Talanta 189 (2018) 411–417

Contents lists available at ScienceDirect

Talanta

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

# Rapid aqueous synthesis of CuInS/ZnS quantum dots as sensor probe for alkaline phosphatase detection and targeted imaging in cancer cells

Fangmei Zhang[a,b], Xu He[c], Pinyi Ma[a,*], Ying Sun[a], Xinghua Wang[a], Daqian Song[a,*]

[a] College of Chemistry, Jilin University, Qianjin Street 2699, Changchun 130012, PR China
[b] Heilongjiang River Fisheries Research Institute of Chinese Academy of Fishery Sciences, Harbin 150070, PR China
[c] Key Laboratory for Molecular Enzymology and Engineering of the Ministry of Education, Jilin University, Qianjin Street 2699, Changchun 130012, PR China

ARTICLE INFO

*Keywords:*
CuInS quantum dots
Inner filter effect
Cell imaging
Alkaline phosphatase

ABSTRACT

Early diagnosis of chronic, critical diseases improves clinical outcomes, and biomarkers play an important role as an indicator of severity or presence of a disease. Alkaline phosphatase (ALP) is one such vital biomarker in the diagnosis of several diseases. Herein we introduce a facile, sensitive fluorescent assay, based on the inner filter effect (IFE), for ALP activity determination in serum and in living cells. It is well known that the key to maximize the sensitivity of an IFE-based fluorescence assays is to broaden the overlap between the absorption of an absorber and the excitation/emission of a fluorophore. We employed CuInS/ZnS quantum dots (CIS/ZnS QDs) and *p*-nitrophenylphosphate (PNPP) as the fluorescent indicator and the substrate, respectively, for ALP activity assessment. Due to the CIS/ZnS QDs have an efficient excitation at 405 nm, meanwhile with a large Stokes shift emission at 588 nm, *p*-nitrophenol (PNP) with absorption peak at 405 nm, the hydrolyzed produce of PNPP and ALP, can act as a competitive absorber to absorb the excitation light of CIS/ZnS QDs, resulting in noticeable quenching of CIS/ZnS QDs. The proposed sensor detects ALP activity in human serum samples (sample consumption: 20 μL) with detection limit of 0.01 U $L^{-1}$. Excellent biocompatibility of CIS/ZnS QDs enables the sensor to monitor endogenous ALP in living cells. Furthermore, because the surface modification or the linking between the receptor and the fluorophore is no longer required, this fluorescent sensing system has the potential to simplify ALP clinical measurement, thereby improving diagnostics of relevant diseases.

## 1. Introduction

The clinical consequence of an ageing world includes rising prevalence of chronic diseases. Early detection of chronic and critical diseases (e.g., cancers and diabetes) [1,2] can prevent disease progression, decrease mortality, and improve quality of life. Therefore, development of robust but simple screening techniques is of crucial importance. Disease screening by extracting biomarkers from biofluids (e.g., plasma or serum) or monitoring biomarkers by a non-invasive measurement would be effective, and techniques based on spectroscopy provide an option in this regard.

Alkaline phosphatase (ALP) is composed of a group of isoenzymes that catalyzes hydrolysis and transphosphorylation of diverse phosphoryl esters and are widespread in tissues (intestine [3,4], liver [5,6], bone [7], kidney [8], and prostate [9]. As a biomarker in various chronic diseases at early stage diagnosis, overexpression of ALP correlate to various diseases including prostate cancer, bone disease (osteoblastic bone cancer) [7], liver dysfunction (liver cancer, hepatitis, and obstructive jaundice) [10], diabetes and other diseases. Even though ALP has been studied for years, its pathological and physiological functions have not yet been explicated. Thus, developing convenient, reliable methods for monitoring ALP activity/level in vivo/in vitro become extremely important not only for clinical diagnoses but also for biomedical research. Moreover, while ALP activity in extracellular fluid suggests the viability of local cells, ALP activity in intercellular indicates an increased risk of disease. As a result, real-time analyzing of ALP expressing cell viability is also crucial to discriminate normal and abnormal behavior of cells (e.g., hyperproliferation) [11,12].

