A Fluorescence Strategy for Silver Ion Assay via Cation Exchange Reaction and Formation of Poly(thymine)-templated Copper Nanoclusters

Xiu WANG,* Pingyue HU,* Zhipeng WANG,* Qiuyun LIU,* Ting XI,* Mengqian KOU,* Ke HUANG,*† and Piaopiao CHEN**†

*College of Chemistry and Material Science, Sichuan Normal University, Chengdu, Sichuan 610068, China
**Department of Laboratory Medicine, State Key Laboratory of Biotherapy and Cancer Center, West China Hospital, Sichuan University and Collaborative Innovation Center for Biotherapy, Chengdu, Sichuan 610041, China

The detection of Ag⁺ ions in the environment and biological systems is important to both environmental monitoring and modern medicine. Herein, a novel and label-free method was developed for Ag⁺ detection, which utilizes a fluorescence strategy combining DNA-templated copper nanoclusters (Cu NCs) with cation exchange reactions. The method is primarily based on the effective detection of an Ag⁺-triggered cation exchange reaction and the release of free Cu²⁺ from CuS nanoparticles (CuS NPs), while the probe T30 serves as an effective template for the formation of fluorescence-inducing Cu NCs. Under optimal conditions, this sensing system displays high sensitivity with a 50 nM limit of detection and a range from 0 - 100 μM. In addition, the proposed method exhibits high selectivity and, therefore, was successfully applied to the analysis of real samples. Overall, these results demonstrate that our established method has advantages of design and operation simplicity, as well as cost-effectiveness.

Keywords Silver ion, CuS NPs, Cu NCs, cation exchange reaction, fluorescence

(Received February 9, 2019; Accepted April 16, 2019; Advance Publication Released Online by J-STAGE April 26, 2019)

Introduction

Silver is indispensable to human life and has increasingly widespread uses in industries like photography, electronic equipment, ornamentation, and pharmaceuticals.¹ In medicine, silver is recognized as a broad-spectrum antimicrobial agent for clinical therapies.² Recently, Ag has been rapidly gaining focus for cancer therapy as a drug delivery agent.³ However, the extensive consumption of Ag has led it to become one of the most prevalent environmental pollutants.⁴,⁵ Therefore, there is great urgency to develop a simple, sensitive, selective and effective strategy to quantitatively analyze Ag⁺ in the surrounding environment. To date, numerous techniques have been developed to detect Ag⁺, including atomic absorption spectroscopy (AAS),⁶ inductively coupled plasma mass spectrometry (ICP-MS),⁷ chemical vapor generation-atomic fluorescence spectroscopy (CVG-AFS),⁸ colorimetry,⁹ fluorescence spectroscopy,¹⁰ surface enhanced Raman scattering (SERS),¹¹⁰ and electrochemistry.¹¹ Some of the aforementioned methods have excellent performance, but still suffer from high energy consumption and high cost. Alternatively, the fluorescence spectroscopy strategy has been developed as a simple and rapid method for multi-analyte detection, and has recently drawn widespread attention. However, some of these methods require the synthesis of fluorescent dyes with complicated synthesis procedures.

As an important genetic material, nucleic acid exhibits excellent stability and programmability, which makes it a relatively promising candidate for fabrication in desired lengths, sequences, and even structures.¹³,¹⁴ Similar to proteins and small molecules, nucleic acids have been used as templates to synthesize various fluorescent nanomaterials,¹⁵-¹⁷ such as Ag NCs and Cu NCs; these new nanomaterials have been used for the analysis of various targets like metal ions,¹⁸,¹⁹ small molecules,²⁰,²¹ enzymes,²² nucleic acids,²³ proteins,²⁴ and even cells,²⁵ due to their advantages of experimental simplicity, procedural rapidity, and cost-effectiveness. Regarding the specific detection of Ag⁺, some catalytic beacon biosensors and label-free catalytic DNAzyme biosensors have been designed.²⁶,²⁷ In addition, several biosensors based on cytosine (C)-Ag⁺-(C) have been developed with a fluorescence sensing method.²⁸-²⁹ Cation exchange reactions, such as Ag⁺ and CuS NPs,³⁰ Ag⁺ and CdSe nanocrystals,³¹ and Ag⁺ and ZnS nanocrystal clusters,³² have been widely used to detect various metal ions, nucleic acids, and proteins due to their advantages such as mild reagent properties, room temperature reactions, neutral conditions, and relative rapidity. However, to our knowledge, the combination of Cu NCs formation with a cation exchange reaction for detection of Ag⁺ has not yet been reported.

