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ORIGINAL ARTICLE

ETHANOL DETECTION BIOSENSOR DESIGN BY HORSERIDASH PEROXIDASE ENZYME AND MODIFIED ELECTRODE WITH MGO NANOPARTICLES

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ABSTRACT: Alcoholic toxicity and the ethanol *in vivo* content in increased levels result in severe metabolic and behavioral perturbations. Thus, design and development of a biosensor for more precise and prompt detection of which attributed to available methods is of great importance. In the present study, developement of ethanol biosensor by *Horseridash peroxidase* and modified electrode with MgO nanoparticles were investigated. Electrochemical studies were carried out on basis of cyclic voltammogram method. A triple electrode system was used in electrochemical cell in a way that bare or modified carbon paste electrode with MgO nanoparticles was applied as working electrode; Ag/AgCl electrode was used as reference electrode and the platinum one as the counter electrode. Designed biosensor was able to detect ethanol within the range of 5 to 60 M. As the modified carbon paste electrode with MgO nanoparticles and HRP located at 4˚C, it maintained its initial activity by 92% in a 21-day period.

Keywords: Horseridash peroxidase, biosensor, MgO nanoparticles, ethanol

INTRODUCTION

Nanotechnology is the study of particles in atomic scale to achieve control on them. The principle objective of most nanotechnology researches is the advent of new combinations or perform modifications in constitutive particles [1-2]. Sensors are tools to measure variable physical and chemical quantities that transport data to signals as well as understandable electrical signals for computer after the measurement process. Nanosensors are specific group of sensors to transfer nanomaterials' signals to macroscopic world. Their function in medicine raises from their ability to specify different cells exact location and type by measuring the modification in variable quantities such as volume, density, displacement and velocity, temperature and magnetic, electric and gravity forces. Bio nanosensors which are the specific type of biosensors, are able to penetrate into the cell without any lesion regarding to their nano size, monitor transportation of definite molecules, demonstrate the effect of different medicines on body in molecular level, and evaluate blood glucose, lactose, sucrose, galactose, and cholesterol daily by contribution of enzyme probes with no blood sampling necessity [3-5]. Ethanol is produced from glucose metabolism by fermentation of some specific types of yeasts in absence of oxygen; ethanol concentration will be enhanced via distillation at the end of fermentation process, the absorbance of which is one of the observed liver malignancies in alcoholics [6]. MgO is used in variable industrial applications such as isolating and resistant materials at the presence of extra temperatures as well as an additive to petroleum. Further, MgO is used as a glass composite resistant to heat in liquid crystal display panels, electro-luminance display panels, plasma display panels, and display florescent tubes [7]. Horseridash enzyme is widely used in biochemical experiments; initially, it is potent to amplify a weak signal and to find and detect targeting molecule. HRP is ideal in many aspects of application because, it is smaller, more stable and cheaper than other putative alternatives such as alkaline phosphatase which develops potent signals in short periods. Recently, neurologists make advantage of that to mark neurons. The structure of this enzyme is shown in figure 1-2 [8]. The objective of this study is development of feasible, economic, and prompt solution to specify abnormal ethanol levels, chemical MgO nanoparticles synthesis, and application of this substance in design of interested biosensor, identifying ethanol levels in specified ranges by modified electrode with MgO nanoparticles and HRP enzyme with electrochemical methods and in accompany with other sciences inclusive of biology and nanotechnology.

MATERIALS AND METHODS

REAGENTS

HRP enzyme (5g) was provided from Sigma Corporation. Graphite and Paraffin to fabricate carbon paste electrode and also raw materials to produce phosphate buffer solution (PBS) 0.1 M including disodium mono-hydroxy phosphate (Na_2HPO_4) and mono-sodium dihydroxy phosphate (NaH_2PO_4), all of which were provided from Merec Corporation. Prepared buffer phosphate solution pH=7. Magnesium acetate ($Mg(CH_3COO)_2$) 0.14M, Polyvenil pyrroline ($(C_6H_9NO)n$), Tri-methyl ammonium hydroxide ($(CH_3)_4NOH$) and Ethanol (C_2H_6O) were used all of which provided from Merec Corporation. Double distilled and deionized water was used because whether double distilled water is not used, there were a possibility for water ions to impair direct transference of electrons and undermine the standards of research.

EQUIPMENT

Triple electrode system was used in applied electrochemical cell in a way that carbon paste electrode (4 mm diameter) was used in forms of bare or modified with MgO nanoparticles; Ag/AgCl electrode was used as the reference electrode and platinum electrode as the counter one. Notably, Ag/AgCl and platinum electrodes were provided from Auroumieh Azar Electrode Corporation. Electrochemical studies were carried out on basis of cyclic voltammogram method and by utilizing potentioacetate-galvanoacetate device in a way that one end of which was connected to the three noted electrodes and the other end to a PC for analyzing data and drawing oxidative and reductive charts. Electrochemical analysis was carried out by potentioacetate-galvanoacetate device (263A, EG&G, USA) (range 700-1500Mv). Morphological studies and observation of synthesized MgO nanoparticles' levels were accomplished by double beam visible/ultraviolet spectrophotometer TU-1901, Broker X-ray diffraction device with CuKα radiation, and transmission electron microscope (TEM) JEM-200CX.

