Template method synthesis of highly fluorescent duplex oligonucleotide copper nanomaterials for Fe$^{3+}$ sensing

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Abstract

Herein, we reported a simple template method for preparation of fluorescent copper nanomaterials, using Duplex oligonucleotide (dsDNA) as the template. The as-prepared copper nanomaterials had good sensing performance, excellent stability and ultrafine size through the characterization of UV–vis absorption spectroscopy, fluorescence spectroscopy, transmission electron microscopy (TEM). Experimental results showed that the fluorescence of copper nanomaterials was linearly quenched by the Fe$^{3+}$ concentrations in the range of 5–100 μM. The detection limit was 5 μM. And when the temperature is between 25 °C and 70 °C, the fluorescence intensity of copper nanomaterials presents a good linear relationship.

1. Introduction

People focus on the exploration of new and efficient fluorescent materials [1, 2]. Copper is the same main group element as gold and silver in the periodic table, and it is relatively rich, cheap and easy to obtain. At present, there are more reports on gold nanomaterials and silver nanomaterials, followed by copper nanomaterials. Compared with gold and silver nanomaterials, copper nanomaterials not only have similar optical properties, such as tunable emission wavelength and higher quantum yield, but also have high biocompatibility and are easier to obtain. On this basis, copper nanomaterials based on oligonucleotide DNA as template have the characteristics of easy access to raw materials, simple synthesis conditions and easy operation, so they are considered to have great application prospects. As an attractive fluorescent probe [3–6], copper nanomaterials have attracted much attention due to their low toxicity, good biocompatibility and stability, good solubility and excellent luminescent properties [7]. Cunps have been used in some research fields, such as bioimaging [8, 9], ion detection sensors [10, 11] and biological transport sensors [12–14]. Copper dust or smoke can be absorbed by the digestive tract and become a allergen; copper compounds mainly enter the human body through the digestive tract, and copper is mainly distributed in the liver, kidney, brain, bone marrow, red blood cells and muscles, and excreted with feces through the bile duct. Copper smoke and dust can stimulate skin and mucosa, causing metal fume fever, dermatitis and eczema. Contact with high concentration of copper compound solution can cause skin mucosal necrosis. Eating food contaminated by copper or copper sulfate can lead to poisoning. However, it is worth noting that when used within concentration limits, an iodide detection method was developed using SSDNA-templated copper nanomaterials as fluorescent probes. The detection limit of this method is as low as 15 nM, and this technique can accurately analyze the content in mice [15]. Iodine plays an important role in the human body and is the main component of thyroid hormone [16].

It is well known that the deficiency or overexposure of some elements has a significant impact on human health. The iron balance is fragile; iron deficiency and iron overload are harmful. Iron homeostasis disorder is one of the most common human diseases. Generally, Fe$^{3+}$ plays significant roles in many biological processes, such as oxygen transport to tissues, electron transfer, and transcriptional regulation [17]. The disorders of Fe$^{3+}$
in its metabolism would induce anemia, liver and kidney damage, diabetes, and heart failure [18]. Thus, it is fundamentally important to monitor and precisely detect Fe$^{3+}$.

As a novel fluorescent probe, high fluorescence nanomaterials have been widely used in various fields, including the detection of metal cations and cations [19], biological imaging [20], protein [21] and DNA bitterness [22] and small molecules detection [23]. Nowadays, copper nanomaterials have attracted much attention due to their unique properties. Under the condition of pH = 6.5, using dithiothreitol (DTT) as a protective and reducing agent, copper nanoclusters (CuNCs) with orange fluorescence was synthesized by Huang’s research group [24]. The maximum emission wavelength was 590 nm, and the excitation wavelength was 360 nm. In the presence of Al$^{3+}$, DTT- CuNCs showed aggregation induced fluorescence enhancement, which could be used for the detection of Al$^{3+}$ in food samples. Zhang Research Group [25] synthesized red emitting (L-amino acid oxidase) by ‘one pot method’ LAAOx@AuNCs. The addition of Hg$^{2+}$ and Cu$^{2+}$ can quench the fluorescence of gold nanoclusters(AuNCs), but the fluorescence of AuNCs-Cu$^{2+}$ system can be recovered after adding ethylene diamine tetraacetic acid(EDTA). Due to the special affinity between Au$^+$ - Hg$^{2+}$, the fluorescence of AuNCs-Hg$^{2+}$ cannot be recovered. Therefore, a method for the detection of Hg$^{2+}$ was established. Lu Group [26] synthesized lysozyme (Lys) - protected AuNCs with red fluorescence and used for selective detection of cyanide (CN$^-$). The combination of CN$^-$ and auncs to form Au(CN)$^{2-}$ complex can significantly quench the fluorescence of Lys AuNCs. The concentration of CN$^-$ is linearly related to the fluorescence intensity within a certain range. Lin et al [27] used the interaction between Hg$^{2+}$ and Au$^+$ to quench the fluorescence of AuNCs protected by chicken protein (EW), and the addition of melamine restored the fluorescence quenching of EW-AuNCs by Hg$^{2+}$. Based on this phenomenon, the method can be used to simultaneously detect melamine and Hg$^{2+}$. Xue Research Group [28] used double emission ratio type fluorescence SiNPs@GSH-AuNCs To detect proteamine and trypsin. When proteamine is added to SiNPs@GSH-AuNCs, the results show that cationic proteamine can compete with SiNPs and adsorb on the surface of GSH-AuNCs, thus inhibiting the self-assembly of GSH-AuNCs and resulting in fluorescence quenching at 507 nm; while trypsin can catalyze the hydrolysis of proteamine, the self-assembly of GSH-AuNCs starts again, and the aggregation induced emission enhancement recovery.

