www.dergipark.gov.tr
ISSN:2148-3736
El-Cezerî Fen ve Mühendislik Dergisi
Cilt: 7, No: 3, 2020 (1191-1200)

El-Cezerî Journal of Science and Engineering
Vol: 7, No: 3, 2020 (1191-1200)
DOI:10.31202/ecjse.729462

ECJSE

Research Paper / Makale

Green Synthesis of Zinc Oxide Nanoparticles Using *Zingiber Officinale* Root Extract and Their Applications in Glucose Biosensor

Soner DÖNMEZ*

Burdur Mehmet Akif Ersoy University, Bucak School of Health, Burdur, TURKEY
sdonmez@mehmetakif.edu.tr

Received/Geliş: 29.04.2020 **Accepted/Kabul:** 11.08.2020

Abstract: Green synthesis of nanoparticles via plant extracts has become an important research field in nanotechnology. In the present study, a novel amperometric glucose biosensor based on the green synthesized zinc oxide (ZnO) nanoparticles by using *Zingiber officinale* root was fabricated. Glucose oxidase (GOx) was immobilized onto the ZnO-modified carbon paste electrode (CPE) via cross-linking with glutaraldehyde. The prepared biosensor (GOx-ZnO/CPE) exhibited a good electrocatalytic ability to the determination of glucose. The biosensor also showed a low detection limit (14.7 μM), a rapid response (less than 1 second), high sensitivity (15.98 μA mM^{-1} cm^{-2}), and higher biological affinity (the Michaelis−Menten constant was estimated as 0.99 mM). Moreover, the prepared biosensor exhibited good anti-interference capability in relation to ascorbic acid (AA) and uric acid (UA). These results demonstrated that a simple and a cost effectiveness biosensor was fabricated for the determination of glucose.

Keywords: Green synthesis; zinc oxide nanoparticle; *Zingiber officinale; g*lucose biosensor

Zencefil (*Zingiber Officinale*) Kök Ekstresi Kullanılarak Çinko Oksit Nanoparçacıkların Yeşil Sentezi ve Glikoz Biyosensörü Olarak Uygulaması

Öz: Nanopartiküllerin bitki özleri yoluyla yeşil sentezi nanoteknolojide önemli bir araştırma alanı haline gelmiştir. Bu çalışmada, Zencefil kökü kullanılarak yeşil sentezlenmiş çinko oksit (ZnO) nanopartikülleri aracılığıyla yeni bir amperometrik glikoz biyosensörü üretildi. Glukoz oksidaz (GOx), glutaraldehit ile çapraz bağlanma yoluyla ZnO ile modifiye edilmiş karbon pasta elektrot (CPE) üzerine immobilize edildi. Hazırlanan biyosensör (GOx-ZnO/CPE) glikoz tayini için iyi bir elektrokatalitik özellik gösterdi. Biyosensör, düşük bir tespit limiti (14,7 μM), hızlı cevap süresi (1 saniyeden daha az), yüksek hassasiyet (15,98 μA $mM^{-1}cm^{-2}$) ve yüksek biyolojik afinite (Michaelis−Menten sabiti 0,99 mM olarak hesaplandı) gösterdi. Ayrıca hazırlanan biyosensör, askorbik asit ve ürik asit gibi girişim yapan maddelere karşı iyi bir seçicilik sergiledi. Bu sonuçlar, glikoz tayini için basit ve uygun maliyetli bir biyosensörün hazırlandığını göstermiştir.

Anahtar kelimeler: Yeşil sentez; çinko oksit nanoparçacık; zencefil; glikoz biyosensör

1. Introduction

One of the most popular biosensors, the glucose biosensor, has been extensively studied for its importance in the food, environment industry, and in clinics [1]. Many techniques, such as colorimetric, spectroscopic, and electrochemical methods, have been introduced for this purpose [2, 3]. Amperometric biosensors based on glucose oxidase (GOx) are now widely used for glucose monitoring because of their reliability, low cost, and simplicity [4-6]. GOx is a flavoprotein that catalyzes the oxidation of β-D-glucose to hydrogen peroxide (H_2O_2) and D-glucono-1,5-lactone [7].

How to cite this article
Dönmez, S, "*Green Synthesis of Zinc Oxide Nanoparticles Using Zingiber Officinale Root Extract and Their Applications in Glucose Biosensor*" El-Cezerî Journal of Science and Engineering, 2020, 7 (3); 1191-1200.

Bu makaleye atıf yapmak için
Dönmez, S, *"Zencefil (Zingiber Officinale) Kök Ekstresi Kullanılarak Çinko Oksit Nanoparçacıkların Yeşil Sentezi ve Glikoz Biyosensörü Olarak Uygulaması"* El-Cezerî Fen ve Mühendislik Dergisi 20XX, 7 (3); 1191-1200.
ORCID: 0000-0003-0328-6481

For a functioning biosensor, the GOx enzyme should be immobilized onto the surface of the electrode to increase durability and stability of the enzyme; however, the immobilization matrix should be biocompatible and should not interfere with the enzyme structure [8].

