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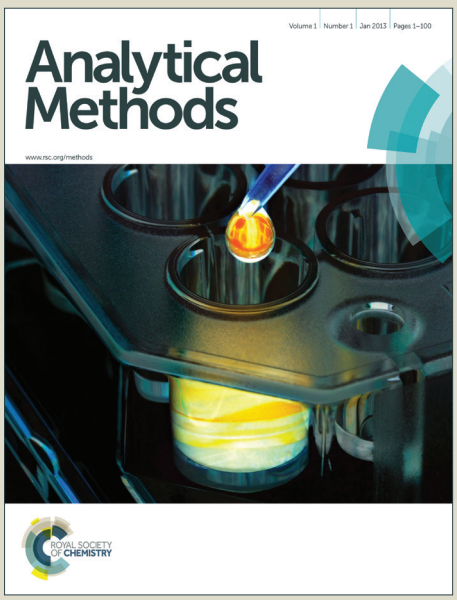

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PAPER

Colorimetric detection of Ag(I) ions using dCTP-stabilized gold nanoparticles

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This work presented a simple colorimetric method for Ag(I) ions detection based on the gold nanoparticles (AuNPs) with unique distance-dependent optical properties. It was the first time that the determination of Ag(I) ions by inducing aggregation of the dispersed dCTP-stabilized AuNPs was reported. Unlike DNA-functionalized AuNP-based colorimetric approaches, this method did not require to design and synthesize DNA strands used to functionalize AuNPs, which simplified the operation process and reduced the cost. The method was validated by recovery tests using tap water and river water samples, and exhibited high sensitivity and selectivity attributing to the strong and specific binding effect of Ag(I) ions and dCTPs. The present strategy provides a good candidate of colorimetric sensing for Ag(I) ions in environmental water samples.

1 Introduction

The rapid and sensitive determination of Ag(I) ions in environmental water is important since Ag(I) ion, which can inactivate sulfhydryl enzymes and accumulate in the body, is a severe environmental pollutant and has medical effects on human health.[1-4] It was demonstrated that some metal ions can selectively bind to the native or artificial bases in DNA duplexes to form metal-mediated base pairs[5]. Based on Ag(I) ion selectively binding with Cytosine–Cytosine (C-C) mismatches to form stable C–Ag(I)–C base pairs in DNA duplexes, several approaches for the sensitive and specific detection of Ag(I) ion have been reported by using surface plasmon resonance [6], fluorescence [7, 8], electrochemistry [9, 10], colorimetry [2, 11], etc. Generally, surface plasmon resonance has mainly focused on interaction between large biomolecules rather than on metal ion detection[6, 12]. Fluorescence detection has high sensitivity, but usually needs the labeling of fluorescent materials. Furthermore, many fluorophore-based sensors are not suitable to detect silver contamination in aqueous solutions because most fluorescence reagents only react in organic or organic/water media. [6, 13] Electrochemistry methods for Ag ion detection are also highly sensitive, while usually demanding the fabrication of modified electrodes. In contrast, colorimetric detection of silver ion based on metal-mediated base pairs and optical properties of gold nanoparticles (AuNPs) features ease of use, low cost and high speed, and the signals can be easily detected by UV-vis absorption spectra, or even naked eyes, instead of complicated instruments. It mainly benefits from the AuNPs with high extinction coefficients and unique distance-dependent optical properties, which allows the AuNPs to be utilized as ideal color reporting groups for colorimetric detection.[14-19] However, the stability of the unmodified AuNPs is subject to such factors as salt concentrations and pH. DNA strands were usually chosen to stabilize AuNPs, such as DNA duplex [5, 20], single-stranded DNA (ssDNA) [2, 21], aptamer[22] and DNAzmye[11]. And Ag(I) ion was successfully detected based on AuNP aggregation resulting from the Ag(I) ion binding to the C–C base pairs in DNA strands. In these methods, DNA sequences were designed and prepared in vitro from native sources or even artificial evolution [22, 23], which is time-consuming and costly. Luckily, some researchers have demonstrated several methods to increase the stability of unmodified AuNPs by mixing the AuNPs with an oligonucleotide or a mononucleotide.[24-26] Therefore, there seems to be great potential in developing the Ag(I) ion colorimetric detection with further simpler process and lower cost. According to the phenomenon that deoxyribonucleotides (dNTPs) could be used to effectively stabilize AuNP solutions by the electrostatic repulsion among AuNPs [25], it is possible to develop a simple colorimetric detection method of Ag(I) ion using mononucleotides and unmodified AuNPs. In this work, we attempt to use the deoxycytidine triphosphates (dCTPs) to stabilize bare AuNPs and to perform the colorimetric detection of Ag(I) ions in aqueous solutions based on the binding effect of Ag(I) ions and dCTPs.

