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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

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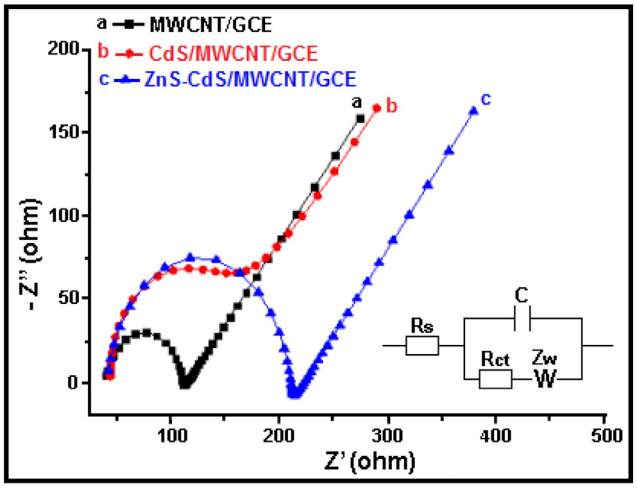

Fig. 1. Electrochemical impedance spectra of modified electrodes in 0.10 M KCl containing 10.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. (Inset: Electrochemical impedance circuit model and representative faradaic signal. Rs, solution resistance; Rct, charge transfer resistance; C, pure capacitance; W, Warburg impedance).

GDH/CdS-ZnS/MWCNT/GCE was recorded in 0.10 M PBS (pH 7.0) containing 10.0 mM NAD^+ in the potential range between −200 and 1000 mV vs. Ag/AgCl at a scan rate of 20 mV/s in the absence of glucose. To see the response of the biosensor toward glucose, cyclic voltammograms of modified electrodes were recorded under the same conditions but in the presence of 40.0 mM glucose. Cyclic voltammograms also were recorded for GDH immobilized onto MWCNT/GCE. Finally, photoelectrochemical biosensor studies were carried out under irradiation of the working electrode surface by a fiber optic illuminator with a 250 W halogen bulb.

2.5. Photoelectrochemical biosensor studies in the FIA system

Electrochemical and especially photoelectrochemical biosensor studies in FIA system were performed by using the home-made photoamperometric flow cell which was constructed for GCE and previous flow set up [10,11,13,14,24]. In all FIA experiments, 0.10 M PBS (pH 7.0) containing 1.0 M KCl was used as carrier solution. After GDH/ZnS-CdS/MWCNT/GCE had been inserted into the photoamperometric flow cell, the optimization studies were performed in FIA system. After a steady-state background current was obtained in optimum conditions (sample loop, flow rate, applied potential, length of tubing) the various concentrations of glucose including 10.0 mM NAD^+ were injected into the system (successive three injections) and the current–time curves were recorded. The current–time curves were also recorded for the photoamperometric FIA study by irradiation of the electrode surface throughout the experiment. All supporting electrolytes were deaerated by allowing highly pure argon to pass through for 5 min before the electrochemical experiments.

In order to show the practical applicability of the proposed biosensor, a real sample (commercial dextrose solution including 5% glucose) and a spiked serum sample were selected for determination of glucose as described in literature [24].

3. Results and discussion

3.1. Characterization of CdS-ZnS/MWCNT/GCE

Electrochemical impedance spectroscopy (EIS) is a useful technique providing detailed information on the impedance changes of the electrode surface which give useful information about the modification of the electrode surfaces as well as using electrochemical sensing applications [31]. Therefore, EI spectra of MWCNT/GCE, CdS/MWCNT/GCE and also ZnS-CdS/MWCNT/GCE were recorded in the dark in 10.0 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.10 M KCl (Fig. 1). While the semicircle diameter was about 450 Ω for bare GCE (data not shown), it was about 122 Ω for MWCNT/GCE (Fig. 1-a) indicating that the charge transfer was relatively facile at MWCNT modified GCE. However, the R_{ct} value slightly increased after CdS (about 180 Ω, Fig. 1-b) and also CdS-ZnS (about 210 Ω, Fig 1-c) were modified on the MWCNT/GCE surface indicating that the CdS and also ZnS on the MWCNT/GCE surface decreased the electron transfer rate between the redox probe and the electrode surface. Because QDs have semiconductor properties and their conductivities are lower than that of MWCNT/GCE. These results indicated that CdS and ZnS was electrochemically precipitated MWCNT/GCE surface.

