Biosensors and Bioelectronics 99 (2018) 332–337

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

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

Profuse color-evolution-based fluorescent test paper sensor for rapid and visual monitoring of endogenous Cu^{2+} in human urine

Yueqing Cai, Junhui You, Zhengyi You, Fang Dong, Shuhu Du*, Liying Zhang*

School of Pharmacy, Nanjing Medical University, Nanjing, Jiangsu 211166, China

ARTICLE INFO

Keywords:
Fluorescent test paper
Sensor
Tricolor probe
Visual detection
Cu^{2+}

ABSTRACT

The fluorescent paper for colorimetric detection of metal ions has been widely fabricated using various sensing probes, but it still remains an elusive task to design a test paper with multicolor variation with target dosages for accurate determination. Herein, we report a profuse color-evolution-based fluorescent test paper sensor for rapid and visual monitoring of Cu^{2+} in human urine by printing tricolor probe onto filter paper. The tricolor probe consists of blue-emission carbon dots (bCDs), green-emission quantum dots (gQDs) and red-emission quantum dots (rQDs), which is based on the principle that the fluorescence of gQDs and rQDs are simultaneously quenched by Cu^{2+}, whereas the bCDs as the photostable internal standard is insensitive to Cu^{2+}. Upon the addition of different amounts of Cu^{2+}, the ratiometric fluorescence intensity of the tricolor probe continuously varied, leading to color changes from shallow pink to blue with a detection limit of 1.3 nM. When the tricolor probe solution was printed onto a sheet of filter paper, as-obtained test paper displayed a more profuse color evolution from shallow pink to light salmon to dark orange to olive drab to dark olive green to slate blue to royal blue and to final dark blue with the increase of Cu^{2+} concentration compared with dual-color probe-based test paper, and dosage scale as low as 6.0 nM was clearly discriminated. The sensing test paper is simple, rapid and inexpensive, and serves as a visual platform for ultrasensitive monitoring of endogenous Cu^{2+} in human urine.

1. Introduction

Recently, the quantification of metal ions is extensively performed by using conventional techniques, including atomic absorption spectroscopy (NG and Garner, 1993), mass spectrometry (Richardson, 2001), inductively coupled plasma-mass spectrometry (ICP-MS) (Bings et al., 2006), and so on. However, these techniques usually require ponderous instruments, the sophisticated sampling and professional operation by well-trained personnel, making it difficult to on-site monitor metal ions in environments and biological fluids. Therefore, there is an urgent demand for developing a simple, economical and portable method for *in vitro* and *in vivo* metal ions' assay, motivating considerable researchers to construct new miniature chemical sensors.

Semiconductor, such as quantum dots (QDs) or carbon dots (CDs), is a promising optical label for chemo/biosensing applications since it offers distinct advantages. These include (1) good optical properties, (2) good photochemical stability, (3) long fluorescence lifetime, and (4) good water solubility (Wang and Guo, 2009). Thus, it is a suitable candidate for fluorescent probe, which can be linked a recognition element to generate fluorescent "turn on", "turn off" or "ratiometric" response. Unfortunately, it still relies on fluorescent instrument, for example, fluorescent spectrometer or confocal microscope. To solve this problem, fluorescent test paper has been developed because it possesses an excellent and unparalleled merit, that is, its visualization capability for the detection of target analyte by the naked eye with the aid of a portable ultraviolet (UV) lamp. Generally, the fluorescent test paper is prepared by printing various fluorescent probes onto filter paper or microporous membrane. To date, fluorescent test paper utilizing a fluorescent single-color probe has been reported in many previous literatures, such as graphene oxide paper for the detection of peptide, protein, and DNA (Mei and Zhang, 2012), QDs paper for the assay of catechol and glucose (Yuan et al., 2012) and CDs paper for the analysis of mercuric ions (Yuan et al., 2014). But they can only exhibit the variation of fluorescence brightness by either "turn on" or "turn off" mode with analytes, which greatly limits their quantitative capability. More recently, test paper based on dual-emission fluorescent probe has been fabricated for the assay of trinitrotoluene by QDs@SiO_2-QDs paper (Zhang et al., 2011), the detection of sulfur dioxide by ratiometric QDs@SiO_2-QDs paper (Yan et al., 2015), the monitoring of pesticides by coumarin-3-carboxylic acid-QDs@SiO_2 paper (Li et al., 2015), the quantification of glucose by

