Talanta 147 (2016) 315–321

Contents lists available at ScienceDirect

Talanta

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

Biosensing of glucose in flow injection analysis system based on glucose oxidase-quantum dot modified pencil graphite electrode

Özlem Sağlam [a], Bayram Kızılkaya [b], Hüseyin Uysal [c], Yusuf Dilgin [a,*]

[a] Çanakkale Onsekiz Mart University, Faculty of Art and Science, Department of Chemistry, Çanakkale, Turkey
[b] Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology, Çanakkale, Turkey
[c] Çanakkale Onsekiz Mart University, Faculty of Art and Science, Department of Molecular Biology and Genetics, Çanakkale, Turkey

ARTICLE INFO

Article history:
Received 24 April 2015
Received in revised form
15 September 2015
Accepted 20 September 2015
Available online 25 September 2015

Keywords:
Glucose oxidase
Quantum dots
Glucose biosensor
Flow injection analysis
Pencil graphite electrode

ABSTRACT

A novel amperometric glucose biosensor was proposed in flow injection analysis (FIA) system using glucose oxidase (GOD) and Quantum dot (ZnS–CdS) modified Pencil Graphite Electrode (PGE). After ZnS–CdS film was electrochemically deposited onto PGE surface, GOD was immobilized on the surface of ZnS–CdS/PGE through crosslinking with chitosan (CT). A pair of well-defined reversible redox peak of GOD was observed at GOD/CT/ZnS–CdS/PGE based on enzyme electrode by direct electron transfer between the protein and electrode. Further, obtained GOD/CT/ZnS–CdS/PGE offers a disposable, low cost, selective and sensitive electrochemical biosensing of glucose in FIA system based on the decrease of the electrocatalytic response of the reduced form of GOD to dissolved oxygen. Under optimum conditions (flow rate, 1.3 mL min^{-1}; transmission tubing length, 10 cm; injection volume, 100 μL; and constant applied potential, −500 mV vs. Ag/AgCl), the proposed method displayed a linear response to glucose in the range of 0.01–1.0 mM with detection limit of 3.0 μM. The results obtained from this study would provide the basis for further development of the biosensing using PGE based FIA systems.

1. Introduction

Nanomaterials have received great attention due to their unique chemical, physical and electronic properties that provide their broad applications in many areas such as catalysis, electrochemical and photoelectrochemical sensing and biosensing, electronics and photonics [1–3]. The unique properties of these materials have also been effectively exploited in the immobilization of biomolecules, the catalysis of electrochemical reactions, the enhancement of electron transfer between electrode and substrate, labeling of biomolecules and acting as a reactant [1,2]. Facilitation of the direct electron transfer between the redox center of the enzyme and the electrode is one of the most important features of nanomaterials therefore mainly used in the construction of the third generation biosensors. Many kinds of nanomaterials such as metal nanoparticles, carbon nanotubes, graphene, metal oxide nanoparticles, semiconductor nanoparticles and hybrid nanoparticles have been widely used in direct electrochemistry of proteins, catalytic activity of many biomolecules and also construction of electrochemical sensors and biosensors [1,2]. Among them, recently, semiconductor quantum dots (QDs) such as CdS, CdTe, CdSe, ZnS etc. have received considerable interest in the fields of electrochemical and especially photoelectrochemical biosensors due to their interesting optical and electronic properties [2,3]. One of the important applications of QDs is the construction of glucose oxidase (GOD) based electrochemical and especially photoelectrochemical glucose biosensors [3–14]. The QDs enhance the electron transfer reactivity of GOD due to direct electron transfer capability [3–9]. However, hybrid or core–shell 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 QDs or nanomaterials in the electrochemical studies [10–14]. The core–shell QDs or hybrid nanomaterials exhibit better charge separation than a single QD or nanomaterial based systems. The core–shell QDs and hybrid materials are ideal candidates for sensing and biosensing applications due to higher quantum yield, increased photostability in photoelectrochemical reactions, extremely large surface-to-atom ratio and good sensitivity to surface ligands [8,15,16]. Therefore, CdS–ZnS modified pencil graphite electrode (PGE) was preferred in this study.

The selection of the electrode material is very important in the construction of the electrochemical biosensor since the type of the electrode generally determines the selectivity, sensitivity, stability and the cost of the biosensor. When compared with the other carbon based electrodes, PGEs have the same advantages such as the high electrochemical reactivity, commercial availability, good

* Corresponding author.
E-mail address: ydilgin@yahoo.com (Y. Dilgin).

http://dx.doi.org/10.1016/j.talanta.2015.09.050
0039-9140/

mechanical rigidity, disposability, lower cost and the ease of modification [17–27]. In addition, 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 [17]. Thus PGEs have been extensively used in various electroanalytical studies [17–27].

A useful approach for the construction of electrochemical sensors and biosensors is using of Flow Injection Analysis (FIA) in electrochemical techniques [28,29]. FIA has some advantages for routine analytical determinations, such as low sample consumption, short analysis time based on a transient signal measurement in a flow-through detector, and using online method for difficult operations of separation and chemical conversion of analyses into detectable species. The GOD immobilization onto various modified electrodes have been proposed in FIA in order to enhance the accuracy, reproducibility, stability and response rates in the analysis [30–35]. However, according to our search of the literature, electrochemical biosensing of glucose using FIA system depending on GOD immobilized onto quantum dot modified PGE has not been reported. This study shows a combination of QDs, PGE and FIA for biosensing of glucose which offers some advantages such as (i) a disposable, practical, easy-to-use and low cost biosensing 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) Efficient immobilization of GOD, good selectivity and sensitivity due to the unique functions of QDs.

