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A fast, highly sensitive and selective assay of iodide ions with single-stranded DNA-templated copper nanoparticles as a fluorescent probe for its application in Kunming mice samples†

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The development of fast, sensitive, selective and flexible methods for the detection of iodide is highly demanded and is of great significance. In this work, single-stranded DNA-templated copper nanoparticles (ssDNA-CuNPs) generated by sodium ascorbate reduction of Cu^{2+} along the single-stranded DNA of poly-T were utilized as a fluorescent probe for the determination of iodide ions (I^-). The detection scheme is based on the instant quenching of the fluorescence of ssDNA-CuNPs by iodide ions. I^- can be quantified in the concentration range from 0.050 to 40 μM and from 40 to 80 μM , and the limit of detection is as low as 15 nM. This method provides a simple and convenient strategy for the biochemical assay of I^- , which is also helpful for early diagnosis of related diseases. The establishment of a low cost and fast detection method would be particularly important in developing countries where medical supplies are lacking.

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Introduction

Iodine is an essential trace element for life and is the primary constituent of thyroid hormones. It plays a vital role in the normal function of the thyroid gland.¹ During prenatal and early development periods, lack of iodine will result in some diseases,² such as mental disabilities and cretinism. The addition of iodide into edible salt is the most efficient method to prevent iodine deficiency disorders. However, excessive intake of iodide may lead to hyperthyroidism and goiter.³ Therefore, the determination of iodide is very important for the early stage monitoring of the corresponding health problems.

Many spectrophotometric methods have been proposed for the detection of iodide such as ultraviolet visible spectrophotometry,⁴ chemiluminescence,^{5,6} reverse flow injection spectrophotometry,⁷ kinetic spectrophotometry,⁸ and so on. Although these methods are very sensitive, a complex pro-

cedure is often required. Efforts dedicated to the feasible and efficient design of iodide testing are still urgently needed. Besides, because of the limited healthcare conditions in developing countries, the establishment of a low cost and fast detection method has special significance.

Compared with the above-mentioned methods, a fluorescence assay provides a promising method which is simple, low cost, and fast track with high sensitivity and specificity.^{9–13} Fluorescent nanoparticles (especially metal nanoparticles) with strong fluorescence emission have shown great potential in biological applications, and have attracted special attention due to their physical properties of luminescence, good biocompatibility, target identification and signal amplification capability.^{14–19} As a new type of functional biological probe, fluorescent copper nanoparticles show great potential, and their production with the single-stranded DNA as a template (ssDNA-CuNPs) is quick and convenient.^{20–25} The large Stokes shift of ssDNA-CuNPs ($\lambda_{\text{ex}} = 345 \text{ nm}$, $\lambda_{\text{em}} = 637 \text{ nm}$, $\Delta\lambda = 292 \text{ nm}$) can be efficiently used to avoid the strong background signal of complex biological systems, thus providing a new possibility for detection in complex biological media. Many important biologically active substances such as glucose,²⁶ insulin²⁷ and amino acids²⁸ have been detected utilizing ssDNA-CuNPs with good sensitivity and selectivity, demonstrating their excellent application prospect.

In an investigation aimed at the development of a convenient and cost-effective procedure for the assay of I^- in bio-

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logical and related circumstances, we established a fluorimetric method with ssDNA-CuNPs for the detection of iodide ions, based on the instant fluorescence quenching of ssDNA-CuNPs by iodide ions. Due to the importance of iodide ions in growth and development, we selected Kunming mice as a model to detect iodide,^{29,30} and the results are satisfactory, which provided a scientific basis for the detection of iodide ions in biological systems and diagnosis of diseases. The work presented in this paper affords a highly sensitive and efficient method for the detection of iodide ions.

Experimental

Chemicals and apparatus

The T-rich ssDNA (poly T) was purchased from Shanghai Sangon Biological Engineering Technology and Services Co. Ltd (China). Sodium ascorbate and potassium iodide were purchased from Sigma-Aldrich (Shanghai, China). 3-(*N*-Morpholino)propanesulfonic acid (MOPS) and other reagents of at least analytical grade were commercially purchased from Aladdin (Shanghai, China), and used without further treatment. The MOPS buffer solution (10 mM, pH 7.6) was prepared with 150 mM NaCl. All reagents used in this work were dissolved in deionized water. All measurements were carried out at room temperature.