Enzyme immobilization on nanostructures facilitates the direct electron transfer between active sites of the enzyme and the electrode, the formation of a desirable microenvironment, and the creation of an extensive surface area for greater enzyme loading [9]. Among the all metal nanostructures, ZnO nanoparticles have attracted considerable attention for the fabrication of efficient amperometric biosensors due to their unique features that include high catalytic efficiency, high isoelectric point, nontoxicity, biocompatibility, excellent electron transfer capability, and good stability [10-12].

The synthesis of large quantities of metal nanoparticles has been carried out by chemical methods; however, toxic substances used in chemical synthesis can be adsorbed onto the surface of nanoparticles and have adverse effects in biomedical applications. [13]. Various techniques have been proposed for the synthesis of ZnO nanoparticles, including thermal evaporation, organo-metallic synthesis, sol-gel processing, homogeneous precipitation, microwave methods, and green synthesis [14-19]. Among these synthesis methods, green chemistry routes using plant extracts are commonly preferred for the synthesis of ZnO nanoparticles due to the environmentally friendly, cost effective, and safe nature of the resulting nanoparticles for human therapeutic use [20].

Various glucose biosensors have been prepared previously with chemically synthesized ZnO nanoparticles [21-23]; only limited investigations have focused on glucose biosensors prepared with green synthesized ZnO nanoparticles. The present work describes, the green synthesis of ZnO nanoparticles using *Zingiber officinale* root extract and the use of these nanoparticles to fabricate a glucose biosensor.

2. Experimental Methods

2.1 Materials

Fresh *Z. officinale* roots were purchased from a local store. Glucose oxidase (derived from *Aspergillus Niger*), the graphite powder, and Zinc acetate dihydrate [Zn $(CH_3COO)_2.2H_2O$] were provided from Sigma-Aldrich. All other reagents used in this study were of analytical grades.

2.2 Instrumentation

All the electrochemical experiments in this study were performed using a CHI 1230B electrochemical workstation. The three-electrode systems consisted of an Ag/AgCl reference electrode, a ZnO nanoparticle modified carbon-paste electrode as the working electrode, and a platinum electrode as an auxiliary electrode.

UV-Visible (UV-Vis) spectroscopy (Pg instrument, T60 Uv-Visible Spectrophotometer) and scanning electron microscope (SEM) with EDX (Energy dispersive X-ray) (JEOL SEM-7100-EDX) was used the characterization of ZnO nanoparticles.

2.3 Green Synthesis of the ZnO Nanoparticles

$Zn(CH_3COO)_2.2H_2O$ was dissolved in 50 mL double distilled water to give a final concentration of 0.02M. Then, 20 mL of *Z. officinale* root extract (0.25 g/mL in double distilled water) was slowly added into the $Zn(CH_3COO)_2.2H_2O$ solution. This mixture was heated at 80 °C under continuous stirring with a magnetic stirrer for about 2 h, until a white precipitate was formed. This ZnO nanoparticle precipitate was centrifuged for 10 min at 10,000 rpm, washed with distilled water, and

then washed with methanol to remove the unwanted impurities. The ZnO nanoparticles were dried (at 60 °C for overnight) and then calcined at 450°C in a muffle furnace for 2 h.

2.4 Fabrication of the GOx-ZnO/GCE Biosensor

A ZnO nanoparticle-modified carbon paste electrode (ZnO/CPE) was constructed by hand mixing of 40 mg graphite powder, 16 μL mineral oil, and 4 mg ZnO nanoparticles. The carbon paste was used to fill the cavity of an insulin syringe and a copper wire was added to provide electrical contact. The syringe plunger was pressed until a smooth surface was obtained, and the surface was then polished on a weighing paper. The enzyme solution was prepared by mixing the 5 μL of GOx enzyme (10 mg/mL in phosphate buffer solution (PBS), pH:7.0), 1.5 μL of 2.5% glutaraldehyde, and 1.0 μL (5 mg/250 μL) of bovine serum albumin. The prepared enzyme solution was dropped onto the surface of the ZnO/CPE and then, the prepared biosensor was left to dry at room temperature for 2 h. When not in use, the biosensor was kept in a dry condition at 4 °C.

2.5 Electrochemical Measurement of Glucose

Glucose was quantified by electrochemical detection of enzymatically released H_2O_2. GOx catalyzes the oxidation of β-D-glucose to H_2O_2 and D-glucono-δ-lactone. Application of a voltage causes the electrooxidation of H_2O_2, which forms two electrons and two hydrogen ions that can then be used to monitor the glucose concentration, as in following reactions (Equation 1,2, and 3).

