

Preparation of carbon paste electrodes including poly(styrene) attached glycine–Pt(IV) for amperometric detection of glucose

Soner Dönmez^a, Fatma Arslan^{b,*}, Nurşen Sarı^b, Nurdan Kurnaz Yetim^{b,c}, Halit Arslan^b

^a Department of Chemistry, Faculty of Sciences and Art, Nevşehir University, 50300 Nevşehir, Turkey

^b Department of Chemistry, Faculty of Sciences, Gazi University, 06500 Teknikokullar, Ankara, Turkey

^c Department of Chemistry, Faculty of Sciences and Art, Kırklareli University, Kırklareli, Turkey

ARTICLE INFO

Article history:

Received 5 August 2013

Received in revised form

28 October 2013

Accepted 29 October 2013

Available online 8 November 2013

Keywords:

Biosensor

Carbon paste

Glucose oxidase

Nanoparticle

Pt(IV) complexes

ABSTRACT

In this study, a novel carbon paste electrode that is sensitive to glucose was prepared using the nanoparticles modified (4-Formyl-3-methoxyphenoxymethyl) with polystyren (FMPS) with L-Glycine–Pt (IV) complexes. Polymeric nanoparticles having Pt(IV) ion were prepared from (4-Formyl-3-methoxyphenoxymethyl) polystyren, glycine and PtCl₄ by template method. Glucose oxidase enzyme was immobilized to a modified carbon paste electrode (MCPE) by cross-linking with glutaraldehyde. Determination of glucose was carried out by oxidation of enzymatically produced H₂O₂ at 0.5 V vs. Ag/AgCl. Effects of pH and temperature were investigated, and optimum parameters were found to be 8.0 and 55 °C, respectively. Linear working range of the electrode was 5.0×10^{-6} – 1.0×10^{-3} M, $R^2=0.997$. Storage stability and operational stability of the enzyme electrode were also studied. Glucose biosensor gave perfect reproducible results after 10 measurements with 2.3% relative standard deviation. Also, it had good storage stability (gave 53.57% of the initial amperometric response at the end of 33th day).

© 2013 Elsevier B.V. All rights reserved.

1. Introduction

Determination of glucose is important in many areas, from clinical studies to industrial productions. Rapid determination of blood sugar for treatment and control of diabetes is significant. Thus; numerous efforts have devoted to develop glucose biosensor with fast and accurate response (Kulys et al., 1995; Çolak et al., 2012).

Composite carbon paste electrodes have been extensively used for electroanalytical applications since their introduction by Adams (1958) due to their intrinsic advantages including low background current, renewable or disposable electrochemical interface and low cost of fabrication (Boujita et al., 1999).

For determination of glucose, electrochemical glucose biosensors using carbon paste electrodes have been prepared (Comba et al., 2010; Jianping et al., 2009; Xiaoli et al., 2005; Arslan et al., 2012). Most of the electrochemical glucose biosensors are based on glucose oxidase (GOx) enzyme, which catalyzes the oxidation of glucose to glucono lactone which is hydrolyzed to gluconic acid and hydrogen peroxide. Quantification of glucose can be achieved via electrochemical detection of the enzymatically released

H₂O₂ (Arslan et al., 2011; Yoo and Lee, 2010; Norouzi et al., 2010).

Glucose + O₂ → H₂O₂ + gluconic acid

H₂O₂ → O₂ + 2H⁺ + 2e[−]

Glucose oxidase enzyme is immobilized on a material to prepare glucose biosensor. To date, different methods have been used for enzyme immobilization (Janeček 1993; Moyo et al., 2012). In view of these, nanocomposite and nanoparticle structures have attracted great attention in preparing advanced materials. Among them, Chengyan Wang and co-workers reported gold nanocomposite films for a glucose biosensor which had high activity (Wang et al., 2011). Noble metal nanoparticles have been extensively utilized due to their extraordinary catalytic activities in both oxidation and reduction reactions. Among them, Pt nanoparticles were one of the mostly used for immobilization of enzymes for the construction of biosensors due to their unique properties such as electrocatalytic ability (Yen et al., 2011). Li et al. (2010, 2011) decorated the magnetic composite of ferric oxide with Pt nanoparticles to construct an amperometric glucose biosensor. Because of excellent performance of Pt in the detection of hydrogen peroxide, platinum electrode and platinum nanostructure modified electrodes have been widely used to immobilize enzyme for the fabrication of biosensors (Li et al., 2011). Among recent advances in nanomaterials for biosensing applications, polymeric nanoplateform materials have gained significant interest for the potential

