One pot synthesis of highly fluorescent N doped C-dots and used as fluorescent probe detection for Hg^{2+} and Ag^+ in aqueous solution

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Abstract: A kind of biocompatible fluorescent nitrogen doped carbon quantum dots (NCDs) has been synthesized using uric acid as raw material through a facile, effective and green thermal treatment method in one pot. The surface morphology and optical properties of the synthesized NCDs were characterized. Without further chemical modification, the NCDs emit bright blue fluorescence with a high quantum yield of 56% due to the enhancement effect of N dopant atoms on a surface of carbon quantum dots. Owing to the strong blue fluorescence, negligible toxicity and the special response of heavy metal ions, the NCDs have been used as a fluorescence probe for sensitive and selective detection of Hg^{2+} and Ag^+ ions with detection limit of 4.8 nM and 1 nM, respectively. Moreover, it was successfully applied to determination of Hg^{2+} and Ag^+ in real environmental water samples, which offers the advantages of simplicity and cost efficiency.

Keywords: Carbon dots, fluorescence, probe, Hg^{2+} and Ag^+

1. Introduction

Heavy metal ions pollution has become a critical worldwide issue. Among them, mercury ions (Hg^{2+}) as one of the most toxic and dangerous heavy metal ion, which can pose severe threats to both human health and environment due to it is not biodegradable [1]. Hg^{2+} may damage many

organs and immune system of human body even at very low concentrations, and it can accumulate in vital organs and tissues and lead to a diversity of diseases [2-4]. Though the silver ion (Ag^+) is less harmful than the mercury ion to living organisms, a large amount of silver is being released into the environment annually from industrial wastes and emissions due to its wide employment in industry, such as electronics, photography, and pharmacy. Otherwise, despite not being a bioaccumulative toxin, silver can inactivate sulfhydryl enzymes bind with amine, imidazole and carboxyl groups of various metabolites, and displace essential metal ions such as Ca^{2+} and Zn^{2+} in hydroxyapatite in bone [5-7]. And recent studies have reported indicated that the grave problems facing in both human health and environment due to the acknowledged antibacterial activity of Ag nanoparticles could be attributed to toxic Ag^+ , which is released from the nanoparticles [8, 9]. So, silver ions have been assigned to the highest toxicity class of heavy metal pollutants [10]. Interest in monitoring toxic metal ions in aquatic ecosystems continued because these contaminants adversely affected the environment and had serious medical effects. Thus, detecting Hg^{2+} and Ag^+ is undoubtedly a significant step in environment and health monitoring. And it's vitally important to develop efficient methods for the selective and sensitive detection of Hg^{2+} and Ag^+ . Current approaches to detecting these two metal ions include inductively coupled plasma mass spectrometry (ICP-MS) [11], atomic absorption spectrometry [12], and stripping voltammetry [13]. Although these methods offer excellent sensitivity and multielement analysis, they are rather complicated, costly, time consuming for sample pretreatment procedures and nonportable [5]. Therefore, developing simple methods for selective and sensitive determination of heavy metal ions is still a great challenge. Among many methods, the fluorescent sensor has drawn much more attention due to its advantages of the operation simplicity, low cost, high sensitivity, intuitive and

fast response. So far, various fluorescent probes are currently being actively developed, such as organic dye molecules, metal nanoparticles and semiconductor quantum dots (QDs). However, the above fluorescent materials are limited by the fact that they are toxic, low sensitive, low selective, hydrophobic or costly.

Carbon dots (CDs) have attracted great interest in chemo- and bio-sensing, theranostic, photocatalysis, bioimaging, light emitting diodes and solar cells [14-17] due to their attractive fluorescence properties, green and economical synthetic strategy, low cytotoxicity, and feasibility for facile large scale production. Amongst them, the applications of CDs used in biosensing, chemical analysis and environment monitoring especially for potential sensor development were caught much attention due to their favorable photostability, high solubility in aqueous medium, biocompatibility and cost effective [18-22]. Currently, many CDs successfully applied to detect heavy ions in aqueous solutions. For example, fluorescent carbon dots with a high quantum yield (82.40%) were synthesized by citric acid as the carbon source and used as fluorescent probe to detecting Hg^{2+} and I^- demonstrated good selectivity [23]. An off-on fluorescent nanosensor for Hg^{2+} and L-cysteine based on C-dots was designed by Yan et al, which demonstrated the detection of Hg^{2+} based on quenching of fluorescence of Cdots, while the detection of L-cysteine owing to the binding preference of L-cysteine with Hg^{2+} [24]. Fu et al reported co-doped carbon nanodots for rapid, sensitive, and label-free detection of Hg^{2+} [25]. And silicon doped C-dots have been synthesized as a fluorescent probe and applied in sensing intracellular Ag^+ and cell imaging [26]. Cayuela et al reported for the first time the direct determination of silver ion (Ag^+) using luminescent carbon quantum dot hydrogels and so on [27]. The C dots have become a fluorescent sensor superior to fluorescent dyes, semiconductor quantum dots and noble metal nanoparticles.

Lots of simple and inexpensive methods for preparation of C-dots have been recently reported. However, most of the resultant C-dots suffer from low quantum yields (always less than 10%), which severely limits their availability for practical applications [28]. And, low cost, highly sensitive sensors based NCDs for the determination of Hg^{2+} and Ag^+ are highly demanded. In this work, we synthesized the fluorescent carbon dots using uric acid with bright blue fluorescence, which can be used as fluorescent detection probe without any further functionalization. The quantum yields of NCDs can reach as high as 56%. We examined the efficient and effective quenching of fluorescence emissions in carbon dots by heavy metal ions. The NCDs can be obviously quenched by Hg^{2+} and Ag^+ at fairly low concentrations. On this basis, the selective and sensitive fluorescence probes have been developed for the detection of trace Hg^{2+} and Ag^+ in the aqueous media. Furthermore, the NCDs can be used for visual identification of Ag^+ and Hg^{2+} in real water samples under UV lamp.

