Int. J. Electrochem. Sci., 13 **(2018)** 2263 – 2271, doi: 10.20964/2018.03.28

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Electrochemical Sensing Platform for Glutamic Pyruvic Transaminase Determination

Fangfang Yao[1,2], *Pengyuan Zheng*[1,*], *Huang Huang*[1], *Yong Yu*[1], *Lu Mei*[1] *and Runping Jia*[2]

[1] The Fifth Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, 450052, P.R. China
[2] The Second Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, 450014, P.R. China
[*]E-mail: zhengypfifth@sina.com

Received: 4 November 2017 / *Accepted:* 17 December 2017 / *Published:* 5 February 2018

The activity of glutamic pyruvic transaminase (GPT) is a clinically significant factor for some acute diseases (e.g., acute hepatopathy and myocardial infarction), necessitating the fast and small format analysis of GPT at point-of-care. In the present study, a graphene–Nafion nanohybrid film coated sensor was used to detect GPT. A simple drop-casting method was used for the preparation of Nafion graphene oxide-decorated screen-printed electrode (SPE), while an electrochemical strategy was employed to reduce the graphene oxide on the SPE surface. Our proposed sensor can determine GPT over the range of 5–300 U/L, with the limit of detection (LOD) of 2.2 U/L.

Keywords: Electrochemical sensing; Glutamic pyruvic transaminase; Graphene; Liver disease; Clinic diagnosis

1. INTRODUCTION

Glutamic-pyruvic transaminase (GPT; synonym alanine aminotransferase, ALT), a significant amino transferase in a majority of body cells, can catalyse the transfer of the amino group from L-alanine to α-ketoglutarate [1-4]. The maximum concentration of GPT is found in the liver. Based on the previous literature, GPT has been recognized as a biomarker for liver diseases, because GPT is released into the blood in the case of liver cell necrosis [5, 6]. In serious cases, the level of GPT can be elevated to *ca.* 400 U/L (50 times higher than the normal) [7, 8]. These results confirm the significance of GPT activity determination in the diagnostics of diseases. Traditional GPT detection techniques are mainly based on the chromatography, colorimetric, and spectrophotometry methods [9, 10] that normally show poor accuracy and require sophisticated equipment and relatively expensive chemicals, such as cofactors and enzymes [11-13]. Thus, it is necessary to develop other techniques for the

detection of GPT to address the above problems. Rietz and Guilbault [14] presented the detection of GPT activity on a solid-surface and in solution using the fluorimetric technique, with the linear range obtained as 5–106 U/L. I. Moser and co-workers [15] reported the detection of GPT with a miniaturized liquid handling system based on a thin film biosensor array. The above system was found linearly related to the concentration of GPT (6-192 U/L). Suzuki and co-workers [16] presented the fabrication of a microfluidic system to measure the activity of GPT (up to 250 U/L) based on a Y-shaped flow channel equipped with an on-chip L-glutamate sensor. Ruey-JenYang and co-workers [17] recently reported the detection of GPT activity using a developed paper-based analytical tool over the concentration range of 0-125 U/L. Despite the substantial effort in the study of the mechanism and structure of GPT [18-20], it is still urgent to develop fast and sensitive strategies for GPT detection with broad working range, to enable the routine clinical detection of GPT activity in laboratories, especially for the diagnosis of liver diseases.

Electrochemical determination of GPT is highly sensitive, selective, and inexpensive; it requires less sample consumption and has therefore attracted substantial attention in recent years [21-23]. To study the GPT activity, the electroactive analytes generated from the enzyme reaction system were determined, considering the function of GPT in the human body as a biological catalyst. He and Chen [24] reported the detection of human serum GPT by assessing the production rate of β-nicotinamide adenine dinucleotide (NAD^+) using a differential pulse voltammetry technique. For the GPT activity, a linear range was 0–200 U L^{-1}; the LOD was 0.3 U/L; and the sensitivity was 0.0567 nA/min/U L. Paraíso and co-workers [25] presented the determination of H_2O_2 using an electrochemical indicator, i.e., a poly(4-aminophenol) and 4-aminoantipyrine coated graphite electrode, providing remarkable detection of GPT, where the LOD and linear range were 2.68 × 10^{-5} U/L and 3.0 × 10^{-5} to 3.0 U/L, respectively. Generally, the current response for the NAD^+ electrochemical detection is quite low, making the measurement more difficult. In spite of the extensive use of electrochemical determination of H_2O_2, the GPT analysis could be affected by the instability of H_2O_2 because the production of H_2O_2 is dependent on the oxygen amount [26].

