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VOLTAMMETRIC DETERMINATION OF RESVERATROL USING POLY(L-PHENYLALANINE)-MODIFIED GOLD ELECTRODE

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In this study, we investigated the electrochemical modification of a gold electrode using poly(L-phenylalanine) and its applicability for the quantification of resveratrol (RESV). The gold electrode was modified with L-phenylalanine employing cyclic voltammetry (CV) in aqueous solution. The detection of RESV with the modified electrode was investigated by square wave voltammetry (SWV) in a phosphate buffer solution (PBS) (pH = 1.2). The analytical calibration curve for RESV showed a linear response with concentration in the oxidation peak current range from 50 to 1000 μM, with a limit of detection (LOD) of 35.16 μM and limit of quantitation (LOQ) of 105.5 μM. The application of the prepared electrochemical sensor was carried out with a standard addition method in red wine samples.

Keywords: L-phenylalanine; resveratrol; electropolymerization; square wave voltammetry

ВОЛТАМЕТРИСКО ОПРЕДЕЛУВАЊЕ НА РЕСВЕРАТРОЛ СО ЕЛЕКТРОДА ОД ЗЛАТО МОДИФИЦИРАНА СО ПРИМЕНА НА ПОЛИ(L-ФЕНИЛАЛАНИН)

Во оваа студија ја испитавме електрохемиската модификација на електрода од злато со примена на поли(L-фенилаланин) и нејзината применливост за квантификација на ресвератрол (RESV). Електродата од злато беше модифицирана со L-фенилаланин со користење на циклична волтаметрија (CV) во водна средина. Детекцијата на RESV со модифицираната електрода беше испитана со квадратно-бранова волтаметрија (SWV) во раствор на фосфатен пуфер (PBS) (pH = 1,2). Аналитичката калибрациона крива за RESV покажа линеарен одговор со концентрација на оксидацискиот пик во областа од 50 до 1000 μM, со граници на детекција (LOD) од 35,16 μM и граници на квантификација од (LOQ) од 105,5 μM. Применливоста на подготвениот електрохемиски сензор беше испитана со методот на стандардни адиции на примероци од црвено вино.

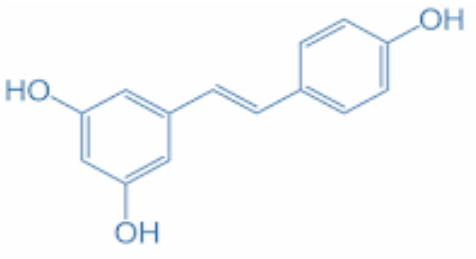
Клучни зборови: L-фенилаланин; ресвератрол; електрополимеризација; квадратно-бранова волтаметрија.

1. INTRODUCTION

RESV (3,5,4'-trihydroxystilben) is a natural polyphenolic compound [1] with a stilbene derivative [2] (Scheme 1). Polyphenols, as antioxidants, have the ability to remove free radicals formed in the human body for various reasons. They are also known to reduce the risk of cardiovascular disease and cancer due to their reduction of oxidative stress [3]. RESV has various anti-inflammatory, anti-proliferative, anti-tumoral, biochemical, and physiological effects [4].

In addition, RESV is an effective antimicrobial and antifungal compound produced by plants

against microbial, fungal, and physical stimuli [5], and it is present in significant amounts in grapes, berries, peanuts and red wine.



Scheme 1. Chemical structure of trans-RESV

RESV recently attracted researchers' attention due to its nutraceutical and therapeutic potential for many diseases. For example, it could play a potential protective role against cardiovascular diseases [6]. RESV has several biological and pharmacological effects including anticancer, anti-inflammatory, cardio-protective and antioxidant activities [7, 8]. Also, its beneficial effects on diseases related to age, such as type two diabetes, cardiovascular diseases, cancer, and neurological diseases, are of great interest [5].

