Simple and Sensitive Multi-components Detection Using Synthetic Nitrogen-doped Carbon Dots Based on Soluble Starch

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Abstract
Although carbon dots (CDs) as fluorescent sensors have been widely exploited, multi-component detection using CDs without tedious surface modification is always a challenging task. Here, two kinds of nitrogen-doped CDs (NCD-m and NCD-o) based on soluble starch (SS) as carbon source were prepared through one-pot hydrothermal process using m-phenylenediamine and o-phenylenediamine as nitrogenous dopant respectively. Through fluorescence “on–off” mechanism of CDs, NCD-m and NCD-o could be used as a fluorescence sensor for detection of Fe³⁺ and Ag⁺ with LOD of 0.25 and 0.51 μM, respectively. Additionally, NCD-m could be used for indirect detection of ascorbic acid (AA) with LOD of 5.02 μM. Moreover, fluorescence intensity of NCD-m also exhibited the sensitivity to pH change from 2 to 13. More importantly, Both NCD-m and NCD-o had potential application for analysis of complicated real samples such as tap water, Vitamin C tablets and orange juice. Ultimately, the small size of NCD-m could contribute to reinforcing intracellular endocytosis, which allowed them to be used for bacteria imaging. Obviously, these easily obtainable nitrogen-doped CDs were able to be used for multi-components detection.

Keywords Carbon dots · Metal ions · Ascorbic acid · Sensor · Bioimaging

Introduction
Metal ions are essential for versatile physiological processes. Dysregulation of specific metal ion levels in living organisms is known to have an adverse effect on normal biological events. Among them, Iron ions (Fe³⁺), as an essential element in human body, is basic structure of hemoglobin, myoglobin and many cofactors involved in enzyme activities and plays crucial role in cytochrome and oxygen transportation exchange at the cellular level. Excessive concentration of Fe³⁺ has adverse effect to human health and immunity and results in severe mental illness, hemochromatosis, and neurodegenerative diseases [1]. As a broad-spectrum antibacterial agent, silver is one of the most commonly used antibacterial agents at present [2, 3]. However, there are concerns about whether silver can be applied in the food field efficiently and safely because it is a kind of heavy metal which can endanger human bodies [4]. Hadrup et al. evaluated silver to be genotoxic in vitro in mammalian cells [5].
When silver ions (Ag$^{+}$) are used in food packing or contact materials, there will be potential risks.

Owing to the pathophysiological significance of metal ions, sensitive and selective methods to detect these ions in biological systems, environment, food and water are in high demand. Fluorescent sensors have attracted increasing attention for detection of metal ions in vitro and vivo, owing to their rapidity, simplicity, high selectivity and sensitivity, and possibility for real-time monitoring [6, 7]. Generally, most widely used fluorescent sensors include organic dyes [8], semiconductor quantum dots (QD) [9], fluorescent metal nanoclusters [10] and fluorescent metal organic frameworks [11], etc. However, their defects such as photo instability, complex equipment and treatment processes, high costs, environmental unfriendliness and cytotoxicity also hinder their practical applications.

As a new class of zero-dimensional spherical nanomaterials in carbon family, carbon dots (CDs) have attracted wide attention due to their merits of multiple optical and electrochemical properties, chemical stability, photostability, biocompatibility, easy functionalization and low toxicity. Therefore, CDs are applied in the fields of sensing, electrochemistry, nano-catalysis, and bioimaging [12–14], etc. However, there is still a mutual defect due to lack of sufficient theoretical and experimental knowledge on fluorescence origins in CDs, which is low quantum yields (QYs) among most as-prepared CDs and significantly limits the development and application of CDs as fluorescence materials [15, 16]. By far, one of the most striking ways is the doping of atoms (like N, S, P and B) [17]. Plenty of works have been focused on the N or S-doped CDs. In the process of N doping, π-conjugate domain of pyridone-like structures formed by condensation reaction are most likely cause of quantum yield (QYs) [18].