2. Experimental

2.1 Chemicals and characterization

All chemicals used were of analytical grade or of the highest purity available. Uric Acid was purchased from Urchem and diethylenetriamine (DETA) was purchased from Aladdin. The used metal salts $\text{Pb}(\text{NO}_3)_2$, $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, $\text{Hg}(\text{NO}_3)_2 \cdot 1/2\text{H}_2\text{O}$, $\text{Cr}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$, $\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, $\text{Mn}(\text{Ac})_2$, $\text{K}_2\text{Cr}_2\text{O}_7$, $\text{Zn}(\text{Ac})_2 \cdot 2\text{H}_2\text{O}$, $\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, AgNO_3 . All glassware was thoroughly cleaned with freshly prepared 3:1 HCl/HNO_3 (aqua regia) and rinsed thoroughly with Milli-Q ($18 \text{ M}\Omega \cdot \text{cm}^{-1}$ resistance) water prior to use. Milli-Q water was used to prepare all the solutions in this study.

The morphology and size of the NCDs were characterized by transmission electron

microscopy (TEM) using a JEOLFETEM-2100 transmission electron microscope operated at an accelerating voltage of 200 kV. Absorption spectra were recorded on a UV-vis spectroscopy was performed with a U-3010 spectrophotometer (Hitachi, Japan) at room temperature. Fluorescence spectra were performed with Eclipse fluorescence spectrophotometer (Varian, USA) and a PerkinElmer LS-55 fluorescence spectrometer. The X-ray photoelectron spectra were recorded using an AXIS Ultra DLD spectrometer. FTIR spectra were obtained with a JASCOFT/IR-420.

2.2 NCDs synthesis

The NCDs were synthesized according to a reported method earlier with some minor modifications by a simple thermal treatment of uric acid (UA) [29]. Simply, 0.1 g of UA was dissolved into 645 μ L of DETA, then the solution was heated from room temperature to 170 $^{\circ}$ C in an oil bath and held at 170 $^{\circ}$ C for 30 min. The solution was taken out of the oil bath and naturally cooled to room temperature, after washed by acetone, the result precipitation was collected by centrifugation. Finally, NCDs were freeze-dried to a yellow solid powder.

2.3 Detection of ions

The fluorescent detection probe was prepared by dissolving 0.004 g of NCDs powder into 4 mL water to form a solution. The fluorescence emission intensity at 438 nm of the blank sample excited at 370 nm was measured and marked as I_0 . For the determination of Hg^{2+} , the solution of Hg^{2+} with different concentration was added (150 μ L, concentration range, 0–100 μ M) into the above NCDs solution, mixed well and allowed to equilibrate for 1 min. After 1 min, the fluorescent intensity (I) of the samples was measured respectively [30]. When detecting for Ag^+ , the same procedure was followed for Hg^{2+} ions. The selectivity was confirmed by adding other related analogues (including Zn^{2+} , Pb^{2+} , Fe^{3+} , Cu^{2+} , Ni^{2+} , Fe^{2+} , Ba^{2+} , Cr^{6+} , Mn^{2+} , Cd^{2+} , Ca^{2+} , Mg^{2+}

and Cr^{3+}) instead of Ag^+ and Hg^{2+} in a similar way. All experiments were performed at room temperature.

3. Results and discussion

3.1. Synthesis and characterization of NCDs

This work suggested that the N doped CDs was synthesized and used as a fluorescent sensor to heavy metal ions Hg^{2+} and Ag^+ . The process of synthesis and application of NCDs were demonstrated in the scheme 1. The thermal treatment of uric acid with DETA at 170 °C led to a yellow dispersion which exhibited bright blue fluorescence under UV-lamp. The NCDs existed stable for at least three months at room temperature. When in presence of Hg^{2+} or Ag^+ , the fluorescence emission was quenched quickly.

3.2. Characterization of the NCDs

The morphology of NCDs was obtained by TEM that exhibited spherical in shape and a uniform dispersion of the NCDs in water. As seen in Fig. 1, the particle size distribution of NCDs was 2.0–3.0 nm, with an average diameter of 2.52 nm. By the high-resolution TEM (inset of Fig. 1), most particles were observed to have a discernible lattice structure. The representative HRTEM image of NCDs (inserted in Fig. 1) show that the lattice spacing was 0.21 nm, which was consistent with the (100) facet of graphitic structure and suggests sp^3 diamond-like carbon or sp^2 graphitic carbon [31-33]. Whereas a few particles possessed well-resolved lattice fringes, which is consistent with the previously reported results [34].

The surface functional groups of the as-prepared NCDs were characterized by FT-IR spectroscopy. In the FT-IR spectra of NCDs (Fig. 2), the absorption bands at 3328 cm^{-1} corresponding to stretching vibration of N–H, and stretching and bending vibrations of CH_3 and

CH₂ at around 2975 cm⁻¹ and 1380 cm⁻¹ were observed. In addition, the intense peak centered at 1641 cm⁻¹ was attribute to characteristic absorption bands of C=C. Otherwise, the C–O vibrations at 1250 cm⁻¹, the asymmetric and symmetric stretching vibrations of C–O–C at 1020 cm⁻¹, and the N–H deformation vibration at 800 cm⁻¹ and the C=O stretching vibrations at 1668 cm⁻¹ can also be observed respectively. All these functional groups contribute to the excellent solubility of NCDs [35].

