

Synthesis of Carbon dots-based Fluorescent Probe for the High Selective Detection of Mercury Ions

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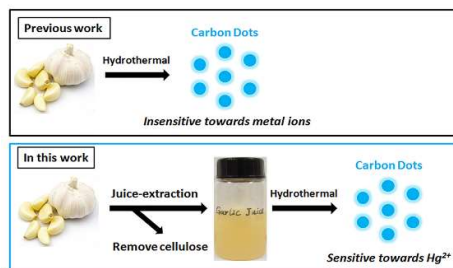
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ABSTRACT: Fluorescent carbon dots (C-dots) were synthesized by one pot hydrothermal treatment of garlic juice at 180 °C for 7h. The as-prepared C-dots with an average diameter of 2.13 ± 0.71

nm can be well dispersed, and show absorbance in the fluorescence with a quantum yield of 8.9% in water. The C-dots were fully characterized by TEM, XPS, XRD, and FTIR, respectively. Additionally, the as-prepared C-dots reveal nearly nontoxic to SW480 cells and can be used for cell imaging. The water solution of C-dots show sensitivity to pH values and metal ions, and also have a high selectivity to the Hg^{2+} ion among other transition metal ions, and achieve a high sensitive detection with the detection limit of $0.45 \mu\text{M}$. The fluorescent C-dots are expected to be useful for the evaluation of mercury ions in environmental water system.

Keywords: Fluorescent carbon dots, hydrothermal treatment, characterization, cell imaging, mercury ions, selective and sensitive detection



1. Introduction

Compared with common small-molecular fluorophore, the fluorescent carbon dots (C-dots) possess several superior photophysical and biological properties, such as the high photostability, facile preparation, excellent water solubility, interfacial activity, low cytotoxicity, and biocompatibility *in vitro* and *in vivo*. [1-7]

Therefore, the C-dots have been intensively and extensively investigated in biochemical sensing, fluorescent probes, photocatalytic technology, environment testing,

and drug carriers.[8-16]

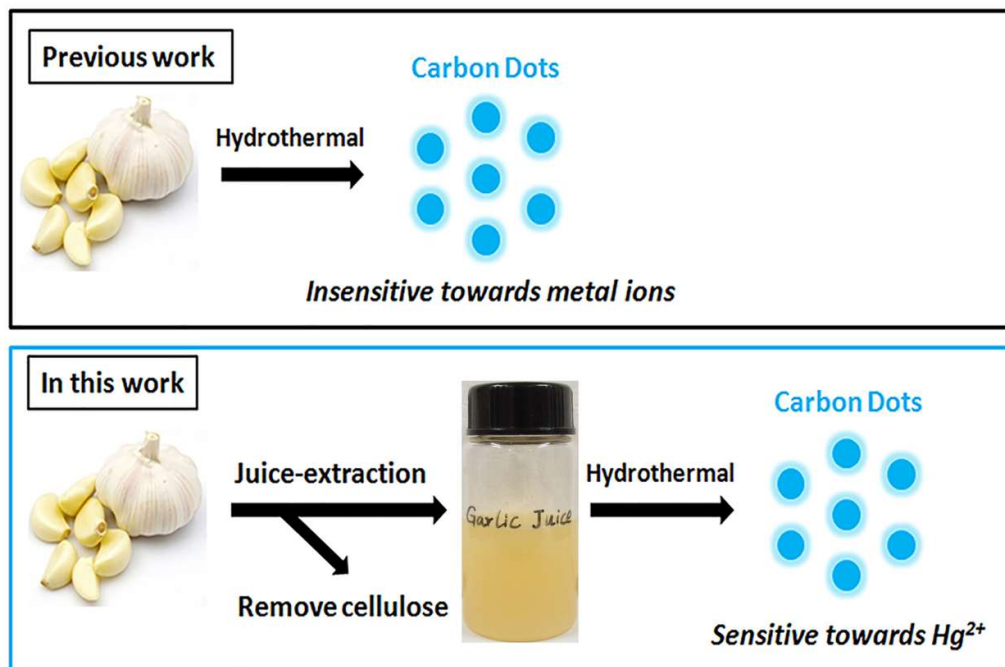
On the sensing of metal ions by C-dots based probes, the detection of Hg^{2+} is attracting great attention from the community. Mercury ions is considered a typical health hazard and show highly toxic for human liver and central nervous system, which results an exposure to high level of mercury ions can lead to cerebral injury and cerebral death.[17-19] Therefore, to develop a sensitive and selective detection method for Hg^{2+} is of great importance for environment protection and human health.[20, 21]

