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Detection of protamine in aqueous solution based on CDs and fluorescent technique

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Abstract. In this paper, one kind of protamine sensor based on the fluorescence quenching of CDs synthesized from bagasse was fabricated. The detection conditions were optimized. The concentration of protamine in the range of 0.6-4.2 mg L^{-1} exhibited a linear relationship, and the limit of detection (LOD) for protamine was as low as 0.094 mg L^{-1}. The excellent performance of the proposed probe shows that this method is sensitive, facile, rapid, low cost and eco-friendly. This study has demonstrated food waste can be converted into valuable optical sensor.

1. Introduction

Protamine is a kind of basic protein extracted from fresh fish mature sperm.[1] It is used for excessive bleeding or spontaneous hemorrhage caused by injection heparin, especially for the postoperative patient, and no other alternative.[2] In addition, protamine in neutral or alkaline medium shows strong antibacterial ability, and higher thermal stability.[3] Therefore, protamine is widely used in food preservation. The dosage of the protamine must be controlled within a suitable rang due to adverse effect such as hypotension and idiosyncratic fatal cardiac arresting at high concentration.[4] So, it is of great importance to the realization of rapid, sensitive and selective detection of protamine in food and pharmaceutical products. Reverse phase-high performance liquid chromatography (RP-HPLC),[5] gold nanoparticles[6], and fluorescence[7] have been used to quantify the levels of protamine. However, these methods require long operation time, high cost, sophisticated instrumentation or multi-step sample preparation.

Fluorometry offered a simple, low cost analysis method widely used in biomedical, environmental science, and so on. Carbon dots (CDs) is a new type of fluorescent nanomaterials having similar optical properties with quantum dots.[8] Because of its excellent optical performance, small sizes, low cytotoxicity and biocompatibility, it has been applied in a variety of areas including biological image, biochemical analysis, energy efficient displays and lighting.[9-10] Thus far, several optical sensors based on CDs have been developed in sensing picric acid, immunoglobulin G, dopamine, and so on.[11-13] The fluorescence probe based on CDs for protamine-sensing has not been reported. In this study, a detection method for protamine has been successfully established based on direct interaction between CDs and protamine with fluorescence quenching.

2. Experimental section

2.1. Materials and Instrumentation

Bagasse was purchased from the market. All reagents were purchased from Sinopharm Chemical Reagent Co., Ltd.All reagents used are of analytical grade. Fluorescence property of CDs was performed with F-4500 fluorescence spectrophotometer (Hitachi Ltd., Japan) and the UV-Vis absorption spectra were measured by a UV-5500 spectrometer (SHYX instrument co., Ltd., China).

2.2. Synthesis of CDs

Appropriate mass of pre-dried bagasse was put into a crucible ,then transferred it into a muffle furnace. The sample was heated to 350 ℃ keeping 80 min. After the product cooled down to room temperature, 1 g of ash was put into a flask and dispersed with 100 mL of double distilled water. The mixture was then homogenized under sonication for 1 h, a brownish solution was obtained by filtration with a 0.22 μm filter membrane to remove the water insoluble impurities, then centrifuged to remove large or agglomerated particles. The supernatant was dialyzed against for 3 days.

2.3. Fluorescence measurements

Firstly, 3 mL of B-R buffer solution (pH 7.0), 2 mL of CDs solution (0.02 g L^{-1}) and different volumes of protamine solution (0.6 g L^{-1}) were successively pipetted into a 10 mL of colorimetric tube. Subsequently, the doubly distilled water was added with a nalvolume of 10 mL. After reacting at 30 ℃ for 20 min, the mixtures were subjected to fluorescence measurements at λ ex 320 nm.

3. Results and discussion

3.1. Optimizing detection conditions

The fluorescence of CDs decreases apparently in the presence of protamine (Fig.1A). The fluorescence intensity of CDs in B-R buffer solutions was stronger than in Tris buffer solutions. The effect of pH, time and temperature on the fluorescence intensity of the CDs was shown respectively in Fig.2B, Fig. 2C, and Fig. 2D ($\Delta F = F_0 - F$, F_0 and F are the fluorescence intensities recorded in absence and presence of protamine, respectively). The optimum conditions were decided as pH=7.0, 20 min, and 30℃.

