One-step synthesis of nitrogen and chlorine co-doped fluorescent carbon nanodots for the sensitive detection of Ag⁺

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Abstract. Nitrogen and chlorine co-doped fluorescent carbon nanodots (CDs) were prepared using a facile and eco-friendly solvothermal process in which N-Chlorosuccinimide(NCS) was used as a carbon source. The resulting CDs were characterized by UV-visible absorption spectroscopy, fluorescence spectroscopy, high-resolution transmission electron microscopy (HR-TEM), Fourier transform infrared and X-ray photoelectron spectroscopy (XPS). It shows emission of blue light at 410 nm when excited at 340 nm. High quantum yield of 21.7% and good biocompatibility were also observed. Importantly, the fluorescence intensity of CDs was selectively quenched after addition of Ag⁺. Based upon the aforementioned phenomenon, a new fluorescent biosensor for the detection of Ag⁺ was proposed. The linear range and detection limit was 5-70 μM and 1.06 μM, respectively. Eventually, these superior properties demonstrated that the CDs have promising applications in the field of environmental and biomedicine research.

1. Introduction
In recent years, the problem of heavy metal pollution has erupted frequently, which led to serious issues in public health and thus caused widespread public concerns [1]. As an important transitional and noble metal, silver has a wide range of applications in the fields of catalysis, medical care and materials [2], and so on. However, the rapid development of heavy industry has led to large amounts of silver ions discharged to the environment with industrial wastes, which poses a serious threat to human health. A recent study reported that silver nanomaterials could bind to sulfhydryl groups in proteins and led to deactivation of proteins, which had bactericidal effect but produced toxic silver ions [3]. Therefore, developing a sensing system which can effectively quantitative detect Ag⁺ is significant.

Carbon nanodots (CDs) are new kind of emerging carbon nano-materials with a size of below 10 nm. Currently, CDs have been applied in many fields, such as biological imaging, biosensing [4], photoelectric catalysis and light emitting devices due to their instinct properties [5] including chemical inertness, anti-photobleaching, good biocompatibility, easy to be large-scale synthesized and functionally modified. Among them, biosensing is one most important application of CDs. Element doping is an effective way to optimize the fluorescence performance of CDs [6]. Atoms doped into CDs change the functional groups and the electron orbit on the surface of CDs, which makes structural functionalization and improve the performance of CDs.
Herein, we report a simple and one-step method to prepare nitrogen and chlorine co-doped fluorescent carbon nanodots from N-chlorosuccinimide (NCS). The synthesis conditions were optimized. The obtained CDs have a high quantum yield (21.7%), good biocompatibility. Remarkably, the bright fluorescence of CDs was effective quenched by \( \text{Ag}^+ \). Based on the aforementioned phenomenon, a facile and rapid sensing platform for the determination of \( \text{Ag}^+ \) was fabricated. The experimental parameters were investigated in detail. Moreover, linear range and detection limit was explored.

2. Experimental

2.1. Apparatus and reagents

UV-visible absorption spectroscopy was performed on a TU-1950 UV-vis spectrometer (Persee, Beijing). Fluorescence spectra and fluorescence lifetime were conducted using a FLS920 fluorescence spectrometer (Edinburgh). X-ray photoelectron spectroscopy (XPS) was obtained from an Escalab 250Xi X-ray photoelectron spectrometer (Thermo Fisher Scientific, USA). High-resolution transmission electron microscopy (HRTEM) images were acquired with a Tecnai G2 F20 S-TWIN microscope (FEI, USA).

NCS, Dulbecco’s modified Eagle’s medium (DMEM), Fetal bovine serum (FBS) and reagents for 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sangon Biotech Co., Ltd (Shanghai, China). Quinine sulfate, \( \text{AgNO}_3 \) and other inorganic salts were obtained from Aladdin Bio-chem Technology Co., Ltd (Shanghai, China). All the other chemicals involved in this work were analytical grade and can be used without further purification. Ultrapure water was applied in the whole experimentation.