Fluorescence spectra were recorded on an Edinburgh FLS-920 spectrophotometer (Edinburgh Instruments Ltd, UK) equipped with a 450 W xenon lamp excitation source, using a quartz cell of 1.0 cm path length. The fluorescence emission spectra were collected from 520 nm to 660 nm at room temperature with a 345 nm excitation wavelength. The high-resolution transmission electron microscopy images (HR-TEM) were taken with a JEOL JEM2100F microscope operated at 200 kV (Japan Electron Optics Laboratory Co.)

Synthesis and characterization of ssDNA-CuNPs

ssDNA-CuNPs were synthesized in aqueous solution according to a previous publication.²⁰ 30.0 μ L of DNA (10 μ M), 60.0 μ L of Cu^{2+} (1 mM) and 90.0 μ L of sodium ascorbate (10 mM) were added to 120 μ L of MOPS buffer (10 mM, 150 mM NaCl, pH 7.6) and mixed. After five minutes at room temperature (25 $^{\circ}$ C) in the dark, the fluorescence with the excitation wavelength of 345 nm was measured. The morphological characterization of the ssDNA-CuNPs was performed by HR-TEM.

Sensitivity and selectivity of ssDNA-CuNPs for the detection of I^{-}

For the fluorescence detection of I^{-} , appropriate amounts of different concentrations of iodide ions were added into ssDNA-CuNP (180 μ L) solution to give a final volume of 300 μ L with MOPS buffer. The mixtures were incubated at room temperature for 5 min before the spectral measurements. To investigate the selectivity of ssDNA-CuNPs for I^{-} , the following anions and cations were used: SO_3^{2-} , Cl^{-} , $\text{H}_2\text{PO}_4^{-}$, CO_3^{2-} , $\text{CH}_3\text{COO}^{-}$, NO_3^{-} , HCO_3^{-} , NH_4^{+} , Fe^{2+} , SO_4^{2-} , Cu^{2+} , HPO_4^{2-} ,

Pb^{2+} , Cr^{3+} , $\text{B}_4\text{O}_7^{2-}$, Zn^{2+} and S^{2-} . The fluorescence intensities of the test solution (F) and the blank solution (F_0) were recorded, in which F and F_0 are the maximum emission intensities of the ssDNA-CuNP system in the presence and absence of I^{-} , respectively.

Preparation and analysis of Kunming mice samples

The preparation of Kunming mice body fluid samples was as follows: three male Kunming mice were utilized, and each mouse was intraperitoneally injected with I^{-} to effectively extract fluid samples for iodide ion determination, while the normal male Kunming mice were used as the control. The mice were anesthetized with anesthetic ether and sacrificed by the cervical dislocation method. Then the chest skin of the mice was cut open, the viscera were taken out, and urine and heart blood were collected. The samples were double diluted for every fluid. The samples were centrifuged at 1000 rpm for 5 min, and the resulting supernatants were collected for the following detection experiments.

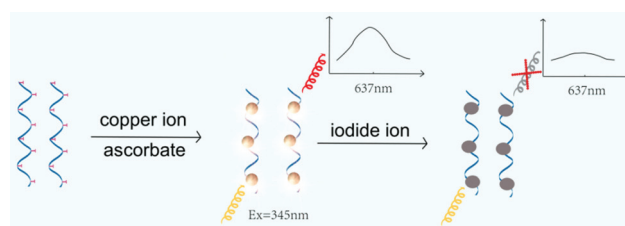
As for the fluorescence detection of iodide ions in biological samples, an appropriate amount of sample solution was first mixed with 250 μ M Zn^{2+} , and then the mixture was added into an aliquot of MOPS buffer containing ssDNA-CuNPs to give a final volume of 300 μ L. Then the fluorescence intensity was recorded using the Edinburgh FLS-920 spectrophotometer.

Data were expressed as the mean \pm standard deviation. All experiments were repeated three times, and the data were calculated with Microsoft Excel.

