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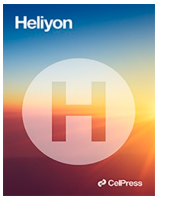

Research article

Electrochemical study of the effect of radiofrequency on glutamate oxidase activity using a glutamate oxidase-based biosensor

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ABSTRACT

A biosensor based on glutamate oxidase (GluOx) was developed to measure glutamate concentration. The main function of this type of biosensor is related to the structure and catalytic activity of GluOx. Since radiofrequency, as the widest spectrum of electromagnetic fields, can affect the catalytic activity and structure of GluOx, in this study, the effect of these fields on the analytical parameters of the fabricated biosensor was investigated. To build the biosensor a sol-gel solution of chitosan and native GluOx were prepared and then immobilized on the surface of the platinum electrode. Similarly, to investigate the effect of radiofrequency fields on the analytical parameters of the biosensor, instead of the native GluOx, irradiated GluOx was used to build the biosensor. To evaluate the biosensor responses, cyclic voltammetry experiments were performed and voltammograms were considered as biosensor responses. To determine the analytical parameters including detection limit, linear range, and saturation region of the responses, calibration curves were drawn for each of the biosensors. Also the long-term stability and selectivity of the fabricated biosensor were evaluated. Thereafter, the optimum pH and temperature for each of these two biosensors were examined. The results showed that radiofrequency waves harmed the detection and response of biosensors in the saturation region, while they had little effect on the linear region. Such results could be due to the effect of radiofrequency waves on the structure and function of glutamate oxidase. In general, the results indicate that when a glutamate oxidase-based biosensor is used to measure glutamate in radiofrequency fields, corrective coefficients for this type of biosensor should be considered to accurately measure glutamate concentration.

1. Introduction

Glutamate is a non-essential amino acid and the primary excitatory neurotransmitter found abundantly in the central nervous system [1]. It helps not only normal brain functions such as cognition, learning, and memory but also plays a prominent role in biological metabolisms and the immune system [2]. Additionally, glutamate is essential to regulate many body functions and its high concentration can be toxic and causes serious health problems, especially neurodegenerative diseases. Due to the important role that glutamate plays in body functions, the exact and precise measurement of glutamate concentration is very important in medical treatment and medical diagnosis [3,4].

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In the last decades, various analytical methods such as Uv-Vis spectroscopy, potentiometric titration, and chromatography have been widely used for glutamate measurement [5–8]. Although conventional methods of glutamate measurement have some advantages, such methods are expensive, time-consuming, and have complicated sample preparation. Such difficulties led to the focus on electrochemical methods for glutamate measuring both in vitro and in vivo. On the other hand, simplicity, online and continuous analysis, fast response, less complex instrumentation, low cost, high sensitivity, and high selectivity, different types of glutamate oxidase (GluOx-based) electrochemical biosensors have been extensively exploited to determine glutamate concentration. The results of the conducted research confirm the appropriate replacement of enzyme-based biosensors with other measurement methods, which is consistent with the results of our studies [9–14].

GluOx as one of the redox enzymes converts glutamate to NH_3 and α-ketoglutarate in the presence of O_2 and H_2O_2 [14–16]. It should note that GluOx plays an important role in the function of GluOx-based biosensors, its catalytic activity is affected by environmental conditions. So far, the effect of temperature, pH, buffer, and electrode type on the performance of biosensors has been studied and reported [15]. But the effect of electromagnetic waves on the GluOx, which in turn has a significant effect on the performance of GluOx-based biosensors, has not been studied. Due to the widespread use of these waves in various fields, they can affect biological systems, including the structure and function of enzymes [17–24].

One hand, some studies have shown that electromagnetic wave exposure influences fine conformational changes of proteins through the direct interaction with the surface charges. These waves strongly affect the polarity of water and due to the important role of water in the catalytic activity of enzymes, electromagnetic waves easily affect the function and structure of the proteins. Even with such a mechanism, electromagnetic waves can easily change the pathway of protein folding and consequently lead to protein misfolding. On the other hand, many studies have reported alterations in the release, metabolism, and transport of some neurotransmitters such as glutamate. Additionally, exposure the radiation can induce the expression of excitatory amino acids and imbalance of neurotransmitters parts of the brain [18,19,21–25].

Considering the very wide effects that have been mentioned so far, in this study to measure glutamate, a GluOx-based biosensor was made and the effect of radiofrequency waves, as one of the important environmental interfering factors on the structure and function of this enzyme was investigated electrochemically.