2 Experimental

2.1 Chemicals and materials

Chloroauric acid tetrahydrate, trisodium citrate, sodium hydroxide, EDTA-2Na, 4-hydroxyethyl piperazine ethyl sulfonic acid (HEPES), mercury bichloride and nitrates (Na^+ , Ag^+ , K^+ , Ca^{2+} , Cd^{2+} , Zn^{2+}, Cu^{2+}, Fe^{3+} , Ni^{2+} , Mg^{2+} , Ba^{2+} , Pb^{2+}) were purchased from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. Deoxycytidine triphosphates was obtained from Sangon Biotech Co., Ltd., Shanghai, China. All reagents were of analytical reagent grade except for the ones with special

description. Doubly deionized water was used throughout the experiment.

2.2 Apparatus

UV-vis absorption spectra were recorded on a UV-vis spectrophotometer (T6, Beijing Purkinje General Instrument Co., Ltd., China) at room temperature. Transmission electron microscopy (TEM) images were supplied by a transmission electron microscope (Tecnai G220, Field Emission Inc., USA).

2.3 Synthesis of AuNPs

All glassware used in the following procedures were soaked in freshly prepared solution of HNO_3-HCl (1:3, v/v) for 24 hours, and then cleaned thoroughly in doubly deionized water before use. AuNPs with an average diameter of 13 nm were prepared using the classical method with slight modification. [27] Firstly, a $HAuCl_4$ solution (1.0 mmol/L, 50 mL) in a round-bottom flask was boiled under vigorous stirring and reflux. Then a sodium citrate solution (38.8 mmol/L, 5 mL) was rapidly added to the boiled $HAuCl_4$ solution, heated for another 10 min and further stirred for 15 min. After that, the solution was cooled to room temperature and filtered by a 0.22-μm membrane. Finally, the wine-red solution was stored at 4 °C.

2.4 Colorimetric detection of Ag(I) ions

First, dCTP solution (1.0 mmol/L, 20 μL) was mixed with the AuNP stock solution (980 μL), and incubated for 10 min at room temperature. Second, the obtained AuNPs-dCTPs solution was centrifuged at 12000 rcf for 20 min at room temperature to remove supernate. Third, the AuNPs-dCTPs were redispersed by HEPES buffer (containing 75 mmol/L $NaNO_3$ and 10 mmol/L HEPES, pH=7.2) to the volume of 1.0 mL. Fourth, Ag^+ solutions (50 μL) with different concentrations were respectively added to the redispersed AuNPs-dCTPs suspensions (100 μL), incubated at room temperature for 8 min and detected by the UV-vis absorption spectra. The aggregation kinetics of AuNPs at various concentrations of Ag^+ was obtained by recording the UV-vis spectra at an interval of 2 min. The concentration of Ag^+ was quantified by the absorption ratio (A_{680}/A_{522}).

