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One Step Synthesis of Fluorescent Carbon Dots and its Potential Application in pH Detection, Food Additives and Free Radical Scavenging

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Lemon yellow is a kind of common food additive. Excessive use of food additives and metabolized free radicals in blood may bring potential harm to human health. In this study, we synthesized a kind of green luminescent fluorescent carbon dots by one-step method, which can effectively detect lemon yellow and this kind of material is sensitive to pH. More importantly, this material can effectively remove superoxide anion and hydroxyl radical. The synthesis method is simple and some unique functions will make this material have greater potential applications.
Keywords: lemon yellow, food additive, pH detection.

1. INTRODUCTION

Carbon dots (CDs), as a very useful nano material, have been applied in many studies, such as fluorescent ink, used for anti-counterfeiting [1] and cell imaging [2], etc.

pH directly affects the progress speed and balance of the chemical response. Being one of the important indicators for maintaining biological cells, it is of great significance to identify the pH of the solution [2]. Conventional approaches for pH detection are use of pH meter and pH test paper [3]. Although the instrumental approaches are more accurate, they are not suitable for the detection of pH in the organism and it is more difficult to monitor the pH change in real time. Fluorescence detection is a new method and do not cause damage to the organism, meanwhile the fluorescence materials are easier to enter organisms, therefore, more and more attentions have been paid to this method. As a new fluorescent material, CDs have been used to detect of pH in aqueous solutions [4].

Food additives, such as coloring reagents, flavor reagents, are widely used in the field of food processing. These additives are mostly synthetic and have a lot of potential harms to human health [5]. The existing detection methods mainly use various analytical instruments. Some analytical instruments which have been used are expensive and need to be sampled first and follow by cumbersome preparation methods [6].

Among food additives, antioxidants have been widely applied in the foods production. Some studies have reported that superoxide anions (O_2^-) and hydroxyl radicals (OH) have some influences on the oxidation and modification of foods components [7]. Excessive free radicals might directly lead to food corruption or damage to normal cells in the human body. Therefore, more and more people began to study how to remove the radicals, and some progresses have been made [8, 9].

In this study, we synthesized a kind of green luminescent fluorescent CDs by one-step method, which can effectively detect lemon yellow and this kind of material is sensitive to pH. More importantly, this material can effectively remove superoxide anion and hydroxyl radical. The simplicity of the synthesis method and the extensiveness of the use provide more possibilities for the application of this material.

2. EXPERIMENTAL PROCEDURES

The reagent used in the experiments came from the biopharmaceutical laboratory of University of Science and Technology Liaoning. Ultrapure water was used in all the experiments.

Transmission electron microscopy (TEM, Hitachi H-7700); UV-vis absorption spectra (UV-2000 UV-vis spectrophotometer, Unico China); fluorescence detection (FL-2700 fluorescence spectrometer, Hitachi Japan); X-ray diffraction (XRD) (Bruker AXS, Germany); Fourier transform infrared spectrometer (FTIR) (Mettle, Switzerland) were used for the analysis.

The synthetic method of CDs was described as below: 25 mL of water solution containing 0.2 g m-phenylenediamine, 0.2 g tris, and 1 mL phosphoric acid were mixed with 25 mL pure water, transfered to 50 mL Teflon-lined autoclave and heated for 8 h at 180 ℃. When the reaction temperature returned to room temperature, all the obtained materials were removed large impurities by centrifugation (10,000 rpm), and the supernatant was dialyzed for 72 h (molecular weight cut-off, MWCO: 500–1000 Da). Change the dialysis water every 8 hours during this period. Finally, the CDs were removed excess solvents

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by vacuum distillation at 60 ℃ and acquired solids by freeze drying [10, 11].

Phosphate buffer solution was used for all the detection work. The reaction solution was excited at 440 nm, and the fluorescence intensities at 514 nm were recorded to conduct various analyses.

Before using this material for analysis, we disperse a certain amount of CDs solid powder in the buffer solution, and add the detection substance to be measured into the solution system according to a certain amount, and then detect the substances according to the change of fluorescence intensity.

The recovery experiment of lemon yellow was pursued. Different concentrations of lemon yellow were spiked in tap water and lake water. The larger particles of real water samples were removed by centrifugation, the smaller particles were removed by using a microporous membrane (internal diameter was 0.22 μm) [12].

Antioxidant capacity analysis included of scavenging of superoxide anion and hydroxyl radical. The methods are according to the reference method [13].

