Moroccan Journal of Chemistry
ISSN: 2351-812X
http://revues.imist.ma/?journal=morjchem&page=login
Químicas & al ./ Mor. J. Chem. 6 N°3 (2018) 540-547

Design an Amperometric Biosensor for the Detection of Lactate

Fernando Mancilla-Peña[1], Juan Reyes-Gómez[2*], Luis A. García-De La Rosa[3], Juan M. Flores-Álvarez[1], Reinaldo D. Martínez-Orozco[4], Ulises Páramo-García[4*]

[1] *Facultad de Ciencias Químicas, Universidad de Colima, Km. 9 Carretera Colima-Coquimatlán, 28400, Coquimatlán, Colima, México.*

[2] *Facultad de Ciencias, Universidad de Colima, Bernal Díaz del Castillo No. 340, Col. Villas San Sebastián, 28045, Colima, México.*

[3] *Unidad Profesional Interdisciplinaria de Ingeniería, Campus Guanajuato, Instituto Politécnico Nacional, Av. Mineral de Valenciana No. 200, Fracc. Industrial Puerto Interior, 36275, Silao de la Victoria, Guanajuato, México.*

[4] *Tecnológico Nacional de México / Instituto Tecnológico de Ciudad Madero, Centro de Investigación en Petroquímica, Prol. Bahía de Aldhair y Av. de las Bahías, Parque Tecnia, Altamira, Tamaulipas, México.*

* *Corresponding author:*
reyesgj@ucol.mx
Received 07 Dec 2017,
Revised 25 May 2018,
Accepted 12 July 2018

Abstract

In this study, we present the design of a new electrode material, as a L-lactate selective electrochemical sensor. We identified the conditions under which the mediator DHB-Nafion provides the best reversibility and robustness. We also found that by using a Nafion overlayer 50% of modified material is preserved. With these conditions the electrochemical detection of NADH is carried out. By adding to the molecular electrode's material L-LDH enzyme, the detection is accomplished at different concentrations of L-lactate. Finally we performed the determination of L-lactate under hydrodynamic regime. The working potential under hydrodynamic regime is 0.56 V vs. Ag/AgCl (pH 7.4) in 50 mM phosphate buffer with 50 mM KCl as supporting electrolyte. The results from the calibration curve obtained by linear regression was 0.9826 at concentrations from 0.0 to 0.6 mM of L-lactate, and 0.9738 at concentrations from 0.7 to 0.11 mM of L-lactate.

Keywords: LDH, L-Lactate, Enzyme biosensor, NADH.

1. INTRODUCTION

The chemical characterization of biological samples of interest is tough due to the complexity of the media (matrices) where the samples are inversed. In order to analyze these samples is necessary to take them into the laboratories and pre-process them, with the purpose of separating their components. This fact must be considered when *in-situ* and real-time data is required, to control a determinant chemical spice in medical diagnosis. Because the miniaturization required by the equipment, the ease of implementation in the place where the analysis is made and the operation in real-time is not easy to achieve [1]. The use of electrochemical sensors can be a viable alternative to solve the problem before mentioned. However, the use of conventional electrodes (without modifications) presents some complications, because many compounds have slow electronic transfer kinetics or they produce the passivation of the electrode [2]. In addition of this, the presence of other electroactive substances in the matrix, adds another level of difficulty. This situation causes interference during the determination of the interest analyte [3], resulting in the decrease of specificity in the analysis. The solution for these set-backs is to modify the surface of the electrode with a redox catalytic mediator [4], in order to carry out the electrochemical activation of the interest analyte. In this manner, two tendencies are reported in diverse works in the literature: 1) the use of synthetic compounds [5-12]. Which are usually porphyrins, phthalocyanines and other metallic complexes with a square planar structure and are useful for the assisted electro-activation of analytes and 2) the use of bio-molecules [13-21], like proteins, enzymes and antibodies between others [22], which ensure the selective recognition of the interest spice. In the case of bio-molecules, though they have the capacity of recognize selectively one analyte and frequently they can be redox mediators, they have the limitation of denaturalization of their base structure, which restricted their use in the heterogeneous medium of a modified electrode. In this study we pretend to approach the detection of L-Lactate. Because the monitoring of their levels have an important biological interest in the detection of lactic acidosis and tissue hypoxia [23]. Since these affections are characterized by the rise in the concentration of L-Lactate in the organism. We have the perspective to obtain a new molecular material of electrode, with possible applications like electrochemical selective sensor. The immobilization (over the surface of the electrode) of enzymes with the capacity of selectively recognize the L-Lactate and the L-Lactate Dehydrogenase [24], was made by the use of a membrane of Nafion which basically is a commercial ionophore and gave us a suitable environment to the incorporation of biomolecules in a modified electrode, due to their amphiphilic nature and physicochemical stability.