2. Experimental

2.1. Materials and apparatus

Glucose oxidase (GOD, from *Aspergillus niger*, E.C. 1.1.3.4) was purchased from Sigma. All other chemicals such as $CdCl_2$, $ZnCl_2$, $Na_2S_2O_3$, glucose, chitosan, Na_2EDTA, mercapto acetic acid (MAA), KCl, H_3PO_4, NaH_2PO_4, Na_2HPO_4, CH_3OOH, etc. were commercially available as analytical reagent. 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 [18–21]. 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 flow cell for only PGEs, which was constructed for photoelectrocatalytic studies from TEFLON, was used (Fig. 1A). 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

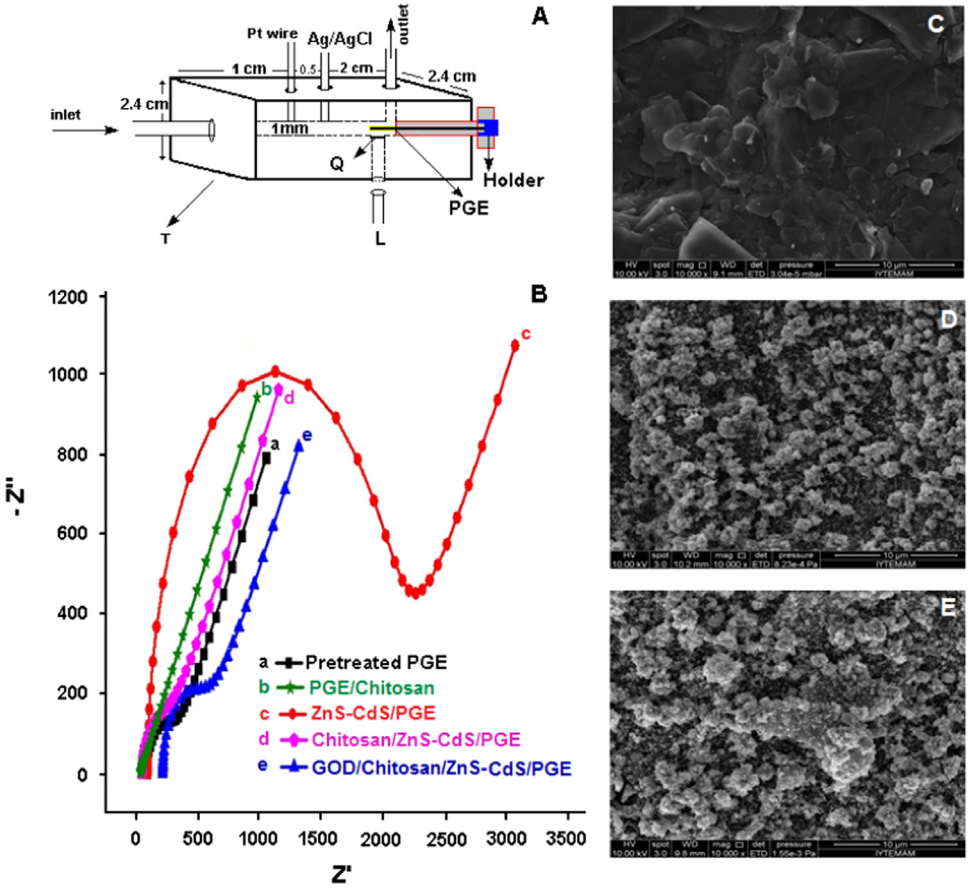

Fig. 1. (A) A home-made electrochemical flow cell for PGE (B) Electrochemical impedance spectra of modified electrodes in 0.1 M KCl containing 10.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and SEM images of (C) Pretreated PGE, (D) CdS/PGE and (E) ZnS–CdS/PGE.

(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.

2.2. Preparation of biosensor

After the surface of the PGE was pretreated by applying a potential of +1.40 V for 60 s in the pH 6.0 phosphate buffer solution (PBS), ZnS–CdS were constructed on PGE by the electrochemical precipitation method according to previous reports with small modifications [36,37]. Briefly, the pretreated PGE was immersed as 10 mm into pH 6.0 PBS containing 15.0 mM $CdCl_2$, 8.0 mM $Na_2S_2O_3$, 8 mM, EDTA and 0.05 mM MAA, which was used minimizing of coagulation of QDs [38], 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 PGE, similarly, the ZnS were prepared on CdS/PGE in the same conditions but in 15 mM $ZnCl_2$. Finally, obtained ZnS–CdS/PGE was immersed in a relatively optimized solution containing 0.5% chitosan prepared from 1.0% CH_3COOH and 200.0 mg mL^{-1} GOD for 1 h at room temperature. Then, GOD/CT/ZnS–CdS/PGE was removed from adsorption solution and dried at 4 °C. Finally, the enzyme immobilized electrode was rinsed with ultrapure water before the electrochemical biosensing experiments.

2.3. SEM images of electrodes

The surface morphologies of the bare and modified PGEs were also examined by recording their Scanning Electron Microscope (SEM) images which were recorded by Philips XL 30S-FEG Scanning Electron Microscope (SEM) in Center for Material Research of Izmir Institute of Technology, Turkey.

2.4. Electrochemical measurements

To see the direct electron transfer between GOD and electrode, cyclic voltammograms of GOD/CT/ZnS–CdS/PGE were recorded in 0.1 M Britton–Robinson buffer solution (BRBS, pH 6.0) in the potential range between −0.7 and −0.1 V vs. Ag/AgCl at various scan rate in the absence of glucose. 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 0.1 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. In order to see response of biosensor towards glucose, cyclic voltammograms of modified electrodes were recorded in the air saturated 0.1 M BRBS (pH 6.0) and 0.5 and 1.0 mM glucose recorded in the potential range between −0.7 and −0.1 V vs. Ag/AgCl at 20 mV s^{-1}.

