A Facile Colorimetric Sensor for 6-Mercaptopurine Based on Silver Nanoparticles

Junling DUAN,[†] Yijing LI, Qin HOU, Wei LV, Li DAI, and Shiyun AI[†]

College of Chemistry and Material Science, Shandong Agricultural University, Tai'an, Shandong 271018, P. R. China

A facile colorimetric method was developed for detecting 6-mercaptopurine (6-MP) using silver nanoparticles (AgNPs). The addition of 6-MP to AgNPs led to the aggregation of AgNPs with a color change from yellow to brown. The ratio between the absorbance at 394 and 530 nm (A_{394}/A_{530}) was used for a quantitative analysis of 6-MP. A linear range of 0 – 0.5 μM was obtained with a detection limit of 10 nM. The developed method is cost-effective and simple.

Keywords Silver nanoparticles, colorimetry, sensor, 6-mercaptopurine

(Received April 13, 2020; Accepted April 20, 2020; Advance Publication Released Online by J-STAGE May 1, 2020)

6-Mercaptopurine (6-MP) is a commonly used anti-cancer drug to treat lymphoblastic leukemia.^{1,2} It can interfere with the synthesis of nucleic acid.^{3,4} However, an excessive dosage of 6-MP can cause side effects, such as anemia and leucopenia.^{5,6} It is thus urgently needed to develop an economic and effective method to detect 6-MP for the protection of human health.

Up to now, different methods including mass spectrometry,⁷ surface-enhanced Raman spectroscopy,^{8,9} chemiluminescence,^{10,11} high-performance liquid chromatography,¹² fluorescence,^{13–16} electrochemistry^{17–19} and UV-Vis spectroscopy²⁰ have been developed to detect 6-MP. Among these methods, the colorimetric method is promising for 6-MP detection because of its economy, simplicity and naked-eye application.^{21–23} Until now, the colorimetric detection of 6-MP has rarely been reported,²⁴ the development of novel colorimetric sensors for 6-MP with low cost and high sensitivity is highly desirable.

Metal nanoparticles (NPs) have usually been developed as colorimetric sensors owing to their high extinction coefficients. Among them, silver nanoparticles (AgNPs) have been widely used for the colorimetric detection of biological and environmental analytes, due to their distance-dependent optical properties.^{25–30} If AgNPs aggregate, the interparticle distance would be decreased. As a result, a UV-Vis spectral change accompanied by a visual color change could be observed.^{31–35} Up to now, AgNPs used as a colorimetric sensor for 6-MP detection has not been reported.

Herein, a facile colorimetric sensor for 6-MP was developed based on AgNPs. The addition of 6-MP to AgNPs resulted in a yellow-to-brown color change. This method could be used for the analysis of real samples. The results suggested the method possesses good sensitivity and selectivity for 6-MP. It was economic, simple and rapid.

Figure 1A illustrates the sensing mechanism of AgNPs used for the colorimetric detection of 6-MP. The prepared citrate-AgNPs were dispersed because of their electrostatic repulsion, induced by negatively charged citrate ions. As shown in Fig. 1B, the obtained AgNPs were spherical with an average size of

about 10 nm. They showed bright-yellow color (see the inset of Fig. S1, Supporting Information). But the AgNPs were irregularly aggregated and the size of the AgNPs increased after adding 6-MP (Fig. 1C). Figure S1 shows the UV-Vis absorption spectra of dispersed citrate-AgNPs and aggregated AgNPs upon adding 0.5 μM 6-MP. It could be seen that the absorption peak of dispersed citrate-AgNPs was at 394 nm (black line), and the absorption peak distribution was narrow, indicating that the size distribution of AgNPs was relatively uniform. However, when 6-MP was added to AgNPs, the solution color of AgNPs turned from bright yellow to brown (see inset of Fig. S1). The absorbance at 394 nm decreased and the maximum absorption wavelength red shifted to 530 nm (see the red line in Fig. S1). The color change of AgNPs and the red shift of the absorption

Fig. 1 Schematic illustration of the sensing mechanism for the colorimetric detection of 6-MP using citrate-AgNPs (A). TEM of citrate-AgNPs in the absence (B) and in the presence (C) of 0.5 μM 6-MP.

