Short Communication

Nitrogen-doped carbon quantum dots as fluorescent probe for “off-on” detection of mercury ions, l-cysteine and iodide ions

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GRAPHICAL ABSTRACT
An “off-on” method for highly sensitive and selective detection of Hg2+ and L-cysteine (L-Cys) or Hg2+ and I− using home-made nitrogen-doped carbon quantum dots (N-CQDs) as fluorescent probe was reported.

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ABSTRACT
The application of fluorescent nanoparticles to the detection of inorganic ions and organic compounds has been attracted wide attention recently. In this paper, an “off-on” method for highly sensitive and selective detection of Hg2+ and l-cysteine (l-Cys) or Hg2+ and I− using home-made nitrogen-doped carbon quantum dots (N-CQDs) as fluorescent probe was reported. The N-CQDs with a fluorescence quantum yield of 42.2% were prepared using tartaric acid, citric acid and ethanediamine as the precursors in the oleic acid media. The fluorescence of the obtained N-CQDs could be quenched selectively and sensitively by the addition of Hg2+ (turn-off) with a detection limit of 83.5 nM. When l-Cys or I− was added into the N-CQDs-Hg2+ system, the fluorescence was recovered effectively (turn-on). This process could be used to the detection of l-Cys or I− with a detection limit of 45.8 and 92.3 nM, respectively.

1. Introduction
The pollution of heavy metal ions has become a serious worldwide problem due to their severe risks to human health and the environment. As one of the most toxic heavy metals, mercury with the feature of strong toxicity and bioaccumulation can cause serious human health problems even at very low concentration [1,2]. On the other hand, l-cysteine, as a small-molecular-weight biological thiol-containing amino acid, is essential in maintaining biological redox homeostasis. An abnormal level of l-Cys has been linked to many diseases, such as slow growth, liver damage, skin lesion, Alzheimer’s disease and cardiovascular disease [3–6]. It is also well-known that the content of iodine is directly related to human health [7]. Iodine deficiency could lead to some serious problems such as intellectual disability. Iodine excess could also cause the disease. It is of great necessity to develop rapid and user-friendly...
method for the detection of mercury ions, L-cysteine and iodide ions.

Recently, the application of fluorescent nanoparticles to the detection of inorganic ions and organic compounds, temperature sensing, bioimaging and nanomedicine has been attracted wide attention [8–12]. Various nanomaterials including fluorescently doped silicas and sol-gels, hydrophilic polymers (hydrogels), hydrophobic organic polymers, semiconducting polymers dots, quantum dots, carbon dots, other carbonaceous nanomaterials, upconversion nanoparticles, noble metal nanoparticles (mainly gold and silver), etc. have been investigated for these applications [13–16]. Among them, carbon dots have received particular concern because of their excellent optical properties, good biocompatibility, great aqueous solubility, and simple synthesis [17]. Heteroatom-doped carbon dots which often possess high fluorescent quantum yield have even become the focus.

Xu et al. developed a new fluorescent probe based on ensemble of gold nanoclusters and polymer protected gold nanoparticles for turn-on sensing of L-cysteine [18]. Li et al. developed a simple and distinctive method for the ultrasensitive detection of Cu²⁺ and Hg²⁺ based on surface-enhanced Raman scattering using cysteine-functionalized silver nanoparticles attached with Raman-labeling molecules [19]. Amjadi et al. investigated a carbon dots-silver nanoparticles fluorescence resonance energy transfer system as a novel turn-on fluorescent probe for selective determination of cysteine [20]. Lin et al. prepared europium-decorated graphene quantum dots as a novel “off-on” fluorescent probe for the label-free determination of Cu²⁺ and L-cysteine [21]. Developing convenient methods based on novel nanomaterials for the detection of metal ions and biological active substances is still of great significance.

