Available online freely at www.isisn.org

Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

Journal by Innovative Scientific Information & Services Network

RESEARCH ARTICLE BIOSCIENCE RESEARCH, 2020 17(2): 775-785. **OPEN ACCESS**

Highly sensitive glucose sensor based on ZnO NPs as a biomimetic enzyme

Hayrunnisa Nadaroglu[1, 2*] and Azize Alayli[2,3]

[1]Department of Food Technology, Vocational School of Technical Science, Ataturk University, 25240 Erzurum, **Turkey**
[2]Department of Nano-Science and Nano-Engineering, Institute of Science and Technology, Ataturk University, 25240 Erzurum, **Turkey**
[3]Department of Nursing, Faculty of Health Sciences, Sakarya University of Applied Sciences, 54187, Sakarya, **Turkey**

***Correspondence:** hnisa25@atauni.edu.tr **Received24-02-2020, Revised: 14-05-2020, Accepted: 17-05-2020 e-Published: 01-06-2020**

In this work, the development of zinc biosensors based on zinc nanoparticles (Zn NPs) biomimetic enzymes were carried out to spectrophotometrically detect glucose in food samples. Briefly; Glucose oxidase enzyme reacts with glucose in food samples to form gluconic acid and H_2O_2. Behind; the peroxide formed can be measured spectrophotometrically using ABTS substrate with the enzyme mimic effect of Zn NPs. In this way, the amount of glucose was determined by a simple, effective and selective method by measuring the change in absorbance of the reaction medium, which changed by oxidation. ZnO NPs were obtained from $Zn(NO_3)_2$ by green synthesis method using raw fig extract. Then, the amount of glucose in some food samples was determined using ZnO NPs. The results showed that the glucose content in the samples was exactly and reproducibly measured with a correlation coefficient of 0.9812 at 3.47 mM-27.78 mM concentrations. This will greatly simplify the design and manufacture of the new biosensor, which can be used for glucose fixation in food samples in an inexpensive, efficient, environmentally friendly and fast manner.

Keywords: ZnO Nanoparticles. Biosensors. Glucose amount. Food samples

INTRODUCTION

The synthesis of metal oxide nanoparticles has now become a very important research area. Nanoparticle synthesis, which has different properties due to the wide area of usage, has become very important especially in the sectors where the area of use is medical, cavern, agriculture, defense, health and energy etc. Another problem we face today is to determine the sensitive, inexpensive and accurate glucose in foods or biological fluids. In the food sector, the amount of glucose in different foods can be determined and the effect on nutrition can be seen. In addition, determination of glucose in biological fluids such as blood and serum can be diagnosed and monitored. Nowadays, monitoring of diabetes is carried out with a biosensor similar to the method we plan to develop. It has been extensively researched for the development of glucose-based biosensors which are widely used in ZnO, Cu (I) / (II) oxides, MnO_2, TiO_2, CeO_2, SiO_2, ZrO_2 and some other metal oxides. (Rahman et al., 2010; Nadaroglu et al., 2017a). The use of nanoparticles in biosensor development is one of the popular subjects that are widely studied. The use of nanoparticles in sensor development resulted in nano-biosensors. Nano-sensors; it has been developed for the specific detection of biological molecules, such as enzymes (proteins) (HU et al., 2009) and nucleic

acids (SIDDIQUEE et al. 2010), as well as infectious agents (LIU; LIN 2007; SINGH et al., 2007). Nanoparticles can support effective electron transfer between the electrodes and the active site of the enzyme, depending on the unique physical properties they gain with their size. Therefore, many nanoparticles show similar properties to oxidoreductases today. Also, some nanoparticles are called nanozyme because of their enzyme-like properties (Rahman et al., 2010; Demirci Gültekin et al. 2016; Nadaroglu et al., 2017b).

Zinc oxide nanoparticles are one of the most important nanomaterials due to their unique electronic, metallic and structural properties. It has been confirmed that biological activity provides direct charge transfer to remain on the surface (LIU; LIN 2007; SINGH et al., 2007). In addition, the working principle of bionsensensors is based on the fundamental principles of potentiometric, amperometric, impedimetric and conductometric, colorimetric, enzymatic, and the combination of these. In this study, we aimed to synthesize nano ZnO molecules which have new properties by using the green synthesis method and the synthesis power of the compounds in Fig plant, from $Zn(NO_3)$ salt (Nadaroglu et al., 2017c).

After the characterization of the new molecular, we planned to use the Zn nanoparticle for the determination of the amount of Glucose by considering that the nano-ZnO particles would be able to mimic the electrochemical properties of the enzyme. For this purpose, we aimed to determine the usage area of the method by analyzing different food samples. We have planned our method to be a simple and reliable method with a wide range of applications based on enzymatic and colorimetric principles.

MATERIALS AND METHODS

Chemicals

Fig (*Ficus carica*) fruits were obtained from Sakarya province in September, 2017. Zinc nitrate ($Zn(NO_3)_2$), Sodium phosphate (Na_2HPO_4), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), hydrochloric acid (HCl) were purchased from Sigma-Aldrich GmbH, (Sternh In Germany). The other chemicals were obtained from Merck. Distilled water was used in all experiments.