The surface morphologies of the bare GCE, MWCNT/GCE, CdS/MWCNT/GCE and ZnS-CdS/MWCNT/GCE were examined also with a scanning electronic microscope (SEM). Fig. 2B shows SEM image of the MWCNT/GCE, which was different from that of the bare GCE (Fig. 2A). Fig.2C and 2D show SEM images of the CdS/MWCNT/GCE and ZnS-CdS/MWCNT/GCE, respectively which were different from that of the MWCNT/GCE (Fig.2B). All SEM images show that the prepared quantum dots were regular in shape and uniformly distributed onto MWCNT/GCE surface, though ZnS-CdS were coagulated, interpretable results were obtained for glucose biosensor.

3.2. Oxidation of NADH at CdS-ZnS/MWCNT/GCE

Before response of enzyme immobilized electrode toward biosensing of glucose, the electrochemical oxidation of NADH at QDs modified MWCNT/GCE has been investigated due to its significance both as a cofactor for dehydrogenase enzyme and its role in the electron transfer chain in biological system and also due to the need develop biosensor dependent on NAD^+/NADH redox couple and dehydrogenase enzymes. To check the electrochemical characteristic of modified electrode toward oxidation of NADH, cyclic voltammograms of the ZnS-CdS/MWCNT/GCE were recorded with/without irradiation of the electrode surface in the absence and presence of 2.0 mM NADH (Fig.3). While the start potential of NADH oxidation was observed at −25 mV and −160 mV without and with illumination of electrode surface respectively, the peak potential was observed at +80 mV for both conditions

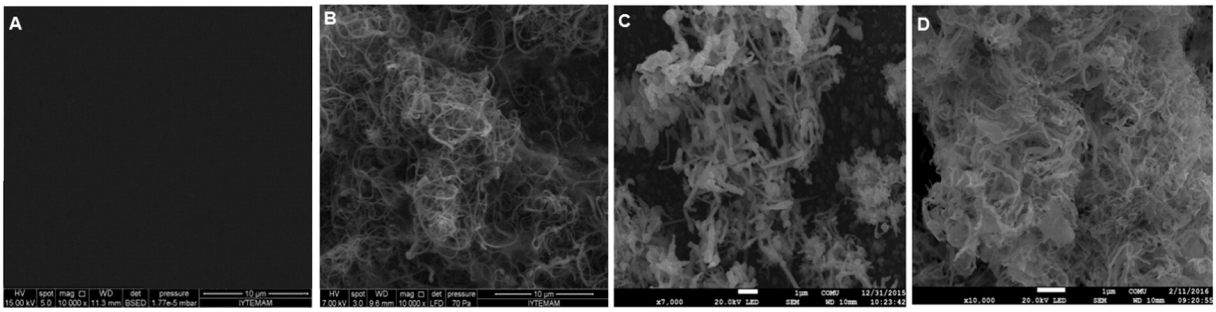

Fig. 2. SEM images of A) bare GCE, B) MWCNT/GCE, C) CdS/MWCNT/GCE D) ZnS-CdS/MWCNT/GCE.

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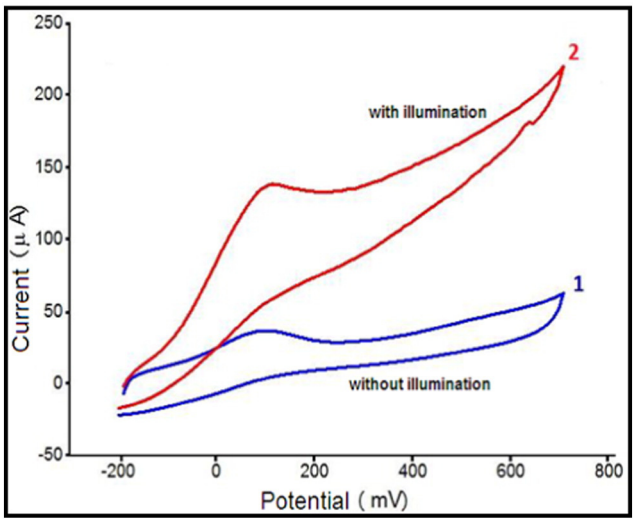

Fig. 3. Cyclic voltammograms of ZnS-CdS/MWCNT/GCE in the presence of 2.0 mM NADH. 1) without light, 2) with light. Supporting electrolyte: 0.10 M PBS (pH 7.0) containing 0.10 M KCl, scan rate: 20 mV/s.

