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Photoelectrochemical glucose biosensor based on dehydrogenase enzyme and NAD^+/NADH redox couple using quantum dot modified pencil graphite electrode

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Abstract

A simple, disposable and economical modified electrode was prepared by electrodeposition of hybrid quantum dot (ZnS-CdS) onto Pencil Graphite Electrode (PGE) surface and following immobilization of glucose dehydrogenase (GDH) onto quantum dot modified electrode (GDH/ZnS-CdS/PGE). The proposed electrode was effectively used for the photoelectrochemical determination of glucose in Flow Injection Analysis (FIA) system using a new home-made flow cell which was designed for PGE for the first time. Results from cyclic voltammetric and FI amperometric measurements have revealed that GDH/ZnS-CdS/PGE is capable of signaling photoelectrocatalytic activity towards NADH when the surface of GDH/ZnS-CdS/PGE was irradiated with a light source with fiber optic cable (250 W Halogen lamp). The currents of NADH produced by enzymatic reaction in the photoamperometric FIA system at optimized conditions (Carrier stream: 0.1 M phosphate buffer solution (pH 7.0) containing 1.0 M KCl and 10.0 mM NAD^+, applied potential: +0.8 V vs. Ag/AgCl/KClsat; flow rate: 0.6 $mL.min^{-1}$, sample loop: 100 μL; transmission tubing length: 10 cm) were linearly correlated with the glucose concentration. The calibration curves were obtained for glucose concentrations in a range from 0.2 to 8.0 mM. The detection limits were found to be 0.09 and 0.05 mM for amperometric and photoamperometric methods, respectively. The relative standard deviations (n=7) for 0.5 mM glucose were 4.5% and 3.5% for photoamperometric and amperometric results respectively. The photoelectrochemical biosensor was applied to the real samples successfully. The results with this biosensor showed good selectivity, repeatability and sensitivity for monitoring glucose in amperometric and photoamperometric FIA studies.

Keywords: Glucose dehydrogenase, CdS-ZnS quantum dots, glucose biosensor, flow injection analysis, photoelectrochemical sensor, electrocatalytic oxidation of NADH.

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INTRODUCTION

Semiconductor quantum nanoparticles (QNPs) have found great attention in the electrochemical and optical sensors/biosensor studies due to their special electronic and photophysical properties, such as broad adsorption, narrow emission and high quantum yield (1-5). Especially, an increasingly growing field of electrochemical sensing studies is the construction of photoelectrochemical sensors/biosensors based on quantum dots (QDs) modified electrodes which exhibit a powerful way for the detection of chemical/biochemical molecules compared to other sensor types.[1,2] The reason can be explained by some significant advantages of QD-based photoelectrochemical sensors such as fast response, remarkable sensitivity, the design of simple, cheap and portable sensor system, easy integration, extension to light addressable sensors etc. [2, 6, 7] Photoelectrochemical 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 electrode 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. If the electrons of electrode transfer to the hole of valence band of QDs and the electrons in conduction band transfer to electron acceptor (oxidant molecules) in the surrounding solution simultaneously, thus a cathodic photocurrent is generated. On the contrary, if electrons of conduction band transfer to electrode and at the same time electron donors in the solution transfer their electron to the hole of valance band, an anodic photocurrent is generated. If the analytes behave as electrons donor/acceptor or reacted with the electron donor/acceptor in the solution, the photocurrent of electrode can be monitored dependent on analyte concentration. Based on this principle many electrochemical photoelectrochemical sensors and biosensors have been developed for the sensitive detection of biologically, environmentally important molecules.[2, 6, 7]

One of the important applications of semiconductor nanoparticles is photoelectrocatalytic oxidation of NADH.[8-13] and the construction of enzyme based electrochemical and photoelectrochemical biosensor dependent on NAD^+/NADH redox couple and dehydrogenase enzymes.[11-14] Moreover, recently core-shell QDs or QNPs such as CdS-ZnS, CdSe-CdS or hybrid nanomaterial such as carbon nanotube-QDs or QNPs, graphene-QDs or QNPs etc. have been preferred instead of using only a QD or a nanomaterial in the electrochemical studies.[2, 8-14] Because core-shell QDs or hybrid nanomaterials submit better charge separation than a single QD or nanomaterial and therefore they are ideal

