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Ratiometric fluorescent detection of copper ions using coumarin-functionalized carbon dots based on FRET

Fanyong Yan[a,*], Zhangjun Bai[a], Yuan Chen[a], Fanlin Zu[a], Xing Li[a], Jinxia Xu[b,*], Liang Chen[c]

[a] *State Key Laboratory of Separation Membranes and Membrane Processes, School of Environmental and Chemical Engineering, Tianjin Polytechnic University, Tianjin 300387, China*
[b] *Tianjin Engineering Center for Safety Evaluation of Water Quality & Safeguards Technology, Tianjin Polytechnic University, Tianjin, 300387, China*
[c] *Graduate School of Life Science, Hokkaido University, Sapporo, 0010024, Japan*

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ABSTRACT

A novel FRET ratiometric fluorescent probe (CMH-GA-CDs) was designed for the detection of Cu^{2+} in aqueous solution. The CMH-GA-CDs were prepared through grafting 7-diethylaminocoumarin-3-carbohydrazide (HCM) on the surface of glyoxylic acid-modified carbon dots (GA-CDs) by amidation reaction. In the absence of Cu^{2+}, a FRET pair from the CDs to CMH units was established, in which the CMH-GA-CDs exhibited dual emission bands centered at 400 nm (CDs) and 458 nm(CMH), respectively, under optimal excitation wavelength of CDs (λ_{ex} = 340 nm). Upon addition of 30 μM Cu^{2+} to CMH-GA-CDs solution, Cu^{2+} coordinated with the heteroatoms N and O, which caused the inhibition of FRET process and the change of fluorescence intensity ratio I_{458}/I_{400} from 1.95 to 0.91. The ratio of I_{458}/I_{400} exhibited a good linear relationship with the concentration of Cu^{2+} in the range of 0–10 μM and the limit of detection was as low as 0.21 μM. Furthermore, The CMH-GA-CDs were used for the detection of Cu^{2+} in tap water and lake water with satisfactory recovery ratio between 91% and 104%, demonstrating the promising applicability for detecting Cu^{2+} in water samples.

1. Introduction

Copper ions (Cu^{2+}), an essential element for living organisms, plays a critical role in physiological and pathological events, such as Alzheimer's disease and Wilson's disease [1]. Excess copper intake in living organisms can induce damage to the liver, kidney and central nervous system [2,3]. In addition, for the widespread use of copper in industry, the heavy metal contamination caused by Cu^{2+} has become an urgent environmental problem to be solved [4,5]. So, it is essential that the development of new methods for determining Cu^{2+} in biological samples and environmental water samples. The conventional methods for monitoring Cu^{2+} include atomic absorption spectrometry (AAS), inductively coupled plasma mass spectrometry (ICP-MS), capillary electrophoresis and voltammetry [6]. These techniques require complicated operation procedures, expensive instrumentations and large sample volumes [7]. In contrast, fluorescence detection methods for Cu^{2+} have been widely exploited and attracted increasing attention by researchers due to its high sensitivity, good selectivity and easy operation [8,9].

Currently, various fluorescent dyes and fluorescent nanoparticles were widely reported for the determination of Cu^{2+} such as coumarin, rhodamine, fluorescein, semiconductor quantum dots (QDs), nanoclusters and carbon dots (CDs) [10–14]. The mechanisms of organic fluorescent probes for the detection of Cu^{2+} mainly contain two types [15,16]. One is the coordinated probe, its fluorescence is usually quenched by the paramagnetic of the Cu^{2+}. The other is reactive probe, its fluorescence is quenched or enhanced by the catalytic hydrolysis of Cu^{2+}. Especially, some specific organic fluorescent probes with similar bisamide structure were developed for sensitive and selective detecting Cu^{2+} [17] (Scheme 1). However, poor water solubility and biological toxicity limit their practical applications. Compared with organic fluorescent dyes and QDs, CDs possessed excellent water dispersibility, good cell permeability and biocompatibility [18,19]. By virtue of these superior properties, the CDs for the recognition of Cu^{2+} have been continuously explored [20,21]. Since most pristine CDs lack of excellent selectivity for specific identification of Cu^{2+}, some functionalized-CDs had been reported for Cu^{2+} through the modification of organic molecules on the surface of CDs [22,23]. These organic molecules as recognition units can arise coordination reaction or chemical reaction with Cu^{2+}, inducing or stimulating changes of fluorescence intensity of the CDs. Meanwhile, the current CDs-based probes for Cu^{2+} mainly are single-intensity-based probe based on fluorescence turn-on or

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[*] Corresponding authors.
E-mail addresses: yanfanyong@tjpu.edu.cn (F. Yan), xujinxiaruth@163.com (J. Xu).

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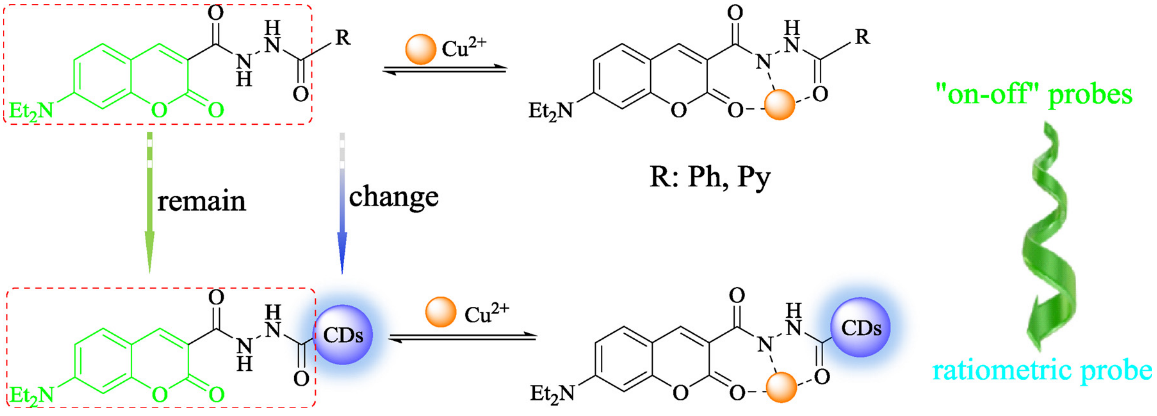