Preparation of plant extract

Fig (*Ficus carica*) fruit was collected from near Sakarya, Turkey and it was identified with the helping of taxonomists. Fig (*Ficus carica*) fruits were washed with distilled water several times for cleaning dust and soil on plants. Small pieces (25 g) were thoroughly shattered to form a homogeneous mixture in blander using 250 mL, 10 mM sodium phosphate buffer (pH: 6.0). Then, it was centrifuged at 5000 *xg* for 10 min and the supernatant was used for green synthesis.

Synthesis of ZnO Nanoparticles

Fig fruit extract was added in sample of $Zn(NO_3)_2$ solution and the reaction mixture was incubated in a closed space for 4 hours. Then, these NPs were washed with alcohol and obtained ZnO NPs were dried at 60 ^oC for 4 hours (Nadaroglu et al., 2017c).

Interaction time

The samples were taken from the reaction medium prepared using $Zn(NO_3)_2$ and fig extract, with a 3-minute interval and they were monitored during 240 minutes by measuring the absorbance changes (255 nm) against the blank solution.

And it was determined when time of the highest formation of the ZnO NPs. Optimum pH;

For this purpose, the reaction medium was formed using related buffer solution (phosphate buffer for pH: 2-3, acetate buffer for pH: 4-6, phosphate buffer for pH: 7-8 and carbonate buffer for pH: 9-11), and the changes in absorbance at 255 nm was measured by spectrophotometer.

Optimum Temperature

In order to determine the temperature at which the ZnO NPs are synthesized more effectively, reactions were made at the range of 10-90 ^oC to determine the optimum temperature by analyzing the sample solutions taken from the reaction medium against the blank solutions.

Characterisation of ZnO NPs

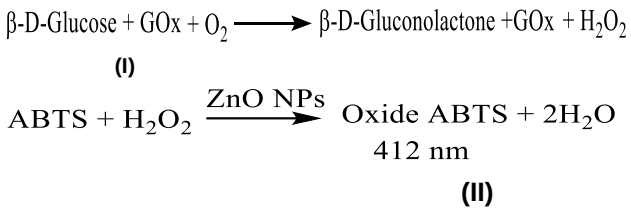
ZnO NPs were firstly synthesized using Fig (*Ficus carica*) fruit extract and they were characterized with scanning at range of 200–1000 nm by using UV–Vis spectrophotometer (Epoch Nanodrop UV–vis spectrophotometer). Secondly, determination of surface topography of ZnO NPs was performed by SEM. The surface morphologies of the ZnO NPs were examined using a Metek, Apollo prime, active area 10 mm^2, Microscope inspect S50, SE detector R580 SEM operated at 20 kV on samples with energy-dispersive X-ray (EDAX) analysis attached to scanning electron microscope (SEM). Then, XRD

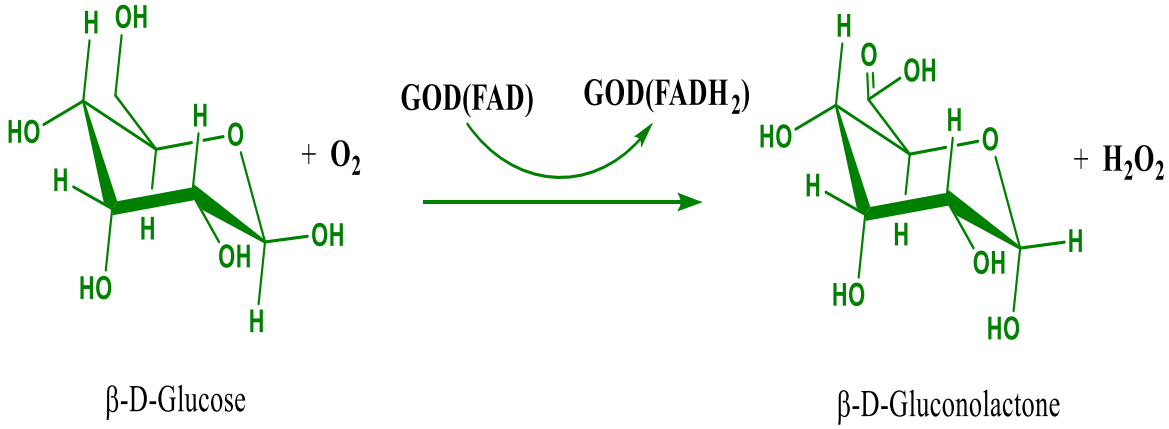
analysis was determined in determination of crystallinity of ZnO NPs. X-ray diffraction (XRD) patterns were performed on Panalytical empyrean equipped with Ni-filtered Cu Kα radiation (λ = 0.1542 nm) in the range of 10°–80° at a scanning rate of 4° min^{-1}. FTIR analysis of ZnO NPs was recorded using Vertex 80 Model FTIR Frontier spectrophotometer with attenuated total reflection (ATR) technique in the 4000-400 cm^{-1} region.