2. EXPERIMENTAL

2.1 Reagents and chemicals

Ion exchange resin perflourinated Nafion®, 3.4-dihydroxybenzaldehyde (DHB), nicotinamide adenine dinucleotide (NADH) L-lactate dehydrogenase enzyme (L-LDH) from rabbit muscle (EC 1.1.1.27), L-lactate lithium, K_2HPO_4 Potassium monohydrogen phosphate and potassium dihydrogen phosphate KH_2PO_4 [25]. All reagents were analytical grade (Sigma-Aldrich).

2.2 Apparatus

We used a three-electrode electrochemical system. A glassy carbon electrode as working electrode, platinum wire as auxiliary electrode and the reference electrode was Ag/AgCl (Cypress Systems Inc.). The electrode potential was controlled with a potentiostat-galvanostat Gamry (Reference 600, ZRA Software v.5) with computational interface.

2.3 Preparation of modified electrodes and glassy carbon biosensors

Glassy carbon electrodes were modified with DHB mediator mixture (100 mM), the cofactor NADH (1 mM), coal dust (0.5 mg/mL), L-LDH (545 ULDH), and 0.05% Nafion 50 mM phosphate buffer with 50 mM KCl. The mixture was gently stirred to avoid denaturing the enzyme. The surface of the glassy carbon electrode was previously polished to reach mirror finish and sonicated to remove excess alumina. Modified electrodes were dried for 12 hrs at room temperature. After drying an overlayer of 50% Nafion was placed, to increase the robustness of the material, and finally was allowed to dry for 12 hrs [25].

2.4 Lactate biosensor design

Our purpose design of the novel biosensor of L-Lactate is shown in the Figure 1. This shows that LDH enzyme depends on NAD^+. Because of this the 3,4-Dihydroxybenzaldehyde is incorporated to the design in order to assure the oxidation of NADH to the NAD^+ cofactor [26-30].

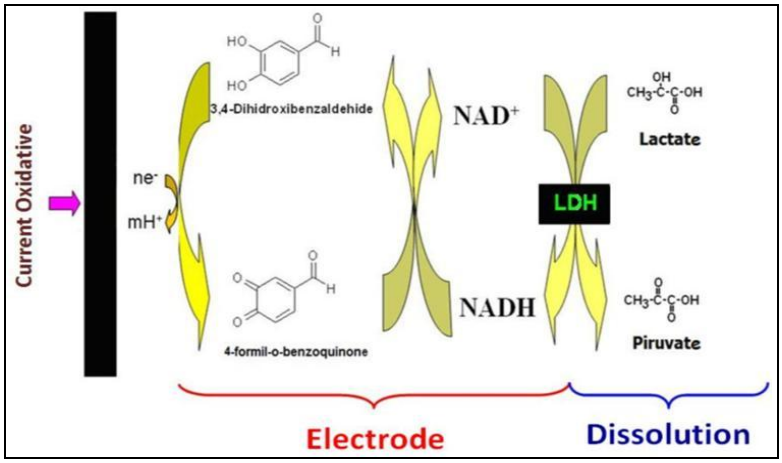

Figure 1. Design scheme of L-lactate biosensor.