There are determination methods for RESV in literature, such as chromatography [9] or ultra violet (UV) analysis. Nevertheless, these methods are expensive and time-consuming. In general, electrochemical methods have been used as alternatives to detect a large number of important analytes, such as food [10], environmental [11], and pharmaceutical [12, 13] components. However, electrochemical methods could be considered promising procedures for the evaluation of RESV properties [14]. Electrochemical methods provide a rapid response, high sensitivity, and low detection limit [15, 16]. RESV, as a phenolic compound, shows electrochemical activity and can be analyzed by electrochemical methods [17]. Some electrochemical sensors for RESV have been reported in literature already [18]. Hitherto, researchers have worked on the electrochemical oxidation mechanism and electrochemical sensing of RESV with unmodified electrodes, such as the glassy carbon electrode (GCE) [19, 20], carbon paste electrode (CPE) [21], and graphite electrode [17, 19]. Only some articles reported the use of modified electrodes [14]. In this work, a gold electrode was modified by poly(L-phenylalanine) using CV in aqueous solution containing 0.1 M potassium nitrate (KNO_3). Electrochemical modification of the gold electrode by poly(L-phenylalanine) was studied [22, 23]. After the gold electrode was modified, its sensitivity towards RESV was tested by SWV. The aim of this work is the development of a method for RESV detection using an electrochemically modified gold electrode. The novelty of this work is the high sensitivity provided by the electrochemical RESV sensor using a simple modified gold electrode.

2. MATERIALS AND METHODS

2.1. *Materials*

L-phenylalanine, potassium ferricyanide ($K_4[Fe(CN)_6]$), potassium dihydrogen phosphate (KH_2PO_4), sodium monohydrogen phosphate (Na_2HPO_4), KNO_3, nitric acid (HNO_3), and RESV were purchased from Sigma-Aldrich. Sodium acetate (CH_3COONa), acetic acid (CH_3COO), sodium perchlorate ($NaClO_4$), potassium chloride (KCl), phosphoric acid (H_3PO_4), boric acid (H_3BO_3), hydrochloric acid (HCl), and sodium hydroxide (NaOH) were purchased from Merck. All aqueous solutions were prepared with ultrapure water.

2.2. *Instrumentation*

A three-electrode cell system was employed in all electrochemical experiments. A platinum wire was used as a counter electrode, and gold electrodes with geometric areas of 0.07 cm^2 were used as working electrodes. The reference electrode was Ag/AgCl in saturated NaCl solution (Ag/AgCl/sat. NaCl)). The electrochemical experiments were done using a potentiostat (Vertex one) controlled by computer software (Ivium soft) for data analysis. Square wave measurements used for RESV detection were performed after 60 s of a pre-concentration step with an accumulation potential of –0.5 V under stirring. After the accumulation step, square wave measurements were recorded from –0.5 to 1 V with an amplitude of 50 mV and a frequency of 70 Hz in the presence of PBS 0.1 M (pH = 1.2) containing 5 µl of Tween 20. All glassware and electrochemical cells were kept in 6 M HNO_3 overnight to remove impurities, and all electrochemical measurements were carried out at room temperature.

2.3. *Electrode modification*

The gold electrode surface was hand polished with a 0.05 µm alumina-water slurry using a polishing cloth. The polished electrode was rinsed

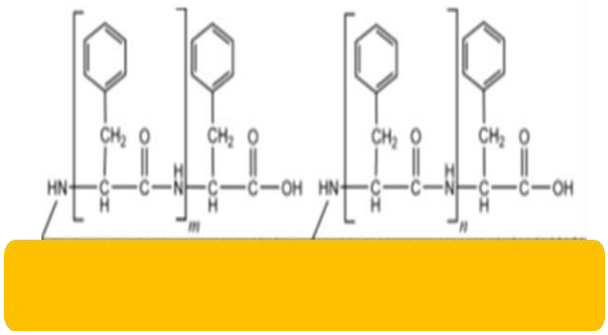
with ultrapure water, sonicated in ultrapure water for 3 min, and, finally, rinsed again with ultrapure water to remove impurities on the electrode surface. Then, the electrode was modified using L-phenylalanine (100 mM) by six reversible potential cycles between +1.5 and +0 V in an aqueous solution containing 100 mM KNO_3 at a scan rate of 80 $mV \cdot s^{-1}$. After the polymerization step, the surface of the modified electrode was washed with ultrapure water to remove surface impurities.