* Corresponding author. Tel.: +903122021518; fax: +90 312 212 22 79.
E-mail address: fatma@gazi.edu.tr (F. Arslan).

application for enzyme immobilization (Kerimov et al., 2011). Especially, due to their biocompatibility and large surface area, they attract attention in biomedical field (Kumari et al., 2010).

In this study, a novel carbon paste electrode that is sensitive to glucose (CPE) was prepared using the nanoparticles modified (4-Formyl-3-methoxyphenoxyethyl) with polystyren (FMPS) with L-Glycine–Pt(IV) complexes. Polymeric nanoparticles having Pt(IV) ion were prepared from (4-Formyl-3-methoxyphenoxyethyl) polystyren, glycine and PtCl₄ by template method. Glucose oxidase enzyme was immobilized to modified carbon paste electrode (CPE) by cross-linking with glutaraldehyde. Optimum working conditions of biosensor with respect to substrate concentration, pH and temperature were investigated. Storage stability and operational stability of the biosensor were investigated.

2. Experimental section

2.1. Equipment and reagents

Electrochemical studies were carried out by using an Epsilon EC electrochemical analyzer with a three-electrode cell. Working electrode was a carbon paste (diameter of 0.8 cm, length of 3 cm glass tubes) electrode. Auxiliary and reference electrodes were a Pt wire and Ag/AgCl electrode (3 M KCl), respectively. pH values of the buffer solutions were measured with an Orion Model 720A pH/ion meter. Temperature control was achieved with a Grant W14 thermostat. Elemental analyses were carried out with a LECO, CHNS-932 instrument. Metal contents were determined by using a Philips PU 9285 atomic absorption instrument. IR spectra were recorded on a Mattson-5000 FT-IR instrument in KBr pellets. Magnetic moments were measured at room temperature with MK-1 model Gouy Balance of Christison Scientific Equipment Ltd. Scanning electron microscopy of the Au–Pd-coated compounds was done by using a JEOL JEM 100 CX II scanning electron microscope (JEOL, Peabody, MA) equipped with a Link analytical system. Electron energy used was 20 keV. Glucose oxidase (EC 1.1.3.22, purified from *Aspergillus Niger* and with an activity of 5204.3 unit mL^{−1}), glucose, (4-Formyl-3-methoxyphenoxyethyl) polystyren, divinylbenzene, glycine, PtCl₄, DMF, acetone and nujol were purchased from Sigma. Graphite powder was supplied from Merck. A stock solution of glucose was allowed to mutarotate for 24 h before use. All other chemicals were obtained from Sigma. All the solutions were prepared by using double distilled water.

2.2. Synthesis of nanoplateforms attached glycine–Pt(IV)

The polymer having Schiff bases were prepared by dropwise addition of (4-Formyl-3-methoxyphenoxyethyl)polystyren [(FMPS); 1 g, 100–200 mesh, 1.0–1.5 mmol/g –CHO loading, 1% cross-linked with divinylbenzene (Aldrich)] in hot DMF (15 mL) to a solution of Glycine and PtCl₄ (1.5 mmol) in DMF (10 mL) while stirring. Stirring was continued until the solution was refluxed ca. three hours later. Then mixture was cooled and was poured into acetone (~100 mL). Resulting solid was collected from acetone (FMPS–GlyPt). The solid was filtered, dried and kept with desiccator over anhydrous CaCl₂.

2.3. Preparation of carbon paste electrodes (CPE)

CPE was prepared by thoroughly mixing 30 µL of nujol with 0.05 g of graphite powder in a mortar. For preparation of CPE, glass tubes (a diameter of 0.6 cm and length of 3 cm) were filled with carbon paste. Height of the paste in the tubes was 0.6 cm. Electric contacts were made using platinum wire. The modified carbon paste electrode (MCPE) was prepared with 2.5 mg of poly(styrene)

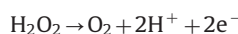
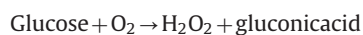
attached glycine–Pt(IV) by thoroughly mixing 30 µL of nujol with 0.05 g of graphite powder in a mortar. Electrode surface was smoothed on a paper to produce a producible working surface.