Some metal ions (Fe$^{3+}$, Ag$^{+}$, Mo$^{6+}$, Cr$^{6+}$, Hg$^{2+}$, Pb$^{2+}$, Cu$^{2+}$, etc.) can interact with functional groups on the surface of some CDs to change their fluorescence properties. For example, Fe$^{3+}$ leads to fluorescence quenching of N-doped CDs (NCDs) [19–25]. And Ag$^{+}$ causes fluorescence quenching of NCDs and N-other atom co-doped CDs [26–29]. Moreover, fluorescence of some NCDs is able to be effectively retained when Fe$^{3+}$ is reduced to Fe$^{2+}$ by reductants such as ascorbic acid [30, 31]. Ascorbic acid (AA), also known as Vitamin C (Vc), is a naturally occurring organic compound and an important food additive. It also plays a significant biological role in anti-oxidation [32], anti-aging [33], anti-cancer [34], immune regulation [35], gene regulation [36], and reduction of atherosclerosis [37]. When added as an antioxidant to foods rich in polysaccharide such as juice, beverage and pasta, AA with low concentrations may exhibit an effect of promoting oxidation thereby affecting the functional properties, such as viscosity of polysaccharide in foods [38, 39]. Therefore, NCDs as fluorescence sensors of metal ions and other analytes have been widely developed.

In this study, two kinds of soluble starch-derived NCDs (NCD-m and NCD-o) were doped by m-phenylenediamine and o-phenylenediamine respectively. Fe$^{3+}$ and Ag$^{+}$ ions were effectively able to quench fluorescence of NCD-m and NCD-o respectively. Therefore, fluorescence sensors based on NCD-m and NCD-o for detecting Fe$^{3+}$ and Ag$^{+}$ ions were respectively established with acceptable detection limits and linear ranges. Additionally, NCD-m could be used indirectly to detect ascorbic acid (AA) on the basis of the reduction of Fe$^{3+}$ to Fe$^{2+}$ by AA. More importantly, these sensors based on as-prepared NCDs could be applied to multi-components detection of Ag$^{+}$, Fe$^{3+}$ and AA in real samples. Moreover, fluorescence intensity of NCD-m was sensitive to pH change, which makes them had a potential as pH sensor. Finally, NCD-m showed their excellent biocompatibility, along with their optical merits, strongly reinforced their applicability potential as bacteria (E. coli) imaging agent.

Materials and Methods

Chemicals

M-phenylenediamine (MDP), o-phenylenediamine (OPD), AgNO$_3$, CaCl$_2$, CdCl$_2$, CoCl$_2$, CuCl$_2$, FeCl$_3$, FeCl$_2$, Hg(NO$_3$)$_2$, Mg(OAc)$_2$, MnCl$_2$, NiCl$_2$, Pb(NO$_3$)$_2$, Zn(OAc)$_2$, Na$_2$SO$_4$, Na$_2$SO$_3$, Na$_2$CO$_3$, Zn(NO$_3$)$_2$ and ascorbic acid (AA) were purchased from Aladin Ltd. (Shanghai, China). Additionally, Vitamin C (ascorbic acid) assay kit (colorimetric method) was purchased from Jiancheng Bioengineering Institute (Nanjing, China) while Vitamin C tablets were purchased from a local pharmacy. Finally, soluble starch (SS) and orange juice were purchased from a local supermarket.

Instrumentation

JEM-F200 transmission electron microscope (TEM) (JEOL, Japan) was used to obtain morphological images of as-prepared NCDs. In addition, F-4600 spectrofluorometer (Hitachi, Japan) was used to measure fluorescence intensity and steady-state emission spectra. UV-2600 spectrophotometer (Shimadzu, Japan) was applied to gather UV–Visible absorption spectra. Moreover, FT-IR spectra were obtained using KBr pellets on IR Affinity-1S FT-IR spectrophotometer (Shimadzu, Japan) to identify functional organic groups within a spectral window of 650–4000 cm$^{-1}$. X-ray diffraction pattern was measured on AXS-D8 X-ray diffractometer (Bruker, Germany). Finally, bacteria imaging was performed.
by A1+/AIR + laser scanning confocal microscope (Nikon, Japan).