Garlic, a valuable natural product, is rich in nutrition, and can prevent tumor and cancer due to its containing an appreciable amount of germanium, selenium and sulfo-species.[22, 23] Abundant production of garlic as carbon source enables a large-scale preparation of C-dots, Zhao *et al.* has reported the S- and N- co-doped C-dots with blue fluorescence prepared by garlic bulblet.[24] In the meanwhile of considering eliminating the difficult decomposing process of cellulose in garlic, herein, we designed and synthesized novel garlic-based C-dots via hydrothermal synthesis method by using garlic juice. Interestingly, the C-dots from garlic juice have unique surface groups, which possess complete different metal ions binding property compared with reported garlic C-dots. The C-dots with the specific recognition ability can be utilized as an effective probe for mercury ions in water medium.[25-27]

2. Experimental

2.1 Chemicals and materials

Ultrapure water was made from ultrapure water purifier (Mini-Q). Garlics were purchased from supermarket. All of the metal-salt were purchased from Sigma-Aldrich. CCK-8 kit was purchased from BOSTER. Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco. SW480 cell line was obtained from Cell Resource Center, IBMS, CAMS/ PUMS.



Scheme 1. Schematic representation of preparing C-dots by hydrothermal treatment of garlic.

2.2 Instrument and characterization.

All fluorescent measurements were performed on a Fluoromax-4 spectrofluorometer (HORIBA JobinYvon Inc.) with excitation slit set at 25 nm band passes in 1cm×1cm quartz cell. In addition, UV-Vis absorption was characterized on a UV5800 spectrophotometer. The morphology and microstructure of the C-dots were examined by transmission electron microscopy (TEM) on JEM-1200EX electron microscopy with an accelerating voltage of 100 kV. For TEM characterization, one drop of C-dots suspension was deposited on a carbon-coated copper grid and dried at room temperature. The optical densities was measured on UV-vis spectra were obtained on a UV5800 spectrophotometer. The FTIR spectra were recorded on an IFS 66V/S (Bruker) IR spectrometer in the range of 600 – 4000 cm⁻¹. X-ray photoelectron spectroscopy (XPS) analysis was measured on an ESCALAB MK II X-ray photoelectron spectrometer using patterns were obtained using Cu K α radiation ($k = 1.54056 \text{ \AA}$).Mg as the exciting source. X-Ray.

2.3 Preparation of luminescent carbon dots.

The skinless garlics were mashed to paste using a mortar and a pestle, the juice was squeezed out from it, and then pure water was mixed into the juice by the ratio of 4:5. Following, 5ml of the carbon source and 12ml ultrapure water were added into Teflon equipped stainless steel autoclave and then the mixture was heated in a constant temperature and humidity drying box at 180 °C for 7 hours, timing in line with the procedure of the reactor but not as soon as the Ptfе autoclave was put into the drying box. When the hydrothermal reaction finished, the autoclave was cooled down naturally and about 15ml C-dots solution was obtained by this way. Finally, the as-prepared C-dots original solution was purified for about 7 hours totally using a dialysis bag in a Bunsen beaker of 1000ml containing 800ml ultrapure water. The purified C-dots solution was dried at 60°C, and then at 80 °C in a vacuum drying oven.

2.4 Sensitivity Detection for pH values.

PL intensity changes of the obtained C-dots in aqueous solution were detected after they were adjusted to different pH values to discover the stability of C-dots in internal environment of human. The diluted C-dots aqueous solution, 10^{-1} times than the original, was adjusted to be pH2, pH3, pH4, pH5, pH6, pH7, pH8, pH9, pH10, pH11, pH12 using phosphate buffer by pH value card. The diluted 10^{-1} times C-dots aqueous solution, pH values of which have been adjusted, were mixed well and placed for about 30 minutes at a room temperature, then mixed again and their PL intensities were examined immediately by a fluorescence spectrophotometer.

