ABSTRACT: In this study, blue-emission carbon dots were prepared from the legumes of the vegetable *Pisum sativum* Linn. by one-step carbonization. The fluorescence of a carbon dot (CDs) solution can be quenched by copper ions and recovered by ethylenediaminetetraacetic acid (EDTA). In addition, two kinds of visual fluorescent filter papers were prepared. Finally, a dual-mode fluorescence and visual fluorescent test paper was employed for the detection of copper ions and EDTA. The simple synthesis method and the high safety enable this material to have more application possibilities.

1. INTRODUCTION

Copper ions are one of the common metal ions in water. In the process of mineral extraction, copper ions are always accompanied by waste water that is simply discharged into natural waters, thus polluting drinking water. Copper ions due to their extremely high toxicity may not only cause acute poisoning in humans but also accumulate slowly in the human body, causing abnormalities in muscles, bones, and other organs.1

Ethylenediaminetetraacetic acid (EDTA) is widely added to food as an antioxidant, antiseptic, and so on. The excessive use or abuse of food additives may cause serious harm to the human body. In some parts of China, EDTA is widely used in some cheap or fast foods. Overuse of EDTA may produce excessive free radicals in the body, making the body prone to obesity, but they also increase the load on the kidneys and other organs.2

As a vegetable, *Pisum sativum* Linn. (*P. sativum* L.) is cultivated over a large area in Asia, of which China has the largest area. At present, only the edible seed part of *P. sativum* L. is mainly used and its legumes are generally used as feed or directly discarded. With the gradual consumption of the Earth’s resources, plant resources are being considered as precious wealth, so how to further improve the utilization rate of plant resources is one of the research hotspots.

Carbon dots (CDs) are very good fluorescent probe, which has been used in drug analysis, food quality control, toxic substance monitoring, and so on. At present, there have been some reports on the synthesis of CDs by using biomass resources.3,4

The preparation of CDs from biomass has been discussed in many reports. First, biomass materials can be widely used in green organic synthesis.5 Typical examples are white light-emitting CDs,6 blue light-emitting fluorescent CDs from waste biomass sources,7 graphene CDs derived from lignin biomass,8 and so on. The CDs made from biomass materials have been reported for detecting metal ions. Typical examples are Cr(III) and Cr(VI),9 Fe(III),10 Hg(II),11 and so on. The significance of this study is to explore the synthesis of green, safe, and environmentally friendly fluorescent materials from biomass and their potential applications.

At present, there are many reports on the detection methods of copper ions and EDTA. Some copper ion detection methods, such as using gold nanorods, gold nanocluster fluorescence, and biological recognition element pyoverdine,12–14 and some EDTA detection methods, such as using zinc powder, test strips, and Zr frameworks as nanotraps, were reported.15–17 Compared with these methods, fluorescent CDs have the advantages of a simple synthesis method, low price, and accurate detection results, according to some reports.18,19

In this study, blue-emission CDs were synthesized using *P. sativum* L. legumes and water. It was found that copper ions can only selectively quench the fluorescence of CDs. EDTA, used as a food additive, can recover the fluorescence of CDs. In addition, two kinds of visual fluorescent test papers were prepared, which
can detect copper ions and EDTA by the change of fluorescence. Based on this, a dual-mode fluorescence and visual fluorescent test paper detection was established for the analysis of copper ions and EDTA (Scheme 1).

2. RESULTS AND DISCUSSION

2.1. Characterization and Optical Properties. The synthetic materials used in this study are *P. sativum* L. legumes and water. The plant fibers of *P. sativum* L. legumes are uniform, and the mechanism of carbonization is assumed to be that the

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**Figure 1.** (a) TEM image, (b) average diameter, (c) FTIR spectrum, and (d) XRD pattern.

**Scheme 1.** Analysis Mechanism of Copper Ions and EDTA
Plant fibers are carbonized at high temperatures to produce fluorescent nanoparticles. The average particle size of the CDs was analyzed by TEM (Figure 1a). Under an electron microscope, it can be seen that the CD particles are black, spherical, and have a similar size (Figure 1b). The average diameter of the CD particles was $1.9133 \pm 0.4508$ nm.