3. RESULTS AND DISCUSSION

3.1 Characterization of DHB-Nafion electrode

The expected operation of the biosensor is while a mixture of L-LDH enzyme remains in the surface of the glassy carbon electrodes, the L-Lactate reacts enzymatically to form L-Pyruvate on solution and NADH on the molecular material of the electrode, all this, through the cofactor NAD^+. The NADH generated, reacts with the 4-formile-o-benzoquinone to form the NAD^+ cofactor y the DHB. By the monitoring of the oxidation currents generated in the process before described, is possible calculate the concentration of L-Lactate in the dissolution. The Figure 2 shows the cyclic voltamperogram of the characterization of DHB-Nafion electrode. The irreversible oxidation signal of the DHB (where the benzoquinone is formed) around a potential of 0.68 mV vs. Ag/AgCl can be observed. This irreversible process do not allows the study of the reduction of the benzoquinone to DHB. This due to the fact that the benzoquinone can react chemically with the medium, and possibly forms benzenodiols. Once the electrochemical signals from the electrode of DHB-Nafion are determined, the next stage is to test if the electrode is capable of oxidize the NADH to the NAD^+ cofactor. Because this, NADH is added to the electrochemical cell and the electrochemical response of the electrode against the oxidation of the NADH is obtained. The Figure 3 shows the cyclic voltammogram of the DHB-Nafion electrode with and without NADH. An increase of 0.5 uA of the anodic current (oxidation zone in blue line), with the addition of NADH can be observed. As well as the contrast, without NADH (red line). The increase of current indicates that the oxidation of the NADH to NAD^+ is taking place.

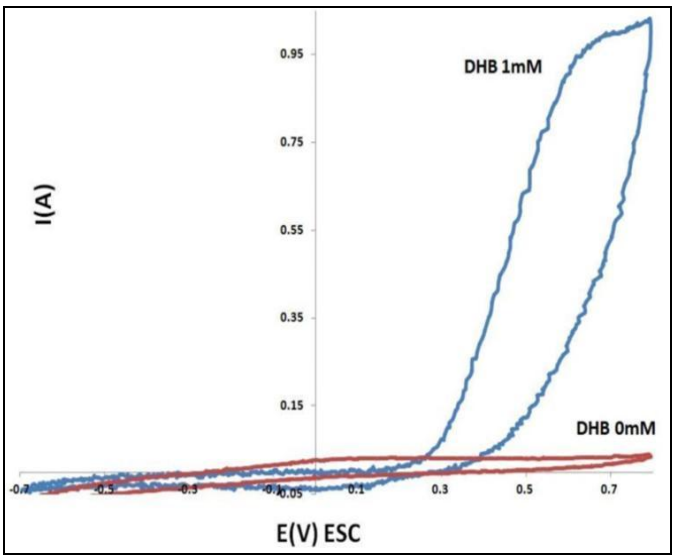

Figure 2. Cyclic voltammogram of DHB-Nafion electrode; 100 mV/s, -0.7 to 0.8 V vs Ag/AgCl, 50 mM phosphate/50 mM KCl buffer, pH = 7.4.

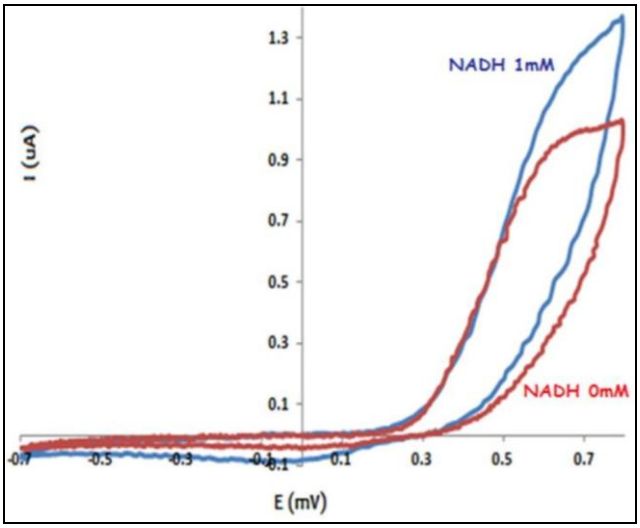

Figure 3. Cyclic voltammogram trace of the DHB-Nafion electrode with and without NADH. 100 mV/s, -0.7 to 0.8 V vs. Ag/AgCl, Buffer of phosphates 50 mM, pH 7.4.

