

## **A simple strategy to fabricate electrochemical sensor based on nickel nanoparticles modified glassy carbon electrode for the determination of glucose in urine**

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### **ABSTRACT**

A non-enzymatic electrochemical sensor has been successfully fabricated using nickel nanoparticles as catalyst. Nickel nanoparticles were electrochemically deposited on a glassy carbon electrode surface by amperometric technique. Nickel nanoparticles modified glassy carbon electrode exhibited high catalytic activity for oxidation of glucose. The morphology of nickel nanoparticles was characterized by scanning electron microscopy (SEM). Electrochemical behaviors of glucose were investigated by cyclic voltammetry and chronoamperometry in alkaline aqueous solution. At the optimum conditions, the calibration curve for glucose determination was linear in the range of 0.05 – 5.00 mM and 5.00 – 17.5 mM and a low detection limit (S/N = 3) of 36  $\mu$ M. Furthermore, the proposed sensor was successfully applied to analyze glucose level in human urine samples when compared with clinical laboratory measurements. The glucose sensor showed a strong potential as a sensitive non-enzymatic and may be used in the future for the clinical diagnostic tool.

**Keywords:** *glucose sensor, nickel nanoparticles, electrodeposition, non-enzymatic*

### **INTRODUCTION**

Glucose determination has gained great attention as a result of its wide application in many areas such as biotechnology, food industry, and clinical diagnostics (Rahman *et al.*, 2010; Fang *et al.*, 2011; Zhu *et al.*, 2012; Wang *et al.*, 2013; Guo *et al.*, 2015). Especially, glucose plays an important analyte in clinical diagnostics because high levels of glucose in the blood (over 6 mM) will increase the risk of diabetes. Diabetes is a serious health care problem, which is a familiar disease and one of the main causes of disability and death in the world. According to the World Health Organization, almost 350 million people are living with diabetes in the world and in 2030, it is estimated that diabetes will be the 7<sup>th</sup> leading cause of death. (Shaw *et al.*, 2010; alwan, 2011; Baghayeri *et al.*, 2016) Blood glucose level has been measured as an effective clinical biomarker for diabetes risk. Nevertheless, drawing blood from fingertip or vein force causes a painful prick. On the other hand, urine is another instructive body liquid, which has been a useful indicator for diagnosis. The glucose level in urine is as well a good pointer for preliminary screening of patients with high level diabetes or having renal glycosuria (Kim *et al.*, 2014; Fan *et al.*, 2015; Baghayeri *et al.*, 2016) However, blood or urine has the complex matrices, therefore, glucose measurement is an analytical challenge.

Electrochemical glucose sensors are generally categorized into two classifications: enzymatic (Ali *et al.*, 2013; Razmi and Mohammad-Rezaei, 2013; Kong *et al.*, 2014; Su *et al.*, 2014) and non-enzymatic (Lin *et al.*, 2013; Guo *et al.*, 2014; Li *et al.*, 2014; Wang *et al.*, 2014). Enzymatic glucose sensors are based on the immobilization of glucose oxidase on various substrates and these enzymatic glucose sensor have been widely developed due to their excellent selectivity and high sensitivity. However, the drawback of these enzymatic sensors is their lack of long-term stability because the glucose oxidase can be easily affected by pH, temperature, ionic detergents, humidity and other types of matrix and also enzyme is expensive. Non-enzymatic glucose sensor has attracted considerable attention to overcome the problems of using enzyme-based glucose sensors.

Various metal nanoparticles have been investigated for electrocatalytic oxidation of glucose in non-enzymatic glucose sensor. Previous studies have focused on the noble metals such as gold (Au), platinum (Pt), palladium (Pd), and silver (Ag) (Xiao *et al.*, 2009; Lorestani *et al.*, 2015; Thanh *et al.*, 2016). However, these metals are very costly. Therefore, nickel nanoparticles are of particular interest, owing to their excellent electrocatalytic activity, low cost and environmental friendly. Nickel based non-enzymatic glucose sensors have shown an outstanding electrocatalytic activity toward glucose oxidation, which is mediated by Ni(II)/Ni(III) redox couples in alkaline aqueous solution (Zhao *et al.*, 2007; Niu *et al.*, 2013; Shervedani *et al.*, 2014). It is well known that nanomaterials exhibit special properties compared to the bulky materials. Thus, nickel nanoparticles are an attractive electrocatalyst for glucose oxidation.