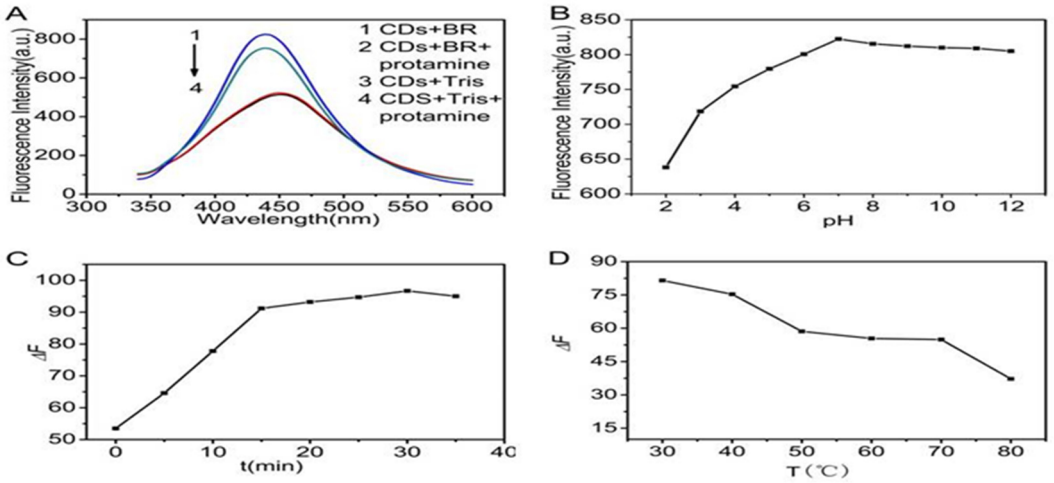

Fig. 1 Effect of different buffer solutions (A), pH (B), time (C) and temperature (D) on fluorescence

3.2. Interference of metal ions

The effect of different metal ions at the concentration of 10 mM respectively(Fig. 2). Cu (Ⅱ) and Al (Ⅲ) were found to be moderate interferences, Ca (Ⅱ) was very poor quenchers for CDs in aqueous solution. The interference effect of Na (Ⅰ), K (Ⅰ) , Zn (Ⅱ), Mg (Ⅱ) on the fluorescence of CDs

were found to be insignificant. Whereas Fe (Ⅱ) was found to be serious effect on the fluorescence of CDs. Fe (Ⅱ) should be avoided when protamine was detected by CDs.

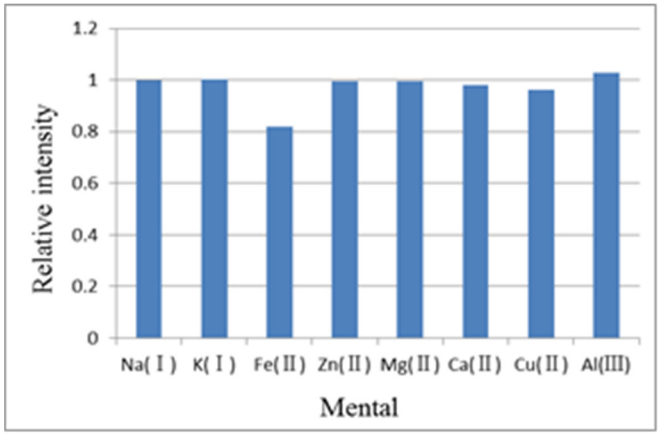

Fig. 2 The effect of different metal ions

3.3 Detection of protamine

Under the optimum experimental conditions established, calibration plot was generated for the detection of protamine (Fig. 3). There is a good linear correlation (R^2=0.9955) between ΔF and the concentration of protamine over the range of 0.6 - 4.2 mg L^{-1} with the following ΔF = 33.10 C – 12.27. LOD of protamine was calculated to be 0.094 mg L^{-1}. The calculation was based on 3σ/s , where σ is the standard deviation of the corrected blank signals of the CDs (n=11) and s is the slope of the calibration curve.

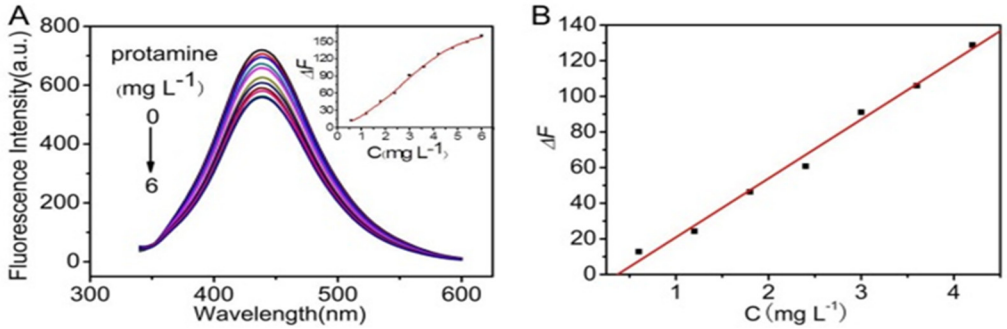

Fig.3 (A) Fluorescence spectra ,(B) A linear region in the range of 0.6 mg L^{-1} to 4.2 mg L^{-1}.

Under the optimum experimental conditions established, the recoveries and relative standard deviation were determined by standard addition method, the results are shown in Table 1. The sample recoveries are in 96.6% -104.0% and the relative standard deviation is less than 3.9%.

Table 1 Analytical results and recovery tests of protamine

Sample (mg L^{-1})	Added (mg L^{-1})	Total found (mg L^{-1})	Recovery (%)	RSD(%)
2.4	0.6	2.89	96.6	3.9
3.0	0.6	3.66	101.7	3.7
3.6	0.6	4.37	104.0	3.6

4. Conclusion

The CDs synthesized from bagasse was employed as a sensor for the detection of protamine, which was based on the fluorescence quenching of CDs in the presence of protamine. The detection method exhibits some advantages, including eco-friendliness, sensitivity, and a low degree of sample matrix interference. As a result, a new model of fluorescence sensor for protamine detection has been constructed.

Acknowledgments

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