(Fig. 3-1). Moreover, the peak current increased significantly under irradiation of electrode surface (Fig. 3-2). Therefore it can be said that ZnS-CdS/MWCNT/GCE showed a good photoelectrocatalytic effect toward the oxidation of NADH due to increasing of peak current.

After cyclic voltammetric experiments, both amperometric and photoamperometric currents vs. various concentration of NADH in FIA system were recorded in the optimized conditions (0.10 M PBS containing 1.0 M KCl (pH 7.0), applied potential: +150 mV; flow rate: 1 mL/min, sample loop: 100 μL; transmission tubing length: 10 cm) using home- made flow cell. Fig. 4 shows the current–time curves for the amperometric and photoamperometric FIA responses to various concentrations of NADH. Although the peak current was increased depending on NADH concentration for both the amperometric and the photoamperometric methods, the responses of the photoamperometric method were higher than that of the amperometric in all concentrations. A linear relationship between the NADH concentration and the peak current was obtained over the concentration range of 0.030–1.0 mM by the amperometric and also photoamperometric FIA method, at the ZnS-CdS/MWCNT/GCE. The linearity of these methods is described by the equations $I(\mu A) = 20.26C\ (mM) + 0.17\ R^2 = 0.9941$ and $I(\mu A) = 37.16C\ (mM) + 0.44$, $R^2 = 0.9976$ for amperometric and photoamperometric studies, respectively, where I is peak current, C is the NADH concentration, and R is the regression coefficient. As these equations are compared in terms of their slopes, it is obvious that the sensitivity of the photoelectrocatalytic FIA procedures better than that of the amperometric method and the ratio of improvement is at about 2 folds.

3.3. Biosensing of glucose at GDH/CdS-ZnS/MWCNT/GCE

Firstly, the electrochemical and photoelectrochemical responses of dehydrogenase immobilized onto ZnS-CdS/MWCNT/GCE toward glucose were investigated by cyclic voltammetry. Cyclic voltammograms of GDH/ZnS-CdS/MWCNT/GCE in the absence and in the presence of 40.0 mM glucose were shown in Fig. 5. It can be seen that no peak was observed (Fig. 5a) in the absence of glucose, however the capacitive current was changed very little under irradiation of electrode surface (Fig. 5b). In the presence of 40.0 mM glucose, the oxidation of the NADH, which was formed from enzymatic reaction between NAD^+ and glucose catalyzed by GDH, was observed at about 500 mV (Fig. 5c). When the surface of GDH/ZnS-CdS/MWCNT/GCE was irradiated (Fig. 5d), the irreversible peak current was significantly increased. These results show that GDH/ZnS-CdS/MWCNT/GCE can be successfully used for photoelectrochemical biosensing of glucose due to increasing of oxidation peak current of enzymatically produced NADH under irradiation of electrode surface even acceptable shifting in oxidation potential was not observed.

Photoelectrochemical biosensor mechanism for overall current was shown in Fig. 6. Photelectrochemical sensors consist of some steps: i) immobilization of QD onto an electrode ii) illumination of electrode surface and iii) generation of photocurrent which depends on the type and concentration of the respective analyte in the supporting electrolyte. When QDs modified MWCNT/GCE surface is illuminated by a light source, photoexcitation of the semiconductor quantum dots yields an electron–hole pairs in the conduction- and the valence-band levels [3]. Electrons of conduction band transfer to MWCNT/GCE surface and at the same time electron donors in the solution (enzymatically produced NADH) transfer their electron to the hole of valance band and oxidized to NAD^+, an anodic photoelectrocatalytic current is generated