2.5 Cell culture, Cytotoxicity assay and Cell imaging.

The SW480 cells were maintained in DMEM /high glucose (1×) medium supplemented with FBS of 10% (v/v) at 37 °C in 5% CO₂ humidified atmosphere. Exponentially growing cells were dissociated with 0.25% Trypsin–EDTA (1×) cell dissociation medium (GIBCO).

The cytotoxicity of C-dots towards SW480 cells was evaluated by the CCK-8 assay. Briefly the SW480 cells were seeded into a 96-well plate for 100μL per well for 24h at 37 °C. After the culture medium was removed, the medium containing C-dots with different concentrations (2mg/ml-0.25mg/ml) was added into subsequently. The cells

were then incubated for another 24h. After that, CCK-8 solution, in the volume of 10 μ L per well, was added to each well and incubated at 37 °C for 1h. The absorbances were measured at 450nm by the Enzyme standard instrument.

Following a bioimaging observing was conducted. The SW480 cells digested by the 0.25% Trypsin-EDTA (1 \times) were plated in glass bottomed culture dishes(Mat Tek) and cultured for 24h, then the cell culture medium in the dishes were removed out of them, the remainder cells were washed for 3 times with PBS (pH 7.3), after that, 1ml C-dots aqueous solution, of which the concentration was 1mg/ml, and 1 ml cell culture medium without FBS were added to different dishes, control group and experimental group respectively, then they were incubated for another 4-6h in the cell culture incubator. When the dishes cultured cells were carried out, the culture medium in them were removed as well, the remainder cells were washed for 1 time, and then 1ml 4% paraformaldehyde (sigma) was added to respectively. The dishes were placed in 4 °C for 30 min to fix the state of cells. At last, the cells fixed well in dishes can be observed by an NIKON TE2000 inverted fluorescence microscope system.

2.6 Quantum yield.

Using the below equation, the QY of the as-prepared C-dots, by measuring the fluorescence intensity in aqueous dispersion, can be calculated,

$$Q_{C-dot} = Q_R \cdot \frac{I_{C-dot}}{I_R} \cdot \frac{A_R}{A_{C-dot}}$$

In this equation, Q is the quantum yield of our sample and I is the measured intensity of luminescent spectra. A is the optical density at excited wavelength. QY was measured choosing quinine sulfate in 0.05M sulfuric acid solution (literature quantum yield 54% at 310nm) as a standard for C-dots. The subscript “R” refers to standard with known quantum yield and “C-dot” for the sample in this equation. In order to minimize reabsorption effects, absorbance of the C-dots solution in the 10 mm fluorescence cuvette was kept ≤ 0.05 at the excitation wavelength (400 nm).

2.7 Detection as the sensing probe of the obtained C-dots for metal ions and Hg²⁺ ion.

Compounding a variety of metal ion aqueous solutions (Ni²⁺, Fe²⁺, Fe³⁺, Na⁺, Cu²⁺, Co²⁺, Ba²⁺, Cd²⁺, Mn²⁺), the concentration of which were all 5mM, and the diluted C-

dots aqueous solution, 10^{-1} times than the original, was mixed with 16 kinds of the pre-compounded metal ions of 5mM with the ratio of 1:9 respectively, the intermixtures were mixed well and placed for about 30 minutes at a room temperature, then mixed again and their PL intensities were examined immediately by a fluorescence spectrophotometer.

2.8 Sensitive detection for Hg^{2+} ion.

Hg^{2+} ion aqueous solutions with a certain concentration gradient from 5mM to 0mM were compounded firstly, then mixed with the diluted C-dots aqueous solution, 5×10^{-1} times than the original with the ratio of 1:9 respectively, following the intermixtures were mixed well and placed for about 30 minutes at a room temperature, then mixed again and their PL intensities were examined immediately by a fluorescence spectrophotometer.

