Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 190 (2018) 360–367

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

journal homepage: www.elsevier.com/locate/saa

Green synthesis of carbon dots from pork and application as nanosensors for uric acid detection

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ARTICLE INFO

Article history:
Received 7 July 2017
Received in revised form 9 September 2017
Accepted 13 September 2017
Available online 15 September 2017

Keywords:
Carbon dots
Uric acid
Pork
Fluorescence quenching

ABSTRACT

In this work, a green, simple, economical method was developed in the synthesis of fluorescent carbon dots using pork as carbon source. The as-prepared carbon dots exhibit exceptional advantages including high fluorescent quantum yield (17.3%) and satisfactory chemical stability. The fluorescence of carbon dots based nanosensor can be selectively and efficiently quenched by uric acid. This phenomenon was used to develop a fluorescent method for facile detection of uric acid within a linear range of 0.1–100 μM and 100–500 μM, with a detection limit of 0.05 μM (S/N = 3). Finally, the proposed method was successfully applied in the determination of uric acid in human serum and urine samples with satisfactory recoveries, which suggested that the new nanosensors have great prospect toward the detection of uric acid in human fluids.

1. Introduction

Uric acid (UA; 2,6,8-trihydroxypurine) is the main product of purine metabolism in human bodies, which mainly exists in urine and serum [1,2]. Normal level of UA is important for body health in urine and serum range from 2.49 to 4.46 mM and 0.13 to 0.46 mM, respectively [3]. Some diseases would be caused when the level of UA is higher or lower than normal level, such as gout, kidney disease, high blood pressure, high blood lipids, atherosclerosis, Parkinson disease, Alzheimer disease and other diseases [4–6]. Therefore, it is highly necessary to monitor the level of UA in body fluids. In recent years, many methods of UA detection have been developed, including electrochemical method [7,8], enzymatic method [9], high performance liquid chromatography [10,11], chemiluminescence [12], and so on. However, these methods exist some disadvantages, such as complicated operation, time consuming and expensive cost. Fluorescence spectrophotometry has rapidly developed because it avoids the disadvantages above, and it attracts more and more attention.

In recent years, nanomaterials including carbon nanomaterials, semiconductor nanomaterials, polymeric nanomaterials, metal nanomaterials have captured intensive attention and were applied in many areas, because of excellent surface effect, small size effect and macroscopic quantum tunneling effect [13–16]. Carbon dots (CDs) is a new member of carbon nanomaterial family with a size of less than 10 nm [17]. In addition to the common characteristics of nanomaterials, carbon dots also showed low cytotoxicity, excellent photostability, high biocompatibility, easy functionalization [18–20]. Therefore, CDs were widely applied in fluorescence sensors, cell imaging, metal detection, organophosphate pesticides detection [21–25] and so on. To date, many methods such as arc discharge [26], microwave digestion [27], ultrasonic oscillation [28], electrochemical method [29], hydrothermal synthesis [30] have been reported to synthesize CDs. In contrast to other methods, the hydrothermal synthesis is the most widely adopted for its simple operation, mild reaction conditions and high quantum yield. Recently, hydrothermal carbonization of aloe, chocolate, bamboo leaves, and rose-heart radish has been successfully applied to synthesize fluorescent CDs, which could be probes for tartrazine, lead ions, copper ion, iron(III) ion and cell imaging [31–34]. All of these showed that taking natural substances as carbon source for simple, economical and green synthesis of CDs is becoming one of the tendencies of CDs research.

In this work, we developed a simple, low-cost and green method for synthesis of CDs from pork. Since pork is a complex that contains a number of organic and biomolecules including fat, proteins, vitamin B, vitamin C, vitamin E, carbohydrates, cholesterol and minerals, which can be useful for doping of multiple heteroatoms in the CDs without addition of any additives. In the experiment, we found that UA could quench the fluorescence of CDs, and the quenching degree was related to the concentration of UA. To our knowledge, there is few studies used CDs

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https://doi.org/10.1016/j.saa.2017.09.037
1386-1425/

from pork for detection of UA, which provides a new method for sensitive detection of UA. In addition, this method has been successfully applied to detect UA in serum and urine samples.

2. Experimental

2.1. Materials and Apparatus

Uric acid (UA) that was shown in Fig. 1, dopamine hydrochloride (DA) and urea were all purchased from Aladdin Chemistry (Shanghai, China). Oxalic acid (OA), ascorbic acid (AA), citric acid (CA) were obtained from Tianjin Zhiyuan Chemical Reagent Co., Ltd. (Tianjin, China). L-Alanine (L-Ala), L-Tyrosine (L-Tyr), DL-Leucine (DL-Leu), DL-Aspartic Acid (DL-Asp), L-Cystine (L-Cys), L-Glutamic acid (L-Glu) and L-Tryptophane (L-Try) were provided by Xinxing Chemical Reagents Co., Ltd. (Shanghai, China). NaCl, KCl, Na_3PO_4, Na_2HPO_4 were purchased from Tianjin Fengchuan Chemical Reagent Co., Ltd. (Tianjin, China). Stock solution of 1.0 mM uric acid was prepared by dissolving in 0.01 M of NaOH solution and diluted to the scale line, then stored below 4 °C. Human serum samples were kindly provided by Yanan Hospital (Kunming, China). Urine samples were provided by laboratory members. All chemicals and solvents were used without further purification. Ultra-pure water was used in each experiment.