The FTIR results are shown in Figure 1c. The peak at 3380 cm$^{-1}$ corresponds to the $\text{−OH}$ stretching vibration region; that at 2941 cm$^{-1}$ corresponds to the $\text{−CH}_2\text{−}$ stretching vibration region; the 1650 cm$^{-1}$ peak corresponds to the C=O region; the 1424 cm$^{-1}$ peak corresponds to the C−N region; and that at 1033 cm$^{-1}$ corresponds the C−C skeleton structure.$^{20,21}$

XRD was used to determine the carbon crystal structure of the CDs (Figure 1d). A single broad peak (2θ) at 20° was observed. The synthesized material has an amorphous carbon structure (002).$^{22}$

XPS is used to analyze the structural composition and elements of synthetic materials. Three peaks at 275, 405, and 535 eV correspond to C 1s, N 1s, and O 1s, respectively (Figure 2a). Figure 2b shows that C 1s has three states, which are C−N (288 eV), C−O (286 eV), and C−C (284 eV). Figure 2c presents the C−N−C structure. Figure 2d proves that the C=O group exists.$^{23,24}$

The fluorescence lifetime analysis of the CDs is shown in Figure 3. The average lifetime of CDs is found to be 24.8 ns, the decay curve has attenuation characteristics, which is consistent with the properties of typical fluorescent materials. This proves that the synthesized CDs are fluorescent materials.$^{11}$

The effect of excitation on emission in the range of 300−420 nm was studied (Figure 4a). It is found that if the excitation wavelength is 340 nm, the fluorescence intensity observed is the highest. Figure 4b shows the normalization of emission wavelengths at different excitation wavelengths. The emission wavelength appears to be equidistantly red-shifted, which shows that the material is excitation wavelength-dependent.$^{25}$ Figure 4c shows the optimum emission and excitation wavelengths are 414 and the 340 nm, respectively. For the CDs, the black line is the UV spectrum, and the transitions of $\pi \rightarrow \pi^*$ (248 nm) and $n \rightarrow \pi^*$ (300−500 nm) are confirmed.$^{26}$ The red line and the green line are the excitation (340 nm) and emission (414 nm)
spectra of the CDs, respectively. The international coordinates ($x = 0.1552, y = 0.1553$) depict that the CDs emit a blue fluorescence (Figure 4d). The quantum yield of the CDs is 18.6% when the excitation wavelength is 340 nm according to the method reported in ref 27.

2.2. Stability and Interference Experiments. The CDs stability was studied under the conditions of temperature, ionic strength, time, and pH. 25 °C is the room temperature, while the suitable temperature for human habitation is below 50 °C. Therefore, the range of 25−50 °C is chosen to measure the fluorescence intensity. The change of fluorescence is very small (Figure 5a). For the usual analytical work, different reagents are commonly added to the solution. Therefore, the influence of ion strength should be studied. The influence of ion strength was analyzed by adding different amounts of NaCl (0−100 mM), and it was found that the fluorescence intensity remained constant even at high salt concentrations (Figure 5b). For the usual analytical work, different reagents are commonly added to the solution. Therefore, the influence of ion strength should be studied. The influence of ion strength was analyzed by adding different amounts of NaCl (0−100 mM), and it was found that the fluorescence intensity remained constant even at high salt concentrations (Figure 5b). Generally speaking, the fluorescence intensity of fluorescent materials gradually decreases after being kept for a period of time, so the effect of this time was compared. It is found that the fluorescence intensity remained almost unchanged for 2 h (Figure 5c). The fluorescence attenuation of the synthesized material was found to be less than 3%, so the synthesized material has very good stability. Very strong acids and bases are rarely used for environmental analyses; therefore, the fluorescence intensities at pH 3−10 were compared. It is found that the fluorescence intensity is the highest under neutral conditions (Figure 5d). We use neutral conditions for the analysis of samples.

As shown in Figure 5, we studied the influence of temperature, ionic strength, time, and pH value. It is found that this material is very stable under neutral conditions and has very high stability to heat, ionization, and time. This is very important for the practical analytical work. First of all, the analysis needs a certain sample separation time, sample preparation time, and detection time. Second, there are some interference impurities in the detection process. This requires that the synthesized material can be stable and has strong anti-interference. Third, because different experimental regions, different seasons, and indoor temperatures may have a certain difference, good temperature stability can ensure correct detection.