3. Results and discussion

3.1 Examinations of the properties of the as-prepared C-dots

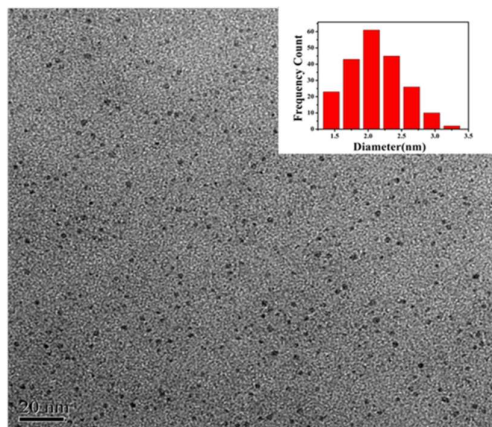


Figure 1. the TEM image of C-dots, scale bar: 10 nm (the inset was histograms of the particle size distribution of the as-prepared C-dots from 210 counts);

The morphology and size of the synthesized C-dots were investigated by TEM. Figure 1 shows TEM image of the as-prepared C-dots combined with the corresponding particle size distribution histograms (Figure 1, inset). The results indicate that the diameters of C-dots are diameter of 2.13nm. The TEM image also reveals that the C-

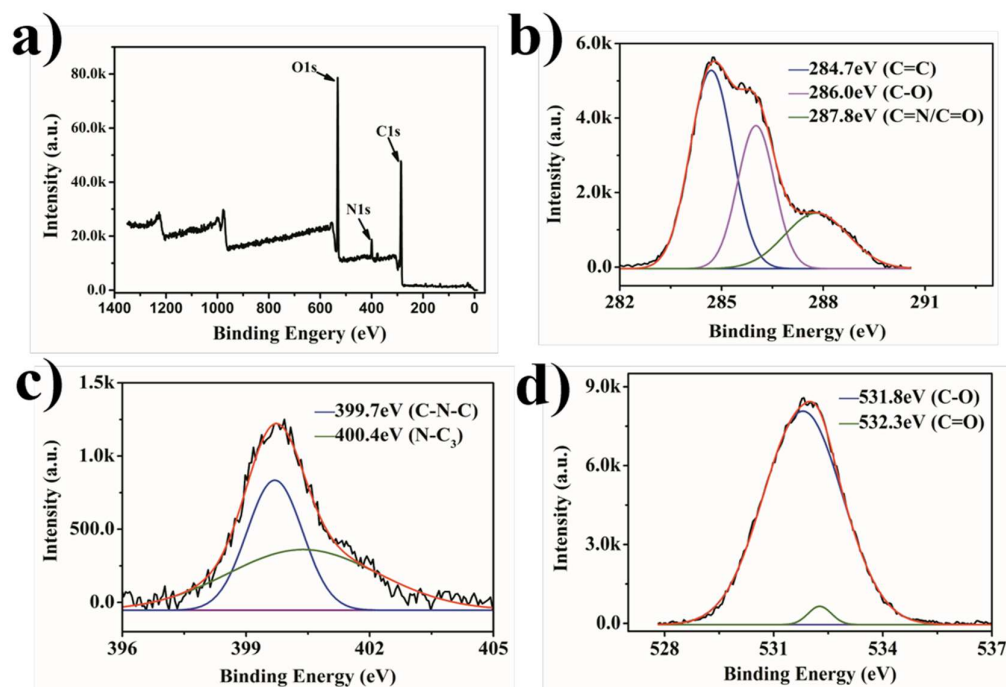


Figure 2. a) XPS survey spectra; b) C1s spectra; c) O1s spectra; d) N1s spectra

dots are almost spherical and monodispersed with a narrow size distribution[28]. C-dots were also characterized by X-ray diffraction (XRD) to describe the chemical nature of the C-dots. The XRD pattern of the C-dots (Figure S1) showed a broad amorphous peak at $2\theta=22.5^\circ$, which clearly indicates the amorphous nature of the as-prepared C-dots[29].