candidates for sensing and biosensing applications due to their high quantum yield, photostability for photoelectrochemical studies, extremely large surface-to-atom ratio and sensitivity to surface ligands.[2, 15-17] For example, dopamine sensitized nanoporous TiO_2 on indium tin oxide (ITO) electrode[8], poly(4,4'-diaminodiphenyl sulfone/nano TiO_2 composite film modified ITO electrode[9] and graphene-TiO_2 nanohybrids modified glassy carbon electrode (GCE)[10] have been successfully used for the photoelectrocatalytic oxidation of NADH under visible light irradiation. In addition, Jafari *et al.* studied electrocatalytic and photoelectrocatalytic oxidation of NADH using reduced graphene oxide/CdS-QDs/Poly-Nile Blue nanocomposite modified GCE and also photoelectrochemical sensing of glucose using glucose dehydrogenase immobilized onto nanocomposite modified electrode surface.[11] They reported that photoelectrochemical sensor is more sensitive than electrochemical sensor for detection of NADH and also glucose at proposed nanocomposite electrodes. In another study, CdSe/ZnS hybrid QDs modified onto gold electrode by chemisorption via benzene dithiol was used for photoelectrochemical sensing of NADH and also glucose.[13] The current signal was triggered by illumination of electrode surface.

In this study, ZnS-CdS hybrid QNPs modified pencil graphite electrode (PGE) was proposed for photoelectrochemical sensing of NADH and glucose for the first time. When compared with the other carbon based electrodes, PGEs have the same advantages such as the high electrochemical reactivity, commercial availability, good mechanical rigidity, disposability, lower cost, lower technology and the ease of modification.[18-30] It was reported that the pencil lead electrodes offer a renewable surface which is simpler and faster than polishing procedures, in common with the solid electrodes, provide useful and reproducible results for the individual surfaces.[18] Thus PGEs have been extensively used in various electroanalytical studies due to the useful properties of PGEs.[18-30] In addition, the novel statement of this study is that photoelectrochemical biosensing of glucose at glucose dehydrogenase(GDH) immobilized onto ZnS-CdS/PGE were performed in FIA system by using new home-made photoelectrochemical flow cell which was designed for PGE for the first time. According to our search of the literature, photoelectrochemical biosensing of glucose in FIA system depending on GDH immobilized onto QDs modified PGE has not been reported yet, although ZnS-CdS modified PGE has been used for the electrochemical biosensing of glucose based on glucose oxidase (GOD) enzyme in FIA system system by using new home-made photoelectrochemical flow cell[31]. The superiority of the proposed study is the construction of photoelectrochemical biosensor in FIA system using GDH modified PGE compared with the study of reference 31, in which only electrochemical

biosensor was constructed. Thus, this study submits a combination of QDs, PGE, photoelectrochemistry and FIA for photoelectrochemical biosensing of glucose which offers some advantages such as i) a disposable, practical, easy-to-use and low cost biosensor due to the useful properties of PGE, ii) fast and economic analysis (FIA exhibits fast analysis and lower cost because of lower consumption of reactant) and iii) a very well immobilization of GDH, construction of photoelectrochemical biosensor with a good selectivity and sensitivity due to the unique functions of QDs and advantages of photoelectrochemistry.

RESULTS AND DISCUSSION

Characterization of CdS-ZnS/MAA/PGE

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.[32] Therefore, EI spectra of bare and QD modified PGEs were recorded in 10.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ containing 0.10 M KCl (Fig. 1A). The semicircle diameter of pretreated bare PGE (about 222 ohm, Fig. 1A-A) was very small, indicating that the charge transfer was relatively facile at bare electrode. However, the Rct value slightly increased, after CdS (about 600 ohm, Fig 1A-B) and also CdS-ZnS (about 900 ohm, Fig 1B-C) was modified on the PGE surface indicating that the CdS and also ZnS on the PGE 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 bare PGE. These results indicated that CdS and ZnS were electrochemically precipitated onto pretreated PGE surface.