PREPARATION OF MGO NANOPARTICLES

Initially, 50 ml of Magnesium acetate 0.14 M was sonicated with appropriate proportion of Polyvinyl pyrroline (PVP) as controlling factor of texture for 30 minutes, subsequently, sufficient extent of Tri-methyl ammonium hydroxide (TMAH) 0.34 M was slowly added to solution. The nanostructure of Magnesium hydroxide was developed from following reaction:

$$Mg\ (CH_3COO)_{2(aq)} + 2(CH_3)4N(OH)_{(aq)} \rightarrow Mg(OH)_{2(s)} + 2(CH_3COO)N(CH_3)_{4(aq)} \quad (1)$$

Finally, once Tri-methyl ammonium hydroxide was added to the compound, it was sonicated for 30 minutes. Magnesium hydroxide residual was filtered and rinsed by ethanol and deionized water for three times, then, the sonicated compound was filtered. The final residual was dehydrated in 550˚C for 4 hours. Following reaction demonstrates Mg dehydration stage:

$$Mg\ (OH)_{2(s)} \rightarrow MgO_{(s)} + H_2O_{(g)} \quad (2)$$

MgO nanoparticles were sonicated in ethanol for 30 minutes to vanish the bulk shape. At final stage, the compound was filtered and dehydrated in 110˚C. The final resulted product was the powder of MgO nanoparticles prepared to be used in next levels.

BARE CARBON PASTE ELECTRODE PREPARATION

Carbon powder (size 50 mm, density 20-30 g/100mL) was blended with binder and silicon oil within a paste made from agate by mortar. Carbon paste electrode was constructed from a proper Teflon tube filled with carbon paste. A copper wire was passed through the prepared paste located at far end of the Teflon tube so the carbon paste electrode could connect to the outer circuit electrically. Initially, the electrode was reconstructed mechanically by some provided paste in upper levels for electrochemical analyses and then glossed by transparent paper.

PREPARATION OF MODIFIED CARBON PASTE ELECTRODE WITH MGO NANOPARTICLES AND HRP ENZYME

Modified carbon paste electrode with MgO nanoparticles provided in previous stage was used to prepare modified carbon paste electrode with MgO nanoparticles and HRP enzyme. At this level, the HRP enzyme was immobilized by infusion of 4 µL out of 10 mg/mL protein solution on the surface of carbon paste electrode modified with MgO nanoparticles and dehydrated at room temperature for 30 minutes. Then, the prepared electrode was slowly rinsed with double

distilled water and kept in refrigerator at 4˚C in idle times.

DATA EXTRACTION METHOD

Cyclic voltammograms concluded from abovementioned experiments were saved by *Echem* and transferred to *Excel* subsequently. Then, their parameters (cathodic peak region, anodic peak region, cathodic current, anodic current) were extracted. Spectroscopic spectrums were also extracted from software and saved and used in *Excel*.

DISCUSSION AND RESULTS
MGO NANOPARTICLES ANALYSIS BY TEM

Transmission electron microscope (TEM) is a type of electron microscope which is able to photograph materials microstructure with magnification of 1000 to 1000000 times and segregation power smaller than 1 nm. TEM is also capable of elemental analysis, identifying structure and crystal orientation of particles as small as 30nm qualitatively and quantitatively. TEM resolution could be limited by spherical deviation, but new generation of deviation regulators could partially eliminate spherical deviation to promote the resolution. Spherical deviation promoter hardware for transmission electron microscope with high resolution (HRTEM) allows producing images with high resolution of 0.5 angstrom (50 pm) and magnification over 50 million times. Capability to determine atoms locus inside materials, has made HRTEM a significant device in research and development of nanotechnology. One of the most important applications of TEM is electron diffraction. An advantage of electron diffraction on X-ray in crystallization is that the model is not in need of a single or even multi-crystal powder, and that the instant transformation of magnified object structure occurs physically and as a result, there is no need to troubleshoot the phase problem faced by X-ray after obtaining crystal image of X-ray diffraction pattern of which in forms of single crystal or multiple ones.