In this work, we developed a facile template method for synthesis of copper nanomaterials in the presence of Duplex oligonucleotide (dsDNA). Moreover, the fluorescence properties, stability and sensing performance of copper nanomaterials were investigated in details, and explored as fluorescent probe for the assay of Fe$^{3+}$ samples.

### 2. Materials and methods

#### 2.1. Materials

Ascorbic acid (AA, C$_{6}$H$_{8}$O$_{6}$), CuSO$_{4}$, Pb(NO$_{3}$)$_{2}$, ZnSO$_{4}$·7H$_{2}$O (Analytically Pure, Tianjin FengChuan Chemical Reagent Technology Co., Ltd); Bi(NO$_{3}$)$_{3}$·3H$_{2}$O, NaCl, Na$_{2}$S$_{2}$O$_{3}$·5H$_{2}$O, KCl (Analytical Pure, Tianjin Beilian Fine Chemicals Development Co., Ltd); AgNO$_{3}$ (Analytically pure, Beijing Fuchen Chemical Reagent Co., Ltd); FeCl$_{3}$·4H$_{2}$O (Analytical pure, Tianjin Shengao Chemical Reagent Co., Ltd); MnSO$_{4}$·H$_{2}$O (Analytical Reagent, No. 4 Chemical Plant, Chaoyang District, Beijing); Ni(NO$_{3}$)$_{2}$·6H$_{2}$O (Analytical Pure, Beijing 5671 Chemical Plant); NaOH(Analytically pure, Tianjin Shengao Chemical Reagent Co., Ltd); Al(NO$_{3}$)$_{3}$·9H$_{2}$O (Analytical Pure, Tianjin Bodi Chemical Co., Ltd); Cd(NO$_{3}$)$_{2}$ (Analytical Pure, Beijing Chemical plant); Na$_{2}$CO$_{3}$ (Analytically Pure, Tianjin Shengao Chemical Reagent Co., Ltd); KCNS (Analytical Pure, Beijing No.1 Chemical Plant); NH$_{4}$Cl (Analytical Pure, Tianjin People’s Chemical plant); Na$_{2}$SiO$_{3}$·9H$_{2}$O (Analytical Pure, Chemical Plant of Beijing Yizhuan Middle School); NaBr·Cd(NO$_{3}$)$_{2}$ (Analyte, Beijing 5671 Chemical Plant); NaNO$_{2}$ (Analytical Reagent, Tianjin Public Private Joint Venture Chemical Reagent Factory No.1); KI (Analytical Reagent, Baoding Chemical Reagent Factory, Hebei Province); NaHCl (Analytical Pure, Tianjin People’s Chemical Plant); Na$_{2}$S$_{2}$O$_{3}$·5H$_{2}$O (Analytical Pure, Chemical Plant of Beijing Yizhuan Middle School); CoCl$_{2}$·6H$_{2}$O (Analysys, Shanghai Public Private Joint Venture China Trade Factory); NaF(Analytical Pure, Beijing Public and Private Chemical Plant); Ultra Pure Water (about 18.25 mΩ).