The fluorescence spectra were obtained using a G9800A Cary Eclipse fluorescence spectrophotometer (Agilent Technologies, USA). Transmission electron microscopy (TEM) images were obtained on a FEI Tecnai G2 F30 transmission electron microscope (FEI, USA). X-ray photoelectron spectroscopy (XPS) analyses were performed on an X-ray photoelectron spectrometer (Kratos, UK). Fourier transform infrared spectra (FTIR) were recorded on a TENSOR27 FTIR spectrometer (Bruker, Germany). Absorbance measurements were performed on a UV-2550 UV–vis spectrophotometer (Shimadzu, Japan). A D8-advance X-ray diffractometer (XRD) (Bruker, Germany), a PHSJ-4A pH meter (Shanghai Instrument Electric Scientific Instrument Co., Ltd., Shanghai, China) and a vortex mixer (Hanuo Instrument Co., Ltd., XH-B, Shanghai, China) were used in the experiment.

2.2. Synthesis of CDs

CDs were prepared by a hydrothermal method using pork as carbon source. 20 g of pork was crushed and dispersed in 150 mL of deionized water, then the mixture was transferred to a Teflon-lined autoclave (200 mL) and heated at 200 °C for 10 h. After cooling down to room temperature naturally, the suspension was centrifuged at 13000 rpm for 20 min, yellow solution was obtained after removing the insoluble substances. In order to get pure CDs, the solution was filtered with a 0.22 μm membrane. Finally, the obtained CDs were stored at 4 °C for further use.

The CDs could be synthesized using pork as carbon source by hydrothermal synthesis method. Compared with other synthetic methods, the hydrothermal synthesis method was simple and convenient. Furthermore, pork was used as the raw material rather than organic compounds during the synthesis process, so this method was more eco-friendly.

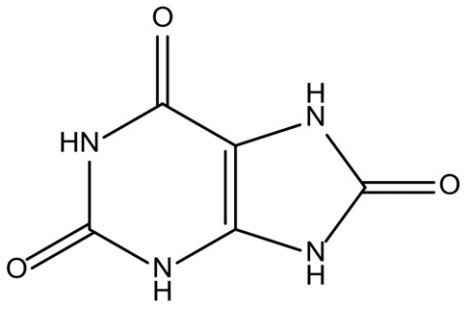

Fig. 1. The molecular structures of UA.

2.3. Measurement of Fluorescence Quantum Yield

The quantum yield (QY) of the as-synthesized CDs was measured on the basis of a procedure described previously [35]. Briefly, a solution of quinine sulfate in 0.1 M H_2SO_4 (QY of 54% at 360 nm, $\eta = 1.33$) was used as a standard. The value of the quantum yield was calculated according to the following equation:

$$Q = Q_R(I_S/I_R)(A_R/A_S)\left(\eta_S^2/\eta_R^2\right)$$

where Q is QY, the subscript 'S' refers to the samples, the subscript 'R' refers to quinine sulfate, A is the absorbance at the excitation wavelength, I is the integrated emission intensity and η is the solvent refraction index.

2.4. Assay Procedures

Typically, 20 μL of CDs solution was diluted to 2 mL in 10 mL centrifuge tubes, then 2 mL of different concentration of UA solution or samples were added into centrifuge tubes, vortex mixed for 5 s and adjusted the pH to 8 with Na_2HPO_4-citric acid. After reaction for 3 min, the fluorescence intensity was recorded at an excitation wavelength of 310 nm and an emission wavelength of 412 nm. The slits for both the excitation and the emission were set to 5 nm.

2.5. Biological Sample Pretreatment

The human serum samples were obtained from Yanan Hospital of Kunming and then diluted 10-fold before experiment. The urine samples were obtained from laboratory members, then decolored with a small amount of activated carbon and centrifuged to remove most of impurities, and diluted 100-fold before experiment.

3. Results and Discussion

3.1. Characterization of CDs

The morphology and microstructure identified by the typical TEM image. As shown in Fig. 2A1, the CDs are nearly spherical and well separated from each other with an average diameter of about 3.5 nm. In addition, Fig. 2A2 showed that the lattice spacing is ca. 0.23 nm, corresponded to that of graphitic carbon, representing the graphitic of the CDs. As shown in Fig. 2B, X-ray diffraction (XRD) pattern of the CDs shows a broad peak at around $2\theta = 23.5°$, it corresponds to the graphitic structure [36].

The surface functional groups of CDs were identified by FTIR spectra and X-ray photoelectron spectroscopy (XPS). As shown in Fig. 2C, the band within the range of 3450–2950 cm^{-1} is attributed to —OH and N—H stretching vibrations. The peaks at 1672 cm^{-1}, 1581 cm^{-1}, 1410 cm^{-1} and 1105 cm^{-1} are assigned to stretching vibrations of C═O, C═C, C—N and C—O, respectively. The full scan XPS spectrum of CDs is shown in Fig. 2D excluded 3 peaks at 287.3, 399.9 and 532.7 eV, which separately correspond to C1s, N1s and O1s. Fig. 2E shows the C1s spectrum of CDs, the four peaks at 284.4 eV, 285.6 eV, 286.5 eV and 288.0 eV could be attributed to C═C, C—N, C—OH and C═O. Fig. 2F shows the N1s spectrum of CDs, the two main peaks at 399.7 eV and 400.9 eV could be linked to N—C—N and N—H. Fig. 2G shows the O1s spectrum of CDs, the two main peaks at 531.2 eV and 532.3 eV could be assigned to C═O and C—OH/C—O—C. The XPS results agreed with FTIR analysis. Therefore, hydrophilic groups such as —COOH, —NH_2, and —OH should exist on the surface of the CDs.

Fig. 3A shows the UV–vis absorption and fluorescence spectra of the CDs, the as-prepared CDs exhibited a small peak at around 235 nm, and