3 Results and discussion

3.1 Mechanism of Ag(I) ion detection based on aggregation of AuNPs

An important feature of AuNPs is their surface plasmon resonance which is related to their size, shape, interparticle distance, surrounding medium and so on. AuNPs with interparticle distances distinctly larger than the average particle diameter appear red; otherwise they appear blue or purple. [15] In this work, the color shifts were easily observed by the UV-vis absorption spectrum or the naked eyes when the AuNPs were transformed between aggregate structures and dispersed states. The dispersed AuNP solution was wine-red, which was prepared by using citrate reduction of $HAuCl_4$ and stabilized by the negative capping agent of citrate. With the addition of electrolyte, the electrostatic repulsion between the negative-charged AuNPs was screened, as a result the AuNPs were induced to aggregate, and the surface plasma resonance absorption peak near 520 nm decreased.[21] Utilization of mononucleotide as a surface-stabilization agent for stabilizing AuNPs in a high salt aqueous solution has been demonstrated.[25] When dCTPs were added into the AuNP solution with high concentration of the electrolyte, the aggregated AuNPs were then redispersed.[25] Based on the affinity of some functional groups (i.e., -CN, -SH, and $-NH_2$) with Au, [27] the added dCTPs were absorbed on the surface of AuNPs, which increased the density of the negative charges on the AuNPs. The strongly electrostatic interactions prevented the AuNPs from salt-induced aggregation at a high salt concentration. [25] Recently, the interaction of Ag(I) ion with cytosine-rich ssDNA oligonucleotides was investigated using dual polarization interferometry [28], and the binding mechanism between Ag(I) ion and single C-C mismatches was studied in a nanopore.[29] As the Ag(I) ions can be selectively captured by C–C mismatches to form C–Ag(I)–C metal-mediated base pairs [30], we attempt to utilize the binding effect of dCTP molecules and Ag(I) ions to induce AuNPs aggregation. A similar principle was demonstrated in colorimetric detection of Hg(II) based on Hg(II)-induced aggregation of mononucleotides-stabilized gold nanoparticles. [31] In practice, when Ag(I) ions were introduced into the dCTP-stablized AuNP solution, the strong binding effect of Ag(I) ion with dCTPs led to the desorption of dCTPs from the AuNPs, which caused aggregation of the AuNPs once again under the solution environment of high ionic strength. The AuNP aggregation resulted in significant color change of the solution from red to blue, which allowed an efficient detection of Ag(I) in aqueous solution. The scheme of colorimetric sensing of Ag(I) ions based on salt-induced aggregation of the dCTPs-stabilized AuNPs was shown in Fig. 1.

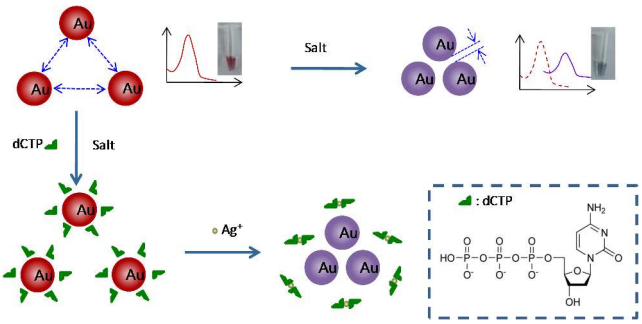

Fig. 1 Schematic description of colorimetric sensing of Ag (I) ions based on the dCTPs-stabilized AuNPs.

3.2 Effect of the concentration of $NaNO_3$

Since the ionic strength of the solution impacts on the electrostatic repulsion between the dCTP-stablized AuNPs, the concentration of salt is an important factor for the stability of dCTPs-AuNPs. [21] For analyzing Ag^+ in aqueous solution based on AuNP aggregation, a salt will be added into the dCTP-stabilized AuNP solution. The salt containing anions that can precipitate or complex with Ag^+ should be completely excluded, such as Br^-, Cl^-, I^-, CO_3^{2-}, SO_4^{2-}, PO_4^{3-}, S^{2-}, etc. In this work, $NaNO_3$ was chosen for inducing AuNP aggregation. It is known that C-Ag(I)-C complexes are stable in a neutral pH medium [7, 21, 23, 32], and strong acidic or alkaline solutions are unfavorable for the determination of Ag(I) ions by inducing aggregation of the dCTP-stabilized AuNPs. Therefore, a pH 7.2 HEPES solution was used as the buffer in this work. $NaNO_3$ solutions with various concentrations were respectively added into the redispersed

AuNPs-dCTPs suspensions to optimize salt concentration. The dCTPs-AuNPs solution was red in color and exhibited a strong surface plasmon resonance absorption peak at 522 nm. The concentrations of $NaNO_3$ solution ranging from 25 mmol/L to 150 mmol/L were investigated. As shown in Fig. 2, the intensities of the maximum absorption peaks decreased as the $NaNO_3$ concentrations increased. When the concentrations were from 25 mmol/L to 75 mmol/L, the peaks were sharp and no peak tailing was observed. However, when the concentration was 100 mmol/L or higher, the peak heights decreased dramatically and peak tailing appeared. The result implied that dCTPs-AuNPs remained stable when the $NaNO_3$ concentration was 75 mmol/L or lower. Taking the sensitivity and the background of Ag(I) ion determination into account, 75-mmol/L $NaNO_3$ was employed in the following experiments.