In the present work, a simple method was developed to fabricate a non-enzymatic glucose sensor. Nickel nanoparticles were electrochemically deposited on the surface of glassy carbon electrode by one step strategy. The morphology of the nickel nanoparticles modified electrode was characterized with scanning electron microscopy (SEM). The electrochemical and electrocatalytic properties of the nickel nanoparticles modified glassy carbon electrode toward the oxidation of glucose were discussed. In addition, the detecting performance of the proposed glucose sensor was evaluated and optimized by chronoamperometry. The results demonstrated that this approach have been shown high catalytic activity for glucose oxidation. Moreover, the proposed glucose sensor can be successfully applied in human urine.

## **MATERIALS AND METHODS**

### **Chemicals and reagents**

Glucose and nickel nitrate hexahydrate were purchased from Sigma-Aldrich Co. Sodium hydroxide was purchased from RCI Labscan Ltd. Sodium acetate trihydrate was purchased from Baker Analyzed. Acetic acid was purchased from Merck. All chemical reagents were of analytical grade and all solutions were prepared with deionized water.

### **Instrumentation**

Electrochemical measurements were performed with a Digi-Ivy model DY2113 potentiostat. A conventional three-electrode system was used, containing an Ag/AgCl electrode as the reference electrode, a Pt wire as the counter electrode, and

the modified glassy carbon electrode as the working electrode. The surface morphological characterization of nickel nanoparticles was examined by means of scanning electron microscope (SEM; LEO, model 1400VP) at an accelerating voltage of 20 kV.

### **Preparation of nickel nanoparticles modified glassy carbon electrode**

Glassy carbon electrode was polished before each experiment with 1.0 and 0.3  $\mu\text{m}$  alumina powder, respectively. Then, the polished electrode was rinsed thoroughly with deionized water and was ultrasonicated in ethanol and deionized water for 10 min each to remove organic and other contaminants.

To prepare the nickel nanoparticles modified glassy carbon electrode, the electrodeposition of nickel nanoparticles on the electrode surface was performed in 0.1 M acetate buffer solution (pH 4.0) containing 5 mM nickel nitrate at -1.1 V for 250 s under the unstirring conditions. After that, the modified electrode was scanned by cyclic voltammetry from 0.1 to 0.8 V in 0.1 M NaOH until a steady state voltammogram was obtained. The final resulting modified electrode was denoted by Ni NPs/GCE

### **Electrochemical measurement**

All electrochemical experiments were performed with a three-electrode system at room temperature of 25 °C. All potentials were measured relative to the Ag/AgCl reference electrode. Cyclic voltammetric scans were performed at the potential range from 0 to 0.8 V at scan rate of 0.1 V/s. The chronoamperometric measurements were performed at 0.54 V.