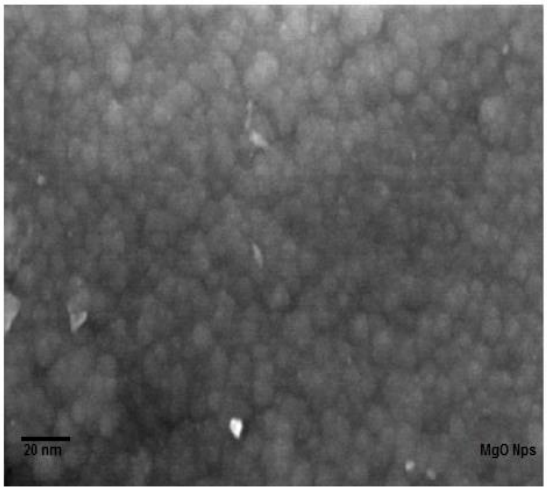


Figure 1. transmission electron image of synthesized MgO nanoparticles

Of most faults of TEM is that extremely thin layers of sample, typically about 100nm are required. Bio samples normally necessitate chemical immobilization, thus, they will be dehydrated and placed in a polymeric resin for immobilizing and allowing them to provide a sufficient thin section. Several bio samples, organic polymers and similar materials could essentially necessitate for staining with heavy atom tag to achieve the required image contrast. Generally, TEM microscopes have many buttons; however, by perception of optical fundamentals, even operating most complicated microscopes will be facile. Today, it is tend to use integrated computer to control the microscope. But, even taking advantage of computer has no effect on microscope optic or number of parameters have to be controlled. According to the concluded results from TEM analyses, synthesized MgO nanoparticles diameter in this study is within the range of 70 nm as shown in figure 1 [9].

X-RAY DIFFRACTION FOR MGO NANOPARTICLES

X-ray pattern of any combination is individual. In diffraction evaluation experiment, the main objective is determination of angles

related to any peak and identification of atomic panels distance (d value). Values of d consist of three decimals at least. Whereas, the peak intensity in XRD pattern is consistent with phase values available in sample, it is possible to accomplish quantitative analysis by this method. In other word, it is not only possible to identify phase types, but also it could be possible to determine their values. However, due to redundant problems and its proximity, XRD method is less used.

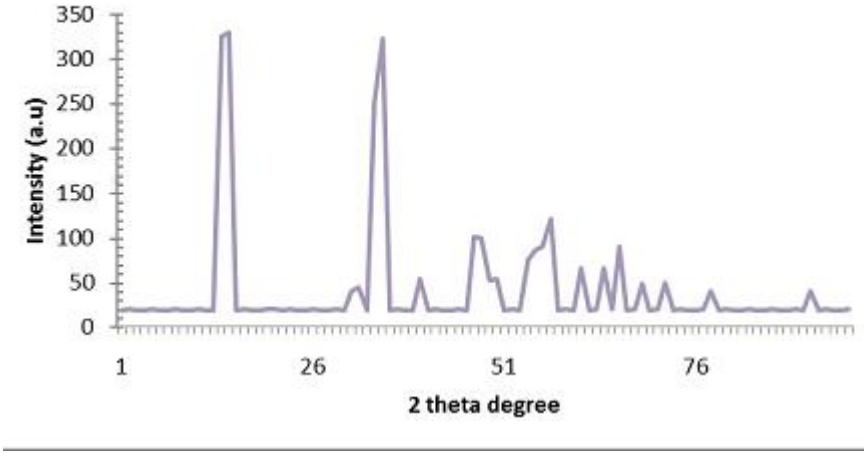


Figure 2. XRD pattern model for synthetic MgO nanoparticles

It is clear that peaks intensity indicates diffracted ray intensity, which is phase-depended in addition to other methods. Initial ray intensity is determined by X-ray developing tube ampere and mediated voltage; thus, they should be considered. On the other hand, particles size, type of available phases in field, evolving sensitivity, and also sample spinning velocity, all of which have effect on peak height. Therefore, these effects should be improved with specific samples manufacture or application of standard materials. Maximum peaks are used in estimation of sample particles by implementing the equation $D=K\lambda/(\beta\cos\theta)$ (Scherrer), K is consonant equal to (0.9). λ is wavelength and β is full width at the half-maximum of line and θ is diffraction angel. Particle size is estimated for MgO nanoparticles as 70 nm and increment in XRD peaks sharpness shows that the nature of particles is crystal-shape. Particle size is estimated from peak intensity relation. MgO nanoparticles are 70 nm in size and increment in XRD peaks sharpness demonstrates that nature of particles is crystal-shaped [10].

UV-VIS ABSORBANCE SPECTRUMS OF MGO NANOPARTICLES

Ultraviolet and visible (UV-vis) absorbance spectrometry is the weakness measurement of a beam either radiating outward the sample or after reflecting from a sample surface. This trial includes a variable of absorptive, transitive, or reflective measurement within ultraviolet (UV) spectrum ranges, visible and infrared near (NIR). These measurements could be carried out in a wavelength or wide range of them. UV-vis spectrophotometry is called in other forms: UV-vis spectrometry, UV-vis spectrophotometry, UV-vis reflective spectrometry. Electron induction in molecules generally takes place in UV and NIR ranges.

