energy positions of Cu 2p3/2 and Cu 2p1/2 of Cu(0).

To elucidate the role of MPA and histidine in the synthesis of Cu nanoparticles, we conducted comparisons using (i) various quantities of Cu2+, MPA, or histidine (ii) alkanethiols of various chain lengths (iii) a variety of amino acids other than histidine, (iv) swapping MPA with ascorbic acid. We also determined the final fluorescence intensity of solutions containing different molar ratios of Cu2+, MPA, or histidine (Fig. S3). A reduction in the concentration of either MPA or histidine caused the fluorescence to disappear, thereby indicating that MPA and histidine are both important to the stability of fluorescent Cu nanoparticles. Fig. S4A illustrates the effect of chain lengths on the final fluorescence intensity. Interestingly, 2-mercaptoacetic acid slightly enhanced fluorescence, whereas longer chain lengths had no apparent effect on the increase of fluorescence intensity. Asparagine, aspartic acid, cysteine, glycine, proline, serine, tryptophan, and tyrosine also used as a substitute for histidine in the synthesis of Cu nanoparticles (Fig. S4B); however, no obvious fluorescent signal was observed in the resulting solutions. When MPA was changed to ascorbic acid, Cu nanoparticles with similar emission were formed (Fig. S4C). Therefore, hisdidine plays an important role as capping agents.

To measure the stability of Cu nanoparticles, samples were mixed with Britton-Robinson buffers of various pH levels for one day. Fig. S5 illustrates that fluorescence quenching does not occur even at high or low pH values. Furthermore, the resulting Cu nanoparticle solution maintained their fluorescence intensity for more than three months without a significant change. These results suggest that the obtained Cu nanoparticles exhibit high stability. As-prepared Cu nanoparticles were then applied as a sensor for the metal ions. Under optimal conditions, we tested the selectivity of the probe toward various metal ions (final concentration 0.1 mM, respectively), including Li^+, Na^+, K^+, Ca^{2+}, Mg^{2+}, Cr^{3+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Pd^{2+}, Pt^{2+}, Cd^{2+}, Hg^{2+}, and Pb^{2+} after being incubated Cu nanoparticle for one day. The fluorescence emission of the as-prepared Cu nanoparticle was quenched in the presence of Mn^{2+}. Fe^{3+} and Pt^{2+} showed a slight degree of fluorescence quenching, whereas no obvious decrease was observed in the presence of other ions. These results demonstrate the selective response of the Cu nanoparticle probe

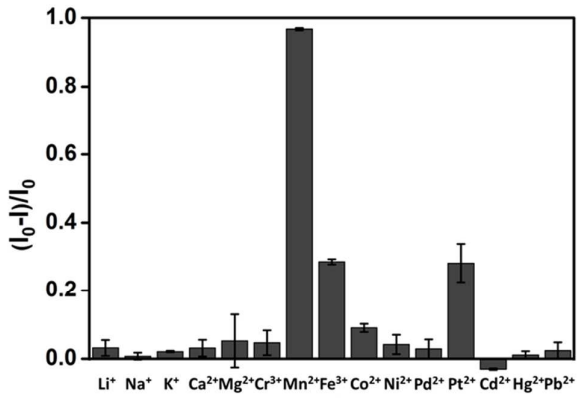

Fig. 3 The quenching effect value $(I_0 - I)/I_0$. (I, I_0 are the corresponding fluorescence intensities at 449 nm in the presence and absence of metal ions, respectively)

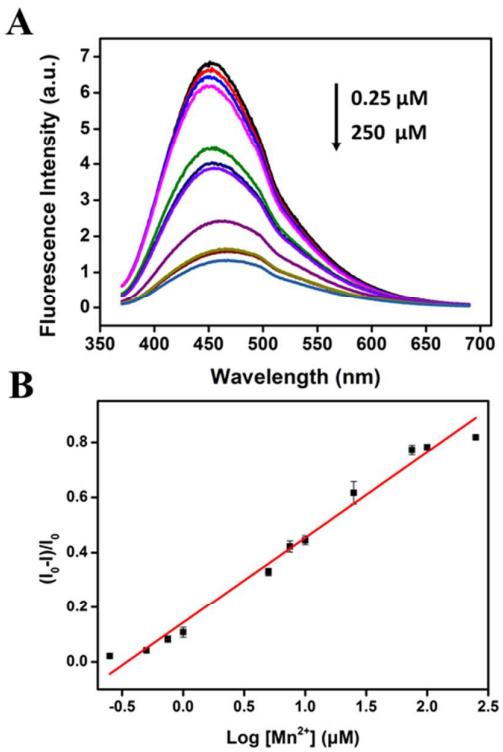

Fig. 4 (A) Fluorescence spectra of Cu nanoparticles in the presence of various concentrations of Mn^{2+} over the range 0.25–250 μM. (B) The linear relationship between quenching effect value $(I_0 - I)/I_0$ and the logarithmic value of Mn^{2+} ions concentration.

to Mn^{2+}; however, the removal of Fe^{3+} and Pt^{2+} was required as sample pre-treatment to avoid the interfere with the detection, such as functionalized mesoporous silica or nanocelluloses.[30, 31]

The sensitivity of the Cu nanoparticle probe was measured by evaluating the fluorescence intensity in the presence of various concentrations of Mn^{2+}. To determine Mn^{2+} effectively, resulting solutions were incubated with Cu nanoparticle at 55 °C for 2 h. The emission intensity gradually decreased with an increase in the concentration of Mn^{2+}. A linear relationship (with a correlation coefficient of 0.99) was observed between the quenching effect value $(I_0 - I)/I_0$ and the logarithmic value of Mn^{2+} ions concentration over the range 0.25–250 μM. The limit of detection (LOD) for Mn^{2+} ions was estimated in the range of 1.6 μM at the signal-to-noise ratio of 3.