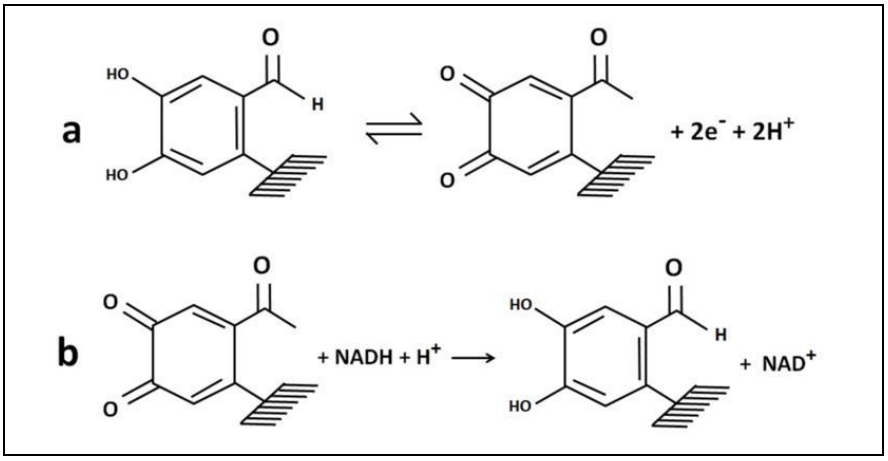

Figure 4. Reaction generated in the matrix and surface of modified glassy carbon electrode.

The oxidation zone of the reaction can be observed in Figure 3, can be explained with the reaction "b" of the Figure 4, when after the generation of benzoquinone (oxidized form of the DHB) a chemical reaction between the benzoquinone and NADH occurs and results in the formation of the NAD^+ and DHB. Because of the last, the NAD^+, not presents a reduction signal. The obtained results until this phase, indicate that is possible generate NAD^+ from NADH, giving the possibility to immobilize DHB, NADH and L-LDH to obtain a L-Lactate biosensor

3.2 Characterization of modified electrodes with DHB, NADH and L-LDH and oxidation of L-Lactate

The electrochemical characterization of the modified electrode with Nafion-DHB-NADH-L-LDH, is observed in Figure 5, where the cyclic voltammogram shows that the resistance of the system increases. This is expected due the low electrical conductivity of the proteins in the dissolution. A signal of irreversible oxidation around a potential of 0.56 V vs. Ag/AgCl, can be observed. This potential is where the most part of transformation of L-Lactate to L-Pyruvate takes place and will be the potential to maintain constant in the amperometric detection of L-Lactate.

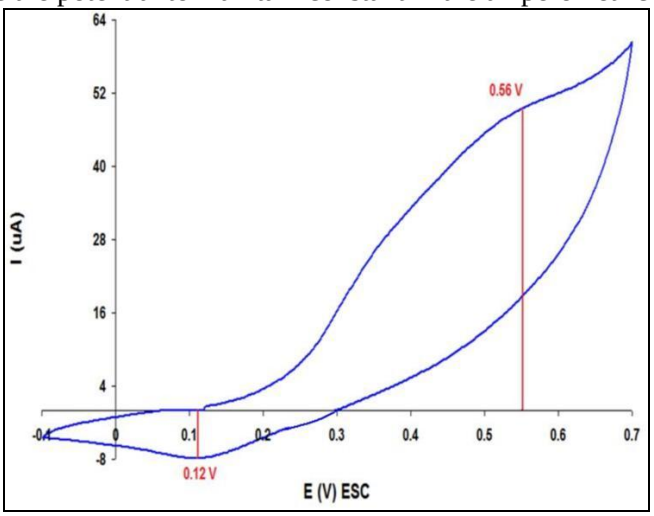

Figure 5. Cyclic voltammogram of the DHB-LDH-NAD^+-C-Nafion electrode. In buffer solution of phosphates 50 mM with KCl 50 mM, pH 7.4, 100 mV/s.

.

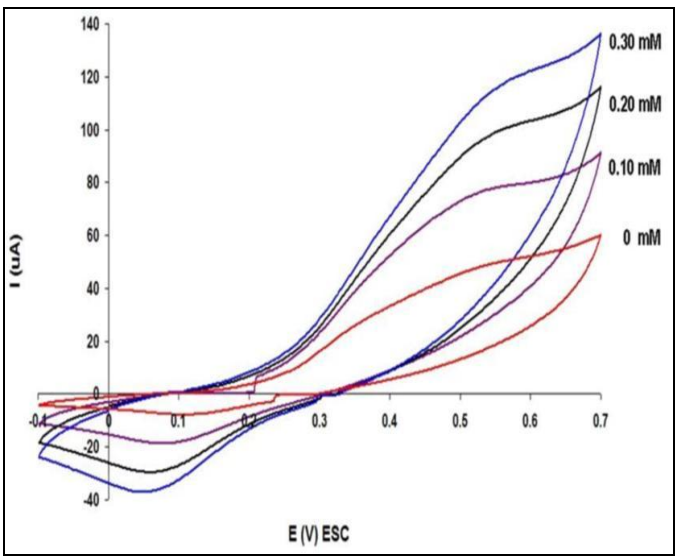

Figure 6. Cyclic voltammogram of the DHB-LDH-NAD^+-C-Nafion with overlayer of 50% Nafion electrode. In buffer of phosphates 50 mM with KCl 50 mM, pH 7.4, 100 mV/s and concentration of Lactate 0, 0.10, 0.20 and 0.30 mM.