Synthesis of NCDs

Nitrogen-doped carbon dots (NCDs) were prepared using one-pot hydrothermal method. In brief, SS (0.25 g) was dissolved in ddH2O (50 ml) with MPD and OPD (0.5 g) respectively before transferring the mixture into a Teflon-lined autoclave and heating at 160 °C for 10 h. After cooling down to room temperature, the supernatant was collected by removing large insoluble dots through centrifugation at 10,000 rpm for 30 min and then filtered using 0.22 μm syringe filters. Subsequently, the pre-processed supernatant was dialyzed against ddH2O for 24 h and then lyophilized. Finally, the residues of NCDs powder were stored at 4°C until use. NCDs prepared from SS and MPD were named NCD-m while those obtained using SS and OPD were labelled NCD-o.

Fluorescence Detection of Fe3+, Ag+ and AA

In order to detect Fe3+ and Ag+, NCD-m and NCD-o (0.2 mg/mL) were respectively mixed with Fe3+ and Ag+ at various final concentrations ranging from 0 μM to 1000 μM (0,0.125,0.25,0.5, 1, 2, 3, 4,5,10,15,20,25,50,75,100,125,150,250,350,450,550,650,750, 850 and 1000 μM) in 2 mL HAc-NaAc buffer (20 mM, pH 500 μM). Their fluorescence spectra were collected at room temperature. Fluorescence quenching of as-prepared NCDs follows Stern–Volmer equation [40]. Therefore, Relative Fluorescence Intensity (F0/F − 1) was calculated to be the signal output value where F0 and F respectively represented fluorescence intensity at Ex = 445 nm of NCD-m or 405 nm of NCD-o in the absence and presence of Fe3+ or Ag+.

In order to detect AA, Fe3+ (500 μM) were mixed with AA at various final concentrations ranging from 0 μM to 850 μM (0,0.125,0.25,0.5, 1, 2, 3, 4,5,10,15,20,25,50,75,100,125,150,250,350,450,550,650,750 and 850 μM) in 2 mL HAc-NaAc buffer (20 mM, pH = 3.0). Afterwards, NCD-m (0.2 mg/mL) were added into Fe3+ + AA and their fluorescence spectra were collected at room temperature. Relative Fluorescence Intensity (F/F0) was calculated to be the signal output value where F0 and F respectively represented fluorescence intensity at Ex = 445 nm of NCD-m in the absence and presence of Fe3+ + AA.

In addition, the selectivity of as-prepared NCDs towards Fe3+, Ag+ and AA was examined by adding different interferents under the same test conditions. Concentration of Fe3+, Ag+, AA and all interferents was 500 μM.

The reaction time in each step of the above experiments was 10 min.

Fluorescence Detection of Fe3+, Ag+ and AA in Real Samples

In order to detect Fe3+ and Ag+ in real samples, tap water obtained from our lab was analyzed using the present methods. The water samples were spiked with Fe3+/Ag+ at different concentrations without any pretreatment. On the other hand, VC tablet sample was prepared by dissolving one Vitamin C Tablet (Instruction: 100 mg of AA/Tablet) in 100 mL ddH2O. It is noteworthy that orange juice (Instruction: 2 mg of AA/100 mL) did not need any pretreatment. VC tablet sample and orange juice were spiked with AA at different concentrations. Testing and data processing were based on the present method.

Bacteria Imaging

Escherichia coli (E. coli DH5α) bacteria were grown overnight at 37 °C in Luria–Bertani medium. Subsequently, 1 ml bacterial culture medium were placed into 2 mL Ep tube. The bacteria were collected by removing the medium through centrifugation at 10,000 rpm for 1 min and then washed twice using ddH2O. The purified bacteria were incubated with NCD-m (0.2 mg/mL) at 37 °C for 12 h in 500 μL mixed solution (v/v phosphate buffered solution: DMSO = 3:2). Afterwards, the bacteria were collected by removing the unbound NCD-m in supernatant through centrifugation at 10,000 rpm for 1 min and then washed twice using ddH2O. Finally, bacteria imaging was performed using confocal fluorescence microscopy.