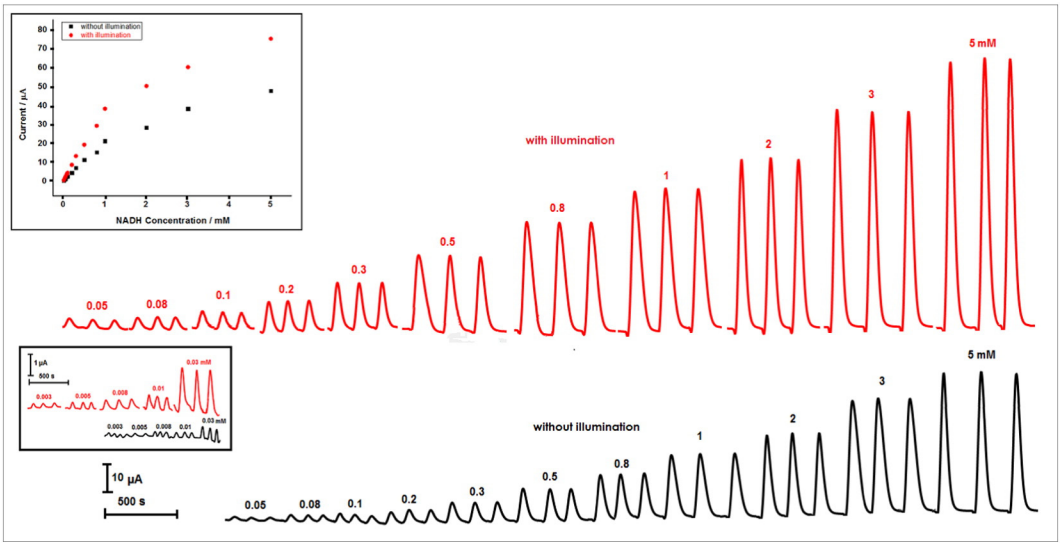

Fig. 4. Current-time curves of NADH with different concentrations using ZnS-CdS /MWCNT/GCE in FIA system for amperometric and photoamperometric methods. (Carrier stream: 0.10 M PBS (pH 7.0) containing 1.0 M KCl, Applied potential: +0.15 V; Flow rate: 1.0 mL/min, sample loop: 100 μL; transmission tubing length: 10 cm). Inset: Plot of peak current versus glucose concentration.

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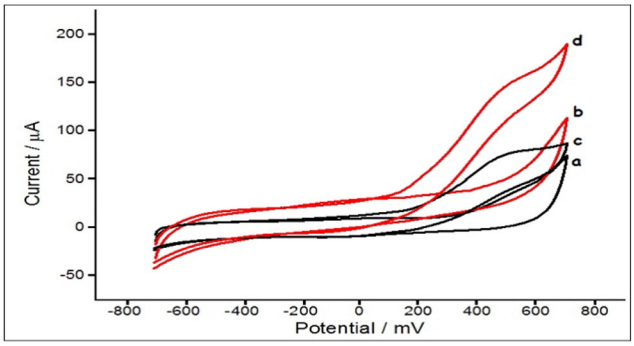

Fig. 5. Cyclic voltammograms of GDH/ZnS-CdS/MWCNT/GCE in the absence (a, b) and in the presence of 40.0 mM glucose (c, d). a and c: without light, b and d: with light. (Supporting electrolyte: 0.10 M PBS (pH 7.0) containing 0.10 M KCl, 10.0 mM NAD^+ scan rate: 20 mV/s.

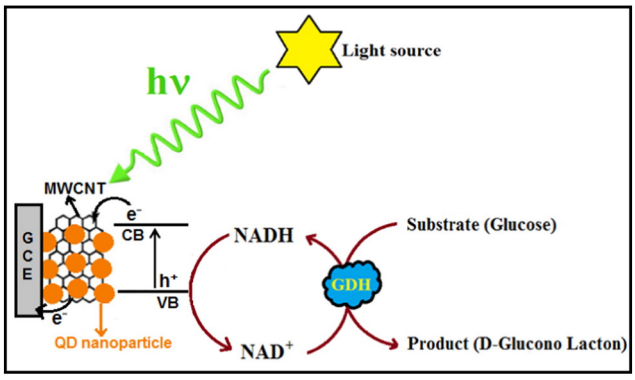

Fig. 6. Schematic representation of the photoelectrochemical glucose biosensor at GDH/ZnS-CdS/MWCNT/GCE.

dependent on glucose concentration. Same mechanism was also presented for glucose, alcohol and lactate biosensor based on dehydrogenase enzyme, NADH/NAD^+ redox couple and quantum dot nanocomposite electrode [21–23].