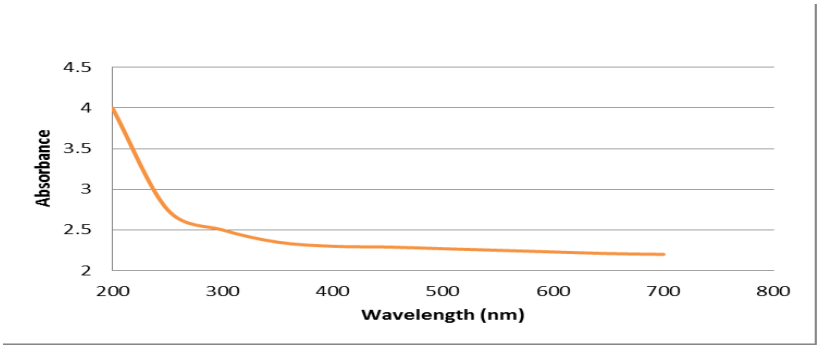


Figure 3. UV-vis spectrum of MgO nanoparticles

Restricted band of a semi-conduct depends on its substance and physical dimensions and is extended from UV to NIR range. A decrease in semi-conduct particle size or its dimensions (less than 10 nm) shifts the band edge to increased shortwaves (with more energy) resulted from quantum confinements. In polymeric and organic materials, UV-vis spectrum contributes in stained groups' detection in electron material. Similarly, absorbance measurements could be associated with bulk properties. For nonorganic complexes, UV-vis spectrum can provide data on oxidative, electron structure, and metal-ligand interactions statuses. For solid materials, UV-vis could measure electron restricted band and specify any local induction or impurities. UV-vis absorbance spectrums of MgO nanoparticles have been shown in figure 3. Although spectrometer wavelength is limited by light source, nanoparticles absorbance band shows a dislocation of blue color which is stemming from available value limitation in sample in contrast with MgO nanoparticles bulk. This optical phenomenon indicates that these nanoparticles illustrate the quantum effect. Acetic acid and ethanol solvent contribute in homogeneous dispersion and slow growth of particles in limited size and prevent the accumulation of particles. Figure 3-3 shows UV-vis spectrum of synthesized nanoparticles chemically. Absorbance peak is within the range of 300-700 nm. These information confirmed quantum effect and specific properties of synthesized MgO nanoparticles [11].

ELECTRON DIRECT TRANSMISSION FROM HRP ENZYME ON SURFACE OF MODIFIED CARBON PASTE ELECTRODE WITH MGO NANOPARTICLES AND VICE VERSA

Cyclic voltammogram studies in this section were carried out to explore and investigate oxidative and reductive peaks in modified carbon paste electrode with MgO nanoparticles and HRP enzyme.

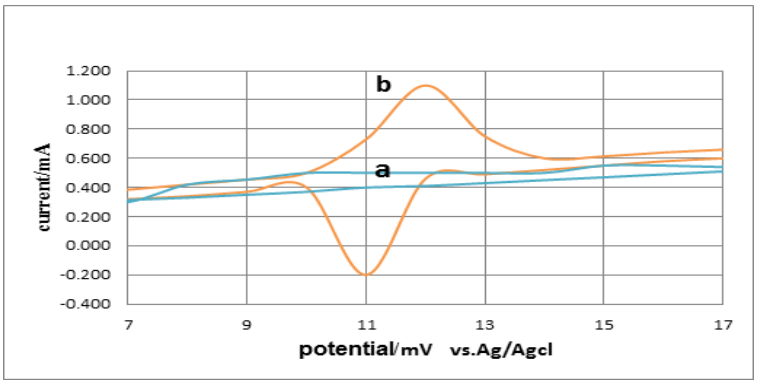


Figure 4. cyclic voltammograms, (a) bare carbon paste electrode and (b) modified carbon paste electrode with MgO nanoparticles and HRP enzyme; scan rate 50 mV/s and in phosphate buffer solution 0.1 M and pH=7.

Moreover, the other important goal in this section has been the determination of difference between bare electrode and the modified one with MgO nanoparticles and HRP enzyme. As it

is shown in diagram b of figure 3-4, a pair of explicit and stable redox peaks is visible for electron direct transfer from HRP enzyme on cyclic voltammogram of modified carbon paste electrode with MgO nanoparticles and HRP enzyme in PBS 0.1M (pH=7). Oxidative and revival peaks were 50 mV/s in 1300 and 1200 mV, respectively. No peak was observed in modified carbon paste electrode with HRP enzyme. Calculated formal redox potential for HRP enzyme is 1250 mV. Formal redox potential is mean : $\frac{1300+1200}{2}$ It was obvious that non-modified electrode showed no cathodic and anodic peak (figure 3-4 diagrams a) which implies that MgO nanoparticles act as facilitator of electron transference from redox to electrode surface and vice versa. These results are consistent with previous studies which demonstrated the role of nanoparticles in electron transference facilitation. In figure 4 (b), it is shown that MgO nanoparticles in nano scale can play a key role in observation of cyclic voltammogram response of HRP enzyme. In an environment that relation of surface to volume increases by size reduction, and raising from the fact that HRP enzyme size is comparable with manufactured nano blocks, this makes nanoparticles a significant mediator in electron transference between HRP enzyme and carbon paste electrode [12].