Cyclic voltammograms of each electrode were also recorded in 10.0 mmol L^{-1} $[Fe(CN)_6]^{3-/4-}$ containing 0.10 M KCl (Fig. 1B). It can be seen that the anodic (+0.288 V) and cathodic (+0.156 V) peaks of Fe^{3+}/Fe^{2+} redox couple recorded with bare PGE were clear and sharp. The anodic peak potentials of QDs modified electrodes were shifted to more positive values (+0.350 V and +0.355 V for CdS/PGE and CdS-ZnS/MAA/PGE respectively), while the cathodic peak potentials of that were shifted to more negative values (+0.140 V for both CdS/PGE and CdS-ZnS/MAA/PGE), also the peak currents of each electrode were decreased compared with bare PGE. The shifting in peak potentials can be attributed to the semiconductor surface properties of QDs.

The surface morphologies of the bare pretreated PGE, CdS/MAA/PGE and CdS-ZnS/MAA/PGE were examined also with a scanning electronic microscope (SEM). Fig. 2B and 2C show SEM image of Cds/MAA/PGE and CdS-ZnS/MAA/PGE, respectively which were different from that of the pretreated PGE (Fig. 2A). A generally porous structure was observed in the SEM image of CdS/MAA/PGE and CdS-ZnS/MAA/PGE and a number of spongy sites were evident on the formation of the QDs. Although ZnS-CdS were coagulated, interpretable results were obtained for glucose biosensor.

Oxidation of NADH at CdS-ZnS/MAA/PGE

Before response of enzyme immobilized electrode toward biosensing of glucose, the electrochemical oxidation of NADH at QDs modified PGE 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 bare PGE and QDs modified PGE were recorded with/without irradiation of the electrode surface in the absence and presence of 2.0 mM NADH (Fig. S1 and S2). In the cyclic voltammograms of pretreated PGE in the absence of NADH, no peaks were observed and irradiation of electrode surface did not affect the voltammogram (Inset of Fig. S1). However, the cyclic voltammogram of CdS-ZnS/PGE in the pH 7.0 PBS was changed under irradiation of electrode surface (Inset of Fig. S2). Moreover, NADH was oxidized at about + 520 mV (Fig. S1-1) and +500 mV (Fig. S2-1) at the bare PGE and CdS-ZnS/PGE, respectively. While a little increment in the oxidation peak current of NADH was observed by irradiation of electrode surface (Fig.S1-2) at bare PGE, the peak current increased significantly (about from 16 μA (without light) to 58 μA (under irradiation, Fig. S2-2)). Therefore it can be said that CdS-ZnS/PGE showed photoelectrocatalytic effect toward the oxidation of NADH due to increasing of peak current even acceptable shifting in oxidation potential was not observed.

In order to obtain the best amperometric and photoamperometric response of CdS-ZnS/PGE towards NADH in FIA system, the effects of the applied potential and flow rate, on the current of 0.10 mM NADH were investigated by recording current–time curves. The results were presented in Figures between S3 and S5 with the plot of peak current versus applied potential and flow rate. From optimization studies, the applied potential, the flow rate, the

sample volume, and the transmission tube length were determined as +600 mV vs. Ag/AgCl, 1.75 mL min^{-1}, 100 μL and 10 cm, respectively. Both amperometric and photoamperometric currents vs. various concentration of NADH were recorded using CdS-ZnS/PGE in these optimum conditions. Fig. S6 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. The inset of Fig. S7 shows a plot of catalytic current vs. NADH concentration. From this figure, a linear relationship between the NADH concentration and the peak current was obtained over the concentration range 8.0×10^{-7}–8.0×10^{-5} M by the amperometric and also photoamperometric FIA method, at the CdS-ZnS/PGE. The linearity of these methods is described by the equations I(μA)= 21.11C (mM) + 0.075 R^2=0.9997 and I(μA) = 38.99C(mM) + 0.058, R^2=0.9991 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 twice fold.

Biosensing of glucose at GDH/CdS-ZnS/MAA/PGE

Firstly the electrochemical and photoelectrochemical responses of dehydrogenase immobilized PGE (GDH/PGE without CdS-ZnS) toward glucose were investigated by cyclic voltammetry. Cyclic voltammograms of GDH/PGE were recorded in 0.10 M PBS (pH 7.0) containing 0.10 M KCl and 10.0 mM NAD^+ in the absence and in the presence of 40 mM glucose at 20 mV s^{-1} scan rate (Fig. 3A). In the first voltammogram (Fig. 3A/a), an irreversible peak was observed at about 720 mV at GDH/PGE, which was attributed to oxidation of enzymatically produced NADH to NAD^+ (reactions 1 and 2). When the electrode surface was irradiated with the light source, the peak current increased a little (Fig. 3A/b).

