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Magnetite Nanoparticles and Prussian Blue Modified Amperometric Biosensor for Lactic Acid Determination

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Abstract: Carbon paste electrode modified with lactate oxidase (LOx) enzyme, Fe_3O_4 nanoparticles and Prussian blue (PB) was investigated as amperometric biosensor for lactic acid. LOx was immobilized by cross-linking with glutaraldehyde (GA) and bovine serum albumin (BSA) into carbon paste matrix. Electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) were used to investigate the electron transfer properties of bare and modified carbon paste electrodes. The parameters affecting the analytical performance of the enzyme electrode have been investigated in detail and optimized. The optimum response to lactic acid was obtained in 0.05 M phosphate buffer at pH 5.5 when polarized at +0.10 V vs. Ag/AgCl. The biosensor provides a linear response for lactic acid in the concentration range of 7.5×10^{-6}–1.3×10^{-4} M with a detection limit of 5.9×10^{-7} M and correlation coefficient of 0.998. The effects of interferents, the operational and storage stabilities of the biosensor were also evaluated. The biosensor showed 5.4% loss in its initial activity after one week when stored at +4°C.

Keywords: Lactic acid, Amperometry, Enzyme electrode, Fe_3O_4 nanoparticles, Prussian blue.

Laktik Asit Tayini için Magnetit Nanopartiküller ve Prusya Mavisi ile Modifiye Edilmiş Amperometrik Biyosensör

Öz: Laktatoksidaz enzimi (LOx), Fe_3O_4 nanopartiküller ve Prusya mavisi (PB) ile modifiye edilmiş karbon pasta elektrot laktik asit tayini için amperometrik biyosensör olarak araştırıldı. LOx karbon pasta matrikse glutaraldehit (GA) ve sığır serum albümin (BSA) kullanılarak çapraz bağlama immobilize edildi. Yalın ve modifiye edilmiş karbon pasta elektrotların elektron transfer özellikleri elektrokimyasal empedans spektroskopisi (EIS) ve dönüşümlü voltametri (DV) kullanılarak incelendi. Enzim elektrodun analitik performansını etkileyen parametreler detaylı olarak incelendi ve optimize edildi. Laktik aside optimum cevap 0.05 M pH 5.5 fosfat tamponu içinde Ag/AgCl elektroda karşı +0.10 V'da elde edildi. Biyosensör laktik aside 7.5×10^{-6}–1.3×10^{-4} M aralığında, 5.9×10^{-7} M gözlenebilme sınırı ve 0.998 korelasyon katsayısı ile doğrusal cevap gösterdi. Bozucu türlerin etkisi, biyosensörün çalışma ve depolama kararlılıkları da değerlendirildi. Biyosensör +4°C de saklandığında 1 hafta sonra başlangıç aktivitesinin %5.4'ünü kaybetti.

Anahtar kelimeler: Laktik asit, Amperometri, Enzim elektrot, Fe_3O_4 nanopartiküller, Prusya mavisi

1. Introduction

Reliable and rapid monitoring of lactic acid is very important in clinical analysis, sports medicine and food industry (Monosík et al., 2012). In clinical diagnosis, blood lactate levels are indicative of various pathological states, including shock, respiratory insufficiencies, and heart and liver diseases (Huang et al., 2008). In sports

medicine, the level of lactic acid during exercise is an indicator for the training status and fitness (Iwuoha et al., 1999). The monitoring of lactic acid levels is also important in the food industry, particularly in determining the quality of dairy products and the control of additives (Perez et al., 2012).

Several techniques have been used for the determination of lactic acid, such as HPLC, ion exclusion chromatography, colorimetric test strips, or enzymatic test kits (Przybyt et al., 2010; Escobal et al., 1998; Monson et al., 1997). However, these methods are often complicated, time-consuming, and expensive; require laboratory equipment and educated personnel. Thus, the development of reliable biosensors for the determination of lactic acid in real samples is of great interest because it provides several advantages, such as high specificity, rapid response and cost effective analysis of samples. Many amperometric enzyme electrodes for lactic acid determination have been reported (Radoi et al., 2010; Çelik et al., 2015; Rahman et al., 2009; Hirst et al., 2013; Shimomuraa et al., 2012).