The X-ray photoelectron spectroscopy (XPS) analysis was performed to investigate the overall composition and the surface elemental analysis of the CDs as shown in Figure 2. The XPS survey spectra (shown in Figure 2a) reveals three peaks at 285.08eV, 399.74eV, and 531.93eV, which are attributed to C_{1s}, N_{1s} and O_{1s}, and the ratio of C, O and N in the C-dots are to be 63.06 %, 29.92 % and 5.435 % (shown in Figure 2a), respectively.[30-32] The C_{1s} spectra (Figure 2b) shows four peaks at 284.7eV, 285.9eV, 286.0eV, 287.4eV, which are attributed to C-C, C-N, C-O, and C=N/C=O bands in C-dots. The O_{1s} spectra (Figure 2c) exhibits two peaks at 531.1 and 532.1eV, which are attributed to C=O and C-OH/C-O-C bands in C-dots. The N_{1s} spectra (Figure 2d) shows two peaks at 399.3 eV and 401.6eV, which are attributed to the C-N and C=N bands in

C-dots[33, 34]. It is not difficult to discover that the as- prepared C-dots have a more C=N band on the surface than the C-dots synthesised using garlic as the direct carbon source[24]. These results indicate that the local structure of the C-dots is composed of a series of CN and CO units, which contain a large number of unsaturated chemical bonds.

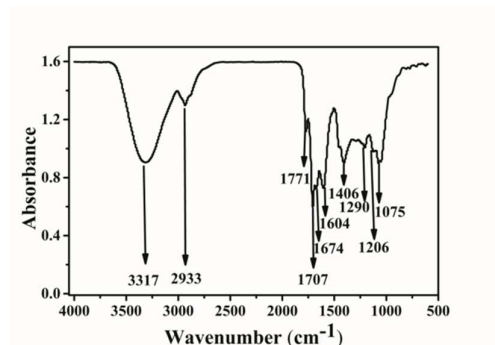


Figure 3. the FTIR spectrum of the as-prepared C-dots

To further identify the functional groups existing in C-dots, FTIR spectroscopy was studied[33]. A broad peak centering at 3317 cm^{-1} (ν -O-H) and a shoulder peak at 2933 cm^{-1} (ν -C-H) reveal the existence of hydroxyl and alkanes groups on the surfaces of C-dots (shown in Figure 3), the characteristic absorptions at 1771 cm^{-1} and 1707 cm^{-1} , demonstrate the existences of -C=O groups. The detection of $\nu_{\text{C}=\text{C}}$ vibration skeletons at about 1674 cm^{-1} indicates the existence of -C=C groups on the surfaces of the C-dots. Moreover, some peaks at 1604 cm^{-1} , 1406 cm^{-1} and 1290 cm^{-1} , reveal that the C-dots contain the groups of -N-H, -C-H, -C-O, and the peaks at about 1206 cm^{-1} and 1075 cm^{-1} suggest the existences of -C-O/-C-O-C groups. These results further confirm that the existence of -COOH, -OH, -NH₂, which is in accord with the results of XPS.

The morphology, size of the as-prepared C-dots and series functional groups on the surface of the C-dots endow the C-dots excellent optical properties and water solubility. Furthermore, the functional groups can provide accepting electron groups, including -C=N, -C=O and -COOH in charge-transfer reactions between them and electron-donating groups to realize sensing function, and the sensing property of the obtained C-dots will be in the following researches. To realize the optical properties of the as-prepared C-dots, the UV-vis absorption spectrum and photoluminescent spectra of the

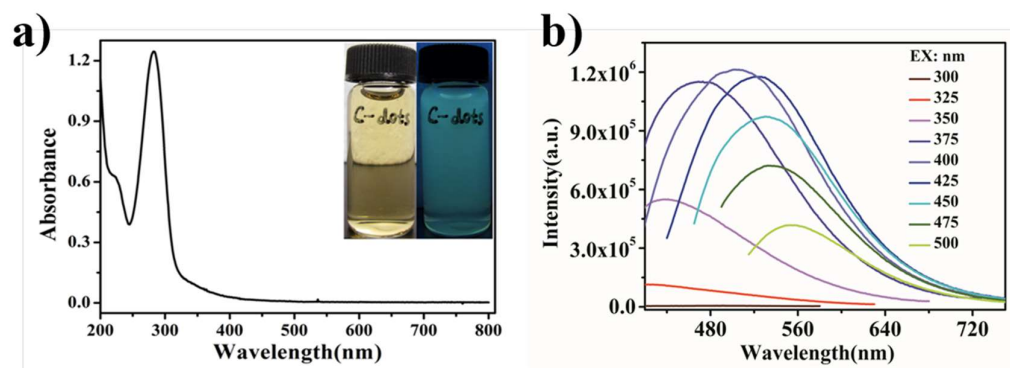


Figure 4. a) UV-visible absorption spectrum of C-dots; inset, under bright light (left) and UV light (right), b) emission spectra of the as prepared C-dots at different excitation spectra