ELECTROCHEMISTRY OF HRP ENZYME ADSORPTION ON MODIFIED CARBON PASTE ELECTRODE WITH MGO NANOPARTICLES.

In next study, the electron transference properties of HRP enzyme on modified carbon paste electrode with MgO nanoparticles were analyzed and the effect of scan rates on cyclic voltammograms of HRP enzyme was studied. In figure 5 (a & b) a linear association between anodic and cathodic currents of HRP enzyme was observed and it was clarified that redox peaks currents increase with scan rate linearly. Correlation ratio will be 0.9925 for cathodic peak and 0.9949 for anodic peak. This phenomenon points to the fact that redox procedure is controlled by relevant peaks on electrode surface and represents stable immobilization of HRP enzyme on carbon paste electrode surface [13].

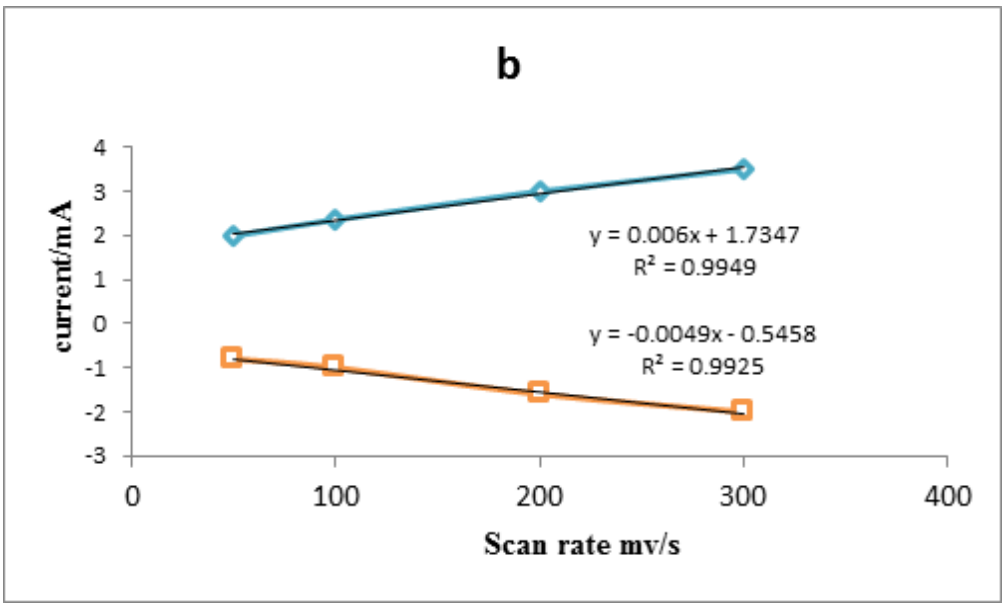


Figure 5. a) Cyclic voltammograms of modified carbon paste electrode with MgO nanoparticles and HRP enzyme, b) slope and equation of line and diagram drawn on basis of scan rate. Scan rates (from inside to outside, respectively) 50, 100, 200, 300 mV/s in PBS 0.1 M (pH=7).

Synthetic of electron direct transference was described in scan rates less than 100 mV/s by benefiting from Laviron model. Diagram of cathodic peak potential in the presence of scan rate logarithm gives transference ratio of electric bar α as 0.53.

$$E_{p,catodic} = E^0 + \frac{RT}{\alpha F} \ln \frac{RTks}{\alpha Fv} \quad (3)$$

$$E_{p,anodic} = E^0 + \frac{RT}{(1-\alpha)F} \ln \frac{RTks}{(1-\alpha)Fv} \quad (4)$$

$$\Delta Ep = Ep,_{anodic} - Ep,_{catodic} = \frac{RT}{\alpha(1-\alpha)F} \quad (5)$$

$$[\log ks = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log\frac{RT}{nFv} - \frac{\alpha(1-\alpha)nF\Delta EP}{2.3\ RT}]$$

Where, α is electron transfer coefficient and n is the number of electrons transferred. *R*, *T*, and *F* are gas consonant, absolute temperature and Faraday consonant, respectively, which the values of these parameters have been defined.

(R = 8.314 J mol^{-1} K^{-1}, F = 96493 C/mol, T = 298 K). Transfer rate constant can be achieved from ΔEp equate ln *v.* In this research, calculated value for electron transfer rate constant (ks) was considered as 1.65 s^{-1}.