Figure 6 shows the increase of anodic current due the formation of the NAD^+ and the L-Pyruvate, with the addition to the solution of lactate (0, 10, 20 and 30 mM). This indicates that the L-LDH enzyme has activity and detects selectively to the L-Lactate. In the cyclic voltammogram, the L-Lactate to L-Pyruvate oxidation at a potential of 0.56 V can be observed. The DHB mediator oxidizes the NADH to NAD+ and together with the L-LDH enzyme resulting in the oxidation reaction previously mentioned (L-Lactate to L-Pyruvate). Due to this products the currents are additive in the anodic signal

3.3 Detection in hydrodynamic regim of L-Lactate

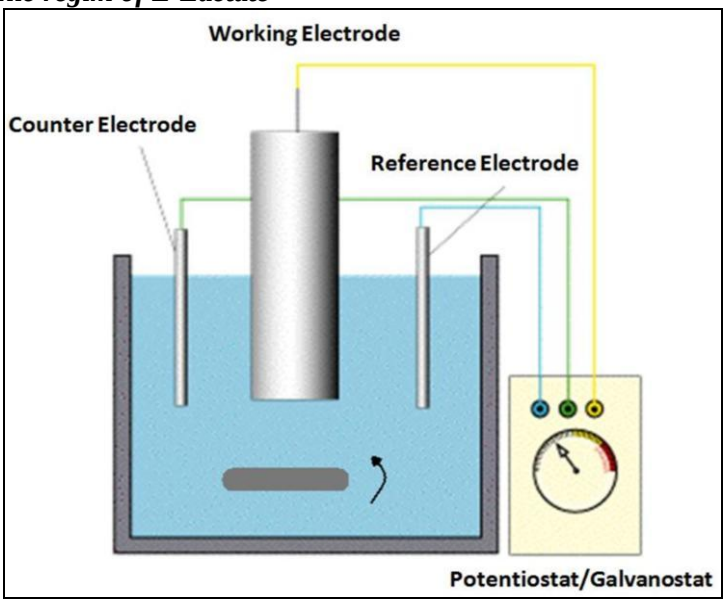

Figure 7. Schematic of the implementation of SSBA technique.

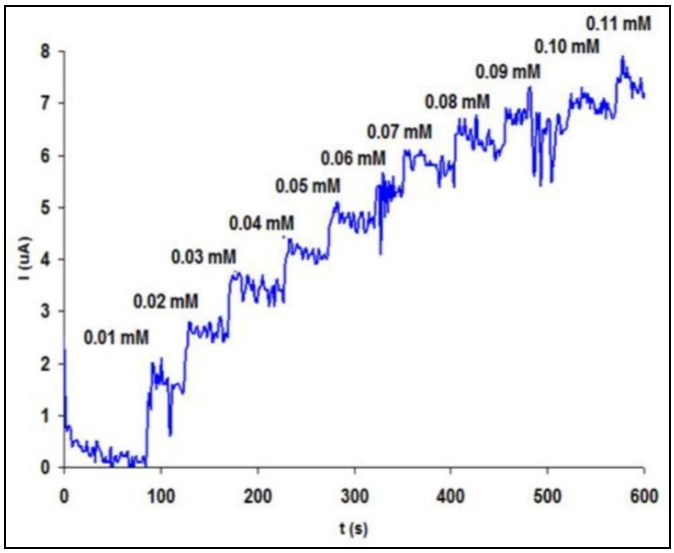

Figure 8. SSBA of the detection of Lactate with the electrode of Nafion-DHB-NADH-L-LDH.

