A highly sensitive sensor for Cu 2+ with unmodified gold nanoparticles and DNAzyme by using the dynamic light scattering technique

ARTICLE *in* THE ANALYST · MAY 2012

Impact Factor: 4.11 · DOI: 10.1039/c2an35217f · Source: PubMed

4 AUTHORS, INCLUDING:

Lian-Sheng Ling
Sun Yat-Sen University
45 PUBLICATIONS **801** CITATIONS

SEE PROFILE

Analyst

Dynamic Article Links

Cite this: *Analyst*, 2012, **137**, 3064

www.rsc.org/analyst

PAPER

A highly sensitive sensor for Cu^{2+} with unmodified gold nanoparticles and DNAzyme by using the dynamic light scattering technique

Xiangmin Miao,[ab] Liansheng Ling,*[a] Du Cheng[a] and Xintao Shuai*[ab]

Received 16th February 2012, Accepted 11th April 2012
DOI: 10.1039/c2an35217f

Copper ion (Cu^{2+}) plays an important role in many biological reactions, and a suitable level of Cu^{2+} is necessary for the regular metabolism of life. Thus developing a sensitive and simple method for determination of Cu^{2+} is essential. Here, a novel and sensitive Cu^{2+} sensor was developed based on detecting the average hydrodynamic diameter of AuNPs by using dynamic light scattering (DLS). Cu^{2+}-specific DNAzyme was double-strand and could not adsorb on the surface of AuNPs, accordingly AuNPs aggregation would occur with the addition of NaCl. However, Cu^{2+} could cleave DNAzyme and release single-stranded DNA (ssDNA) fragments, which could adsorb on the surface of AuNPs and prevent them from aggregation. Such differences in DNA adsorption ability on AuNPs before and after the addition of Cu^{2+} affected the disperse state of AuNPs directly, and then affected their average hydrodynamic diameter, which could be detected with the DLS technique. Based upon the above mentioned principle, detection of Cu^{2+} could be realized over the range from 100 pM to 2.0 nM, with a linear regression equation of $D = 306.73 - 89.66C$ (C: nM, $R = 0.9953$) and a detection limit of 60 pM (3δ/slope). Moreover, satisfactory results were obtained when the assay was applied in the detection of Cu^{2+} in water samples.

Introduction

Copper ion (Cu^{2+}), as one of the heavy metal ions, is essential in electron transfer processes of many biological reactions.[1] However, lower or higher concentration of it can cause adverse effects to biological systems. For instance, a lower level of Cu^{2+} can affect the enzyme activity and inhibit cell metabolism, [2] while excess Cu^{2+} is related to gastrointestinal disturbance and damage to liver or kidney.[3] So, it is vital to develop sensitive and novel methods for routinely and effectively monitoring the concentration of Cu^{2+}. In the past few years, several analytical approaches such as atomic absorption spectrometry (AAS) and inductive coupled plasma atomic emission spectroscopy (ICP-AES) have been used for the detection of Cu^{2+} ions.[4,5] Recently, other complementary analytical strategies were established with organic dyes, semiconductor nanocrystals and metal nanoparticles by detecting the signal of fluorescence,[6–8] surface plasmon resonance,[9,10] electrochemistry[11] and color.[12–15] Thereinto, metal nanoparticles based colorimetric methods have received considerable attention due to their advantages of simplicity, on-site, and real-time detection without instruments.

Metal-dependent DNAzymes are a class of catalytic DNA molecules that can catalyze many chemical reactions in the presence of specific metal ions. Unlike RNA or protein enzymes, most of the DNAzymes are inexpensive and can be denatured and renatured many times without losing binding ability and catalytic activity.[16,17] Based upon the above mentioned properties, DNAzymes have been extensively applied in the development of colorimetric sensors.[18–21] In addition, gold nanoparticles (AuNPs) are a type of attractive material due to their high extinction coefficients, size or distance dependent optical properties. The color of AuNPs can be altered easily by changing their dispersed/aggregated states, and such changes can be directly observed by the naked eye. For these reasons, AuNPs have been widely used in the detection of protein,[22,23] DNA[24,25] and metal ions.[26,27] Comparing with the labeled one, label-free methods have attracted great interest because they are relatively simple and inexpensive, without needing the modification of DNAzyme (or its substrate) onto AuNPs and the separation of modified AuNPs from unmodified DNAzyme (or its substrate). Meanwhile, the diameter of AuNPs depends on their dispersed states, which can be readily detected by the dynamic light scattering (DLS) technique. Based on these, DLS has been applied in the detection of DNA,[28,29] protein,[30,31] As(III)[32] and Pb^{2+} (ref. 33 and 34) successfully. In view of the high sensitivity of the DLS technique and the simplicity of label-free methods, herein a DLS sensor for Cu^{2+} was constructed with unmodified AuNPs for the first time by transducing