2.4. Immobilization of glucoseoxidase to modified carbon paste electrode

25 µL of GOx enzyme (5204.3 Unit/mL), 7.5 µL (5 mg/250 µL) of bovine serum albumin (BSA) and 10 µL of 2.5% glutaraldehyde (GA) were dropped upon MCPE. Electrode was dried at room temperature and washed with buffer solution (0.1 M phosphate buffers of pH 8.0) several times in order to remove the excess nonimmobilized enzyme and glutaraldehyde. The electrode was kept in a refrigerator at 4 °C in phosphate buffer when it was not in use.

2.5. Electrochemical measurements

Quantification of glucose was achieved via electrochemical detection of enzymatically released H₂O₂.



GOx/MCPE was immersed in phosphate buffer (0.1 M) of pH 8.0. The solution contained 0.1 M sodium perchlorate as supporting electrolyte. Electrode was brought to equilibrium by keeping at 0.5 V (vs. Ag/AgCl electrode (3 M KCl)). Steady current (i_a) was recorded. Glucose solution was added to the cell using a micro-pipette, and the mixture was stirred. Current (i_b) values obtained at 0.5 V were recorded, and the values (Δi = i_b – i_a) were plotted against the glucose concentration.

3. Results and discussion

In this study, a novel carbon paste electrode that is sensitive to glucose (CPE) was prepared using the nanoparticles modified (4-Formyl-3-methoxyphenoxyethyl) with polystyren (FMPS) with L-Glycine–Pt(IV) complexes. These nanoparticles were first synthesized for this study. Determination of glucose was carried out by the oxidation of enzymatically produced H₂O₂ at 0.5 V vs. Ag/AgCl. Reaction Scheme 1 showing the glucose determination is shown below.

According to this scheme, a biochemical reaction occurs between glucose in solution and glucose oxidase enzyme which immobilized onto modified carbon paste electrode. In this reaction, glucose is oxidized to gluconic acid. And flavin adenine dinucleotide (FAD), which is a prosthetic group in enzyme structure, is reduced to FADH₂ by taking electron. FADH₂ in enzyme is oxidized by giving its electrons to oxygen in solution to make the reaction reversible. Thus, enzyme comes to its previous form. By taking electron, oxygen is reduced to H₂O₂. H₂O₂, which is produced at the end of biochemical reaction, is oxidized to oxygen molecule on modified electrode surface. Glucose determination was made by measuring the anodic current of hydrogen peroxide on modified electrode surface. The parameters effecting the



Scheme 1. Reaction scheme for the glucose determination.

performance of biosensor and optimum working conditions were investigated.

3.1. Characterization of nanoplateforms attached glycine–Pt(IV)

Analytical data and physical properties of synthesized compounds are summarized in Table S1. The analytical data of the complexes correspond well with suggestion of structure. The average molecular weight (M_w) of the polymer and its metal complexes from this elemental analyses were suggested to be 1817.5 for (FMPS–Gly–Pt(IV)). Elemental analyses are consistent with a studied polymer. However, deviations from these values were noted, which may be attributed to the polymeric nature of the coordination polymers. The number average and weight average molecular weights (M_w and M_n) of PAA–Schiff bases and polydispersity index (M_w/M_n) were given in Table S1. According to GPC, modified polymers have a very narrow molecular weight distribution (PDI: 1.02) for (FMPS–Gly–Pt).