Results and Discussion

Characterization of NCDs

Morphology of as-prepared NCDs were observed using Transmission electron microscopy (TEM). Figure 1a and b show that both NCD-m and NCD-o had good uniformity and dispersibility, and the average particle size was 5 ± 0.5 nm. HRTEM images of NCD-m and NCD-o are shown in the insets of Fig. 1a and b, indicating that lattice spacing was 0.2 nm, which was consistent with the (100) plane spacing of sp2 carbon [41, 42]. Additionally, Fig. 1c indicates that the measured XRD pattern of NCD-m and NCD-o demonstrated the similar peaks centered at 2θ = 22.3 Å, which was corresponding to a representative graphite structure [43]. Moreover, functional
groups on the surface of as-prepared NCDs were identified using FT-IR spectra. NCD-m and NCD-o had similar FT-IR spectra as demonstrated in Fig. 1d. The broad band centered at 3,271 cm$^{-1}$ was contributed by the stretching vibrations of O–H/N–H. On the other hand, the absorption at 2,925 cm$^{-1}$ was attributed to the stretching vibration of C–H due to hydrogen-atom-rich periphery of benzene ring structure to be retained. Additionally, the peak at 1,633 cm$^{-1}$ belonged to C=C stretching vibration of polycyclic aromatic hydrocarbons. Moreover, the bands at 1,330 and 1,014 cm$^{-1}$ were attributed to the C-N(amine) and C-O (alcohols or phenols) stretching vibration, respectively. The above results indicated that NCDs with the lattice structures were successfully synthesized and possessed many chemical groups on the surface including hydroxyl, carboxyl and amine groups.

**Optical Properties of NCDs**

Fluorescence and UV–vis spectra were employed to investigate the typical optical properties of as-prepared NCDs. As shown in the insets of Fig. 2a, b, NCD-m and NCD-o

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**Fig. 1 (a, b) TEM images (Insets: lattice structures), (c) XRD pattern and (d) FT-IR spectra of as-prepared NCDs**
respectively emitted green and yellow fluorescence under 365 nm UV lamp. Fig. S1a, b show that an increase in excitation wavelength resulted in a gradual and slight redshift in emission wavelength of both NCD-m and NCD-o. In addition, emission intensity changed according to variations in excitation intensity in certain ranges. Maximum excitation wavelength for NCD-m and NCD-o was 445 nm and 405 nm respectively while corresponding emission wavelength was 527 nm and 561 nm respectively. Evidently, both NCD-m and NCD-o exhibited the excitation-dependent emission behavior, which is ascribed to different band gaps from various defect/surface states of CDs [44]. Moreover, NCD-m and NCD-o displayed different UV–vis absorptions as illustrated in Fig. 2. UV–vis spectrum of NCD-m indicated an absorption peak at 290 nm, corresponding to n–π* electronic transition. UV–vis absorption spectrum of NCD-o had three characteristic peaks at 237, 257 and 282 nm in UV region and a broad peak at 416 nm in visible region. The two absorption peaks located at 237 nm and 257 nm were attributed to π–π* transitions of aromatic sp² domain from the carbon core typically, and the other two absorption peaks located at 282 nm and 416 nm were assigned to n–π* transitions of C = N/C = O and/or C-N from surface moieties [45–47].

Stability of NCDs

Stability of as-prepared NCDs is important to evaluate their practical applications in chemical and biological sensing. The effect of storage time, ionic strength and pH on stability of as-prepared NCDs was investigated. Fig. S2a shows that there was no significant change in fluorescence intensity of NCD-m and NCD-o aqueous solutions within 1 to 6 months of storage. As shown in Fig. S2b, fluorescence intensity of NCD-m decreased to some extent with an increase of ionic strength, indicating that high ionic strength led to the partial fluorescence quenching of NCD-m. Meanwhile, fluorescence intensity of NCD-o was stable in high salt solutions even when the concentration of NaCl was up to physiological ionic strength (~200 mM), indicating that NCD-o was able to resist comparatively high ionic strength. Moreover, fluorescence intensity of as-prepared NCDs was sensitive to pH variation. Figure 3a and c indicate that fluorescence intensity of NCD-m gradually and significantly decreased with pH change from 2 to 13. However, NCD-o exhibited a different sensitivity toward pH variation. Figure 3b and d demonstrate that fluorescence intensity of NCD-o was comparatively stable under neutral conditions (pH = 7) but significantly decreased under acidic (pH = 6–2) and alkaline conditions (pH = 8–13). Some researchers speculated that protonation–deprotonation occurring on the surface of these CDs with pH variation induced the changes in surface charges, resulting in pH dependence [48–50]. Therefore, NCD-m might have a potential application of monitoring pH change in vitro or vivo. In addition, a neutral pH condition was important to the further application of NCD-o as fluorescence sensor or bioimaging agent.