3. RESULTS AND DISCUSSION

3.1. *Gold electrode modification*

The polished and cleaned gold electrode was modified by six potential cycles between +1.5 and +0.0 V at a scan rate of 80 mV/s. The CVs were taken on the gold electrode both in the background solution (aqueous solution containing 100 mM KNO_3; Fig. 1A) and in the background solution containing 100 mM L-phenylalanine (Fig. 1B).

From Figure 1B, we see the polymerization of L-phenylalanine from changes in the CVs of L-phenylalanine during modification of the gold electrode. In the first cycle, a peak was formed on the gold electrode that could be attributed to the oxidation of L-phenylalanine at 1100 mV, as this peak was not observed in the voltammogram of the background electrolyte solution (Fig. 1A). With the increasing number of cycles, a continuous decrease in peak current was observed, indicating growth of the polymeric film at the gold electrode surface. According to the literature, upon the oxidation of amines on metallic electrodes, the amino group of L-phenylalanine was oxidized to a free radical and, then, covalently immobilized onto the gold electrode surface [24]. The electropolymerized L-phenylalanine film structure is shown in Scheme 2 [22].



Scheme 2. Proposed structure of the poly(L-phenylalanine) film at the gold electrode surface

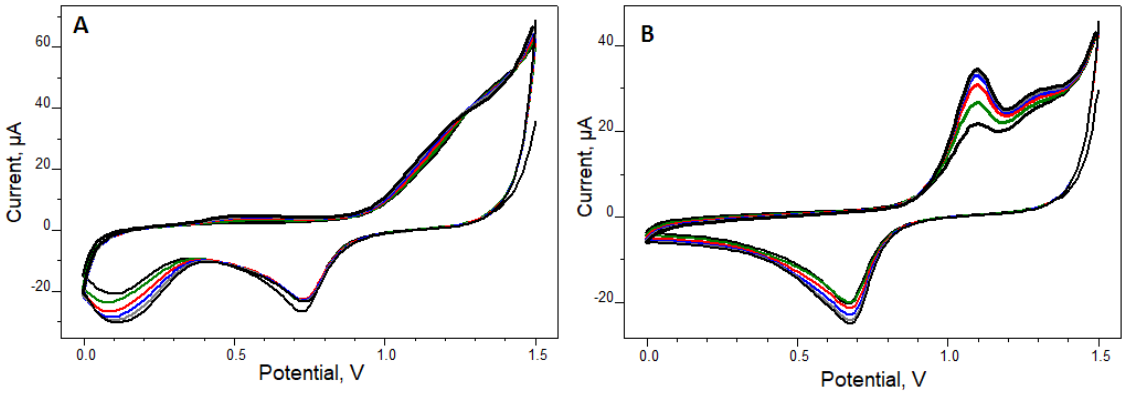


Fig. 1. CVs of the background solution (A) and electrochemical polymerization of L-phenylalanine in the background solution (B) on a gold electrode (cycle number = 6, scan rate = 80 mV/s *vs.* Ag/AgCl/(sat. NaCl))

3.1.1. *Monomer concentration effect*

The monomer concentration used for poly(L-phenylalanine) film preparation plays an important role in the electrochemical properties of the deposited layer. Figure 2 shows a comparison of the RESV current responses obtained with a modified gold electrode after electropolymerization of L-phenylalanine at concentrations from 10 to 150 mM. As seen, 100 mM of L-phenylalanine concentration gives the highest anodic peak current for RESV (Fig. 2).

The current response of RESV on the poly(L-phenylalanine)/gold electrode gradually increased as the concentration of L-phenylalanine increased from 1 to 100 mM and, then, decreased as the L-phenylalanine concentration increased further. Therefore, a 100 mM monomer concentration was used for subsequent experiments.