The present study proposed an electrochemical sensor towards the analysis of GPT by immobilizing reduced graphene oxide (RGO) onto the electrode. The developed sensor was highly sensitive because RGO offered a favourable, high-capacity, and hydrophilic platform for loading GPT. Our proposed strategy was shown to be highly sensitive, simple, and cost-effective, thus outperforming other methods reported previously.

2. EXPERIMENTS

2.1. Chemicals

(Nicotinamide adenine dinucleotide) NADH was commercially available from Roche. Glutamic dehydrogenase (GLDH), β-nicotinamide adenine dinucleotide (NAD+), pyridoxal 5′-phosphate hydrate (PLP), α-ketoglutarate (α-KG), l-alanine, and GPT (lyophilized powder) were commercially available from Sigma-Aldrich. All specimens were prepared in 0.1 M of pH 7.4

phosphate buffer solution (PBS). Distilled water (DW) was used for specimen dilution throughout the experiments.

2.2. Preparation of the graphene modified electrode

After sequential ultrasonic rinsing using ethanol:propanol (1:1) and DW, the bare SPE was surface-coated and then left to dry at the ambient temperature. This was followed by dispersing graphene oxide (GO) in DW (1.0 mg/mL) under 0.5 h ultrasonication or until thorough dispersion. Subsequently, the Nafion–isopropyl-alcohol solution (10 μL, 1.0 wt %) was added into the GO solution (50 μL, 1 mg/mL) and ultrasonicated for approximately 0.5 h. Then, the Nafion/ER-GO/SPE was obtained after coating an aliquot of 10 μL of the as-prepared mixed solution onto the screen-sprinted electrode (SPE), with the solvent evaporated for 120 min at ambient temperature. Nafion/GO/SPE was then obtained after air drying. This was followed by immersing the as-prepared Nafion/ER-GO/SPE into 0.02 M KH_2PO_4 solution, and a cathodic potential of −0.7 V was applied to the GO coated SPE with a potentiostat for *ca.* 10 min.

2.3. Instruments

For voltammetric measurements, an electrochemical geometry was used, where the working, reference and counter electrodes were a screen-printed electrode, a saturated calomel electrode (SCE), and a platinum wire, respectively. On the other hand, a Gamry Reference 600 potentiostat was used for the cyclic voltammetry (CV) measurements. All measurements were carried out at ambient temperature. To remove the residual aluminium oxide particles present on the surface after each treatment, the electrode was washed and ultrasonicated for 5 min in DW. A Hanna HI 221 pH-meter (full range: 0-14) was used for the measurement of solution pH.

2.4. Electrochemical Measurements

NADH generated from the transdeamination was detected for the electrochemical detection of GPT. During the first step, coenzyme pyridoxal 5′-phosphate hydrate (PLP) facilitated the production of l-glutamate. For the second step, the produced l-glutamate was used as a reactant; NADH was formed in the presence of glutamic dehydrogenase (GLDH) which was converted into NAD^+ through oxidization, releasing two electrons on the working electrode in the third step. Therefore, when other reactants were adequate, the NADH quantity was found to be in proportion to the effective quantity of GPT. Electrochemical oxidization of NADH occurred during the third step, suggesting the direct relationship between the EC current and the GPT activity.

3. RESULTS AND DISCUSSION

The surfaces of the bare SPE and the Nafion/ER-GO/SPE were studied. Fig. 1 shows the CV obtained for these two electrodes in an electrochemical probe with $[K_3Fe(CN)_6]$ (1.0×10^{-3}) M and KCl (0.1 M) at the scan rate of 100 mV/s. A reversible one-electron transfer process was suggested by the CV shape. The peak-to-peak separation was obtained as *ca.* 69 mV in the case of the bare SPE, corresponding to the reversible redox process of $[K_3Fe(CN)_6]$. The voltammogram of $[Fe(CN)_6]^{3-}$ was shown against the peak currents. For the ER-GO/SPE, a remarkable increase in the current was observed, corresponding to the increased conductivity of graphene relative to that of graphite. On the other hand, the coated SPE showed shifted cathodic and anodic peak potentials of graphene compared to the bare SPE. In terms of graphene, the Epc (cathodic peak potential) was obtained as 0.283 V and Epa (anodic peak potential) as 0.113 V. On the other hand, the bare SPE showed the Epc of 0.25 V and the Epa of 0.14 V.