2.5. Flow injection analysis (FIA) procedure

Electrochemical biosensor studies in FIA system were performed by using the home-made amperometric flow cell which was constructed for PGE for the first time (Fig.1A). In all FIA experiments, 0.1 M BRBS (pH 6.0) containing 1.0 M KCl was used as carrier solution. After GOD/CT/ZnS–CdS/PGE had been inserted into a flow cell, amperometric responses of glucose were monitored dependent on glucose concentration under optimized conditions (applied potential: −500 mV; flow rate: 1.3 mL min^{-1} sample loop: 100 μL; tubing length: 10 cm). For this, after a steady-state background current of supporting electrolyte, the various concentrations of glucose containing 1.0 M KCl were injected into the system and the current–time curves were recorded.

In order to show the practical applicability of the proposed biosensor, a real sample (commercial dextrose solution including 5% glucose) was selected for determination of glucose as described in the literature. For the determination of glucose, dextrose solution was diluted 555 times with 0.1 M BRBS (pH 6.0) containing 1.0 M KCl. The diluted dextrose solution containing about 0.5 mM glucose was injected into carrier stream in FIA system.

3. Results and discussions

3.1. Characterization of GOD/CT/ZnS–CdS/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. In a typical impedance spectrum, there are two parts: a semicircle portion and a linear portion. The semicircle part at higher frequencies and the linear part at lower frequencies correspond to the electron transfer-limited and diffusion-limited processes, respectively. The charge transfer resistance (Rct) can be estimated by the semicircle diameter of impedance spectra at high frequency which controls the electron-transfer kinetics of redox probe at the electrode interface [13,14]. Fig. 1B shows the Nyquist plot of different modified electrodes obtained from EI spectra in 10.0 mM $[Fe(CN)_6]^{3-/4-}$ containing 0.1 M KCl as a redox prob. The semicircle diameter of pretreated bare PGE (Fig. 1B-a) was very small, indicating that the charge transfer was relatively facile at bare electrode. The charge transfer resistance (Rct) value of pretreated PGE (Fig. 1B-a) decreased after the electrode was modified with CT (Fig. 1B-b). Because CT has a positive charge under acidic conditions due to protonation of its free amino groups. Thus the positively charged groups attracted the negatively charged redox probes ($[Fe(CN)_6]^{3-/4-}$) and the electron transfer between electrolyte and electrode surface accelerated. However, the Rct value slightly increased after ZnS–CdS was modified on the PGE surface (Fig. 1B-c) indicating that the CdS–ZnS on the PGE surface decreased the electron transfer rate between the redox probe and the electrode surface. Because the QDs have semiconductor properties and their conductivities are lower than that of bare PGE. While the Rct value of ZnS–CdS /PGE (Fig. 1B-d) decreased by immobilization of CT, the diameter of the high frequency semicircle increased further after immobilization of GOD onto CT/ZnS–CdS/PGE (Fig. 1B-e). The observed increase in diameter is due to non-conductive properties of GOD which obstructs the diffusion of the redox couple to the electrode surface. These results indicated that GOD was steadily immobilized onto CT/ZnS–CdS/PGE surface.

The surface morphologies of the pretreated PGE, CdS/PGE and ZnS–CdS/PGE were also examined by recording SEM images of all electrodes. Fig. 1D and E shows SEM images of the CdS/PGE and ZnS–CdS/PGE, respectively which were different from that of the pretreated PGE (Fig.1C). All SEM images show that the prepared quantum dots were regular in shape and uniformly distributed onto PGE surface, though ZnS–CdS were coagulated, interpretable results were obtained for glucose biosensor.

3.2. Direct electrochemistry of GOD modified PGE

In order to obtain the best response of the modified electrode, the percentage of CT and concentration of GOD were optimized during the preparation of the electrode. From optimization studies, cyclic voltammograms show that maximum peak currents attributed to oxidation and reduction of redox center of GOD were observed when modified electrodes were prepared with 0.5% CT in 0.1 M CH_3COOH and 200.0 mg mL^{-1} of GOD. After GOD/CT/ZnS–CdS/PGE was prepared under optimum conditions as mentioned in experimental section, the direct electrochemistry of GOD was

investigated by recoding cyclic voltammograms of modified electrode applying various scan rates in Argon (Ar) saturated BRBS at pH 6.0. Cyclic voltammogram of GOD/CT/ZnS–CdS/PGE shows a well-defined redox pair, which attributed to characteristic of reversible electron transfer process of redox active center (Flavin adenine dinucleotide, FAD) in GOD with two electrons and protons, at all studied scan rates. The anodic and cathodic peak currents and peak to peak separation increased gradually with the increase of scan rate. While oxidation peak shifted to more positive potentials, the reduction peak shifted to more negative potentials. Both anodic and cathodic peaks currents increased linearly by increasing scan rate from 20 to 1000 mV s^{-1}which indicates a typical surface controlled process.

The effect of pH on the direct electrochemistry of GOD was also investigated by recording cyclic voltammograms of GOD/CT/ZnS–CdS/PGE into supporting electrolyte with various pH. The stable and well-defined cyclic voltammograms were obtained in the pH range 2.0–10.0. The negative shifts in both cathodic and anodic peak potentials were observed with increasing pH values, which indicates that hydrogen ion is involved in the electrode reaction of GOD. Anodic and cathodic peak potentials of GOD have a linear relationship with pH from 2.0 to 7.0 with slope of −64 and −62 mV pH^{-1}, respectively (Fig. 2), which are very close to the theoretical value of −58.6 mV pH^{-1} for a reversible couple. Therefore, it can be said that equal numbers of electrons and protons are transferred in the direct electrochemistry of GOD at the modified electrode surface. Our results are in accordance with those reported in the literature [3–7], in which the numbers of electrons and protons was found to be two for FAD in GOD as following (Eq. (1)):

$$GOD - FAD + 2e^- + 2H^+ \leftrightarrow GOD - FADH_2 \quad (1)$$

3.3. Electrochemical response of the biosensor to glucose

It is well known that GOD modified electrodes generally show a good electrocatalytic activity toward O_2 and H_2O_2. In order to show the bioelectrocatalytic activity of GOD/CT/ZnS–CdS/PGE toward the reduction of O_2, cyclic voltammograms of modified electrode were recorded in Ar saturated (Fig. 3 curve a), air saturated (Fig. 3 curve b) and air saturated BRBS (pH 6.0) containing 0.5 mM glucose (Fig. 3 curve c) and 1.0 mM glucose (Fig. 3 curve d). As shown in Fig. 3,