ETHANOL DETECTION BIOSENSOR DESIGN BY HRP ENZYME AND MODIFIED ELECTRODE WITH MGO NANOPARTICLES

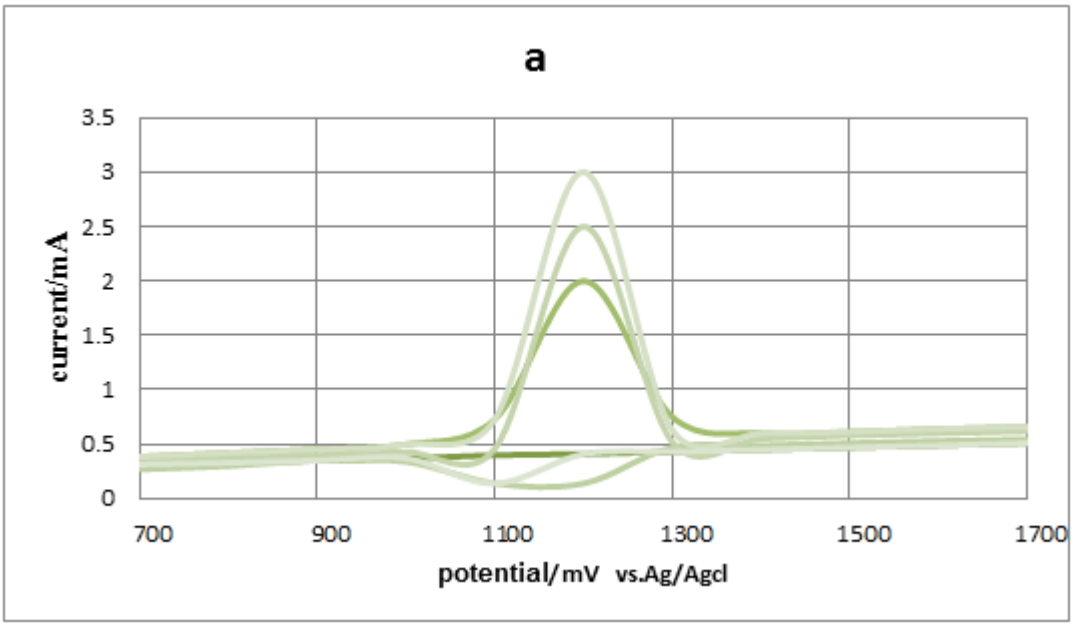
Ethanol detection importance in small ranges such as micro-molar in biologic, medical, and industrial activities is explicit. In alcohol (ethanol) detection, methods including spectrometry, chemiluminescence, and electrochemistry are used. More appropriate and functional method is taking advantage of electrochemical processes; because, they have higher sensitivity and selectivity attributed to other methods. In this research, variable concentrations (5 to 60 mM)

Ethanol were added to PBS 0.1 M (pH=7) at the presence of modified carbon paste electrode with MgO nanoparticles and HRP enzyme and studied from the aspect of cyclic voltammometry (versus Ag/AgCl electrode). The results showed that by increase in ethanol concentration, anodic peak is increased and cathodic peak is decreased. These results have been shown in figure 6 (a). In this section of study, it was found that sensitivity and response to ethanol occurs only when all three factors of carbon paste electrode, MgO nanoparticles and HRP enzyme are used together. It can be concluded that HRP enzyme is immobilized on MgO nanoparticles and indicates proper catalytic activity related to ethanol. Revival peak reduction was accompanied with oxidative peak elevation associated with HRP enzyme and MgO nanoparticles. Electrocatalytic process can be expressed upon following mechanisms:

HRP -Fe (III) + e^- + H^+ ↔ HRP -Fe (II) H^+ on electrode surface (7)

C_2H_5OH + HRP -Fe (II) H^+ → HRP -Fe (III) + H^+ + C_2H_5OH in solution (8)

Availability of such process is a potent evidence for presence of HRP enzyme and its proper immobilization on carbon paste electrode surface which electron transference process has been facilitated by MgO nanoparticles in this procedure. Designed biosensor showed ability to specify ethanol within the range of 5 to 60 M. in figure 3-6 (b) calibration diagrams for designed biosensor to specify ethanol by utilization of HRP enzyme and modified carbon paste electrode with MgO nanoparticles has been shown. Correlation ratio in this section of study was calculated as 0.9634 in linear range. In highest applied concentration of alcohol, elevation of cathodic peak is stopped and remains steady in 60 mM and this maximum value is the designed biosensor response in presence of ethanol. Aforesaid details have been shown in figure 3-6 (b).