In summary, this study provides a facile route for the synthesis of water-soluble Cu nanoparticles at ambient temperature for use as fluorescent probes in the detection of manganese ions. The system exhibited good selectivity to Mn^{2+} as well as a wide-range linear dose-response curve. The low cytotoxicity of the Cu nanoparticles and the abundance of copper in nature, further support the potential applicability of the Cu nanoparticles as fluorescent sensors in biomedical applications.

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Notes and references

1. W. Z. Wei H, Yang L, Tian S, Hou C, Lu Y., *The Analyst.* , 2010, **135**, 1406-1410.
2. J. Chen, J. Liu, Z. Fang and L. Zeng, *Chemical Communications*, 2012, **48**, 1057-1059.
3. S. Liu, F. Lu and J.-J. Zhu, *Chemical Communications*, 2011, **47**, 2661-2663.
4. J. Sun, J. Zhang and Y. Jin, *Journal of Materials Chemistry C*, 2013, **1**, 138-143.
5. Y.-H. Lin and W.-L. Tseng, *Analytical chemistry*, 2010, **82**, 9194-9200.
6. G.-Y. Lan, C.-C. Huang and H.-T. Chang, *Chemical Communications*, 2010, **46**, 1257-1259.
7. C.-C. Huang, Z. Yang, K.-H. Lee and H.-T. Chang, *Angewandte Chemie International Edition*, 2007, **46**, 6824-6828.
8. J. Xie, Y. Zheng and J. Y. Ying, *Chemical Communications*, 2010, **46**, 961-963.
9. X. Yang, M. Shi, R. Zhou, X. Chen and H. Chen, *Nanoscale*, 2011, **3**, 2596-2601.
10. X. Mu, L. Qi, P. Dong, J. Qiao, J. Hou, Z. Nie and H. Ma, *Biosensors and Bioelectronics*, 2013, **49**, 249-255.
11. Y. Chen, W. Li, Y. Wang, X. Yang, J. Chen, Y. Jiang, C. Yu and Q. Lin, *Journal of Materials Chemistry C*, 2014, **2**, 4080-4085.
12. P. Bian, J. Zhou, Y. Liu and Z. Ma, *Nanoscale*, 2013, **5**, 6161-6166.

13. X. Yuan, Y. Tay, X. Dou, Z. Luo, D. T. Leong and J. Xie, *Analytical chemistry*, 2013, **85**, 1913-1919.
14. H.-C. Yeh, J. Sharma, J. J. Han, J. S. Martinez and J. H. Werner, *Nano letters*, 2010, **10**, 3106-3110.
15. T. A. C. Kennedy, J. L. MacLean and J. Liu, *Chemical Communications*, 2012, **48**, 6845-6847.
16. W. Wang, F. Leng, L. Zhan, Y. Chang, X. X. Yang, J. Lan and C. Z. Huang, *The Analyst*, 2014, **139**, 2990-2993.
17. H. Cao, Z. Chen, H. Zheng and Y. Huang, *Biosensors and Bioelectronics*, 2014, **62**, 189-195.
18. Z. Zhou, Y. Du and S. Dong, *Analytical chemistry*, 2011, **83**, 5122-5127.
19. X. J. Zhao and C. Z. Huang, *New Journal of Chemistry*, 2014, **38**, 3673-3677.
20. J. Liu, J. Chen, Z. Fang and L. Zeng, *The Analyst*, 2012, **137**, 5502-5505.
21. X. Yang, Y. Feng, S. Zhu, Y. Luo, Y. Zhuo and Y. Dou, *Analytica chimica acta*, 2014, **847**, 49-54.
22. D. J. Scott, B. M. da Costa, S. C. Espy, J. D. Keasling and K. Cornish, *Phytochemistry*, 2003, **64**, 123-134.
23. D. G. Kehres and M. E. Maguire, *FEMS Microbiology Reviews*, 2003, **27**, 263-290.
24. C. Bowler, L. Slooten, S. Vandenbranden, R. De Rycke, J. Botterman, C. Sybesma, M. Van Montagu and D. Inzé, *The EMBO Journal*, 1991, **10**, 1723-1732.
25. A. W. Dobson, K. M. Erikson and M. Aschner, *Annals of the New York Academy of Sciences*, 2004, **1012**, 115-128.
26. A. Takeda, *Brain Res Brain Res Rev.*, 2003, **41**, 79-87.
27. E. A. Malecki, *Brain Research Bulletin*, 2001, **55**, 225-228.
28. K. B. Narayanan and H. H. Park, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 2014, **131**, 132-137.
29. X. Mao, H. Su, D. Tian, H. Li and R. Yang, *ACS applied materials & interfaces*, 2013, **5**, 592-597.
30. T. Kang, Y. Park and J. Yi, *Industrial & Engineering Chemistry Research*, 2004, **43**, 1478-1484.
31. P. Liu, P. F. Borrell, M. Božič, V. Kokol, K. Oksman and A. P. Mathew, *Journal of Hazardous Materials*, 2015, **294**, 177-185.