The purpose of this work is to design a simple strategy for Ag⁺ detection. Herein, by combining the cation exchange reaction between Ag⁺ and CuS NPs, which generated Cu²⁺ and poly(thymine)-templated Cu NCs fluorescence signals,
the proposed strategy was developed to detect Ag⁺. Therefore, the determination of target Ag⁺ could be realized via monitoring the change of fluorescence signals resulting from the formation of Cu NCs. In addition, the conditions related to this work were studied in detail. The established method exhibits high sensitivity and excellent selectivity. This detection procedure is not only sensitive, but also quite simple; it only requires mixing the reaction solutions with the target analyte and directly detecting the signal using a molecular fluorometer.

**Experimental**

**Reagents and chemicals**

T30 (5’-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT-3’) was synthesized by Sangon Co., Ltd. (Shanghai, China). Highly purified NaNO₃, AgNO₃, NaOH, and CuSO₄·5H₂O were purchased from Kelong Reagent Factory (Chengdu, China). Ascorbic acid (AA) was obtained from Sigma-Aldrich (St. Louis, MO). 3-[N-Morpholino]propanesulfonic acid (MOPS) was purchased from Solarbio Technology Co., Ltd. (Beijing, China). The 10 mM MOPS buffer solution was used in all working solutions (pH 7.4, 100 mM NaNO₃, 2.5 mM Mg(NO₃)₂). And ultra-pure water (18.2 MΩ·cm) was prepared from a Millipore system (Millipore, USA).

**Apparatus**

Fluorescence measurements were performed by scanning from 540 to 650 nm at the excitation wavelength of 340 nm on an F-7100 fluorescence spectrometer (Hitachi, Japan). The pH measurement of the MOPS buffer solution was determined by FE20 pH meter (Mettler Toledo, Shanghai, China). The morphology of the CuS NPs was recorded by JEM-2100F transmission electron microscopy.

**Preparation of CuS NPs**

The common procedure for the synthesis of 3-MPA-stabilized CuS NPs has been reported previously. Briefly, 15 μL 3-MPA was added into a solution of Cu(NO₃)₂ (50 mL, 0.4 mM), and the pH of the solution was adjusted to 7.0 with NaOH. Then, the air in this mixture was removed by a controlled negative pressure (30 min). Subsequently, 1.34 mM Na₂S (50 mL) was slowly added into the mixture solution. The mixture solution was continually reacted for approximately 24 h in the presence of N₂ until obtained a brown solution. After performing more than three times dialysis against ultrapure water for 48 h utilizing a dialysis bag with a MWCO of 7000 to avoid the interference of residual S²⁻, water-soluble CuS NPs were obtained.

**Analytical procedure**

First, 50 μL of different concentrations of Ag⁺ and 50 μL of CuS NPs (stock solution) were added into 150 μL MOPS buffer (pH 7.4, 10 mM NaNO₃, 2.5 mM Mg(NO₃)₂) and reacted for 1.5 h in a dark at room temperature (RT) to perform the cation exchange reaction. Then, 9 μL T30 (20 μM) and 18 μL AA (10 mM) were added into the solution in the dark at RT and reacted for 5 min to form the Cu NCs. Finally, the resulting solution was diluted to 1 mL with high purity water and the fluorescence measurements were performed immediately.

**Pretreatment of water sample**

The river water was collected from the Jin Jiang River, which traverses Chengdu, China, and the tap water was from the Sichuan University campus. The samples were first filtered through 0.22 μm membranes to remove insoluble particles, and then analyzed.

**Results and Discussion**

**Principle of the proposed strategy**

The Ag⁺ sensing mechanism of the fluorescence assay is illustrated in Scheme 1. In the presence of target Ag⁺, the cation exchange reaction with CuS NPs was initiated and an abundance of Cu²⁺ was released. In the reaction mixture, the Cu²⁺ is quickly reduced to Cu⁰ by ascorbic acid in the solution. Subsequently, Cu⁰ becomes strongly bound to the specific DNA template T30, thus synthesizing Cu NCs. Meanwhile, the concentration of Cu NCs can be evaluated through observation of the signal intensity at the excited wavelength of 340 nm. Therefore, the concentration of target Ag⁺ can be quantitatively detected by fluorometer.