Once the characterization of the Nafion-DHB-NADH-L-LDH electrode was carried out, the next stage was in the detection of L-Lactate in hydrodynamic regimen. This was performed through Steady State Batch Amperometry (SSBA) technique, which is based in applying the cronoamperometry technique with agitated medium (Figure 7) and additions of well known concentrations of the analyte. The result are increments in the current that describe a stairway form. This technique can help to prove the detection selective of the L-Lactate. In Figure 8 the response curve of the

SSBA applied to the detection of L-Lactate with the electrode of Nafion-DHB-NADH-L-LDH is shown. The applied potential is 0.56 V by 10 min, with an agitation speed of 800 rpm and additions of 0.01 mM of lactate when the signal remains stable. We propose two detection zones in Figure 8, the first around 0.01 to 0.06 mM and the second around 0.06 to 0.11 mM. This is because in the first addition the sensor doesn´t have resistance problems. However, the passivation begins to be present because of the products of the reaction and also a change in the slope can be observed with a decrease of the sensitivity. The absence of reversibility of the reduction reaction from 4-formile-o-benzoquinone to 3,4- dihydroxybenzaldehyde [31], this is because non-conductor benzaldehydes are being formed [32] and are the cause of the electrical conductivity decrease. This produces passivation of the electrodes modified with this mediator. The calibration curve (Figure 9) of detection (L-Lactate) is obtained when the values of concentration of L-Lactate against the increase the current produced in each addition are plotted. This Figure shows the changes in slope before mentioned. The linear coefficient of the first slope is an acceptable 0.9826, while in the second slope, the linearity is reduced. Because of this, we propose that the designed material is appropriate for the additions of Lactate that don't exceed 0.06 mM of concentration. The evidences discussed above show that is possible to build an efficient device for the Lactate detection.

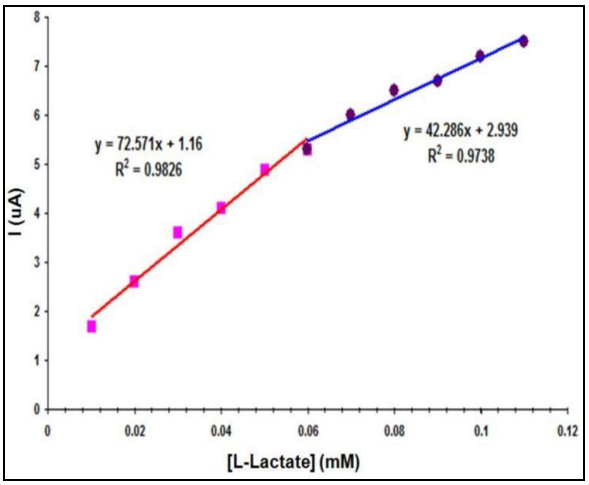

Figure 9. Calibration curve for the detection of L-Lactate.

4. CONCLUSIONS

The modified Nafion-DHB electrode is capable of generating the cofactor NAD^+, which is essential to carry out the enzymatic reaction of L-Lactate to L-Pyruvate. The modified Nafion-DHB-NADH-L-LDH electrode, is capable of detecting selectively the L-Lactate through the increase of concentration of this analyte in the cell due to the increase of anodic signal. Furthermore we accomplished the L-Lactate detection in hydrodynamic regimen. Also their calibration curve was obtained and two linearly zones were observed. The first one is around 0.0 to 0.06 mM while the second one is around 0.07 to 0.11 mM. It is important to note that this second zone, has less sensitivity due the decrease of its slope. Finally, we can conclude that the specific L-Lactate detection is viable and is possible to measure this analyte without the previous preparation of the sample. The detection limits found show lactate concentrations up to 0.11 mM, allowing identifying small quantities in tests of quality of food, medicine, clinical trials, etc. The overlayer of Nafion also provides adequate strength to preserve the modification in the electrode material. It is recommended for each modified electrode standardizing their specific correlation. It is important to note that, the detection area as a logarithmic zone that represents one single equation instead of the two linear areas could be

considered, however, this area would cause more correlation errors that the correlation of the linear equations proposed in this article.