Working Principle of Glucose Nanobiosensor

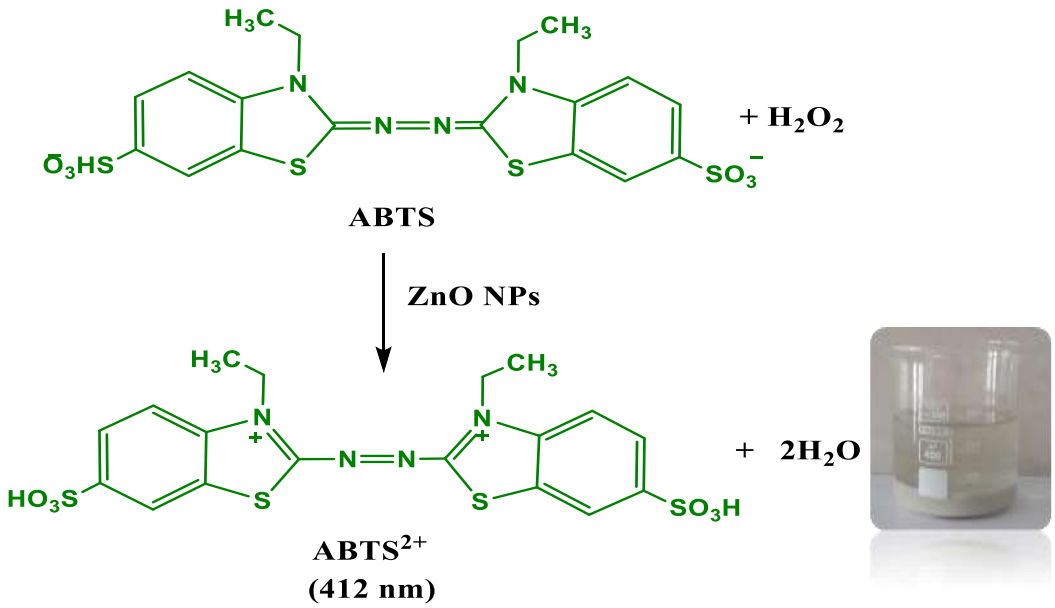
For analysis; the reaction medium was prepared by adding 24 mL of the ABTS solution prepared as 60 mM, 10 µL of homogenous 0.1 mM ZnO NPs, 185 µL of 100 mM H_2O_2 solution and 185 µL of 0.2 M acetate buffer (pH: 4.0). The reaction mixture homogenized in the magnetic stirrer was allowed to incubate in the water bath set at 45 ° C for 10 min. The tubes removed from the water bath were centrifuged and the ZnO magnetic nanoparticles were removed from the solution. Add 100 mL of the reaction solution from which the ZnO NPs were separated and add 900 mL of distilled water and mix. Absorbance change was measured by UV-Vis spectrophotometer (GUNGOR et al.2018).

Glucose oxidase is a stable enzyme, which helps in oxidizing glucose to glucolactone and to convert the oxygen into hydrogen peroxide. Hydrogen peroxide is a simple compound consisting of a single oxygen–oxygen bond. The obtaining hydrogen peroxide reacts with ABTS to form the colored ABTS complex. The color change (412 nm) resulting from the reaction is measured to measure the peroxide and the amount of glucose that formed it and is calculated using the standard graph (Schema 1 and 2).





Schema 1. Reactions used in the determination of glucose amount.



Schema 2. Reaction of the ABTS radical used in the determination of glucose

RESULTS AND DISCUSSION

Glucose oxidase (GOx) based glucose biosensors have been of great importance in the research and development of glucose sensors and the market place over the last 40 years. This is due to the high demand for monitoring the amount of blood glucose, both biologically and clinically, precisely and reliably (RAKOW; Suslick 2000; Koschinsky; Heinemann 2001; LIU; LIN 2007; Oliver et al., 2009; Gündüz et al., 2017). There are still some disadvantages of enzyme-based glucose determination. Examples include critical enzyme immobilization, optimum temperature and critical operating conditions such as pH, chemical instability and high cost (Sachedina; Pickup 2003; Reitz et al., 2008). Metal nanoparticles have become a major area of research nowadays because of their widespread use. Some of these applications are antimicrobial antioxidant properties and their use as biosensors. One of these research areas is the fact that foods or clinical samples can be used as nanosensors for the determination of glucose (Karaduman et al. 2017; Nadaroglu et al., 2017d). In addition, it has been shown that nanoparticles exhibit enzyme-like properties along with the dimensions of the structures (Koschinsky ; Heinemann 2001). For this purpose, optimum conditions were determined and ZnO nanoparticles were synthesized by using fig extract. Follow-up of the green synthesis (370 nm) was also performed with UV-Vis spectrophotometer and the data are shown in Figure 1.

The absorbance values of synthesized Zn NPs were measured against distilled water using UV-VIS spectrophotometer. UV-visible spectra of zinc nanoparticles synthesized by reduction of the compound ZnO and also catalyzed with purified peroxidase enzyme from Fig (*Ficus carica*) fruit were showed in Figure 1. The peaks of synthesized zinc nanoparticles by peroxidase enzyme were in the range of 190 to 600 nm and the sharpest peak of these nanoparticles was observed at 370 nm (Figure 1). The sharp peak at 370nm exhibited the characteristic peak of synthesized ZnO nanoparticles. Khorsand and his group found that ZnO Nanoparticles absorb absorbance at 370 nm as we found in our study (Khorsand Zak et al., 2011).

As a result of studies to optimize the synthesis of ZnO nanoparticles; It was observed that ZnO nanoparticle synthesis reached its maximum at 30 minutes, pH: 4 (Figure 2A), Temperature 40^{o}C, (Figure 2B) and 7 mM $ZnCl_2$ concentration (Figure 2C). In their study, SINGH et al. Reported that the optimal synthesis of ZnO NPs was at 40 ^{o}C. In their study, SINGH et al. Reported that the optimal synthesis of ZnO NPs was at 40 ^{o}C and pH: 7.0 [20]. The optimum synthesis concentration for the starting material $Zn(COOH)_2$ was reported to be 5 mM as a result of their experiments and they stated that their reactions were completed within 75 minutes (Singh et al., 2018).