**Feasibility of silver ion detection**

At the beginning, CuS NPs were synthesized by the common method and the resulting products were characterized using a transmission electron microscope (TEM). The CuS NPs were dispersed with a mean diameter of approximately 5 ± 1 nm (Fig. 1(A)). To verify the feasibility of this method, different...
concentrations of Ag⁺ solutions were added in blank solutions (CuS NPs + T30 + AA) and the changes in fluorescence intensity were monitored (Fig. 1B). The results show that highly fluorescent products were obtained in the experimental group containing Ag⁺, and furthermore, the fluorescence emission increases distinctly with increasing concentrations of Ag⁺. Therefore, this above experimental result has demonstrated that the detection of Ag⁺ can be realized by the sensing method we developed.

Experimental conditions

Cation exchange reaction. The cation exchange reaction of Ag⁺ with CuS NPs was a crucial step, which required the optimization of the Cu NPs volume and the time of cation exchange reaction.
It was found that the fluorescence intensity ($F$) increased significantly as the total volume of CuS NPs increased when Ag$^+$ was added into the reaction (Fig. 2(A)). Although, the fluorescence intensity ($F_0$) of solutions containing differing volumes of CuS NPs showed corresponding incremental changes, the value of $F - F_0$ could still respond to the concentration of Ag$^+$; therefore, it could be applied to quantify the concentration of Ag$^+$ (Fig. 2(B)). In addition, the maximum fluorescence intensity difference was obtained using an optimum volume of 50 $\mu$L CuS NPs.

The appropriate cation exchange reaction time between CuS NPs and Ag$^+$ was also investigated. As the reaction time was extended from 0.25 to 2.5 h, the fluorescence intensity increased noticeably before finally reaching the maximum value (Figs. 2(C) and 2(D)). The results showed an incubating time of 1.5 h ensured the complete cation exchange reaction. Therefore, cation exchange reaction time of 1.5 h was used in subsequent experiments.

**Conditions of Cu NCs formation.** The influences of AA concentration and the volume of T30 on Cu NCs formation were fully investigated. We first fixed a T30 volume of 9 $\mu$L with concentrations of AA varying from 1 to 8 mM. The fluorescence intensity greatly increased initially from 1 to 3.5 mM (Figs. 3(A) and 3(B)). However, with further increases of AA concentration from 3.5 to 8 mM, the fluorescence intensity showed a relatively distinct decline. Therefore, AA concentration of 3.5 mM was adopted in subsequent experiments.

To optimize the quantity of template T30, we similarly fixed the AA concentration at 3.5 mM and varied the volume of T30 (20 $\mu$M). The fluorescence intensity greatly increased initially from 1 to 3.5 mM (Figs. 3(A) and 3(B)). However, with further increases of AA concentration from 3.5 to 8 mM, the fluorescence intensity showed a relatively distinct decline. Therefore, AA concentration of 3.5 mM was adopted in subsequent experiments.

To optimize the quantity of template T30, we similarly fixed the AA concentration at 3.5 mM and varied the volume of T30 (20 $\mu$M). As shown in Figs. 3(C) and 3(D), the optimum volume of template T30 was 9 $\mu$L, and this volume was used throughout the experimentation.

**Analytical performance of Ag$^+$**

The Ag$^+$-dependent curve created using standard concentrations was performed under the optimized experiment conditions. The significant fluorescence signal enhancement generated by Cu NCs in the presence of Ag$^+$ concentrations ranging from 0 to 100 $\mu$M was recorded (Figs. 4(A) and 4(B)). The results demonstrated a relatively strong linear correlation ($R^2 = 0.992$) between the fluorescence intensity and Ag$^+$ concentration with a detection limit of 50 nM ($3\sigma/K$, $\sigma$ represents the value of 11 repeated measurements of blank solution; $K$ represents the slope of the standard curve), which was comparable to the other methods (Table 1). According to the secondary drinking water standards of the United States Environmental Protection Agency (EPA), the admissible limit of Ag$^+$ concentration in drinking water systems is 930 nM. These experiment results demonstrated that the developed strategy for the detection of Ag$^+$ could be applied to the concentrations described in national standards and further indicated that our established method could be applied in real samples.