2.2. Synthesis of CDs

0.2 g of NCS was dispersed into 30 ml anhydrous ethanol. Subsequently, the above mixed solution was added to a 50 mL polyphenylene-lined stainless steel autoclave and heated at 160~240 °C by using automatic electric oven for 4~8 h. After naturally cooled to room temperature, the resulting mixture was filtered using a 0.22 μm microporous membrane to remove residue, and finally turned into a transparent homogeneous solution with a yellow color. The above solution was purified by dialysis using a dialysis membrane (MWCO: 100-500 Da) for 48 h, during which ultrapure water was replaced every 8 h and then stored in a refrigerator at 4 °C.

2.3. Quantum Yield (QY) Calculation

QY of the prepared CDs in aqueous solution was measured using a relative quantum yield measurement method and taking Quinine sulfate (QY 54%) as a standard. First, CDs aqueous solutions of different concentrations were configured so as to obtain 5 gradient absorbance of solution. The absorbance value located at 340 nm was record. Subsequently, the fluorescence (excited at 340 nm) of the above solution was measured and the fluorescence integral was calculated. Then, a standard curve equation of the reference and sample was achieved by using the absorbance as the abscissa and the fluorescence integral as the ordinate. Finally, QY was obtained through the following formula:

\[
\text{QY}_{\text{CDs}} = \text{QY}_{\text{ST}} \frac{G_{\text{CDs}}}{G_{\text{ST}}} \left( \frac{N_{\text{CDs}}}{N_{\text{ST}}} \right)^2
\]

where the subscripts “CDs” and “ST” refer to carbon nanodots and Quinine sulfate, respectively. \( G \) stand for the slope of the above fitted line, and \( N \) stand for the refractive index of the solvent.

2.4. Cytotoxicity Assay

MTT colorimetric assay was performed to assess the cytotoxicity of prepared CDs to HeLa cells according to the following procedure. First, HeLa cells were grown in 96-well plates at 5×10^3 cells/well and then incubated at 37 °C with 5% \( \text{CO}_2 \) for 24 h. Subsequently, the medium was removed
and fresh medium containing different concentration of CDs was added. After incubation for another 24 h, 100 μL medium including MTT reagent (10 μL, 5 mg mL⁻¹) was renewed and continue incubated for 4 h. Six wells were set to repeat. Finally, the absorbance of each well was measured at 570 nm using a microplate reader at 570 nm. The following formula was used to calculate the cell viability:

\[
\text{cell viability(%) = } \frac{\text{Abs(\text{Treated})}}{\text{Abs(\text{Control})}} \times 100
\]

Where Abs(Treated) and Abs(Control) is the absorbance of the well in the presence and absence of CDs.

2.5. Detection of Ag⁺

AgNO₃ was employed as a source of Ag⁺ in the present study. The stock solutions were prepared by dissolving appropriate amount in 100 mL volumetric flask. The working solution of Ag⁺ with different concentrations was freshly prepared by stepwise diluting the stock of Ag⁺ with PBS buffer (pH=7.4, 50 mM). For fluorescence quenching, different concentrations of Ag⁺ were added to the CDs solution. The solution fluorescence intensity was measured at 410 nm with an excitation wavelength at 340 nm.

For selectivity measurements, a series of inorganic salts were employed in the experiments. In the procedure of the detection, 10 mM stock solution was gained by dissolving the above-mentioned inorganic salts with ultrapure water, and then diluted to a relative concentration using PBS buffer. Subsequently, the above salt solution was mixed with CDs, and then measured by fluorescence spectroscopy under the same condition.

3. Results and discussion

3.1. Synthesis of the CDs

Figure 1. Illustration of the formation of CDs derived from NCS using a solvothermal treatment and their Ag⁺ sensing.