In this report, water-soluble N-CQDs prepared from tartaric acid, citric acid and ethanediamine by a facile one-pot solvothermal method were used to the “off-on” detection of Hg²⁺, L-Cys and L-Cys-p₂ domains which cannot be removed large particles product. The N-CQDs were prepared by a facile solvothermal method reported by our group [22,23]. Typically, CA monohydrate (1.5 g) and tartaric acid (1.5 g) were placed in a three neck flask, and then ethanediamine (5 mL) and oleic acid (30 mL) were added. The mixture was heated at 220 °C for 30 min under vigorous magnetic stirring. The colorless solution changed to a clear brownish black solution as the reaction progressed. After the reaction, the solution was cooled at room temperature, and then black solid precipitate was obtained directly. The precipitate was washed sufficiently with n-hexane, dispersed in ultrapure water and centrifuged at 6000 rpm for 30 min to remove large particles product.

2. Experimental

2.1. Chemicals

Citic acid monohydrate (CA), tartaric acid, ethanediamine and oleic acid were all analytical reagents. They were purchased from Shanghai Sinopharm Chemical Reagent Co. Ltd. (China). Quinine sulfate (AR) was purchased from Aladdin Industrial Corporation (Shanghai, China). Ultrapure water was prepared with a Milli-Q system (Millipore, Bedford, MA, USA). Other reagents were all analytical and were used without further purification.

2.2. Characterization of the N-CQDs

The fluorescence spectra were obtained by a Varian Cary Eclipse Fluorescence Spectrophotometer. Transmission electron microscopy (TEM) and high-resolution TEM (HRTEM) images were recorded using FEI Tecnai G2 F20 instruments. The X-ray photoelectron spectroscopy (XPS) spectra were recorded using a Thermo ESCALAB 250Xi multifunctional imaging electron spectrometer. A UV-2550 spectrophotometer was used to record the UV–vis spectra. A Magna-IR 750 Fourier transform infrared spectrometer (Nicolet) was used to record the FTIR spectra. The fluorescence decay experiments were performed using an Edinburgh Instruments FLS920 fluorescence spectrometer.

2.3. Preparation of the N-CQDs

The N-CQDs were prepared according to a simple one-step solvothermal method reported by our group [22,23]. Typically, CA monohydrate (1.5 g) and tartaric acid (1.5 g) were placed in a three neck flask, and then ethanediamine (5 mL) and oleic acid (30 mL) were added. The mixture was heated at 220 °C for 30 min under vigorous magnetic stirring. The colorless solution changed to a clear brownish black solution as the reaction progressed. After the reaction, the solution was cooled at room temperature, and then black solid precipitate was obtained directly. The precipitate was washed sufficiently with n-hexane, dispersed in ultrapure water and centrifuged at 6000 rpm for 30 min to remove large particles product.

3. Results and discussion

The N-CQDs were prepared by a facile solvothermal method with oleic acid as the reaction media. The product was then characterized by TEM, XPS, FTIR, UV–vis and photoluminescence spectrum. From the TEM image (Fig. 1A) we can find that the resulting product has good dispersion with a narrow size distribution in the range of 1 to 4 nm (inset of Fig. 1A). The average diameter of the N-CQDs is about 2.66 nm. The obtained N-CQDs are amorphous as the HRTEM images do not show any discernible lattice fringes.

There are two distinct absorbance bands centered at 239 and 351 nm (Fig. 1B). The peak at 239 nm can be ascribed to the π–nπ transitions of the aromatic C=C sp² domains which cannot produce observed fluorescence signal [24,25]. The other absorption peak at 351 nm can be ascribed to the trapping of excited state energy of the surface states, which can lead to strong fluorescence [26,27]. The fluorescent spectrum for the obtained N-CQDs confirmed to the UV–vis absorption features. Fig. 1C shows that the emission peak remains essentially unchanged with the variation.
of excitation wavelength in the range of 320–400 nm. With the further increase of excitation wavelength, a redshift of emission wavelength happened. The maximum emission peak was observed at 460 nm, at an excitation wavelength of 360 nm.