* Corresponding authors.
E-mail addresses: shuhudu@njmu.edu.cn (S. Du), zly@njmu.edu.cn (L. Zhang).

http://dx.doi.org/10.1016/j.bios.2017.07.072
Received 23 May 2017; Received in revised form 17 July 2017; Accepted 29 July 2017
Available online 31 July 2017
0956-5663/

QDs@SiO_2-CDs paper (Huang et al., 2016), and so on. It is a pity that not only the fabrication procedure of dual-emission probe is complicated, but also the color variation of dual-emission probe is not profuse. So, multicolor-variation-based fluorescent test paper remains immature.

In this work, we present a fluorescent colorimetric method for dosage-sensitive and visual detection of Cu^{2+} by use of the tricolor probe system, which is fabricated by a simple blend of one blue CDs (bCDs) and two 3-mercaptopropionic acid (MPA)-functionalized QDs green QDs (gQDs) and red QDs (rQDs). With the increase of Cu^{2+} amount, photoluminescence (PL) intensity of gQDs and rQDs was gradually quenched, while the PL intensity of bCDs kept constant, accompanying continuous fluorescence color changes. Based on this, we develop a handy test strip by the assembly of the tricolor probe onto the filter paper for rapid monitoring of urinary copper, which is a promising auxiliary index for clinical diagnosis of Menkes (deficiency of copper) and Wilson's diseases (WD) (hyperaccumulation of copper) (Kaler, 2011; Burknead et al., 2011; Merle et al., 2007; Huster et al., 2006). The detail data are displayed below.

2. Experimental section

2.1. Reagents and instruments

3-Mercaptopropionic acid (MPA) was obtained from Sigma-Aldrich. Te powder, $NaBH_4$, $CdCl_2$•$2.5H_2O$, sulfuric acid (98%), NaOH, ethanol (95%), citric acid and ethylenediamine were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Rhodamine and KI were bought from Aladdin. HEPES (pH 7.4) was purchased from Sigma-Aldrich. All chemicals used were analytical grade. Ultrapure water (18.2 MΩ cm) was prepared by a Millipore water purification system.

UV–vis absorption and fluorescence spectra were recorded by Shimadzu UV-2450 (Japan) spectrophotometer and Hitachi F-4600 (Japan) fluorescence instrument, respectively. A JEOL JEM-2100 transmission electron microscope (TEM) instrument (Japan) was used to observe the morphology of nanoparticles. The hydrodynamic sizes of nanoparticles were determined using a Malvern Zetasizer Nano-ZS90 (UK) particle size analyzer. FT-IR spectra were recorded on a TENSOR 27 spectrometer (Bruker, Germany) with a resolution of 2 cm^{-1} and a spectral range of 4000–400 cm^{-1}. X-ray photoelectron spectroscopy (XPS) spectra were collected on a PHI Quantera II spectrometer (Japan). To assess accuracy of the method, urine samples were also analyzed on an ICP-MS (iCAP Q, Thermo Fisher, America). Fluorescent photos were taken under an AGL-9406 UV lamp (China) with a Canon 600D digital camera (Japan).

2.2. Synthesis of CDs

Blue-emission CDs were prepared by the previously reported method (Huang et al., 2016). Briefly, 1.05 g of citric acid and 0.34 mL of ethylenediamine were first dissolved in 20 mL ultrapure water. Then the solution was transferred to a polytetrafluoroethylene autoclave (30 mL) and heated at 200 °C for 5 h. After gradually cooled to room temperature, the resultant bCDs were purified by dialysis for 24 h.