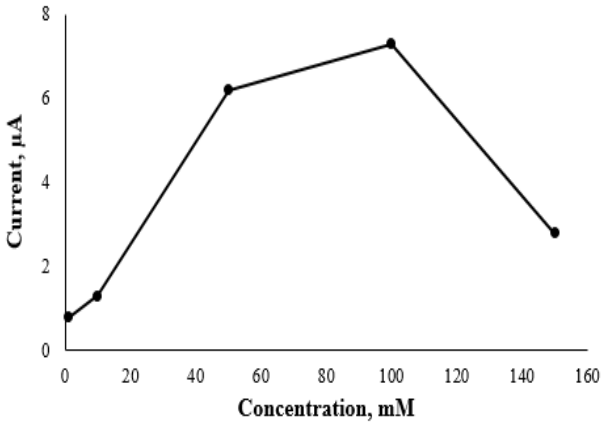


Fig. 2. Relationship between anodic current of 1 mM RESV *vs.* monomer concentration

3.1.2. *Cycle number effect*

The cycle number effect on the anodic current response of 1 mM RESV was tested by the SWV method, and results are shown in Figure 3. The current response of RESV on the modified electrode increased as the number of cycles increased from two to six, and then, it decreased for cycle numbers higher than six. Therefore, six cycles were used for all experiments.

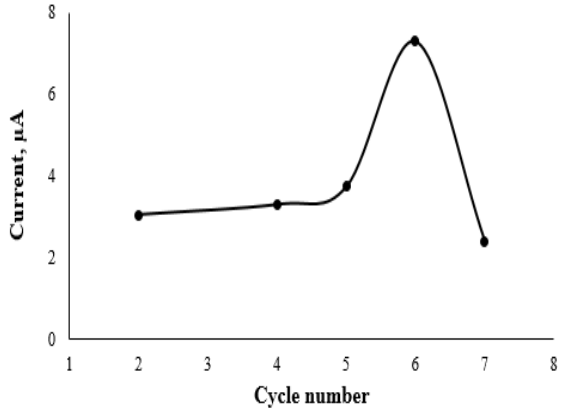


Fig. 3. Effect of cycle number on the current oxidation of RESV

3.1.3. *Gold electrode characterization*

The modified electrode's surface was characterized by CV at a scan rate of 100 mV/s in 6 mM ferrocyanide containing 1.0 M KNO_3. Figure 4 reveals the CVs of the gold electrode before and after modification.

Voltammograms of the redox medium showed reversible behavior for both the bare and modified electrodes. After surface modification of the gold electrode, an increase in oxidation peak current at 310 mV was observed from 32 to 34 µA.