Results and discussion

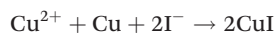
The mechanism of the fluorescent probe for the iodide assay

ssDNA-CuNPs are excellent for bioanalysis due to their small size, excellent stability, easy synthesis and low cost. The working principle of the fluorimetric assay of I^{-} is schematically represented in Scheme 1. The fluorescent ssDNA-CuNP formation is initiated by the reduction of Cu^{2+} to Cu^0 on ssDNA templates, due to the high affinity between thymine and Cu^{2+} , and then Cu^0 is clustered to form CuNPs.²¹ But in the presence of I^{-} , the copper nanoparticles will absorb I^{-} due to their electrical double layer and large surface area.³¹ As a result, a redox reaction occurs on the ssDNA-CuNP surface in



Scheme 1 Schematic representation of the sensing procedure for iodide analysis.

the presence of iodide and a nearby copper ion according to the following equation,³²



Hence, Cu^0 on ssDNA-CuNPs will be etched and the collapse of ssDNA-CuNPs will occur consequently, which will influence the morphology and the fluorescence of ssDNA-CuNPs significantly. Fig. 1 displays the TEM image of ssDNA-CuNPs and the change after the addition of I^- . It can be found that the average size of ssDNA-CuNPs is about 2 nm (Fig. 1A), but the nanoparticles almost totally disappear after the addition of I^- (Fig. 1B), illustrating the breakdown of the initially formed nanoparticles. Thus, it is possible for us to fabricate ssDNA-CuNPs for the detection of I^- ions.³³

Detection of iodide ions using ssDNA-CuNPs

The scheme described above indicated that iodide ions can effectively alter the fluorescence of ssDNA-CuNPs. A new approach based on the iodide-prevented formation of CuNPs thus could be established for I^- detection. We then evaluated the fluorescence change that resulted from the interaction between ssDNA-CuNPs and I^- . As can be seen from Fig. 2A, the emission spectrum of ssDNA-CuNPs displayed an emission peak at 637 nm upon excitation at 345 nm. The addition of I^- significantly quenched the fluorescence of ssDNA-CuNPs (Fig. 2B). The quick response is also observed from the fluorescence kinetics curve of ssDNA-CuNPs with iodide ions (Fig. S1†). Consequently, the fluorescent procedure can be established for the low-cost, fast and simple detection of I^- , using ssDNA-CuNPs as a fluorescent probe. Because the response of the fluorescent probe is usually affected by the media pH and temperature, besides the reaction time, it is necessary to examine the optimum conditions with a better performance, and several responsive conditions were investigated (Fig. S2†). A comparatively neutral medium with pH 7.6 (MOPS buffer 10 mM) and instant detection at room temperature were finally chosen for further experiments, to obtain a high sensitivity for the detection of I^- .

Analytical parameters for the determination of iodide ions

Under the optimum conditions, the capability of the fluorescence approach for the evaluation of I^- was investigated. In

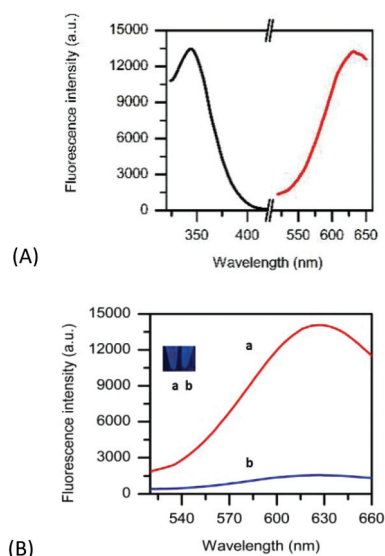


Fig. 2 The excitation and emission spectra of ssDNA-CuNPs (A); and the fluorescence spectra of ssDNA-CuNPs (B) in the presence (a) and absence of $60 \mu\text{M I}^-$ (b).

order to evaluate the sensitivity of this strategy for I^- , a series of different concentrations of I^- ranging from 0 to $80 \mu\text{M}$ were introduced to the system and the fluorescence spectra were