References

1. K. Rajeshwar and J. G. Ibáñez, "Environmental Electrochemistry: Fundamentals and Applications in Pollution Abatement"; Academic Press, San Diego, 1997.
2. M. A. Deyab, H. A. Abo Dief, E. A. Eissa and A. R. Taman, *Electrochim. Acta*, 52 (2007) 8105-8110.
3. S. Pornpen, J. Patcharee, W. Nuanchawee and S. Busarawan, *Clin. Chim. Acta*, 372 (2006) 103-111.
4. M. Biesaga, K. Pyrznska and M. Trojanowicz, *Talanta*, 51 (2000) 209-224.
5. Z. H. Wen and T. F. Kang, *Talanta*, 62 (2004) 351-355.
6. D. Nizam, O. Joshua and W. Schuhmann, *Bioelectrochem*., 66 (2005) 105-110.
7. M. S. Maia, H. Winnischofer, M. Nakamura, K. Araki, H. E. Toma and L. Angnes, *Anal. Chim. Acta*, 539 (2005) 215-222.
8. I. O. Kenneth, Z. Zhao and T. Nyokong, *Electrochem. Commun*., 7 (2005) 679-684.
9. M. Siswana, I. O. Kenneth and T. Nyokong, *Talanta*, 69 (2006) 1136-1142.
10. R. Cássia, A. Benedito, F. Santos, A. Atsushi, L. Tatsuo, *J. Pharm. Biomed. Anal.*, 42 (2006) 184-191.
11. J. R. Wilney, A. L. Sousa, C. S. Luz, F. S. Damos, L. T. Kubota, A. A. Tanaka and M. C. N. Tanaka, *Talanta*, 70 (2006) 588-594.
12. D. Jayasri and S. Narayanan, *Food Chem*., 101 (2007) 607-614.
13. P. Yang, L. Wang, Q. Wu, Z. Chen and X. Lin, *Sens. Act. B: Chem*., 194 (2014) 71-78.
14. B. Cai, M. Zhao, Y. Wang, Y. Zhou, H. Cai, Z. Ye and J. Huang, *Ceram. Int*., 40 (2014) 8111-8116.
15. R. D. Peterson, B. T. Cunningham and J. E. Andrade, *Biosens. Bioelectron*., 56 (2014) 320-327.
16. M. Ligaj, M. Tichoniuk, D. Gwiazdowska and M. Filipiak, *Electrochim. Acta*, 128 (2014) 67-74.
17. M. Jarczewska, R. Ziółkowski, Ł. Górski, E. Malinowska, *Bioelectrochem*., 96 (2014) 1-6.
18. H. Zhang, Y. Sun, S. Gao, H. Zhang, J. Zhang, Y. Bai and D. Song, *Talanta*, 125 (2014) 29-35.
19. N. Nesakumar, S. Sethuraman, U. Krishnan and J. Rayappan, *J. Coll. Int. Sci*., 410 (2013) 158-164.
20. C. W. Hsu and G. J. Wang, *Biosens. Bioelectron*., 56 (2014) 204-209.
21. Z. Fang, W. Wu, X. Lu and L. Zeng, *Biosens. Bioelectron*., 56 (2014) 192-197.
22. A. E. Böyükbayram, S. Kıralp, L. Toppare and Y. Yağcı, *Bioelectrochem*., 69 (2006) 164-171.
23. J. M. De Miguel, J. A. Andueza, V. J. González, L. Pastor and J. Muñoz, *Amer. J. Emerg. Med*., 24 (5) (2006) 553-559.
24. V. A. De La Peña, P. D. Dios and R. T. Sierra, *Arch. Oral Biol*., 52 (2007) 911-915.
25. L. A. García, E. Castro, S. Gutiérrez, F. Bedioui, and A. Alatorre, *Electrochem. Commun*., 7 (2005) 853-856.
26. E. Golemis, Protein-Pritein Interactions, Cold Spring Harbor Laboratory Press, Nueva York, 2002.
27. M. M. Bradford, *Anal. Bioquím*., 72 (1976) 248-254.
28. Y. D. Kim, C. B. Park and D. S. Clark, *Biotech. Bioeng*., 73 (2001) 331-337.
29. K. Rathee, V. Dhull, R. Dhull, S. Singh, *Biochem. Biophys. Rep*., 5 (2016) 35-54.
30. L. Reverté, B. Prieto, M. Campas, *Anal. Chim. Acta*, 980 (2016) 8-21.
31. D. Weiss, M. Dorris and A. Loh, *Biosens. Bioelect*., 22 (2007) 2436-2441.
32. E. Horozova, T. Dodevska, N. Dimcheva, *Bioelectrochem*., 74 (2009) 260-264.