$\text{Glucose} + \text{GOx}(\text{FAD}^*) \rightarrow \text{D-glucono-}\delta\text{-lactone} + \text{GOx}(\text{FADH}_2)$	(1)
$\text{GOx}(\text{FADH}_2) + O_2 \rightarrow \text{GOx}(\text{FAD}) + H_2O_2$	(2)
$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$	(3)

*FAD: flavin adenine dinucleotide

The prepared biosensor was immersed in 0.1 M PBS (pH 7.0) containing 0.1 M KCl as a supporting electrolyte, and its steady-state current (i_a) was measured. The electrochemical cell was stirred after addition of glucose and its current (i_b) was measured again. The current differences ($\Delta i = i_b – i_a$) were plotted against glucose concentrations.

3. Results and Discussion

3.1 UV-VIS Spectroscopic Studies

The UV-Vis spectrum of the green synthesized ZnO nanoparticles using *Z. officinale* root extract is shown in Figure 1. The characteristic absorption peak of ZnO nanoparticles was observed at the wavelength of 365 nm, confirming the formation of ZnO nanoparticles. This result is similar to findings previously reported by Safawo et al. [24].

3.2 FE-SEM and EDX analysis

The morphology of the green synthesized ZnO nanoparticles was analyzed by SEM with an EDX pattern. Figure 2a shows spherical particles ranging about 17–40 nm in size and forming cauliflower-like structures.

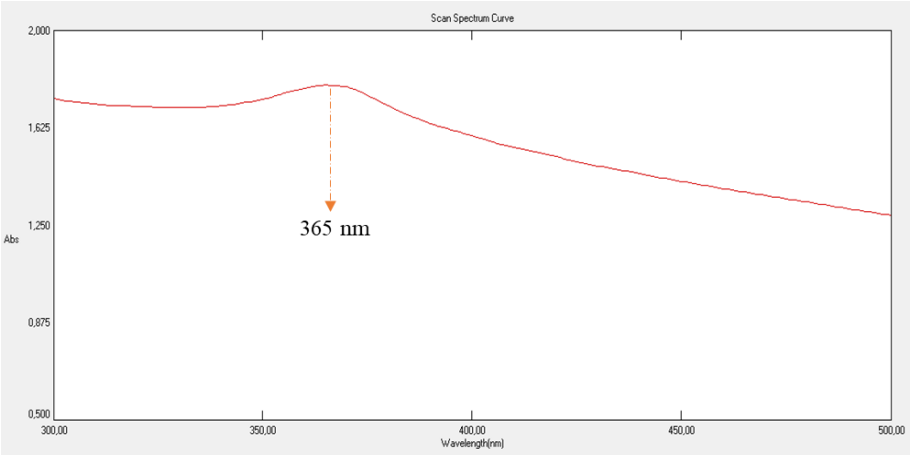

Figure 1. UV–Vis spectrum of ZnO nanoparticles representing a characteristic peak at 365 nm

Sharp peaks related to zinc and oxygen atoms were detected in the EDX spectrum of the ZnO nanoparticles (Figure 2b). The atomic percentages of ZnO nanoparticles were 48.8% for zinc and 51.2 % for oxygen. The FE-SEM and EDX results were similar to those previously reported for green synthesized ZnO nanoparticles [25, 26].

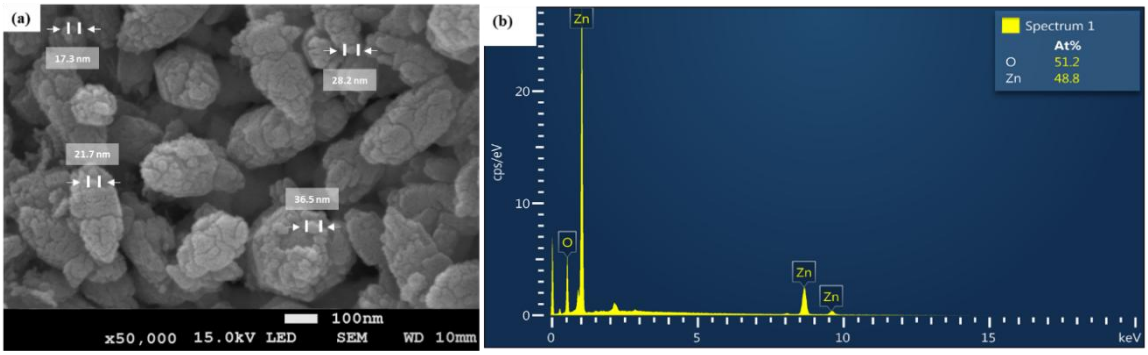

Figure 2. a) FE-SEM images of the ZnO nanoparticles (b) EDX spectrum of ZnO nanoparticles

3.3 Electrocatalytic Property of CPE and ZnO/CPE

The H_2O_2 sensitivity of the prepared electrode is an important parameter in amperometric glucose biosensors, as enzymatically released H_2O_2 is measured. The electrocatalytic activities of both bare CPE and ZnO/CPE during oxidation of H_2O_2 were investigated using cyclic voltammetry (CV) techniques. Figure 3 shows CVs of the CPE and ZnO/CPE in the presence and absence of 10 mM H_2O_2 in 0.1 M PBS (pH 7) at a scan rate of 100 mVs^{-1}. A current-response increment related to the oxidation of H_2O_2 was obtained for both the CPE and the ZnO/CPE, but a significantly greater oxidation peak was observed for the ZnO/CPE. The electrocatalytic activity was also evaluated by amperometry. Amperometric responses of both electrodes (CPE and ZnO/CPE) increased as the H_2O_2 concentration was increased from 0.5 mM to 10 mM (inset of Figure 3). However, an almost four-fold higher current of H_2O_2 was obtained using the ZnO nanoparticle-modified electrode when compared with the CPE. Therefore, the green synthesized ZnO nanoparticles can catalyze and accelerates the oxidation of H_2O_2 and thereby function as excellent glucose biosensor.