[a]*School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou 510275, P.R.China. E-mail: cesllsh@mail.sysu.edu.cn; shuaixt@mail.sysu.edu.cn; Fax: +86-20-84112245; Tel: +86-20-84110156*
[b]*Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou 510275, P.R.China*

Cu^{2+}-catalyzed cleavage of DNAzyme into the average hydrodynamic diameter change of AuNPs.

Experimental section

Materials and reagents

All oligodeoxyribonucleotides were synthesized by Sangon Biotech. Co. Ltd. (Shanghai, China). The substrate strand (50 μM) and the enzyme strand (50 μM) were mixed in 1 : 1 ratio and incubated at 60 °C for 10 min, then cooled to room temperature for the hybridization. All DNA stock solutions were stored in the refrigerator at 4 °C. Gold chloride ($HAuCl_4$) and sodium citrate were obtained from Sigma-Aldrich Co. (St Louis, MO, USA). $Cu(NO_3)_2 \cdot 3H_2O$ was purchased from Guangzhou Chemical Reagent Factory (Guangzhou, China). Gold colloids (11–13 nm) were prepared according to the literature[35] by adding 2.0 mL sodium citrate (38.8 mM) into rapidly stirred and boiling $HAuCl_4$ solution (20 mL, 1.0 mM) and boiled for 20 min. Then it was allowed to cool down to room temperature and was filtered by a 0.45 μm filter membrane. Sterilization water was homemade. All other materials were used at the highest quality available and purchased from regular sources. 25.0 mM Tris–HCl buffer (pH 7.5) was used in the research.

Design of oligonucleotides

Cu^{2+}-dependent DNAzyme, which was used in our study, was designed according to the literature.[36–38] Oligonucleotide 5′-AGCTTCTTTCTAATACGGCTTACCTACCA-3′ was designed as the substrate strand while oligonucleotide 3′-CAAGAA AGAATTTTTCTTTCTCCGGGTCCGAATGGATGGT-5′ was designed as the enzyme strand. Compared to that used in the literature, the substrate strand was extended 5 bases at the 3′ end and the enzyme strand was extended 5 complementary bases at the 5′ end in order to improve the stability of dsDNA on the premise of keeping their DNAzyme activity.

Instrumentations

The absorption spectrum was obtained with a TU-1901 UV-visible absorption spectrometer. The Circular Dichroism spectra were recorded with a J-810 Circular Dichroism spectrometer (JASCO International Co. Ltd., Japan). The TEM images of the nanoparticles were recorded with a JEM-2010HR transmission electron microscope (Japan). DLS measurements were performed by using a Zetaplus/90plus Dynamic Light Scattering instrument (Brookhaven Instrument Co., USA). The DLS instrument was operated under the following conditions: temperature: 25 °C, detector angle: 90°, incident laser wavelength: 683 nm, and laser power: 100 mW. The size we obtained was directly calculated according to the Stokes–Einstein relation with the apparatus Zetaplus/90.[39] All sizes reported here were based on the intensity average, and the particle size was the average value of three measurements.

Pretreatment of water samples

Water samples collected from the South Lake and Pearl River (China), and waste water obtained from GuangHua Sci-Tech Co., Ltd. (ShanTou, China), were pretreated with nitric acid according to the literature[40] with slight modification. First, 25.0 mL samples were digested at 140 °C for about 1 h in a heat-resisting container in the presence of 2.0 mL 70% nitric acid. Then, the obtained suspension was filtered through a 0.45 μm filter membrane. Meanwhile, the heat-resisting container was washed with 2.0 mL nanopure water twice and filtered subsequently. Finally, the filtered fluid was diluted to 25 mL and stored at 4 °C prior to the measurement.

DLS detection of Cu^{2+}

DLS detection of Cu^{2+} with DNAzyme and unmodified AuNPs was performed according to the literature,[14,28] 0.8 μL of DNAzyme (25 μM), 100 μL of AuNPs (6.2 nM) and different concentrations of Cu^{2+} were mixed, and incubated for 15 min at 30 °C. Then, 0.8 μL NaCl was added to investigate the stability of AuNPs in different conditions. After 5 min of incubation, 20 μL of them was diluted to 200 μL with water and mixed thoroughly for DLS detection.