Based on the data obtained, ZnO nanoparticle synthesis was performed and the synthesized

nanoparticles were filtered, washed and dried at 60 ^{o}C.

Characterization of the Synthesized ZnO Nanoparticles

XRD of ZnO NPs

Zinc nanoparticles' XRD which was produced in its peroxidase enzyme catalyst and its crystallographic analysis are given in Figure 3A. Characteristic peaks which belong to XRD spectrum in its at 2θ = 14.15^{o}, 23.55^{o}, 36.09^{o}, 49.22 ^{o} that can be indexed at (111), (200), (220) facets which agree with the values reported for face centered cubic (fcc) zinc nanocrystals (Figure 3A).

Surface Characterization of ZnO NPs

Chemical and mineralogical compositions of synthesized green zinc NPs were determined by scanning electron microscopy (SEM), which was used to examine the surface of the adsorbent. Images of ZnO NPs were magnified 10.000 times by Zeiss, Active area 10 mm^2 (Figure 3B).

The X-ray diffraction (XRD) patterns of the ZnO seed layer and the grown nano-rods are illustrated in Figure 3B. The XRD peaks attributed to ZnO were detected at 2θ values of 31.7°, 34.4° and 36.2°, corresponding to the (100), (002) and (101) planes, respectively, for both samples. This pattern was consistent with wurtzite crystal structure (ICDD 36-1451). The ZnO seed layer pattern had higher intensity of the (101) plane than that of the (002) plane. This finding was due to the random atomic arrangement of glass substrate that may have disturbed the orientation of ZnO seed layer, resulting in a small incline in the orientation of the seed layer.

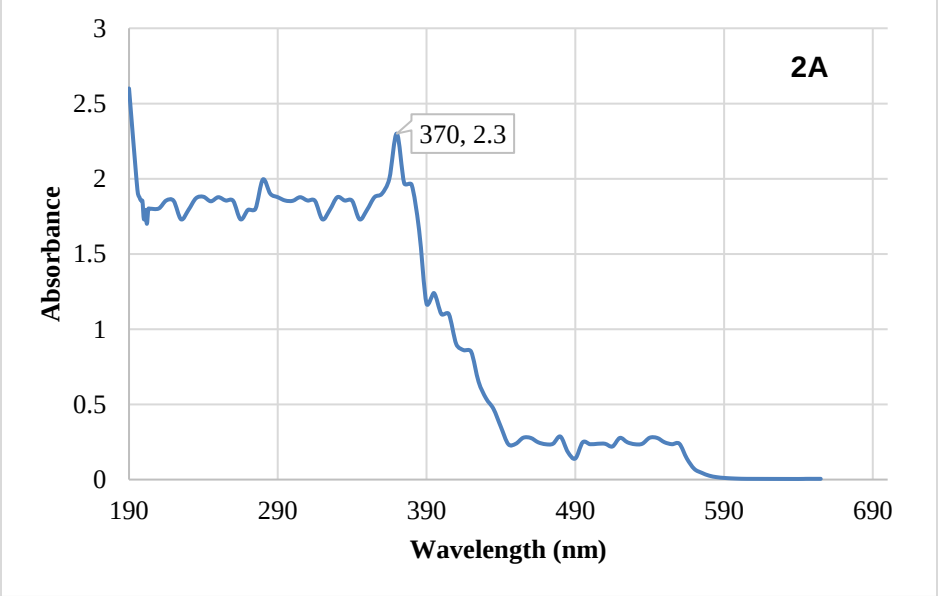
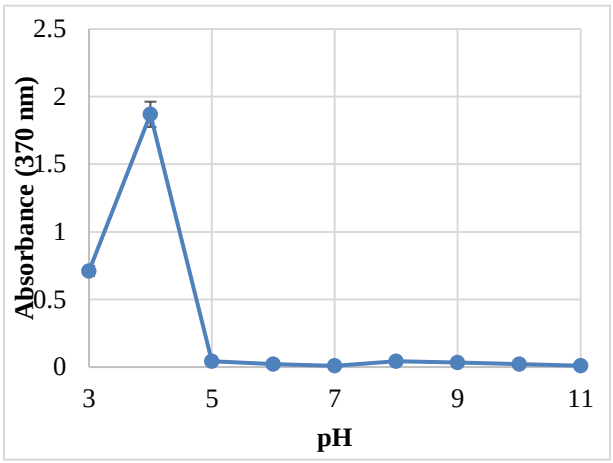
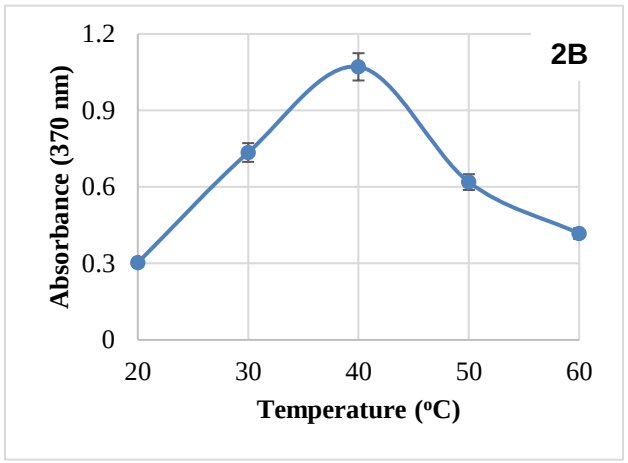