DNA strand: ProbeA: TTT TTT TGA TGG CAT GGA CCG CTG AGG ACA TA

ProbeA": AAA AAA ACT ACC GTA CCT GGC GAC TCC TGT AT

#### 2.2. Preparation of copper nanomaterials

20 μM DNA1 and 20 μM DNA2 were annealed, heated in 95 °C water bath for 5 min, and cooled slowly to room temperature. DNA was reacted with PBS (0.01 M, pH = 7.4) at 40 °C for 1 h to form dsDNA. Then the template was reacted with AA (20 mM) and copper sulfate (2 mM) at room temperature for 5 h to form copper nanomaterials. Keep at 4°C.
2.3. Characterization
UV–vis absorption spectra of copper nanomaterials were taken at a Lambda 950 UV–vis spectroscope (Perkin-Elmer). Fluorescence spectra were recorded on a LS-45 fluorescence spectrophotometer (PerkinElmer). Transmission electron microscopy (TEM) experiments were performed to analyze the size of copper nanomaterials with a transmission electron microscope (JEM-2100F) working at an acceleration voltage of 200 kV.

2.4. Detection of Fe^{3+}
450 µl copper nanomaterials were placed in a 1.5 ml centrifuge tube, and then 80 µl ferric ion solutions with different concentrations were added into the centrifuge tube. The reaction time was 1 h at room temperature. The fluorescence intensity was measured at the excitation wavelength of 400 nm.

2.5. Temperature sensing
500 µl of the prepared copper nanomaterials were placed in a series of 1.5 ml centrifugal tubes. The water bath was adjusted to different temperatures to heat the copper nanomaterials for 20 min, and then the fluorescence intensity of the copper nanomaterials was measured.

3. Results and discussion
3.1. Characterization and optimization of copper nanomaterials
It is generally believed that some metal ions can specifically interact with some nucleotides to form special DNA structures, the sensor system consists of two complementary oligonucleotide chains (DNA1 and DNA2), which can hybridize to form dsDNA templates. In the presence of Cu^{2+} and ascorbic acid, the hybrid dsDNA can be used as an effective template for the formation of fluorescent dsDNA copper nanomaterials. In the absence of dsDNA or ssDNA template, copper nanomaterials are not formed. This is the interaction of T in DNA with copper ions to form complexes, and then the T-Cu complex on the DNA template is reduced to Cu0 by ascorbic acid. We performed a series of characterization of the synthesized copper nanomaterials (figure 1). As shown in figure 1(a), the excitation and emission spectra of copper nanomaterials. It can be seen from the figure that the synthesized copper nanomaterials have strong fluorescence intensity, and the maximum emission wavelength of copper nanomaterials is 420 nm. As shown in figure 1(b). The chemical and surface properties of copper...
nanomaterials were characterized by UV–vis spectroscopy. Copper nanomaterials has a small UV absorption spectrum. A characteristic absorption peak was observed at 310 nm for copper nanomaterials in the UV–vis absorption spectrum due to a quasi-continuous electronic energy band structure and quantum confinement effects of Cu nanomaterials. The synthesized copper nanomaterials were dripped onto the copper mesh of carbon support membrane and dried at room temperature. The samples were tested by TEM. Figure 1(c) is the transmission electron microscope (TEM). It can be seen that the copper nanomaterials are evenly dispersed and the particle size is small. Figure 1(d) shows the particle size distribution of copper nanomaterials. The average diameter of copper nanomaterials is about 11.0 ± 0.1 nm. The above results show that the copper nanomaterials have good fluorescence, uniform dispersion and small particle size, which indicates that the high fluorescence copper nanomaterials have been successfully synthesized. We optimized the synthesis time, temperature, molar ratio and pH of copper nanomaterials. By adjusting the molar ratio of copper sulfate and ascorbic acid, the appropriate drug dosage was determined. As shown in figure 2(a), when the molar ratio of copper sulfate to ascorbic acid solution is 1:30, the fluorescence intensity of the synthesized copper nanomaterials is higher. Therefore, the molar ratio of copper sulfate to ascorbic acid is 1:30. Figure 2(b), shows the optimization of synthesis time and pH value of copper nanomaterials. As shown in figure 2(b), with the increase of time, the good fluorescence of copper nanomaterials appears at the point of synthesis time of 6 h. Therefore, we determined that the optimal synthesis time of copper nanomaterials is 6 h. As shown in figure 2(c) when the pH of the solution is 6.00, the fluorescence of copper nanomaterials is better. For this reason, we determined that the optimum pH of synthesis was 6.00. The determination of the above synthesis conditions will lay a good foundation for the synthesis of copper nanomaterials.

![Figure 2](image2.png)

Figure 2. (a) The optimization diagram of the molar ratio of copper sulfate to ascorbic acid; (b) The optimization diagram of reaction time; (c) The optimization of pH value of reaction system.