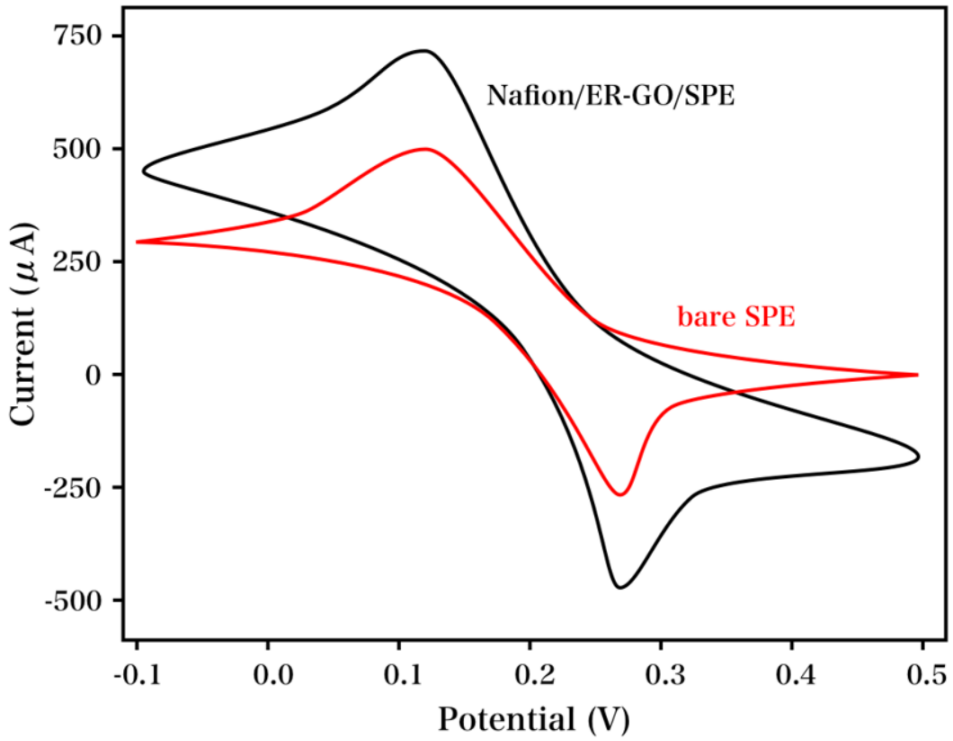

Figure 1. CVs obtained at the bare SPE and Nafion/ER-GO/SPE in 1 mM $[Fe(CN)^6]^{3-}$ that contained 0.1 M KCl at the scan rate of 100 mV/s.

The effect of the scan rate on the redox current of paracetamol was investigated using Nafion/ER-GO/SPE. It can be seen that both the anodic and cathodic peak currents increase with increasing scan rate accompanied by enlarged peak separation. For the bare SPE and Nafion/ER-GO/SPE, the electroactive areas were studied through CVs, with the Randles–Sevcik equation given as $Ip = 2.69 \times 10^5\ n^{3/2}AD_0SC_0v^{1/2}$. The slope obtained from the plot of the voltammetric peak current (Ip) *vs.* the square root of the scan rate (Fig. 2) suggested the correspondence to the electroactive area.

According to Laviron's model [27, 28], the number of electrons involved in the reaction and the charge-transfer coefficient constant can be calculated as 1.959 and 0.442, respectively.