Fluorescence Response of NCDs to a Variety of Analytes

19 kinds of analytes (Ag⁺, Ca²⁺, Cd²⁺, Co²⁺, Cu²⁺, Fe³⁺, Fe²⁺, Hg²⁺, Mg²⁺, Mn²⁺, Ni²⁺, Pb²⁺, Zn²⁺, SO₄²⁻, SO₃²⁻, CO₃²⁻, NO₃⁻, Cl⁻ and AA) at a concentration of 500 μM were chosen to test the selectivity of as-prepared NCDs (0.2 mg/mL) as exhibited in Fig. 4. Compared with the other analytes, fluorescence of NCD-m and NCD-o was effectively quenched by Fe³⁺ and Ag⁺ respectively. Therefore, NCD-m and NCD-o displayed obvious selectivity toward Fe³⁺ and Ag⁺ respectively and this provided the study with a possibility of monitoring Fe³⁺ and Ag⁺ through as-prepared NCDs. It was worth noting that Fe²⁺ could not effectively quench fluorescence of NCD-m, which provided a possible mean of detecting reducing agents those
could reduce Fe$^{3+}$ to Fe$^{2+}$. Moreover, the responsive time of as-prepared NCDs toward Fe$^{3+}$ and Ag$^{+}$ was measured and the result was shown in Fig. S3, which indicates that the response of NCD-m toward Fe$^{3+}$ was balanced within 5 min, and the response of NCD-o toward Ag$^{+}$ was balanced within 6 h.

**Fluorescence Detection of Fe$^{3+}$ and Ag$^{+}$**

Considering that different concentrations of Fe$^{3+}$ and Ag$^{+}$ could result in different levels of fluorescence quenching of as-prepared NCDs, this study tested the capability of NCD-m and NCD-o to detect Fe$^{3+}$ and Ag$^{+}$ respectively.

The findings revealed that fluorescence intensity of as-prepared NCDs gradually decreased with an increase of tests-related metal ions concentration from 0 μM to 1000 μM as shown in Fig. 5a, b. Stern–Volmer equation was used to calculate fluorescence quenching. Notably, the plot of Relative Fluorescence Intensity ($F_0/F – 1$) against the concentration of test-related metal ions did not fit a linear Stern–Volmer equation in their entire concentration ranges. Additionally, Fig. 5c shows that there was a good linear relationship between $F_0/F – 1$ of NCD-m and the concentration of Fe$^{3+}$ within the range of 0.5–20 μM with correlation coefficient ($R^2$) of 0.986. The linear equation was fitted as $F_0/F – 1 = 0.007 c + 0.026$ ($c$ representing the concentration of Fe$^{3+}$) and limit of detection (LOD) was calculated to be 0.25 μM based on three times the standard deviation rule (LOD = 3Sd/s). Moreover, Fig. 5d highlights that there was a good linear relationship between $F_0/F – 1$ of NCD-o and the concentration of Ag$^{+}$ within the range of 5–125 μM with $R^2$ of 0.994. The linear equation was fitted as $F_0/F – 1 = 0.002 c + 0.070$ ($c$ representing the concentration of Ag$^{+}$) and LOD was calculated to be 0.51 μM (LOD = 3Sd/s).