Lactic acid biosensors can be based on two enzymes; lactate oxidase or lactate dehydrogenase. However, lactate dehydrogenase enzyme requires the presence of cofactor NAD^+ that complicates analysis and increases its cost (Radoi et al., 2010). Lactate oxidase catalyzes the oxidation of lactate into pyruvic acid and hydrogen peroxide according to the following equations:

$$\text{L-lactate} + \text{LOx}_{ox} \rightarrow \text{pyruvate} + \text{LOx}_{red}$$

$$\text{LOx}_{red} + \text{O}_2 \rightarrow \text{LOx}_{ox} + \text{H}_2\text{O}_2$$

$$\text{H}_2\text{O}_2 \rightarrow \text{O}_2 + 2\text{H}^+ + 2\text{e}^-$$

The amount of hydrogen peroxide can be measured amperometrically (Çelik et al., 2015). Unfortunately, amperometric determination of H_2O_2 requires high anodic potential. Under these conditions, other electroactive species present in the sample such as ascorbic acid, acetaminophen and uric acid can also be oxidized and interfere in the analysis. A way to decrease the applied potential thus the interference effect is the use of mediators instead of oxygen. Artificial electron transfer mediators usually require lower oxidation potentials that are desirable for avoiding possible interferences from other oxidizable compounds. Artificial mediators carry electrons between the enzyme and the electrode surface by the following reactions where M_{ox} and M_{red} are the oxidized and reduced forms of the mediator. The reduced form is reoxidized at the electrode, giving a current signal (proportional to the lactate concentration) while regenerating the oxidized form of the mediator.

$$\text{L - lactate} + \text{LOx}_{ox} \rightarrow \text{pyruvate} + \text{LOx}_{red}$$

$$\text{LOx}_{red} + \text{M}_{ox} \rightarrow \text{LOx}_{ox} + \text{M}_{red}$$

$$\text{M}_{red} \rightarrow \text{M}_{ox} + e^{-}$$

Cobalt-phtalocyanine (Shimomuraa et al., 2012), tetracyanoquinodimethane (Bassi et al., 1999), meldola's blue (Pereira et al., 2011), ferrocene (Serafín et al., 2013), benzyl viologen (Niu et al., 2000) and tetrathiafulvalene (Molinero-Abad et al., 2014), 1,4-benzoquinone (Erden et al., 2011), benzo(c)cinnoline (Çelik et al., 2015) are some examples of the mediators used in the past for the fabrication of biosensors.

Another approach to lower the working potential is the use of horseradish peroxidase (HRP) together with lactate oxidase to perform lactic acid determination via H_2O_2 reduction (Ghamouss et al., 2006). However, the use of an additional enzyme (HRP) increases the cost of the analysis. Prussian blue (PB) was reported to be an "artificial peroxidase" because it can catalyze electrochemical reduction of H_2O_2 at lower potentials (Banerjee et al., 2013; Karyakin et al., 2000). PB, as an attractive alternative electrocatalyst for H_2O_2 detection, has been widely used in the biosensor construction (Gong et al., 2013a; Gong 2013b; Cinti et al., 2015).

Applications of nanomaterials to biosensors have recently aroused much interest. These interesting materials exhibit large surface-to-volume ratio, high surface reaction activity, high catalytic efficiency, and strong adsorption ability that are helpful in designing high performance biosensors. In addition, nanoparticles have a unique ability to promote fast electron transfer between electrode and the active site of the enzyme (Yang et al., 2009). Among the various nanomaterials, magnetite nanoparticles have recently gained increased interest due to biocompatibility, strong superparamagnetic property and low toxicity (Erden et al., 2013; Wang et al., 2008). The successful applications of magnetic nanoparticles in the immobilization of biomolecules have also been reported (Kaushik et al., 2008). Carbon paste electrodes are widely used in the construction of biosensors due to their low background current, wide range of working potentials and easily renewable surfaces (Erden et al., 2011; Erden et al., 2013).