The XPS measurements were employed for further confirmation of the composition and structures of the as-prepared NCDs. As shown in Fig. 3a, the chemical compositions and structures of the NCDs were further investigated. The full scan XPS spectrum of the NCDs presents 284 eV, 399 eV and 532 eV, which corresponding to carbon (C 1s), nitrogen (N 1s), and oxygen (O 1s) elements, respectively. Furthermore, the high-resolution spectrum of C1s (Fig. 3b) can be separated into four peaks at 284.4, 285.3, 287.1, and 288.2 eV, which were ascribed to the four types of carbon bonds (C–C/C=C, C–N, C–O, and C=O respectively). The high-resolution spectrum of N1s (Fig. 3c) showed three peaks at 398.4, 399.5 and 401.2 eV, associated with N in a graphite-like structure (N-C3), pyridinic-like N, and N–H bond of NH₂ groups, respectively [36], which indicated that N has been successfully doped into the framework of NCDs. The high-resolution O1s spectrum exhibited two peaks at 529.8 and 531.7 eV (Fig. 3d) were assigned to –OH and C=O, respectively. The XPS results were consistent with the results of FTIR spectroscopy.

The optical properties of the NCDs were investigated in details [14, 37]. The UV-vis absorption and PL spectra of NCDs in an aqueous solution were obtained to explore their optical properties in Fig. 4, NCDs exhibited pale transparent color under daylight conditions and bright

blue color under excitation of 365 nm (inset of Fig. 4). From the UV-vis spectra, two obvious absorption peaks at 265 nm and 340 nm are likely assigned to the $\pi - \pi^*$ transition of sp^2 aromatic conjugate domains and the $n-\pi^*$ broadened absorption bands of C=O transitions, respectively. Subsequent fluorescence characterization indicated that the NCDs can emit strong blue fluorescence under excitation at 365 nm with a UV lamp.

The NCDs also had the narrow and symmetry excitation and emission features which were characterized at 290 nm and 450 nm, respectively. The fluorescence spectra of NCDs at different excitation values (290 nm to 410 nm with an increase of 20 nm) showed excitation dependent emission spectra as shown in Fig. 5a. The e_m peak of NCDs was blue-shifted at 425–430 nm when λ_{e_x} was at 290–330 nm. However, it was red-shifted from 430 to 450 nm when e_x moved from 350 to 410 nm. The e_x -dependent PL spectra shifted with the increase in λ_{e_x} , indicating that the PL band can be tuned by adjusting λ_{e_x} . The NCDs have a large Stokes shift, which was benefit to the distinction of the target from the background signal in imaging [38]. The strongest emission of NCDs diluted solution was observed at 450 nm under 370 nm excitation, with a PL quantum yield of 56%, this value was higher than those of CDs without N dopant reported earlier [15]. And the average lifetime of NCDs was 3.74 ns in Fig. 5b. The e_x -dependent PL behavior was common with most of NCDs. The excitation was dependent on emission in longer wavelength regions, which was probably due to sp^2 hybridization of carbon clusters with size effect [39, 40].

3.3. Effect of pH

Firstly, the influence of pH on the fluorescence of NCDs was studied. The pH value of the as prepared of NCDs was about 6. Fluorescence spectra of NCDs were recorded at different pH values by adjusting the pH by 0.1 M solutions of HCl and NaOH. Figure S1a showed the

fluorescence curves of NCDs at different pH values. It was seen that an increase of the pH value from 1.0 to 6.0 led to slight change of PL intensity, but a further increase from 7.0 to 13.0 resulted in a gradual decrease of fluorescence intensity, indicating the fluorescence intensity of NCDs strongly depended on the pH value (Fig. S1b).

Otherwise, after adding 0.1 M solutions of NaOH, the fluorescence intensity of NCDs decreased gradually, it was red-shifted phenomenon (Fig. S1a), due to the ionization of the carboxyl and hydroxyl groups as observed earlier [41, 42]. Furthermore, to confirm the stability of NCDs under high ionic strength environments, the fluorescence intensity of NCDs was measured in a solution containing different concentrations of NaCl. As illustrated in (Fig. S2), only a slight change in the PL intensity was observed even when the concentration of NaCl was as high as 300 mM, which revealed that NCDs were stable even under high ionic strength conditions. The stability of NCDs in high-salt conditions ensured their applications in more complicated conditions. It is found that NCDs have great potential for sensing applications under physiological conditions.

3.4. Selectivity

The fluorescent NCDs were investigated in the application for detection of metal ions. As shown in Fig. 6a, the fluorescence response of NCDs were studied in the presence of different metal ions (including Hg^{2+} , Ag^+ , Zn^{2+} , Pb^{2+} , Fe^{3+} , Cu^{2+} , Ni^{2+} , Fe^{2+} , Ba^{2+} , Cr^{6+} , Mn^{2+} , Cd^{2+} , Ca^{2+} , Mg^{2+} and Cr^{3+}) with the concentration of 100 μM . It was noticeable that the separate presence of other ions results almost no apparent quenching of the fluorescence in comparison with the blank. However, serious decrease in the fluorescence intensity of the NCDs occurred in presence of Hg^{2+} and Ag^+ within 1 min and 10 min respectively, implying that the addition of Hg^{2+} and Ag^+ could

induce the quenching of fluorescence emission of NCDs in an aqueous solution. Thus, NCDs could be used to detect Hg^{2+} and Ag^+ with representative metal ions at the same concentration of 100 μM .