A comparison between the proposed methods based on as-prepared NCDs with the previously reported methods based on other CDs are listed in Tables S1 and S2. The comparison suggested that the proposed methods for detection of Fe$^{3+}$ and Ag$^{+}$ were comparable or even better than those previously reported in literatures.
Given that as-prepared NCDs might be applied for detection of Fe $^{3+}$ and Ag $^{+}$ in real samples, tap water samples were spiked with Fe $^{3+}$/Ag $^{+}$ at different concentrations without any pre-treatment. Afterwards, the spiked samples were analyzed by the proposed methods based on as-prepared NCDs. Tables 1 and 2 shows that recoveries from the spiked Fe $^{3+}$ samples were in the range of 96.0–111.0% with Relative Significant Difference (RSD) of 2.8–4.2% and recoveries of the spiked Ag $^{+}$ samples were in the range of 96.6–106.0% with RSD of 2.1–4.5%. This result suggested that NCD-m and NCD-o could be employed as the promising fluorescence sensors for quantitative detection of Fe $^{3+}$ and Ag $^{+}$ in real water samples, respectively.

**Possible Mechanism of Fluorescence Quenching**

Fluorescence of CDs can be generally quenched by dynamic or static quenching. These two quenching can be theoretically described by Stern–Volmer equation by ground-state complex formation model [40]:  

$$F_0/F - 1 = K_{sv}c.$$  

where $F_0$ and $F$ are fluorescence intensity before and after quencher addition, respectively. $K_{sv}$ is Stern–Volmer constant, and $c$ is the concentration of quencher.

In this study, there were good linear relationships between Relative Fluorescence Intensity ($F_0/F - 1$) of as-prepared NCDs and the concentration of Fe $^{3+}$/Ag $^{+}$ (Fig. 5c and d), indicating that quenching mechanism was dynamic or static quenching. Combined with FT-IR results of NCD-m and Fe$^{3+}$-NCD-m, it was able to infer that NCD-m could form stable complex with Fe $^{3+}$ through these hydroxyl,carboxyl and amine groups on their surface (Fig. S4), resulting in fluorescence quenching due to photo-induced electron or energy transfer and high selectivity of NCD-m to Fe $^{3+}$ [51, 52]. Moreover, the mechanism by which Ag $^{+}$ quenched fluorescence of NCD-o was also dynamic or static quenching [53, 54]. To further understand this kind of fluorescence quenching mechanism, fluorescence lifetime experiments were performed. As shown in Fig. 6, fluorescence lifetime of NCD-m and NCD-o barely changed after they were reacted with Fe $^{3+}$ and Ag $^{+}$ respectively. This suggested that the mechanism by which test-related metal ions quenched fluorescence of as-prepared NCDs could be attributed to static quenching. In detail, static quenching occurred when the fluorescent material formed a complex in the ground state with the quencher. Fluorescent quenching behavior involved the transfer of electrons from the excited state of as-prepared NCDs to the unfilled orbital of test-related metal ions, leading to a nonradiative electron transfer process [55].

**Fluorescence Detection of AA**

Most of current methods for detecting AA based on CDs are “on–off–on” strategies where fluorescence of CDs is first quenched by Fe $^{3+}$ then recovered from the reduction of Fe $^{3+}$ to Fe $^{2+}$ in the presence of AA [56–59]. But Fig. S5b suggests that fluorescence of NCD-m quenched by Fe $^{3+}$ (500 μM) couldn’t be recovered significantly even when...
the concentration of AA was up to 1000 μM. This undesirable result might be due to that Fe$^{3+}$ bound to the surface of NCD-m did not react with AA adequately (Fig. S5a). Therefore, in this study, the order of reaction was adjusted to ensure a more complete reduction reaction between AA and Fe$^{3+}$ (Fig. S5c). This “on–off” strategy shows that when Fe$^{3+}$ first reacts with AA, most fluorescence of NCD-m was retained with the increase in AA concentration (Fig. S5d).