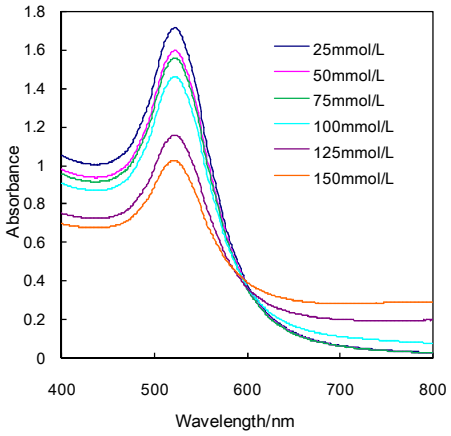

Fig. 2 Absorption spectra of the dCTP-stabilized AuNPs with different $NaNO_3$ concentrations in HEPES buffer.

3.3 Aggregation kinetics of AuNPs

The aggregation kinetics of AuNPs caused by different concentrations of Ag(I) ions is shown in Fig. 3. The results were obtained by using UV-vis spectrophotometer, and the A_{680}/A_{522} values were recorded at an interval of 2 min. At low concentrations of Ag(I) ions (20 μmol/L and 40 μmol/L), the absorbance ratio A_{680}/A_{522} values of the solutions increased gradually within 8 min, and then remained constant as time went on. At the high concentrations of Ag(I) ions (60 μmol/L and 80 μmol/L), the A_{680}/A_{522} values rose sharply and then reached a plateau within 4 min. Compared with the effect of low concentrations of Ag(I) ions, the interaction of Ag (I) ions and dCTPs was a faster kinetic process at high concentrations of Ag(I) ions. The results also suggested that Ag(I) ion concentration can affect the kinetics of AuNP aggregation. It could be inferred that Ag(I) ions at low concentrations did not completely desorb the dCTP molecules absorbed on the surface of AuNPs, and the AuNPs could not aggregate completely owing to some dCTPs remaining on AuNPs. While with the increment of Ag(I) ion concentration, increasingly more dCTP molecules departed from the surface of AuNPs, and more AuNPs aggregated under high ionic strength as a result of losing the protection from dCTPs. The aggregation of AuNPs triggered by Ag(I) ions could be completed within 8 min, and therefore the absorption spectra were recorded after the addition of Ag(I) ion solution for 8 min in all the experiments.

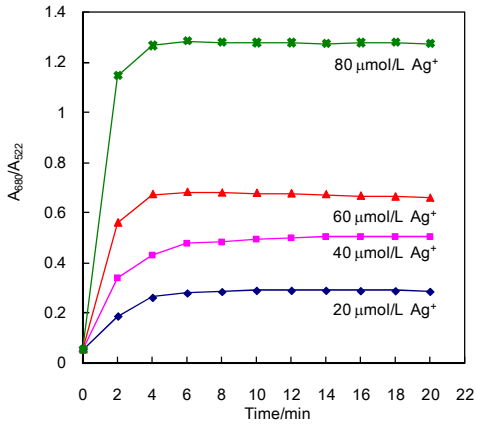

Fig. 3 Plots of A_{680}/A_{522} of dCTPs-AuNPs vs. time in the presence of different concentrations of Ag^+. The data were recorded at an interval of 2 min.

To further verify the Ag(I) -induced dCTPs-AuNPs aggregation, the TEM images of dCTPs-AuNPs before and after their reaction with Ag(I) ions (80 μmol/L) were observed, as shown in Fig. 4. The dCTPs-AuNPs exhibited homogeneous dispersion while the AuNPs treated by Ag(I) ions appeared obvious aggregation, which clearly demonstrated that Ag(I) ions broke the protection of AuNPs from dCTPs and induced the aggregation of AuNPs.