3. RESULTS AND DISCUSSION

3.1. Characterization

The carbonized principle was deduced that some amino groups of m-phenylenediamine dehydrated with the hydroxyl group of Tris(Hydroxymethyl)aminomethane (Tris), the role of phosphoric acid was to dop phosphorus in the reactants, some literatures have reported that doping phosphorus would give CDs better properties [13]. The morphology of the CDs was analysed by TEM. For CDs, whose size was irregular and aggregated within a certain range (Fig. 1 a), the average diameter was measured to be 2.87 ± 0.35 nm (Fig. 1 b).

We analysed the surface groups by FTIR (Fig. 1 c). The peaks at 1389, 1509, and 1636 cm^{-1} belonged to –C–N, –N–H, and –C=O, respectively [10]. The appearance of 987 cm^{-1} and 1162 cm^{-1} assigned to vibration of P–O–C and P=O phosphate group, suggests the presence of phosphoric acid. The broad peak at 3487 cm^{-1} was –OH [14].

Inner three-dimensional structure was analysed by XRD (Fig. 1 d). A broad peak whose central position was at 27.6°, similar to 002 facets of graphitic carbons [11, 15, 16].

3.2. Optical analysis

There were two peaks at 360 nm and 440 nm in the absorption spectrum (Fig. 2 a). Fluorescence intensities were determined from 340 to 460 nm (Fig. 2 b). When the excitation wavelength was 440 nm, the fluorescence intensity could reach to maximum, the wavelength position here was 514 nm. The CIE coordinate of the CDs is (X, Y) =(0.1482, 0.1709), belonged the green fluorescence region. The quantum yields were calculated to be 22.2 % using Rhodamine 6G method [10].

The photostability of CDs was compared within 60 min, it indicating that CDs exhibited optical stability (Fig. 3 a). The differences of fluorescence intensities were compared by adding different amounts of NaCl from 0 to 400 mM (Fig. 3 b), the result depicted that the detection would not be disturbed by ionic strength. Meanwhile, the CDs exhibited good thermal stability (Fig. 3 c).

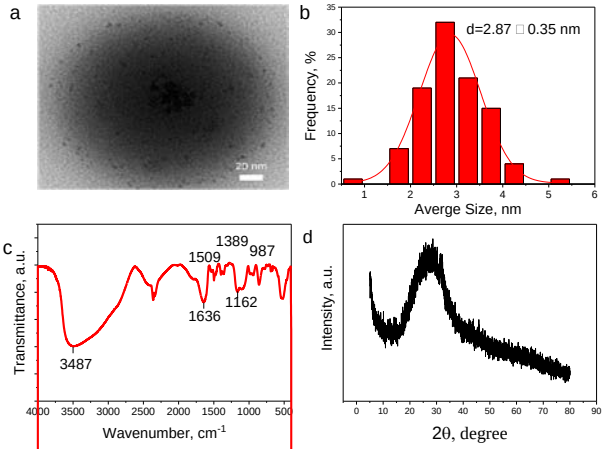

Fig. 1. a – TEM image; b – averge diameter; c – FTIR; d – XRD

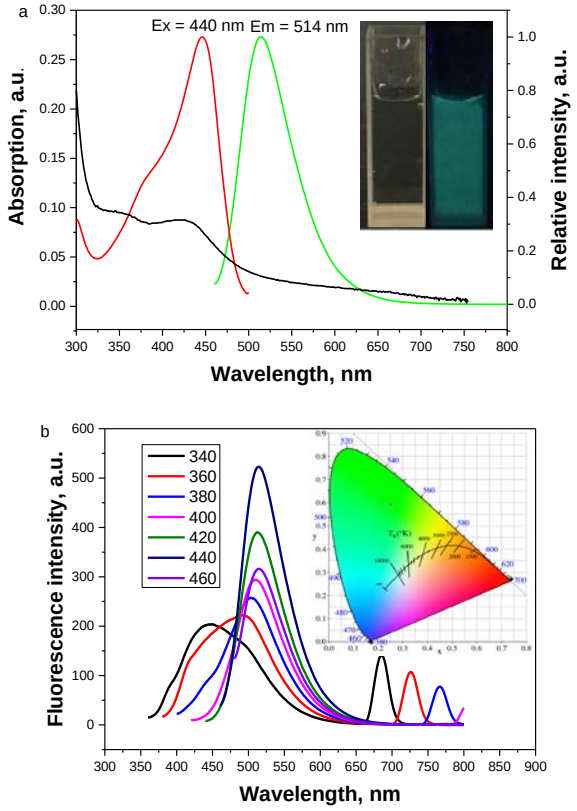

Fig. 2. a – UV-Vis spectrum, excitation and emission spectra, inset: the CDs solution and its fluorescence;
b – fluorescence spectra when the excitation wavelength is changed, inset: the CIE coordinate