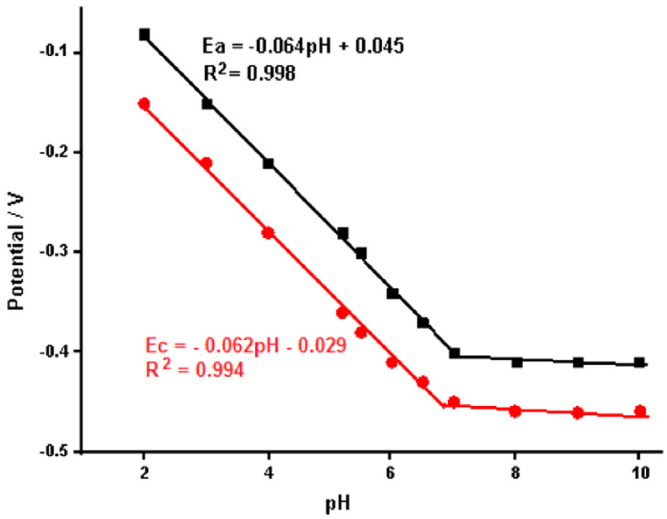

Fig. 2. The plot of pH vs. anaodic and cathodic potentials which were calculated from cyclic voltammograms of GOD/CT/ZnS–CdS/PGE recorded in the supporting electrolyte with pHs varied between 2.0 and 10.0 at 50 mV s^{-1} in the potential range of −0.7 to −0.1 V vs. Ag/AgCl.

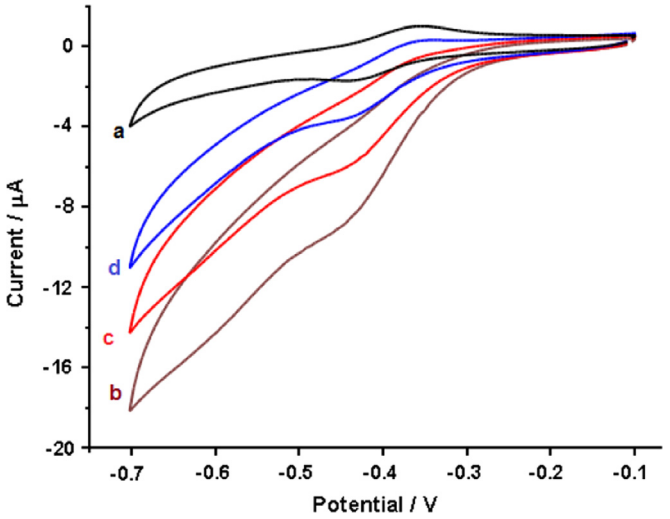

Fig. 3. Cyclic voltammograms of GOD/CT/CdS–ZnS/PGEs in Ar- saturated (a) and Air-saturated buffer (BRBS, pH 6.0) in the absence of glucose (b), and in the presence of 0.5 mM (c) and 1.0 mM (d) glucose at scan rate of 20 mV s^{-1}.

above mentioned redox couple for FAD (Eq. (1)) in Ar saturated solution was observed, attributed that the reversible electrochemical reaction of GOD as shown in Eq. (1). In the air saturated buffer solution, cathodic peak current increases while anodic peak current decreases in the absence of glucose as compared to the corresponding peaks in Ar saturated solution. This is because the electrochemically formed GOD-$FADH_2$ electrocatalyzed the reduction of O_2 which expressed as follows:

$$GOD\text{-}FADH_2 + O_2 \longrightarrow GOD\text{-}FAD + H_2O_2 \quad \cdots\cdots (2)$$

Upon addition of glucose (0.5 and 1.0 mM), the reduction peak current decreases dependent on the concentration of glucose because of the decrease of O_2 on the electrode surface caused by enzyme catalyzed reaction between the oxidized form of GOD and glucose as expressed the following reaction (Eq. (3)):

$$Glucose + GOD - FAD \leftrightarrow Glucanolactone + GOD - FADH_2 \quad (3)$$

Cyclic voltammograms of GOD/CT/ZnS–CdS/PGE were recorded in the presence of glucose and also H_2O_2 in Ar saturated pH 6.0 BRBS (data not shown). While no changes in anodic and cathodic peaks of enzyme modified electrode were observed in the presence of glucose, the reduction of H_2O_2 was observed in the presence of H_2O_2. Onset reduction potential of H_2O_2 is about −550 mV and increased by decreasing potential, which was also observed cyclic voltammograms of enzyme modified electrode in air saturated buffer solution (see Fig. 3 curve b). However, onset reduction potential of O_2 is about −300 mV and peak current reached maximum value at about −440 mV (Fig. 3 curve b) and this current decrease by adding glucose due to enzymatic reaction. These results further supported that the current decreased upon consumption of dissolved O_2 and thus could be applied in the construction of oxidase-based electrochemical biosensing of glucose.

