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Photoamperometric flow injection analysis of glucose based on dehydrogenase modified quantum dots-carbon nanotube nanocomposite electrode

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ABSTRACT

In this work, a core-shell quantum dot (QD, ZnS-CdS) was electrodeposited onto multiwalled carbon nanotube modified glassy carbon electrode (ZnS-CdS/MWCNT/GCE) and following glucose dehydrogenase (GDH) was immobilized onto QD modified electrode. The proposed electrode (GDH/ZnS-CdS/MWCNT/GCE) was effectively used for the photoelectrochemical biosensing of glucose in flow injection analysis (FIA) system using a home-made flow cell. Results from cyclic voltammetric and FI amperometric measurements have revealed that GDH/ZnS-CdS/MWCNT/GCE is capable of signaling photoelectrocatalytic activity toward NADH when the surface of enzyme modified electrode was irradiated with a light source (250 W Halogen lamp). Thus, photoelectrochemical biosensing of glucose was monitored by recording current-time curve of enzymatically produced NADH at optimized conditions. The biosensor response was found linear over the range 0.010–2.0 mM glucose with detection limits of 6.0 and 4.0 μM for amperometric and photoamperometric methods, respectively. The relative standard deviations (n = 5) for 0.5 mM glucose were 5.8% and 3.8% for photoamperometric and amperometric results, respectively. The photoelectrochemical biosensor was successfully applied to the real samples. The results with this biosensor showed good selectivity, repeatability and sensitivity for monitoring glucose in amperometric and photoamperometric FIA studies.

1. Introduction

Recently, photoelectrochemical (PEC) sensors and biosensors, which uses light to induce electron transfer between photoelectrochemically active molecules, ions or materials and electrode for generating the detectable photocurrent signal, have been received considerable attention in analytical science and technology [1–24]. Because, PEC techniques have some significant advantages such as fast response, remarkable sensitivity, the design of simple, cheap and portable sensor system, easy integration, extension to light addressable sensors etc. [1–5]. As can be seen from previously published PEC sensor studies [1–24], organic material- and inorganic semiconductor nanoparticles-based PEC have been extensively used. Especially, semiconductor materials have found great attention due to their special electronic and photophysical properties, such as broad adsorption, narrow emission and high quantum yield [3,6–9,16–18,20–23]. Moreover, recently core-shell quantum dots (QDs) such as CdS-ZnS, CdSe-CdS or hybrid nanomaterial such as carbon nanotube-QDs, graphene-QDs etc. have been preferred instead of using only a QD or a nanomaterial in the electrochemical studies [3,16–18, 20–23]. Because core-shell QDs and hybrid nanomaterials submit better charge separation than a single QD and nanomaterial and therefore they are ideal candidates for photoelectrochemical sensor and biosensor applications due to their high quantum yield, photostability for photoelectrochemical studies, extremely large surface-to-atom ratio and sensitivity to surface ligands [3,25–27].

One of the important applications of PEC sensors is photoelectrocatalytic oxidation of NADH [12–18] and the construction of enzyme based photoelectrochemical biosensor dependent on NAD^+/NADH redox couple and dehydrogenase enzymes [19–24]. Recently, many researchers have focused on hybrid nanomaterial modified electrodes for this aim [16–18,20–23]. For example, dopamine sensitized nanoporous TiO_2 on indium tin oxide (ITO) electrode [16], poly(4,4′-diaminodiphenyl) sulfone/nano TiO_2 composite film modified ITO electrode [17] and graphene-TiO_2 nanohybrids modified glassy carbon electrode (GCE) [18] have been successfully used for the photoelectrocatalytic oxidation of NADH under visible light irradiation. These studies reported that the sensitivity of electrochemical determination of NADH was improved by irradiation of electrode surfaces. In addition, Jafari et al. studied electrocatalytic and photoelectrocatalytic oxidation of NADH using reduced graphene