After cyclic voltammetric studies, photoelectrochemical biosensing of glucose was also investigated using amperometric technique in FIA system. To establish that a reliable analytical response could be achieved for the glucose, under optimized conditions (Carrier stream: 0.10 M PBS containing 1.0 M KCl (pH 7.0), applied potential: +400 mV; flow rate: 0.6 mL/min, sample loop: 100 μL; transmission tubing length: 10 cm) using a GDH/ZnS-CdS/MWCNT/GCE, a calibration study was carried out over the range from 0.010 mM to 2.0 mM glucose concentration, with two injections of each concentration. Fig. 7 shows the diagrams for amperometric and photoamperometric FI responses to various concentrations of glucose. Although the peak currents increased depending on glucose concentration for both the amperometric and the photoamperometric methods, the responses of photoamperometric method were higher than those of amperometric in all concentrations. A linear relationship between the glucose concentration and the peak current was obtained over the concentration range from 0.010 to 2.0 mM glucose for both the photoamperometric and amperometric FIA method at the glucose biosensor. The linearity of these methods were described by the equations $I(\mu A) = 2.57C\ (mM) + 0.033$, $R^2 = 0.9971$, and $I(\mu A) = 7.80C\ (mM) + 0.049$, $R^2 = 0.9985$ for amperometric and photoamperometric studies respectively, where I is the peak current and C is the concentration of glucose. When these equations are compared in terms of their slopes, it is clear that the sensitivity of the photoelectrocatalytic FIA procedure is better than that of the amperometric method and the ratio of improvement is about 3.0 folds.

The limit of detection (LOD) was calculated as 6.0 μM and 4.0 μM glucose for amperometric and photoamperometric glucose biosensors, respectively, based on $3s_b/m$ where s_b is the standard deviation of the blank response and m is the slope of the calibration curve.

The precision of electrochemical and photoelectrochemical biosensor was investigated by making 5 repeat injections of 0.5 mM glucose solution. The RSD for electrochemical and photoelectrochemical biosensors were calculated to be 3.8% and 5.8% respectively. These results indicate GDH/ZnS-CdS/MWCNT/GCE has very good repeatability for electrochemical and photoelectrochemical biosensing of glucose.

The various analytical parameters, such as electrode type, electrochemical technique, linearity ranges, sensitivity, detection potential, and LOD values obtained from proposed methods were compared with other enzyme modified electrodes in previously-published reports for the photoelectrochemical biosensing of glucose