3.4 Optimization of the amount of ZnO nanoparticles in the CPE

Different amounts of ZnO-nanoparticles, ranging from 2–12 mg, were added to the carbon paste for fabrication of the glucose biosensor. As shown in Figure 4, the amperometric responses of the

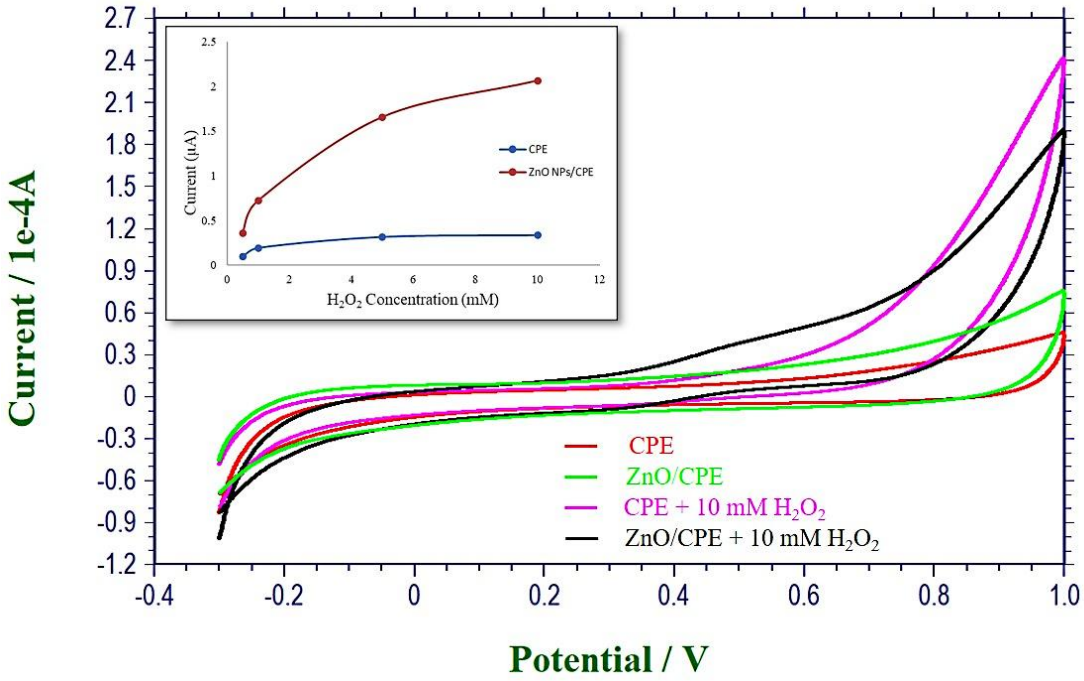

Figure 3. Cyclic voltammograms of bare CPE and ZnO/CPE in 0.1 M PBS (pH 7.0) in presence and absence of 10 mM H_2O_2. Inset: Amperometric responses of the CPE and the ZNO/CPE to different concentration of H_2O_2 from 0.5 mM to 10 mM.

biosensor to 1.0 mM glucose increased with increasing amounts of the nanoparticles from 2–4 mg, whereas a further increase in the nanoparticle amount caused a decrease in the amperometric responses. For this reason, 4 mg of nanoparticles were added to the carbon paste in subsequent experiments.

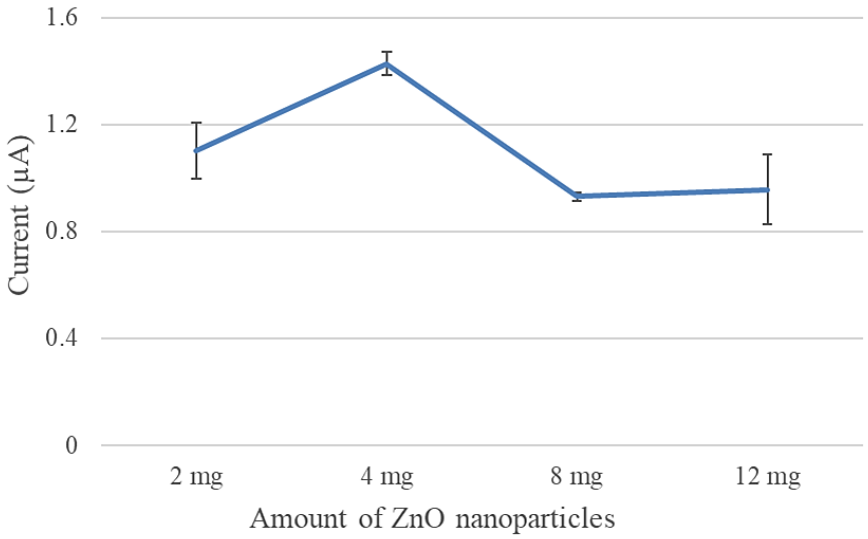

Figure 4. Influence of ZnO nanoparticles amount in the carbon paste on the biosensor response