**Selectivity**

To evaluate the selectivity of our proposed assay, various metal ions including monovalent and divalent cations were investigated for their fluorescence response. 250 $\mu$M K$^+$, Ca$^{2+}$, Co$^{2+}$, Mg$^{2+}$, Ni$^{2+}$, Fe$^{2+}$, Cu$^{2+}$, Pb$^{2+}$, Na$^+$, Cd$^{2+}$, Hg$^{2+}$, and Zn$^{2+}$ were detected at 10 times of the Ag$^+$ concentration. As shown in Fig. 4(C), only Ag$^+$ caused a significant increase in the value...
of the ratio of fluorescence intensity of Cu NCs and blank ($F/F_0$). We also detected the fluorescence responses of the mixture of Ag$^+$ and coexisting ions. Figure 6(D) shows that the selective detection of Ag$^+$ is hardly affected in presence of these commonly coexisting ions. It is worth noting that obvious interference from Hg$^{2+}$ was detected when the concentration is higher than 10-fold of Ag$^+$. Hydride generation was used here to reduce the interference of Hg$^{2+}$. Fortunately, a satisfactory result was obtained by adding KBH$_4$ (0.05%, m/v) to reduce the Hg$^{2+}$ to Hg$^0$ (gas), which can be separated from the solution. These results demonstrated that only Ag$^+$ could cause the formation of Cu NCs along the T30 scaffold with an obvious fluorescence intensity change in this proposed strategy. Meanwhile, these results also suggested the good selectivity of this method for Ag$^+$ assay, which helps in its use for the detection of actual samples.

**Samples analysis**

In order to investigate the feasibility of our established approach in real samples, the direct determination of Ag$^+$ based on cation exchange reaction and Cu NCs was studied in real water samples. Tap water and river water were selected as two real samples. The experiment result displayed the recoveries of a mixed known quantity of Ag$^+$ to the spiked real water samples to be 95 to 104% (as shown in Table 2), which shows that our proposed strategy has potential for practical applications.
Conclusions

In summary, an innovative and label-free homogeneous Ag⁺ fluorescent analysis method was developed by combining a cation exchange reaction and poly T-templated Cu NCs. The established approach displayed several significant advantages, including simple operation and design, cost-effectiveness, and the use of non-toxic chemicals. Moreover, due to the relatively simple process, the design and fabrication of a commercial detection kit would be feasible. The Ag⁺ detection strategy proposed in this work further extends the application range of Cu NCs and selective cation exchange reactions.

Acknowledgements

The authors thank the National Natural Science Foundation of China (No. 21605108), the China Postdoctoral Science Foundation (2019M653391) and the Foundation of Sichuan Normal University No ZZYQ2018-04 and SYJS2018-05 for financial support.