Results and discussion

Scheme of the assay

The scheme of the assay was shown in Fig. 1, DNA hybridization occurred between the enzyme strand and the substrate strand to form a duplex structure, which could not adsorb onto the surface of AuNPs to prevent them from the salt-induced aggregation. However, the substrate strand of DNAzyme could be cleaved and released some ssDNA fragments by Cu^{2+}, accordingly adsorbed onto the surface of AuNPs and prevented them from aggregation in the presence of NaCl. Therefore, the dispersed state of AuNPs was controlled by the concentration of Cu^{2+}, which could be estimated with the average hydrodynamic diameter of AuNPs by using the DLS technique.

DLS, TEM and UV-vis absorption assay of AuNPs

Fig. 2 demonstrated the size distribution, TEM image and UV-vis absorption spectrum of AuNPs under different conditions. As shown in Fig. 2A, the average hydrodynamic diameter of AuNPs and DNAzyme mixture was increased to 308.3 nm (a) with the addition of 40 mM NaCl, which illustrated that DNAzyme could not improve the stability of AuNPs in high-salt concentration,

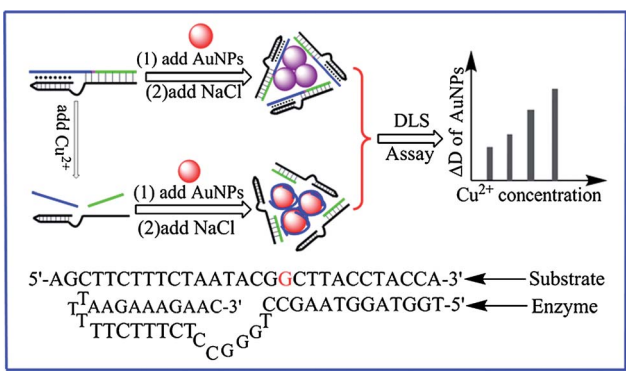

Fig. 1 Schematic illustration of the DLS sensor for Cu^{2+}.

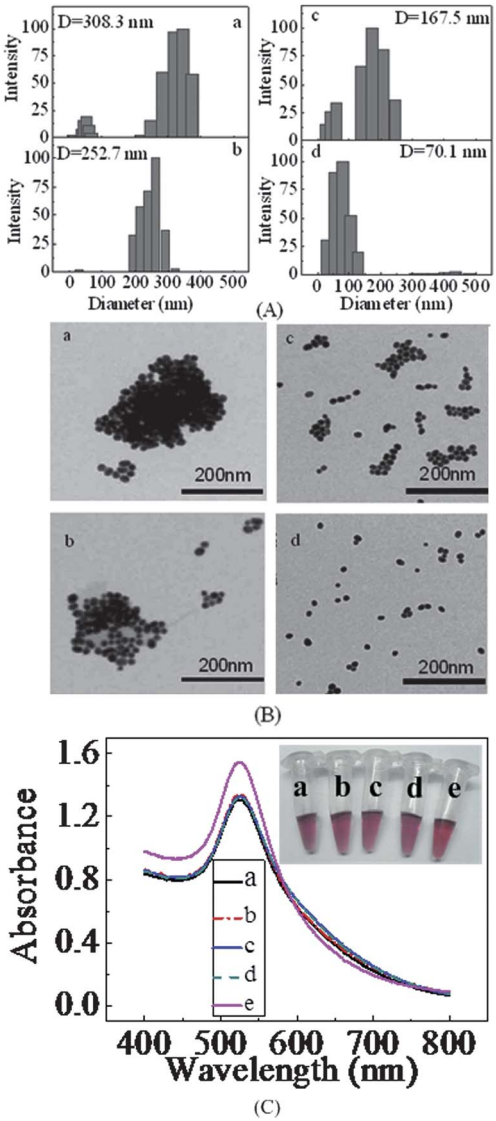

Fig. 2 The size distribution (A) and TEM image (B) of nanoparticles in the presence of 40 mM NaCl. a: 6.2 nM AuNPs + 200 nM DNAzyme; b: a + 500 pM Cu^{2+}; c: a + 5.0 nM Cu^{2+}; d: a + 10 nM Cu^{2+}. The UV-vis absorption spectrum (C) of nanoparticles in the presence of 40 mM NaCl. a: 6.2 nM AuNPs; b: 6.2 nM AuNPs + 200 nM DNAzyme; c: a + 500 pM Cu^{2+}; d: a + 5.0 nM Cu^{2+}; e: a + 10 nM Cu^{2+}. All experiments were conducted in 25 mM Tris-buffer (pH 7.5).