Cyclic voltammograms of GDH/CdS-ZnS/MAA/PGE in the absence and in the presence of 40 mM glucose were also shown in Fig 3. It can be seen that no peak was observed (Fig. 3B/a1) in the absence of glucose, however the capacitive current was changed very little under irradiation of electrode surface (Fig. 3B/a2). In the presence of 40 mM glucose, the

oxidation of the NADH, which was formed from enzymatic reaction between NAD^+ and glucose catalyzed by GDH, was observed at about 720 mV (Fig. 3B/b1). When the surface of GDH/CdS-ZnS/MAA/PGE was irradiated (Fig. 3B/b2), the irreversible peak current was significantly increased. These results show that GDH/CdS-ZnS/PGE can be successfully used for photoelectrochemical biosensing of glucose due to increasing of oxidation peak current under irradiation of electrode surface even acceptable shifting in oxidation potential was not observed.

After cyclic voltammetric studies, photoelectrochemical biosensing of glucose was also investigated using amperometric technique in FIA system. In order to obtain the best amperometric and photoamperometric response of GDH/CdS-ZnS/MAA/PGE toward glucose in FIA system, the effect of the applied potential and flow rate on the current of 0.5 mM glucose containing 10.0 mM NAD^+ and 1.0 M KCl was investigated by recording fiagrams which were presented in figures between S8 and S10 with the plot of peak current versus applied potential and flow rate. The best currents for electrocatalytic and photoelectrocatalytic oxidation of NADH produced by enzymatic reaction of glucose in FIA system were found at about 800 mV. In addition, the current values obtained from the photoamperometric method were about 50-40% higher than that obtained from amperometric method. Thus an applied potential of 800 mV was selected as optimum potential value. The maximum peak current was observed at the flow rate of 0.6 mL min^{-1} since biosensors and photoelectrochemical biosensors could find enough time for the occurrence of enzymatic reaction and also photoexcitation of mediator in the low flow rate. The peak currents decreased by increasing the flow rate after 0.6 mL min^{-1}. Thus, the flow rate of 0.6 mL min^{-1} was selected as optimum flow rate even though sample frequency is very low.

To establish that a reliable analytical response could be achieved for the glucose, under optimized conditions using a GDH/CdS-ZnS/MAA/PGE, a calibration study was carried out over the range from 0.2 mM to 30 mM glucose concentration, with two injections of each concentration being made via a 100 μL sample loop. Figure 4 shows the fiagrams 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.

Figure 5A shows a plot of catalytic current versus glucose concentration. From this figure, a linear relationship between the glucose concentration and the peak current was obtained over the concentration range from 0.2 to 8.0 mM glucose for both the

photoamperometric and amperometric FIA method at the glucose biosensor (Fig. 5B). The linearity of these methods were described by the equations I(μA)= 0.118C (mM) + 0.052, R^2=0.9923, and I(μA) = 0.245C (mM) + 0.089, R^2=0.9971 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 2.0 folds.

The limit of detection (LOD) was calculated as 0.09 mM and 0.05 mM 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 7 repeat injections of 0.5 mM glucose solution. The RSD for electrochemical and photoelectrochemical biosensors were calculated to be 3.5% and 4.5% respectively. These results indicate GDH/CdS-ZnS/MAA/PGE has very good repeatability for electrochemical and photoelectrochemical biosensing of glucose.

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/CdS-ZnS/MAA/PGE in FIA system under optimized conditions toward glucose (0.5 mM) 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 and glutamic acid at the concentration one hundred fold and for galactose ten folds 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, equmolar of AA, DA, UA and L-Cyst 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 towards glucose by using Nafion as an over layer cost coated at the biosensor surface[33, 34] or using ascorbate oxidase. 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.[35]

Real Sample Analysis

One real sample (commercial dextrose solution) and one spiked serum sample were used for determination of glucose at GDH/CdS-ZnS/MAA/PGE as described in literature .[36, 37] For this, 250 μL of spiked serum samples were diluted to 5.0 mL with 0.1 M PBS (pH 7.0) containing 10 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 (including 5% glucose), it was diluted 555 times (about 0.5 mM glucose) with 0.1 M PBS (pH 7.0) containing 10 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 1. It can be seen that acceptable recoveries were obtained for spiked glucose in serum plasma and commercial dextrose solution samples.