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oxide/CdS-QDs/Poly-Nile Blue nanocomposite modified GCE [21] and electrogenerated chlorpromazine sulfoxide/grapheme/CdS-QD/Ionic liquid modified GCE [22] and also photoelectrochemical sensing of glucose [21] and ethanol [22] using glucose dehydrogenase alcohol dehydrogenase immobilized onto nanocomposite modified electrode surface, respectively. They reported that photoelectrochemical sensor is more sensitive than electrochemical sensor for detection of NADH and also glucose and ethanol at proposed nanocomposite electrodes. In another study, CdSe/ZnS QDs modified onto gold electrode by chemisorption via benzene dithiol and proposed electrode was used for photoelectrochemical sensing of NADH and also glucose [20]. The current signal was triggered by irradiation of electrode surface. Liu et al. developed a photoelectrochemical lactate biosensor using lactic dehydrogenase/multiwalled carbon nanotube (MWCNT)-TiO_2 nanoparticle composite/ITO electrode [23]. They reported that the PEC biosensor showed superiority over the electrochemical biosensor in lactate detection, which could be attributed to the excellent biocompatibility and PEC performance of MWCN-TiO_2 nanoparticle composite.

Although hybrid or composite nanomaterials have been successfully used for the construction of photoelectrochemical biosensor studies, according to our search of the literature, electrochemical biosensing of glucose in flow injection analysis (FIA) system depending on GDH immobilized onto QDs modified electrodes has not been reported, yet. Only one study has been reported for photoelectrochemical glucose biosensing in FIA system using poly-hematoxyline modified GCE [24] as well as a few studies have been published for photoelectrocatalytic oxidation of NADH in FIA system [13,14]. In this study, ZnS-CdS/MWCNT nanocomposite modified GCE was proposed for photoelectrochemical sensing NADH and glucose. The novel statement of this study is that photoelectrochemical biosensing of glucose at GDH immobilized ZnS-CdS/MWCNT/GCE were performed in FIA system by using a home-made photoelectrochemical flow cell which was designed for GCE in our previous studies [10,11,13,14,24]. Thus, this study proposes a combination of QDs, MWCNT, photoelectrochemistry and FIA for photoelectrochemical biosensing of glucose.

This offers advantages such as: i) a sensitive and selective biosensor due to the properties of MWCNT, ii) fast and economical analysis (FIA achieves faster analysis at reduced cost because of lower consumption of reactant), iii) superior immobilization of GDH and construction of a photoelectrochemical biosensor with good selectivity and sensitivity due to the unique functions of QDs and MWCNT, and iv) sensitivity of photoelectrochemistry.

2. Experimental

2.1. Chemicals

Glucose dehydrogenase (from *Pseudomenas* sp. 338.7 U/mg), β-nicotineamide adenine dinucleotide sodium salt (from *Saccharomyces cerevisiae*, $C_{21}H_{26}N_7NaO_{14}P_2$, $NaNAD^+$, MW: 685.41 g/mol), bovine serum albumin (BSA), glutaraldehyde (GA, d: 1.061 g/mL, MW: 100.12 g/mol, 25% w/w in water),D-(+)-glucose, reduced β-nicotinamide adenine dinucleotide disodium salt (MW: 709.40 g/mol $C_{21}H_{27}N_7Na_2O_{14}P_2$, $NADHNa_2$) KCl, H_3PO_4, NaH_2PO_4, Na_2HPO_4, CH_3OOH, NaOH, HCl, mercapto acetic acid (MAA), $Na_2S_2O_3$, $ZnCl_2$, sodium salt of EDTA and $CdCl_2 \cdot 2.5H_2O$ were supplied from Merck, Sigma or Carlo Erba, multiwalled carbon nanotube (MWCNT) was supplied from Dropsens.

The stock solutions of glucose (1.0 M) and NAD^+ (10.0 mM) were freshly prepared with deionized water daily. The stock solution of NADH (5.0×10^{-2} M) was daily prepared in the pH 7.0 phosphate buffer solution (PBS). The concentration of NADH in the diluted solutions was checked by using a Perkin Elmer Lambda 35 UV–VIS Spectrometer. The absorbance of the solution was monitored at 340 nm considering a molar extinction coefficient of 6600 L/mol·cm [28].