The product emitted bright blue\(^1\) color under 365 nm UV light (Fig. 1B, inset). The fluorescence quantum yield of the obtained N-CQDs was calculated to be 42.2\% using quinine sulfate as a reference.

The FT-IR spectrum (Fig. 2A) shows a broad band around 3403 cm\(^{-1}\), which can be attributed to N–H and O–H stretching vibrations. The intense peaks at 1654 and 1560 cm\(^{-1}\) can be assigned to asymmetric C=O and C=N stretching vibrations. The XPS spectrum (Fig. 2B) exhibits three peaks at 286.05, 399.87 and 532.34 eV, which can be assigned to C\(_{1s}\), N\(_{1s}\), and O\(_{1s}\) respectively. Besides, the C\(_{1s}\) spectrum can be fitted into four peaks at 284.67, 285.32, 285.88 and 287.64 eV (Fig. 2C), showing the presence of four types of carbon bonds: sp\(^2\) C\(\equiv\)C or sp\(^3\) C\(\equiv\)C, C\(\equiv\)N or C\(\equiv\)O, sp\(^2\) N–C=O, and C–O. The fitting of the N\(_{1s}\) spectrum showed that there existed three types of nitrogen bonds: C–N–C (399.34 eV), N–(C)= (399.82 eV), and C–N–H groups (400.58 eV) (Fig. 2C) [28]. Above characterizations indicated that the obtained N–CQDs have abundant functional groups such as hydroxyl and amino groups due to the relatively low carbonization temperature. These polar functional groups endowed the product with perfect water solubility.

3.1. Selectivity and sensitivity of Hg\(^{2+}\) detection

The N-CQDs with a final concentration of 2.1 \(\mu\)g mL\(^{-1}\) was used to evaluate the selectivity towards various metal ions. Different metal ions (including Cu\(^{2+}\), Ni\(^{2+}\), Co\(^{2+}\), Cu\(^{2+}\), Zn\(^{2+}\), Mg\(^{2+}\), Sr\(^{2+}\), Pb\(^{2+}\), Ba\(^{2+}\), Al\(^{3+}\), K\(^+\), Na\(^+\), Fe\(^{2+}\), and Hg\(^{2+}\)) were added into the N-CQDs solution with a final concentration of 100 \(\mu\)M, then the FL spectra were recorded under excitation at 360 nm to study the selectivity. Fig. 3A shows that the FL intensity decreases remarkably with the addition of Hg\(^{2+}\), whereas other ions had negligible or less impact on the intensity. This indicates that the obtained N-CQDs have high selectivity towards Hg\(^{2+}\) ions.

In another control experiment, 30 \(\mu\)M of Hg\(^{2+}\) alone (red bars, Fig. 3B) and the mixtures of 30 \(\mu\)M of Hg\(^{2+}\) and 30 \(\mu\)M of the above mentioned metal ions (blue bars, Fig. 3B) were added into the N-CQDs aqueous solution respectively, then the quenching effects were examined. The interference tests show that the coexistence of other ions only has a small or negligible effect on the fluorescence quenching.

The concentration experiment (Fig. 3C and D) shows that the FL intensity decreases with the increase of Hg\(^{2+}\) concentration. There exists a good linear relationship (R\(^2\) = 0.9979) between the FL intensity and Hg\(^{2+}\) concentration in the range of 0–18 \(\mu\)M. The limit of detection (LOD) was estimated to be 83.5 nM based on three times the standard deviation rule. It should be noted that the obtained LOD of the as-prepared N-CQDs for Hg\(^{2+}\) detection is much lower or comparable to those previously reported with other fluorescent probes [12,29–31]. The results show that the as-prepared N-CQDs may be useful in environmental applications for Hg\(^{2+}\) detection.

The method was then used to assay the concentration of Hg\(^{2+}\) in real water samples. The tap water samples obtained from our lab without any pretreatment were spiked with Hg\(^{2+}\) at different concentration levels and then analyzed with the proposed method. Similarly, the FL intensity decreased with increasing the concentration of Hg\(^{2+}\) in tap water. Good linear relationship between the PL

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\(^1\) For interpretation of color in Fig. 1, the reader is referred to the web version of this article.
intensity and the concentration of Hg\textsuperscript{2+} ions was observed in the range of 0–18 μM.