EXPERIMENTAL

Chemicals

Glucose dehydrogenase (from *Pseudomenas sp.* 338.7 U/mg), β- Nicotinamide adenine dinucleotide sodium salt (from *Saccharomyces cerevisiae*, $C_{21}H_{26}N_7NaO_{14}P_2$, $NaNAD^+$, MW: 685.41 $g.mol^{-1}$), Bovine serum albumin (BSA), glutaraldehyde (GA, d: 1.061 $g.mL^{-1}$, MW: 100.12 $g.mol^{-1}$, 25% w/w in water),D-(+)-glucose, reduced β-nicotinamide adenine dinucleotide, disodium salt (MW: 709.40 g mol^{-1} $C_{21}H_{27}N_7Na_2O_{14}P_2$, $NADHNa_2$) KCl, H_3PO_4, NaH_2PO_4, Na_2HPO_4, CH_3COOH, NaOH, HCl, mercapto acetic acid (MAA), $Na_2S_2O_3$, $ZnCl_2$, sodium salt of EDTA and $CdCl_2.2.5H_2O$ were supplied from Merck, Sigma or Carlo Erba.

The stock solutions of glucose (1.0 M) and NAD^+ (0.01 M) 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 $cm^{-1}.M^{-1}$.[38]

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 PGE as the working electrode.[19-24] A pencil lead with a diameter of 0.5 mm (Ultra-Polymer, 2B) and a total length of 60 mm (Tombow, Japan), and a mechanical pencil Model T 0.5 (Rotring, Germany), which was used as the holder for the pencil lead, were purchased from a local bookstore. Electrical contact to the lead was obtained by wrapping a metallic wire to the metallic part of the holder. For each measurement, a total of 10 mm of lead (area is about 15.9 mm^2) was immersed into the solution. Cyclic voltammograms and electrochemical impedance curves were recorded in a static cell while amperometric experiments were performed in a FIA system. A new home-made photoelectrochemical flow cell for only PGEs, which was constructed from TEFLON, was used.[31] Deepness of pencil lead in this flow cell was arranged as 10 mm. 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.), 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.

Preparation of the modified electrodes and the glucose biosensor

After the surface of the PGE was pretreated by applying a potential of +1.40V for 60 s in the pH 7.0 PBS, ZnS-CdS were constructed on PGE by the electrochemical precipitation method according to previous reports with small modifications.[39,40] Briefly, the pretreated PGE was immersed as 10.0 mm into pH 6.0 PBS containing 15.0 mmol L^{-1} $CdCl_2$, 8.0 mmol L^{-1} $Na_2S_2O_3$, 8.0 mmol L^{-1} EDTA and 0.05 mmol L^{-1} MAA, which was used for minimizing of coagulation of QDs[41], for electrochemical deposition with a deposition potential of -1.00 V vs. Ag/AgCl for 1000 s at 30 ^{0}C. After CdS was prepared on PGE, similarly, the ZnS were

prepared on CdS/PGE in the same conditions but in 15 mmol L^{-1} $ZnCl_2$. The modified electrode will be hereafter designated as CdS-ZnS/MAA/PGE.

The GDH enzyme was immobilized onto modified PGE by the cross-linking procedure. Typically, 1.0% bovine serum albumin (BSA) was first mixed with GDH (80.0 $mg.mL^{-1}$ in PBS, pH 7.0) in a volume ratio of 1 to 1, and then 5 μL of this mixture was mixed with 4 μL of 20.0 mM glutaraldeyde (GA) as cross linking reagent. CdS-ZnS/MAA/PGE was immersed into the final solution for 1 h and then dried for 10 min at +4 ^{0}C. Finally, obtained GDH/CdS-ZnS/MAA/PGE was stored at +4 ^{0}C.

Electrochemical Procedure

In order to characterize modified electrode, electrochemical impedance spectra of bare and modified PGEs 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 10.000–0.05 Hz and a signal amplitude of 5 mV (Fig. 1). In addition, cyclic voltammograms were also 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 between -0.5 and +0.8 V at 50 $mV.s^{-1}$. The surface morphologies of the bare and modified PGEs were also examined by recording their scanning electron microscope (SEM) images.