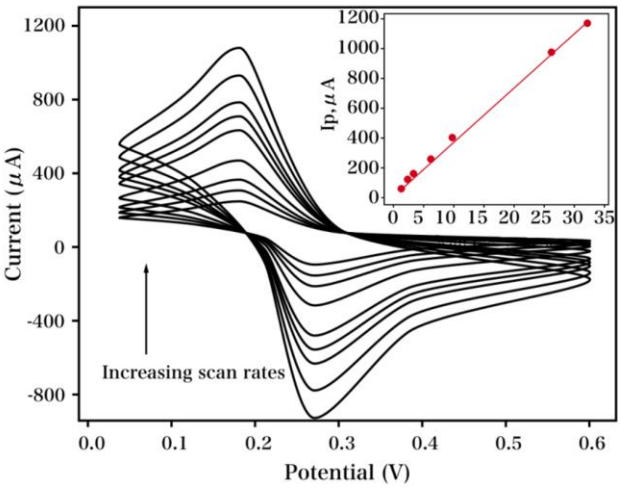

Figure 2. CVs obtained at the Nafion/ER-GO/SPE in 0.1 M $[Fe(CN)_6]^{3-}$ that contained 0.1 M KCl at varying scan rates.

A linear increase of the redox peak currents for the graphene-modified SPE with the increasing scan rate (5-1000 mV/s) was observed, as predicted by the Randles–Sevcik equation. The surface confined property of the coated electrode reaction was indicated by the corresponding results. CV experiments were carried out to study the electrochemical features of NADH on Nafion/ER-GO/SPE in PBS (pH 4). Two voltammograms were recorded for Nafion/ER-GO/SPE before and after the addition of NADH (10 μM) in PBS (pH 4), as displayed in Fig. 3.

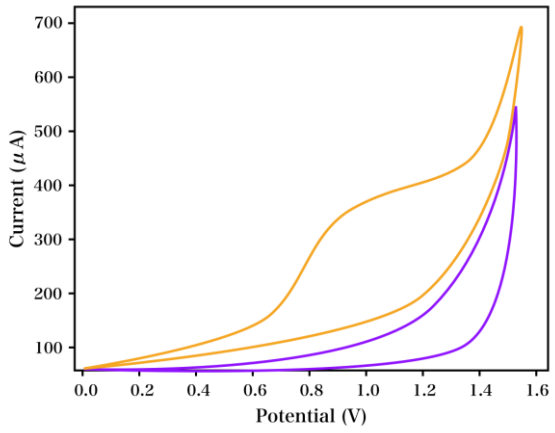

Figure 3. CVs obtained at Nafion/ER-GO/SPE in the absence of 10 μM ASA and in the presence of 10 μM ASA in PBS (pH 4).

Nafion/ER-GO/SPE showed no redox peaks in the absence of the aspirin electroactive species over the potential range of 0 - 1.5 V, suggesting the non-electroactivity of graphene in the scanned

potential window. On the other hand, Nafion/ER-GO/SPE showed an extremely sensitive anodic peak with NADH (10 μM) in PBS (pH 4) at the current of 780 mV. These phenomena may be attributed to the excellent electrocatalytic activity, high conductivity, large specific surface area and synergistic effect of the ER-GO and Nafion.

Considering the direct dependence of GPT detection on the NADH analysis, we first studied the detection ability of NADH. Fig. 4 shows the CVs of NADH (2.5 mM) in the PBS solution. For the EC oxidation where NADH was converted into NAD^+ through electrochemical oxidization, only one current peak was observed, suggesting the irreversibility of the electrochemical reaction of NADH. The peak current was found to be linearly related to the square root of the scan rate (10-70 mV/s), with the R^2 of 0.998. This indicated the diffusion-controlled property of the reaction process.