3.4. Amperometric detection of glucose on GOD/CT/ZnS–CdS/PGE in FIA system

3.4.1. Optimization of the experimental parameters

The decrease in the reduction current of O_2 is proportional to the increase of glucose concentration due to enzyme catalyzed reaction and has been employed for glucose determination. Thus, after the investigation of electrochemical biosensing of glucose using cyclic

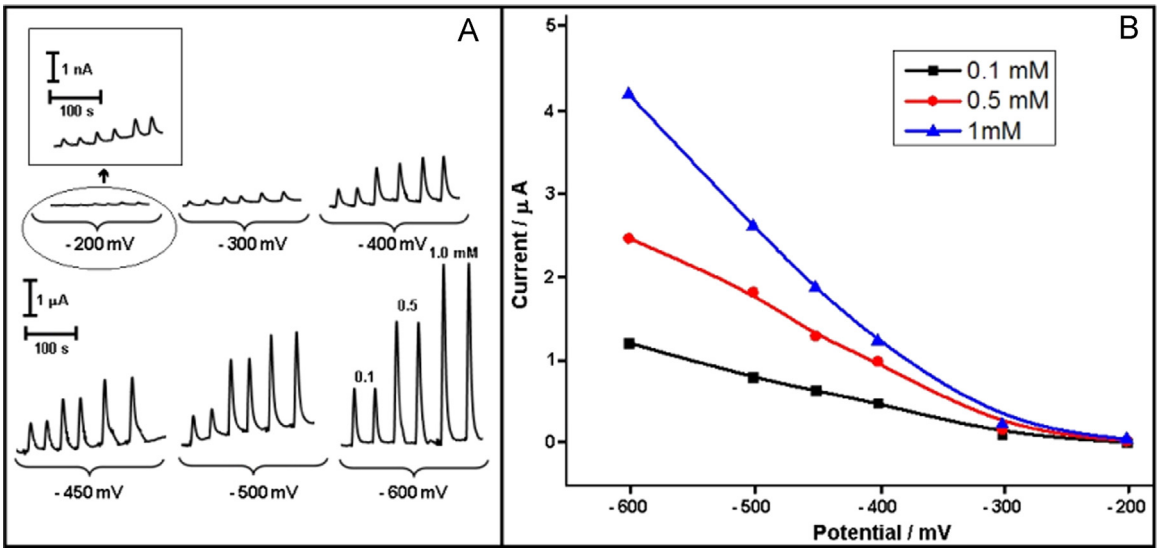

Fig. 4. (A) Current–time curves of air saturated 0.1, 0.5 and 1.0 mM glucose solutions containing 1.0 M KCl at various applied potential using GOD/CT/ZnS–CdS/PGE. (Carrier stream: air saturated 0.1 M BRBS (pH 6.0) containing 1.0 M KCl; flow rate: 1.3 mL min^{-1}; sample loop: 100 μL; tubing length: 10 cm). (B) The effect of applied potential on peak currents obtained fiagrams using GOD/CT/ZnS–CdS/PGE.

voltammetric technique, it was also investigated using amperometric technique in FIA system by using the new home made flow cell. The application of PGE in FIA was reported for the first time.

In order to obtain the best amperometric response of GOD/CT/ZnS–CdS/PGE towards glucose in FIA system, the effect of the applied potential and flow rate on the current of glucose containing 1.0 M KCl was investigated. Firstly, the applied potential was optimized under the conditions of 1.3 mL min^{-1} flow rate, 100 μL sample loop, 10 cm tubing length and air saturated 0.1 M BRBS (pH 6.0) containing 1.0 M KCl as carrier stream. Then, the current–time curves were recorded at various applied potential. After a steady background current was obtained, air saturated 0.1, 0.5 and 1.0 mM glucose solutions containing 1.0 M KCl were injected into the carrier stream. The obtained current–time curves of GOD/CT/ZnS–CdS/PGE at various applied potentials were shown in Fig. 4A. In addition, Fig. 4B shows the plot of electrocatalytic currents of glucose vs. the applied potential. Although peak currents increased by decreasing the applied potential, −500 mV was selected as the optimum applied potential due to the possibility of H_2 formation.

In the second optimization step, the effect of flow rate on electrocatalytic currents of glucose was investigated. For this purpose, the current–time curves were recorded at various flow rates using GOD/CT/ZnS–CdS/PGE in air saturated 0.1 M BRBS (pH 6.0) containing 1.0 M KCl using sample loop of 100 μL, tubing length of 10 cm and applied potential of −500 mV. In these conditions, the obtained diagrams for air saturated 0.5 mM glucose containing 1.0 M KCl at various flow rates were shown in Fig. 5A and the plot of the electrocatalytic currents vs. flow rate were shown in Fig. 5B. As can be seen in these figures, the peak currents were increased by increasing the flow rate from 0.25 to 1.3 mL min^{-1}. At low flow rates than 1.3 mL min^{-1} (especially at lowest flow rates), broadened and small peaks were observed due to dispersion of injected analyte. After the flow rate of 1.3 mL min^{-1} the peak currents slowly decreased by increasing flow rates. This can be attributed to the fact that the biosensing signal produced by GOD decreases which is proportional to the enzyme substrate incubation time.

On the other hand the peak height increased linearly by increasing the sample volume (20, 50, 100 μL) while the peak width was broadened (Data not shown). The effect of transmission tube length on currents was also examined. For an optimal measurement, the shortest transmission tube length was found to be