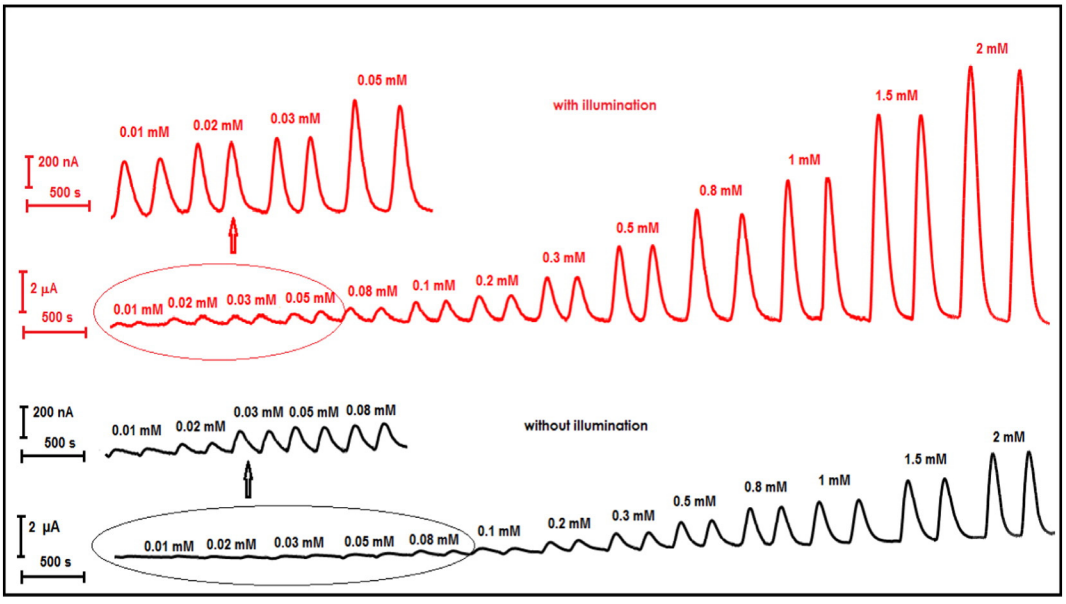

Fig. 7. Current-time curves of glucose with different concentrations using GDH/ZnS-CdS/MWCNT/GCE in FIA system for amperometric and photoamperometric methods. (Carrier stream: 0.10 M PBS (pH 7.0) containing 1.0 M KCl, 10.0 mM NAD^+, applied potential: +0.4 V; Flow rate: 0.6 mL/min, sample loop: 100 μL; transmission tubing length: 10 cm).

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Table 1
Comparison of analytical parameters obtained from GDH/ZnS-CdS/MWCNT/GCE with different electrodes in the literature for photoelectrochemical biosensing of glucose dependent on GDH and NAD^+/NADH redox.

Electrode type	Method	DP[a]	Sensitivity	LR[b]	LOD[c]	Ref.[d]
TH cross-linked MWNTs[e] and Au NPs multilayer functionalized ITO[f] electrode	Amp.[g] in the dark	0.2 V vs. Ag/AgCl (sat.KCl)	7.8 μA/mM	10 μM–2.56 mM	5.0 μM	19
	Amp under irradiation		8.5 μA/mM	1 μM–3.25 mM	0.7 μM	
CdSe/ZnS nanocrystals (QDs) modified Au electrode by chemisorption via a dithiol compound	Amp. under irradiation	0.05 V vs. Ag/AgCl (sat.KCl)	–	Upto 1 mM	–	20
Reduced graphene oxide-cadmium sulfide quantum dots/poly-nile blue nanocomposite	Amp. without irradiation	−0.02 V vs. Ag/AgCl (sat.KCl)	0.4407 μA/cm^2. mM[1]	Upto 20 mM	–	21
	Amp under irradiation		0.7276 μA/cm^2. mM			
Poly-hematoxylin modified GCE[h]	FIA[i] Amp. without irradiation	0.3 V vs. Ag/AgCl	0.76 μA/mM	10–1000 mM	3.0 μM	24
	FIA Amp. with irradiation		1.9 μA/mM	5–1000 mM	1.5 μM	
QDs (ZnS-CdS) modified MWCNT/GCE	FIA Amp. without irradiation	0.4 V vs. Ag/AgCl	2.57 μA/mM	0.01–2.0 mM	6.0 μM	This work
	FIA Amp. with irradiation		7.80 μA/mM	0.01–2.0 mM	4.0 μM	

[a] Detection potential.
[b] Linearity range.
[c] Limit of detection.
[d] Reference.
[e] Multi-walled carbon nanotube.
[f] Indium tin oxide.
[g] Amperometry.
[h] Glassy carbon electrode.
[i] Flow injection analysis.

dependent on NAD^+/NADH redox couple and dehydrogenase enzyme (Table 1). As can be seen from Table 1, the sensitivity of proposed photoamperometric method was found to be 7.46 μA/mM, which is better than that of some previously reported biosensors [21,24] and however it is lower than that of reference of [19]. On the other hand, the detection potential of glucose at proposed electrode is higher than previously reported modified electrodes [19–24]. Therefore, it can be said that CdS-ZnS modified MWCNT/GCE is a very useful electrode material for immobilization of GDH and photoelectrochemical biosensing of glucose in FIA system.