Figure 1: Scanning of ZnO NPs' wavelength





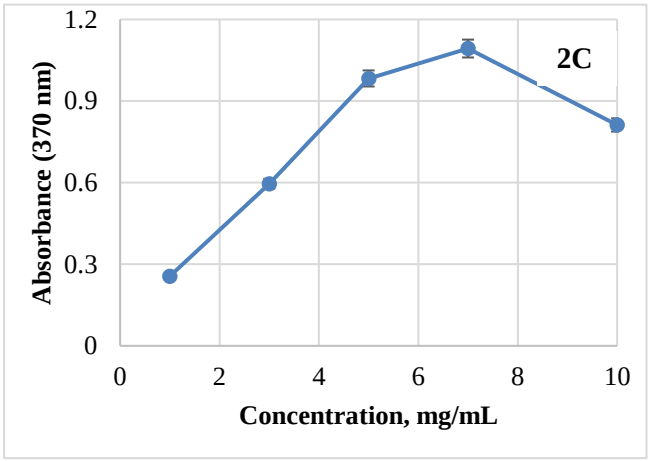
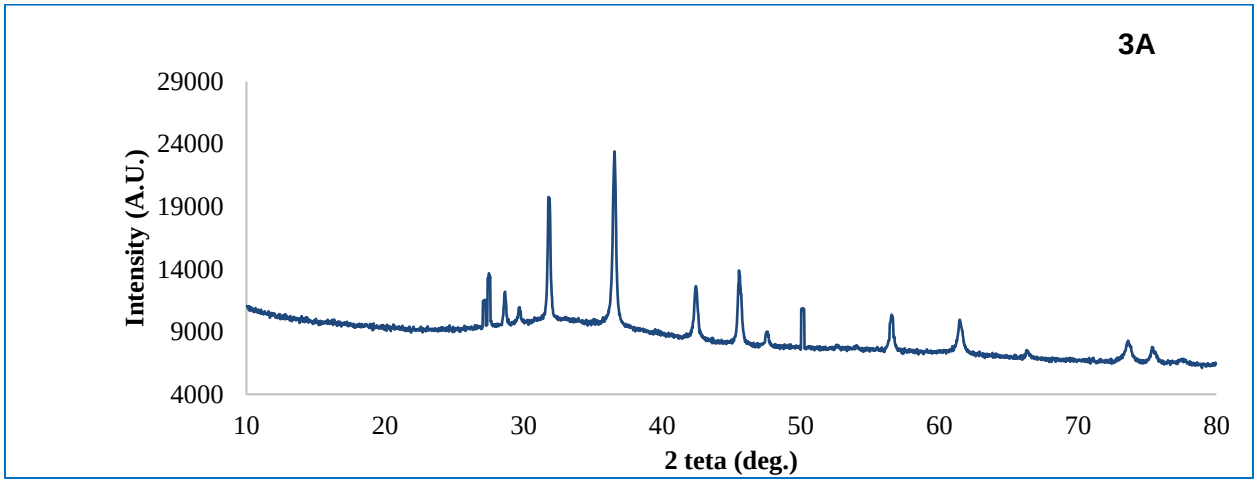


Figure 2: The effect of pH, (A) Temperature (B) and concentration of metal ion (C) on the synthesis ZnO NPs using green synthesis