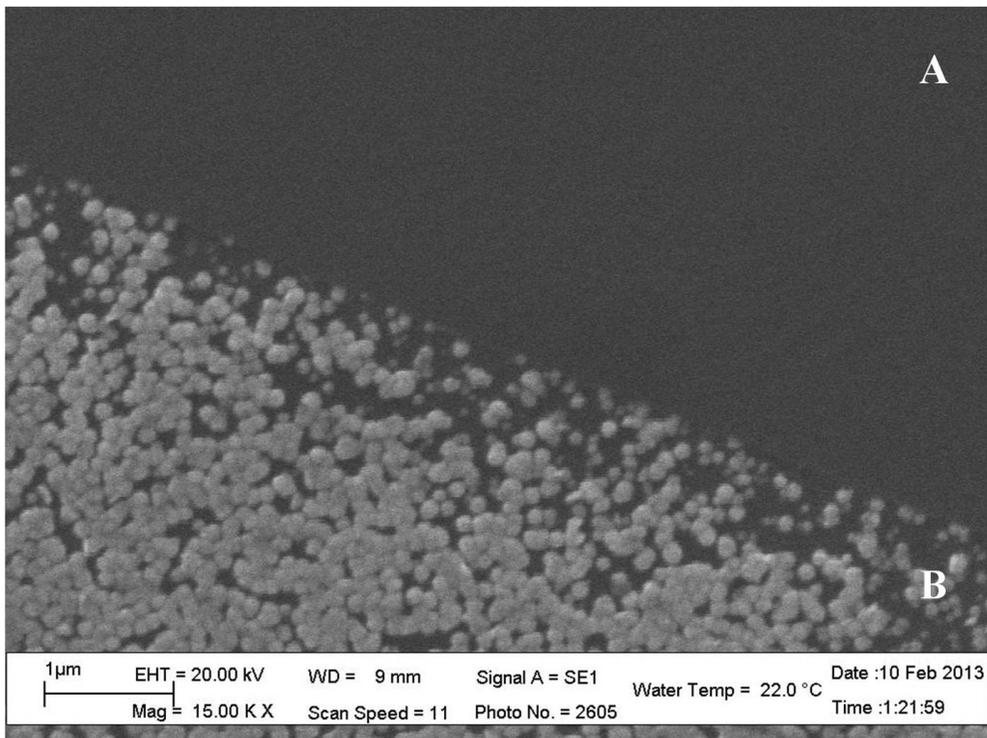
### **Real sample analysis**

The urine samples were collected from a normal healthy female volunteer. The utilization of the Ni NPs/GCE for glucose determination in urine sample analysis was also investigated by direct analysis. Urine samples were diluted 10 times with 0.1 M NaOH to reduce the matrix effect of urine samples.

## **RESULTS AND DISCUSSION**

### **Characterization of nickel nanoparticles modified glassy carbon electrode**

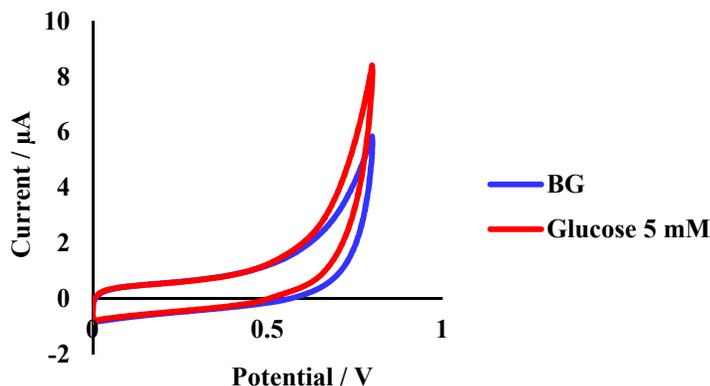
For the modified electrode, nickel nanoparticles were electrochemically deposited on the glassy carbon electrode surface by chronoamperometry. The nickel nanoparticles were investigated using SEM. The surface morphology of the unmodified glassy carbon substrate and the electrodeposited nickel nanoparticles on glassy carbon substrate are shown in Figure 1. The result in the SEM image indicated that nickel nanoparticles were electrodeposited on the glassy carbon substrate surface and were well distributed on the surface with diameters in the range of 60 – 150 nm.



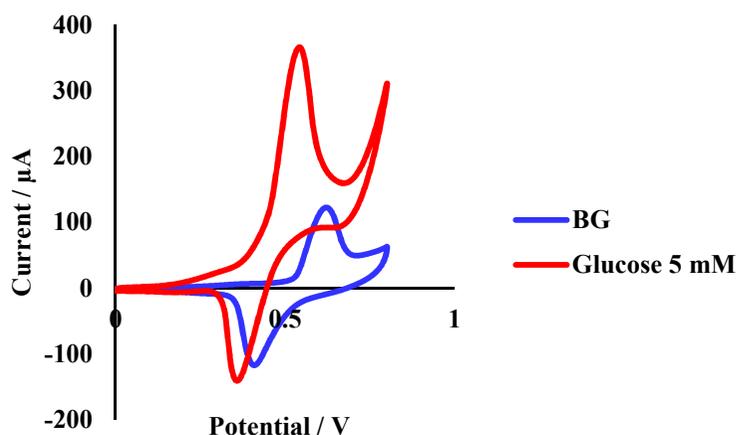
**Figure 1** Scanning electron microscope image of the unmodified glassy carbon substrate (A) and the electrodeposited nickel nanoparticles on glassy carbon substrate (B).