and resulted in the formation of AuNPs dimers, trimers and larger aggregates, which accordingly increased the average diameter of the whole nanoparticle solution. However, the average diameter decreased to 252.7 nm, 167.5 nm and 70.1 nm upon the addition of 500 pM, 5.0 nM and 10 nM of Cu^{2+} respectively. Moreover, the TEM images in Fig. 2B provided more direct information about the disperse state of AuNPs under different conditions, which was in good accordance with that of the size distribution measured by DLS. As a comparison, when the UV-vis absorption spectrum of AuNPs was measured under different conditions, the color of the mixture of AuNPs–DNAzyme did not change until the concentration of Cu^{2+} reached 10 nM. These results indicated the high sensitivity of the DLS technique.

The CD spectroscopy of DNAzyme

Many studies have reported that CD spectroscopy was the effective method for monitoring DNA structure.[41–46] Here, to reveal the mechanism of the average diameter change of AuNPs and DNAzyme mixture, the effect of Cu^{2+} on the structure of DNAzyme was studied by measuring the CD spectroscopy of DNAzyme under different conditions. As shown in Fig. 3, the CD spectrum of the enzyme strand (curve a) was almost the same as that of the substrate strand (curve b). It had a weak positive Cotton effect peak around 278 nm that was due to the weak base stacking and a weak negative Cotton effect peak around 245 nm that was due to the weak helicity, which represented the

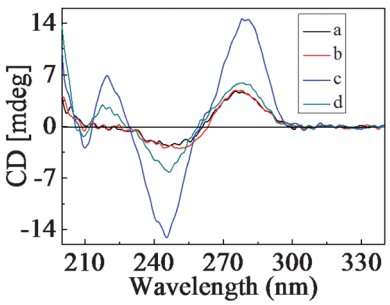

Fig. 3 CD spectra of the enzyme strand under different conditions: a: the enzyme strand (10.0 μM); b: the substrate strand (10.0 μM); c: a + b (10.0 μM); d: c + 1.0 μM Cu^{2+}.

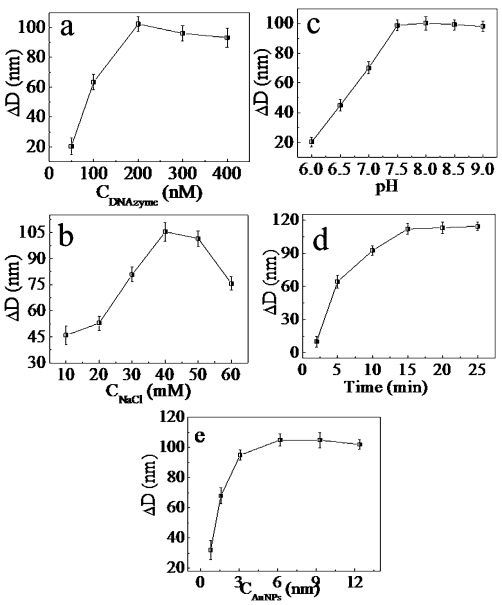

Fig. 4 Effect of DNAzyme concentration (a), NaCl concentration (b), pH value (c), cleavage time (d) and AuNPs concentration (e) on the ΔD of AuNPs from the mix of DNAzyme and AuNPs to the finish of 5 min incubation with NaCl. Here ΔD was defined as $\Delta D = -(D_{AuNPs-Cu^{2+}} - D_{AuNPs})$. $D_{AuNPs-Cu^{2+}}$ denoted the average diameter of AuNPs in the presence of DNAzyme and 400 pM of Cu^{2+}, D_{AuNPs} denoted the average diameter of AuNPs in the presence of DNAzyme and without Cu^{2+}. All experiments were conducted in 25 mM Tris-buffer (pH = 7.5). The error bars represented the standard deviation for a series of three measurements.

single-stranded structure.[47,48] But the positive peak around 278 nm and the negative peak around 245 nm increased dramatically when the enzyme strand and the substrate strand were mixed, which revealed the formation of duplex DNA (curve c).[49,50] Upon the addition of Cu^{2+}, the CD spectrum of the mixture was almost the same as that of the substrate (curve d), which indicated that DNAzyme was cleaved into single-stranded fragments by Cu^{2+}.