The selectivity of NCDs toward Hg^{2+} and Ag^+ was tested in detail, and to evaluate the selectivity of the present sensing system, the values of I/I_0 of fluorescent intensity of all samples were calculated to compare the specificity of all ions directly. From the Fig. 6b, the intuitive comparison with other samples indicated that Hg^{2+} and Ag^+ induced a great extent of quenching in fluorescence, and the quenching efficiency was over 50%, indicating good selectivity for Hg^{2+} and Ag^+ response. In contrast, no obvious decrease was observed by addition of other ions into the NCDs solutions. As shown in Fig. 6c, other samples exhibited bright blue fluorescence, it was clear that only Hg^{2+} and Ag^+ resulted in a significant FL quenching of NCDs under UV lamp, which was consistent with the results of fluorescence spectra. These results clearly demonstrated that the NCDs as fluorescent sensor were highly selective toward Hg^{2+} and Ag^+ over the other metal ions. The high selectivity of NCDs to Hg^{2+} and Ag^+ may be attributed to the fact that the interaction between ions and the carboxylate or hydroxyl groups made NCDs close to each other, which accelerated the non-radiative recombination of the excitons through an effective electron transfer process, leading to a substantial decrease of the fluorescence of NCDs [43].

3.5. Sensitivity

Under the condition mentioned above, we evaluated the capability of this analytical system for quantitative detection of Hg^{2+} and Ag^+ . There was a phenomenon of Hg^{2+} concentration-dependent quenching of the fluorescence of NCDs. Fig. 7a presented the FL data versus the concentration of Hg^{2+} ions. With the increase of the concentration of Hg^{2+} , the

fluorescence intensity of NCDs decreased gradually. And when the concentration of Hg^{2+} was added up to 1 mM, the FL intensity can be considered quenched completely, which can be detected from the image under UV lamp (inset of Fig. 7a). The relative PL intensity (I/I_0 , I and I_0 are the PL intensities of NCDs at 440 nm in the presence and absence of Hg^{2+} ion) of the NCDs is linear to the Hg^{2+} concentration in the range of 0.01–1 μM with a linear equation: $I/I_0 = -0.03104c + 0.9843$ ($R^2 = 0.993$) (Fig. 7b). And the detection limit of 4.8 nM was obtained based on a three times standard deviation, which was comparable to other previously reported values [23, 24] and lower than the maximum level of 1 ppb (5 nM) for Hg^{2+} in drinking water permitted by the European Union and the US Environmental Protection Agency (10 nM) [44]. Fig. 7c showed the corresponding fluorescent images of detection, the fluorescence emissions of samples can be observed quenching gradually with the addition of the Hg^{2+} , which was consistent with the results of fluorescence. The results showed that the proposed FL sensor could be an appropriate tool for the detection of Hg^{2+} .

The same phenomenon was followed for Ag^+ ions (Fig. 8a), it was seen that the PL intensity decreases with increased concentration of Ag^+ ions, and when met 1 mM of Ag^+ , the PL intensity direct turned-off (inset of Fig. 8a). A good linear correlation (linear equation: $I/I_0 = -0.05158c + 0.98$ ($R^2 = 0.984$)) was observed over the concentration range of 0.001–1 μM and the detection limit of 1 nM was obtained based on a $S/N \geq 3$ (Fig. 8b). And Fig. 8c showed the corresponding photo images of detection under UV lamp (orientation of the arrowhead: (0, 0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 15, 20, 40, 60, 80, 100 μM). These images showed that the fluorescence emission was decreased accompanied with the addition of the concentration of Ag^+ (Fig. 8c), which was agreed well with the results of fluorescence spectra. Compared with some reported fluorescent

CDs probes (Table S1), the lowest detectable concentration of Hg^{2+} and Ag^+ by NCDs were much lower, indicated that the NCDs was more sensitive probe.

In order to deduce the possible mechanism of the detection for Hg^{2+} and Ag^+ of NCDs, the FTIR spectra (Fig. S3) and UV-vis absorption spectra (Fig. S4) of samples before and after testing were recorded and compared. Compared with the NCDs probe, the FTIR spectra for NCDs after sensor performance did not change in any obvious manner by mixing different ions. Although the accurate fluorescence quenching mechanism was still unclear at present, we could deduce the possible mechanism based on the obtained results and previous reports. According to the previous reports, the fluorescence quenching of CDs by metal ions (e.g. Fe^{3+} ions and Au^{3+}) is attributed to the nonradiative electron-transfer from the CDs to the metal ions [45-47]. The Hg^{2+} and Ag^+ may be have strong binding affinity and fast chelating kinetic with the carboxyl, hydroxyl and amino group of NCDs, which change the electronic structure of NCDs and affect the distribution of excitons, which facilitating the non-radiative recombination of the excitons through an effective electron transfer process. The Hg^{2+} and Ag^+ -induced conversion of the functional group ($-\text{CONH}-$) from diethylenetriamine structure to an opened-ring amide may also make important contribution to the fluorescence quenching [47, 48].

In this case, the fluorescence quenching was possibly caused by the synergetic effect of electron-transfer [49]. For validating the quenching mechanisms, the NCDs with Hg^{2+} and Ag^+ systems were studied by UV-vis absorption spectra in Fig. S4. As shown in Fig. S4, the significantly enhanced adsorption band suggests that the Hg^{2+} and Ag^+ could work as an acceptor to absorb the fluorescence of NCDs. The Hg^{2+} and Ag^+ ions can cause electron-transfer from the NCDs to the Hg^{2+} and Ag^+ ions which resulting in the fluorescence quenching effect [45, 46].

3.6 Reversibility

The reversibility of NCDs was evaluated due to reversibility of the analytical system after being used as sensor was important key for practical applications. As seen in Fig. 9a-I, when the NCDs were mixed with Hg^{2+} , the fluorescence emission of NCDs turned off (Fig. 9a-II), after recovered by saturated EDTA solution, the fluorescence emission renewed completely (Fig. 9a-III). The corresponding photoimages of three samples were listed in the inset of Fig. 9a, which showed the process of blank, detection and recover clearly, which were in agreement with the fluorescence spectra. Similar with the Hg^{2+} , the emission of NCDs in process of detection for Ag^+ underwent the same quenching in detection and recovering after adding the saturated EDTA solution, which were exhibited in the Fig. 9b and inset of Fig. 9b. The fluorescence intensities of the EDTA-containing CDs- $\text{Hg}^{2+}/\text{Ag}^+$ samples were close to those of the reference solutions NCDs respectively (Fig. 9a and Fig. 9b). Therefore, both of NCDs can be classified as a reversible chemosensor not a chemodosimeter for $\text{Hg}^{2+}/\text{Ag}^+$ ions. The result indicates that the chemosensor may be a promising sensing platform in real environmental.

