Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 195 (2018) 230–235

Contents lists available at ScienceDirect

Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy

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

A ratiometric nanoprobe based on silver nanoclusters and carbon dots for the fluorescent detection of biothiols

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ARTICLE INFO

Article history:
Received 23 October 2017
Received in revised form 24 January 2018
Accepted 30 January 2018
Available online 01 February 2018

Keywords:
Ratiometric nanoprobe
Combinatorial probe
Fluorescence analysis
Visual semi-quantitation

ABSTRACT

Ratiometric fluorescent probes could eliminate the influence from experimental factors and improve the detection accuracy. In this article, a ratiometric nanoprobe was constructed based on silver nanoclusters (AgNCs) with nitrogen-doped carbon dots (NCDs) and used for the detection of biothiols. The fluorescence peak of AgNCs was observed at 650 nm with excitation wavelength at 370 nm. In order to construct the ratiometric fluorescent probe, NCDs with the excitation and emission wavelengths at 370 nm and 450 nm were selected. After adding AgNCs, the fluorescence of NCDs was quenched. The mechanism of the fluorescence quenching was studied by fluorescence, UV–Vis absorption and the fluorescence lifetime spectra. The results indicated that the quenching could be ascribed to the inner filter effect (IFE). With the addition of biothiols, the fluorescence of AgNCs at 650 nm decreased due to the breakdown of AgNCs, and the fluorescence of NCDs at 450 nm recovered accordingly. Thus, the relationship between the ratio of the fluorescence intensities (I_{450}/I_{650}) and biothiol concentration was used to establish the determination method for biothiols. Cysteine (Cys) was taken as the model of biothiols, and the working curve for Cys was $I_{450}/I_{650} = 0.60C_{Cys} - 1.86$ (C_{Cys}: μmol/L) with the detection limit of 0.14 μmol/L (S/N = 3). Then, the method was used for the detection of Cys in human urine and serum samples with satisfactory accuracy and recovery ratios. Furthermore, the probe could be applied for the visual semi-quantitative determination of Cys by naked eyes.

1. Introduction

The sensitivity, selectivity and accuracy of fluorescence analysis depend greatly on the property of fluorescent probes. Most of fluorescent probes only have a single-wavelength emission. Due to the lack of internal standard, the fluorescence signal of these probes is susceptible to the fluctuation of experimental conditions, such as stability of excitation intensity, concentration error of probes, and change of solution environment. The ratiometric fluorescent probes which have two or more emission bands at different wavelengths can overcome the above mentioned problems. The quantitative analysis by ratiometric probes is based on the intensity ratio of two emission peaks, thus the ratiometric probes have the self-calibration function. They can eliminate the influence of experimental factors and improve accuracy [1].

The traditional ratiometric probes were comprised of organic molecules with two different fluorescence peaks. The active groups of organic molecule probes could react with the analyte through coordination [2], redox, and so on [3]. The occurrence of these reactions could lead to the simultaneous changes of fluorescence intensities and wavelengths. The changes of emissions spectra were usually attributed to the mechanisms such as fluorescence resonance energy transfer (FRET) [4] and intramolecular charge transfer (ICT) [5]. Ma group had done remarkable jobs in ratiometric fluorescent probes. Therein, a ratiometric probe was designed by using propylamine and 1, 8-naphthalimide for monoamine oxidase A (MAO-A) detection. After amino of the probe reacted with MAO-A through amine oxidation and β-elimination, a new emission peak at around 550 nm appeared except original 454 nm. According to the relationship between the intensity ratio of these two fluorescence peaks and the concentration of MAO-A, the quantitative determination for MAO-A was carried out [6]. Besides, Ma group synthetized other ratiometric probe to detect hydrogen sulfide through reducing azido to amino [7]. Nonetheless, the construction of these ratiometric probes usually involved elaborate design, tedious synthesis, complex separation and purification [8–10].

As a new type of probes, nanoparticle fluorescent probes have recently received intense attention. Because most nanoprobes had only single emission peak, they could not serve as ratiometric probes. In

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https://doi.org/10.1016/j.saa.2018.01.078
1386-1425/

recent years, a few of ratiometric nanoprobes were successfully constructed by combining two kinds of fluorescence units. For instance, Wang utilized CdTe quantum dots (QDs) of two sizes to obtain a dual-emission fluorescent hybrid sphere [11]. The red-emitting CdTe QDs were entrapped in the silica sphere as the reference unit, and the green-emitting CdTe QDs were covalently bonded to the silica surface as the response part. Tian developed a hybrid ratiometric nanoprobe by modifying the red-emitting CdTe QDs (640 nm) with the blue-emitting benzothiazole fluorophore (420 nm) [12]. Ouyang group used the positively charged aggregation-induced emission organic fluorescence nanoparticles (AIE-based OFNs) and negatively charged Au nanoclusters to establish a dual-emission ratiometric probe via electrostatic attraction for the detection of Hg^{2+} and melamine [13]. The AIE-based OFNs@AuNCs nanoprobes had the advantages of simple preparation and label-free. In this paper, a ratiometric nanoprobe was subtly constructed by combining two kinds of common nanoparticles with different emission wavelengths.