2. Materials and methods

2.1. Materials

GluOx (Glutamate oxidase) (*EC 1.4.3.11*, from *Streptomyces* sp) and Glutamate were purchased from Sigma-Aldrich (St. Louis, Missouri, USA). Potassium phosphate, KH_2PO_4, and dipotassium phosphate K_2HPO_4 were obtained from Merck Ltd. They used to prepare phosphate buffer solution as a supporting electrolyte in all electrochemical experiments. Chitosan (high molecular weight, MW~1000 kDa; ~80% deacetylation) was obtained from the Aldrich Company. All aqueous solutions were prepared in double-distilled water with a resistance of 18.0 MΩ cm^{-1}. Double-distilled deionized water was prepared using an ion-exchange system (Millipore, France). Cyclic voltammetry and chronoamperometry experiments were carried out using Galvanostat/Potentiostat apparatus (Autolab 302 N, Holland) by a conventional three-electrode system at controlled temperature conditions.

2.2. Construction of a GluOx -based biosensor

The GluOx-based biosensor was constructed by coating the platinum electrode (PE, 2 mm diameter) with an aqueous mixture containing chitosan (Chit) and GluOx with a certain ratio. To ensure the health of GluOx used in the construction of GluOx–based biosensor, its catalytic activity was evaluated using spectrophotometric measurement (Unico, UV-2100, USA). Various steps were taken to build a biosensor [14,26]. At first, the surface of the electrode was polished by alumina slurry with diameters of 10 μm and 0.3 μm, respectively followed by washing distilled water twice at least to achieve a mirror-like surface. Second, to remove the remaining alumina and other pollutants, PE was ultra-sonicated (Elmasonic, S30H, Germany) in water and ethanol for about 5 min. To ensure the absence of any contamination, cyclic voltammetry was performed at a 5 mM concentration of ferrocyanide in 100 mM buffer phosphate, pH 7.4 [27]. Third, 0.1% (W/V) chitosan was prepared in HCl 0.1 M solutions at pH 4.5. Fourth, a stock sol-gel solution was provided by mixing 20 μL of chitosan and 15 μL of GluOx in phosphate buffer 100 mM at pH 7.4. Fifth, 5 μL of this suspension was dropped on the PE surface and dried at room temperature. In the last step, the modified electrode was thoroughly immersed in phosphate buffer to remove residual unbounded matter from the electrode surface [14]. In the continuation of the article, when a native enzyme is used in the structure of the biosensor, it is named PE/Chit/GluOx, and when the irradiated enzyme is used, it is named PE/Chit/$GluOx_{irr}$.

2.3. The effect of electromagnetic waves

Electrochemical methods were used to investigate the effect of electromagnetic waves on the structure and function of GluOx. For this purpose, first, the sol-gel composition made of chitosan and native GluOx was deposited on the surface of the PE and the biosensor responses were recorded. To investigate the effect of electromagnetic waves, the GluOx packets (that is previously prepared in 100 mM phosphate buffer with a pH 7.4) were then irradiated to different frequencies of electromagnetic waves inside the GTEM 1000 (Gigahertz Transverse Electromagnetic) waveguide. The frequencies used in the RF (radiofrequency) range were 915, 1800, 2450, and 4000 MHz for 1 h. It is noteworthy to mention that many devices such as mobile phones, microwave home ovens and wireless systems

that are used in everyday life are exploited in such a frequency range. In the next step, to make a GluOx-based biosensor, a sol-gel composition made of chitosan and irradiated GluOx was deposited at the surface of PE. The differences in biosensor responses in these two different conditions (PE/Chit/GluOx and PE/Chit/GluOx$_{irr}$) were considered as the effect of electromagnetic waves on the structure and function of GluOx. All the steps of the PE/Chit/GluOx making and irradiation process are clearly shown in Fig. 1 as a graphical abstract.

2.4. Enzyme activity assay

The activity of GluOx was assayed using a standard method that is described in the literatures [28,29]. Briefly, the assay was based on the quantification of H_2O_2 that is produced by the L-glutamate oxidation in the presence of GluOx/GluOx$_{irr}$. For this purpose, a cocktail was prepared containing a mixture of 300 units/ml catalase, 100 mM L-glutamate in 100 mM buffer phosphate. Then, GluOx/GluOx$_{irr}$ was added to the cocktail and was incubated at 30 °C for 20 min. In the next stage, 25% (w/v) trichloroacetic acid, 1 M acetate buffer, pH 5.0, and 0.10% (w/v) 3-methyl-2-benzothiazolinone hydrazone solution (MBTH) was added to cocktail and incubate at 50 °C for 30 min. Ultimately, the final solution was placed at room temperature for 20 min and a sample of it was transferred into a cuvette and the absorption was recorded at 316 nm.