Scheme 1. The design strategy of CMH-GA-CDs for detecting Cu^{2+}.

fluorescence turn-off, which is usually affected by the interferences of the instrumental efficiencies and environmental conditions [24]. By contrast, ratiometric fluorescence probe can provide built-in correction for the environmental effects and reliable method for visualizing detection at the molecular level [25,26].

Inspired by the previous reported, a novel ratiometric fluorescent probe (CMH-GA-CDs) was designed, which endows with the capability of CMH-GA-CDs for the ratiometric detection of Cu^{2+} in aqueous solution (Scheme 1). Herein, CDs with hydroxyl groups were synthesized and covalently linked to glyoxylic acid molecules to form glyoxylic acid-modified carbon dots (GA-CDs). Then, 7-diethylaminocoumarin-3-carbohydrazide (CMH) were modified on the surface of GA-CDs to obtain fluorescence probe CMH-GA-CDs (Scheme 2). The mechanism of this probe for Cu^{2+} is that Cu^{2+} coordinated with the heteroatoms N and O [15–17], and then reducing the energy transfer efficiency from CDs to CMH units [27,28]. The experimental results displayed that CMH-GA-CDs possessed good sensitivity, excellent selectivity and strong anti-interference ability for the detection of Cu^{2+}. The limit of detection was calculated can be reached to 0.21 μM, which was much lower than the maximum level of Cu^{2+} (20 μM) in drinking water by Environmental Protection Agency (EPA). Furthermore, the CMH-GA-CDs were successfully applied in tap water and lake water with satisfactory recovery results.

2. Experimental

2.1. Apparatus

Transmission electron microscopy (TEM) measurements were carried out by a JEM–2011 microscope (JEOL, Japan) (at 200 kV). Fourier transform infrared (FT-IR) spectra were recorded on a TENSOR37 Fourier transform infrared spectrometer from Tianjin Gangdong scientific and technological development Co., Ltd. Fuorescence spectra were recorded with an F-380 fluorescence spectrophotometer from Tianjin Gangdong scientific and technological development Co., Ltd. UV–vis absorption spectra were obtained by using a TU–1901 UV–vis spectrometer (Beijing Puxi Inc., China). Zeta potential were measured with Zetasizer nano ZS90 (Edinburgh, UK).

2.2. Materials

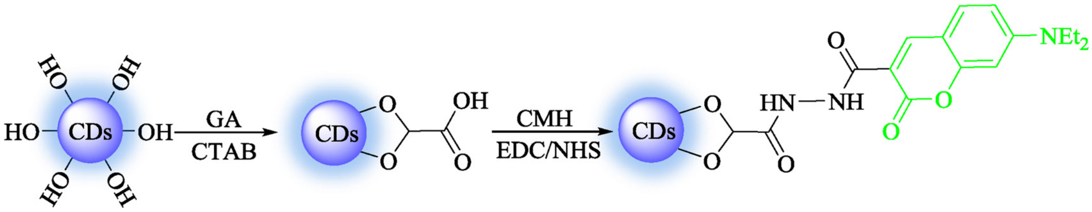
Anhydrous citric acid (CA, 99.5%), Tris(hydroxymethyl)methyl aminomethane (Tris, 98%), 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 98%), N-hydroxysulfosuccinimide (NHS, 98%) andHexadecyl trimethyl ammonium Bromide (CTAB, 98%) were purchased from Aladdin Industrial Inc. 4-(Diethylamino)salicylaldehyde (98%), glyoxylic acid (GA, 40%), diethylmalonate (99%), piperidine (99%), hydrazine hydrate (98%) were purchased from Sigma-Aldrich. All other chemicals were of analytical reagent grade and used without further purification. Double-distilled water was used throughout all experiment.

2.3. Synthesis of CDs

CDs were prepared by hydrothermal method according to the previous report by our group [29]. Firstly, CA (5 mmol, 0.96 g) and Tris (5 mmol, 0.60 g) were dissolved in distilled water (30 mL). Next, the solution was transferred to poly(tetrafluoroethylene) autoclave (50 mL) preheated at 90 °C for 1 h and then heated at 200 °C for 6 h. When the reactors cooled to ambient temperature naturally, the deep-brown aqueous dispersion liquid formed was centrifuged at medium speed (4000 rpm/min) for 15 min in order to remove insoluble particulates. Then, the solution was subjected to dialysis (MW = 3500 Da) to remove unreacted reagent and small molecular species. Finally, CDs were dried in a freeze-drying oven (yield: 0.75 g, 48.1%).

2.4. Synthesis of GA-CDs

GA-CDs were prepared according to a literature procedure with a slight modification [30]. A mixture of CDs (0.8 g), GA (1.46, 20 mmol) and CTAB (0.1 g) in 30 ml of CH_3OH/cyclohexan (v/v = 1/2) solution was stirred at 60 °C for 6 h under N_2 atmosphere. After cooling to room temperature, the solid was obtained by rotary evaporation. Then, the crude product was purified by silica-column chromatography using methylene chloride and methanol as eluents. Finally, the GA-CDs were dried under vacuum at 50 °C overnight.



Scheme 2. The schematic illustration of the synthetic process of CMH-GA-CDs.