The characteristic peak of IR spectra of (FMPS–Gly–Pt(IV)) support polymer is given in Table S1. Three overtone peaks showed in 1943, 1873, 1800 cm^{-1} of (FMPS–Gly–Pt(IV)) support polymer. IR bands in the 3433, 3011, 2923, 556 and 541 cm^{-1} regions are characteristic of $\nu(\text{OH})$ (for H_2O in Pt(IV) complexes), $\nu(\text{CH})$ aromatic, $\nu(\text{CH})$ aliphatic, $\nu(\text{CH})$ buckling out of plane and $\nu(\text{CH})$ buckling, respectively. Imine band was observed in 1638 cm^{-1} . Also weak bands appeared in the 461 and 537 cm^{-1} region, belonging to $\nu(\text{Pt–N})$ and $\nu(\text{Pt–O})$, respectively. These are confirmed formation of coordinate covalent bond through one nitrogen and carboxyl oxygen for all modified complexes (Sari et al., 2012). Nanoplateforms attached Glycine–Pt(IV) were found to be diamagnetic. This reason suggested Pt(IV) complex is octahedral (Sari et al., 2012).

SEM images of nanoplateforms attached Glycine–Pt(IV) are shown in Table 1. This image indicates that protects structure are of modified polymers from (APS). Table 1 presents the elemental compositions of nanoplateforms attached Glycine–Pt(IV) as obtained from EDX analysis. EDX is not the technique of choice for analyzing polymers. Because intensities of the carbon atom is often erratic in modified polymers. However, an EDX spectrum gives an excellent elemental analysis for all elements in the Periodic Table above beryllium in modified polymer. Table 1 shows that hydrogen atoms are not taken into account composition of all studied polymer. EDX analysis shows the presence of Pt(IV) ions in the prepared coordination polymer. The combined information from SEM and EDX indicate the genuine modified polymer formation with Glycine group and Pt(IV) ions.

3.2. Amperometric responses of CPE and MCPE to hydrogen peroxide

Amperometric responses of the CPE and MCPE to hydrogen peroxide were determined at different hydrogen peroxide concentrations. According to Fig. 1, it can be seen that when H_2O_2 concentrations of both carbon paste and modified carbon paste electrodes are increased, anodic currents of H_2O_2 are also increased. This situation shows that both two electrodes are sensitive to H_2O_2 . However as shown in Fig. 1, when MCPE was used, differences in anodic current were obtained higher than those obtained by using CPE. From this, it can be said that sensitivity of MCPE electrode to H_2O_2 is higher.

3.3. The working potential

After preparing modified carbon paste electrode (MCPE), the hydrogen peroxide oxidation was carried out at different potentials (0.2, 0.3, 0.4, 0.5, 0.6, 0.7 V) (Fig. S1). When Fig. S1 is examined, it is also seen that the variation in current was higher

Table 1

SEM imaging (mag 200 $\times$ and 1000 $\times$) EDX spectra nanoplateforms attached Gly–Pt(IV).

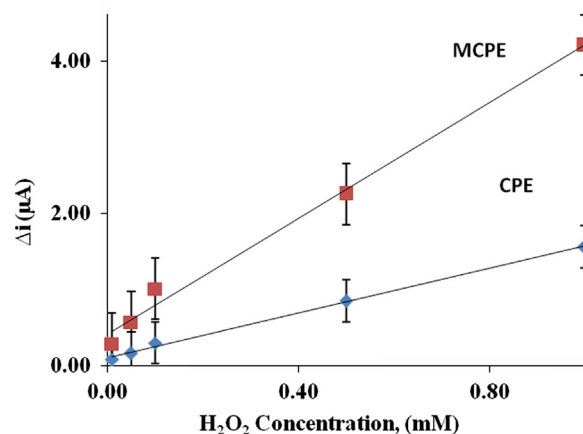
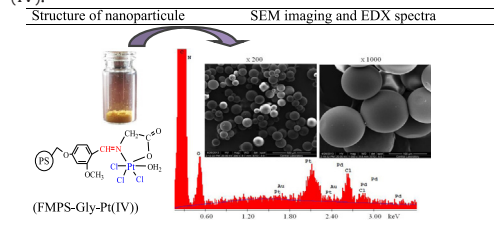


Fig. 1. Amperometric responses of carbon paste electrode (CPE) and modified carbon paste (MCPE) to hydrogen peroxide (at 25 $^{\circ}\text{C}$, 0.1 M phosphate buffer of pH=8.0, 0.5 V operating potential).

in high potentials than low potentials. It is seen that oxidation currents of H_2O_2 are too low and close to each other at 0.2 V, 0.3 V and 0.4 V. The interference effects of substances present in body fluids (e.g. ascorbic acid and uric acid) could be more in high potentials (Zhang et al., 2007). Therefore 0.5 V was used as working potential.