2.5. Biosensor responses

Electrochemical measurements using the cyclic voltammetry method were carried out based on a traditional three-electrode system. In this system Pt wire, Ag/AgCl, and modified electrode (PE/Chit/GluOx) were utilized as an auxiliary, reference, and working electrodes, respectively. For all experiments, voltammograms were recorded between the potential ranges −0.6 to 1 V at a 10 mV/s scan rate at 37 °C. A uniform concentration of oxygen within the phosphate buffer was essential for the enzymatic reaction of GluOx in the presence of glutamate. For this reason, in all experiments buffer phosphate is continually stirred in the open air with a magnetic stirrer to reach a constant oxygen concentration. It should be noted that the voltammograms obtained in the cyclic voltammetry experiments were considered PE/Chit/GluOx responses. In addition, all experiments were performed three times using the same electrodes, and the averages along with the relative standard deviations of peak currents were calculated and reported as biosensor responses.

2.6. The effect of pH and temperature

The pH effects on biosensors' responses of the native and irradiated GluOx were investigated. To determine optimal pH, the pH varied between 4.5 and 10.5. Similarly, the temperature effect on the response of PE/Chit/GluOx on the native and irradiated GluOx was evaluated in the range of 25 °C–75 °C. All experiments were performed in buffer phosphate 100 mM in the presence of glutamate as the substrate of GluOx.

2.7. Linearity Range and responses saturation

To determine the linear region and saturation limit of biosensor responses, cyclic voltammetry experiments were performed in the presence of different concentrations of glutamate at optimum pH and temperature. Then, the changes in the anodic peak currents at

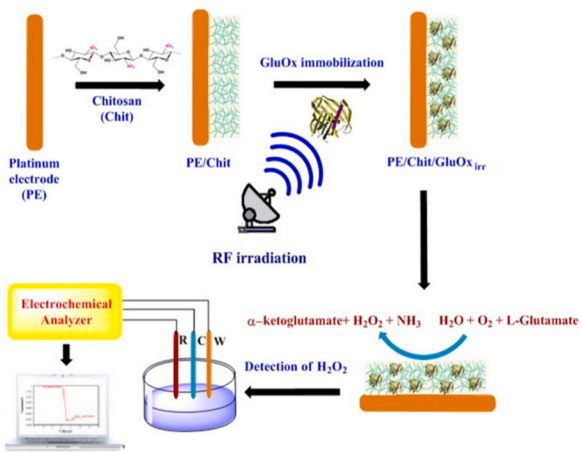

Fig. 1. Graphical abstract. In this scheme, the manufacturing and irradiation processes of PE/Chit/GluOx are illustrated. PE/Chit/GluOx$_{irr}$ represents platinum electrode, Chitosan, and irradiated GluOx. R, C, and W represent the reference electrode, counter electrode, and working electrode, respectively.

different concentrations of glutamate were plotted.

2.8. Selectivity

To check the selectivity of PE/Chit/GluOx, the biosensor responses in the presence of 1 mM concentration of glutamate alone and together with ascorbic acid, glutathione and uric acid were analyzed separately, and the anodic currents created at 0.6 V were investigated [16,30,31].

2.9. Long-term stability

The long-term stability of PE/Chit/GluOx and PE/Chit/$GluOx_{irr}$ were evaluated in the presence of a 1 mM glutamate. The responses of both biosensors at optimum temperature and pH were investigated in five days for 30 days [12,16,31].

3. Results and discussions

3.1. The effect of radiation on the GluOx activity

As shown in this Table 1, the activity of the irradiated enzyme is significantly reduced compared to the native enzyme, so the amount of this reduction depends on the radiation frequency. The survey of the catalytic activity showed that the activity of the irradiated enzyme at 4000 MHz has decreased by 70% compared to the native enzyme. The noteworthy point is that these data are completely consistent with what was obtained in this study by the electrochemical experiments.

3.2. Platinum electrode response

After the preparation of the PE, its voltammogram was examined in ferrocyanide solution. Fig. 2 shows the voltammograms of the PE at 5 mM concentration of ferrocyanide solution. The absence of additional peaks indicates that the electrode preparation steps were well performed. According to Fig. 2, the anodic and cathodic peaks are generated at potentials of 0.32 and 0.15 V, respectively. The ratios of anodic and cathodic peak currents are approximately equal to one. Therefore, considering the proximity of the potentials of anodic and cathodic peaks and the ratio of these two currents, the reactions of ferrocyanide on the electrode surface can be considered reversible. This result is fully consistent with many similar studies [27].