diluted aqueous solution of C-dots were measured respectively. UV-visible absorption of the C-dots aqueous solution could be seen from Figure 4a) clearly, showing the as prepared C-dots have a weak absorption band at ~ 300 nm, which is attributed to the $n - \pi^*$ electron transition.[36-38] In addition, the diluted aqueous solution of the as-prepared C-dots can be excited at 400 nm wavelength exciting light to obtain a strongest absorption peak with a 500 nm emission wavelength (as shown in Figure 4b), which is very stable, when the C-dots aqueous solution was prepared at 37 °C from the initial reaction solution, it also can emit fluorescence while being exposed to an ultraviolet lamp unit at 365 nm, in comparison to non-fluorescent aqueous solution of C-dots under sun-light, which confirmed the formation of fluorescent production, being shown in Figure. 1a. The QY of the as-prepared C-dots was estimated to be 8.9%.[30] C-dots are considered much safer for fluorescent probes in biological application, and they are also sensitive for metal ions detections and pH responses in microenvironment of organism tissues. [39, 40] At the same time, C-dots are nanometer size fluorescent nanomaterials, they are able to pass into cells, which make them to be applied for cell imaging, and then drug loading[30, 41, 42]. At present, much effort has been put into developing green approaches to prepare fluorescent C-dots. Some raw materials have been used as carbon sources to synthesize C-dots, such as activated carbon, natural

products and so on[43, 44].

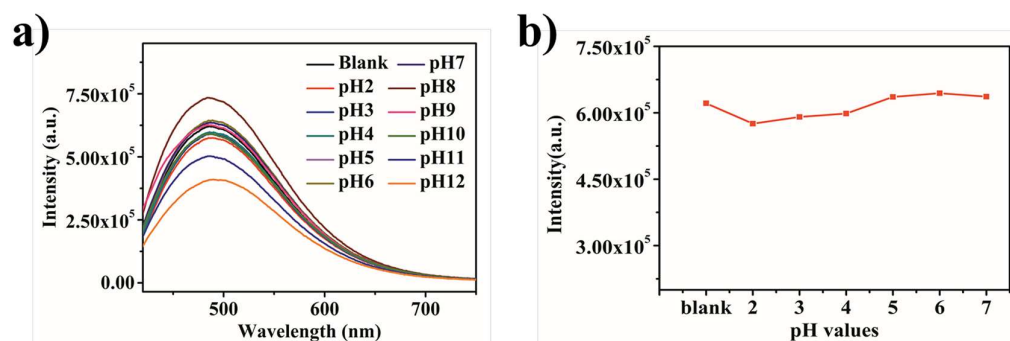


Figure 5. a) Sensitivity of the as-prepared C-dots aqueous solution toward different pH values; b) line graph of the intensity of C-dots solution quenched at different pH

Moreover, by detecting changes in fluorescence intensity caused by pH values having a range of 2-12, we can also judge the stability of C-dots in internal environment by PL intensity quenching of C-dots aqueous solution having different pH values. From Figure 5a, the strongest PL intensity is pH 8 of the C-dots solution, a significant fluorescence enhancing phenomenon, when the pH values is pH2, pH3, pH4. As being illustration, the spectrum was detected showing there are a series of function groups at the surface of the C-dots, such as -COOH, -OH, -NH₂, and the fluorescence quenching may due to electron transfer between these groups of C-dots at different pH values[33, 34, 45]. A line graph of Figure 5b shows PL intensities changing trend at pH2-pH7, the changing is slight, from which a conclusion can be obtained that the C-dots are stable in human internal environment of pH2-pH7[46].