### **Electrochemical behavior of the Ni NPs/GCE**

To study the electrochemical behavior, cyclic voltammograms of glucose using the Ni NPs/GCE were observed, and the bare GCE was also observed for comparison. Figure 2 shows the cyclic voltammograms of the bare GCE in 0.1 M NaOH containing 5 mM glucose in the potential window ranging from 0.0 to 0.8 V with scan rate of 0.1 V/s. It was found that the bare GCE exhibits no electrochemical response in the presence of glucose. As shown in Figure 3, a pair of redox peaks can be observed with the anodic peak at around 0.5 V and the cathodic peak at around 0.3 V because the effect of nickel nanoparticles on the GCE exhibits high electrocatalytic activity towards glucose detection.

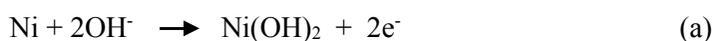


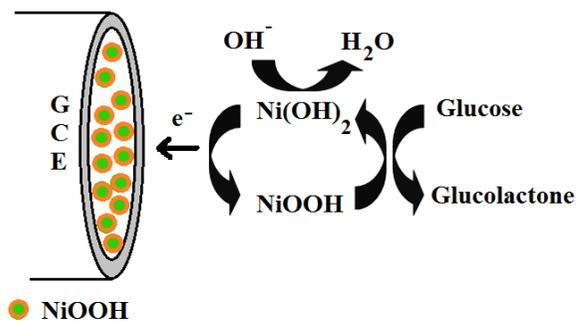
**Figure 2** Cyclic voltammograms of the bare GCE in the absence (blue line) and presence (red line) of 5 mM glucose in 0.1 M NaOH, at scan rate of 0.1 V/s



**Figure 3** Cyclic voltammograms of the Ni NPs/GCE in the absence (blue line) and presence (red line) of 5 mM glucose in 0.1 M NaOH, at scan rate of 0.1 V/s

As shown in Scheme 1, glucose sensing mechanism at Ni NPs/GCE is schematically presented. To the electrochemical preparation of active and stable Ni(OH)<sub>2</sub>, the nickel nanoparticles on the electrode surface were conditioned in 0.1 M NaOH by potential cycling from 0.0 to 0.8 V at a scan rate of 0.1 V/s for 10 cycles. The electrochemical reaction of the Ni<sup>2+</sup>/Ni<sup>3+</sup> couple in 0.1 M NaOH, which was consistent with the published report (Zhao *et al.*, 2007; Niu *et al.*, 2013; Shervedani *et al.*, 2014), may be as follows:



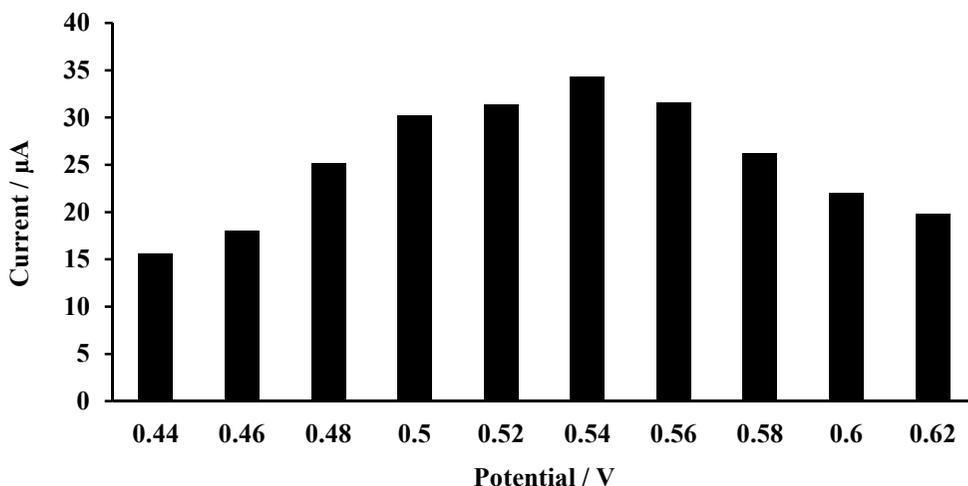


**Scheme 1** The schematic illustration for the sensing mechanism of electrocatalytic glucose by Ni NPs/GCE.

### The effect of the detection potential

The detection potential is important for selectivity and sensitivity of glucose sensor. For electrochemical detection of sensor, the detection potential was investigated using chronoamperometry. To optimize the detection potential, 5 mM glucose was examined in the range from 0.44 to 0.62 V. The results indicated that the chronoamperometric response of glucose increased as the detection potential increased. Nevertheless, the background response also increased when the detection potential increased. Therefore, the background must be substrated from the total current. It can be concluded that the decreasing of current higher than 0.56 V leading to the effect of background current.

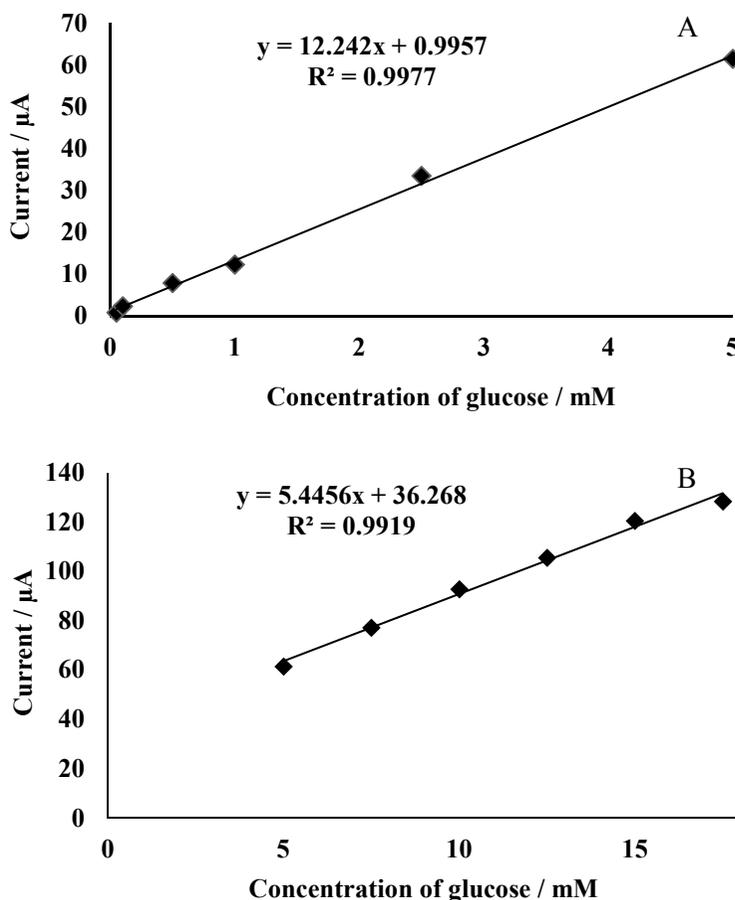
As shown in Figure 4, the current of detection potential of 0.54 V is higher than other current. Thus, the potential of 0.54 V was chosen as the detection potential in this work.



**Figure 4** Schematic presentation of the effect of detection potential for glucose sensor.

### Electroanalytical sensing of glucose

Under the optimum conditions, the proposed non-enzymatic sensor was used to detect glucose. The relationship between current and time at the various concentrations of glucose was obtained by chronoamperometry. The concentration of glucose was studied in the range from 0.05 to 17.5 mM in 0.1 M NaOH. The results indicated that the chronoamperometric response is proportional to the concentration of glucose. It can be seen from Figure 5, the calibration curve shows two linear segments: the first linear segment increases from 0.05 to 5.00 mM with a correlation coefficient of 0.997 (Figure 5A), while the second linear segment increases up to 17.50 mM with a correlation coefficient of 0.991 (Figure 5B). Furthermore, the low limit of detection and quantification were found to be 36  $\mu$ M and 0.12 mM, respectively. The proposed sensor showed a good analytical performance with a relative standard deviation of 4.17%.