3.3. PE/Chit/GluOx responses

After the stabilization of GluOx with chitosan on the surface of the platinum electrode, biosensor responses were evaluated in the presence of different concentrations of glutamate at the optimal condition of temperature and pH. Also, the effect of variation in the coating of the chitosan on the change of biosensor current was investigated, and the results indicated no effect. Then, the obtained voltammograms were considered PE/Chit/GluOx and PE/Chit/$GluOx_{irr}$ responses. Fig. 3A shows PE/Chit/GluOx voltammograms at different concentrations of glutamate. As shown in this figure, with increasing glutamate concentration the amount of enzyme access to the substrate has also increased, as a result the biosensor response also increased. In other words, increasing the concentration of the substrate makes redox reactions easier on the surface of the biosensor.

The anodic peak current at each of the glutamate concentrations was then obtained using Fig. 3A at 0.4 V. Fig. 3B shows the anodic peak currents at various concentrations of glutamate. As shown in this figure, the overall characteristic curve of PE/Chit/GluOx is sigmoidal similar to many of the usual characteristic curves of most biosensors [12,16,30,31]. The analytical parameters of the PE/Chit/GluOx were obtained using this figure. These parameters include linear range, the concentration of response saturation, low detection limit, and sensitivity. All analytical parameters of the PE/Chit/GluOx are presented in Table 2. It is necessary to pay attention to this important point, although the effect of concentration on PE/Chit/GluOx responses has been studied between 0 and 16 mM, and more than twenty voltammogram curves have been recorded, only a limited number of them have been reported in Fig. 3A.

Table 1
Enzyme Activity of GluOx and $GluOx_{irr.}$ Each value represents the mean ± SD of three measurements.

Enzyme	Frequency (MHz)	Enzyme Activity (units/ml)
GluOx	–	0/13 ± 0.0065
$GluOx_{irr}$	915	0/112 ± 0.0056
$GluOx_{irr}$	1800	0.081 ± 0.004
$GluOx_{irr}$	2450	0.067 ± 0.0033
$GluOx_{irr}$	4000	0.041 ± 0.002

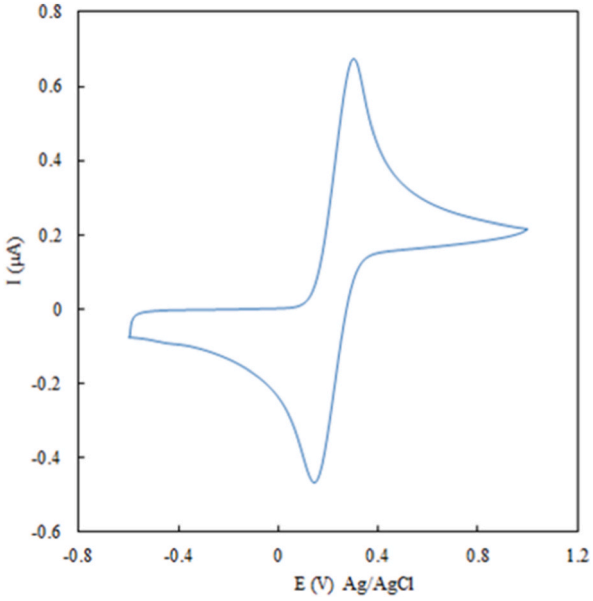

Fig. 2. Cyclic voltammograms of PE in the 5 mM concentration of ferrocyanide at 37 °C, pH 7.4, and scan rate 10 mV/s.

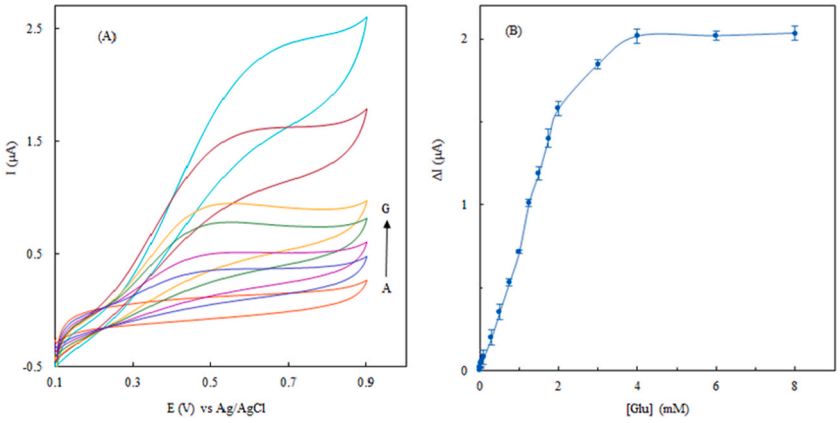

Fig. 3. Avoltammograms as biosensor responses at optimum pH and temperature in different concentrations of glutamate. Each of the voltammograms is obtained in A: 0.05 mM; B: 0.3 mM; C: 0.5 mM; D: 0.75 mM; E: 1 mM; F: 1.5 mM; G: 2 mM; in 100 mM buffer phosphate, pH 7.4, 37 °C and scan rate of 10 mV/s. B. Calibration curve of the PE/Chit/GluOx. In this curve, each point represents the anodic peak current at 0.4 V.