[†] To whom correspondence should be addressed.

E-mail: duanj1@sdau.edu.cn (J. D.); ashy@sdau.edu.cn (S. A.)

spectrum indicated that 6-MP made the dispersed AgNPs aggregate. The reason for the 6-MP-induced aggregation might be that 6-MP was rich in sulfur atoms. The sulfur atoms showed higher affinity with AgNPs than the carboxylic group of the original citrate ions on the surface of the AgNPs. Thus, 6-MP could replace citrate ions through forming stronger Ag-S covalent bond,³⁶ while decreasing the distance between AgNPs. Consequently, the aggregation of AgNPs was induced.

We used a citric acid-sodium citrate buffer solution (pH 3.0 – 6.6) to investigate the effect of 6-MP on the aggregation of AgNPs. As shown in Fig. S2A (Supporting Information), the absorbance ratio at 530 and 394 nm (A_{530}/A_{394}) was applied to measure the aggregation degree of AgNPs. The higher was the absorbance ratio, the greater was the aggregation degree of AgNPs obtained. The results showed that AgNPs aggregated easily under pH < 4, suggesting that AgNPs could not exist stably in a strong acidic environment. On the contrary, AgNPs could exist stably at relatively high pH (pH > 4) values. It could be seen that the A_{530}/A_{394} value of the system changed little between pH 4 – 6 after adding 6-MP, indicating that the aggregation degree of AgNPs in this pH range was nearly the same, while the aggregation degree of AgNPs was relatively small at pH 6.6. A possible reason might be that the stability of the prepared AgNPs was due to the negatively charged citrate ions on the surface. Citrate has three pK_a values (3.1, 4.7 and 6.4).³⁵ When the pH value was below 6.4, the carboxylic groups of citrate were protonated. While the carboxylic groups were deprotonated at pH 6.6. Thus, with adding the same concentration of 6-MP which could replace citrate, the aggregation degree was smaller at pH 6.6 than that at pH < 6.4. Considering the wide applications under neutral conditions, pH = 6.6 was selected for use in this experiment. At the same time, the influence of the buffer volume on the detection was studied (Fig. S2B). Different volumes of buffer were added to a 1-mL solution of AgNPs in the presence of 0.3 μM 6-MP. The final solution volume was 3 mL. It was found that the AgNPs still maintained good dispersion after adding 50 – 200 μL of the buffer solution. When 6-MP was present in the system, the A_{530}/A_{394} value increased rapidly with the addition of 120 – 150 μL of the buffer solution. When the buffer volume was higher than 150 μL , the change in the A_{530}/A_{394} value gradually slowed down. The larger was the A_{530}/A_{394} value obtained after adding a larger volume of the buffer solution, indicating an increased aggregation degree of AgNPs. Considering the stability of the system, a 80- μL buffer solution (100 mM, pH 6.6) was chosen for use.

Under the optimized conditions, a series of titration experiments were carried out *via* changing the 6-MP concentration. As can be seen in Fig. 2A, the solution color of the AgNPs turned from yellow to brown with increasing 6-MP concentration. The UV-Vis spectra of the AgNPs upon the addition of various concentrations of 6-MP are shown in Fig. 2B, the absorbance at 394 nm decreased while that at 530 nm increased with increasing 6-MP concentration. The absorbance ratio, A_{394}/A_{530} , was used to detect 6-MP quantitatively. As can be seen in Fig. 2C, the A_{394}/A_{530} value decreased linearly in the range of 0 – 0.5 μM ($R^2 = 0.99741$). The calculated detection limit was 10 nM, which was comparable, or lower than, that of other reported methods (see Table S1, Supporting Information).

The effect of different organic molecules on the 6-MP detection was tested to study the selectivity. As shown in Fig. 3A, only 6-MP enabled AgNPs to aggregate, showing a yellow-to-brown color change. Figure 3B shows the absorbance ratio of A_{530}/A_{394} after adding different organic molecules.