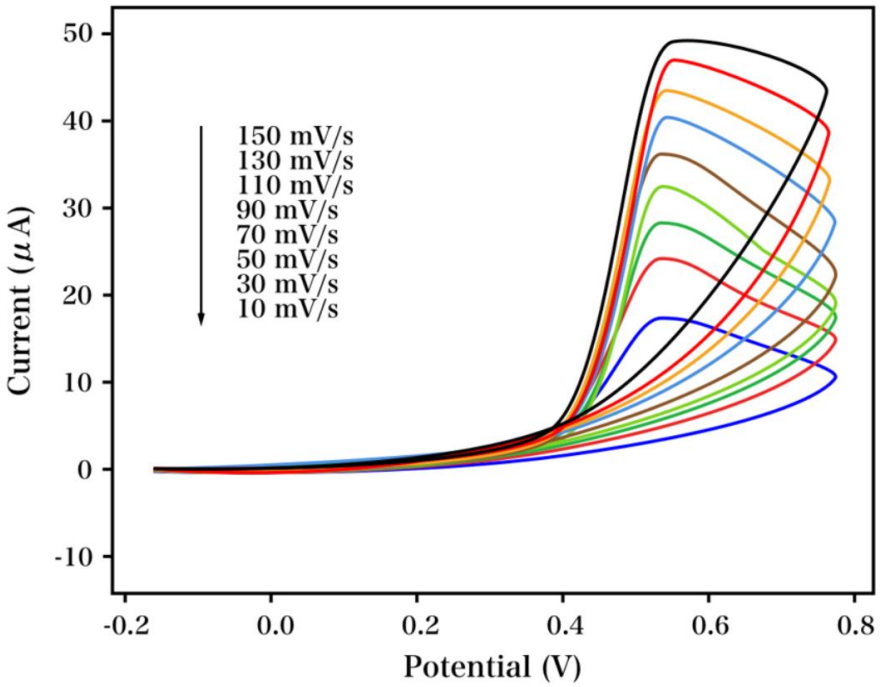

Figure 4. CVs obtained in the presence of 2.5 mM NADH at different scan rates from 10 to 150 mV/s with the quiescent solution.

The nanocomposite structure formed by the combination of ER-GO and Nafion with an excellent conductivity coupled with this effect increased the activity and sensitivity of the newly prepared modified electrode towards the electroanalysis [29]. The developed electrochemical sensor was used to detect the activity of GPT (5-300 U/L). For each detection, the consumed GPT solution volume was *ca.* 20 μL. The concentrations of GLDH, NAD^+, PLP, α-KG, and l-alanine were 3.5 U/L, 7.5 mM, 0.1 mM, 0.75 mM, and 83.3 mM, respectively. All test chemicals were dissolved in the PBS solution at 25 °C for 120 min. After the injection of the GPT solution, NADH formed through the reaction was determined using CV measurement and the scan rate of 50 mV/s. All GPT specimens were detected three times. Fig. 5A displayed the dependence of the peak current on the GPT activity. The oxidation peak currents of each GPT activity were averaged. Fig. 5B displays the relationship

between the GPT activity and the peak current, with two linear ranges observed. The two separate linear ranges resulted from the electrochemical reaction and the enzymatic reaction kinetics of NADH. The LOD was calculated to be 2.2 U/L. Table 1 presents the performance comparison of our proposed sensor and those reported previously towards the GPT analysis.