Table 2
For easy comparison, the analytical parameters are presented in Table 2.

Enzyme	Frequency (MHz)	LOD (experimental) (μM)	LOD (computational) (μM)	Saturation point (mM)	Linear region range (μM)
GluOx	–	10	10.1	4	30–3000
$GluOx_{irr}$	915	25	25.19	3.5	50–2000
$GluOx_{irr}$	1800	45	45.42	3	100–2000
$GluOx_{irr}$	2450	50	52.96	2	150–1750
$GluOx_{irr}$	4000	100	108.3	1.75	300–1500

3.4. The effect of radiation on biosensor responses

The effect of $GluOx_{irr}$ on the responses of the biosensor, especially its effect on the cathodic potential shifting, is well shown in Fig. 4. As indicated in this figure, when the $GluOx_{irr}$ was used instead of the native GluOx in the biosensor structure, not only the cathodic potential decreased but also shifted to more negative values. After investigating the effect of Glu on PE/Chit/GluOx responses

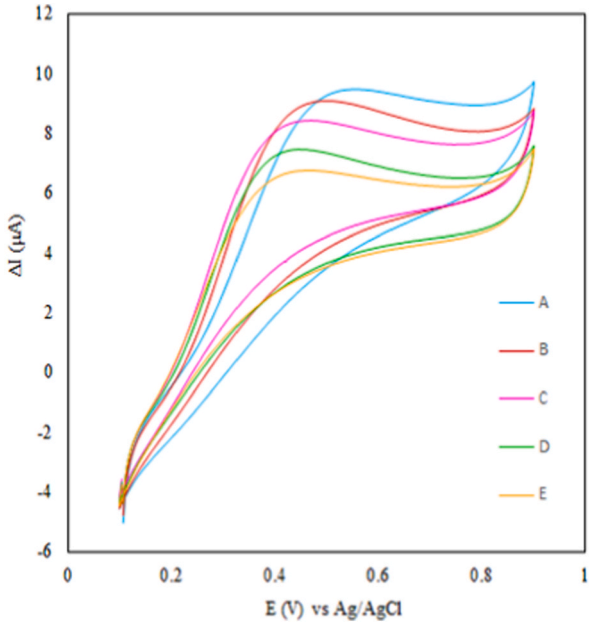

Fig. 4. Biosensor responses when native (GluOx) or irradiated ($GluOx_{irr}$) enzymes were used. A: PE/Chit/GluOx; and PE/Chit/$GluOx_{irr}$ at B: 915 MHz; C: 1800; D: 2450 MHz and E: 4000 MHz. All measurements were performed at a constant glutamate concentration (1 mM) in 100 mM buffer phosphate, pH 7.4, 37 °C, and a scan rate of 10 mV/s.

and plotting the calibration curve reported in Fig. 4A and B, a similar study was performed when the irradiated GluOx was used in the fabricated PE/Chit/$GluOx_{irr}$. Regardless of the voltammograms, in Fig. 5A. Only calibration curves in the case of the native and irradiated GluOx at various frequencies have been reported. As can be seen in this figure, the use of irradiated GluOx in the PE/Chit/$GluOx_{irr}$ did not affect the linear range of the biosensor. However, the electromagnetic radiation affected the limit of detection (LOD), so that, the LOD was 10 μM for PE/Chit/GluOx, while for PE/Chit/$GluOx_{irr}$ at frequencies 915, 1800, 2450, and 4000 MHz, were 25, 45, 50 and 100 μM, respectively. The values of the LODs indicate a deterioration in the PE/Chit/$GluOx_{irr}$ responses. Also, the responses of the PE/Chit/GluOx and PE/Chit/$GluOx_{irr}$ were examined in the saturation region (4 mM). As can be seen in Fig. 5B, different frequencies of electromagnetic radiation had a profound effect on the PE/Chit/GluOx responses. Comparing the native GluOx, these responses decreased significantly when the irradiated enzymes were used. As shown in Fig. 5A, the starting saturation for the native and the irradiated enzyme at the frequencies of 915, 1800, 2450, and 4000 MHz, are 4, 3.5, 3, 2, and 1.75 mM, respectively. In other words, the irradiated enzyme enters the saturation zone more readily than the native enzyme.