3.4. Effect of amount of the nanoparticle to selectivity

Selectivity is an important challenge in the development of a biosensor. In order to obtain the best compromise between sensitivity and selectivity, we evaluated the response of the electrode toward ascorbic and uric acids, and acetaminophen (Luque et al., 2005). Firstly, 5.0×10^{-4} M hydrogen peroxide was added to the cell. After the addition of hydrogen peroxide; 3.0×10^{-4} M uric acid, 1.0×10^{-4} M ascorbic acid and 1.0×10^{-4} M acetaminophen were also added to the cell.

The interference was evaluated at 0.5 V using electrodes prepared with 2.5, 7.5, and 10 mg of nanoparticles. It was observed that interference effect increased when amount of polymer was increased. When the amount of polymer was lower than 2.5 mg, the electrode became very weak. Because of this polymer, the amount was limited to 2.5 mg. The lowest percentage ratio of interference was found when 2.5 mg composite was used. Therefore, an amount of 2.5 mg of this nanoparticles was used in future studies (Fig. S2).

3.5. Determination of optimum pH

Since enzyme activity is dependent on the ionization state of the amino acids in the active site, pH plays an important role in

maintaining the proper conformation of an enzyme. The effect of pH on the response of the glucose biosensor was determined in 0.1 M phosphate buffer at pH range of 6.0–9.0. The measurements were taken at a constant glucose concentration of 1.0×10^{-2} M. Fig. S3 shows that the maximum response was obtained at pH 8.0. For glucose biosensor, pH values different from 8.0 were employed in the literature (pH 5.0; 9.0; 7.5 and 6.2) (Çolak et al., 2012; Arslan et al., 2012; Arslan et al., 2011; Nenkova et al., 2010). This was attributed to the fact that the used polymer and type of immobilization were different. GOx enzyme kept its activity even at high pH, when it was immobilized to poly(styrene) attached glycine-pt(IV).

3.6. Determination of optimum temperature

Enzymes are known to be sensitive to changes in temperature. The relationship between reaction rate of an enzyme and temperature is exponential. Temperature's influence on the response of glucose enzyme electrode was tested between 20 °C and 70 °C at pH 8.0 using constant glucose concentration of 1.0×10^{-2} M. As seen in Fig. S4, the current difference increases with temperature 55 °C and decreases afterward. The highest electrode response was obtained at 55 °C. For glucose biosensor, temperature values were employed in the literature (65, 60, 50 and 55 °C). (Çolak et al., 2012; Arslan et al., 2012; Zhou et al., 2005; Sulak et al., 2006). The study was carried out at 25 °C due to the difficulties involved in working at 55 °C. Temperature of 25 °C was chosen as working temperature for all further experiments. GOx enzyme kept its activity even at high temperatures, when it was immobilized to poly(styrene) attached glycine-pt(IV).

3.7. Effect of substrate concentration on response of biosensor and calibration curve

The effect of the substrate concentration on the reaction rate, catalyzed by immobilized GOx, was studied using varying concentrations (5.0×10^{-6} – 1.0×10^{-1} M) of glucose (Fig. 2). The linear working range of the electrode was 5.0×10^{-6} – 1.0×10^{-3} M, $R^2=0.997$ (Fig. 3). When compared with literature (4.0×10^{-5} – 2.8×10^{-4} M and 1.0×10^{-4} – 1.0×10^{-3} M respectively for Liu and Ju (2003) and Luque et al., 2005), linear working range of biosensor is wider. It was shown that the linearity of graphs was highly satisfactory and it could be used for the quantitative determination of glucose. The detection limit of the biosensor was 1.0×10^{-6} M and the response time of the biosensor was 200 s. Detection limit of biosensor is lower than detection