Optimization of the assay conditions

The aggregation of AuNPs was decided by the concentration and conformation of DNAzyme, and the maximum concentration of ssDNA fragments was controlled by the concentration of DNAzyme. As shown in Fig. 4a, the ΔD of AuNPs increased with the concentration of DNAzyme from 50 nM to 200 nM, and kept at a plateau over the range from 200 nM to 400 nM. This might be because the amount of ssDNA that adsorbed onto the surface of AuNPs reached a maximum at 200 nM of DNAzyme, and thus the excess DNAzyme could not increase the amount of adsorbed ssDNA fragments. Therefore, 200 nM of DNAzyme was used for further research.

NaCl plays an important role in the aggregation of AuNPs, and thus the effect of its concentration on the ΔD of AuNPs was investigated over the range of 10–60 mM. As shown in Fig. 4b, the ΔD of AuNPs was proportional to the concentration of NaCl over the range of 10–40 mM, then reached a plateau between 40 and 50 mM, and finally declined with further additions of NaCl because excess NaCl could trigger the aggregation of AuNPs as well even in the presence of ssDNA. In view of the sensitivity of the proposed sensor, 40 mM of NaCl was used in the whole research.

The pH value of Tris–HCl buffer can affect the DNA hybridization, DNA cleavage and the aggregation of AuNPs. Furthermore, it is of importance to the Cu^{2+} induced diameter change of AuNPs in the presence of DNAzyme. Therefore, the effect of the pH value of Tris–HCl buffer on the ΔD of AuNPs was investigated over the pH range from 6.0 to 9.0. As shown in Fig. 4c, the ΔD of AuNPs increased with the pH value from 6.0 to 7.5, and kept at a plateau over the pH range of 7.5–9.0. Thereby pH 7.5 was chosen for the research. The time for Cu^{2+} catalyzed DNA cleavage was studied as well, as shown in Fig. 4d, and the ΔD of AuNPs increased with the cleavage time and reached a plateau at 15 min, which indicated that the substrate strand of DNAzyme could be completely cleaved within 15 min. Thus 15 min was selected as the optimum cleavage time.

The detection signal of the sensor was based on the disperse states change of AuNPs. Thus the concentration of them could affect the sensitivity of the system. As shown in Fig. 4e, the ΔD of AuNPs was increased along with the concentration of AuNPs in the range from 0.98 nM to 6.2 nM and then kept at a plateau, which might be explained by the fact that the size of the aggregates depended on the concentration of AuNPs within this range, and the ΔD was independent of the concentration of AuNPs when it was higher than 6.2 nM. Therefore, 6.2 nM AuNPs was selected for the research.

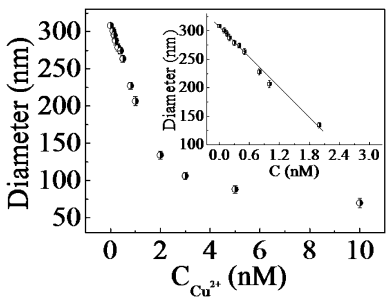

Fig. 5 Calibration plots of Cu^{2+} under the conditions of 6.2 nM of AuNPs, 200 nM of DNAzyme and 40 mM of NaCl. All experiments were conducted in 25.0 mM of Tris-buffer (pH = 7.5). The error bars represented the standard deviation for a series of three measurements.

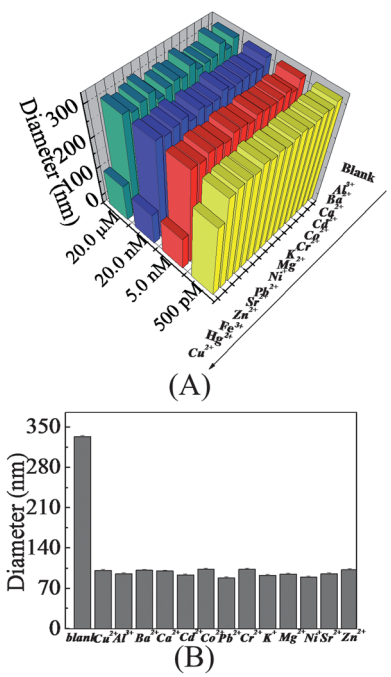

Fig. 6 (A) The size change of AuNPs before and after the addition of different metal ions in the presence of 200 nM DNAzyme and 40 mM NaCl (Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{2+}, K^{+}, Mg^{2+}, Ni^{+}, Pb^{2+}, Sr^{2+}, Zn^{2+}, Fe^{3+}, Hg^{2+} and Cu^{2+}). (B) The average diameter of AuNPs in the presence of 200 nM DNAzyme (blank), other data were obtained from the solution that contained a different interference cation (5.0 nM) and the same concentration of DNAzyme (200 nM) and Cu^{2+} (5.0 nM). The error bars represented the standard deviation for a series of three measurements.