**Figure 5** Calibration curve of glucose at concentration 0.05, 0.10, 0.50, 1.00, 2.50, and 5.00 mM (A) 5.00, 7.50, 10.00, 12.50, 15.00 and 17.50 mM in 0.1 M NaOH.

### Real sample analysis

The developed non-enzymatic glucose sensor was investigated to determine the concentration of glucose in real human urine sample by adding glucose in the 0.1 M NaOH. Table 1 exhibits the results of glucose detection in real urine samples, the results are satisfying and good agreement with clinical laboratory measurements.

**Table 1** The detection of glucose in real urine sample.

Samples No.	Glucose concentration of urine (mM)	Glucose Added (mM)	Glucose concentration obtained		Recovery (%)
			Present sensor (mM)	Clinical laboratory (mM)	
1	N.D.	2.5	2.18	2.20	87.20
2	N.D.	5.0	4.15	4.33	83.00

**Note:** N.D. means non-detected.

### CONCLUSIONS

In summary, the proposed sensor has demonstrated the successfully preparation of the non-enzymatic glucose sensor. The electrodeposited nickel nanoparticles on the GCE were simple, rapid, inexpensive and efficient. The proposed sensor showed high sensitivity, low detection limit (LOD = 36  $\mu$ M, S/N=3) and wide concentration range (0.05 to 17.50 mM). Moreover, the proposed sensor was effectively to quantify glucose in real human urine samples and the results are in good agreement with clinical laboratory measurements.

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### REFERENCES

- Ali Kamyabi, M., Hajari, N., Turner, A., and Tiwari, A. (2013). A high-performance glucose biosensor using covalently immobilised glucose oxidase on a poly(2,6-diaminopyridine)/carbon nanotube electrode. *Talanta*, 116, 801-808.
- Alwan, A. (2011). Global status report on noncommunicable diseases 2010. World Health Organization. Switzerland.
- Baghayeri, M., Amiri, A., and Farhadi, S. (2016). Development of non-enzymatic glucose sensor based on efficient loading Ag nanoparticles on functionalized carbon nanotubes. *Sensors And Actuators B: Chemical*, 225, 354-362.
- Fan, Y., Tan, X., Liu, X., Ou, X., Chen, S., and Wei, S. (2015). A novel non-enzymatic electrochemiluminescence sensor for the detection of glucose based on the