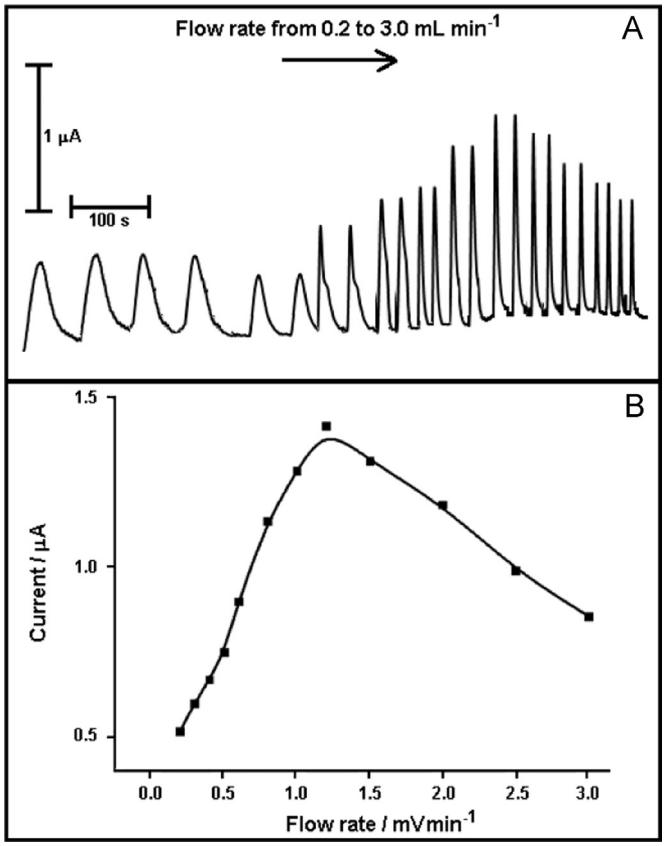

Fig. 5. (A) The fiagrams of air saturated 0.5 mM glucose containing 1.0 M KCl at various flow rate using GOD/CT/ZnS–CdS/PGE (Carrier stream: 0.1 M BRBS (pH 6.0) containnig 1.0 M KCl; applied potential: −500 mV; sample loop: 100 μL; tubing length: 10 cm). (B) The effect of flow rate on peak currents obtained fiagrams using GOD/CT/ZnS–CdS/PGE.

10 cm. When the transmission tube length was increased, amperometric current peaks were broadened, therefore sample frequency was decreased.

From optimization studies, the applied potential, the flow rate, the sample volume, and the transmission tube length were determined as −500 mV, 1.3 mL min^{-1}, 100 μL and 10 cm, respectively.

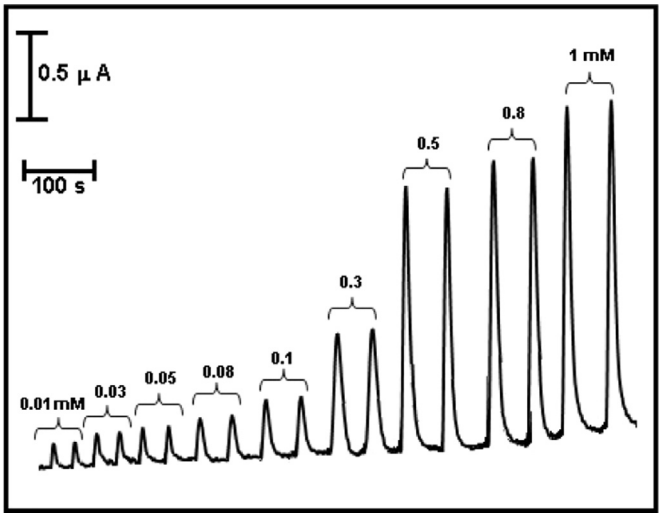

Fig. 6. Current–time curves of glucose with different concentrations in the range from 0.01 mM to 1 mM glucose using GOD/CT/ZnS–CdS/PGE in FIA system (Carrier stream: pH 6.0 BRBS containing 1.0 M KCl; applied potential: −500 mV; flow rate: 1.3 mL min^{-1} sample loop: 100 µL; tubing length: 10 cm).

3.4.2. Calibration curve and amperometric response in FIA

Under optimized conditions using a GOD/CT/ZnS–CdS/PGE, the electrochemical responses of glucose which dependent on its concentration were monitored in FIA system. Thus a calibration study was carried out over the range 0.01–1.0 mM glucose concentration with two injections of each concentration. After a steady-state background current of supporting electrolyte under optimum conditions was obtained, air saturated glucose solutions containing 1.0 M KCl in various concentrations were injected into the system and the current–time curves were recorded (Fig. 6). With an increase in glucose concentration, the amperometric response obtained from diagram also increased linearly in the range from 0.01 to 1.0 mM. The linear equation was $I(\mu A)=1.830\ C\ (mM)+0.146$, with a correlation coefficient of 0.998 and detection limit of 3.0 µM, which was obtained from the equation of LOD = 3 sb/m, where sb is standard deviation of injected buffer solution and m is slope of calibration plot. The sensitivity of the proposed biosensor was estimated to be 11.5 µA mM^{-1} cm^{-2}.

The various analytical parameters, such as electrode type, voltammetric technique, linearity ranges, sensitivity, applied potential, K_m and LOD values obtained from proposed methods were compared with other quantum dot or hybrid modified electrodes in previously-published reports for the electrochemical biosensing of glucose (Table 1). As can be seen from Table 1, the sensitivity of modified PGE was found to be 11.5 µA mM^{-1} cm^{-2}, which is better than that of some previously reported biosensors [3–7,12,14] and however is lower than that of references of [8,11,13]. LOD value of proposed method is better than that of reference of 9. In addition, PGE offers a wide linearity range compared with some modified electrodes. Although the sensitivities of some modified electrode [8,11,13] is better than that of quantum dot modified PGE, these electrodes have some disadvantages compared with PGE, such as the time-consuming polishing procedure for

Table 1
Comparison of analytical parameters obtained from GOD/CT/CdS–ZnS/PGE with different electrodes in the literature for electrochemical biosensing of glucose dependent on GOD using quantum dot modified electrodes.