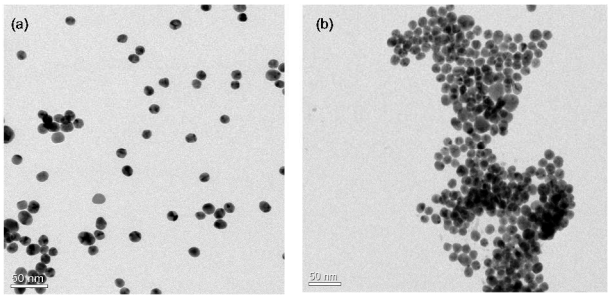

Fig. 4 TEM images of dCTPs-AuNPs solution before (a) and after (b) treated with 80 μmol/L Ag^+.

3.4 Selectivity of the colorimetric assay

The selectivity of the colorimetric sensing was investigated by respectively adding metal ions (150 μmol/L), including K^+, Na^+, Mg^{2+}, Ca^{2+}, Ba^{2+}, Fe^{3+}, Ni^{2+}, Zn^{2+},Cu^{2+}, Cd^{2+}, Pb^{2+} and Hg^{2+}, into the dCTPs-AuNPs solution. In this system, the interference of Pb^{2+} could not be ignored. To eliminate the interference of Pb^{2+}, EDTA-2Na (100 μmol/L), a chelating ligand, was individually added into the Pb^{2+} solution. Photographs and the A_{680}/A_{522} response were monitored respectively, as shown in Fig. 5. The dCTPs-AuNPs solution turned to blue in the presence of 80 μmol/L Ag^+, and the color of solutions with other metal ions barely changed. The A_{680}/A_{522} value of the solution with Ag^+ was found to be at least 12 times higher than the values of solutions with other metal ions although the concentrations of the other metal ions were greater than the concentration of Ag(I) ions. The results revealed that the addition of certain amount of other metal ions had no significant effect on AuNP aggregation in the dCTPs-AuNPs system, which indicated the colorimetric assay had high selectivity for Ag(I) ions detection.

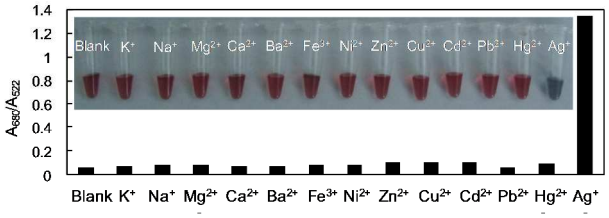

Fig. 5 A_{680}/A_{522} values and photographic images of dCTPs-AuNPs solutions in the presence of different metal ions. The concentration of Ag^+ is 80 μmol/L, the concentration of Pb^{2+} is 150 μmol/L mixed with EDTA-2Na 100 μmol/L, and the concentration of all other metal ions is 150 μmol/L. Incubation time of dCTPs-AuNPs and metal ions was 8 min.

3.5 Analytical performance and sample detection

The sensitivity of the colorimetric system for Ag^+ was evaluated under the optimized conditions, including 10-min incubation of dCTPs and AuNPs, 75-mmol/L $NaNO_3$ in HEPES buffer, 8-min reaction of Ag^+ and dCTPs-AuNPs. A series of Ag^+ standard solutions were detected respectively, and the images and UV-vis spectra of the solutions were shown in Fig. 6. The dCTPs-AuNPs solution exhibited strong surface plasma resonance absorption peak at 522 nm. As the concentration of Ag^+ solution increased, the absorption peak at 522 nm decreased and shifted to red gradually. Meanwhile, a new absorption peak between 600 nm and 700 nm appeared and grew. Moreover, the solution color dramatically changed from red to purple, then to blue along with the increase of Ag^+ concentration. The results confirmed that the addition of Ag^+ triggered the aggregation of AuNPs, which is closely related to the concentration of Ag^+ solution. The A_{680}/A_{522} values depended linearly on the concentrations of Ag^+ in the range of 10-80 μmol/L. And regression equation of the calibration curves is y=0.0164x-0.1045 with the correlation coefficient R^2 of 0.9887 (y representing A_{680}/A_{522} value, and x standing for Ag^+ concentration). The limit of detection is 0.083 μmol/L ($3\sigma/k$). The experiment results demonstrated that the method was sensitive for the analysis of Ag^+ in aqueous solution.