The influence of common metal ions is compared. It is found that only copper ions can quench the fluorescence (Figure 6a). Anions usually coexist with cations. The effect of common anions on the fluorescence intensity is compared. The anions had little effect on the fluorescence intensity (Figure 6b). We hope to find a reagent to recover the fluorescence of CDs by

Figure 4. (a) Emission spectra; (b) normalized emission spectra; (c) UV spectrum (black line), excitation spectrum (red line), and emission spectrum (blue line); and (d) international coordinate.
screening some of the common reagents, and luckily EDTA was found to be able to recover the fluorescence of CDs (Figure 6c). As shown in Figure 6d, the fluorescence is quenched and recovered by copper ions and EDTA, respectively.

2.3. Mechanism of Selective Detection of Copper and EDTA. There are many reports that heavy metal ions can selectively quench the fluorescence of CDs, for example, in some reports on mercury ions, the quenching mechanism was considered to be surface oxidation due to energy transfer. In reports on iron ions, the mechanism is the neutralization of the negative charges of CDs by Fe ions. The quenching mechanism of Cu ions is static quenching or a combination of static and dynamic quenching.

Selective detection of EDTA is mainly due to its unique properties. EDTA as a chelate can be cross-linked with metal ions, so that the metal ions cannot work, thus realizing the recovery of fluorescence. Most organic substances are not as strongly adsorbed or cross-linked as EDTA and thus selective testing of EDTA is achieved.

2.4. Analytic Results of Copper Ions and EDTA. 2.4.1. Effect of Quenching Time. Before testing, we need to analyze the effect of time on the reaction. The fluorescence intensity decreased rapidly in the first 10 min and decreased slowly at the beginning of the next 10 min until equilibrium was reached (Figure 7a). When EDTA was added, the fluorescence intensity increased rapidly, and after 15 min, it tended to balance (Figure 7b).

2.4.2. Results of the Copper Ion Test. When copper ions were added, the fluorescence intensity decreased gradually (Figure 8). The inset of Fig. 8 shows the correlation between the quenching rate and the concentration of copper ions.

2.4.3. Results of the EDTA Test. The recovery of fluorescence by EDTA is based on the quenching of fluorescence intensity by copper ions. It was observed that EDTA gradually recovered the quenched fluorescence; when the addition of EDTA concentration was different, the recovery effect was also different (Figure 9).

2.4.4. Fluorescence Recovery Switching Experiments. First, the fluorescence was quenched by the copper ion. Second, when EDTA was added, the fluorescence was recovered. When the copper ions were added again, the fluorescence was quenched again, and the fluorescence was recovered again upon the addition of EDTA (Figure 10). It is therefore concluded that the fluorescence quenching and recovery have good repeatability.

2.4.5. Detection Mechanism of Copper Ions and EDTA. The detection mechanism is that copper ions can interact with the functional groups on the surface of the CDs to form complex compounds by coordination. The fluorescence of CDs was quenched by the change of the surface state. When EDTA was added, the chelation between copper ions and edetic acid resulted in the recovery of fluorescence.

2.5. Recovery Rate in Real Samples. The recovery of copper ions and EDTA in real samples was between 98 and 101%, and the relative standard deviation (RSD) was very small.
The limits of detection (LODs) for copper and EDTA were 0.13 and 0.07 μM, respectively (Tables 1 and 2).

2.6. Results of the Visual Fluorescent Paper Test. First, we prepared different concentrations of copper ion solutions. Then, a suitable amount of copper ion solution was added to the CD solution for quenching and then the test paper was immersed in the solution. It can be seen that under sunlight, the appearance of the test paper was the same (Figure 11a). However, the fluorescence intensity under ultraviolet (UV) light decreased with the increase of the copper ion concentration (Figure 11b).

For the detection using the EDTA test paper, under sunlight irradiation, the appearance should be consistent (Figure 12a). Under UV light irradiation, when the amount of EDTA was different, the fluorescence intensity was different (Figure 12b). Therefore, a correlation was found between the fluorescence intensity and the concentration of EDTA, and the correlation was visible to the naked eye.

3. CONCLUSIONS

Biological resources are one of the precious resources for human beings. A lot of resources are wasted in our day-to-day life. For example, in this study, P. sativum L. legumes are used; because of their poor taste, their utilization rate is very low, and they are even abandoned.