Given that fluorescence of NCD-m was quenched by approximately 55.6% in the presence of Fe$^{3+}$ (500 μM) and the continuous increase in the concentration of Fe$^{3+}$ was not able to significantly change fluorescence, Fe$^{3+}$ (500 μM) was chosen to react with AA (Fig. 5a). Figure 7a indicates that fluorescence intensity of Fe$^{3+}$ + AA + NCD-m gradually increased with an increase in AA concentration from 0 μM to 850 μM. In addition, the plot of Relative Fluorescence Intensity ($F/F_0$) against the concentration of AA did not exhibit a linear correlation in the entire concentration range. However, Fig. 7b shows that there was a good linear relationship between $F/F_0$ and the concentration of Ag$^+$ within the range of 5–125 μM.

The concentration of Fe$^{3+}$ within the range of 0.5–20 μM. (d) Linear relationship between Relative Fluorescence Intensity of NCD-o and the concentration of Ag$^+$ within the range of 5–125 μM.

**Table 1** Detection of Fe$^{3+}$ using NCD-m in real samples (n = 5)

| Samples   | Add (μM) | Total found (μM) | Recovery (%) | RSD (%) |
|-----------|----------|------------------|--------------|---------|
| Tap water | 5.0      | 4.8              | 96.0         | 3.1     |
| Tap water | 10.0     | 11.1             | 111.0        | 4.2     |
| Tap water | 15.0     | 16.3             | 108.7        | 2.8     |

**Table 2** Detection of Ag$^+$ using NCD-o in real samples (n = 5)

| Samples   | Add (μM) | Total found (μM) | Recovery (%) | RSD (%) |
|-----------|----------|------------------|--------------|---------|
| Tap water | 10.0     | 10.6             | 106.0        | 2.7     |
| Tap water | 50.0     | 48.3             | 96.6         | 2.1     |
| Tap water | 100.0    | 97.6             | 97.6         | 4.5     |
concentration of AA). LOD was calculated to be 5.02 μM (LOD = 3Sd/s). According to the above results, NCD-m had a potential as a fluorescence sensor in detection of AA.

In order to study the selectivity of NCD-m toward AA, this study selected 15 analytes including Na⁺, K⁺, Ca²⁺, Glu, Fru, Suc, Chol, BSA, Lys, Trp, Tyr, Cys, TA, CA, and GSH at a concentration of 500 μM and observed the changes in fluorescent intensity after NCD-m were added into analyte + Fe³⁺ solution. The result (Fig. 8) revealed that cations (Na⁺, K⁺, Ca²⁺), carbohydrates (Glu, Fru, Suc), amino acids (Lys, Trp, Tyr), Chol and BSA had ignorable reducibility of Fe³⁺ to Fe²⁺, which made most fluorescence of NCD-m to be quenched by Fe³⁺. Additionally, the chemical structures of AA, Cys, TA, CA and GSH contained reducing groups such as carboxyl and thiol [60], which results in the striking conversion of Fe³⁺ to Fe²⁺. Due to the different reducibility of these reductants, fluorescence of NCD-m was preserved to varying degrees. Therefore, Cys, TA, and CA have some interference since they are usually used as reducing agents. In particular, GSH, a well-known reducing agent, has better reducibility of Fe³⁺ to Fe²⁺ compared to AA. This phenomenon was previously employed in designing fluorescence sensors for GSH detection [61–63].

A comparison between the proposed method based on NCD-m with the previously reported methods based on other CDs are listed in Table S3, which suggests that the
The proposed method for detection of AA was comparable or even better than those previously reported in literatures.

Practical application of NCD-m for detection of AA in real samples including VC tablets and orange juice were carried out using a VC assay kit for comparison. The samples were pretreated as indicated in Sect. 2.5. and spiked with AA at different concentrations. Fluorescence intensity of Fe$^{3+}$ (500 μM) + real samples + NCD-m (0.2 mg/mL) was recorded at 445 nm (Fig. 9a). The concentrations of AA were then calculated using the linear equation (Fig. 9b). It was shown that the data detected using NCD-m was comparable to that calculated from the instructions compared to that detected using VC assay kit.