3.5 Interference studies

The interference of electroactive compounds such as ascorbic acid (AA) and uric acid (UA), which are commonly present in blood, can cause problems in correct glucose determinations. The anti-interference capability of the biosensor is demonstrated in Figure 5. The amperometric responses of

the biosensor in the presence of physiologically normal levels (0.1 mM UA and 0.1 mM AA [27]) were compared with the glucose response obtained with a 1.0 mM glucose solution. UA and AA at physiological levels both gave negligible signals, and a well-defined glucose response was observed for the biosensor, supporting the high glucose selectivity of the biosensor.

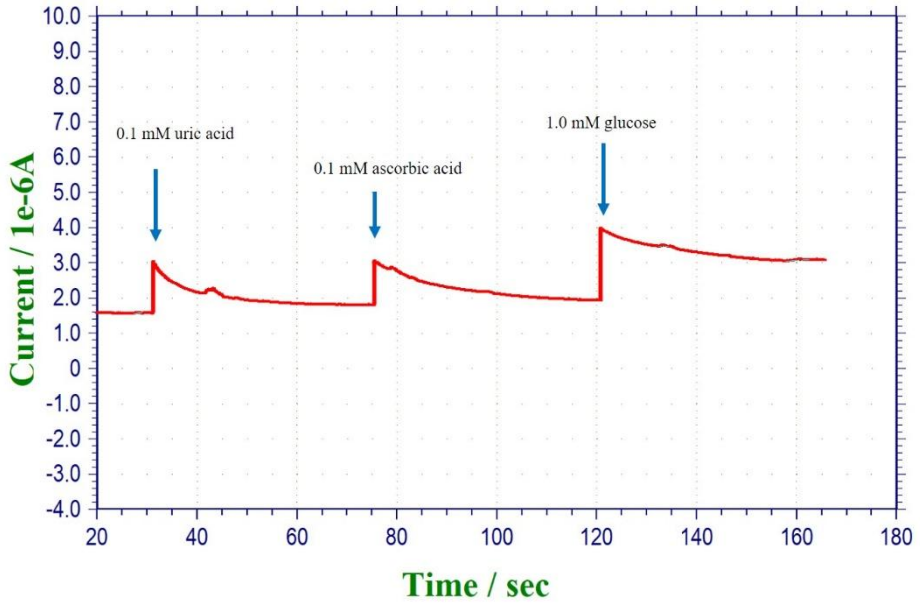

Figure 5. Amperometric response of the biosensor with addition of 0.1 mM uric acid, 0.1 mM ascorbic acid, and 1.0 mM glucose in 0.1 M PBS (pH 7) at the applied potential of +0.7 V.

3.6 Amperometric Determination of Glucose by the Fabricated Biosensor

The performance of the biosensor was also examined for different concentrations of glucose into PBS. A fast and sensitive response to glucose was obtained with the biosensor. The amperometric response of the biosensor reached 95% of its maximum steady-state current within 1 second (Figure 6).

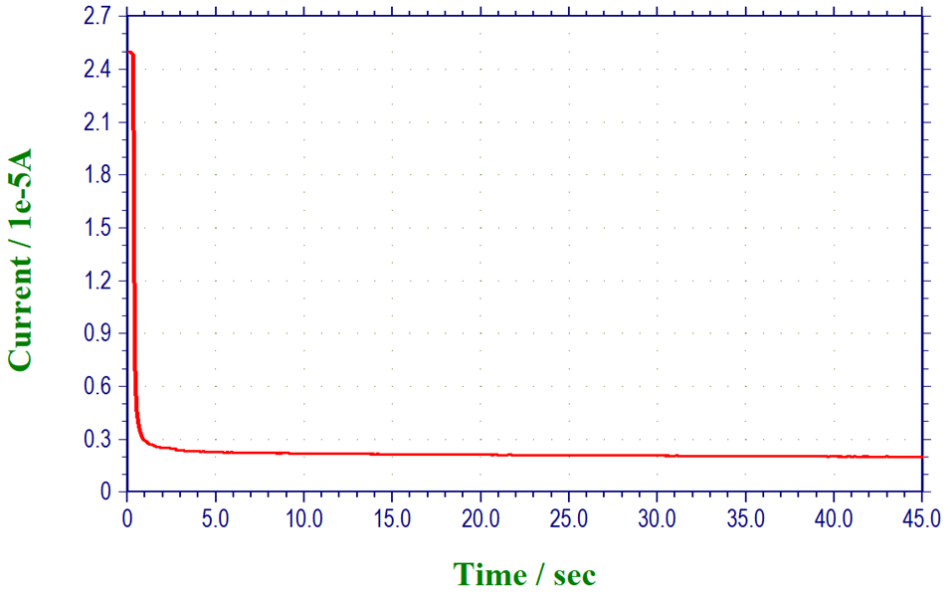

Figure 6. The response time of the glucose biosensor in 1.0 mM glucose concentration in 0.1 M PBS (pH 7) at the working potential of +0.7 V.