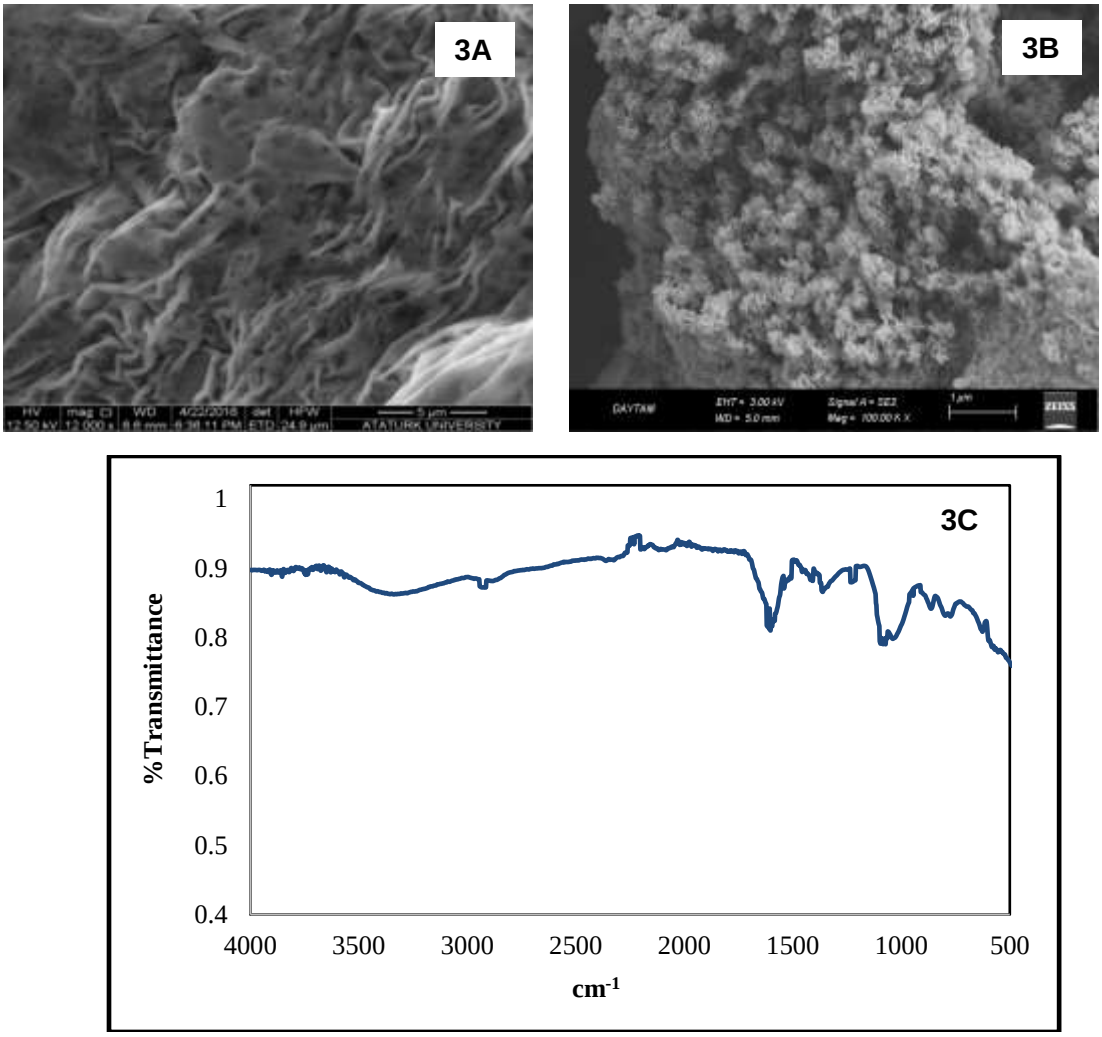


Figure 3: (A) XRD image (b) SEM image (C) FTIR image of Zn NPs

This result agreed with that of Chakrabarti et al., indicating that the substrate used can influence the preferred orientation in sol–gel-derived ZnO films due to the glass substrate with non-bridging oxygen. The intensity of the (002) plane in ZnO nanorods increased after hydrothermal reaction compared with ZnO seed layer because of the preferred growth in the c-axis direction. However, the intensity of the (101) plane was slightly similar to that of the (002) plane due to initial nuclei alignment. When the (101) plane of ZnO nanorods was initially grown on the (002) axis of ZnO seed layer, ZnO nanorods grew in the c-axis and along the length but slightly inclined. This result agreed with the FESEM images indicating the growth of ZnO nano-rods with inclined alignment (Figure 3(A and B).

Fourier Transform Infrared Spectroscopy (FTIR) Analysis

Figure 3C shows the FTIR of the ZnO-NPs prepared by the solvo thermal method, in the range of 1000–200 cm^{-1}. There were several absorption bands at araund 912, 723, 628, 588, 470 and 391 cm^{-1}. A broad absorption band was observed at around 3391 cm^{-1}. The band at 391 cm^{-1} corresponds to cubic structure. The FTIR results show the high purity of the obtained ZnO-NPs (Figure 3C).

The characterized ZnO nanoparticles were then used to design the nano-sensor to determine Glucose. Therefore, a standard graph at different glucose concentrations was initially plotted in Figure 4.

In our study, high correlation between 3.47 mM – 27.78 mM was obtained and it was thought that the method could be used by diluting the values above this range. The fabricated electrode also showed a high sensitivity of 48.75 μA/mM•cm^2 within the linear range of 0.05 to 1 mM and good resistance towards interfering species. The modified electrode showed comparable results with a well-established glucometer to determine glucose in blood samples at different concentrations. In his study, Ridhuan and his group reported a good correlation between the 0.05 - 1 mM glucose concentration range. They made all measurements using Nafion/GOx/ZnO NR electrode (TUKOMP 2018). In his study, Ridhuan and his group reported a good correlation between the 0.05 - 1 mM glucose concentration range. They made all measurements using Nafion / GOx / ZnO NR electrode (TUKOMP 2018).

The obtained calibration curve was used to determine the amount of glucose in different foods. For this purpose; the amount of glucose was measured spectrophotometrically after pre-treatment for both the comparison standard kit and the newly developed method in Cherry juice, Orange juice, peach juice, molasses, honey, milk, energy drink, coke and bread samples (Table 1).