3.7. Application of NCDs in real samples

The excellent specificity combined with high sensitivity and fast response of NCDs to Hg^{2+} and Ag^+ suggested that this method might be directly applied for detecting Hg^{2+} and Ag^+ in real samples. Before using in the real samples, the cytotoxicity of the NCDs was first evaluated by methylthiazoleterazolium (MTT) method. The results of MTT assays were carried out to evaluate the cytotoxicity of the NCDs probes to A-549 cells. As expected, the cell viability was estimated to be greater than 85% upon addition of the NCDs over a wide concentration range of 17–100 $\mu\text{g}\cdot\text{mL}^{-1}$ (Fig. S5). High cell viability confirmed the low toxicity of the NCDs. The results proved

that the NCDs can be using in environmental detection without any pollution and side effects. With respect to this, we further evaluated the practicality of the detection by distinguishing Hg^{2+} and Ag^+ from natural water sources (lake water). In order to evaluate the practical feasibility of our proposed fluorescent sensor NCDs towards the determination of Hg^{2+} and Ag^+ , the lake water samples were filtered through a 0.22 μm membrane and then spiked different concentrations of metal ions with lake water according to earlier method [50].

The selectivity was evaluated and shown in Fig. 10, Fig. 10a showed fluorescence spectra of NCDs containing 100 μM all ions lake water samples (blank, Fe^{2+} , Fe^{3+} , Ca^{2+} , Ni^{2+} , Cu^{2+} , Ba^{2+} , Zn^{2+} , Mn^{2+} , Cr^{3+} , Mg^{2+} , Pb^{2+} , Cr^{6+} , Cd^{2+}) compared with Hg^{2+} and Ag^+ in lake water. Relative fluorescence intensity ($\lambda_{\text{ex}}=370\text{ nm}$) of aqueous NCDs solution in the presence of 100 μM of various metal ions in the lake water was calculated and listed in Fig. 10b. The corresponding photo images of detection under UV lamp showed the clearly cancellation in the lake water sample of Hg^{2+} and Ag^+ compared with other ions (Fig. 10c). In spite of the interference from numerous minerals and organics existing in lake water, this sensing system can highly selective toward the practical Hg^{2+} and Ag^+ detection in real samples.

The sensitivity of lake water samples was also investigated and results were summarized in Fig. 11 and Fig. 12. It is seen that the PL intensity decreases with increased concentration of Hg^{2+} and Ag^+ ions. This fluorescent assay possesses enough high sensitivity for Hg^{2+} and Ag^+ level detection in practical sample, and is capable to monitor Hg^{2+} and Ag^+ level in a continuous and real-time way. Relative fluorescence intensity ($\lambda_{\text{ex}}=370\text{ nm}$) of aqueous NCDs solution in the presence of 100 μM of various metal ions in the lake water (Fig. 11a and 12a). Fig. 11b and 12b showed the corresponding photo images of detection under UV lamp. These results clearly

demonstrated that the NCDs probe was likely to be capable of practically useful Hg^{2+} and Ag^+ detection upon further development. The fact further verified the potential applications of NCDs in Ag^+ and Hg^{2+} detection in an aqueous solution. More importantly, the new strategy eliminated the need to use QDs, organic dyes, and organic solvents, which is much more environmentally friendly.

4. Conclusion

We have developed a convenient and low-cost method for the synthesis of highly fluorescent N doped carbon dots. The as-prepared NCDs have good photostability and a quantum yield as high as 56%. Such NCDs have been further used as a novel sensing probe for label-free, sensitive detection of Hg^{2+} and Ag^+ ions with a detection limit at 4.8 nM and 1 nM, respectively. This fluorescent probe also possesses high selectivity toward Hg^{2+} and Ag^+ analysis and has been successfully used for the analysis of a lake water sample. We expect that this strategy may offer a new approach for developing low-cost and sensitive sensors in biological and environmental applications.

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References

[1] E.M. Nolan, S.J. Lippard, Tools and Tactics for the Optical Detection of Mercuric Ion, Chem.

Rev. 108 (2008) 3443–3480.