Fig. 2 (A) Photographic images of the citrate-AgNPs after adding various concentrations of 6-MP. (B) UV-Vis spectra of citrate-AgNPs upon the addition of 6-MP. (C) Plot of A_{394}/A_{530} against the 6-MP concentration.

The A_{530}/A_{394} value in the presence of 6-MP (0.7 μM) was much larger than the blank, while the A_{530}/A_{394} value upon the addition of other organic molecules (70 μM) was similar to the blank, while keeping AgNPs in a dispersion state. The effect of coexisting organics on the determination of 6-MP was also investigated. As depicted in Fig. 3C, in the presence of mixtures of different organic molecules with 6-MP, the color of AgNPs solutions remained the same. This indicated that other organic molecules did not interfere with the 6-MP detection, suggesting that this method had high selectivity for the determination of 6-MP.

To test the practicality of this facile colorimetric method, human urine samples spiked with 6-MP were analyzed. The results are depicted in Table S2 (Supporting Information). It can be seen that the found values are similar to the added values. The recovery results (95 – 102.5%) were good and the RSDs were lower than 3.1%, indicating that this method was credible for detecting 6-MP in real samples.

In this paper, a facile colorimetric sensor for 6-MP detection using AgNPs has been developed. The addition of 6-MP resulted in the aggregation of AgNPs with a yellow-to-brown color change. The factors influencing the 6-MP detection were studied. The 6-MP concentration could be easily determined using UV-Vis spectroscopy. The developed colorimetric method was convenient and economic. It showed good sensitivity and selectivity for 6-MP detection. Besides, it could be used for 6-MP recognition in real samples.

Fig. 3 (A) Photographic images and (B) corresponding A_{530}/A_{394} of citrate-AgNPs upon the addition of various organic molecules. (C) Photographic images of the citrate-AgNPs adding mixtures of different organic molecules with 6-MP. The 6-MP concentration was $0.7 \mu\text{M}$ and the concentrations of other organic molecules were $70 \mu\text{M}$.

Acknowledgements

We appreciate financial support, including the National Natural Science Foundation of China (No. 21607096, 41771342), National Key Research and Development Plan (No. 2017YFD0801504) and Funds of Shandong “Double Tops” Program (SYL2017XTTD15) and Promotive Research Fund for Excellent Young and Middle-aged Scientists of Shandong Province (No. BS2015CL008, ZR2017BB064).

Supporting Information

This material is available free of charge on the Web at <http://www.jsac.or.jp/analsci/>.