Calibration curve and detection limit

Under the conditions of 6.2 nM AuNPs, 200 nM DNAzyme, 40 mM NaCl and 15 min of cleavage time, the function of Cu^{2+} concentration on the diameter change of AuNPs was investigated. As shown in Fig. 5, the average diameter of AuNPs was decreased linearly along with the increase of Cu^{2+} concentration over the range from 100 pM to 2.0 nM, the linear regression equation was $D = 306.7 - 89.66C$ (C: nM), with a correlation coefficient of 0.9953, and the detection limit was 60 pM (3δ/slope), which was about 4800 times lower than that of the colorimetric method.[14] Merits of the colorimetric method were cost-effectiveness and independence of instrument, but it could not be used to detect Cu^{2+} in the sample when its

Table 1 Detection of Cu^{2+} in water samples

Samples		Added (pM)[a]	Found (pM)[b]	Recovery (%)	RSD (%)
South Lake		0.0	—	—	—
		150.0	136.3	90.9	4.27
		200.0	189.3	94.7	3.58
		300.0	310.8	103.6	3.81
		500.0	510.7	102.1	2.44
		1000.0	970.1	97.0	3.05
Pearl River		0.0	—	—	—
		150.0	142.7	95.1	3.42
		200.0	205.3	102.7	2.61
		300.0	293.6	97.9	3.01
		500.0	530.9	106.2	2.92
		1000.0	982.1	98.2	2.34
Waste liquid from GuangHua (diluted 100 times)	By AAS	0.0	156.0	—	—
	By DLS	0.0	152.3	—	—
		500.0	487.7	97.5	3.37
	By AAS	0.0	828.0	—	—
	By DLS	0.0	816.9	—	—
		500.0	459.1	91.8	4.23
	By AAS	0.0	1560.0	—	—
	By DLS	0.0	1542.0	—	—
		500.0	556.7	111.3	3.98

[a] The addition of Cu^{2+} to water samples. [b] The detection of Cu^{2+} in water samples.

concentration was lower than 290 nM. Therefore, the proposed method was a good complement to the colorimetric method; people could select the suitable method for the detection of Cu^{2+} according to the possible concentration in the real sample and usable instruments. The higher sensitivity and lower detection limit were mainly due to the amplification property of AuNPs and the sensitive DLS technique. Although the hydrodynamic diameter of DNAzyme might change during the process of DNA cleavage, high concentration was needed for detection in the absence of AuNPs. While AuNPs had strong light scattering properties, Cu^{2+} induced DNA cleavage could be transduced into the change of the average diameter of AuNPs because single-stranded and double-stranded DNA had different adsorption abilities on the surface of AuNPs, which could be detected with the dynamic light scattering technique.[30]

Selectivity of the assay

The selectivity of the proposed assay was investigated by detecting the ΔD of the mixture of 6.2 nM AuNPs, 200 nM DNAzyme and 40 mM NaCl before and after the addition of different concentrations (500 pM, 5.0 nM, 20.0 nM and 20.0 μM) of different cations such as Al^{3+}, Ba^{2+}, Ca^{2+}, Cd^{2+}, Co^{2+}, Cr^{2+}, K^{+}, Mg^{2+}, Ni^{+}, Pb^{2+}, Sr^{2+}, Zn^{2+}, Fe^{3+}, Hg^{2+}, and Cu^{2+} respectively. As shown in Fig. 6A, the size change of AuNPs was manifest upon the addition of 500 pM Cu^{2+}, and it was much higher than that of other cations under the concentration of 20.0 μM, which indicated the good selectivity of the assay. Moreover, the effect of different cations on the average diameter of the mixture of Cu^{2+} (5.0 nM), DNAzyme (200 nM) and AuNPs (6.2 nM) was investigated respectively. As demonstrated in Fig. 6B, the variance of the average diameter of the mixture of AuNPs, DNAzyme and Cu^{2+} was less than 9.2% upon the addition of different cations. Such results suggested that the other cations had little effect on the assay.