- [2] A.H. Stern, A review of the studies of the cardiovascular health effects of methylmercury with consideration of their suitability for risk assessment, *Environ. Res.* 98 (2005) 133–142.
- [3] D.Y. Su, X. Yang, Q.D. Xia, Q. Zhang, F. Chai, C.H. Wang, F.Y. Qu, Folic acid functionalized silver nanoparticles with sensitivity and selectivity colorimetric and fluorescent detection for Hg^{2+} and efficient catalysis, *Nanotechnology* 25 (2014) 355702 (11pp).
- [4] Y.Y. Zhang, H. Jiang, X.M. Wang, Cytidine-stabilized gold nanocluster as a fluorescence turn-on and turn-off probe for dual functional detection of Ag^+ and Hg^{2+} , *Anal. Chim. Acta* 870 (2015) 1–7.
- [5] C.Y. Lin, C.J. Yu, Y.H. Lin, W.L. Tseng, Colorimetric Sensing of Silver (I) and Mercury(II) Ions Based on an Assembly of Tween 20-Stabilized Gold Nanoparticles, *Anal. Chem.* 82 (2010) 6830–6837.
- [6] T. Lou, Z. Chen, Y. Wang, L. Chen, Blue-to-red colorimetric sensing strategy for Hg^{2+} and Ag^+ via redox-regulated surface chemistry of gold nanoparticles, *ACS Appl. Mater. Interfaces* 3 (2011) 1568–1573.
- [7] J.F. Zhang, Y. Zhou, J. Yoon, J.S. Kim, Recent progress in fluorescent and colorimetric chemosensors for detection of precious metal ions (silver, gold and platinum ions), *Chem. Soc. Rev.* 40 (2011) 3416–3429.
- [8] Z.M. Xiu, Q.B. Zhang, H.L. Puppala, V.L. Colvin, P.J. Alvarez, Negligible particle specific antibacterial activity of silver nanoparticles, *Nano Lett.* 12 (2012) 4271–4275.
- [9] Y. Yin, M. Shen, X. Zhou, S. Yu, J. Chao, J. Liu, G. Jiang, Photoreduction and stabilization capability of molecular weight fractionated natural organic matter in transformation of silver ion to metallic nanoparticle, *Environ. Sci. Technol.* 48 (2014) 9366–9373.
- [10] H.T. Ratte, Bioaccumulation and toxicity of silver compounds: a review, *Environ. Toxicol. Chem.* 18 (1999) 89–108.
- [11] J.L. Barriada, A.D. Tappin, E.H. Evans, E.P. Achterberg, Dissolved silver measurements in seawater. *Trends Anal Chem, Anal. Chem.* 26 (2007) 809–817.
- [12] M. Chamsaz, M.H. Arbab-Zavar, J. Akhondzadeh, Triple-phase single-drop microextraction of silver and its determination using graphite-furnace atomic-absorption spectrometry, *J. Anal. Sci.* 24 (2008) 799–801.

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- [13] R. Mikelova, J. Baloun, J. Petrlova, V. Adam, L. Havel, J. Petrek, A. Horna, R. Kizek, Electrochemical determination of Ag-ions in environment waters and their action on plant embryos, *Bioelectrochemistry* 70 (2007) 508–518.
- [14] S.Y. Lim, W. Shen, Z.Q. Gao, Carbon quantum dots and their applications, *Chem. Soc. Rev.* 44 (2015) 362–381.
- [15] S.N. Qu, D. Zhou, D. Li, W.Y. Ji, P.T. Jing, D. Han, L. Liu, H.B. Zeng, D.Z. Shen, Toward Efficient Orange Emissive Carbon Nanodots through Conjugated sp²-Domain Controlling and Surface Charges Engineering, *Adv. Mater.* 28 (2016) 3516–3521.
- [16] L. Wang, B.Q. Li, F. Xu, X.Y. Shi, D.M. Feng, D.Q. Wei, Y. Li, Y.J. Feng, Y.M. Wang, D.C. Jia, Y. Zhou, High-yield synthesis of strong photoluminescent N-doped carbon nanodots derived from hydrosoluble chitosan for mercury ion sensing via smartphone APP, *Biosens. Bioelectron.* 79 (2016) 1–8.
- [17] I.P.J. Lai, S.G. Harroun, S.Y. Chen, B. Unnikrishnan, Y.J. Li, C.C. Huang, Solid-state synthesis of self-functional carbon quantum dots for detection of bacteria and tumor cells, *Sens. Actuators B: Chem.* 228 (2016) 465–470.
- [18] Y.D. Ye, L. Xia, D.D. Xu, X.J. Xing, D.W. Pang, H.W. Tang, DNA-stabilized silver nanoclusters and carbon nanoparticles oxide: A sensitive platform for label-free fluorescence turn-on detection of HIV-DNA sequences, *Biosens. Bioelectron.* 85 (2016) 837–843.
- [19] L.L. Pan, S. Sun, L. Zhang, K. Jiang, H.W. Lin, Near-infrared emissive carbon dots for two-photon fluorescence bioimaging, *Nanoscale* 8 (2016), 17350–17356.
- [20] Y. Zhou, Z.F. Ma, A novel fluorescence enhanced route to detect copper(II) by click chemistry-catalyzed connection of Au@SiO₂ and carbon dots, *Sens. Actuators B: Chem.* 233 (2016) 426–430.
- [21] H. Miao, L. Wang, Y. Zhuo, Z.N. Zhou, X.M. Yang, Label-free fluorimetric detection of CEA using carbon dots derived from tomato juice, *Biosens. Bioelectron.* 86 (2016) 83–89.
- [22] J. Liu, Y. Liu, N.Y. Liu, Y.Z. Han, X. Zhang, H. Huang, Y. Lifshitz, S.T. Lee, J. Zhong, Z.H. Kang, Metal-free efficient photocatalyst for stable visible water splitting via a two-electron pathway, *Science* 347 (2015) 970–974.
- [23] J.L. He, H.R. Zhang, J.L. Zou, Y.L. Liu, J.L. Zhuang, Y. Xiao, B.F. Lei, Carbon dots-based fluorescent probe for “off-on” sensing of Hg(II) and I⁻, *Biosens. Bioelectron.* 79 (2016)

