Analytical Biochemistry 486 (2015) 102–106

Contents lists available at ScienceDirect

Analytical Biochemistry

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

# Development of tyrosinase biosensor based on quantum dots/chitosan nanocomposite for detection of phenolic compounds

En Han [a,*], Yi Yang [a], Zheng He [a], Jianrong Cai [a], Xinai Zhang [a], Xiaoya Dong [b]

[a] School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, People's Republic of China
[b] Key Laboratory of Modern Agricultural Equipment and Technology (MOE), Institute of Agricultural Engineering, Jiangsu University, Zhenjiang 212013, People's Republic of China

ARTICLE INFO

*Article history:*
Received 26 April 2015
Received in revised form 30 June 2015
Accepted 1 July 2015
Available online 6 July 2015

*Keywords:*
Quantum dots
Nanocomposite
Phenolic compounds
Amperometric detection

ABSTRACT

A sensitive and simple amperometric biosensor for phenols was developed based on the immobilization of tyrosinase into CdS quantum dots/chitosan nanocomposite matrix. The nanocomposite film with porous nanostructure, excellent hydrophilicity and biocompatibility resulted in high enzyme loading, and the tyrosinase (Tyr) immobilized in this novel matrix retained its activity to a large extent. The CdS quantum dots/chitosan nanocomposite film was characterized by scanning electron microscopy and electrochemical impedance spectroscopy, and the parameters of the various experimental variables for the biosensor were optimized. Under the optimal conditions, the designed biosensor displayed a wide linear response to catechol over a concentration range of $1.0 \times 10^{-9}$ to $2.0 \times 10^{-5}$ M with a high sensitivity of 561 ± 9.7 mA $M^{-1}$ and a low detection limit down to 0.3 nM at a signal-to-noise ratio of 3. The CdS quantum dots/chitosan nanocomposites could provide a novel matrix for enzyme immobilization to promote the development of biosensing and biocatalysis.



Phenolic compounds, including phenol and simple substituted phenol derivatives, are widely used in various production processes such as pesticides, plasticizers, petrochemical products, and wood preservatives [1]. Many of these compounds as a large group of pollutants are present in the medical, water, and food matrices, and most of them are extremely harmful to human health through oral, dermal, or respiratory tracks [2–4]. Therefore, it is important to develop a simple method for rapid determination of trace phenolic compounds due to their toxicity and persistency in the environment.

Some methods, including spectrophotometry [5], liquid chromatography [6,7], and gas chromatography [8], have been employed to detect the phenolic compounds in water samples. However, some of these techniques are time-consuming, complex to perform, and expensive, which limits their application for rapid and real-time detection. Electrochemical biosensors have been considered to be a promising method for in situ and rapid determination of phenols due to high sensitivity, simplicity, and ease of miniaturization [9–12]. One of the crucial factors during these biosensors' fabrication is the effective immobilization of enzyme in a suitable matrix on electrode surface. Therefore, it is critical to search for materials as effective enzyme immobilization matrices to design a phenol biosensor with high analytical performance.

Since the discovery of carbon nanotubes, nanomaterials such as graphemes [13,14], magnetic nanobeads [15], gold nanoparticles [16,17], polymers [18,19], and quantum dots (QDs)[1] [20,21] have received wide research interest in bioassays due to their electronic, optical, and mechanical properties. Among these nanomaterials, QDs have been extensively used for optical and electrochemical biosensing because of their unique advantages such as nanoscale size similar to proteins, much higher specific surface, and versatility in surface modification, which are favorable for the immobilization of biomolecules [22–26]. Chitosan (Chit) is a nontoxic natural biopolymer with a large amount of positive charges. It has been widely used for protein immobilization to develop biosensors due to its special properties, including excellent film-forming ability and good biocompatibility [27–29].

In the current work, we developed a nanocomposite film based on CdS QDs and Chit as a host matrix for tyrosinase immobilization to design an amperometric biosensor for sensitive and rapid detection of phenols. This nanocomposite film displayed several advantages for enzyme immobilization such as good hydrophilicity and

[1] *Abbreviations used:* QD, quantum dot; Chit, chitosan; PBS, phosphate buffer solution; SEM, scanning electron microscope; Tyr, tyrosinase; GCE, glassy carbon electrode; EIS, electrochemical impedance spectroscopy; RSD, relative standard deviation.

* Corresponding author. Fax: +86 511 88780201.
*E-mail address:* enhan182@163.com (E. Han).

http://dx.doi.org/10.1016/j.ab.2015.07.001
0003-2697/

biocompatibility, high specific surface, and good film-forming capability. The designed biosensor based on the nanocomposite matrix exhibited excellent analytical performances for the detection of phenolic compounds such as high sensitivity, wide linear range, subnanomolar detection limit, and good repeatability.

## Materials and methods

### *Reagents and chemicals*

Tyrosinase (EC 1.14.18.1, 1000 U $mg^{-1}$) from mushroom and mercaptopropionic acid were purchased from Sigma–Aldrich. Catechol, *p*-cresol, phenol, *m*-cresol, *p*-chlorophenol, and Chit (90% deacetylation) were obtained from Sinopharm Chemical Reagent (Shanghai, China). Other reagents were of analytical grade and were used without further purification. All aqueous solutions were prepared with twice-distilled water. Phosphate buffer solution (PBS) was 0.1 M $Na_2HPO_4$ and $NaH_2PO_4$, and its pH was adjusted with $H_3PO_4$ or NaOH solutions. Phenolic solutions in 0.1 M PBS were prepared daily.