Figure 2. Effects of (a) the ratio of ethanol, (b) treatment temperature, (c) treatment time on the normalized quantum yield. (d) Effect of pH on the normalized fluorescence intensity.
The procedure employed for the synthesis of CDs derived from NCS through a solvothermal method and subsequently for Ag⁺ sensing is demonstrated in figure 1. In order to achieve best luminescent properties, several key factors governing the synthesis procedure were systematically investigated. First of all, the ratio of ethanol was altered from 0 to 100%. As depicted in figure 2a. When using anhydrous ethanol solvent, CDs has the highest normalized QY. This is due to ethanol modification on CDs surface. The higher the ratio of ethanol, CDs are modified by more surface modifiers while forming, the stronger the fluorescence properties. It is generally known that the treatment temperature is a key parameter for the process of carbonization. As demonstrated in figure 2b, the normalized QY increased gradually with raising treatment temperature and started to level off after 200 °C. This may be due to the fact that low QY was obtained at low temperature due to the incomplete carbonization process. As the temperature was increased, the extent of carbonization process and the conversion rate was also increased. Thus, QY was improved and reached a maximum value. After that, the reaction temperature will damage the surface structure of CDs, and bring about a decreased QY. Therefore, 200 °C was designed. As another key factor, reaction time was also optimized. QY was enhanced with increasing reaction time. After the time was up to 6 h, a maximum was gained. It is possible that a longer reaction time promotes the carbonization process that leads to high QY. Hence, 6 h was chosen. Finally, the pH of solution was changed. As shown in figure. 2d, acidic, neutral and weakly alkaline conditions have almost no effect on fluorescence. In strong alkaline conditions, CDs fluorescence was weakened. The reason was that the presence of acidic functional groups on the surface of CDs, such as carboxyl, react with OH⁻ in the external environment.

3.2. Characterization of CDs

Above all, HRTEM was used to investigate the size and morphology of the prepared CDs. The HRTEM micrographs of prepared CDs are shown in figure 3a. It is obvious that the well-dispersed CDs are with near spherical morphologies. They are separated with each other. Most of the CDs possess a diameter around 2-3 nm.

Subsequently, FTIR and XPS were used to investigate the structure, elementary analysis and surface composition of CDs. As shown in figure 3b, the CDs exhibited the dull and intense band at 3428, 3162 and 2800 cm⁻¹ which was respectively assigned to the stretching vibration of O-H, N-H and C-H. The bands at 1772, 1697, 1294, 1191 and 821 cm⁻¹ was ascribed to C=O, C=O, C-O, C-N
and C-Cl, respectively. It reveals the presence of a large number of hydroxyl, carboxyl and amino groups on the surface of CDs. As demonstrated in figure 3c, three pivotal peaks at 204.6, 293.8, 407 and 539.5 eV, which are respectively assigned to C1s, N1s, and O1s, were observed in the full-scan XPS spectra. Further treatment to the C1s spectrum of CDs was performed. Four peaks at 284.6, 285.6, 286.6, and 288.8 eV, which are assigned to C-C, C-N, C-O, and C=O, respectively, appeared in figure 3d by deconvoluting the high-resolution C1s spectrum. The above results are in aligned with the antecedent FTIR analysis.

3.3. Optical properties of CDs

To gain deeper insight into the optical properties of CDs, UV-Vis absorption, excitation and emission spectra of CDs were conducted. Strong absorption was observed in the near ultraviolet region and the absorption decreased with increasing wavelength. Under the optimized excitation wavelength, the CDs displayed a strong emission peak at 400 nm showing excellent fluorescence properties. Figure 4b shows the excitation-dependent photoluminescence behaviors of CDs by tuning the excitation wavelength from 300 to 400 nm. The emission peak was red-shifted from 400 to 450 nm due to the “edge red-shift effect” [7]. Finally, time-resolved fluorescence decays of CDs were acquired at 340 nm and are shown in figure 4c. Fitted using a bi-exponential function, the lifetime of τ1 and τ2 was 21.05 and 13.72 ns, respectively. As a consequence, the average lifetime was calculated to be 4.48 ns.