Besides, by evaluating the cytotoxicity of the as-prepared C-dots, the cell viabilities are all $\geq 80\%$ when the concentration of the as-prepared C-dots is ≤ 1 mg/ml from Figure s2, indicating the as-prepared C-dots has a low cytotoxicity to SW480 cell in vitro, consequently this kind of C-dots are able to be used for biological organism researches, such as cell imaging and biosensing of metal ions in vitro[44, 47, 48]. Sequentially, while the SW480 cell they were exposed at a UV excitation light, and the overly of their imaging in bright-field and UV excitation light respectively was shown in Figure 6c[4, 49]. Therefore, the as-prepared C-dots having passed into cancer cells are able to emit

absinthe fluorescence stably when they were exposed at UV-light, indicating an application as a kind of nanoparticles to realize the sustainable observation of SW480 cells morphology.

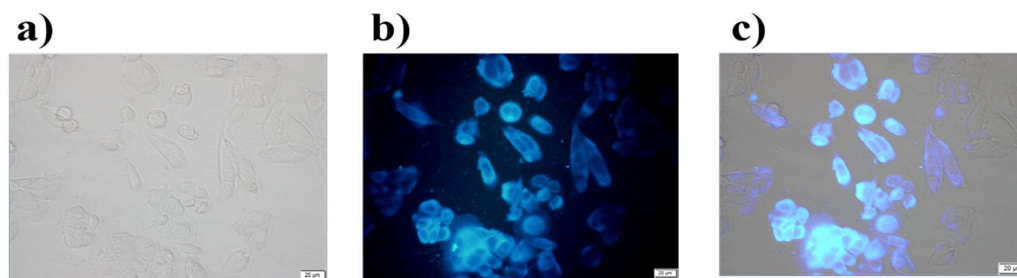


Figure 6. a) SW480 cell imaging in bright-field; b) SW480 cell PL imaging obtained with a 365nm excitation wavelength; c) the overly of a) and b). Scale bar = 20μm.

3.2 Detection as the sensing probe of the as-prepared C-dots for metal ions and Hg^{2+} ion.

By detecting changes in fluorescence intensity caused by external physical or chemical stimuli, which may be metal ions, such as Ni^{2+} , Mg^{2+} , Fe^{2+} , Ca^{2+} , Hg^{2+} , Mn^{2+} , Na^{+} , Cu^{2+} , Mn^{2+} , Co^{2+} , Cd^{2+} , a selectivity of the C-dots solution for metal ions with different charges can be obtained[28, 50].

Concretely, by the detection for 11 kinds metal ions at a concentration of 5mM respectively, PL intensity of C-dots solutions quenched by every metal ion which was used here all come to change, and as compared with other metal ions, the fluorescence of C-dots solution mixed with Hg^{2+} ion is quenched largely, which shows the most obvious quenching effect on the PL intensity from the fluorescent intensity spectra shown in Figure 7a, moreover, from Figure 7b, the value of F/F_0 about C-dots solution quenched by Hg^{2+} illustrates the changes of PL intensities between the quenched C-dots ultrapure

water solution and the blank group, besides, there is also a biggest change of Hg^{2+} among the other quenched C-dots solutions by the other metal ions[9, 12, 51]. Following, Figure 7c is the quenched fluorescence spectra of Hg^{2+} at different concentrations, it is observed that the quenched degree of C-dots solution by Hg^{2+} is