- competitive reaction between glucose and phenoxy dextran for concanavalin A binding sites. *Electrochimica Acta*, 180, 471-478.
- Fang, B., Zhang, C., Wang, G., Wang, M., and Ji, Y. (2011). A glucose oxidase immobilization platform for glucose biosensor using ZnO hollow nanospheres. *Sensors And Actuators B: Chemical*, 155(1), 304-310.
- Guo, M., Wang, P., Zhou, C., Xia, Y., Huang, W., and Li, Z. (2014). An ultrasensitive non-enzymatic amperometric glucose sensor based on a Cu-coated nanoporous gold film involving co-mediating. *Sensors And Actuators B: Chemical*, 203, 388-395.
- Guo, M.-M., Xia, Y., Huang, W., and Li, Z. (2015). Electrochemical fabrication of stalactite-like copper micropillar arrays via surface rebuilding for ultrasensitive nonenzymatic sensing of glucose. *Electrochimica Acta*, 151, 340–346.
- Kim, H., Jang, K., Veerapandian, M., Kim, H., Seo, Y., Lee, K., and Lee, M. (2014). Reusable urine glucose sensor based on functionalized graphene oxide conjugated Au electrode with protective layers. *Biotechnology Reports*, 3, 49-53.
- Kong, F., Gu, S., Li, W., Chen, T., Xu, Q., and Wang, W. (2014). A paper disk equipped with graphene/polyaniline/Au nanoparticles/glucose oxidase biocomposite modified screen-printed electrode: Toward whole blood glucose determination. *Biosensors And Bioelectronics*, 56, 77-82.
- Li, M., Bo, X., Mu, Z., Zhang, Y., and Guo, L. (2014). Electrodeposition of nickel oxide and platinum nanoparticles on electrochemically reduced graphene oxide film as a nonenzymatic glucose sensor. *Sensors And Actuators B: Chemical*, 192, 261-268.
- Lin, K., Lin, Y., and Chen, S. (2013). A highly sensitive nonenzymatic glucose sensor based on multi-walled carbon nanotubes decorated with nickel and copper nanoparticles. *Electrochimica Acta*, 96, 164-172.
- Lorestani, F., Shahnavaz, Z., Mn, P., Alias, Y., and Manan, N. (2015). One-step hydrothermal green synthesis of silver nanoparticle-carbon nanotube reduced-graphene oxide composite and its application as hydrogen peroxide sensor. *Sensors And Actuators B: Chemical*, 208, 389-398.
- Niu, X., Lan, M., Zhao, H., and Chen, C. (2013). Highly Sensitive and Selective Nonenzymatic Detection of Glucose Using Three-Dimensional Porous Nickel Nanostructures. *Analytical Chemistry*, 85(7), 3561-3569.
- Rahman, M.M., Ahammad, A.J.S., Jin, J.H., Ahn, S.J., and Lee, J.J. (2010) A comprehensive review of glucose biosensors based on nanostructured metal-oxides, *Sensors*, 10, 4855–4866.
- Razmi, H., and Mohammad-Rezaei, R. (2013). Graphene quantum dots as a new substrate for immobilization and direct electrochemistry of glucose oxidase: Application to sensitive glucose determination. *Biosensors And Bioelectronics*, 41, 498-504.
- Shaw, J.E., Sicree, R.A. and Zimmet., P.Z. (2010). Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res. Clin. P. R.*, 87(1), 4-14.
- Shervedani, R., Karevan, M., and Amini, A. (2014). Prickly nickel nanowires grown on Cu substrate as a supersensitive enzyme-free electrochemical glucose sensor. *Sensors And Actuators B: Chemical*, 204, 783-790.
- Su, S., Sun, H., Xu, F., Yuwen, L., Fan, C., and Wang, L. (2014). Direct electrochemistry of glucose oxidase and a biosensor for glucose based on a glass carbon electrode modified with MoS<sub>2</sub> nanosheets decorated with gold nanoparticles. *Microchimica Acta*, 181(13-14), 1497-1503.
- Thanh, T., Balamurugan, J., Hwang, J., Kim, N., and Lee, J. (2016). In situ synthesis of graphene-encapsulated gold nanoparticle hybrid electrodes for non-enzymatic glucose sensing. *Carbon*, 98, 90-98.

- Wang, J., Gao, H., Sun, F., and Xu, C. (2014). Nanoporous PtAu alloy as an electrochemical sensor for glucose and hydrogen peroxide. *Sensors And Actuators B: Chemical*, 191, 612-618.
- Wang, Z., Lei, H., and Feng, L. (2013) A facil channel for d-glucose detection in aqueous solution. *Spectrochim. Acta A.*, 114, 293-297.
- Xiao, F., Zhao, F., Mei, D., Mo, Z., and Zeng, B. (2009). Nonenzymatic glucose sensor based on ultrasonic-electrodeposition of bimetallic PtM (M=Ru, Pd and Au) nanoparticles on carbon nanotubes–ionic liquid composite film. *Biosensors And Bioelectronics*, 24(12), 3481-3486.
- Zhao, C., Shao, C., Li, M., and Jiao, K. (2007). Flow-injection analysis of glucose without enzyme based on electrocatalytic oxidation of glucose at a nickel electrode. *Talanta*, 71(4), 1769-1773.
- Zhu, Z., Garcia-Gancedo, L. A., Flewitt, J., Xie, H., Moussy, F., and Milne, W. I. (2012) A critical review of glucose biosensors based on carbon nanomaterials: carbon nanotubes and grapheme, *Sensors*, 12, 5996–6022.