A calibration curve for the glucose biosensor revealed a hyperbolic dependence on glucose concentrations ranging from 0.01 to 15 mM under optimal experimental conditions (Figure 7a). The linear region of the biosensor response is shown in the inset of Figure 7a. A good linearity was obtained within the range of 0.01– 1.0 mM (R^2 = 0.9997). The limit of detection was estimated at 14.7 µM based on a signal-to-noise ratio of 3 (S/N=3). The sensitivity of the biosensor was estimated as 15.98 µA mM^{-1} cm^{-2}. After nine sequential measurements of 1.0 mM glucose, the relative standard deviation (RSD) and the standard deviation (SD) of the prepared biosensor were 3.425% and, 0.032, respectively, indicating that the biosensor had good repeatability.

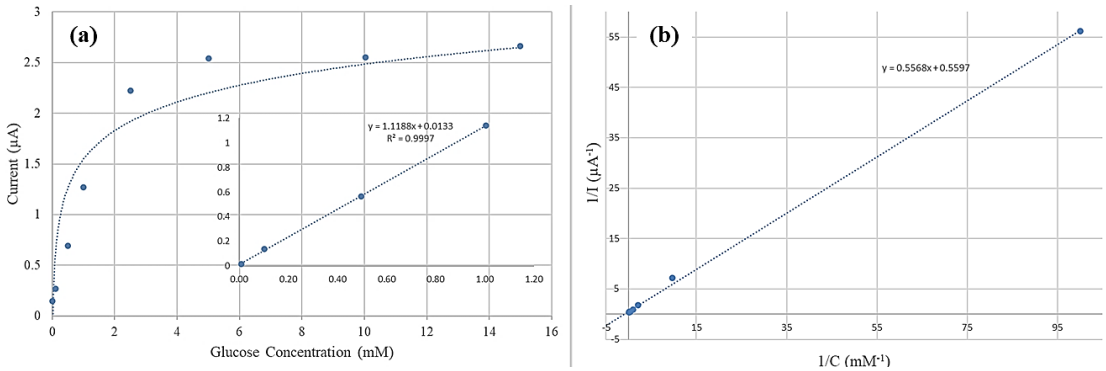

Figure 7. (a) Calibration curve of the fabricated biosensor. Inset figure: the linear range of the fabricated biosensor from 0.01-15 mM glucose. (b)The Lineweaver–Burk plot of 1/*I* vs. 1/*C* for the fabricated biosensor.

The apparent Michaelis–Menten constant (Km) was used to assess the biological activity of the immobilized enzyme and to determine the enzyme substrate kinetics [28]. It can be estimated from the Lineweaver–Burk equation [29] as follow (Equation 4):

$$\frac{1}{i} = \frac{K_m}{i_{max} \times C} + \frac{1}{i_{max}} \quad (4)$$

where C is the substrate concentration, i_{max} is the maximum current obtained under substrate saturation and i_{ss} is the steady-state current after the addition of substrate. Figure 7b depicts the Lineweaver–Burk plot of the prepared biosensor. The K_m and i_{max} values for the fabricated biosensor were calculated as 0.99 mM and 1.79 µA, respectively. The low K_m value can be attributed to the excellent affinity between the electrode and the enzyme. When compared with other ZnO nanoparticle-based glucose biosensors, the prepared biosensor has a much smaller Km (Table 1) and therefore a high biological affinity for glucose.

Table 1. Comparison of the analytical performance of ZnO nanoparticles-based glucose biosensors

Electrode material	Km	LOD (µM)	Sensitivity (µA cm^{-2} mM^{-1})	Response Time (s)	Ref.
ZnO nanowires	-	46	1.2 ± 0.2	5–10	[11]
ZnO nanorod array	2.9	10	23.1	<5	[30]
ZnO nanorod	1.95	10	25.7	<2	[31]
ZnO:Co nanoclusters	21	20	13.3	8	[32]
ZnO nanocombs	2.19	20	15.3	<10	[33]

ZnO nanoparticle	0.99	14.7	16.0	<1	This work

4. Conclusions

In this study, ZnO nanoparticles were synthesized by an eco-friendly approach using a *Z. officinale* root extract. The synthesized ZnO nanoparticles were incorporated into a glucose biosensor, which showed a good performance. The incorporation of ZnO nanoparticles can significantly increase the electrocatalytic activity of the GOx. The experimental results indicated that the green synthesized ZnO nanoparticle-modified glucose biosensor performs much better than many previously published ZnO nanoparticle–based biosensors in terms of its sensitivity, response time, detection limit, cost-effectiveness, and low K_m value. The lower K_m indicates a higher enzymatic activity of the immobilized GOx. The biosensor also showed satisfactory anti-interference capability in the presence of the common interfering substances such as UA and AA.