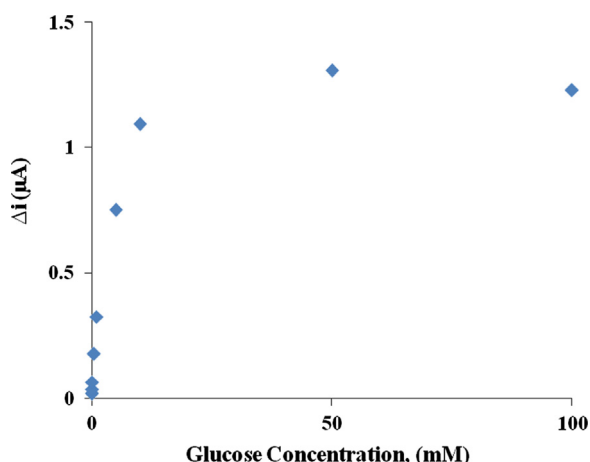


Fig. 2. The effect of glucose concentration upon the amperometric response of the biosensor (in pH 8.0 phosphate buffer and at a 0.5 V operating potential, 25 °C).

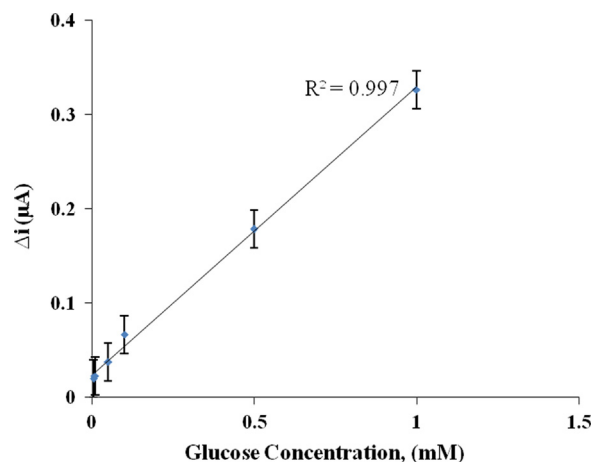


Fig. 3. The calibration curve of the glucose biosensor (in pH 8.0 phosphate buffer and at a 0.5 V operating potential, 25 °C) ($n=3$).

limit in literature (1.0×10^{-5} M and 2.0×10^{-5} M respectively for Liu and Ju (2003) and Luque et al., 2005). Because of the reasons that prepared glucose biosensor has a wide linear working range, this range includes low concentrations and determination limit is low; glucose determination can be made with variety of different samples with our biosensor. Kinetic parameters maximum current ($I_{\max(\text{app})}$) and Michaelis–Menten constant ($K_{m(\text{app})}$) for enzyme biosensor were calculated as 11.70 μA and 0.019 mM, respectively, from Lineweaver–Burk plots (Fig. S5). K_m values for immobilized GOx presented in the literature are 0.0063 mM, 20.38 mM and 14.4 mM (Arslan et al., 2012; Zhou et al., 2005; Zheng et al., 2002). This was attributed to the fact that the used polymer and the type of immobilization were different.

The new nanomaterial used in this study increased the affinity of glucose oxidase enzyme to glucose. It can be understood from the K_m value which is lower than the K_m values in literature.

3.8. Operational stability of the enzyme electrode

Operational stability of the biosensor was studied by performing the activity assay (under optimum conditions) 10 times in the same day (Fig. S6). The relative standard deviation obtained after 10 measurements at a constant glucose concentration of 5.0×10^{-4} M was found to be 2.3%.

3.9. Storage stabilization of the enzyme electrode

Storage stability of the biosensor was determined by performing activity assays within 29 days. As shown in Fig. S7, during the first 19 days, a decrease was obtained in the response of biosensor. Between the 19th and 29th days, no change was seen in the response of biosensor. Immobilized enzyme retained 53.77% of its initial activity after 29 days.

4. Conclusions

In this study, a novel carbon paste electrode that is sensitive to glucose was prepared using the nanoparticles modified (4-Formyl-3-methoxyphenoxymethyl) with polystyren (FMPS) with L-Glycine–Pt(IV) complexes. These nanoparticles were first synthesized for this study. Glucose biosensor prepared is useable in a large linear working range. It has very low detection limit and acceptable response time for a biosensor. Therefore, glucose determination can be made with variety of different samples with our biosensor. Glucose

biosensor gives perfect reproducible results. Also, it has good storage stabilization. Thus, Glucose determination can be made with our biosensor for many times. Glucose biosensor prepared in this study is easy to prepare and highly cost effective.