- [24] F.Y. Yan, D.C. Shi, T.C. Zheng, K.Y. Yun, X.G. Zhou, L. Chen, carbon dots as nanosensor for sensitive and selective detection of Hg^{2+} and l-cysteine by means of fluorescence “off-on” swithcing, *Sens. Actuators B: Chem.* 224 (2016) 926–936.
- [25] Z. Fu, M.H. Yao, X.Q. Niu, F.L. Cui, Facile synthesis of highly luminescent co-doped carbon nanodots for rapid, sensitive, and label-free detection of Hg^{2+} , *Sens. Actuators B: Chem.* 226 (2016) 486–494.
- [26] Y.L. Jiang, Z.Y. Wang, Z.H. Dai, Preparation of Silicon–Carbon-Based Dots@Dopamine and Its Application in Intracellular Ag^+ Detection and Cell Imaging, *ACS Appl. Mater. Interfaces* 8 (2016) 3644–3650.
- [27] A. Cayuela, M.L. Soriano, S.R. Kennedy, J.W. Steed, Fluorescent carbon quantum dot hydrogels for direct determination of silver ions, *Talanta* 151 (2016) 100–105.
- [28] C.Y. Xiong, W.B. Liang, H.J. Wang, Y.N. Zheng, Y. Zhuo, Y.Q. Chai, R. Yuan, *Chem. Commun.* 52 (2016) 5589–5592.
- [29] M. Zheng, Z.G. Xie, D. Qu, D. Li, P. Du, X.B. Jing, Z.C. Sun, On–Off–On Fluorescent Carbon Dot Nanosensor for Recognition of Chromium(VI) and Ascorbic Acid Based on the Inner Filter Effect, *ACS Appl. Mater. Interfaces* 5 (2013) 13242–13247.
- [30] Q. Xu, J.F. Wei, J.L. Wang, Y. Liu, N. Li, Y.S. Chen, C. Gao, W.W. Zhang, T.S. Sreeprased, Facile synthesis of copper doped carbon dots and their application as a “turn-off” fluorescent probe in the detection of Fe^{3+} ions, *RSC Adv.* 34 (2016) 28745–28750.
- [31] X.J. Gong, W.J. Lu, M.C. Paau, Q. Hu, X. Wu, S.M. Shuang, C. Dong, M.M.F. Choi, Facile synthesis of nitrogen-doped carbon dots for Fe^{3+} sensing and cellular imaging, *Anal. Chim. Acta* 861 (2015) 74–84.
- [32] H.T. Li, Z.H. Kang, Y. Liu, S. T. Lee, Carbon nanodots: synthesis, properties and applications, *J. Mater. Chem.* 22 (2012) 24230–24253.
- [33] S. C. Ray, A. Saha, N. R. Jana and R. Sarkar, Fluorescent Carbon Nanoparticle: Synthesis, Characterization and Bio-imaging Application, *J. Phys. Chem. C* 113 (2009) 18546–18551.
- [34] Z. Gao, L.B. Wang, R.X. Su, R.L. Huang, W. Qi, Z.M. He, A carbon dot-based “off-on” fluorescent probe for highly selective and sensitive detection of phytic acid, *Biosens. Bioelectron.* 70 (2015) 232–238.

-
- [35] H. Nie, M.J. Li, Q.S. Li, S.J. Liang, Y.Y. Tan, L. Sheng, W. Shi, S.X. A. Zhang, Carbon Dots with Continuously Tunable Full-Color Emission and Their Application in Ratiometric pH Sensing, *Chem. Mater.* 10 (2014) 3104–3112.
- [36] K.G. Qu, J.S. Wang, J.S. Ren, X.G. Qu, Carbon dots prepared by hydrothermal treatment of dopamine as an effective fluorescent sensing platform for the label-free detection of iron(III) ions and dopamine, *Chem. Eur. J.* 19 (2013) 7243–7249.
- [37] J. Yu, C.X. Xu, Z.S. Tian, Y. Lin, Z.L. Shi, Facilely synthesized N-doped carbon quantum dots with high fluorescent yield for sensing Fe^{3+} , *New J. Chem.* 3 (2016) 2083–2088.
- [38] Y. Guo, L.L. Yang, W.W. Li, X.F. Wang, Y.H. Shang, B.X. Li, Carbon dots doped with nitrogen and sulfur and loaded with copper(II) as a “turn-on” fluorescent probe for cystein, glutathione and homocysteine, *Microchim Acta* 183 (2016) 1409–1416.
- [39] H.P. Liu, T. Ye, C.D. Mao, Fluorescent carbon nanoparticles derived from candle soot, *Angew. Chem. Int. Ed.* 34 (2007) 6473–6475.
- [40] G. Eda, M. Chhowalla, Chemically derived graphene oxide: towards large-area thin-film electronics and optoelectronics, *Adv. Mater.* 22 (2010) 2392–2415.
- [41] A. Sachdev, I. Matai, P. Gopinath, Implications of surface passivation on physicochemical and bioimaging properties of carbon dots, *RSC Adv.* 4 (2014) 20915–20921.
- [42] L. Zhou, Y.H. Lin, Z.Z. Huang, J.S. Ren, X.G. Qu, Carbon nanodots as fluorescence probes for rapid, sensitive, and label-free detection of Hg^{2+} and biothiols in complex matricesw, *Chem. Commun.* 48 (2012) 1147–1149.
- [43] Y.M. Guo, Z. Wang, H.W. Shao, X.Y. Jiang, Hydrothermal synthesis of highly fluorescent carbon nanoparticles from sodium citrate and their use for the detection of mercury ions, *Carbon* 52 (2013) 583–589.
- [44] L. Ma, Y. Li, L. Li, J. Sun, C. Tian, Y. Wu, A protein-supported fluorescent reagent for the highly-sensitive and selective detection of mercury ions in aqueous solution and live cells. *Chem. Commun.* 47 (2008) 6345–6347.
- [45] S.J. Zhu, Q.N. Meng, L. Wang, J.H. Zhang, Y.B. Song, H. Jin, K. Zhang, H.C. Sun, H.Y. Wang, B. Yang, Highly Photoluminescent Carbon Dots for Multicolor Patterning, Sensors, and Bioimaging, *Angew. Chem. Int. Ed.* 125 (2013) 4045–4049.
- [46] H.J. Zhang, Y.L. Chen, M.J. Liang, L.F. Xu, S.D. Qi, H.L. Chen, X.G. Chen, Solid-Phase

Synthesis of Highly Fluorescent Nitrogen-Doped Carbon Dots for Sensitive and Selective Probing Ferric Ions in Living Cells, *Anal. Chem.* 86 (2014) 9846–9852.