Electrode type	DP[a]	Sensitivity	LR[b]	LOD[c]	K_m[d]	Ref.[e]
Au, CdS, ZnS modified ITO electrode		0.29 µA mM^{-1} for AuNPs/ITO 0.35 µA mM^{-1} for CdSNPs/ITO 0.54 µA mM^{-1} for ZnSNPs/ITO	–	–	7.5 mM 7.0 mM 3.8 mM	[3]
ZnS nanoparticles modified ITO[f] electrode	– (with CV[g])	0.549 µA mM^{-1}	0.2–5.5 mM	0.04 mM	3.8 mM	[4]
CdS modified pyrolytic graphite electrode	About −450 mV vs. SCE[h]	7.0 µA mM^{-1} (electrode area is 9.4 mm^2)	0.5–11.1 mM	0.05 mM	–	[5]
CdS nanoparticles modified Pt electrode	−500 mV vs. Ag/AgCl	1.83 µA mM^{-1} cm^{-2}	1 µM to 2.5 mM	1 µM	–	[6]
CdS nanorod and Au nanoparticles modified ITO	−400 mV vs. SCE	5.9 µA mM^{-1} (electrode area is 30 mm^2)	50–500 µM	38 µM	–	[7]
CdSe–CdS quantum dots and MWCNT[i] modified GCE[j]	+260 mV vs. Ag/AgCl	31.13 µA mM^{-1} cm^{-2}	0.16–5.6 mM	0.025 mM	0.46	[8]
CdTe qunatum dot modified FTO[k] electrode	−200 mV vs. SCE	–	0.2–8 mM	0.04 mM	–	[9]
CdSe/ZnS nanoparticles modified Au electrode	−350 mV vs. Ag/AgCl	–	0.1–5 mM	–	–	[10]
CdTe–CdS core shell quantum dot graphene Au nanoparticle modified Au electrode	−200 mV vs. Ag/AgCl	5762.8 nA nM^{-1} cm^{-2}	1×10^{-11}–1×10^{-8} M	3×10^{-12} M	5.24×10^{-6} mM	[11]
CdTe quantum dot and CNT[l] modified GCE	– (with CV)	1.018 µA mM^{-1}	Up to 0.7 mM	–	5.1 mM	[12]
Graphene quantum dots modified CCE[m]	−420 mV vs. SCE	0.085 µA μM^{-1} cm^{-2}	5–1270 µM	1.73 µM	0.76 mM	[13]
Graphene–CdS nanocomposite modified GCE	– (with CV)	1.76 µA mM^{-1} cm^{-2}	2–10 mM	0.7 mM	1.6 mM	[14]
CdS–ZnS quantum dot modified PGE[n]	−500 mV vs. Ag/AgCl	11.5 µA mM^{-1} cm^{-2}	0.01–1.0 mM	3 µM	0.69 mM	This work

[a] Detection potential.
[b] Linearity range.
[c] Limit of detection.
[d] Michaelis–Menten constant.
[e] Reference.
[f] Indium tin oxide.
[g] Cyclic voltammetry.
[h] Saturated calomel electrode.
[i] Multi-walled carbon nanotube.
[j] Glassy carbon electrode.
[k] Florinated tin oxide.
[l] Carbon nanotube.
[m] Carbon ceramic electrode.
[n] Pencil graphite electrode.

electrode surface, low reproducibility in the preparation of modified electrodes, expensive electrode materials, etc. Therefore, it can be said that CdS–ZnS modified PGE is a very useful electrode material for immobilization of GOD and electrochemical biosensing of glucose. Because, PGE having important advantages such as high electrochemical reactivity, commercial availability, good mechanical rigidity, disposability, low cost, ease of modification, lack of need for polishing, and renewable surface.

The Michaelis–Menten constant (K_m) which is an important parameter for revealing enzyme-substrate reaction kinetics, was also calculated using the Lineweaver–Burk equation as follows [39]:

$$1/I_{ss} = 1/I_{max} + K_m/I_{max} \cdot 1/C$$

where C is the glucose concentration, I_{ss} is the steady-state response current under saturated substrate conditions, I_{max} is maximum electrocatalytic current. From the curve of the I_{ss}^{-1} vs. C_s^{-1} (data not shown), the apparent Michaelis–Menten constant K_m and the maximum current response I_{max} were estimated to be 0.69 mM, 0.36 μA mM^{-1}, respectively. The obtained K_m value was compared with those of other glucose biosensors dependent on GOD and quantum dot modified electrodes (Table 1). As can be seen in Table 1, K_m value obtained from proposed method was smaller than the reported values of K_m for some constructed biosensors [3,4,12–14] dependent on GOD. Smaller K_m values show that the electrochemical biosensors possess higher biological affinity to glucose and have a superior enzymatic activity.

The repeatability of modified electrode was also tested by detecting 0.1 mM and 0.3 mM glucose for five successive injections in FIA system and their relative standard deviations (RSD) are found to be 4.5% and 3.3%, respectively, which demonstrate the modified electrodes had good repeatability.

3.4.3. *Real sample analysis*

Before real sample analysis, the possible interference species such as ascorbic acid (AA), uric acid (UA), dopamine (DA), L-Cysteine (L-Cyst), Glutamic acid (GA) on the detection of glucose were studied. Experimental results show that no significant change of reduction peak current could be observed for AA, UA, DA, L-Cyst. and GA at the concentration ten fold as high as that of glucose (data not shown), which shows the good selectivity of the enzyme electrode.

In order to show the practical applicability of the proposed biosensor, a real sample (commercial dextrose solution) was selected for determination of glucose as described in literature [40]. For the determination of glucose in commercially supplied dextrose solution (including 5% glucose which is about 277.5 mM), dextrose solution was diluted 555 times with 0.1 M BRBS (pH 6.0) containing 1.0 M KCl. Thus diluted dextrose solution containing about 0.5 mM glucose was obtained. The measured glucose concentration in the test solution was found to be 280.8 ± 5.7 mM. It can be concluded that acceptable glucose values were obtained for commercial dextrose solution samples.

4. Conclusion

In this study, the development of a simple and highly sensitive electrochemical glucose biosensor based on a new modified electrode, which could be easily prepared by electrochemical precipitation of ZnS–CdS onto the surface of PGE, was described for the first time. In addition, PGE was used in FIA system using a newly constructed home-made photoelectrochemical flow cell for the electrochemical biosensing of glucose 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 electrochemical biosensors in the future.

Acknowledgment

We thank The Scientific and Technological Research Council of Turkey (**TÜBİTAK**) for financial support (Project number: 112T375).