3.4. Cytotoxicity Assay of CDs

In order to apply the prepared CDs as a fluorescent probe, we need to ensure its favorable biocompatibility. MTT colorimetric assay was conducted to assess the cytotoxicity of CDs using a
HeLa cell line. Figure 5 shows the concentration-dependent cell viability of CDs. As illustrated in figure 5, over 87.5% cell viability was found after 24 h incubation of HeLa cells even with a concentration of CDs up to 400 mg L$^{-1}$. It demonstrated that the cell cytotoxicity of CDs is minimal, which makes them great potential for biological imaging, cell tracking, and fluorescence sensing.

3.5. Detection of Ag$^+$

![Graph showing relative fluorescence intensity of CDs in the presence of 1 mM of various metal ions.](image)

To evaluate the feasibility of CDs for detection of Ag$^+$, relative fluorescence intensity of CDs in the presence or absence of 1 mM Ag$^+$ was further studied. As showed in figure 6a, the fluorescence of CDs was effectively quenched by Ag$^+$. Because that Ag$^+$ interacts strongly with the multifunctional groups of CDs. Subsequently, the selectivity was investigated by introducing the following metal ions into the sensing system. Similar experiments were carried out. As shown in figure 6a, no obvious changes of relative fluorescence intensity were observed to other metal ions. Based on the aforementioned quenching effect, it is possible to employ a rapid and facile strategy for the determination of Ag$^+$. Based on fluorescence analysis, we found that the Stern-Volmer equation was compliant for the above quenching data: $F_0/F = 1 + K_{sv}[C]$, where $F_0$ and $F$ represent the fluorescence intensity of CDs in the absence and existence of Ag$^+$, respectively. $K_{sv}$ and $C$ mean the Stern-Volmer quenching constant and the concentration of Ag$^+$, respectively. As shown in figure 6b, quite good linear relationship existed between the $F_0/F - 1$ and the concentration of Ag$^+$ within the limit of 5-70 μM, which the equation of linear regression was

$$F_0/F - 1 = 0.00226C_{Ag^+} - 0.0082(R^2 = 0.99).$$

Figure 6. (a) Relative fluorescence intensity of CDs in the presence of 1 mM of various metal ions. (B) Relationship between the fluorescence intensity of CDs and the concentration of Ag$^+$. To evaluate the feasibility of CDs for detection of Ag$^+$, relative fluorescence intensity of CDs in the presence or absence of 1 mM Ag$^+$ was further studied. As showed in figure 6a, the fluorescence of CDs was effectively quenched by Ag$^+$. Because that Ag$^+$ interacts strongly with the multifunctional groups of CDs. Subsequently, the selectivity was investigated by introducing the following metal ions into the sensing system. Similar experiments were carried out. As shown in figure 6a, no obvious changes of relative fluorescence intensity were observed to other metal ions. Based on the aforementioned quenching effect, it is possible to employ a rapid and facile strategy for the determination of Ag$^+$. Based on fluorescence analysis, we found that the Stern-Volmer equation was compliant for the above quenching data: $F_0/F - 1 = 0.00226C_{Ag^+} - 0.0082(R^2 = 0.99)$. The detection limit is calculated to be 1.06 μM by the formal of $3 \sigma/S$, where $\sigma$ and $S$ stand for the standard deviation of the blank signal and the slope of the linear calibration equation, respectively. It may be noticed that this method owns the properties of lower detection of limit and wider linear range.

4. Conclusion

In summary, we have developed a facile and eco-friendly solvothermal route for the synthesis of fluorescent CDs using NCS as carbon source. The synthesis conditions were investigated, and the structure and optical properties were characterized. Under optimal conditions, the obtained CDs exhibit favorable quantum yield (i.e., 21.7%), low cytotoxicity and good biocompatibility, which enable them to be a suitable candidate for biosensing. Moreover, the fluorescence of CDs could be quenched by Ag$^+$, which made the CDs quite suitable to work as fluorescent probe for environmental Ag$^+$ sensor. Thus, this approach shows great promise for environmental and biomedicine applications in the light of simple synthesis, excellent optical properties, and good biocompatibility.

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