Reducing the detection limit, reducing the responses in the saturation region, and reducing saturation points (Table 2), all indicate that the performance of the irradiated GluOx is not optimal compared to the native GluOx. This could be due to a change in the structure of the GluOx, which results in a decrease in the catalytic activity of the GluOx. The effect of electromagnetic radiation on the

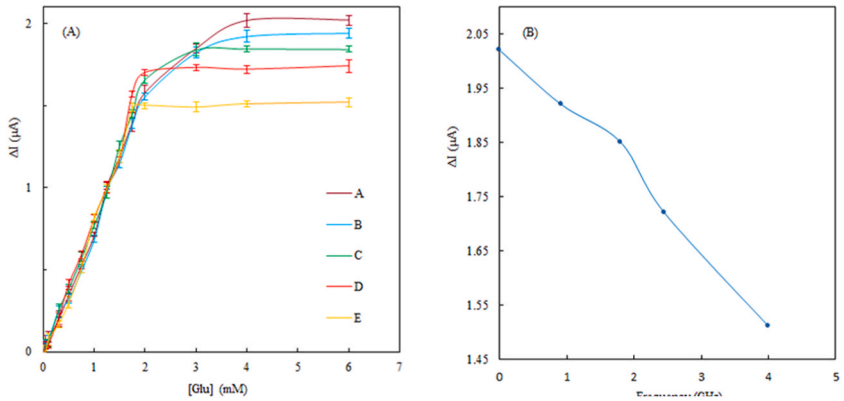

Fig. 5. A. Radiation effect on the calibration curves. Calibration curves of the A: PE/Chit/GluOx; and PE/Chit/$GluOx_{irr}$ at B: 915 MHz; C: 1800; D: 2450 MHz and E: 4000 MHz. All voltammograms are obtained in the presence of 1 mM glutamate in the 100 mM buffer phosphate, pH 7.4, 37 °C, and scan rate of 10 mV/s. B. The effect of radiofrequency on biosensor responses. At each of the frequencies (0 for PE/Chit/GluOx) and 0.915, 1.8, 2.45, and 4 GHz (for PE/Chit/$GluOx_{irr}$), the responses are obtained from Fig. 3A at a saturated concentration of glutamate (4 mM).

structure, catalytic activity, and stability of enzymes has been reported and the results of this study are also in agreement with these references [21–24]. In other words, these results demonstrate that any changes in the structure and function of the enzyme (native or irradiated GluOx), completely affected the PE/Chit/GluOx or PE/Chit/GluOx$_{irr}$ responses and, in turn, their analytical parameters.

3.5. The effects of pH and frequencies on biosensor responses

Since any change in the conformation and/or in the catalytic activity of the GluOx can be affected by its optimal pH, so the effect of pH on biosensor responses in the range of 3.4–10.4 in the presence of 1 mM Glu, was examined and the results are shown in Fig. 6. These results show that the native enzyme had the best performance at pH 7.4 which is consistent with many references [15,32]. Then, to study the effect of electromagnetic waves on the PE/Chit/GluOx$_{irr}$ responses, the optimal pHs of biosensor performance at different frequencies were investigated. As can be seen in this figure not only the PE/Chit/GluOx$_{irr}$ responses are reduced, but also the pHs are shifted to lower values compared to the optimal pH of the native enzyme. Based on these results, it seems that the shift of pH and reduction of anodic peak currents are due to the disruption of the aquatic environment around the enzyme and the change in the conformation, especially in the active site.

3.6. Effects of temperature and frequencies on biosensor responses

The effect of temperature on the structure and function of GluOx or GluOx$_{irr}$ in the range of 25 °C–75 °C was investigated at optimal pH (7.4). As shown in Fig. 7. It can be seen that 37 °C is the optimum temperature for the PE/Chit/GluOx responses in which the anodic peak current is equal to 1.87 μA. This is the best temperature for the catalytic activity of the native enzyme [15,32].

A similar study was then performed to determine the optimum temperature of the PE/Chit/GluOx$_{irr}$. Fig. 7 clearly shows that the anodic peak currents have significantly decreased. This decrease is completely dependent on the radiation frequency. So that by increasing the frequency from 915 MHz to 4000 MHz, the anodic peak currents decreased from 1.83 μA to 1.48 μA. This reduction compared to the native GluOx indicates a 21% decrease in the PE/Chit/GluOx responses. Again, such a reduction could be due to the effect of electromagnetic radiation on both the conformation and function of the GluOx.

3.7. The effect of interferers

The effects of ascorbic acid, glutathione and uric acid on the PE/Chit/GluOx responses are shown in Fig. 8. As shown in this figure, ascorbic acid and glutathione were decreased the responses by 56% and by 33%, respectively, while uric acid increases the biosensor response by 13%. These results are consistent with other reports. It should be noted that to reduce the interference effects, some methods such as pretreatment of working electrodes have been developed before enzyme immobilization [12,16,30,31].