Application of the proposed method in water samples

To investigate the potential application of the proposed method in real samples, the assay was applied to detect Cu^{2+} in water samples from South Lake and Pearl River (China), respectively. As shown in Table 1, Cu^{2+} could not be detected in the samples from South Lake and Pearl River. The recovery of them varied between 90.9% and 106.7% in the addition and recovery experiment. Meanwhile, three types of waste water from GuangHua Sci-Tech Co., Ltd. (ShanTou, China) were investigated and the concentration of Cu^{2+} detected by our method was 152.3, 816.9 and 1542.0 pM after being diluted 100 times. So, the Cu^{2+} concentration in the wastewater was 15.2, 81.7 and 154.2 nM separately, which was in good accordance with the results from GuangHua Sci-Tech Co., Ltd. In addition, the recovery of them varied between 91.8% and 111.3% in the addition and recovery experiment. These results indicated that the present method could be accepted for the detection of Cu^{2+} in real water samples from nature.

Conclusion

In conclusion, a new method for detection of Cu^{2+} was constructed with the unmodified AuNPs and DNAzyme by using the DLS technique. The detection limit reached 60 pM, which was due to the amplification property of AuNPs and the high sensitivity of the DLS technique. Moreover, the proposed method was simple and timesaving. These advantages enable DLS to become an important analytical technique, which is worthy of being extended to other bioassays in the future.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (no.: 20975116, 50830107).