References

1. Z. K. He, X. L. Liu, Q. Y. Luo, X. M. Yu, and Y. E. Zeng, *Anal. Sci.*, **1995**, *11*, 415.
2. W. W. Tang, W. H. Li, Y. F. Li, M. Zhang, and X. P. Zeng, *New J. Chem.*, **2015**, *39*, 8454.
3. Z. Li, Y. N. Ni, and S. Kokot, *Biosens. Bioelectron.*, **2015**, *74*, 91.
4. Z. J. Sun, Y. L. Liu, and Y. F. Li, *Spectrochim. Acta, Part A*, **2015**, *139*, 296.
5. L. Wang and Z. J. Zhang, *Talanta*, **2008**, *76*, 768.
6. Y. S. Yuan, Y. L. Wang, S. P. Liu, Y. F. Li, R. L. Duan, H. Zhang, and X. L. Hu, *RSC Adv.*, **2016**, *6*, 52255.
7. U. Hofmann, G. Heinkele, S. Angelberger, E. Schaeffeler, C. Lichtenberger, S. Jaeger, W. Reinisch, and M. Schwab, *Anal. Chem.*, **2012**, *84*, 1294.
8. L. X. Zhang, H. L. Li, G. Chu, L. Luo, J. Jin, B. Zhao, and Y. Tian, *Colloids Surf., A*, **2016**, *508*, 309.
9. L. Yang, Y. H. Chen, H. L. Li, L. Luo, Y. Zhao, H. Q. Zhang, and Y. Tian, *Anal. Methods*, **2015**, *7*, 6520.
10. P. Biparva, S. M. Abedirad, and S. Y. Kazemi, *Spectrochim. Acta, Part A*, **2015**, *145*, 454.
11. H. W. Sun, T. Wang, X. Y. Liu, and P. Y. Chen, *J. Lumin.*, **2013**, *134*, 154.
12. H. Mawatari, Y. Kato, S. Nishimura, N. Sakura, and K. J. Ueda, *J. Chromatogr. B*, **1998**, *716*, 392.
13. K. A. Rawata, R. K. Singhal, and S. K. Kailasa, *Sens. Actuators, B*, **2017**, *249*, 30.
14. Z. G. Chen, G. M. Zhang, X. Chen, J. H. Chen, J. B. Liu, and H. Q. Yuan, *Biosens. Bioelectron.*, **2013**, *41*, 844.
15. M. X. Gao, J. L. Xu, Y. F. Li, and C. Z. Huang, *Anal. Methods*, **2013**, *5*, 673.
16. Z. Li, Y. Wang, Y. N. Ni, and S. Kokot, *Biosens. Bioelectron.*, **2015**, *70*, 246.
17. H. Karimi-Maleh, A. F. Shojaei, F. Karimi, K. Tabatabaeian, S. Shakeri, and R. Moradi, *Biosens. Bioelectron.*, **2016**, *86*, 879.
18. H. Karimi-Maleh, F. Tahernejad-Javazmi, N. Atar, M. L. Yola, V. K. Gupta, and A. A. Ensafi, *Ind. Eng. Chem. Res.*, **2015**, *54*, 3634.
19. H. Beitollahi, S. G. Ivani, and M. Torkzadeh-Mahanic, *ECS J. Solid State Sci.*, **2017**, *6*, M29.
20. C. Q. Tu and X. R. Wen, *Adv. Mater. Res.*, **2014**, *881-883*, 479.
21. J. L. Duan, H. Z. Yin, R. R. Wei, and W. W. Wang, *Biosens. Bioelectron.*, **2014**, *57*, 139.
22. J. L. Duan and J. H. Zhan, *Sci. China Mater.*, **2015**, *58*, 223.
23. X. D. Wang, Y. Y. Yang, J. Dong, F. Bei, and S. Y. Ai, *Sens. Actuators, B*, **2014**, *204*, 119.
24. S. Diamai, W. Warjri, D. Saha, and D. P. S. Negi, *Colloids Surf., A*, **2018**, *538*, 593.
25. Jigyasa and J. K. Rajput, *Biosens. Bioelectron.*, **2018**, *120*, 153.
26. S. A. Ghoto, M. Y. Khuawar, and T. M. Jahangir, *Anal. Sci.*, **2019**, *35*, 631.
27. S. Rostami, A. Mehdinia, A. Jabbari, E. Kowsari, R. Niroumand, and T. J. Booth, *Sens. Actuators, B*, **2018**, *271*, 64.
28. M. Okochi, T. Kamiya, T. Omasa, M. Tanaka, and H. Honda, *Anal. Sci.*, **2016**, *32*, 93.
29. Y. Zhou, W. Huang, and Y. He, *Sens. Actuators, B*, **2018**, *270*, 187.
30. M. L. Firdaus, I. Fitriani, S. Wyantuti, Y. W. Hartati, R. Khaydarov, J. A. Mcalister, H. Obata, and T. Gamo, *Anal. Sci.*, **2017**, *33*, 831.
31. M. N. Karim, S. R. Anderson, S. Singh, R. Ramanathan, and V. Bansal, *Biosens. Bioelectron.*, **2018**, *110*, 8.
32. N. Abe and N. Iki, *Anal. Sci.*, **2017**, *33*, 1141.
33. S. Mohammadi and G. Khayatyan, *Spectrochim. Acta, Part A*, **2017**, *185*, 27.
34. L. Zhan, T. Yang, S. J. Zhen, and C. Z. Huang, *Microchim. Acta*, **2017**, *184*, 3171.
35. W. Trisaranakul, A. Chompoosor, W. Maneeprakorn, D. Nacapricha, N. Choengchan, and S. Teerasong, *Anal. Sci.*, **2016**, *32*, 769.
36. Y. S. Shon and E. Cutler, *Langmuir*, **2004**, *20*, 6626.