- [47] Y. Zhang, P.P. Cui, F. Zhang, X.T. Feng, Y.L. Wang, Y.Z. Yang, X.G. Liu, Fluorescent probes for “off-on” highly sensitive detection of Hg^{2+} and L-cysteine based on nitrogen-doped carbon dots, *Talanta* 152 (2016) 288–300.
- [48] J. Ju, W. Chen, Synthesis of highly fluorescent nitrogen-doped graphene quantum dots for sensitive, label-free detection of Fe (III) in aqueous media, *Biosens. Bioelectron.* 58 (2014) 219–225.
- [49] J. Liao, Z.H. Cheng, L. Zhou, Nitrogen-Doping Enhanced Fluorescent Carbon Dots: Green Synthesis and Their Applications for Bioimaging and Label-Free Detection of Au^{3+} Ions, *ACS Sustainable Chem. Eng.* 4 (2016) 3053–3061.
- [50] F. Chai, C.G. Wang, T.T. Wang, Z.F. Ma, Z.M. Su, L-cysteine functionalized gold nanoparticles for the colorimetric detection of Hg^{2+} induced by ultraviolet light, *Nanotechnology* 21 (2010) 025501.

Figure captions

Scheme 1. Schematic illustration of synthesizing NCDs and their application in label-free detection of Ag^+ and Hg^{2+} .

Fig. 1. TEM images of NCDs. The inset of TEM shows a HRTEM image of an individual as-prepared NCDs and the size distribution of the NCDs.

Fig. 2. FT-IR spectra of the NCDs.

Fig. 3. (a) XPS full survey spectra of NCDs. The high-resolution XPS spectra of C1s (b), O1s (c) and N1s (d).

Fig. 4. UV–vis absorption (black line) and PL emission (blue line) spectra of the aqueous dispersion of the nanoparticles. Inset: the photograph of NCDs dispersion under UV light (365 nm).

Fig. 5. (a) PL spectra of NCDs at λ_{ex} 290–410 nm, (b) Fluorescence decay profiles of NCDs at 370 nm excitation, respectively.

Fig. 6. (a) Fluorescence spectra of solutions of NCDs on addition of 100 μM other ions and 100 μM Ag^+ . (b) Relative fluorescence intensity (λ_{ex} =370 nm) of aqueous NCDs solution in the

presence of 100 μM of various metal ions. (c) The corresponding photo images of detection under UV lamp (orientation of the arrowhead: blank, Ag^+ , Hg^{2+} , Zn^{2+} , Pb^{2+} , Fe^{3+} , Cu^{2+} , Ni^{2+} , Fe^{2+} , Ba^{2+} , Cr^{6+} , Mn^{2+} , Cd^{2+} , Ca^{2+} , Mg^{2+} , Cr^{3+}).

Fig. 7. (a) Fluorescence spectra of solutions of NCDs on addition of Hg^{2+} solution (0–100 μM). (b) shows a plot of fluorescent intensity versus the concentrations of Hg^{2+} in the range of 0.001–1 μM . The error bars represent the standard deviations based on three replicate measurements. (c) The corresponding photo images of detection under UV lamp (orientation of the arrowhead: (0, 0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 15, 20, 40, 60, 80 and 100 μM).

Fig. 8. (a) Fluorescence spectra of solutions of NCDs on addition of Ag^+ solution (0–100 μM). (b) shows a plot of fluorescent intensity versus the concentrations of Ag^+ in the range of 0.001–1 μM . The error bars represent the standard deviations based on three replicate measurements. (c) The corresponding photo images of detection under UV lamp (orientation of the arrowhead: (0, 0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 15, 20, 40, 60, 80 and 100 μM).

Fig. 9. Fluorescence spectra for the binding of the NCDs with $\text{Hg}^{2+}/\text{Ag}^+$, the quenching of NCDs fluorescence by $\text{Hg}^{2+}/\text{Ag}^+$, and fluorescence recovery as result of EDTA addition. Reversible investigation of NCDs+ Hg^{2+} (a) and NCDs+ Ag^+ (b) with addition of EDTA.

Fig. 10. Selectivity of detection for lake water samples (a) fluorescence spectra of NCDs containing 100 μM other ions (blank, Hg^{2+} , Fe^{2+} , Fe^{3+} , Ca^{2+} , Ni^{2+} , Cu^{2+} , Ba^{2+} , Zn^{2+} , Mn^{2+} , Cr^{3+} , Mg^{2+} , Pb^{2+} , Cr^{6+} and Cd^{2+}) compared with Ag^+ 100 μM in lake water. (b) Relative fluorescence intensity ($\lambda_{\text{ex}}=370\text{ nm}$) of aqueous NCDs solution in the presence of 100 μM of various metal ions in the lake water. (c) The corresponding photo images of detection under UV lamp.

Fig. 11. Sensitivity of detection for lake water samples (a) fluorescence spectra of NCDs on addition of Hg^{2+} solution (0–100 μM) (b) The corresponding photo images of detection under UV lamp (orientation of the arrowhead: (0, 0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 15, 20, 40, 60, 80 and 100 μM).

Fig. 12. Sensitivity of detection for lake water samples (a) fluorescence spectra of NCDs on addition of Ag^+ solution (0–100 μM) (b) The corresponding photo images of detection under UV lamp (orientation of the arrowhead: (0, 0.001, 0.01, 0.05, 0.1, 0.5, 1, 5, 10, 15, 20, 40, 60, 80 and 100 μM).

Scheme 1

Fig. 1

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