The selectivity was evaluated. For example, metal ions (Fig. 4 a), included of 18 common metal ions, 9 kinds of anions (Fig. 4 b), and some interferences (Fig. 4 c), included of Galactose, Uric acid, Vitamin C, Valine, Thiourea, Dopamine, Maltose, Lysine, Phenylalanine, Malic acid, Threonine, Alanine, Serine, Imidazole, Tyrosine, Glutamate, Glutathione, Glucose, Glycine, Aspartic acid, Proline, Xylose. The above results showed that no matter metal ions, anions, or interferences, had little effect on the CDs.

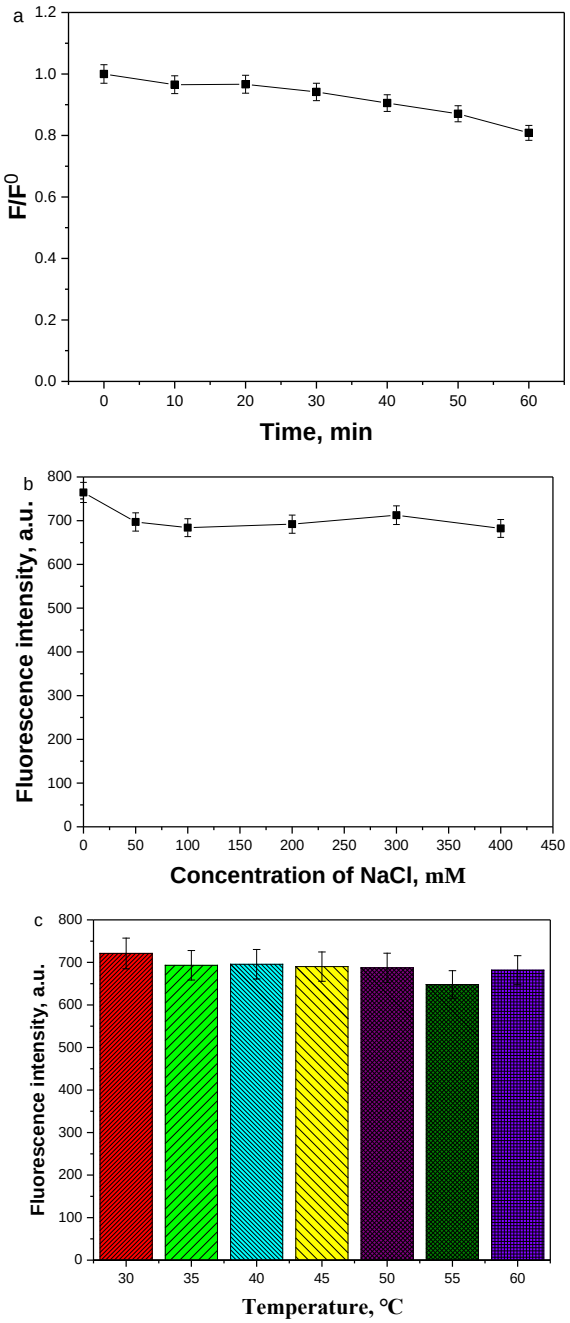

Fig. 3. a – the photostability of the CDs; b – the influence of ionic strength; c – the influence of temperature