Appendix A. Supplementary materials

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.bios.2013.10.059>.

References

- Adams, R., 1958. *Anal. Chem.* 30, (1576–1576).
- Arslan, F., Ustabas, S., Arslan, H., 2011. *Sensors* 11, 8152–8163.
- Arslan, H., Özdemir, M., Zengin, H., Zengin, G., 2012. *Int. J. Electrochem. Sci.* 7, 1025–10214.
- Boujita, M., Boitard, M., El Murr, N., 1999. *Biosens. Bioelectron.* 14, 545–553.
- Çolak, Ö., Arslan, H., Zengin, H., Zengin, G., 2012. *Int. J. Electrochem. Sci.* 7, 6988–6997.
- Comba, F.N., Rubianes, M.D., Herrasti, P., Rivas, G.A., 2010. *Sens. Actuators B: Chem.* 149, 306.
- Janeček, Š., 1993. *Process Biochem.* 28, 435–445.
- Jianping, L., Xiaoping, W., Yonghai, Y., 2009. *Sens. Actuators B: Chem.* 139, 400–406.
- Kerimov, M.K., Kurbanov, M.A., Tatardar, F.N., Mekhtili, A.A., 2011. *Surf. Eng. Appl. Electrochem.* 47, 76–83.
- Kulys, J., Krikstopaitis, K., Ruzgas, T., Razumumas, V., 1995. *Mat. Sci. Eng. C* 3, 51–56.
- Kumari, A., Yadav, S.K., Yadav, S.C., 2010. *Colloids Surf. B: Biointerfaces* 75, 1–8.
- Li, H., He, J., Zhao, Y., Wu, D., Cai, Y., Wei, Q., Yang, M., 2011. *Electrochimica Acta* 56, 2960–2965.
- Li, J., Yuan, R., Chai, Y., Che, X., 2010. *J. Mol. Catal. B: Enzym.* 66, 8–14.
- Liu, S., Ju, H., 2003. *Biosens. Bioelectron.* 19, 177–183.
- Luque, L., Rodriguez, M.C., Rivas, G.A., 2005. *Talanta* 66, 467–471.
- Moyo, M., Okonkwo, J.O., Agyei, M.N., 2012. *Sensors (Basel)* 12, 923–953.
- Nenkova, R., Ivanova, D., Vladimirova, J., Godjevargova, T., 2010. *Sens. Actuators B: Chem.* 148, 59–65.
- Norouzi, P., Faridbod, F., Larijani, B., Ganjali, M.R., 2010. *Int. J. Electrochem. Sci.* 5, 1213–1224.
- Sari, N., Antepli, E., Nartop, D., Yetim, N.K., 2012. *J. Mol. Sci.-IJMS* 13, 11870–11880.
- Sulak, M., Gökdoğan, Ö., Gülce, A., Gülce, H., 2006. *Biosens. Bioelectron.* 21, 1719–1726.
- Wang, C., Chen, S., Xiang, Y., Li, W., Zhong, X., Che, X., Li, J., 2011. *J. Mol. Catal. B: Enzym.* 69, 1–7.
- Xiaoli, C., Guodong, L., Yuehe, L., 2005. *Nanotechnol. Biol. Med.* 1, 130–135.
- Yen, M.-Y., Teng, C.-C., Hsiao, M.-C., Liu, P.-I., Chuang, W.-P., Ma, C.-C.M., Hsieh, C.-K., Tsai, M.-C., Tsai, C.-H., 2011. *J. Mater. Chem.* 21, 12880–12888.
- Yoo, E.-H., Lee, S.-Y., 2010. *Sensors* 10, 4558–4576.
- Zhang, Y., Wen, G., Zhou, Y., Shuang, S., Dong, C., Choi, M.M.F., 2007. *Biosens. Bioelectron.* 22, 1791–1797.
- Zheng, H., Xue, H., Zhang, Y., Shen, Z., 2002. *Biosens. Bioelectron.* 17, 541–545.
- Zhou, Z., Qiao, L., Zhang, P., Xiao, D., Choi, M.M.F., 2005. *Analy. Bioanal. Chem.* 383, 673–679.