The aim of this work was to develop an amperometric biosensor for lactic acid measurement that possesses properties such as low cost, good stability, easy modification technique, rapid response time and good anti-interference ability. Preparation of a carbon paste enzyme electrode based on magnetite nanoparticles and Prussian blue to our best knowledge has not been reported. The parameters that influence the electrode performance and the analytical characteristics of the enzyme

electrode for lactic acid determination were also investigated.

2. Material and Methods

2.1. Chemicals

LOx (*Pediococcus* sp), Fe_3O_4 nanoparticles, Prussian blue, uric acid, ascorbic acid and glutaraldehyde were purchased from Sigma (St. Louis, MO, USA). Sodium monohydrogenphosphate and sodium dihydrogenphosphate were supplied from Riedel-de Haën (Seelze, Germany). Lactic acid, glucose, bovine serum albumin (BSA), graphite powder, paraffin oil and glucose were from Fluka (Buchs, Switzerland). All other chemicals were obtained from Merck (Darmstadt, Germany).

2.2. Equipment and method

An IVIUM electrochemical analyzer (Ivium Technologies, Netherlands) and a three-electrode cell stand (Bioanalytical Systems, Inc., USA) were used for electrochemical measurements. The working electrode was a modified carbon paste electrode. The counter and the reference electrodes were a Pt wire (BAS MW 1034) and Ag/AgCl (BAS MF 2052) electrode, respectively (Bioanalytical Systems, Inc., USA). The pH values of the buffer solutions were measured with ORION Model 720A pH/ion meter and ORION combined pH electrode (Thermo Scientific, USA). All solutions were prepared with water from Elga, Ultra-Pure Water System.

All amperometric experiments were carried out in 0.05 M pH 5.5 phosphate buffer solution (PBS). For the amperometric measurements, the biosensor was immersed in the stirred buffer solution, applying the proper potential. When the stable baseline was obtained, the proper volume of lactic acid was added, followed by recording the steady-state current. CV experiments were performed at a scan rate of 50 mV s^{-1} over the relevant potential range (−0.2 to +0.6 V) using 5.0 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.10 M KCl. EIS measurements were performed at the frequency range of 10^5–0.05 Hz with 10 mV amplitude under open circuit potential (E_{OCP}) conditions in 5.0 mM $[Fe(CN)_6]^{3-/4-}$ solution containing 0.10 M KCl. All the measurements were carried out at room temperature. Solutions were deoxygenated with high-purity nitrogen for at least 5 min prior to CV measurements, and the gas flow was kept over the solution during the measurements.

2.3. Preparation of the biosensor

Carbon paste was prepared in the following proportions for bare electrode

(CPE): 77.1% (30 mg) graphite powder and 22.9% (8.9 mg) paraffin oil. Fe_3O_4 nanoparticles modified carbon paste electrode (Fe_3O_4/CPE) was composed of 59.1% (23 mg) graphite powder and 17.9% (7 mg) Fe_3O_4 nanoparticles 22.9% (8.9 mg) paraffin oil. Fe_3O_4 nanoparticles and Prussian blue modified carbon paste electrode Fe_3O_4/PB/CPE) was composed of 51.4% (20 mg) graphite powder, 17.9% (7 mg) Fe_3O_4 nanoparticles, 7.7% (3 mg) PB and 22.9% (8.9 mg) paraffin oil. The modified electrodes were prepared by hand-mixing graphite powder with nanoparticles and PB then adding paraffin oil and thoroughly mixing for approximately 30 minutes to form homogeneous carbon paste.