The sensitivity and selectivity for Hg\textsuperscript{2+} ions towards quenching of fluorescence of the N-CQDs are possibly due to several reasons, such as greater affinity of Hg\textsuperscript{2+} ions towards nitrogen, larger radius of Hg\textsuperscript{2+} ions and its ability to form a stable non-fluorescent complex with the N-CQDs \cite{32}. Another possible explanation is aggregate-induced quenching \cite{29}. The Hg\textsuperscript{2+} ion may simultaneously bind to multiple nitrogen and oxygen atoms of the N-CQDs. This complexation process may induce the formation of aggregates, change the electronic structure of the N-CQDs and then lead to non-radiative electron/hole recombination annihilation.

3.2. Detection of L-Cys

It is reported that L-Cys could react with Hg\textsuperscript{2+} due to the existence of thiol group \cite{19,33}. The Hg\textsuperscript{2+}-S bond would be formed in the process. Thus, Hg\textsuperscript{2+} would be removed from the surface of the N-CQDs and the fluorescence intensity would be recovered due to avoidance of fluorescence quenching by Hg\textsuperscript{2+} species. Therefore, the N-CQDs-Hg\textsuperscript{2+} system was explored to probe L-Cys. We found that the quenched fluorescence of the N-CQDs was recovered immediately upon the addition of L-Cys to the solution of N-CQDs-Hg\textsuperscript{2+}. The fluorescence signal stabilized after 1 min, indicating a fast recovery of the system. Several relevant amino acids including L-Cys, L-Ser, L-Tyr, D-Asp, L-Trp, L-Phe and D-Phe showed only a small or negligible recovering effect. In the control experiment, 30 μM of L-Cys alone (black bars, Fig. 4A) and the mixtures of 30 μM of L-Cys and 300 μM of the above mentioned amino acids (red bars, Fig. 4A) were added into the N-CQDs-Hg\textsuperscript{2+} aqueous solution respectively. Then the FL recovering effects of L-Cys and the mixtures of L-Cys and other amino acids on the N-CQDs-Hg\textsuperscript{2+} were examined. The results showed that the influence of other coexisting amino acids was negligible. These observations indicate that the N-CQDs-Hg\textsuperscript{2+} sensor is insensitive to other amino acids but selective to L-Cys in the mixtures.

As shown in Fig. 4B, the FL intensity of the N-CQDs-Hg\textsuperscript{2+} at 460 nm increases gradually with the increase of L-Cys concentration, indicating that addition of L-Cys can effectively recover the fluorescence of the N-CQDs-Hg\textsuperscript{2+}. Fig. 4C shows the effect of the amount of L-Cys on the fluorescence recovery of the system. It can be seen that the enhancement of fluorescence is proportional to the concentration of L-Cys in the range of 0 to 40 μM (R\textsuperscript{2} = 0.9990). The limit of detection (LOD) was estimated to be 45.8 nM based on three times the standard deviation rule. This sensitivity is higher than that of other reported methods \cite{18,21,34}. The good repeatability of the sensing system can also be observed from the calculated error bars.