Acknowledgments

I wish to thank Dr. Ömer Gürkan Dilek and Dr. Erhan Keyvan for their valuable technical support on this study.

Kaynaklar

[1]. Yang, Z., Z. Ye, B. Zhao, X. Zong, and P. Wang, "A rapid response time and highly sensitive amperometric glucose biosensor based on ZnO nanorod via citric acid-assisted annealing route," Physica E Low Dimens. Syst. Nanostruct., 2010, 42(6),1830-1833.
[2]. Galant, A.L., R.C. Kaufman, and J.D. Wilson, "Glucose: Detection and analysis," Food Chem., 2015, 188,149-160.
[3]. Dönmez, S., F. Arslan, N. Sarı, N. Kurnaz Yetim, and H. Arslan, "Preparation of carbon paste electrodes including poly(styrene) attached glycine–Pt(IV) for amperometric detection of glucose," Biosensors Bioelectron., 2014, 54,146-150.
[4]. Guan, H., D. Gong, Y. Song, B. Han, and N. Zhang, "Biosensor composed of integrated glucose oxidase with liposome microreactors/chitosan nanocomposite for amperometric glucose sensing," Colloids Surf. Physicochem. Eng. Aspects, 2019, 574,260-267.
[5]. Nazemi, Z., E. Shams, and M.K. Amini, "Construction of a biointerface for glucose oxidase through diazonium chemistry and electrostatic self-assembly technique," J. Solid State Electrochem., 2016, 20(2),429-438.
[6]. Anusha, J.R., C.J. Raj, B.-B. Cho, A.T. Fleming, K.-H. Yu, and B.C. Kim, "Amperometric glucose biosensor based on glucose oxidase immobilized over chitosan nanoparticles from gladius of Uroteuthis duvauceli," Sensors Actuators B: Chem., 2015, 215,536-543.
[7]. Suzuki, N., J. Lee, N. Loew, Y. Takahashi-Inose, J. Okuda-Shimazaki, K. Kojima, et al., "Engineered Glucose Oxidase Capable of Quasi-Direct Electron Transfer after a Quick-and-Easy Modification with a Mediator," Int. J. Mol. Sci., 2020, 21(3),1137.
[8]. Anusha, J., C.J. Raj, B.-B. Cho, A.T. Fleming, K.-H. Yu, and B.C. Kim, "Amperometric glucose biosensor based on glucose oxidase immobilized over chitosan nanoparticles from gladius of Uroteuthis duvauceli," Sensors Actuators B: Chem., 2015, 215,536-543.
[9]. Ahmad, M., C. Pan, Z. Luo, and J. Zhu, "A Single ZnO Nanofiber-Based Highly Sensitive Amperometric Glucose Biosensor," J. Phys. Chem. C, 2010, 114(20),9308-9313.
[10]. Aini, B.N., S. Siddiquee, K. Ampon, K.F. Rodrigues, and S. Suryani, "Development of glucose biosensor based on ZnO nanoparticles film and glucose oxidase-immobilized eggshell membrane," Sens Biosensing Res, 2015, 4,46-56.