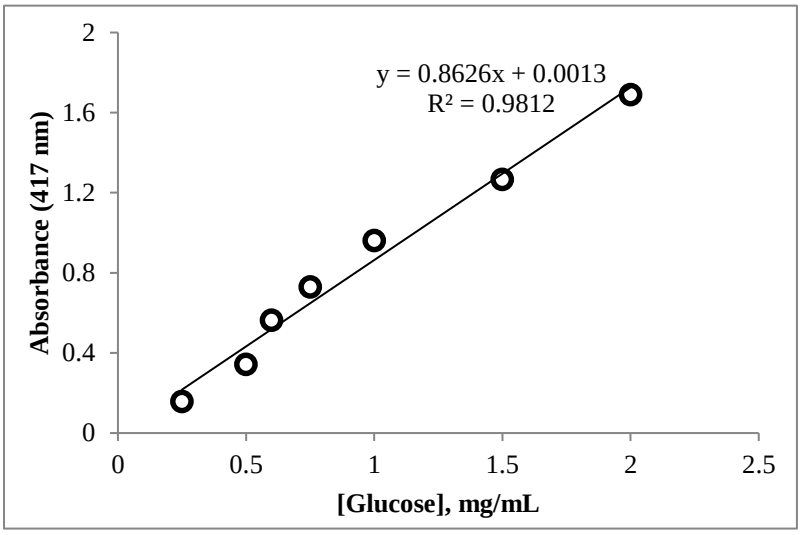


Figure 4: Calibration curve of glucose at 0.1 mM ZnO concentration (50 mM phosphate buffer solution (pH: 7.0) and 25 °C).

Table 1: The amount of glucose in different foods.

Food sample	ZnO NPs Based Spectrophotometric Method (g/L)	Glucose Analysis Kit with Spectrophotometric Method (g/L)	The Amount of Glucose in Foods from the Literature HPLC (g/L)
Cherry juice	158.2 ± 1.12	113.2 ± 1.05	106.2 g
Orange juice	48.1 ± 1.6	42.8 ± 1.4	46.4
Peach juice	62.2± 3.12	58.2 ± 2.22	56.1
Molasses	14.76 ± 4.05	14.8 ± 1.06	10.0
Honey	238.4 ± 1.11	242.5 ± 1.0	283.9
Milk	43.3 ± 0.9	46.9 ± 1.13	50.0
Energy drink	246.1± 3.2	217.99 ± 2.6	215.5
Coke	54.16 ± 0.5	53.03 ± 0.9	52.3
Bread	26.07 ± 0.14	26.99 ± 0.63	-

When the results were compared, both methods were compared by using the same biosensor-based spectrophotometric measurement in the market and the glucose levels in the food were compared from the TURKOMP data bank (TUKOMP 2018). According to the results, it was concluded that Zn NPs glucose levels were similar to the results obtained by using the commercial kit and both findings were consistent with the findings obtained by TURKOMP.

CONCLUSION

Currently, nanotechnology, one of the fastest growing areas, the high surface area and sole physicochemical properties of many nanomaterials create it's potential to produce new structures, systems, nano platforms or devices with possible uses in a extensive variety of disciplines. ZnO NPs have little toxicity and biodegradability properties. Various nanostructured ZnOs have been reported for glucose monitoring and they showed excellent sensitivity and selectivity.

ZnO nanoparticles are among the limited number of studied nanoparticles in the determination of glucose up to date. It has been found that ZnO nanoparticles are preferred for such a method due to the potential for electron reduction and the methods are placed on electrical based sources.

The biosensors produced with ZnO produced in this way were used to determine the glucose in the solution medium in an electrically sensitive manner. In addition, the ZnO nanoparticles were immobilized with the GOx enzyme and the sensor was developed (Kim et al., 2006; Rahman et al., 2010).

New nano materials were synthesized by using ZnO and GOx together with different methods (Zhao et al., 2007). Most of these sensors are based on electrochemical potential difference. One of the methods for the determination of glucose is by HPLC. All of these methods, which we have seen so far, are generally cost-sensitive and the range of precision is variable. When the values obtained from the new method based on the spectrophotometric basis used to determine the glucose in the market and the values in the TURKOMP database were compared, it was understood that a new method with high sensitivity was developed. It is thought that ZnO nanoparticles can be used to selectively and selectively determine glucose rate in clinical samples.

ZnO NPs could be important components in bio analytical devices since they clearly enhance the performances in terms of sensitivity. Zn NPs could be used for determination of glucose at the low amount in food samples. Furthermore, ZnO NPs could be combined with different nanomaterial to increase even more the performances of biosensors is a well-accepted strategy.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

ACKNOWLEGEMENT

This research has been performed under the project numbered FAD-2018-6321 and supported by the Research Development Center of Ataturk University. The authors acknowledged the support of Ataturk University, Turkey, for this work. The authors, Hayrunnisa Nadaroglu and Azize Alayli Gungor, hereby confirm that this manuscript is performed according to guidelines and has not already been published nor is it under consideration for publication elsewhere. This article does not contain any studies with human or animal subjects.

AUTHOR CONTRIBUTIONS

The authors (HN and AA) participated in the preparation of the article. All the authors have read and approved final version of the article.

REFERENCES

Gultekın, D. D, Alaylı gungor A, Onem H, Babagıl, A., Nadaroglu, H. Synthesis of copper nanoparticles using a different method: determination of their antioxidant and antimicrobial activity. Journal of the Turkish Chemical Society, Section A: Chemistry v. 3 n. 3 p. 623-623, 2016. doi.org/10.18596/jotcsa.287299

Gunduz,S., Nadaroglu, H., Turgut, E., Gungor, A.A. Determination of hydrogen peroxide in foods using enzyme-like behaviors of nano iron-oxide. Internatıonal Journal of Innovative Research and Reviews, v. 1 p. 1–5, 2017.

Gungor, A.A., Nadarglu, h., Babagıl, a., ONEM, H. Green synthesis of Nanoceria (CeO_2) and evaluation of enzyme like characteristics. Journal of Advanced Materials and Processing, v. 6 p. 13–25, 2018.