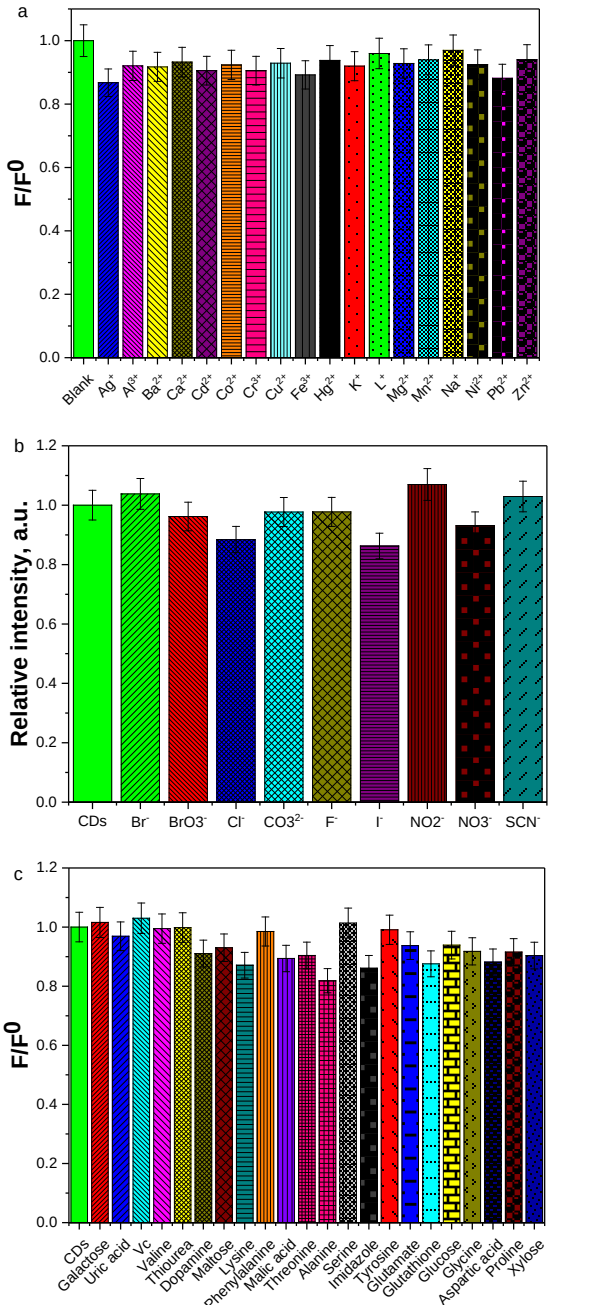

Fig. 4. The selectivity in the presence of:a – metal ions; b – anion; c – other interferences. (F^0 is defined as the fluorescence intensity of CDs, F is defined as the fluorescence intensity of different interference)

3.3. Sensing of pH in the actual water samples

The effect of pH on the CDs is depicted in Fig. 5. With the increase of pH value, the fluorescence intensity decreased linearly (the scope is 3 to 10). According to the sensitivity of CDs to pH, we used CDs to detect pH in water samples (Table 1).

Table. 1. Detection of pH value in real samples

Sample	pH value by pH meter	pH value by CDs	RSD, %[a]
Tap water	8.06	7.82 ± 0.19	3.02
Lake water	7.56	7.23 ± 0.24	4.46
Mineral water	7.73	7.44 ± 0.13	3.82

[a] Relative standard deviation (RSD) was defined as (standard deviation/mean) × 100%.

We chose tap water, lake water and mineral water for testing, using the pH meter for reference. By experiments, we measured the pH values of three kinds of water samples were 8.06, 7.56, and 7.73, respectively. By comparing with the pH meter, the relative standard deviations (RSD) of three water samples were less than 5 %. Similar results can be obtained when the pH fluorescence test paper was used to measure the pH of three water samples, so we initially believe that the synthesized CDs can be used to evaluate the pH in water.

In this study, the CDs which we synthesized are sensitive to pH, we made it be a fluorescent quantitative paper (Fig. 6). It was seen that when the pH value was increased from 3 to 7, the color of the paper was changed

from green to yellow and the fluorescence intensity was decreased gradually. It can be concluded that the pH value of the solution can be identified by the color and fluorescence intensity of the test paper.

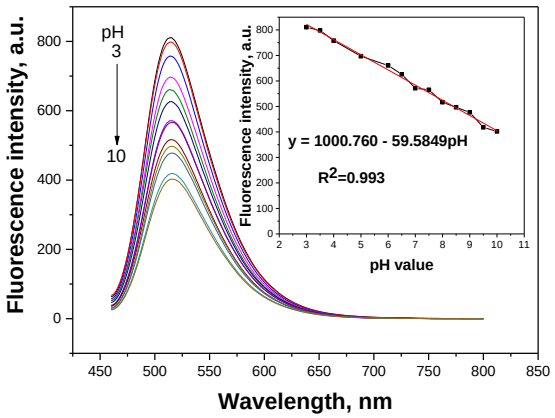

Fig. 5. Relationship between pH and fluorescence intensity of solution

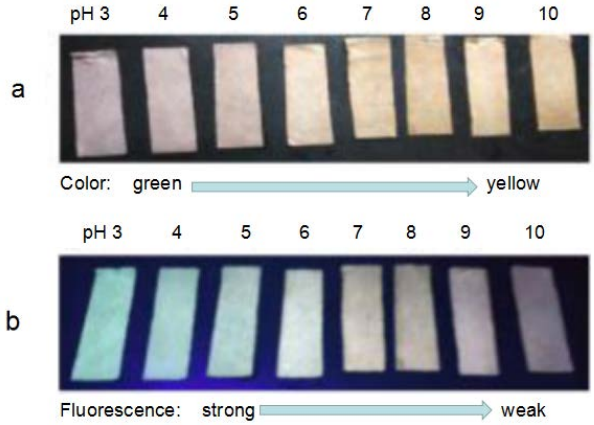

Fig. 6. pH fluorescent test paper: a – differences in color when pH changes; b – differences in fluorescence when pH changes