![Figure 3](image3.png)

Figure 3. (a) The fluorescence intensity showed that the fluorescence intensity changed little within three weeks; (b) Stability test of copper nanomaterials in different concentrations of sodium chloride solution.
3.2. Stability of copper nanomaterials

In the experiment, we studied the stability of the synthesized copper nanomaterials. As shown in figure 3(a), With the migration of time, the synthesized copper nanomaterials were detected. The results showed that the fluorescence intensity changed little within three weeks. Therefore, the synthesized copper nanomaterials can be

Figure 4. The selective detection of common cations (A) and anions (B) by copper nanomaterials shows that the concentration of all metal ions is 100 μmol·L⁻¹.

Figure 5. (a) The fluorescence spectra of Cu nanomaterials with different concentrations of Fe³⁺ (from top to bottom: 5, 30, 50, 70, 90, 100 μmol·L⁻¹); (b) Linear relationship between fluorescence intensity and Fe³⁺.

Figure 6. (a) The TEM image after adding Fe³⁺; (b) The fluorescence intensity before and after Fe³⁺ was compared.
stored for about three weeks at 4 °C. The above experimental data show that the synthesized nanomaterials have good storage and fluorescence stability.

3.3. Selectivity analysis of copper nanomaterials for ferric ion
20 kinds of metal cations (K⁺, Na⁺, Ca²⁺, Hg²⁺, Cr³⁺, Fe³⁺, Pb²⁺, Bi³⁺) and inorganic anions (F⁻, Cl⁻, Br⁻, I⁻, S²⁻, etc) were determined in this experiment. The concentration of all ions is 100 μmol · l⁻¹. As shown in figure 4, only trivalent iron ions have strong quenching effect on the fluorescence of copper nanomaterials, Mn²⁺, Cr²⁺ have weak effects on copper nanomaterials, and other ions have almost negligible effects on copper nanomaterials. These results indicate that the copper nanomaterials have good selectivity as a sensor for ferric ions.

3.4. Analysis of sensing properties of copper nanomaterials
In this experiment, the quenching degree of Cu nanomaterials by different concentrations of Fe³⁺ was investigated. As shown in figure 5(a), the fluorescence of copper nanomaterials is quenched in varying degrees with the increase of ferric ion concentration. As shown in figure 5(b) and table 1, there is a good linear relationship between the fluorescence quenching degree of copper nanomaterials in the range of 5 μmol · l⁻¹–100 μmol · l⁻¹, and the correlation coefficient is 0.9117. As shown in figures 6(a) and (b), the copper nanomaterials aggregate after the addition of ferric ions and the fluorescence of copper nanomaterials is caused by aggregation of nano materials particle size. This is due to the interaction between ferric ion and ligand (ascorbic acid).

3.5. A measurement relating to temperature
In addition, the temperature dependent fluorescence measurement of copper nanomaterials was also studied. As shown in figure 7(a), the fluorescence intensity of copper nanomaterials changes at different temperatures. When the temperature is between 25 °C and 70 °C, the fluorescence intensity of copper nanomaterials presents a

| Fluorescent probe         | Linear range (M) | Detection limit (M) | References |
|---------------------------|------------------|---------------------|------------|
| Graphene quantum dots     | 0–400 M          | 7.22 M              | [29]       |
| Au NPs                    | 40–80 M          | 0.015 M             | [30]       |
| Au NCs                    | 5–1280 M         | 3.5 M               | [31]       |
| Ag NCs                    | 0.5–20 M         | 0.12 M              | [32]       |
| Au/Ag NCs                 | 1–80 M           | 0.5 M               | [33]       |
| dsDNA-CuNPs               | 5–100 μM         | 5 μM                | This work  |

Figure 7. (a) Fluorescence spectra of copper nanomaterials at different temperatures; (b) Linear relationship between fluorescence intensity and temperature.

Table 1. Other fluorescent nanomaterials for the detection of Fe³⁺
good linear relationship, and the correlation coefficient is 0.9309 (as shown in figure 7(b)). The temperature dependent fluorescence measurement of Cu nanomaterials is well demonstrated.

4. Conclusion

In this paper, DNA was used as a template to synthesize high fluorescent copper nanomaterials. Ferric ions can quench the fluorescence of copper nanomaterials with high selectivity. In the range of 5 μmol · L⁻¹–100 μmol · L⁻¹, the fluorescence quenching degree of Cu nanomaterials has a good linear relationship, and the correlation coefficient is 0.9117. The detection limit was 5 μM. The copper nanomaterials can be used for selective detection of ferric ions. At the same time, we found that the fluorescence intensity of Cu nanomaterials changed with the temperature (25 °C–70 °C), and the correlation coefficient was 0.9309. The nanomaterials have potential applications in the application of nano thermometers.

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