[11]. Fung, C.M., J.S. Lloyd, S. Samavat, D. Deganello, and K.S. Teng, "Facile fabrication of electrochemical ZnO nanowire glucose biosensor using roll to roll printing technique," Sensors Actuators B: Chem., 2017, 247,807-813.
[12]. Muthuchamy, N., R. Atchudan, T.N.J.I. Edison, S. Perumal, and Y.R. Lee, "High-performance glucose biosensor based on green synthesized zinc oxide nanoparticle embedded nitrogen-doped carbon sheet," J. Electroanal. Chem., 2018, 816,195-204.
[13]. Janaki, A.C., E. Sailatha, and S. Gunasekaran, "Synthesis, characteristics and antimicrobial activity of ZnO nanoparticles," Spectrochim. Acta A, 2015, 144,17-22.
[14]. Al Abdullah, K., S. Awad, J. Zaraket, and C. Salame, "Synthesis of ZnO Nanopowders By Using Sol-Gel and Studying Their Structural and Electrical Properties at Different Temperature," Energy Procedia, 2017, 119,565-570.
[15]. Kim, J.H., W.C. Choi, H.Y. Kim, Y. Kang, and Y.-K. Park, "Preparation of mono-dispersed mixed metal oxide micro hollow spheres by homogeneous precipitation in a micro precipitator," Powder Technol., 2005, 153(3),166-175.
[16]. Kahn, M.L., M. Monge, V. Colliere, F. Senocq, A. Maisonnat, and B. Chaudret, "Size-and shape-control of crystalline zinc oxide nanoparticles: a new organometallic synthetic method," Adv. Funct. Mater., 2005, 15(3),458-468.
[17]. Dai, Z.R., Z.W. Pan, and Z. Wang, "Novel nanostructures of functional oxides synthesized by thermal evaporation," Adv. Funct. Mater., 2003, 13(1),9-24.
[18]. Hasanpoor, M., M. Aliofkhazraei, and H. Delavari, "Microwave-assisted Synthesis of Zinc Oxide Nanoparticles," Procedia Materials Science, 2015, 11,320-325.
[19]. Vijayakumar, S., S. Mahadevan, P. Arulmozhi, S. Sriram, and P.K. Praseetha, "Green synthesis of zinc oxide nanoparticles using Atalantia monophylla leaf extracts: Characterization and antimicrobial analysis," Mater. Sci. Semicond. Process., 2018, 82,39-45.
[20]. Yuvakkumar, R., J. Suresh, A.J. Nathanael, M. Sundrarajan, and S. Hong, "Novel green synthetic strategy to prepare ZnO nanocrystals using rambutan (Nephelium lappaceum L.) peel extract and its antibacterial applications," Mater. Sci. Eng. C, 2014, 41,17-27.
[21]. Anusha, J.R., H.-J. Kim, A.T. Fleming, S.J. Das, K.-H. Yu, B.C. Kim, et al., "Simple fabrication of ZnO/Pt/chitosan electrode for enzymatic glucose biosensor," Sensors Actuators B: Chem., 2014, 202,827-833.
[22]. Shamsazar, A., F. Shamsazar, A. Asadi, and S. Rezaei-Zarchi, "A glucose biosensor based on glucose oxidase enzyme and ZnO nanoparticles modified carbon paste electrode," Int. J. Electrochem. Sci, 2016, 11,9891-9901.
[23]. Kumar, S.K.N., K. Kavyashree, B.C. Pallavi, S. Kiran, N.S. Kumar, and P. Sharanappa. Fabrication and characterization of enzymatic glucose sensor based on ZnO nanoparticles. in International Conference on Advanced Nanomaterials & Emerging Engineering Technologies. 2013.
[24]. Safawo, T., B. Sandeep, S. Pola, and A. Tadesse, "Synthesis and characterization of zinc oxide nanoparticles using tuber extract of anchote (Coccinia abyssinica (Lam.) Cong.) for antimicrobial and antioxidant activity assessment," OpenNano, 2018, 3,56-63.
[25]. Bala, N., S. Saha, M. Chakraborty, M. Maiti, S. Das, R. Basu, et al., "Green synthesis of zinc oxide nanoparticles using Hibiscus subdariffa leaf extract: effect of temperature on synthesis, anti-bacterial activity and anti-diabetic activity," RSC Adv., 2015, 5(7),4993-5003.
[26]. Chen, L., I. Batjikh, J. Hurh, Y. Han, Y. Huo, H. Ali, et al., "Green synthesis of zinc oxide nanoparticles from root extract of Scutellaria baicalensis and its photocatalytic degradation activity using methylene blue," Optik, 2019, 184,324-329.
[27]. Numnuam, A., P. Thavarungkul, and P. Kanatharana, "An amperometric uric acid biosensor based on chitosan-carbon nanotubes electrospun nanofiber on silver nanoparticles," Anal. Bioanal. Chem., 2014, 406(15),3763-3772.
[28]. Chen, X., J. Chen, C. Deng, C. Xiao, Y. Yang, Z. Nie, et al., "Amperometric glucose biosensor based on boron-doped carbon nanotubes modified electrode," Talanta, 2008, 76(4),763-767.

[29]. Nenkova, R., D. Ivanova, J. Vladimirova, and T. Godjevargova, "New amperometric glucose biosensor based on cross-linking of glucose oxidase on silica gel/multiwalled carbon nanotubes/polyacrylonitrile nanocomposite film," Sensors Actuators B: Chem., 2010, 148(1),59-65.
[30]. Wei, A., X.W. Sun, J. Wang, Y. Lei, X. Cai, C.M. Li, et al., "Enzymatic glucose biosensor based on ZnO nanorod array grown by hydrothermal decomposition," Appl. Phys. Lett., 2006, 89(12),123902.
[31]. Yang, Z., Z. Ye, B. Zhao, X. Zong, and P. Wang, "A rapid response time and highly sensitive amperometric glucose biosensor based on ZnO nanorod via citric acid-assisted annealing route," Physica E Low Dimens. Syst. Nanostruct., 2010, 42(6),1830-1833.
[32]. Zhao, Z.W., X.J. Chen, B.K. Tay, J.S. Chen, Z.J. Han, and K.A. Khor, "A novel amperometric biosensor based on ZnO:Co nanoclusters for biosensing glucose," Biosensors Bioelectron., 2007, 23(1),135-139.
[33]. Wang, J., X.W. Sun, A. Wei, Y. Lei, X. Cai, C.M. Li, et al., "Zinc oxide nanocomb biosensor for glucose detection," Appl. Phys. Lett., 2006, 88(23),233106.