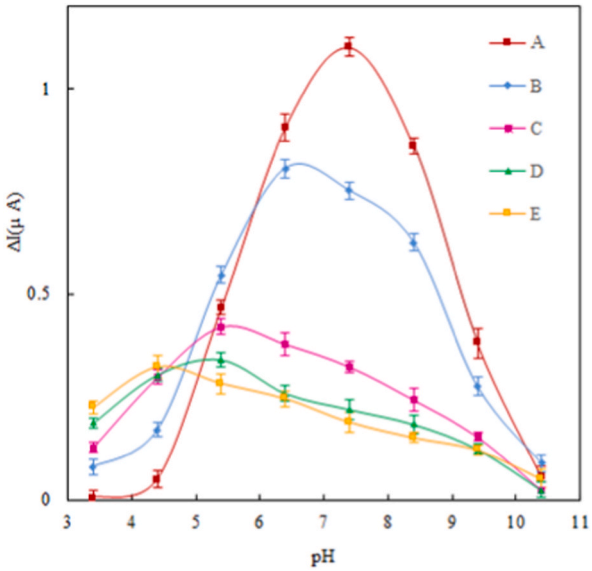

Fig. 6. The effect of radiofrequency on the optimal pH of biosensors performance. All measurements were performed at a constant glutamate concentration (1 mM) in 100 mM buffer phosphate, 37 °C, and a scan rate of 10 mV/s (A; 0) for PE/Chit/GluOx, (B; 915 MHz), (C; 1800 MHz), (D; 2450 MHz), and (E; 4000 MHz) for PE/Chit/GluOx$_{irr}$, respectively.

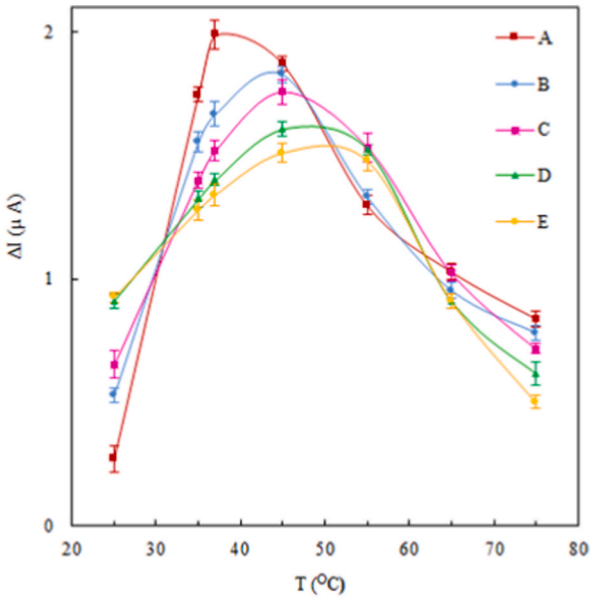

Fig. 7. The effect of radiofrequency on the optimal Temperature of biosensors performance. All measurements were performed at a constant glutamate concentration (1 mM) in 100 mM buffer phosphate, pH 7.4, and a scan rate of 10 mV/s (A; 0) for PE/Chit/GluOx, (B; 915 MHz), (C; 1800 MHz), (D; 2450 MHz), and (E; 4000 MHz) for PE/Chit/GluOx$_{irr}$, respectively.

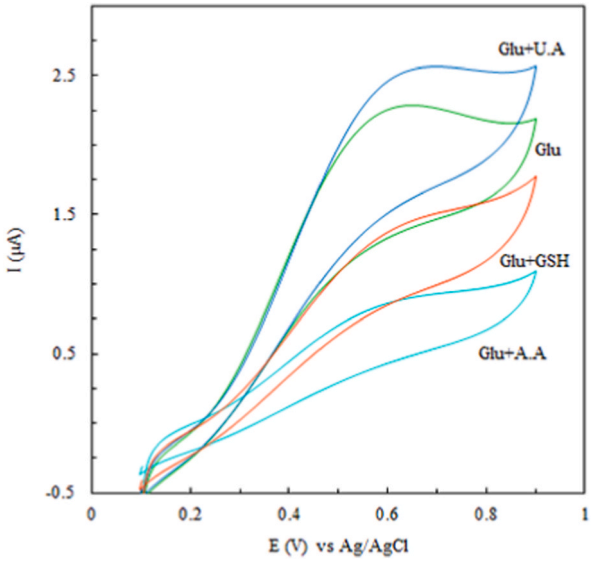

Fig. 8. Interferes effect on PE/Chit/GluOx responses. Cyclic voltammetry experiments were carried out in the presence of Glu (1 mM) + A.A (0.5 mM), Glu (1 mM) + GSH (0.5 mM), Glu (1 mM) + U.A (0.5 mM) in 100 mM buffer phosphate, pH 7.4, 37 °C and scan rate of 10 mV/s. Glu (glutamate); Ascorbic Acid (A.A); Uric Acid (U.A); Gluthatione (GSH).