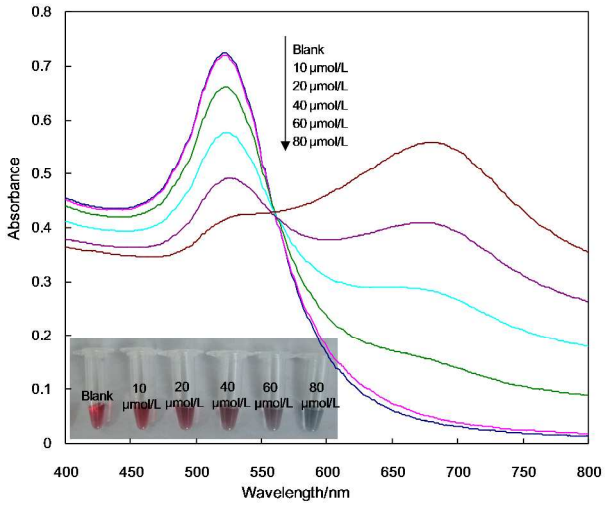

Fig. 6 Photographic images and UV-vis absorption spectra of AuNPs treated with Ag^+ of various concentrations

To evaluate the feasibility of the presented method for analyzing real samples, Ag^+ in tap water and river water samples was measured. The river water was sampled from Nan Canal in Shenyang City, China. Ag(I) ions in the samples were not detected. The recoveries were 97.7-103.9% for tap water samples and 97.8-106.7% for river water samples (see Table 1). These results indicated that this colorimetric assay can be applied to the detection of Ag^+ in environmental samples.

Table 1 Recoveries for the determination of Ag^+ in real water samples (n = 3).

Samples	Spiked (μmol/L)	Found (μmol/L)	Recovery (%)	RSD (%)
Tap water	30	29.3±0.11	97.7	4.5
	50	51.9±0.06	103.8	2.6
	70	72.7±0.17	103.9	6.8
River water	30	30.2±0.14	100.7	5.5
	50	48.9±0.07	97.8	2.9
	70	74.7±0.19	106.7	7.5

4 Conclusions

We developed a colorimetric detection method for Ag(I) ions in aqueous phase using dCTPs and unmodified AuNPs. Ag^+ in aqueous solutions could be easily quantified by monitoring the absorbance of the AuNPs. The dispersed AuNPs exhibited red color due to the electrostatic repulsion of dCTPs on AuNPs. With the addition of Ag^+, the dCTPs desorbed from the surface of AuNPs owing to the strong binding effect of Ag(I) ions and dCTPs and the aggregation of the AuNPs was induced. The colorimetric assay could be performed by a UV-vis spectrometer, or even by the naked eyes, and the operation process was simple. The use of commercialized dCTPs instead of expensive reagents, such as DNA duplex, reduced the cost. The method showed good sensitivity and selectivity for the determination of Ag^+. It was demonstrated to be quite promising for simple and rapid detection of Ag^+ in aqueous solution.

Acknowledgements

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Notes and references

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1. H. T. Ratte, *Environ. Toxicol. Chem.* , 1999, **18**, 89-108.
2. X. H. Zhou, D. M. Kong and H. X. Shen, *Anal. Chim. Acta.*, 2010, **678**, 124-127.
3. G. D. Huy, M. Zhang, P. Zuo and B. C. Ye, *Analyst*, 2011, **136**, 3289-3294.
4. R. Freeman, T. Finder and I. Willner, *Angew. Chem. Int. Ed.*, 2009, **48**, 7818-7821.
5. G. H. Clever, C. Kaul and T. Carell, *Angew. Chem. Int. Ed.*, 2007, **46**, 6226-6236.
6. C. C. Chang, S. h. Lin, S. C. Wei, C. S. Yu and C. W. Lin, *Anal. Bioanal. Chem.* , 2012, **402**, 2827-2835.
7. Y. H. Lin and W. L. Tseng, *Chem. Commun.* , 2009, 6619-6621.