Nanosilver including silver nanoparticle and silver nanocluster was usually used to build probes for the detection of metal ions such as Cd^{2+} [14], Al^{3+} [15], Pb^{2+} [16], Ni^{2+} [17] and biothiols such as cysteine, homocysteine, glutathione [18,19]. Biothiols were small molecules with sulfydryl. They could be detected by nanosilver probes through the coordination between sulfydryl and Ag atom. Herein, in order to reduce the influence from the experimental factors, a ratiometric nanoprobe (AgNCs/NCDs) was constructed for detecting biothiols based on AgNCs and environmental-friendly NCDs. By addition of biothiols such as Cys, the decrease in fluorescence intensity of AgNCs was accompanied by a fluorescence recovery of NCDs. Then, the quantitative relationship between the intensity ratio of the two fluorescence peaks and the concentration of Cys was established. Furthermore, the probe could be used for the semi-quantitative analysis of biothiols by naked eyes. In this way, a simple and visual detection method for biothiols was established based on the AgNCs/NCDs probe.

2. Experimental

2.1. Materials and Instruments

Bovine serum albumin (BSA) was purchased from Aladdin Chemical Company. Amino acids (cysteine, leucine, phenylalanine, histidine, serine, methionine, alanine, glycine, aspartic acid, arginine, tryptophan and glutamic acid) and glutathione were obtained from Macklin Reagent Company. Citric acid, urea, acetonitrile (ACN), $AgNO_3$, $NaBH_4$, NaOH, $Na_2HPO_4 \cdot 12H_2O$ and $NaH_2PO_4 \cdot 2H_2O$ were obtained from Tianjin Zhiyuan Chemical Factory. All reagents were analytic grade unless otherwise stated. Double-distilled water was used throughout the experiments.

Fluorescence spectra were obtained using an F-4600 fluorescence spectrophotometer (Hitachi, Japan). UV–Vis absorption spectra were measured with a 2700 UV–Vis spectrophotometer (Shimadzu, Japan). Zeta potential was collected with Nano ZS nanometer particle size analyzer (Malvern, UK). Fluorescence lifetime experiments were performed by an FLS-920 combined fluorescence lifetime and steady-state spectrometer (Edinburgh, UK). The pictures were obtained under ultraviolet lamp (50W, Gongyi, China).

2.2. Synthesis of a Ratiometric Nanoprobe

AgNCs were prepared using a modified method [20]. Briefly, 5.0 mL of 0.01 mol/L $AgNO_3$ solution was gently added to 5.0 mL of 50 mg/mL BSA solution at room temperature under vigorous stirring. After 15 min, 0.3 mL of 1.0 mol/L NaOH was added into the solution under continuous stirring, followed by dropwise addition of 1.0 mL of fresh $NaBH_4$ (0.01 mol/L). Then the resulting solution was stirred for 2 h. The obtained solution was dialyzed using 8–14 kDa dialysis bag until the conductivity of the dialysate was near that of double-distilled water. The reddish brown AgNCs solution was diluted to a constant

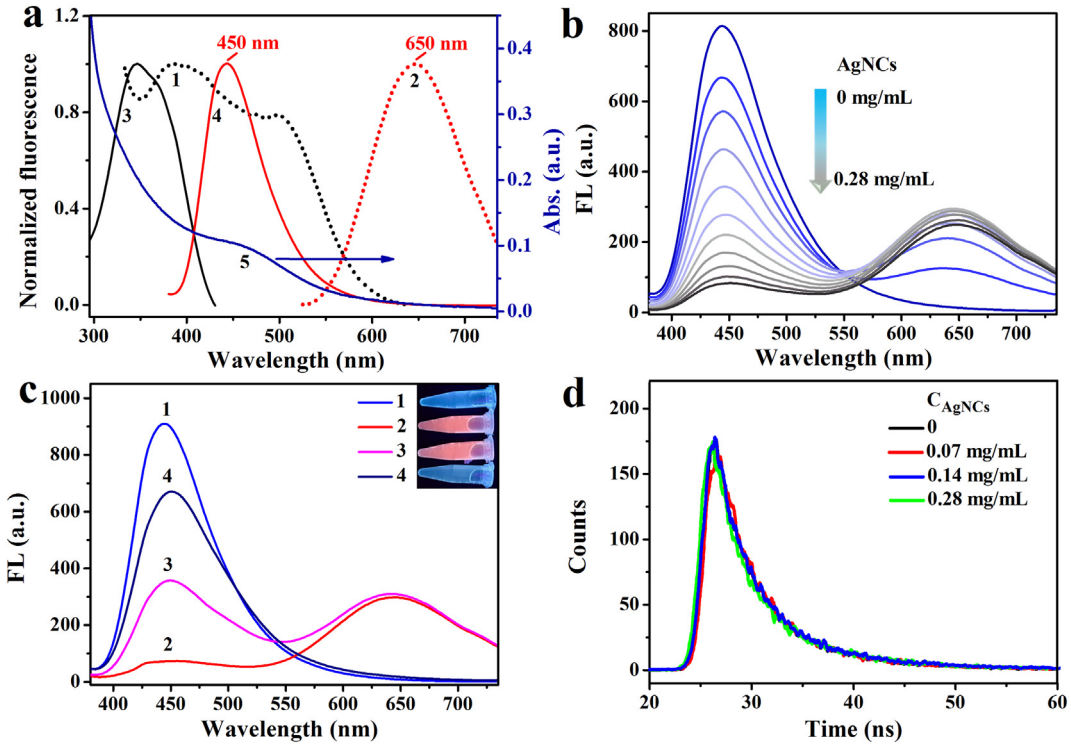

Fig. 1. (a) The excitation and emission spectra of AgNCs (1, 2) and NCDs (3, 4). (b) The fluorescence spectra of the AgNCs/NCDs probe with constant concentration of 0.28 mg/mL NCDs and different concentrations of AgNCs. (c) The fluorescence spectra of NCDs (1), AgNCs (2), AgNCs/NCDs (3), AgNCs/NCDs with the addition of Cys (4). (d) Time-resolved decay curves of NCDs in the absence and presence of AgNCs.