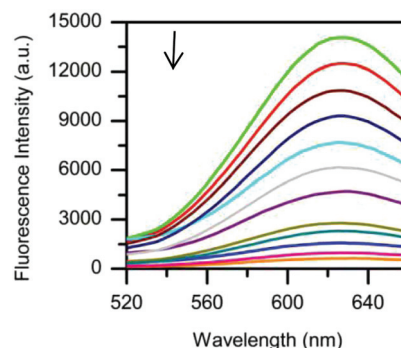


Fig. 3 Fluorescence responses of ssDNA-CuNPs to different concentrations of I^- in MOPS buffer: 0, 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, and $80 \mu\text{M}$ (from top to bottom). The final concentrations of DNA, Cu^{2+} , and ascorbic acid are $1 \mu\text{M}$, 0.2 mM , and 3 mM , respectively.

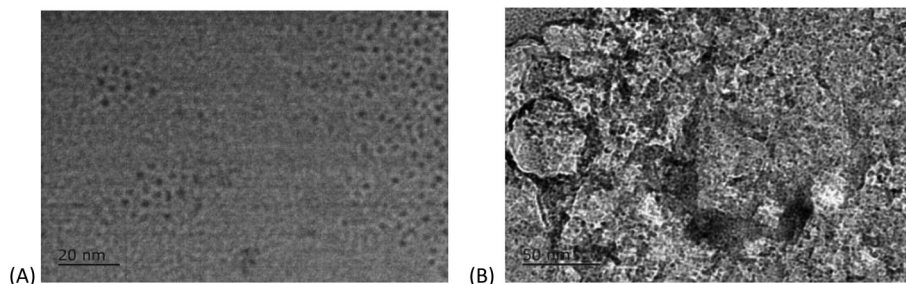


Fig. 1 TEM image of ssDNA-CuNPs before (A) and after (B) the addition of I^- .

Table 1 Analytical figures of merit for I^- detection

Linear range (μM)	R^2	Linear regression equation	RSD	LOD (μM)	LOQ (μM)
0.050–40	0.9971	$\Delta F = 136.54 + 295.80 \text{ C}_1^-$	0.46 ^a	0.015	0.050
40–80	0.9969	$\Delta F = 9727 + 47.98 \text{ C}_1^-$	0.76 ^a		

^a Relative standard deviation of ΔF corresponding to 8.0 and 45 μM I^- , respectively.

recorded after incubation for 5 min (Fig. 3). As illustrated, the fluorescence intensities decreased gradually with increasing concentrations of I^- . Linear ranges of I^- concentrations were obtained from 0.050 to 40 μM and from 40 to 80 μM with the

limit of detection (LOD) down to 15 nM, and Fig. S3† shows the calibration curve of the assay system for I^- detection. The corresponding regression equation of the working curve, correlation coefficient (R^2), relative standard deviation (RSD), separately determined six times in parallel, LOD (calculated by $3S_b/k$, which referred to the quotient between three times the blank reagent's standard deviation, where $S_b = 0.05$, $n = 11$, and k is the slope of the working curve) and the limit of quantification (LOQ, calculated by $10S_b/k$) of the fluorescent sensor are all listed in Table 1. Accordingly, with the biocompatible fluorescent probe of ssDNA-CuNPs, the present fluorimetric method can allow for the sensitive, repeatable and stable evaluation of I^- .

Selectivity of the fluorescent probe for iodide ions

To evaluate the potential interference toward I^- detection with ssDNA-CuNPs, the fluorescence response of I^- (25 μM or none) together with ssDNA-CuNPs and MOPS buffer was then investigated in the presence of different anions and cations. It is clear from Fig. 4 that representative ions except S^{2-} did not affect the fluorescence intensity and cause interference (the signal perturbation on I^- detection was generally less than $\pm 5.0\%$). But the addition of zinc ions into the detection system will efficiently avoid the interference of S^{2-} . Therefore, it is possible to use ssDNA-CuNPs as a fluorescent probe for selective I^- detection in complex biological samples.