The enzyme was immobilized into modified graphite powder employing the cross-linking method using bovine serum albumin and glutaraldehyde. For this purpose, graphite powder, Fe_3O_4 nanoparticles and PB were mixed together and enzyme solution (30 µL LOx (200 Unit/mL), 1.5 mg BSA and 10 µL 1.25% GA) was added to construct LOx/ Fe_3O_4/PB/CPE. Paraffin oil was added after the evaporation of water and mixed for approximately 30 minutes until a uniform paste was obtained. In all cases, the paste was placed into the bottom of the working electrode body and the electrode surface was polished with a weight paper to have a smooth surface. The electrodes were only washed with distillated water and working buffer between measurements. Electrodes were stored in refrigerator at +4 $^{\circ}$C in when not in use.

3. Results and Discussion

3.1. Electrochemical characterization of the electrodes

CV was used to study the function of the magnetite nanoparticles included in the carbon paste matrix in the electrochemical characteristics of the resultant electrode. The electron transfer properties of bare and modified carbon paste electrodes (CPE, Fe_3O_4/CPE) were investigated in 0.1 M KCl solution containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ at 50 mV s^{-1}. The cyclic voltammograms are shown in Figure 1. At the bare CPE (curve a) well-defined oxidation and reduction peaks of $[Fe(CN)_6]^{3-/4-}$ were observed. After the electrode was modified with Fe_3O_4 it was observed that Fe_3O_4 nanoparticles increased current intensity and decreased peak-to-peak separation for $[Fe(CN)_6]^{3-/4-}$ waves (curve b). This enhanced electrochemical behavior indicates that Fe_3O_4/CPE is able to drive the electron transfer reaction faster than bare CPE. The obtained results are in good agreement with the results reported in the literature, indicating the fast electron transfer behavior of magnetite nanoparticle modified electrodes (Erden et al., 2013; Yang et al.,

2009).

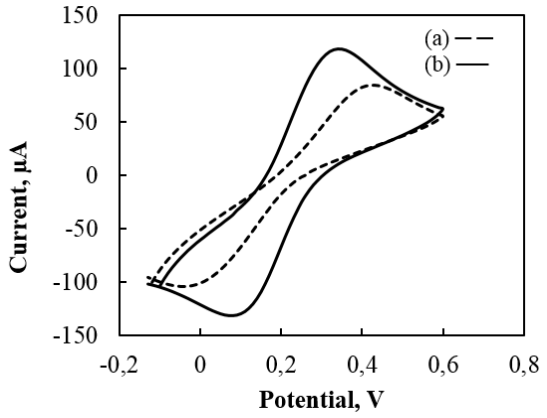

Figure 1. Cyclic voltammograms of (a) CPE, (b) Fe_3O_4/CPE at 50 mV s^{-1} in 0.1 M KCl solution containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$

Electrochemical impedance spectroscopy was used for following the effect of carbon paste composition on the electron transfer resistance. Figure 2 displays the Nyquist plots for bare CPE, Fe_3O_4/CPE and Fe_3O_4/PB/CPE. The Nyquist curves include a depressed semicircle portion and a linear portion. The semicircle portion at high frequencies corresponds to the electron transfer limited process, and the linear portion at low frequencies corresponds to the diffusion process. The diameter of the semicircle is equal to the electron transfer resistance (R_{ct}) value (Kaçar et al., 2014). The diameter of the semicircle for Fe_3O_4/CPE (curve b) is smaller than the one for bare CPE (curve a). This result indicates that the Fe_3O_4 nanoparticles increase electron transfer at solution/electrode interface. The smallest semicircle was observed with Fe_3O_4/PB/CPE (curve c) suggesting that Fe_3O_4 and PB have a synergetic effect and this composite can make the electron transfer easier.