In the past decade, numerous methods, including colorimetric [13], chromatographic [14], fluorometric [15,16], electrochemical approaches [17] and surface-enhanced Raman scattering [18,19], have been developed for detecting ALP activity. Even though each of them possesses their own advantages, these methods usually accompany with some shortcomings which limit their application, such as complicated synthesis procedure, poor photostability and water solubility, requiring

* Corresponding authors.
*E-mail addresses:* mapinyi@jlu.edu.cn (P. Ma), songdq@jlu.edu.cn (D. Song).

https://doi.org/10.1016/j.talanta.2018.07.031
Received 24 April 2018; Received in revised form 2 July 2018; Accepted 11 July 2018
Available online 20 July 2018
0039-9140/ 

surface modification or low sensitivity. The inner filter effect (IFE) has emerged as an efficient and valuable strategy for the design and development of novel sensors [20,21], because it does not require the surface modification or establishing any covalent linking between a fluorophore and analyte [22,23]. However, it is difficult to find exactly spectral overlapped absorber and fluorophore, which is vital point in sensitivity of IFE-based sensor.

Semiconductor quantum dots (SQDs), which combine efficient broadband absorption with narrowband fluorescence spectrum, can overcome the drawbacks mentioned above. As ternary quantum dots (QDs), CuInS (CIS) QDs have been gaining increased attention as a promising fluorescence probe. Due to their advantages such as emission wavelength can across the visible to near infrared spectroscopy (NIR) spectral region, excellent water-dispersible, photo-stability, biocompatibility, and chemical inertness. Although CuInS QDs (CIS QDs) [24,25] can be employed as biosensors, the synthesis of water soluble, photo-stable CIS QDs without any further phase transition remains a challenge. Chen et al. demonstrated dramatic improvement in the photo luminescent (PL) intensity of the CIS QDs by coating them with ZnS shell [26]. CIS/ZnS QDs have also been applied for in vivo imaging [27–29]. Consequently, our team conducted the preparation of CIS/ZnS QDs in aqueous phase by the microwave-assisted method successfully.

In view of this, we proposed here a new IFE-based approach ALP sensing ALP (Fig. 1). The sensor was fabricated exploiting the ALP-catalyzed hydrolysis reaction of an ALP substrate, *p*-nitrophenyl phosphate (PNPP). The maximum absorption wavelength of the hydrolysis product *p*-nitrophenol (PNP) significantly overlaps with excitation wavelength of the prepared CIS/ZnS QDs. In the presence of ALP, the excitation of QDs was significantly weakened by the competitive absorption, thus resulting in the efficient quenching of QDs. The developed method exhibits various merits including rapidity, simplicity, low cost, high sensitivity, and excellent selectivity. We further investigated the possibility of applying the established fluorescence approach for cell imaging. To the best of our knowledge, this is the first report where CIS/ZnS QDs were employed for fabricating an IFE-based fluorescent assay.

## 2. Experimental

### 2.1. Materials and reagents

Indium chloride ($InCl_3{\cdot}4H_2O$, 99.9%) and 3-mercaptopropionic acid (MPA, ≥ 99%) were purchased from J&K Chemical company Ltd., China. Sodium sulfide ($Na_2S{\cdot}9H_2O$, 98%), zinc acetate ($Zn(OAc)_2{\cdot}2H_2O$, 99.0%), copper chloride ($CuCl_2{\cdot}2H_2O$, 95.0%), sodium hydroxide (NaOH, 96%), ethanol ($C_2H_5OH$, 99.7%), and ammonium hydroxide solution ($NH_3{\cdot}OH$, 25.0–28.0%) were purchased from Tianjing Guangfu Chemical Company Ltd., China. Bovine serum albumin (BSA) was purchased from Ding Guo Biotechnology Company Ltd., China. Horse IgG was purchased from Beijing Biosynthesis Biotechnology Company Ltd., China. Alkaline phosphatase (ALP) from bovine intestinal mucosa, acetylcholinesterase (AChE), horseradish peroxidase (HRP), glucose oxidase (GOX), cysteine (Cys), and glutathione (GSH) were purchased from Shanghai Yuanye Biotechnology Company Ltd., China. All chemicals were used as received without further purification. Ultrapure water was obtained from a Millipore purification device (18.2 MΩ cm). Human hepatoma cell line (BEL-7402) was purchased from Shanghai Chinese Academy of Science's cell bank.