3.3. Detection of I\textsuperscript{-}

It was reported that iodide ions can also be effectively bound to Hg\textsuperscript{2+} to form the Hg\textsuperscript{2+}-I\textsuperscript{-} complex. Thus, Hg\textsuperscript{2+} may be removed from the N-CQDs-Hg\textsuperscript{2+}, which makes the fluorescence increase due to avoidance of fluorescence quenching from Hg\textsuperscript{2+} species \cite{30,32}. Various environmentally relevant anions, including Br\textsuperscript{-}, Cl\textsuperscript{-}, F\textsuperscript{-}, SO\textsubscript{4}\textsuperscript{2-}, C\textsubscript{6}H\textsubscript{5}O\textsubscript{2}\textsuperscript{-}, Ac\textsuperscript{-}, CO\textsubscript{3}\textsuperscript{2-}, HCO\textsubscript{3}\textsuperscript{-} and I\textsuperscript{-}, were added into the N-CQDs-Hg\textsuperscript{2+} solution. Significant FL recovering effect was observed with the addition of I\textsuperscript{-}, while other anions showed no or only a slighter recovered effect. In the control experiment, 90 μM of I\textsuperscript{-} alone (black bars, Fig. 5A) and the mixtures of 90 μM of I\textsuperscript{-} and 900 μM of the above mentioned anions (red bars, Fig. 5A) were added into the N-CQDs-Hg\textsuperscript{2+} aqueous solution respectively, then the FL recovering effects of I\textsuperscript{-} and the mixtures of I\textsuperscript{-} and other anions on the fluorescence of the N-CQDs-Hg\textsuperscript{2+} were examined. The results clearly show that the influence of other coexisting anions on the FL recovering effect is negligible. These observations further indicate that the N-CQDs-Hg\textsuperscript{2+} sensor is selective to I\textsuperscript{-} in the mixtures.
Fig. 5B shows the effect of the amount of I/C0 on the fluorescence recovery of the system. It can be seen that the fluorescence intensity of the system increases with the increase of I/C0 concentration.

Fig. 5C shows the effect of the amount of I/C0 on the fluorescence recovery of the system. The enhancement of fluorescence is proportional to the concentration of I/C0 in the range of 0–90 μM (R² = 0.9962). The limit of detection (LOD) was estimated to be 92.3 nM based on three times the standard deviation rule. This sensitivity is higher than that of other reported methods [35–37]. The good repeatability of the sensing system for the detection of I/C0 can be observed from the calculated error bars.

Though the mechanism of “off-on” fluorescence for the obtained N-CQDs was not entirely clear at this point, some characterizations have been performed in order to deepen the understanding of this process. The UV–vis spectral analysis showed that the UV–vis spectra had no significant change when Hg²⁺ ions were added into the N-CQDs aqueous solutions. The intensity and position of the absorbance bands centered at 239 and 351 nm did not change nearly. Besides, the fluorescence decay experiments showed that the fluorescence life time of the N-CQDs decreased obviously in the process of fluorescence quenching with Hg²⁺ ions (from 15.5 ns to 11.2 ns). These results indicated that the fluorescence quenching belonged to dynamic quenching process, and electron transfer process between the excited states of the N-CQDs with Hg²⁺ ions may be the main reason for fluorescence quenching. When L-Cys or I⁻ was added into the N-CQDs-Hg²⁺ system, the UV–vis spectra mainly reflected the feature of Hg²⁺-L-Cys or Hg²⁺-I⁻ complexes. So we deduced that Hg²⁺ ions can be effectively carried off from the surface of the N-CQDs by L-Cys or I⁻, and then the fluorescence of the N-CQDs was retrieved.

4. Conclusions

In conclusion, we have prepared highly fluorescent N-CQDs with a quantum yield of 42.2% via a facile one-pot solvothermal method. The as-prepared N-CQDs have been successfully used for “off-on” detection of Hg²⁺ and L-Cys or Hg²⁺ and I⁻ with high sensitivity and selectivity. Hg²⁺ can effectively quench the fluorescence of the obtained N-CQDs through the charge transfer process. Upon the addition of L-Cys or I⁻ into N-CQDs-Hg²⁺, the fluorescence can be recovered. The possible mechanism relies upon L-Cys or I⁻ being able to effectively shield the quenching due to the binding of Hg²⁺ and L-Cys or I⁻, which removes Hg²⁺ from the surface of the N-CQDs. Detection limits of 83.5 nM for Hg²⁺, 45.8 nM for L-Cys, and 92.3 nM for I⁻ were achieved. The established method has a promising prospect for the detection of real samples.

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References

References omitted for brevity.