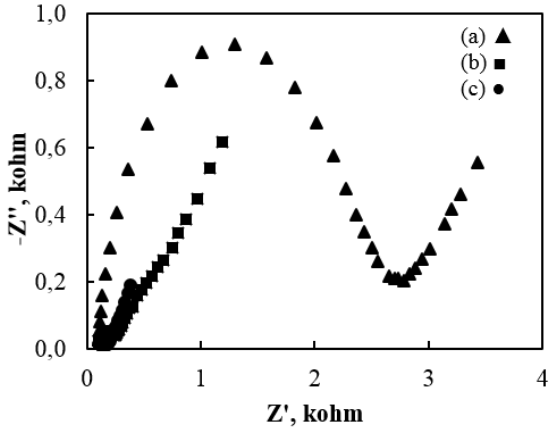

Figure 2. The Nyquist curves of (a) CPE, (b) Fe_3O_4/CPE and (c) Fe_3O_4/PB/CPE in 0.1 M KCl solution containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$

3.2. Optimum working conditions and electrode composition of LOx/Fe_3O_4/PB/CPE

It is important to evaluate the amount of LOx incorporated in the carbon paste matrix. The response of the LOx/Fe_3O_4/PB/CPE to lactic acid was measured at four different enzyme amounts as 2 U; 4 U; 6 U and 8 U by keeping the other parameters constant. Maximum sensitivity was observed at the loading of 6 U (Figure 3a). Higher enzyme loadings led to the current decrease, which is likely due to the blocking of the electrode surface by the large amount of immobilized protein. On the other hand, the linearities and the working ranges of the calibration graphs recorded for electrodes containing 2 U, 4 U and 8 U enzymes were not satisfactory. 6 U of enzyme loading was consequently used for

biosensor construction in further experiments. The effect of nanoparticle amount on the electrode response was investigated by means of different lactic acid calibrations in phosphate buffer at pH 5.5. Fe_3O_4 amount was varied as 10.2%, 12.8%, 15.4% and 17.9% while the graphite, PB and paraffin oil amount kept constant. The highest sensitivity was obtained with the carbon paste electrodes containing 17.9% Fe_3O_4 (Figure 3b). The stability of the carbon paste matrix was not satisfactory at higher nanoparticle amounts. Therefore, 17.9% was selected as optimum nanoparticle amount for biosensor construction.

The effect of buffer pH is important to improve the enzyme activity, and consequently, the response to lactic acid. Thus, the dependence of enzymatic activity of the immobilized LOx on pH was investigated in order to find the optimal pH for the lactic acid biosensor. This study was carried out at 0.09 mM lactic acid concentration over the pH range from 5 to 8.5 (Figure 3c). The response current increases with increasing pH from 5 to 5.5 and then decreases as pH increases further. Results in Figure 3c showed that at pH 5.5, the higher current was obtained corresponding to the highest activity of the enzyme. Therefore, pH 5.5 was chosen as the optimum value to work with the presented biosensor. The selected pH is in good agreement with the data reported in the literature (Gorton et al., 2001). However, different pH values such as 6.8 (Wang et al., 2011), pH 7.0 (Zanini et al., 2011; Çelik et al., 2015) and pH 7.4 (Zhao et al., 2014) were also reported for lactic acid enzyme electrodes. The optimum pH for the free lactate oxidase from *Pediococcus* species was reported as 7.5 (Suman et al., 2005). The optimum pH of the presented biosensor is 2 pH unit less basic than the optimum pH of the free LOx. Such a shift in optimum pH may be attributed to that the microenvironment of the enzyme has been changed by the immobilization procedure, leading to a change of physicochemical characteristics of the enzyme (Lillis et al., 2000).

Once the optimal enzyme amount and the pH of buffer solution were established, the effect of applied working potential was studied in the range of (−0.20) V to (+0.20) V against Ag/AgCl reference electrode. The highest response was obtained at +0.10 V. Thus, this potential was selected for the further experiments. The low working potential of +0.10 V allows good sensitivity and simultaneously avoids from undesired interferences.