Electrochemical and also photoelectrochemical biosensing of glucose at GDH/CdS-ZnS/MAA/PGE were investigated using cyclic voltammetric techniques. Firstly, a cyclic voltammogram of GDH/CdS-ZnS/MAA/PGE was recorded in 0.10 M PBS (pH 7.0) containing 10.0 mM NAD^+ in the potential range between -200 and 1000 mV versus Ag/AgCl at a scan rate of 20 mV s^{-1} in the absence of glucose. To see the response of the biosensor towards glucose, cyclic voltammograms of modified electrodes were recorded under the same conditions but in the presence of 40.0 mM glucose. Cyclic voltammograms were also recorded for GDH immobilized bare PGE. 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.

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 PGE for the first time[31]. In all FIA experiments, 0.10 M PBS (pH 7.0) containing 1.0 M KCl was used as the carrier solution. After GDH/MAA/ZnS-CdS/PGE had been inserted into a flow cell, the optimization studies for GDH/CdS-ZnS/MAA/PGE 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.[36,37]

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 PGE. Although, photoelectrochemical biosensor dependent on NAD^+/NADH redox couple-dehydrogenase enzymes has been reported,[11-13, 36, 42] according to our search of the literature, the uses of PGE for photoelectrochemical biosensor in FIA system have not been reported, yet. GDH/CdS-ZnS/MAA/PGE exhibited a good photoelectrocatalytic response for the detection of glucose and a linear range was obtained between 0.2 and 8.0 mM glucose with a detection limit of 0.05 mM. 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/PGE can be successfully used for photoelectrochemical biosensing of glucose due to increasing of oxidation peak current under irradiation of electrode surface even oxidation potential of enzymatically produced NADH is very high (about -800 mV) and an acceptable shifting in oxidation potential is not observed. In addition, PGE was successfully used for photoelectrochemical biosensing of glucose in FIA system by using new home-made photoelectrochemical flow cell for the first time. 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 cheap, sensitive, selective, disposable and faster photoelectrochemical biosensors in the future.

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Table 1. The results of glucose determination in spiked serum samples (n=3).

Sample	Spiked (mM)	FIA amperometric			FIA photoamperometric		
		Found (mM)	Recovery	RSD%	Found (mM)	Recovery	RSD%
Serum	0.25	0.24±0.01	93	4.1	0.26±0.01	101	3.7
	0.50	0.46±0.05	91	9.4	0.51±0.02	98	3.9
	0.75	0.77±0.04	101	5.4	0.74±0.015	98	2.0
Dextrose Solution	Labelled Claim (%5 = 277.5mM)	255.5±5.5	–	–	283±5.4	–	–

List of Figures

Fig 1. A) Cyclic voltammograms (Scan rate: 50 mV/s), and **B)** Electrochemical impedance spectra of modified electrodes in 0.1 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, capacitance; W, Warburg impedance)

Fig. 2. SEM images of **A)** bare PGE, **B)** CdS/MAA/PGE, **C)** CdS-ZnS/MAA/PGE

Fig. 3. Cyclic voltammograms of GDH/CdS-ZnS/MAA/PGE in the absence (a1, a2) and in the presence of 40 mM glucose (b1, b2). a1 and b1: without light, a2 and b2: with light. (inset: cyclic voltammograms of GDH/PGE in the presence of 40 mM glucose a: without light, b: with light) Supporting electrolyte: 0.10 M PBS (pH 7.0) containing 0.1 M KCl, 10.0 mM NAD^+ scan rate: 20 $mV.s^{-1}$.

Fig. 4. Current- time fiagrams of glucose with different concentrations using GDH/CdS-ZnS/MAA/PGE in FIA system for amperometric and photoamperometric methods. (Carrier stream: 0.1 M PBS (pH 7.0) containing 1.0 M KCl, 10 mM NAD^+, Applied potential: +0.8 V; Flow rate: 0.6 $mL.min^{-1}$, sample loop: 100 µL; transmission tubing length: 10 cm).

Fig 5. The catalytic current curve vs. the concentration of injected glucose solution for amperometric (a) and photoamperometric (b) methods.

Figures

Fig. 1.