2.2. Apparatus

All the solutions were prepared with ultrapure water from Elga Option Q7B water purification system (18.2 MΩ cm). All electrochemical experiments were carried out using an Autolab PGSTAT 128N Potentiostat/Galvanostat equipped with a FRA2 frequency response analyzer. A traditional three-electrode system was used with a platinum wire as the counter electrode, an $Ag/AgCl/KCl_{(sat.)}$ as the reference electrode, and a MWCNT modified GCE as the working electrode. Cyclic voltammograms and electrochemical impedance curves were recorded in a static cell while amperometric experiments were performed in a FIA system. The home-made photoelectrochemical flow cell, which was constructed from TEFLON for GCE [10, 11, 13 14, 24], was used. The pH values of the solutions were adjusted using a HI 221 Hanna pH-meter with a combined glass electrode (Hanna Instrument HI-1332). In order to perform FIA experiments, an eight-channel Ismatec, Ecoline peristaltic pump with polyethylene tubing (0.75 mm i.d.), and a Rheodyne 8125 sample injection valve were used. In order to perform the photoelectrochemical experiments, a fiber optic illuminator 250 W halogen bulb with Foi-5 Light Guide (Titan Tool Supply Inc., USA) was used to illuminate the electrode surface. A Bandelin Sonorex RK 100H Ultrasonic bath was used for cleaning procedure of the GCEs before their modification.

2.3. Preparation of the modified electrodes and the glucose biosensor

GCE (3 mm diameter) were polished with 1.0-, 0.3- and 0.05-μm alumina slurry, respectively. After rinsing thoroughly with deionized water, all polished GCEs were sonicated in absolute ethanol and deinozed water for about 5 min., respectively. MWCNTs were functionalized by sonicating in a mixture of concentrated HNO_3 and H_2SO_4 (v/v 1:3) for 1 h followed by extensive washing in deionized water until — filtrate was neutral. Then, a suspension of 1.0 mg/mL MWCNT was prepared into DMF and 10.0 μL of this suspension was dropped onto GCE and dried under IR lamp for 10 min. ZnS-CdS were electrodeposited onto MWCNT/GCE according to previous reports with small modifications [29,30]. Briefly, the MWCNT/GCE immersed into pH 6.0 PBS containing 15.0 mM $CdCl_2$, 8.0 mM $Na_2S_2O_3$, 8.0 mM, EDTA and 0.05 mM MAA, which was used minimizing of coagulation of QDs [9], for electrochemical deposition with a deposition potential of −1.00 V vs. Ag/AgCl for 1000 s at 30 °C. After CdS was prepared on MWCNT/GCE, similarly, the ZnS were prepared on CdS/MWCNT/GCE in the same conditions but in 15 mM $ZnCl_2$. The modified electrode will be hereafter designated as CdS-ZnS/MWCNT/GCE.

GDH enzyme was immobilized onto modified electrode by the cross-linking procedure. Typically, 1.0% BSA was mixed with GDH (20.0 mg/mL in PBS, pH 7.0) in a volume ratio of 1 to 1, and then 5.0 μL of this mixture was mixed with 4.0 μL of 20.0 mM glutaraldeyde as crosslinking reagent. CdS-ZnS/MWCNT/GCE was immersed into the final solution for 1 h and then dried for 10 min. at +4 °C. Finally, obtained GDH/CdS-ZnS/MWCNT/GCE was stored at +4 °C.

2.4. Electrochemical procedure

In order to characterize modified electrode, electrochemical impedance spectra of MWCNT/GCE and QD modified MWCNT/GCE were recorded in pH 7.0 PBS containing 10.0 mM $K_3Fe(CN)_6$, 10.0 mM $K_4Fe(CN)_6$ and 0.10 M KCl at the formal potential of 180 mV with a frequency, range of 150.000–1 Hz and a signal amplitude of 5 mV (Fig. 1). The surface morphologies of the bare GCE, MWCNT/GCE and QD modified MWCNT/GCE were also examined by recording their scanning electron microscope (SEM) images.

Electrochemical and also photoelectrochemical biosensing of glucose at GDH/CdS-ZnS/MWCNT/GCE were investigated using cyclic voltammetric techniques. Firstly, a cyclic voltammogram of