3.4. Interference studies

The effects of common interfering species such as ascorbic acid (AA), uric acid (UA), dopamine (DA), L-cysteine (L-Cyst), galactose, saccharose, glutamic acid, which may affect the response of electrode were investigated. Amperometric responses of GDH/ZnS-CdS/MWCNT/GCE toward glucose (0.5 mM) in FIA system under optimized conditions were recorded in the presence of these interference species. Experimental results show that no significant change of oxidation peak current could be observed for saccharose, galactose and glutamic acid at the concentration one hundred fold and for L-cysteine at equimolar as high as that of glucose (data not shown), which shows the good selectivity of the enzyme electrode in the presence of other monosaccharides and also disaccharides. However, equimolar of AA, DA and UA with glucose concentration showed serious interference (increased the oxidation current), because their oxidation potentials were close to that of NADH. It is reported that the interference of AA can be eliminated and enhance the biosensor selectivity toward glucose by using nafion as an over layer cost coated at the biosensor surface or using ascorbate oxidase [32,33]. Another possible way of removing of interfering effect of all these compounds is that very small amount of lead(IV) acetate as an oxidizing agent can be added to nafion layer due to preoxidation reaction of these interfering compounds before they reach the electrode surface [34].

3.5. Real sample analysis

One real sample (commercial dextrose solution) and one spiked serum sample were used for determination of glucose at GDH/CdS-ZnS/MWCNT/GCE as described in literature [24]. For this, 250.0 μL of spiked serum samples were diluted to 5.0 mL with 0.10 M PBS (pH 7.0) containing 10.0 mM NAD^+ and 1.0 M KCl. Glucose detection was performed by spiking a known volume and concentration of glucose standard solution into the diluted serum samples in order to obtain various concentrations, and by measuring of the amperometric and photoamperometric response of electrode in FIA system. For the determination of glucose in commercial dextrose solution (containing 5% glucose), it was diluted 555 times (about 0.5 mM glucose) with 0.10 M PBS (pH 7.0) containing 10.0 mM NAD^+ and 1.0 M KCl. Glucose detection was also performed for this sample as described spiked serum sample. The results for the recovery test were given in Table 2. It can be seen that acceptable recoveries were obtained for spiked glucose in serum plasma and commercial dextrose solution samples.

4. Conclusions

In this study, the constructing photoelectrochemical glucose biosensor dependent on NAD^+/NADH redox couple and GDH in FIA system was proposed using enzyme modified MWCNT/GCE. Photoelectrochemical biosensors dependent on NAD^+/NADH redox couple-dehydrogenase enzymes have been reported in previous studies [19–24] and one of them is for photoelectrochemical glucose biosensing

Table 2
The results of glucose determination in real samples and a dextrose solution (n = 3).

Sample	Glucose (mM)	FIA amperometric			FIA photoamperometric		
		Found (mM)	Recovery	RSD%	Found (mM)	Recovery	RSD%
Spiked serum	0.25	0.22 ± 0.015	88	6.8	0.25 ± 0.050	100	2.3
	0.50	0.48 ± 0.015	96	3.1	0.49 ± 0.010	98	2.0
	0.75	0.76 ± 0.010	103	1.3	0.74 ± 0.010	99	1.3
Dextrose solution	Labeled claim (%5 = 277.5 mM)	266.6 ± 4.6	–	–	261.1 ± 4.0	–	–

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in FIA system using poly-hematoxyline modified GCE [24]. However, according to our search of the literature, the uses of QDs modified MWCNT/GCE has not been reported for photoelectrochemical biosensing of glucose in FIA system yet. GDH/CdS-ZnS/MWCNT/GCE exhibited a good photoelectrocatalytic response for the detection of glucose and a linear range was obtained between 0.010 and 2.0 mM glucose with a detection limit of 4.0 μM. The sensitivity of the photoamperometric biosensor in FIA system was improved about two folds in compared with that of amperometric procedure. It can be concluded that GDH/CdS-ZnS/MWCNT/GCE can be successfully used for photoelectrochemical biosensing of glucose due to increasing of oxidation peak current under irradiation of electrode surface. As a result, a novel, fast and facile sensor was reported in this study which is expected to offer new prospects for the development of sensitive, selective and faster photoelectrochemical biosensors in the FIA system.

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