Detection of iodide ions in Kunming mice samples

The fluorescent probe described here was further utilized to monitor I^- in complex samples such as Kunming mice, encouraged by the above-mentioned investigations. The Kunming mice are an outbred strain of laboratory animals with high disease resistance, resilience, and survival and breeding rates, which have been widely utilized in related pharmaceutical and genetic studies. We obtained the male Kunming mice model and determined the I^- content after the masking of Zn^{2+} , and then a certain amount of the standard solution of I^- was added into the corresponding sample solutions for recovery testing. The results are shown in Table 2. It can be seen that a good recovery of 96.2–102.6% was obtained, showing the good selectivity and high accuracy of the method which displayed great potential to the point of early diagnosis and treatment of related diseases.

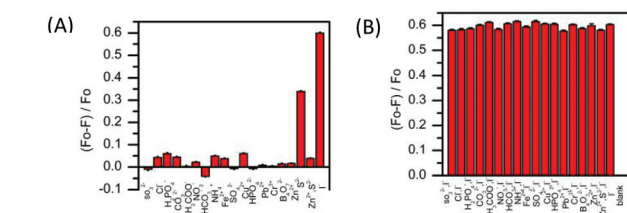


Fig. 4 Selectivity of the fluorescence sensor for I^- over other representative ions in aqueous solution. The concentration of all the detected ions is 250 μM . The concentrations of DNA, Cu^{2+} , and ascorbic acid are 1 μM , 0.2 mM, and 3 mM, respectively. (A) ssDNA-CuNPs with representative ions; (B) ssDNA-CuNPs, I^- and representative ions. The error bars represent the standard deviation of three measurements.

Table 2 The results for the determination of I^- in samples

No. ^a	Found (μM)	Added (μM)	Measured (μM)	Recovery (%)
1	1.41 ± 0.03			
2	1.32 ± 0.05			
3	1.43 ± 0.07			
4	4.33 ± 0.04	10	13.99 ± 0.03	96.6
5	4.54 ± 0.07	10	14.59 ± 0.07	100.5
6	4.28 ± 0.10	10	13.90 ± 0.09	96.2
7	8.47 ± 0.03	10	18.11 ± 0.06	96.4
8	8.96 ± 0.03	10	18.94 ± 0.07	99.8
9	8.27 ± 0.07	10	18.53 ± 0.02	102.6

^a Samples 1–3, normal serum sample as the control; 4–6, the urine model with I^- ; 7–9, the thyroid gland model with I^- . Each sample was measured three times.

Table 3 Comparable methods for the determination of iodide ions

Methods	Analytical ranges	LODs	Optimum pH value	Applicability to specific samples	Ref.
Polymer-stabled silver nanoparticles	Not given	250 μM	Not given	Click bile acid polymers	14
Gold-nanoparticle based fluorescent probes	0–1.0 μM	50 nM	10	Water samples, edible salt samples	17
Polyethyleneimine-templated Ag nanoclusters	0.05–6 μM	40 nM	7.0–8.0	Water	18
Histidine-mediated gold nanoclusters	0.8–60 μM	118 nM	7.4	Lake water	34
	1.2–50 μM	215 nM		Human urine	
Fluorescent probe: ssDNA-CuNPs	0.05–40 μM 40–80 μM	15 nM	7.4	Male Kunming mice	Our work

Comparison with other methods

We compared our experimental results with those using different nanoscale probes to detect Γ^- reported in recent years. It can be concluded from Table 3 that our method showed relatively low LODs, wide linear range and good applications for the iodide ions compared to other methods. Thus, our method is favorable to the sensitive determination of iodide ions in biological and other samples.

Conclusions

In summary, we have developed a simple, fast, low cost, selective and sensitive detection assay for Γ^- sensing by the fluorescence quenching of single-stranded DNA-templated copper nanoparticles (ssDNA-CuNPs). Under the optimized conditions, the practical application of this assay was proved by measuring the level of Γ^- of the male Kunming mice, indicating the acceptable accuracy of the method. This new method offered several advantages over the current Γ^- detection techniques. First, the method did not require complicated and expensive instruments, which simplified operations and reduced the cost. Second, the method produced a detection concentration as low as 15 nM, which was useful for the rapid and ultrasensitive detection of Γ^- . Finally, this strategy exhibited excellent selectivity for Γ^- over other anions; its advantages make this method quite promising for the rapid detection of Γ^- in bioassays and clinical diagnostics.

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