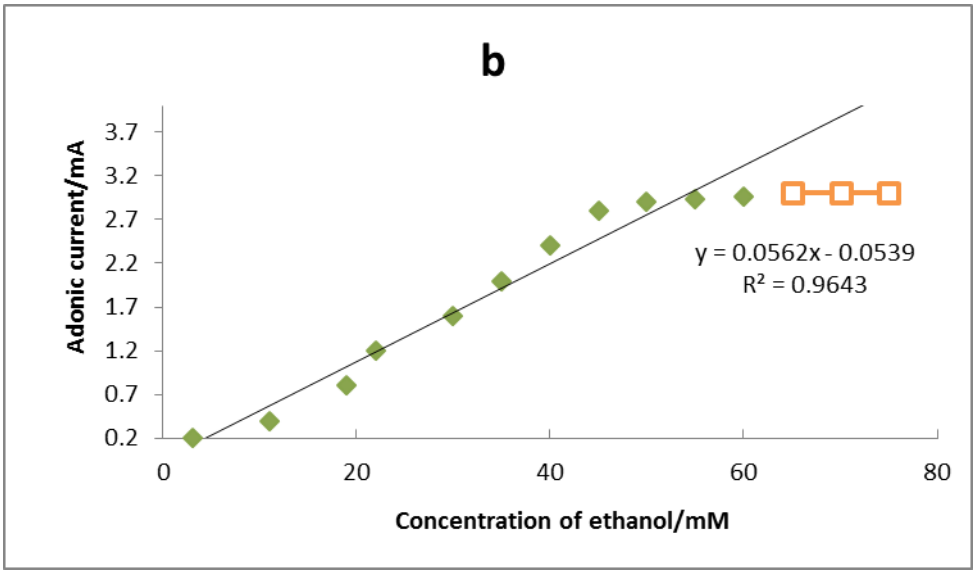


Figure 6. (a) cyclic voltammograms of modified carbon paste electrode with MgO nanoparticles and HRP enzyme in variable ethanol concentrations and, (b) relation between cathodic peak current of HRP enzyme and different concentrations of ethanol; scan rate is equal to 50 mV/s in PBS 0.1 and pH=7.

PH EFFECT ON ETHANOL DETECTION BIOSENSOR

In next study, pH effect on ethanol biosensor was studied on basis of modified carbon paste electrode with MgO nanoparticles and HRP enzyme; this study was implemented within the range of 4 to 12 pH and it was found that the activity of biosensor will be decreased by emerging of acidic properties and the maximum activity of this biosensor was reported in pH=7. Details have been shown in figure 7 [14].

As regards acidic process and alkaline state is ion exchange. PH changes in the electron transfer process directly affect. According to the HRP enzyme PH effect on the enzymes that have already been identified the maximum activity of the biosensor had been diagnosed with PH = 7.

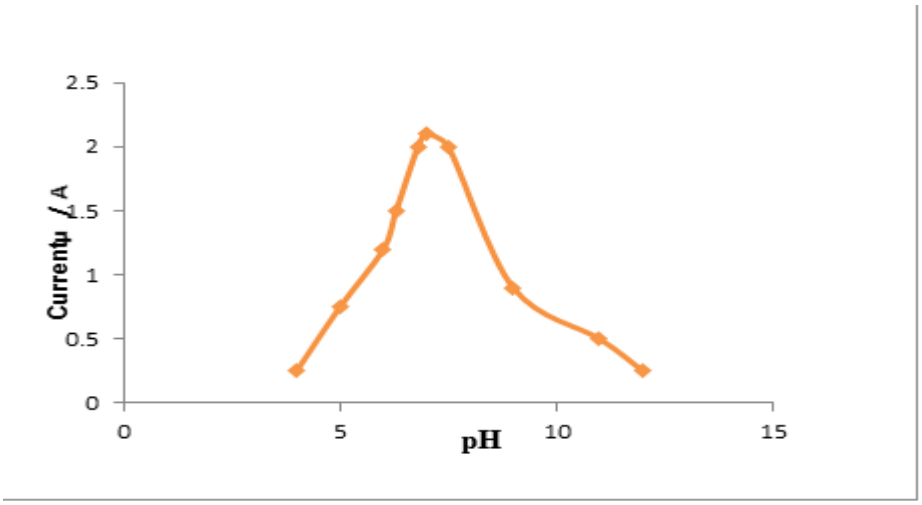


Figure 7. pH effect on ethanol detection biosensor on basis of modified carbon paste electrode with MgO nanoparticles and HRP enzyme; in PBS 0.1 M.

TEMPERATURE EFFECT ON ETHANOL DETECTION BIOSENSOR

Temperature is an effective and significant factor in electrocatalytic activity of proteins and enzymes. Figure 8 demonstrates temperature effect on designed biosensor and its response. An increase in temperature from 10˚C to 55˚C led to increase in biosensor activity and the maximum thermal effect and activity increment was reported in 32˚C. This implicates successful immobilization of HRP enzyme on carbon paste electrode and thermal stability of this enzyme structure [15].