2.3. Synthesis of CdTe QDs

QDs were prepared by the method of previous literature (Zhou et al., 2016). Typically, 0.06 g Te powder and 0.1 g $NaBH_4$ were first mixed in 2 mL of ultrapure water under nitrogen atmosphere in an ice bath, then stirred for 6 h to get the NaHTe solution. Meanwhile, 0.228 g of $CdCl_2$•$2.5H_2O$ and 210 μL of MPA were dissolved in 100 mL of ultrapure water and adjusted pH to 9 with 1.0 M NaOH, and the mixing solution was deoxygenated by bubbling nitrogen for 30 min. Subsequently, 5 mL of H_2SO_4 (0.5 M) was injected into the as-obtained NaHTe solution and the timely produced H_2Te gas was inlet into the above mixing solution of $CdCl_2$ and MPA until the solution color transformed from colorless to orange. After refluxing for 1 h, gQDs with an emission at 510 nm were synthesized. The synthesis of rQDs with an emission at 600 nm was performed in a similar way, but varying the time of reflux (24 h). The prepared QDs were rinsed with acetone and dispersed in 100 mL of ultrapure water for further use.

2.4. Preparation of fluorescent test papers

The tricolor probe-based fluorescent test papers were prepared as described in our previous work (Zhou et al., 2016). Briefly, a common cartridge of a commercial inkjet printer was washed with ultrapure water until the ink powder was cleared away completely, followed by drying in an oven at 50 °C for 6 h. The tricolor probe solution as ink was into the vacant cartridge. A rectangle pattern of 7 × 3 cm^2 was printed on a piece of filter paper by the printer connected to a computer, and the printing was repeated for 30 times. After air-drying for 15 min, the pattern was cut into 3 × 1 cm^2 pieces for the visual detection of Cu^{2+}. For comparison, the dual-color probe-based fluorescent test papers were also fabricated using the same method, but replacing the tricolor probe with dual-color probe.

2.5. Colorimetric detection of Cu^{2+} in human urine

With the patients' informed-consent, 24-h urine samples of four patients with WD were obtained from the First Affiliated Hospital of Anhui University of Chinese Medicine. Moreover, normal urine samples were also collected from three healthy volunteers in a period of 24 h. Prior to the assay, all urine samples were filtered through 0.45 μm Supor filters to remove any particulate suspension, then stored in refrigerator at 4 °C until further analysis.

Fluorescent measurement of Cu^{2+}: Different amounts of Cu^{2+} were added into the tricolor probe solution with HEPES buffer (pH 7.4) and thoroughly mixed for 30 s, and the fluorescent spectra were recorded with a spectrometer.

Detection of Cu^{2+} on test paper: Each urine sample (500 μL) was dropped onto the as-obtained test paper, and after that the corresponding color of test paper was observed under a 365 nm UV lamp.

3. Results and discussion

3.1. Characterization of QDs and CDs

Fig. 1 shows the fluorescence spectra of bCDs, gQDs, rQDs and tricolor probe. The bCDs, gQDs and rQDs display a maximal emission

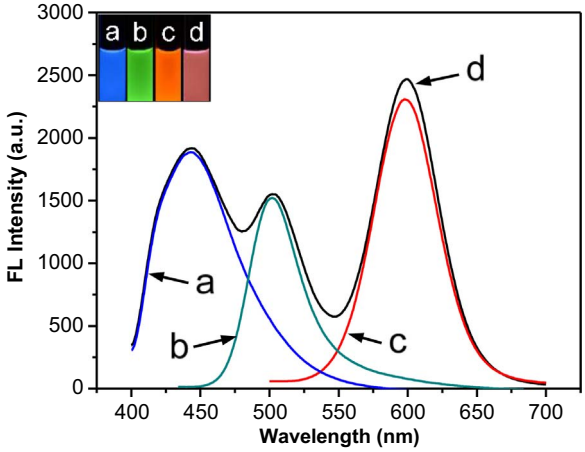

Fig. 1. Fluorescence emission spectra (λ_{ex} = 360 nm) of (a) bCDs, (b) gQDs, (c) rQDs and (d) the tricolor probe (the inset photos were taken under 365 nm UV lamp).