### *Apparatus and characterization*

Electrochemical studies were performed with a CHI660D electrochemistry workstation (Shanghai CH Instrument, China). The electrochemical measurements were carried out with a three-electrode system. A saturated calomel electrode and a Pt foil electrode were used as the reference electrode and the counter electrode, respectively, and modified glassy carbon electrode was used as the working electrode. Micrographs were obtained with a JSM-7001F scanning electron microscope (SEM). The static contact angles were measured with a contact angle meter (ramé–hart model 100) using droplets of deionized water at 25 °C. Electrochemical impedance spectroscopy was studied in 0.1 M PBS containing 10 mM $Fe(CN)_6^{3-/4-}$ and 0.1 M KCl with a frequency range of 0.01 Hz to 10 kHz.

### *Preparation of CdS QDs/Chit nanocomposite and enzyme electrodes*

The water-soluble CdS QDs were prepared using mercaptopropionic acid as stabilizing agent according to a reported method [30]. Chit solution (0.1%, w/w) was prepared by dissolving Chit flakes in 1.0% acetic acid and was then diluted into 0.1% solution by adding twice-distilled water. Then 40 μM CdS QDs was mixed with Chit solution at a 1:1 volume ratio and sonicated for 10 min. Subsequently, 4.0 mg of tyrosinase (Tyr) was dissolved in 1.0 ml of CdS QDs/Chit mixture solution. The glassy carbon electrode (GCE) was polished carefully using 0.3 and 0.05 μm alumina slurry, followed by rinsing thoroughly with distilled water. After successive sonication in 1:1 nitric acid, acetone, and distilled water, the electrode was allowed to dry at room temperature. Then 15 μl of the mixed solution (CdS QDs/Chit/Tyr) was spread on the surface of the GCE and dried at 4 °C in a refrigerator.

### *Protocols and statistical data treatment*

In this experiment, all electrochemical measurements were carried out in a thermostated cell at 25 °C containing 0.1 M PBS. The current response of the biosensor was obtained by injecting catechol into the PBS at −0.2 V. The average results of the tables were obtained with three replicates based on three different biosensors prepared by the same steps. The data of the figures were acquired using three different electrodes constructed by the same procedure and tested with the same reactants and the same sample. The error bars are standard deviations.

## Results and discussion

### *Characterization of Chit and QDs/Chit nanocomposite films*

The surface morphologies of Chit and QDs/Chit composite films were characterized by SEM imaging. As shown in Fig. 1A, the morphology of the pure Chit film exhibited an excellent film-forming character with irregular wrinkle. Compared with pure Chit film, the morphology of QDs/Chit nanocomposite film displayed a porous structure (Fig. 1B). This obvious difference could be attributed to the interaction between the negatively charged CdS QDs and the positively charged Chit molecules to produce aggregation. This nanocomposite film might integrate the merits of its precursors and would be suitable for enzyme immobilization with advantages such as good biocompatibility and adhesion and controlled porosity.

The biocompatibility of a host matrix for loading of enzymes and preserving their bioactivity is positively related to its hydrophilicity, which can be studied by the contact angle measurement of the material. As shown in the insets of Fig. 1, the contact angle of CdS QDs/Chit nanocomposite film was measured to be 30°, which was smaller than that of pure Chit film (57°). This demonstrated that the QDs/Chit nanocomposite film possessed better hydrophilicity than that of pure Chit film due to the abundant carboxylic acid groups on QDs surface. Thus, the high activity of the entrapped enzymes could be kept on this nanocomposite film, which is highly advantageous to the preparation of biosensors.

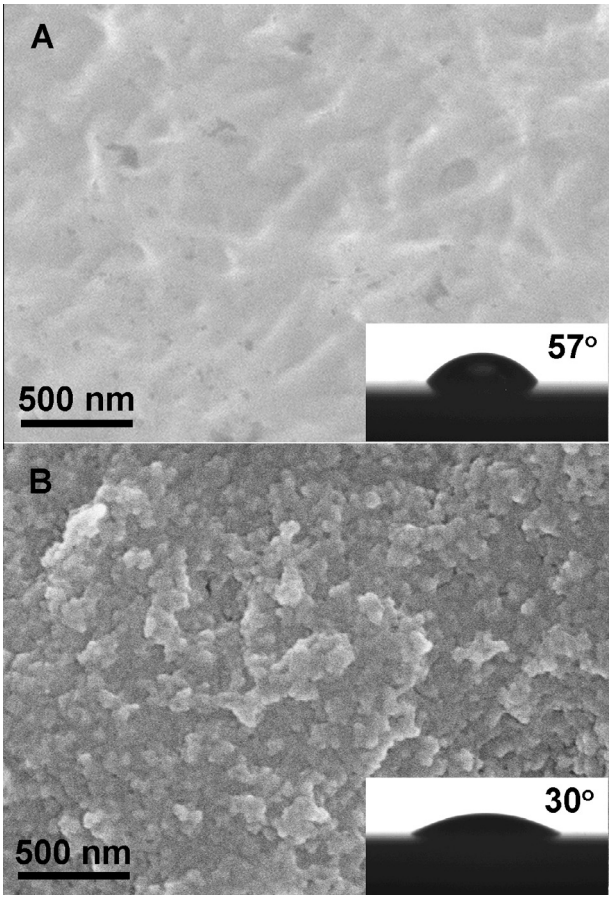


**Fig.1.** SEM images and contact angles (insets) of Chit film (A) and CdS QDs/Chit nanocomposite film (B).