HU, J., Zheng, P.C., Jıang, J.H., Shen, G.L., LIU, G.K. Electrostatic interaction based approach to thrombin detection by surface-enhanced raman spectroscopy. Analytical Chemistry, v. 81 n.1 p. 87-93, 2017. doi: 10.1021/ac801431m

Karaduman, I., Gungor, A.A., Nadaroglu, H., Altundas, A., Acar, S. Green synthesis of γ-Fe_2O_3 nanoparticles for methane gas sensing. Journal of Materials Science Materials in Electronics, v. 28(21) p. 16094–16105, 2017. doi: 10.1007/s10854-017-7510-5

Khorsand Zak A, razali R, ABD Majıd W.H., Darroudi M (2011) Synthesis and characterization of a narrow size distribution of zinc oxide nanoparticles. International Journal of Nanomedicine, v. 6 p. 1399-1403, 2011. doi: 10.2147/IJN.S19693

Kım, J.S., Park, W., IL Lee, C., YI, G. ZnO nanorod biosensor for highly sensitive detection of specific protein binding. Journal of the Korean Physical Society, v. 49 n.4 p. 1635-1639, 2006.

KOSCHINSKY, T., HEINEMANN, L. Sensors for glucose monitoring: Technical and clinical aspects. Diabetes-Metabolism Research and Reviews, v. 17 n.2 p. 113-23, 2001.

LIU, G., LIN, Y. Nanomaterial labels in electrochemical immunosensors and immunoassays. Talanta, 2007 v. 15 74 n. 3 p. 308-17. doi: 10.1016/j.talanta.2007.10.014.

Nadaroglu, H., Gungor, A.A., INCE, S. Synthesis of Nanoparticles by Green Synthesis Method. International Journal of Innovative Research and Reviews, v. 1 n. 1, p. 6-9, 2017a.

Nadaroglu, H., Gungor, A.A., INCE, S., Babagıl, A. Green synthesis and characterisation of platinum nanoparticles using quail egg yolk. Spectrochim Acta - Part A and Biomolecular Spectroscopy, v. 172 p. 43-47, 2017b. doi: 10.1016/j.saa.2016.05.023

Nadaroglu, H., INCE, S., Gungor, A.A. Green synthesis of gold nanoparticles using quail egg yolk and investigation of potential application areas. Green Process Synthesis, v. 6 p. 43-48, 2017c. doi: 10.1515/gps-2016-0091

Nadaroglu, H., Onem, H., Gungor, A.A. Green synthesis of Ce_2O_3 NPs and determination of its antioxidant activity. IET Nanobiotechnology, v. 11 n. 4 p. 411-419, 2017d. doi: 10.1049/iet-nbt.2016.0138

National Food Composition Database (TURKOMP) http://www.turkomp.gov.tr/database10.04.2018. http://www.injirr.com/article/view/4

Olıver, N.S., ToumazoU, C., CASS, A.E.G., Johnston, D.G. Glucose sensors: A review of current and emerging technology. Diabetic Medicine 26(3): 197-210, 2009.

Rahman, M.M., Ahammad, A.J.S., JIN, J.H., AHN, S.J., LEE, J.J. A comprehensive review of glucose biosensors based on nanostructured metal-oxides. Sensors, 2010. doi: 10.3390/s100504855

Rakow, N.A., Suslıck, K.S. A colorimetric sensor array for odour visualization. Nature, 406(6797): 710-713, 2000. doi: 10.1038/35021028

Reıtz, E., JIA, W., Gentile, M., Wang, Y., LEI, Y. CuO nanospheres based nonenzymatic glucose sensor. Electroanalysis, 20 (22) (2008) 2482–2486, 2008. doi: 10.1002/elan.200804327

Sachedına, N., PICKUP, J.C. Performance assessment of the Medtronic-MiniMed Continuous Glucose Monitoring System and its use for measurement of glycaemic control in Type 1 diabetic subjects. Diabetic Medicine, 20: 1012-1015 2003. doi: 10.1046/j.1464-5491.2003.01037.x

Sıddıquee, S., Yusof, N.A., Salleh, A.B., TAN, S. G., bakar, F. A., Lee Y. H., DNA hybridization based on Trichoderma harzianum gene probe immobilization on self-assembled monolayers on a modified gold electrode. Sensors Actuators, B Chemistry, 147(1): 198-205, 2010. doi: 10.1016/j.snb.2010.02.014

Sıngh, A.K., Pal, P., Gupta, V., Yadav, T.P., Gupta, V., Singh, S.P., Green synthesis, characterization and antimicrobial activity of zinc oxide quantum dots using *Eclipta alba*. Materials Chemistry and Physics, 203: 40-48, 2018. doi:10.1016/j.matchemphys.2017.09.049

Sıngh, S.P., Arya, S.K., Pandey, P., Malhotre, B.D., Cholesterol biosensor based on rf

sputtered zinc oxide nanoporous thin film. Applied Physics Letters, 2007. doi: 10.1063/1.2768302

Zhao, Z.W., Chen, X.J, TAY, B.K., TAY, B.K., Chen, J.S., Han, Z.J., Khor, K.A., (2007) A novel amperometric biosensor based on ZnO:Co nanoclusters for biosensing glucose. Biosensors and Bioelectronics, 23: 135–139, 2007. doi: 10.1016/j.bios.2007.03.014