It is well known that Prussian blue (PB) shows peroxidase-like activity due to its close similarity with peroxidase, so it can be employed to catalyze the reduction of hydrogen peroxide. To assess the electrocatalytic activity of Fe_3O_4/PB

modified carbon paste electrode, the electrocatalytic reduction of H_2O_2 at Fe_3O_4/PB/CPE was investigated. With the addition of H_2O_2 into the electrochemical cell, the reduction peak current increased at about +0.10 V and the anodic peak current decreased dramatically, indicating a typical electrocatalytic reduction process of H_2O_2. Furthermore, the reduction peak current increased with the increasing H_2O_2 concentration. Moreover, the electrocatalytic reduction peak current at the Fe_3O_4/PB/CPE modified electrode was much higher than that at Fe_3O_4/CPE with the same concentration of H_2O_2. Therefore, it can be concluded that incorporation of PB acted as an artificial peroxidase, into carbon paste matrix, provided lactic acid determination via H_2O_2 reduction at very low potentials.

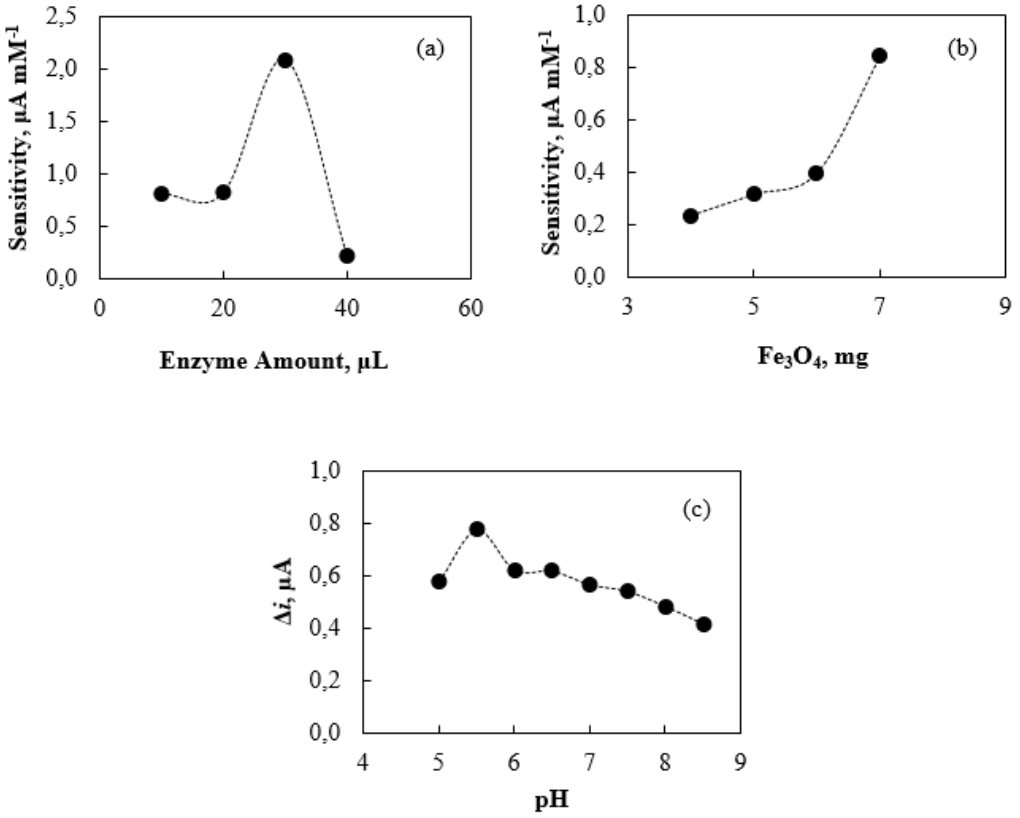

Figure 3. The effect of (a) enzyme amount (pH 5.5) (b) nanoparticle amount (pH 5.5) and (c) pH on the response of lactic acid biosensor (0.05 M PBS).