**Fig. 8** Selectivity of NCD-m to various analytes

**Fig. 9** (a) Fluorescence intensity of Fe$^{3+}$ (500 μM)+real samples+NCD-m (0.2 mg/mL). (b) Concentration of AA calculated from product description, detected using NCD-m as sensor, and detected using VC assay kit.
Moreover, Table 3 indicates that when NCD-m were used to analyze the spiked samples, recoveries from the spiked VC tablet samples ranged between 92.6–106.5% with RSD of 2.6–3.8%. On the other hand, recoveries from the spiked orange juice samples ranged between 98.2–108.5% with RSD of 2.7–4.5%. Therefore, NCD-m displayed an acceptable precision of AA detection in real samples.

### Bacteria Imaging

Because of their interesting properties such as high optical absorptivity, chemical stability, biocompatibility, nano-size, and low toxicity, CDs have been widely applied to cell imaging in recent years [64, 65]. In this study, to evaluate whether green-emitting NCD-m was applicable to bacteria imaging, E. coli DH5α were used as the model bacteria. Considering that the thick cell wall of E. coli mainly consists of peptidoglycan, a certain amount of DMSO was added to destroy bacterial cell wall when NCD-m was incubated with DH5α then made these CDs entered into bacteria more easily. Fluorescent signal in DH5α was then confirmed by confocal fluorescence microscope. Bright green fluorescence in DH5α and the uniform morphology of E. coli bacteria were observed in Fig. 10, which indicated high quantum yield of NCD-m and high vitro cellular uptake of NCD-m by DH5α. This fluorescence image of DH5α also shows that these bacterial cells had normal appearance, which could explain the biocompatibility of NCD-m.

### Conclusions

This study used a hydrothermal method to synthesize Fe $^{3+}$-sensitive NCD-m and Ag $^+$-sensitive NCD-o from soluble starch as carbon source and two isomers as nitrogen dopants. These as-prepared NCDs had the potential as fluorescence sensors to detection of Fe $^{3+}$ and Ag $^+$ in aqueous solution. This is because NCD-m and NCD-o were selective toward Fe $^{3+}$ and Ag $^+$ in aqueous solutions with fluorescence “on–off” mode, respectively. Additionally, a good linear relationship was observed between Relative Fluorescence Intensity ($F/F_0$) of NCD-m/NCD-o and the concentration of Fe $^{3+}$/Ag $^+$ within the available range. Besides, Fe $^{2+}$ which resulted from the reduction of Fe $^{3+}$ by ascorbic acid (AA) was not able to effectively quench fluorescence of NCD-m. This phenomenon was therefore applied in detection of AA. In addition, a good linear relationship was observed between Relative Fluorescence Intensity ($F/F_0$) of NCD-m and the concentration of AA within the available range. In real samples, as-prepared NCDs showed acceptable precision in detection of Fe $^{3+}$, Ag $^+$ and AA. Finally, NCD-m were able to be successfully taken by E. coli DH5α bacteria and used for fluorescence imaging. Therefore, the sensors based on NCD-m and NCD-o, which utilized the economical materials, extremely simple preparation and fluorescence “on–off” strategy, provided the simple methods for multi-component (Fe $^{3+}$, Ag $^+$ and AA) detection in real samples and bacteria imaging.

### Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1007/s10895-021-02764-7.

### Authors’ Contributions

Yuanyuan Hu conceived and designed the study. Jun Luo conducted the literature search. Yuanyuan Hu and Wenxuan Ji performed the related experiments. Yuanyuan Hu, 

![Fluorescence image (magnification 60×) of E. coli DH5α bacteria treated with NCD-m](image)
Wenxuan Ji, Jinjuan Qiao, Heng Li, Yun Zhang, and Jun Luo were involved in the analysis and interpretation of data. Yuanyuan Hu and Jun Luo drafted the manuscript. The study was supervised by Jinjuan Qiao, Heng Li and Yun Zhang. All authors read and approved the final manuscript.

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**Data Availability** All data generated or analyzed during this study are included in this published article and its supplementary materials.

**Declarations**

**Consent to Participate** This research did not involve clinical trials and animal experiments which were related to humans and animals as the experimental subjects. Currently, our institutions only require employees to get approval from the ethics committee before carrying out the above two kinds of experiments.

**Competing Interests** The authors declare that they have no competing interests.

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