3.4. Lemon yellow sensor based on the CDs

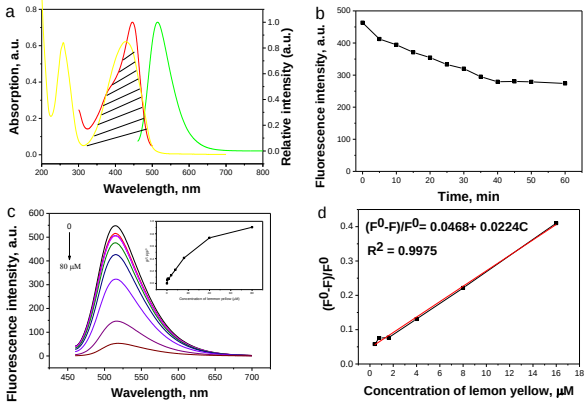

Fig. 7. a – the fluorescence spectra of lemon yellow excitation and emission spectra; b – the quenching time of lemon yellow; c – quenching of fluorescence intensity by lemon yellow; inset: quenching rate with the addition of lemon yellow; d – linear relationship between quenching rate and lemon yellow

It was seen that there were two peaks (260 nm, 430 nm) (Figure 7a). The excitation wavelength (440 nm) was coincidence with UV-vis band (430 nm) of lemon yellow. When lemon yellow was spiked into the CDs solution, the excitation fluorescence was shielded by the UV-vis band of the lemon yellow, which caused the inner filter effect (IFE) occurred. Fig. 7 b depicts the quenching time of lemon yellow, it is seen that quenching reaction needs a shorter time.

The effects of different concentrations (0 to 80 μM) of lemon yellow on CDs were shown in Fig. 7 c. It was seen that CDs were gradually quenched with the increase of lemon yellow. Within the scope of 0 to 16 μM, With the increase of lemon yellow concentration, the quenching rate increases gradually (Fig. 7 d), where F^0 is defined as the fluorescence intensity in the initial state, F is defined as the fluorescence intensity at different lemon yellow concentrations, and C is the concentration of lemon yellow, respectively. The limit of detection (LOD) was calculated to be 0.13 μM. The determination of lemon yellow in real water samples is shown in Table 2. We choose tap water and lake water as the test objects. It is proved that lemon yellow can be detected by fluorescence of the CDs.

Table. 2. Detection of lemon yellow value in real samples

Sample	Spiked concentration, μM	Total found, μM	Recovery, % N = 3	RSD, % N = 3
Tap water	3.8	3.671	96.61	0.66
	8.0	8.007	100.09	0.89
	10	9.990	99.90	0.61
Lake water	3.8	3.769	99.18	0.61
	8.0	8.069	100.86	0.10
	10	10.626	106.26	4.09

3.5. Evaluation of the antioxidant of the CDs

3.5.1. Scavenging of superoxide anions

When the CDs solution was added, the scavenging rate of superoxide anions was increased, finally, the scavenging rate of CDs on superoxide anions was close to equilibrium (Fig. 8).

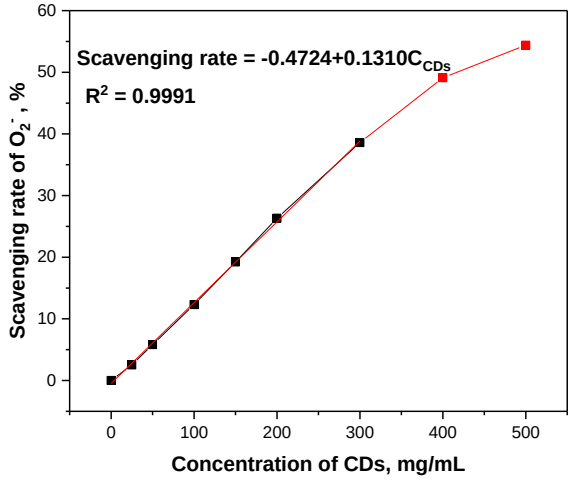

Fig. 8. Scavenging rate of superoxide anions (O_2^-) under different concentrations of CDs