3.3. Performance parameters of LOx/Fe_3O_4/PB/CPE

The current response of the Fe_3O_4/PB/CPE to lactic acid was investigated under stirring in 0.05 M PBS (pH 5.5). Figure 4 shows the calibration curve corresponding to the lactic acid biosensor. The response current is linear to the concentration of lactic acid over the range from 7.5×10^{-6} M to 1.3×10^{-4} M with a correlation coefficient of 0.998 and detection limit of 5.9×10^{-7} M.

The repeatability of LOx/Fe_3O_4/PB/CPE was studied. Four calibration curves were plotted by the use of the same electrodes sequentially. The relative standard deviation of the sensitivities was found as 4.1%. Enzymes have a tendency to lose their activity when not stored in appropriate conditions. Thus, the immobilization method and storage conditions play an important role in the stability of lactic acid biosensors. Measurements for the study of storage stability were performed over a period of 7 days (Figure 5). Between measurements biosensor was stored at 4 °C under dry conditions. After one week the biosensor lost 5.4% of its initial activity. Consequently, it is convenient to work with presented biosensor within the first days after its construction. However, this is not a serious drawback since the biosensor preparation is fast and the repeatability of the biosensor is high for the same day.

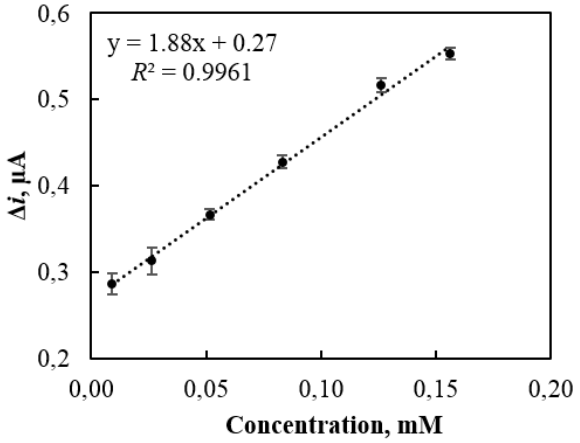

Figure 4. Effect of lactic acid concentration on the response of LOx/Fe_3O_4/PB/CPE (0.05 M, pH 5.5 PBS, +0.10 V).

The interferences from ascorbic acid (1×10^{-5} mol L^{-1}), uric acid (1×10^{-5} mol L^{-1}) and glucose (1×10^{-5} mol L^{-1}) in the detection of 1×10^{-5} mol L^{-1} lactic acid were studied. None of the analyzed substances presented a significant electrochemical response on lactic acid response of the biosensor (data not shown). These results indicate that detecting lactic acid at the low working potential of +0.10 V provides practically interference free biosensor response.

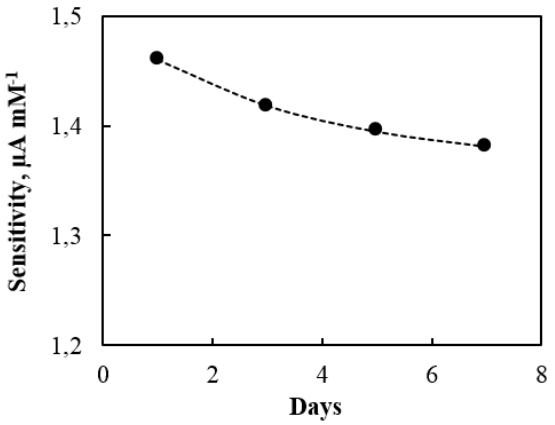

Figure 5. Effect of storage stability on the response of LOx/Fe_3O_4/PB/CPE (0.05 M, pH 5.5 PBS, +0.10 V).