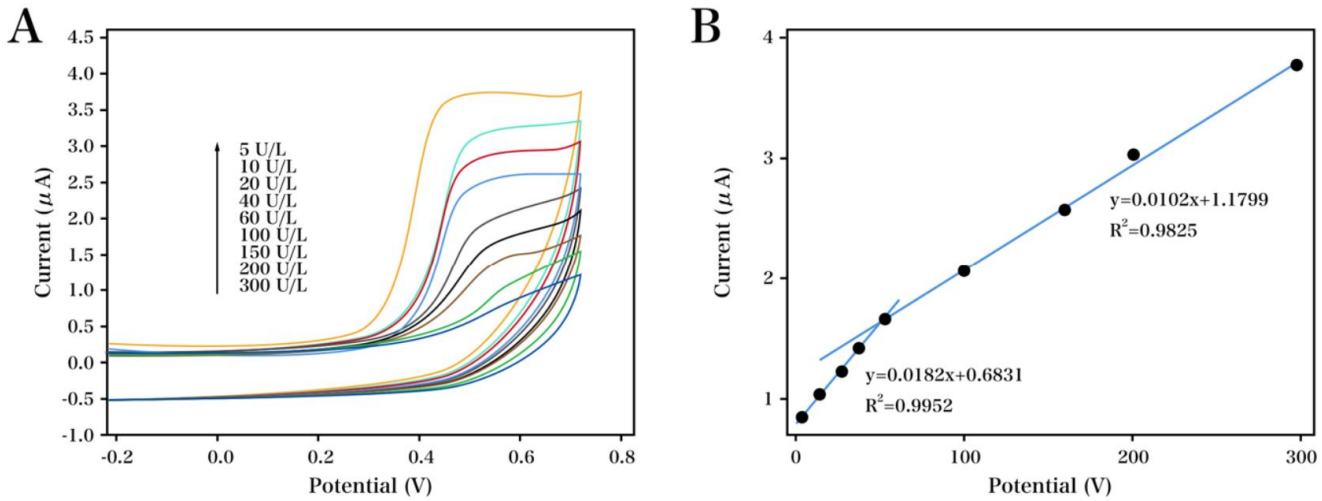

Figure 5. (A) CVs obtained for enzymatic reaction systems in the presence of varying activities of GPT with the quiescent solution (5, 10, 20, 40, 60, 100, 150, 200, 300 U/L). (B) Calibration curves recorded for enzymatic reaction systems in the presence of varying GPT activities.

Table 1. Performance of our proposed sensors and other sensors towards the analysis of GPT.

Electrode	Linear detection range	Detection limit	Reference
Microfluidic chip	50–250 U/L	9.25 U/L	[4]
Fluorescence quenching of bovine serum albumin	5-400 U/L	3 U/L	[30]
Unmodified screen-printed carbon electrode	60-300 U/L	—	[31]
L-glutamate oxidase/photo-crosslinkable polymer	8–250 U/L	4.4 U/L	[32]
Microfludic system	10-100 U/L	—	[33]
Microfluidic system	9-250 U/L	—	[34]
Nafion/ER-GO/SPE	5-300 U/L	2.2 U/L	This work

Table 2 shows the results and relative deviations obtained using the proposed Nafion/ER-GO/SPE and ALT Activity ELISA Assay (Sigma). The relative errors between the two techniques ranged from 4.45% to 8.84%, indicating that the results obtained from the Nafion/ER-GO/SPE were acceptably close to those obtained by the ELISA kit. Therefore, the accurate determination of GPT in clinical diagnosis by as-prepared electrochemical method was achieved with satisfactory results.

Table 2. Experimental results of GPT concentration in serum samples obtained by proposed electrochemical and ALT Activity Assay.

Serum samples	1	2	3
Nafion/ER-GO/SPE (U/L)	0	0	0
ALT Activity Assay	0	0	0
Added (U/L)	50	100	150
Found (U/L)- Nafion/ER-GO/SPE	49.65	98.99	152.48
Found (U/L)- ALT Activity Assay	50.11	98.50	151.61
Recovery (%)	99.30	98.99	101.65
Relative deviation (%)	8.84	4.45	5.54

4. CONCLUSIONS

In the present study, Nafion/ER-GO/SPE was used for sensitive and selective detection of GPT. The developed sensor showed distinct stability and reproducibility towards the routine analysis of GPT, where no maintenance or pretreatment was required. A linear response was obtained in a buffered solution over the range from 5 to 300 U/L, and the LOD was 2.2 U/L.