Meanwhile, environmental pollution is one of the most important problems to the human society. Due to the extensive use of food additives in recent years, some food safety problems have been appearing frequently. Assessment of the content of food additives has become a key part of food safety.

P. sativum L. is very cheap and easy to obtain in China. The synthetic raw materials of this study are only P. sativum L. and water, without any chemical reagent, and the new material formed after carbonization is very safe, which can be used for the detection of metal ions in food samples and for biological applications.

Biomass is a very broad resource, and we will continue to develop more applications for biomass.

4. MATERIALS AND METHODS

4.1. Materials. P. sativum L. was bought from the market, whose legumes were peeled by hand and mechanically crushed to a particle size of 0.85 mm. 0.5 g of P. sativum L. powder and 10 mL water were mixed and carbonized in an oven at 200 °C for 8
Figure 7. Quenching time for (a) copper ions and (b) EDTA.

Figure 8. Quenching of fluorescence intensity. Inset: correlation between the copper ion concentration and the quenching rate \((F_0 - F)/F_0\) and the concentration of EDTA.

Figure 9. Recovery of fluorescence intensity by EDTA. Inset: linear correlations between \((F - F_0)/F_0\) and the concentration of EDTA.

Figure 10. Fluorescence switching of copper ion quenching and EDTA recovery.

Table 1. Detection Results for Copper Ions

| sample       | spiked concentration (μM) | total found (μM) | recovery (%) | RSD (%) |
|--------------|---------------------------|-----------------|--------------|---------|
| river water  | 16                        | 15.81           | 98.81        | 1.02    |
|              | 24                        | 23.73           | 98.88        | 1.33    |
|              | 32                        | 31.81           | 99.41        | 0.55    |
| tap water    | 16                        | 16.10           | 100.63       | 0.76    |
|              | 24                        | 23.89           | 99.54        | 0.64    |
|              | 32                        | 31.92           | 99.75        | 0.58    |

Table 2. Detection Results for EDTA

| sample     | spiked concentration (μg mL⁻¹) | total found (μg mL⁻¹) | recovery (%) | RSD (%) |
|------------|--------------------------------|-----------------------|--------------|---------|
| bread      | 30                             | 29.82                 | 99.40        | 0.67    |
|            | 40                             | 39.59                 | 98.98        | 0.62    |
|            | 50                             | 49.48                 | 98.96        | 0.48    |
| biscuit    | 30                             | 29.61                 | 98.73        | 0.79    |
|            | 40                             | 39.56                 | 98.90        | 0.47    |
|            | 50                             | 49.61                 | 99.22        | 0.43    |
After cooling, the supernatant was purified using a water membrane (molecular weight cut-off, 1000 Da).

4.4. Detection of Copper Ions. The concentration of CDs used was 0.08 mg·mL⁻¹. In the whole process of analysis, we considered the influence of the reaction time, sample order, and other factors on the reaction results.

4.5. Detection of EDTA. The order of the experiment was the preparation of the CD solution, followed by the addition of copper ions to the CD solution, and the addition of EDTA after the completion of the reaction.

4.6. Testing of Real Samples. River water and tap water were chosen as the real samples to analyze copper ions. Bread and biscuit were chosen as real samples to analyze EDTA. The river water and tap water were filtered using a water filter paper. Bread and biscuit were ground into powder separately. Then, deionized water was added and sonicated, and the supernatant was used for analysis.

4.7. Preparation of Fluorescent Filter Paper. In this study, two kinds of fluorescent filter papers were prepared. One was a copper ion test paper and the other an EDTA test paper. First, a common filter paper was cut into a rectangle of 5 cm length and 1.5 cm width. Then, the filter paper was soaked in CD solution and dried naturally. This paper was called the copper ion detection paper. For the preparation of the EDTA test paper, the common filter paper was first immersed in the CD solution and dried naturally. Then, it was soaked in 50 μM copper ion solution and dried naturally.

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Notes
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■ ABBREVIATIONS

carbon dots, CDs; ethylenediaminetetraacetic acid, EDTA; X-ray diffraction, XRD; transmission electron microscopy, TEM; X-ray photoelectron spectroscopy, XPS; Fourier transform infrared spectroscopy, FTIR; ultraviolet, UV; relative standard deviation, RSD; limit of detection, LOD

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