For the CDs, within the scope of 0 to 300 mg/mL, a linear equation was fitted to:

Scavenging rate = $-0.4724 + 0.1310C_{CDs}$ ($R^2 = 0.9991$) (1)

3.5.2. Scavenging of hydroxyl radical

The CDs solution has some scavenging effects on hydroxyl radicals (Fig. 9). Within the scope of 0 to 14 mg/mL, a linear equation was fitted to:

Scavenging rate = 4.6714 + 4.4325C_{CDs}, (R^2 = 0.9924) (2)

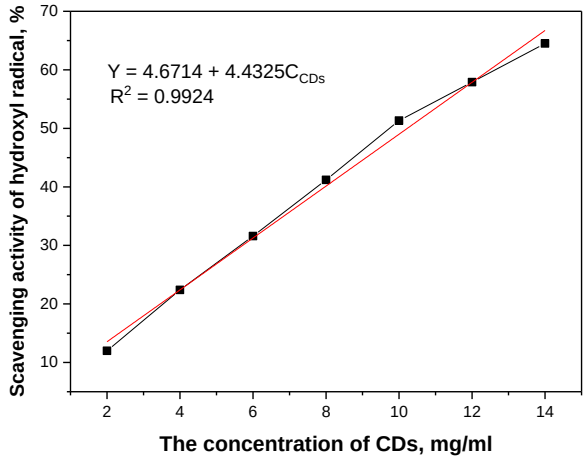

Fig. 9. Scavenging rate of hydroxyl radical (·OH) under different concentrations of CDs

3.6. Mechanism analysis for antioxidant

The mechanism of free radical detection is depicted in Fig. 10. The mechanism for scavenging of superoxide anions was deduced that the superoxide anion was very oxidizing, but when the CDs were added into the superoxide anions solution, the superoxide anions would passivate the functional groups of CDs. The functional groups play a big role in the generation of fluorescence, because the surface functional groups were oxidized, so the fluorescence of CDs was quenched [10]. The mechanism for scavenging of hydroxyl radical was deduced that green-emission CDs used the phosphoric acid as a raw material, phosphoric acid contains a lot of protons, they are easily absorbed the surface of the CDs. When these protons encountered hydroxyl radicals, these protons could react with the hydroxyl radicals to produce water [7]. Due to the depletion of protons, the surface of the the CDs was modified, so the fluorescences were quenched.

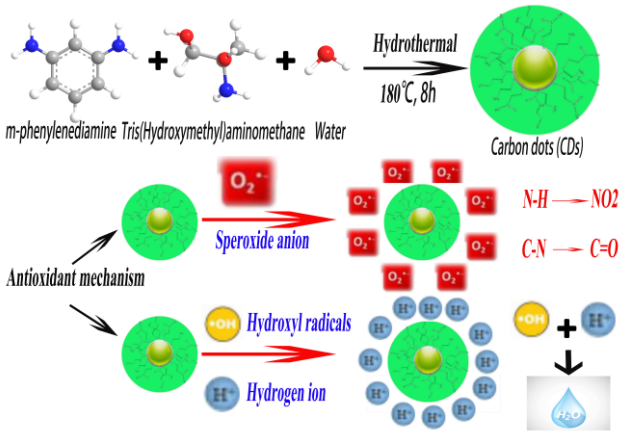

Fig. 10. Synthetic mechanism and application of the CDs

4. CONCLUSIONS

So far, the method for rapid determination of pH in water is often used the test paper or instrument. Compared with conventional filter paper, our fluorescent filter paper has higher accuracy, just use a simple ultraviolet lamp to preliminarily measure the pH value of water. In the future, this material might even enter into the blood of animals or people for the determination of pH value in the blood. What's rare is that our synthetic materials can remove free radicals, we hope that this kind of material can not only detect the pH value of blood, but also can scavenge the excessive free radicals in blood, and play some roles in blood purification. This kind of material is sensitive to pH, can effectively remove superoxide anion and hydroxyl radical. Under certain conditions, this material can detect lemon yellow in foods, its versatility makes it have more potential applications.

So far, all we have done is laboratory research, how to further expand production, how to put this material into practical use, and more research is needed, and we will continue to do that, and hopefully by our research, more suggestions are provided for the application of fluorescent carbon quantum dots.

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