### 2.2. Instruments

UV–Vis and fluorescence spectra were recorded on a Cary 60 spectrometer (Agilent Technologies, USA) and a F-2700 spectrofluorophotometer (HITACHI Co., Ltd., Japan), respectively. Morphologies of CIS/ZnS QDs were captured by a JEOL JEM-1200EX transmission electron microscope (TEM) operated at 200 kV (JEOL Co., Ltd., Japan). FT-IR spectra were recorded using KBr pellets on a Nicolet Avatar360 FT-IR spectrophotometer (Thermo Fisher Scientific Inc., USA) at the wavenumber range of 4000–400 $cm^{-1}$. Cell imaging was carried out by laser scanning confocal microscope (LSM710, CarlZeiss, Oberkochen, Germany). The X-ray photoelectron spectroscopy (XPS) measurements were performed using a Thermo Escalab 250 spectrometer with monochromatized Al Kα excitation (Thermo Fisher Scientific, Inc., USA).

### 2.3. Synthesis of CIS/ZnS QDs

CIS/ZnS QDs was synthesized as following steps. Briefly, 0.25 mL $InCl_3{\cdot}4H_2O$ stock solution (0.1 M) was dissolved in 20 mL ultrapure water with MPA (0.2 mmol), and the pH was adjusted to 9.0 by adding 1 M NaOH dropwise. Next, 0.5 mL of ammonium hydroxide solution containing $CuCl_2{\cdot}2H_2O$ (0.15 mL 0.1 M) with MPA (0.3 mmol) was added into the above mentioned $InCl_3$ solution. The molar ratio of $Cu^{2+}$ to $In^{3+}$ was 5:3 in the reaction mixture. Subsequently, $Na_2S$ solution (40 μL 1 M) was injected into the above reaction mixture at room temperature with vigorous stirring for 2 min. The reaction mixture was heated to 100 °C by microwave assisted heating. After 5 min of microwave irradiation, the reaction mixture was cooled below 50 °C. In successive steps, 1 mL 0.04 M $Zn(OAc)_2$ and 1 mL 0.04 M $Na_2S$ were dropwise injected into the reaction mixture, which was further irradiated at 100 °C for 5 min to obtain CIS/ZnS QDs. The color of the reaction mixture progressively changed from colorless through yellowish, fawn and finally to adobe brown. The QDs were further purified by precipitating with ethanol, and a highly water-soluble CIS/ZnS QDs in powder form was obtained.

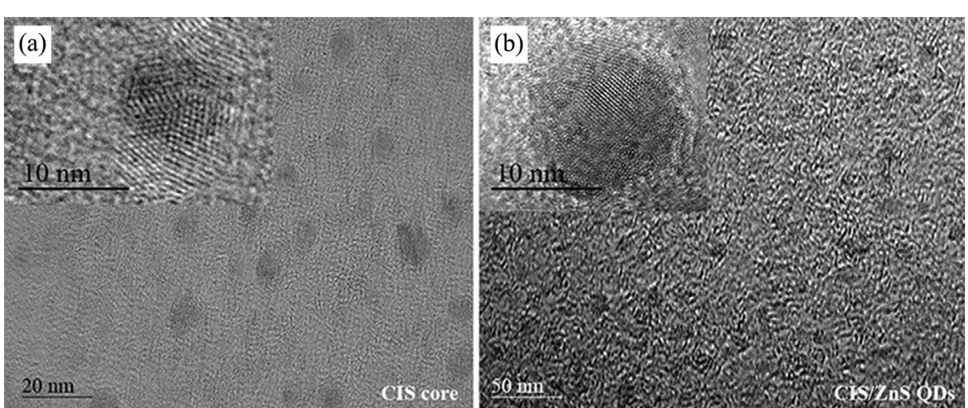


**Fig. 1.** TEM image of CIS core (a) and CIS/ZnS QDs (b).