3.8. PE/Chit/GluOx and PE/Chit/GluOx$_{irr}$ stability

The results of the long-term stability of both PE/Chit/GluOx and PE/Chit/GluOx$_{irr}$, are illustrated in Fig. 9. As shown in this figure the response of the PE/Chit/GluOx is reduced by about 15% whereas the PE/Chit/GluOx$_{irr}$ responses were reduced about 20%. It should be noted that, only the long-term stability results of the PE/Chit/GluOx$_{irr}$ at 4000 MHz as a sample is reported. The stability of PE/Chit/GluOx that is reported in this paper is quite consistent with many similar biosensors [12,16,31].

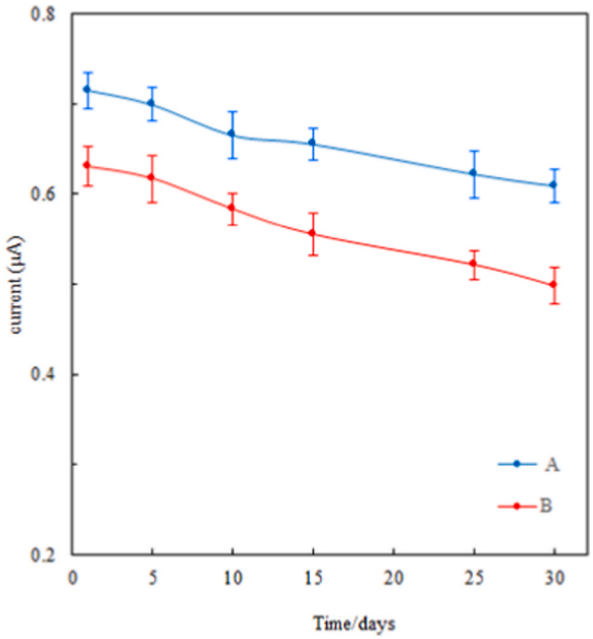

Fig. 9. Long-term stability of the biosensor responses to 1 mM glutamate in five days for 30 days, at 37 °C, pH 7.4, and scan rate 10 mV/s (A; 0) for PE/Chit/GluOx and (B; 4000 MHz) for PE/Chit/$GluOx_{irr.}$

4. Conclusions

Due to the widespread use of electromagnetic waves in the fields of communication and telecommunications, medicine, both diagnostic and therapeutic, and in various fields of research, in the last decade, its biological effects have been investigated in several studies. The effect of these waves on biological macromolecules such as DNA, proteins, and particularly enzymes, has been the focus of most researchers. In this study, the effect of electromagnetic waves in the radiofrequency range on glutamate oxidase (GluOx) as an important macromolecule has been investigated for the first time using the electrochemical method. For this purpose, a biosensor based native enzyme (PE/Chit/GluOx) and irradiated enzyme (PE/Chit/$GluOx_{irr}$) were made and their analytical parameters were accurately measured. The many advantages of chitosan have caused the use of this natural polysaccharide in the structure of the (PE/Chit/GluOx) or (PE/Chit/$GluOx_{irr}$) biosensor. The most important of these features are non-toxicity, the appropriate functional groups to create a crosslink, low cost, availability, good film-forming ability, and biocompatibility [26].

The results showed that the lowest detection limit, saturation point of responses, changes in responses in the saturation region, optimum temperature and pH of the enzyme, and Glu concentration at linear region range are all affected by electromagnetic radiation in the range of radio frequencies (RF). However, these waves did not have much effect on the linear region of the biosensor (PE/Chit/GluOx or PE/Chit/$GluOx_{irr}$). Also, long-term stability study showed that the biosensors responses were reduced after about one month.

Based on the results, it should be noted that when a glutamate oxidase-based biosensor is used to quantitatively measure the glutamate, the analytical parameters of the biosensor are significantly affected by radiofrequency electromagnetic waves. Such effects require that correction coefficients for glutamate oxidase-based biosensors should be considered when used in environments containing radiofrequency waves. Such effects on the structure and function of glutamate oxidase at other frequencies, especially in the extremely low frequency (ELF) range, should also be considered.

Author contribution statement

Faezeh Faraji; Hassan Tavakoli: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mahvash Jafari: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data.

Akram Eidi; Adeleh Divsalar: Conceived and designed the experiments.

Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare no conflict of interest.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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