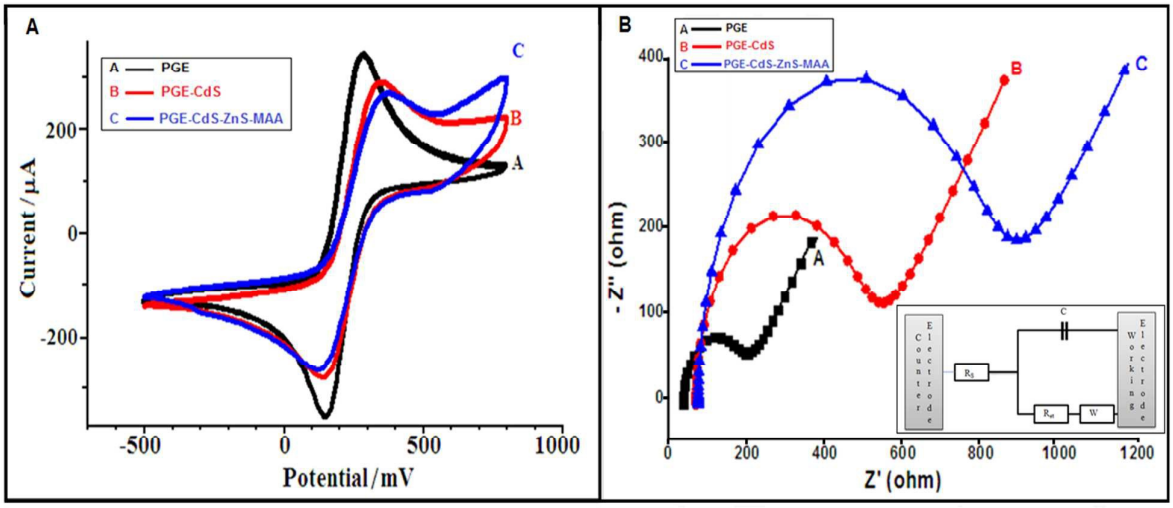

Fig. 2.

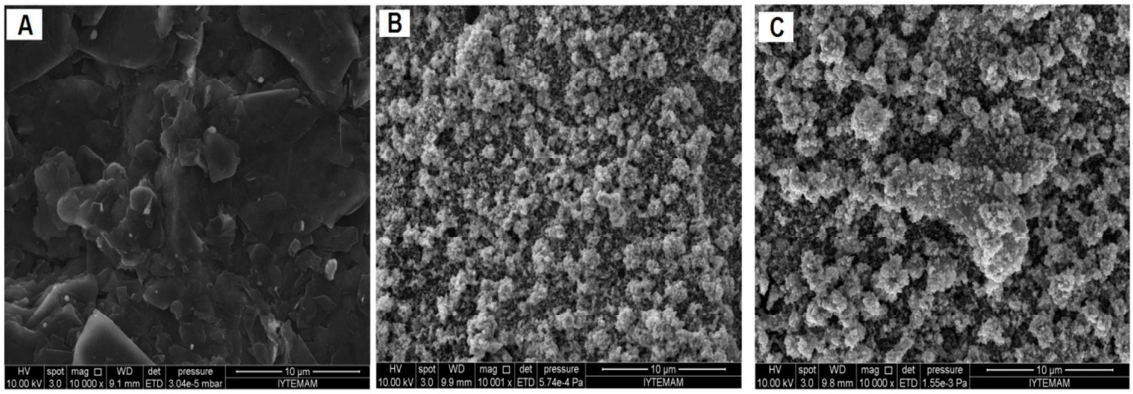

Fig. 3.