References

1. M. Yeh, Y. Shih, H. Chung, J. Chou, H. Lu, C. Liu, J. Liu, W. Huang, S. Peng and L. Wu, *Experimental and Therapeutic Medicine*, 11 (2016) 1300.
2. M. Yeh, Y. Shih, H. Chung, J. Chou, H. Lu, C. Liu, J. Liu, W. Huang, S. Peng and L. Wu, *Mol. Med. Rep.*, 16 (2017) 2483.
3. K. Honma, M. Kamikubo, K. Mochizuki and T. Goda, *Metabolism*, 71 (2017) 118.
4. J. Wang, Z. Xu, H. Zou, S. Hu, D. Wang, J. Liu and L. Wang, *Microfluidics and Nanofluidics*, 21 (2017) 27.
5. P. Katiyar, A. Kumar, A.K. Mishra, R.K. Dixit and A.K. Gupta, *International Journal of Research in Medical Sciences*, 3 (2017) 2429.
6. J. Rani and D. Raju, *International Journal of Research in Medical Sciences*, 4 (2016) 1564.
7. V. Goyal, K. Chugh and Y. Agrawal, *Journal of Health Specialties*, 2 (2014) 169.
8. A. Siddique, K. Bose, S. Saha, K. Das, C. Kundu, S. Ganguly and P. Mondal, *Sri Lanka Journal of Child Health*, 46 (2017) 243.
9. M. Díaz-Rubio, J. Pérez-Jiménez, M. Martínez-Bartolomé, I. Álvarez and F. Saura-Calixto, *Plant Foods for Human Nutrition*, 70 (2015) 9.
10. M. Circu, R. Maloney and T. Aw, *Chemico-Biological Interactions*, 264 (2017) 16.
11. D. Chen, X. Xu, W. Wang, L. Zhang and L. Chang, *Journal of Hainan Medical University*, 22 (2016) 143.
12. D. Craig and Z. Hiebert, *BioMetals*, 30 (2017) 449.
13. H. Wang, C. Chu, S. Tsai and R. Yang, *Micromachines*, 7 (2016) 9.
14. B. Rietz and G. Guilbault, *Anal. Chim. Acta.*, 77 (1975) 191.
15. I. Moser, G. Jobst, P. Svasek, M. Varahram and G. Urban, *Sens. Actuators, B* 44 (1997) 377.
16. N. Ohgami, S. Upadhyay, A. Kabata, K. Morimoto, H. Kusakabe and H. Suzuki, *Biosens. Bioelectron.*, 22 (2007) 1330.
17. W. Liang, C. Chu and R. Yang, *Talanta*, 145 (2015) 6.

18. W. Jiang, Y. Cheng, M. Yen, Y. Chang, V. Yang and O. Lee, *Biomaterials*, 35 (2014) 3607.
19. M. Fan, L. Lu, C. Su, M. Xue, J. Dou, P. Li, H. Feng and Y. Fan, *Asian Pacific Journal of Tropical Medicine*, 9 (2016) 694.
20. K. Park, J. Kim and K. Cho, *Molecular Nutrition & Food Research*, 58 (2014) 1537.
21. J. Wang, *Talanta*, 56 (2002) 223.
22. M. Breadmore, *Electrophoresis*, 28 (2007) 254.
23. S. Wu, Z. Zhou, L. Xu, B. Su and Q. Fang, *Biosens. Bioelectron.*, 53 (2014) 148.
24. Y. He and H. Chen, *Anal. Chim. Acta.*, 353 (1997) 319.
25. L. Paraíso, L. Paula, D. Franco, J. Madurro and A. Brito-Madurro, *Int. J. Electrochem. Sci*, 9 (2014) 1286.
26. Z. Li, N. Ping, H. Zhang and J. Zhang, *Chinese Journal of Analytical Chemistry*, 37 (2009) 617.
27. E. Laviron, *J. Electroanal. Chem.*, 52 (1974) 355.
28. E. Laviron, *J. Electroanal. Chem.*, 101 (1979) 19.
29. C. da Silva, F. Veregue, L. Aguiar, J. Meneguin, M. Moisés, S. Fávaro, E. Radovanovic, E. Girotto and A. Rinaldi, *New J. Chem.*, 40 (2016) 8872.
30. X. Chen, M. Chen and L. Rong, *Rsc Advances*, 5 (2015) 103557.
31. Z. Xu, S. Hu, W. Tian, D. Wang, J. Liu, C. Liu and L. Wang, *Analytical Methods*, 7 (2015) 9421.
32. K. Chang, W. Hsu, H. Chen, C. Chang and C. Chen, *Anal. Chim. Acta.*, 481 (2003) 199.
33. N. Ohgami, S. Upadhyay, H. Kusakabe and H. Suzuki, Microfluidic system for the determination of the activities of glutamic oxaloacetic transaminase and glutamic pyruvic transaminase, The International Conference on Solid-State Sensors, Actuators and Microsystems, 2005. Digest of Technical Papers. Transducers, 2007, pp. 1596.
34. N. Ohgami, S. Upadhyay, A. Kabata, K. Morimoto, H. Kusakabe and H. Suzuki, *Biosens. Bioelectron.*, 22 (2007) 1330

© 2018 The Authors. Published by ESG (www.electrochemsci.org). This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution license (http://creativecommons.org/licenses/by/4.0/).