Table 1. Characteristics of various carbonaceous material-based lactic acid biosensors

Working Electrode/Enzyme	Working Potential, V	Detection Limit, M	Linear range, M	Repeatability (RSD), %	pH	Ref.
BCC-MWCNT-CPE/LOx	-0.40 vs. Ag/AgCl	7.0×10^{-8}	2.0×10^{-7}–1.1×10^{-4}	2.5	7.0	Çelik et al., 2015
MWCNT (Nanocomposite Type A) AuPE-PGELS-CS/LOx MWCNT (Nanocomposite Type B) AuPE-PGELS-CS/LOx	-0.05 vs. Ag/AgCl	1.6×10^{-6} 1.7×10^{-6}	5.0×10^{-6}–3.4×10^{-4}	4.5 3.7	7.0	Monosík et al., 2012
PDDA-ZnO-MWCNT-PG/LOx	+0.40 vs. Ag/AgCl	6.0×10^{-6}	2.0×10^{-4}–2.0×10^{-3}	2.8	6.8	Wang et al., 2011
MWCNT-MB-PE/LDH	0.0 vs.SCE	7.5×10^{-6}	1.0×10^{-4}–1.0×10^{-2}	2.3	7.5	Pereira et al., 2007
PB-CPE/LOx	-0.05 vs. Ag/AgCl	1.0×10^{-6}	Up to 8.0×10^{-4}	-	5.5	Garjonyte et al., 2001
TBO-modified CPE/LDH	0.0 vs. Ag/AgCl	5.0×10^{-4}	1.5×10^{-3}–8.0×10^{-3}	-	7.2	Molina et al., 1999
pTTCA-MWNT-Au/LDH	+0.30 vs. Ag/AgCl	1.0×10^{-6}	5.0×10^{-6}–9.0×10^{-5}	4.3	6.8	Rahman et al., 2009
SPCE–HRP/LOx	0.0 vs. Ag/AgCl	1.0×10^{-6}	1.0×10^{-5}–2.0×10^{-4}	3.0	7.2	Ghamouss et al., 2006
TiO_2-PRG-GC/LOx	+0.25 vs. Ag/AgCl	6.0×10^{-7}	2.0×10^{-6}–4.0×10^{-4}	3.2	7.0	Casero et al., 2014
Fe_3O_4-PB-CPE/LOx	+0.10 vs. Ag/AgCl	5.9×10^{-7}	7.5×10^{-6}–1.3×10^{-4}	4.1	5.5	This work

AuPE: Au planar electrodes, PGELS: Planar glass-epoxy-laminate substrate, CS: Chitosan, MWCNTs: Multiwalled carbon nanotubes PDDA: polydiallyldimethyl-ammonium chloride, PG: pyrolytic graphite, MLGE: Multi layered graphene electrode, PRG: Photocatalytically reduced graphene, TiO_2: titanium dioxide nanoparticles, ZnO: zinc oxide nanoparticles, LOx: lactate oxidase, LDH: lactate dehydrogenase, TBO: Toluidine blue-O, CP:Carbon paste, MB: Meldola's Blue, PE: paste electrode, pTTCA: poly-5,20-50,20 0-terthiophene-30-carboxylic acid , SPCE: Screen printed carbon electrode, HRP: Horseradish peroxidase

Table 1 compares the characteristics of various carbonaceous material-based lactic acid biosensors reported in literature with the presented biosensor. According to data in this table, the presented lactic acid biosensor is better in some cases or comparable with the other sensors reported so far.

4. Conclusion

In this study, we have presented the use of magnetite nanoparticles and Prussian blue for the development of amperometric lactic acid biosensor for the first time. The LOx/Fe_3O_4/PB/CPE has demonstrated a good catalytic activity towards lactic acid determination. The magnetite nanoparticles increased electron transfer at solution/electrode interface resulting in an enhanced sensitivity of the biosensor. In addition, one of the main advantages of this work in comparison to others was the incorporation of PB into carbon paste matrix, which was acted as an artificial peroxidase, and provided a lactic acid determination via H_2O_2 reduction. The low operating potential of the biosensor (+0.10 V) minimized the risk of interference. Moreover, the cost of the presented biosensor is lower than that of the conventional techniques. The preparation procedure of the biosensor is very simple and it shows a good repeatability. It can be concluded that Fe_3O_4 and PB modified enzyme electrode is a good promise for

practical applications in real samples and the purposed strategy can be extended for the development of other enzyme based biosensors.

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