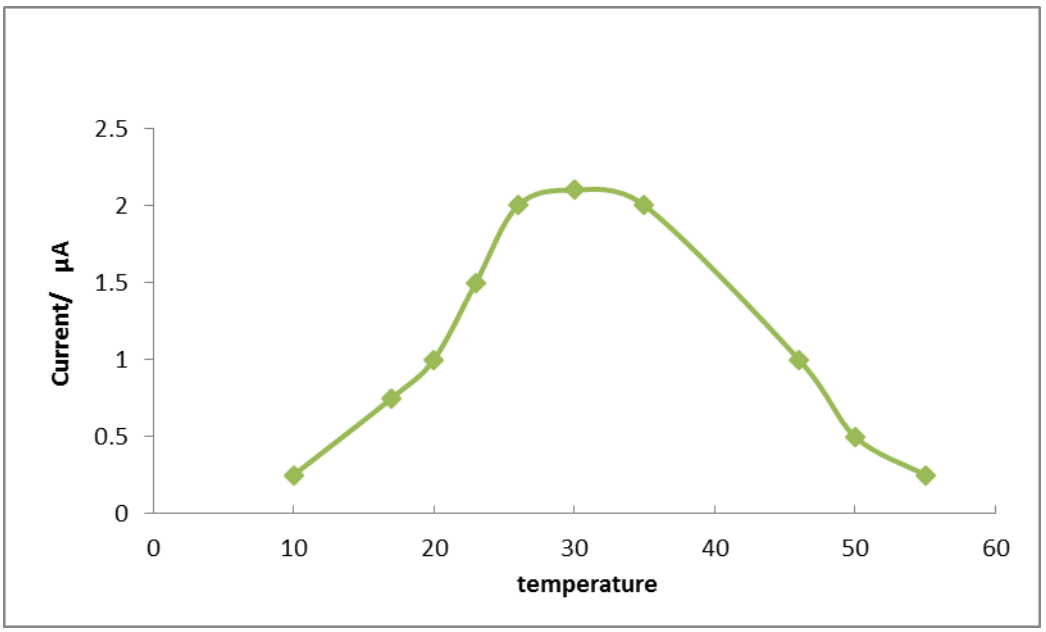


Figure 8. Temperature effect on ethanol detection biosensor on basis of modified carbon paste electrode with MgO nanoparticles and HRP enzyme; in PBS 0.1 M and pH=7.

STABILITY OF ETHANOL DETECTION BIOSENSOR BY HRP ENZYME AND MODIFIED ELECTRODE WITH MGO NANOPARTICLES

As modified carbon paste electrode with MgO nanoparticles and HRP enzyme was situated in 4˚C temperature, it maintained its initial activity within a 21-day period. This biosensor is reproducible by infusion of HRP enzyme drop by drop on its surface as the electrode tip was rubbed slowly on a clean piece of paper and then on a glass plain surface to obtain a homogenous and flat surface. Therefore, this method was prompt, facile, significant and repeatable in ethanol detection. Interference effects were analyzed by testing 0.5 mM ethanol responses in the presence of uric acid or ascorbic acid with gradual increase in concentration. Uric acid 0.25 mM or ascorbic acid 0.3 mM resulted in increments of 12% and 21% in revival current respectively, thus, the design of this biosensor was successful and the interference factors barely caused any interference in this biosensor response [11].

REFERENCES

Tomsia AP, Lee JS, Wegst UG, Saiz E. Int J Oral Maxillofac Implants. 28. 2013: 535.

Long NV, Thi CM, Yong Y, Cao Y, Nogami M. Recent Pat Nanotechnol.13 2013 4799.

Gao PX, Ding Y, Mai W, Hughes WL, Lao CS, Wang ZL. Science, 309 (2005)1700.

Hill HAO. Coordination Chemistry Reviews. 151, 1996, 115.

Song S, Clark RA, Bowden EF, Tarlov MJ.. Journal of Physical Chemistry. 97(1993) 6564.

Riley EP. Psychopharmacol (Berl).62,1979 47.

Aghebati-maleki L, Salehi B, Behfar R, Saeidmanesh H, Ahmadian F, Sarebanhassanabadi M, Negahdary M. Int. J. Electrochem. Sci. 8,2013: 257.

PDB WY, Carlsson GH, Nicholls P, Svistunenko D, Berglund GI, Hajdu J. Biochemistry. 44,2005: 635.

Karlík M. Materials Structure, 8,2001:3.

Li W, Hsiao GS, Harris D, Nyffenegger RM, Virtanen JA, Penner RM, J. Phys. Chem., 100,1996: 20103.

Negahdary M, Rezaei-Zarchi S, ISRN Biophysics. 2012: 1.

Xu X, Liu S, Ju H. Sensors, 3,2003: 350.

Negahdary M, Asadi A, Mehrtashfar Sh, Imandar M, Akbari-dastjerdi H, Salahi F, Jamaleddini A, Ajdary M. Int. J. Electrochem. Sci., 7,2012: 5185.

M Mohammadian, Farzampanah L, Behtash-oskouie A, Majdi S, Mohseni Gh, Imandar M, Shirzad M, Soleimani R, Negahdary M. Int. J. Electrochem. Sci. 8,2013: 11215.

Negahdary M, Rezaei-Zarchi S, Rousta N, Samei Pour S. International Scholarly Research Network ISRN Biophysics. 4,2012: 231.

Salimi A, Sharifi E, Noorbakhsh A, Soltanian S, Biophys. Chem. 125,2007: 540.