References

1. J. H. Kang, J. B. Chae, and C. Kim, R. Soc. Open Sci., 2018, 5, 180293.
2. W. Sim, R. Barnard, M. Blaskovich, and Z. Ziota, Antibiotics, 2018, 7, 93.
3. X. F. Zan, Q. Z. Li, Y. T. Pan, D. J. Morris, P. Zhang, P. Li, H. Z. Yu, and M. Z. Zhu, ACS Appl. Nano Mater., 2018, 1, 6773.
4. L. Y. He, Y. X. Lu, F. Y. Wang, W. J. Jing, Y. Chen, and Y. Y. Liu, Sens. Actuators, B, 2018, 254, 468.
5. M. Rana, M. Balcioglu, N. M. Robertson, M. S. Hizir, S. Yumak, and M. V. Yigit, Chem. Sci., 2017, 8, 1200.
6. M. Ghaedi, A. Shokrollahi, K. Niknam, E. Niknam, A. Najibi, and M. Soylak, J. Hazard. Mater., 2009, 168, 1022.
7. F. Laborda, J. Jiménez-Lamana, E. Bolea, and J. R. Castillo, J. Anal. At. Spectrom., 2011, 26, 1362.
8. K. Huang, K. L. Xu, J. Tang, L. Yang, J. R. Zhou, X. D. Hou, and C. B. Zheng, Anal. Chem., 2015, 87, 6584.
9. X. Y. Li, Z. T. Wu, X. D. Zhou, and J. M. Hu, Biosens. Bioelectron., 2017, 92, 496.
10. M. Nagy, D. Rácó, Z. L. Nagy, P. P. Fehér, J. Kalmár, I. Fábián, A. Kiss, M. Zsuga, and S. Kéli, Sens. Actuators, B, 2018, 255, 2555.
11. P. Zheng, M. Li, R. Jurevic, S. K. Cushing, Y. X. Liu, and N. Q. Wu, Nanoscale, 2015, 7, 11005.
12. Y. Fu, Y. J. Yang, T. Tuersun, Y. Yu, and J. F. Zhi, Analyst, 2018, 143, 2076.
13. H. M. Meng, H. Liu, H. L. Kuai, R. Z. Peng, L. T. Mo, and X. B. Zhang, Chem. Soc. Rev., 2016, 45, 2583.
14. R. Z. Peng, X. F. Zheng, Y. F. Lyu, L. J. Xu, B. Z. Zhang, G. L. Ke, Q. L. Liu, C. J. You, S. Y. Huan, and W. H. Tan, J. Am. Chem. Soc., 2018, 140, 9793.
15. Y. L. Cheng, Y. Zhang, R. J. Pei, Y. F. Xie, W. R. Yao, Y. H. Guo, and H. Qian, Anal. Sci., 2018, 34, 415.
16. D. R. Liu, X. Y. Pan, W. Mu, C. Li, and X. J. Han, Anal. Sci., 2019, 35, 367.
17. H. Zhang, Y. N. Guan, X. S. Li, L. Lian, X. Y. Wang, W. X. Gao, B. Zhu, X. Y. Liu, and D. W. Lou, Anal. Sci., 2018, 34, 1155.
18. J. Y. Li, W. X. Fu, J. C. Bao, Z. Y. Wang, and Z. H. Dai, ACS Appl. Mater. Interfaces, 2018, 10, 6965.
19. J. Lee, J. Park, H. H. Lee, H. Park, H. I. Kim, and W. J. Kim, Biosens. Bioelectron., 2015, 68, 642.
20. Z. G. Mao, Z. H. Qing, T. P. Qing, F. Z. Xu, L. Wen, X. X. He, D. G. He, H. Shi, and K. M. Wang, Anal. Chem., 2015, 87, 7454.
21. L. Zhang, Q. Y. Cai, J. Li, J. Ge, J. Y. Wang, Z. Z. Dong, and Z. H. Li, Biosens. Bioelectron., 2015, 69, 77.
22. D. W. Yang, Z. Z. Guo, Y. G. Tang, and P. Miao, ACS Appl. Nano Mater., 2017, 1, 168.
23. J. Oblisoca, C. Liu, R. Batson, M. Babin, J. Werner, and H. C. Yeh, Biosens. Bioelectron., 2013, 3, 185.
24. F. He, J. Wang, B. C. Yin, and B. C. Ye, Anal. Chem., 2018, 90, 8072.
25. Y. C. Shiang, C. C. Huang, W. Y. Chen, P. C. Chen, and H. T. Chang, J. Mater. Chem. B, 2012, 22, 12972.
26. R. Saran and J. W. Liu, Anal. Chem., 2016, 88, 4014.
27. H. Wang, Y. Liu, and G. Liu, Nanomaterials, 2018, 8, 258.
28. Z. Y. Wang, J. Zhao, Z. J. Li, J. C. Bao, and Z. H. Dai, Anal. Chem., 2017, 89, 6815.
29. S. Bi, B. Ji, Z. P. Zhang, and J. J. Zhu, Chem. Sci., 2013, 4, 1858.
30. X. R. Zhang, H. X. Liu, R. J. Li, N. B. Zhang, Y. Xiong, and S. Y. Niu, Chem. Commun., 2015, 51, 6952.
31. D. H. Son, S. M. Hughes, Y. Yin, and A. P. Alivisatos, Science, 2004, 306, 1009.
32. J. J. Yao, X. G. Han, S. Zeng, and W. W. Zhong, Anal. Chem., 2012, 84, 1645.
33. Y. D. Zhu, J. Peng, L. P. Jiang, and J. J. Zhu, Analyst, 2014, 139, 649.
34. L. Y. He, Y. X. Lu, F. Y. Wang, W. J. Jing, Y. Chen, and Y. Y. Liu, Sens. Actuators, B, 2018, 254, 468.
35. Z. Y. Wang, J. Zhao, Z. J. Li, J. C. Bao, and Z. H. Dai, Anal. Chem., 2017, 89, 6815.
36. Z. S. Wu, M. K. Feng, X. X. Chen, and X. J. Tang, J. Mater. Chem. B, 2016, 4, 2086.
37. M. Zhang, H. N. Le, X. Q. Jiang, B. C. Yin, and B. C. Ye, Anal. Chem., 2013, 85, 11665.
38. Y. H. Lin and W. L. Tseng, Chem. Commun., 2009, 6619.