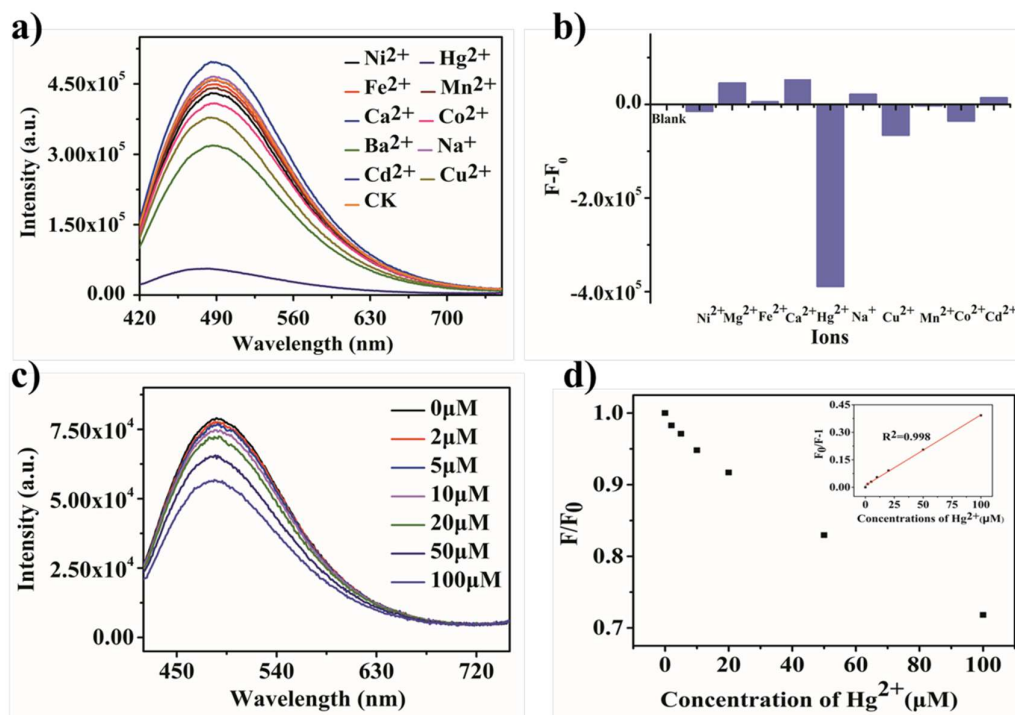


Figure 7. a) spectra of fluorescence being quenched for the addition of different metal ions; b) histogram of $F - F_0$ from different metal ions quenching C-dots solution; c) spectra of quenched fluorescence of C-dots solution by Hg^{2+} at different concentrations; d) Sensitivity of Hg^{2+} detection: F/F_0 and a fitting line for calculating the limit

becoming more and more obvious following a rising trend of the Hg^{2+} concentrations. Figure 7d showed the F/F_0 values of the as-prepared C-dots solution treated with a concentration gradient of Hg^{2+} ions, indicating the quenching degree compared with reference solution, and its detecting limit is 0.45 μM calculated by the formula of $y = 0.004x + 0.01$ from the fitting line shown in the inset graph of Figure 7d, $R^2 = 0.998$. In conclusion, water solution of the as-prepared C-dots has good selectivity and sensitivity to Hg^{2+} when it is used to detecting metal ions as a sensing probe[16, 52]

4. Conclusions

In summary, we developed a green, concise and low-cost method to synthesize photoluminescent C-dots using the garlic as the precursor by hydrothermal treatment. From the results of characterization, the as-prepared C-dots are a kind of nanoparticle

having an average diameter of 2.13 ± 0.71 nm and a series functional groups including some unsaturated groups which are able to obtain electrons, otherwise, the fluorescence of the as prepared C-dots aqueous solution can be quenched by being adjusted with different pH values, giving rise to a strongest fluorescence quenching degree of pH 12 and an enhanced fluorescence intensity of pH 8. These results illustrate the C-dots solution is stable in human internal environment with a pH range of 2 to 7. The as-prepared C-dots solution can emit absinth-green fluorescence under UV-light by good fluorescent properties. In addition, this kind of C-dots having passed into cancer cells are able to emit absinth fluorescence stably when they are exposed to UV light, then they were used for cell imaging to observe the morphology of SW480 cell sustainably and can be applied as drug carriers to load drugs for its small particle size and nontoxicity. The fluorescence can be quenched by Hg^{2+} from 5mM to 10nM with a detecting limit is $0.45 \mu\text{M}$ at a concentration range of $0 \mu\text{M}$ - $100 \mu\text{M}$, which illustrate the sensitivity and selectivity of the as-prepared C-dots for Hg^{2+} , resulting the C-dots synthesized by one pot hydrothermal treatment can be used as fluorescent probe to detecting Hg^{2+} in natural environments and human internal environments having a high level of Hg^{2+} .

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NOTES AND REFERENCES

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