References

[1] X. Luo, A. Morrin, A.J. Killard, M.R. Smyth, Electroanalysis 18 (2006) 319–326.
[2] Z. Yue, F. Listad, W.J. Parak, S.G. Hickey, L. Tu, N. Sabir, D. Dorfs, N.C. Bigall, ACS Appl. Mater. Interfaces 5 (2013) 2800–2814.
[3] J. Du, X. Yu, J. Di, Biosens. Bioelectron. 37 (2012) 88–93.
[4] J. Du, X. Yu, Y. Wu, J. Di, Mater. Sci. Eng. C 33 (2013) 2031–2036.
[5] Y. Huang, W. Zhang, H. Xiao, G. Li, Biosens. Bioelectron. 21 (2005) 817–821.
[6] J. Sun, Y. Zhu, X. Yang, C. Li, Particuology 7 (2009) 347–352.
[7] J. Qian, S. Yan, Z. Xiao, J. Colloid Interface Sci. 366 (2012) 130–134.
[8] F. Huang, F. Wang, S. Feng, Y. Li, S. Li, Y. Li, J. Solid State Electrochem. 17 (2013) 1295–1301.
[9] W. Wang, L. Bao, J. Lei, W. Tu, H. Ju, Anal. Chim. Acta 744 (2012) 33–38.
[10] J. Tanne, D. Schafer, W. Khalid, W.J. Parak, F. Lisdat, Anal Chem. 83 (2011) 7778–7785.
[11] G. Zhiguo, Y. Shuping, L. Zaijun, S. Xiulan, W. Guangli, F. Yinjun, L. Junkang, Electrochim. Acta 56 (2011) 9162–9167.
[12] Q. Liu, X. Lu, J. Li, X. Yao, J. Li, Biosens. Bioelectron. 22 (2007) 3203–3209.
[13] H. Razmi, R.M. Rezaei, Biosens. Bioelectron. 41 (2013) 498–504.
[14] K. Wang, Q. Liu, Q.M. Guan, J. Wu, H.N. Li, J.J. Yan, Biosens. Bioelectron. 26 (2011) 2252–2257.
[15] X. Peng, M.C. Schlamp, A.V. Kadavanich, A.P. Alivisatos, J. Am. Chem. Soc. 119 (1997) 7019–7029.
[16] J. Li, Y.A. Wang, W.Z. Guo, J.C. Keay, T.D. Mishima, M.B. Johnson, X. Peng, J. Am. Chem. Soc. 125 (2003) 12567–12575.
[17] J. Wang, A.N. Kawde, E. Sahlin, Analyst 125 (2000) 5–7.
[18] E. Dede, O. Sağlam, Y. Dilgin, Electrochim. Acta 127 (2014) 20–26.
[19] Z.O. Uygun, Y. Dilgin, Sens. Actuators B 188 (2013) 78–84.
[20] Y. Dilgin, B. Ertek, B. Kızılkaya, D.G. Dilgin, H.İ. Gökçel, Sci. Adv. Mater. 4 (2012) 920–927.
[21] Y. Dilgin, B. Kızılkaya, B. Ertek, N. Eren, D.G. Dilgin, Talanta 89 (2012) 490–495.
[22] E. Hur, G.A. Varol, A. Arslan, Synth. Met. 184 (2013) 16–22.
[23] A. Ozcan, Electroanalysis 26 (2014) 1631–1639.
[24] A.N. Kawde, M. Aziz, N. Baig, T. Yassien, J. Electroanal. Chem. 740 (2014) 68–74.
[25] J. Zhu, X.Y. Wu, D. Shan, P.X. Yuan, X.J. Zhang, Talanta 130 (2014) 96–132.
[26] K.K. Tadi, R.V. Motghare, V. Ganesh, Electroanalysis 26 (2014) 2328–2336.
[27] S. Tajik, M.A. Taher, H. Beitollahi, J. Electroanal. Chem. 720 (2014) 134–138.
[28] E.H. Hansen, J. Mol. Recognit. 9 (1996) 316–325.
[29] M. Mayer, J. Ruzicka, Anal. Chem. 68 (1996) 3808–3814.
[30] J. Yu, S. Liu, H. Ju, Biosens. Bioelectron. 19 (2003) 401–409.
[31] Ö. Yılmaz, D.O. Demirkol, S. Gülcemal, A. Kılınç, S. Timur, B. Çetinkaya, Colloid Surf. B. 100 (2012) 62–68.
[32] E. Turkusic, J. Kalcher, E. Kahrovic, N.W. Beyene, H. Moderegger, E. Sofic, S. Begic, K. Kalcher, Talanta 65 (2005) 559–564.
[33] J.S.Y. Chia, M.T.T. Tan, P.S. Khiew, J.K. Chin, C.W. Siong, Sens. Actuators B. 210 (2015) 558–565.
[34] P. Kotzian, P. Brázdilová, S. Řezková, K. Kalcher, K. Vytřas, Electroanalysis 18 (2006) 1499–1504.
[35] İ. Çakar, K.V. Özdokur, B. Demir, E. Yavuz, D.O. Demirkol, S. Kocak, S. Timur, F. N. Ertaş, Sens. Actuators B. 185 (2013) 331–336.
[36] C. Lu, X.F. Wang, J.J. Xu, H.Y. Chen, Electrochem. Commun. 10 (2008) 1530–1532.
[37] Z. Qian, H.J. Bai, G.L. Wang, J.J. Xu, H.Y. Chen, Biosens. Bioelectron. 25 (2010) 2045–2050.
[38] A. Salimi, R. Rahmatpanah, R. Hallaj, M. Roushani, Electrochim. Acta 95 (2013) 60–70.
[39] R. Kamin, G. Wilson, Anal. Chem. 52 (1980) 1198–1205.
[40] D.G. Dilgin, H.İ. Gökçel, Anal. Methods 7 (2015) 990–999.