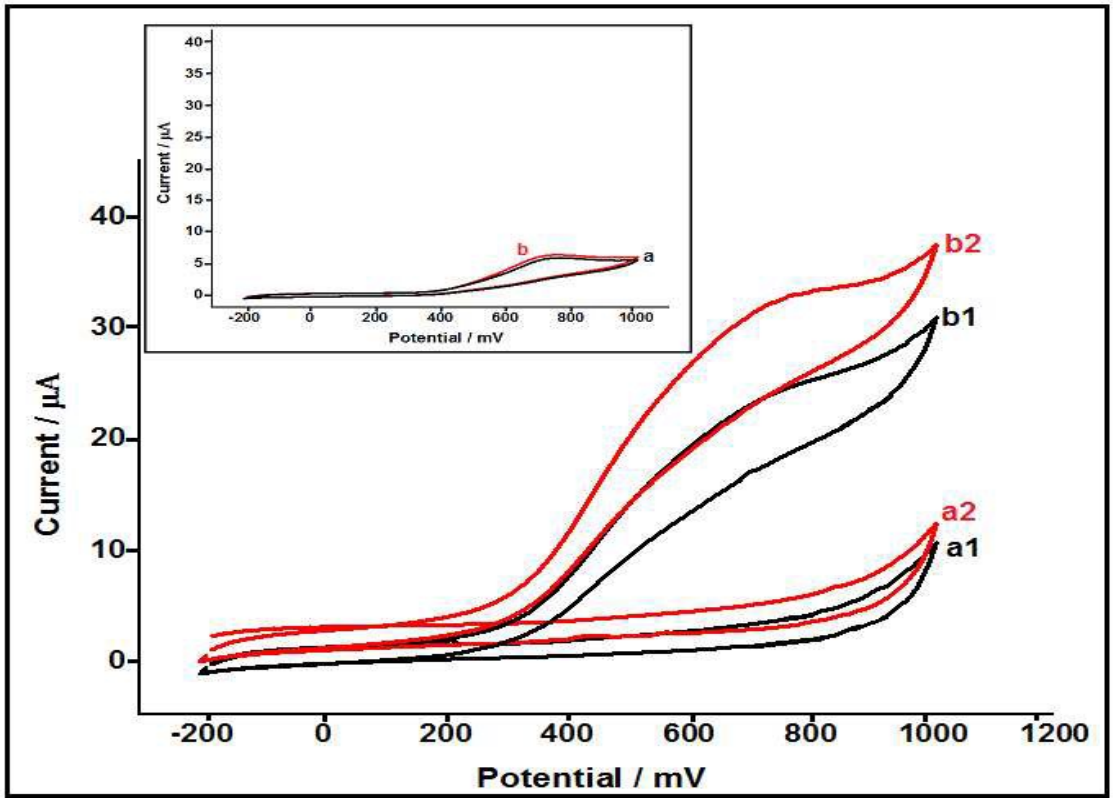

Fig. 4.

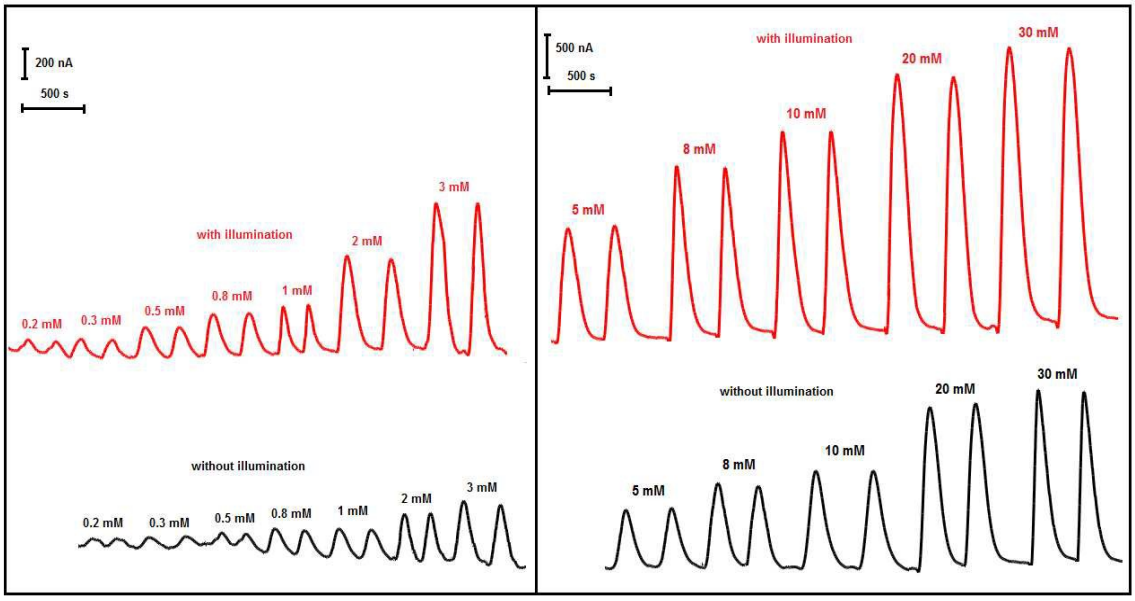

Fig. 5.