References

1 E. L. Que, D. W. Domaille and C. J. Chang, *Chem. Rev.*, 2008, **108**, 1517–1549.
2 W. D. Wang, A. Fu, J. S. You, G. Gao, J. B. Lan and L. J. Chen, *Tetrahedron*, 2010, **66**, 3695–3701.
3 P. G. Georgopoulos, A. Roy, M. J. Yonone-Lioy, R. E. Opiekun and P. J. Lioy, *J. Toxicol. Environ. Health, Part B*, 2001, **4**, 341–394.
4 T. Kato, S. Nakamur and M. Mirita, *Anal. Sci.*, 1990, **6**, 623–626.
5 M. S. Chan and S. D. Huang, *Talanta*, 2000, **51**, 373–380.
6 B. C. Yin, P. Zuo, H. Huo, X. Zhong and B. C. Ye, *Anal. Biochem.*, 2010, **401**, 47–52.
7 Q. T. Meng, X. L. Zhang, C. He, G. He, P. Zhou and C. Duan, *Adv. Funct. Mater.*, 2010, **20**, 1903–1909.
8 A. Dhir, V. Bhalla and M. Kumar, *Tetrahedron Lett.*, 2008, **49**, 4227–4230.
9 T. Kang, S. Hong, J. Moon, S. Oh and J. Yi, *Chem. Commun.*, 2005, 3721–3723.
10 S. Lee, I. Choi, S. Hong, Y. I. Yang, J. Lee, T. Kang and J. Yi, *Chem. Commun.*, 2009, 6171–6173.
11 J. F. Huang and B. T. Lin, *Analyst*, 2009, **134**, 2306–2313.
12 X. Y. Xu, W. L. Daniel, W. Wei and C. A. Mirkin, *Small*, 2010, **6**, 623–626.
13 B. C. Yin, B. C. Ye, W. H. Tan, H. Wang and C. C. Xie, *J. Am. Chem. Soc.*, 2009, **131**, 14624–14625.
14 Y. Wang, F. Yang and X. R. Yang, *Nanotechnology*, 2010, **21**, 1–6.
15 J. W. Liu and Y. Lu, *Chem. Commun.*, 2007, 4872–4874.
16 R. R. Breaker, *Nat. Biotechnol.*, 1997, **15**, 427–431.
17 D. Sen and C. R. Geyer, *Curr. Opin. Chem. Biol.*, 1998, **2**, 680–687.
18 D. Li, A. Wieckowska and I. Willner, *Angew. Chem., Int. Ed.*, 2008, **47**, 3927–3931.
19 M. Hollenstein, C. Hipolito, C. Lam, D. Dietrich and D. M. Perrin, *Angew. Chem.*, 2008, **120**, 4418–4422.
20 X. R. Yang, J. Xu, X. M. Tang, H. X. Liu and D. B. Tian, *Chem. Commun.*, 2010, **46**, 3107–3109.
21 J. H. Lee, Z. D. Wang, J. W. Liu and Y. Lu, *J. Am. Chem. Soc.*, 2008, **130**, 14217–14226.
22 Y. Zhuo, Y. Q. Chai, R. Yuan, L. Mao, Y. L. Yuan and J. Han, *Biosens. Bioelectron.*, 2011, **26**, 3838–3844.
23 X. Liu, Q. Dai, L. Austin, J. Coutts, G. Knowles, J. H. Zou, H. Chen and Q. Huo, *J. Am. Chem. Soc.*, 2008, **130**, 2780–2782.
24 A. P. Xin, Q. P. Dong, C. Xiong and L. S. Ling, *Chem. Commun.*, 2009, 1658–1660.
25 H. L. Yan, C. Xiong, H. Yuan, Z. X. Zeng and L. S. Ling, *J. Phys. Chem. C*, 2009, **113**, 17326–17331.
26 T. Li, L. L. Shi, E. K. Wang and S. J. Dong, *Chem.–Eur. J.*, 2009, **15**, 3347–3350.
27 K. W. Huang, C. J. Yua and W. L. Tseng, *Biosens. Bioelectron.*, 2010, **25**, 984–989.
28 Q. Dai, X. Liu, J. Coutts, L. Austin and Q. Huo, *J. Am. Chem. Soc.*, 2008, **130**, 8138–8139.
29 X. Liu and Q. Huo, *J. Immunol. Methods*, 2009, **349**, 38–44.
30 H. Jans, X. Liu, L. Austin, G. Maes and Q. Huo, *Anal. Chem.*, 2009, **81**, 9425–9432.
31 J. R. Kalluri, T. Arbneshi, S. A. Khan, A. Neely, P. Candice, B. Varisli, M. Washington, S. McAfee, B. Robinson, S. Banerjee, A. K. Singh, D. Senapati and P. C. Ray, *Angew. Chem.*, 2009, **121**, 9848–9851.
32 X. M. Miao, L. S. Ling and X. T. Shuai, *Chem. Commun.*, 2011, **47**, 4192–4194.
33 L. Beqa, A. K. Singh, S. A. Khan, D. Senapati, S. R. Arumugam and P. C. Ray, *ACS Appl. Mater. Interfaces*, 2011, **3**, 668–673.
34 X. M. Miao, C. Xiong, W. W. Wang, L. S. Ling and X. T. Shuai, *Chem.–Eur. J.*, 2011, **17**, 11230–11236.
35 K. C. Grabar, R. G. Freeman, M. B. Hommer and M. J. Natan, *Anal. Chem.*, 1995, **67**, 735–743.
36 N. Carmi, H. R. Balkhi and R. R. Breaker, *Proc. Natl. Acad. Sci. U. S. A.*, 1998, **95**, 2233–2237.
37 N. Carmi, L. A. Shultz and R. R. Breaker, *Chem. Biol.*, 1996, **3**, 1039–1046.
38 J. W. Liu and Y. Lu, *J. Am. Chem. Soc.*, 2007, **129**, 9838–9839.
39 R. Pecora, *J. Nanopart. Res.*, 2000, **2**, 123–131.
40 J. Sastre, A. Sahuquillo, M. Vidal and G. Rauret, *Anal. Chim. Acta*, 2002, **462**, 59–72.
41 Z. L. Zhang, W. M. Huang, J. L. Tang, E. K. Wang and S. J. Dong, *Biophys. Chem.*, 2002, **97**, 7–16.
42 W. B. Gratzer, *Eur. J. Biochem.*, 1970, **15**, 209–214.
43 C. F. Jordan, L. S. Lerman and J. H. Venable, *Nature, New Biol.*, 1972, **236**, 67–70.
44 E. J. Ramm, V. I. Vorob'ev, T. M. Birshtein, I. A. Bolotina and M. V. Voleenshtein, *Eur. J. Biochem.*, 1972, **25**, 245–253.
45 C. H. Spink and J. B. Chaires, *J. Am. Chem. Soc.*, 1997, **119**, 10920–10928.
46 C. S. Braun, G. S. Jas, S. Choosakoonkriang, G. S. Koe, J. G. Smith and C. R. Middaugh, *Biophys. J.*, 2003, **84**, 1114–1123.
47 Y. Sang, L. Zhang, Y. F. Li, L. Q. Chen, J. L. Xu and C. Z. Huang, *Anal. Chim. Acta*, 2010, **659**, 224–228.
48 M. Rosa, R. Dias, M. G. Miguel and B. Lindman, *Biomacromolecules*, 2005, **6**, 2164–2171.
49 M. Etienne, J. Bessiere and A. Walcarius, *Sens. Actuat. B*, 2001, **76**, 531–538.
50 J. F. Wu and E. A. Boyle, *Anal. Chem.*, 1997, **69**, 2464–2470.