8. H. L. Li, J. F. Zhai and X. P. Sun, *Langmuir*, 2011, **27**, 4305-4308.
9. J. Zhao, Q. Fan, S. Zhu, A. Duan, Y. Yin and G. Li, *Biosens. Bioelectron.*, 2013, **39**, 183-186.
10. G. Xu, G. Wang, X. He, Y. Zhu, L. Chen and X. Zhang, *Analyst*, 2013, **138**, 6900-6906.
11. T. Li, L. Shi, E. Wang and S. Dong, *Chem. Eur. J.*, 2009, **15**, 3347-3350.
12. D. Habauzit, J. Chopineau and B. Roig, *Anal. Bioanal. Chem.* , 2007, **387**, 1215-1223.
13. L. Basabe-Desmonts, D. N. Reinhoudt and M. Crego-Calama, *Chem. Soc. Rev.* , 2007, **36**, 993-1017.
14. C. A. Mirkin, R. L. Letsinger, R. C. Mucic and J. J. Storhoff, *Nature*, 1996, **382**, 607-609.
15. R. Elghanian, J. J. Storhoff, R. C. Mucic, R. L. Letsinger and C. A. Mirkin, *Science*, 1997, **277**, 1078-1081.
16. J. Yguerabide and E. E. Yguerabide, *Anal. Biochem.* , 1998, **262**, 137-156.
17. J. J. Storhoff, A. A. Lazarides, R. C. Mucic, C. A. Mirkin, R. L. Letsinger and G. C. Schatz, *J. Am. Chem. Soc.* , 2000, **122**, 4640-4650.
18. J. W. Liu and Y. Lu, *J. Am. Chem. Soc.* , 2004, **126**, 12298-12305.
19. J. S. Lee, M. S. Han and C. A. Mirkin, *Angew. Chem. Int. Ed.*, 2007, **46**, 4093-4096.
20. D. M. Kong, L. L. Cai and H. X. Shen, *Analyst*, 2010, **135**, 1253-1258.
21. B. Li, Y. Du and S. Dong, *Anal. Chim. Acta.*, 2009, **644**, 78-82.
22. S. C. B. Gopinath, T. Lakshmipriya and K. Awazu, *Biosens. Bioelectron.*, 2014, **51**, 115-123.
23. Y. Xiang and Y. Lu, *Chem. Commun.* , 2013, **49**, 585-587.
24. H. X. Li and L. J. Rothberg, *J. Am. Chem. Soc.* , 2004, **126**, 10958-10961.
25. W. Zhao, T. M. H. Lee, S. S. Y. Leung and I. M. Hsing, *Langmuir*, 2007, **23**, 7143-7147.
26. S. Kim, J. Kim, N. H. Lee, H. H. Jang and M. S. Han, *Chem. Commun.* , 2011, **47**, 10299-10301.
27. K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, *Anal. Chem.* , 1995, **67**, 735-743.
28. Y. Zheng, C. Yang, F. Yang and X. R. Yang, *Anal. Chem.* , 2014, **86**, 3849-3855.
29. Y. Wang, B. Q. Luan, Z. Y. Yang, X. Y. Zhang, B. Ritzo, K. Gates and L. Q. Gu, *Sci. Rep. ,* 2014, **4**, 5883.
30. A. Ono, S. Cao, H. Togashi, M. Tashiro, T. Fujimoto, T. Machinami, S. Oda, Y. Miyake, I. Okamoto and Y. Tanaka, *Chem. Commun.* , 2008, 4825-4827.
31. Y. Y. Xu, L. Deng, H. Wang, X. Y. Ouyang, J. Zheng, J. H. Li and R. H. Yang, *Chem. Commun.* , 2011, **47**, 6039-6041.
32. C. K. Wu, C. Xiong, L. J. Wang, C. C. Lan and L. S. Ling, *Analyst*, 2010, **135**, 2682-2687.

Table of Contents Entry

A simple colorimetric detection method for Ag(I) ions based on dCTP-stabilized gold nanoparticles was proposed.