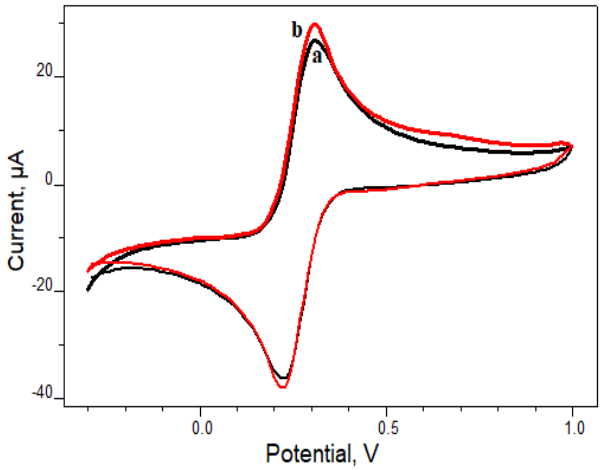
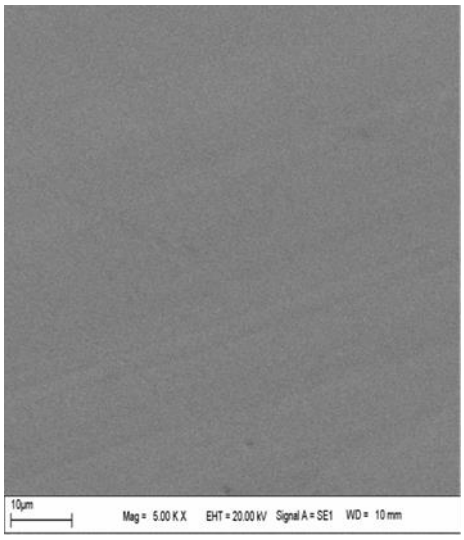
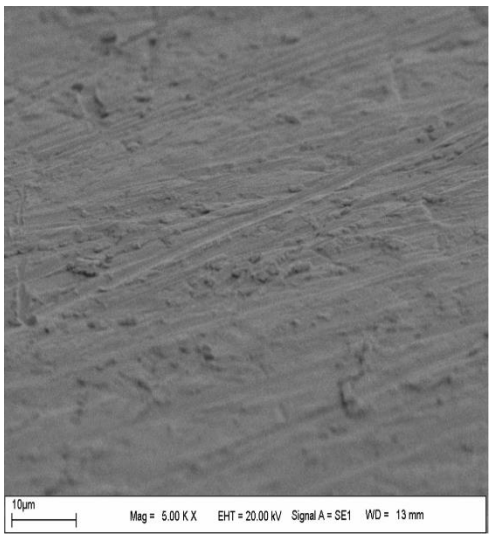


Fig. 4. CVs of 3 mM ferrocyanide in 1.0 M KNO_3 on a bare gold electrode **(a)** and on the poly(L-phenylalanine)/gold electrode **(b)** at a scan rate of 100 mV/s *vs.* Ag/AgCl/(sat. NaCl)



(A)



(B)

Fig. 5. SEM images of the **(A)** bare electrode and **(B)** poly(L-phenylalanine)-modified electrode

On the other hand, the reduction peak current at 220 mV increased from 15 µA in the bare electrode to 17 µA in the modified electrode. This current increase is an expected since poly(L-phenylalanine) is a conductive polymer. To illustrate the modification of the gold electrode surface, Scanning electron microscope (SEM) images are presented in Figure 5. This figure compares the results of the SEM analysis, showing morphological changes on the gold electrode surface from electropolymerization of L-phenylalanine. The SEM of the bare gold electrode (Fig. 5A) presents a uniform surface. From Figure 5B, a uniform and compact layer is observed on the electrode surface, indicating that a poly(L-phenylalanine) film was successfully electropolymerized on the electrode surface with the formation of a well-adhered polymeric film.

3.1.4. *Electrolyte effect*

The effect of the electrolytic solution on the electro-activity of the modified electrode was studied in presence of 1 mM RESV, and the results are summarized in Figure 6. Phosphate buffer solution (PBS) gives a higher oxidation current for RESV compared to other buffer solutions and electrolytes. Thus, PBS buffer was chosen for electrochemical detection of RESV.

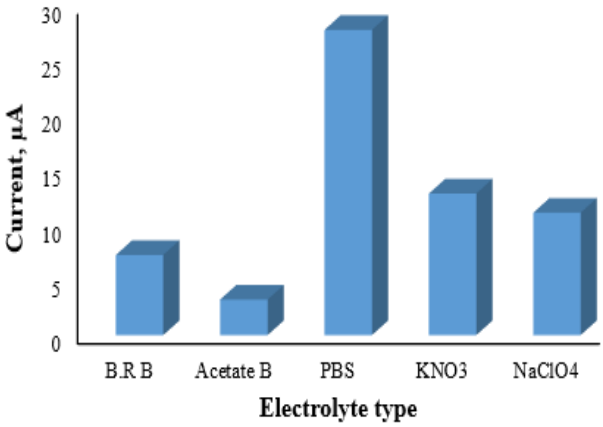


Fig. 6. Electrolyte effect for 1 mM RESV on the poly(L-phenylalanine) gold electrode

3.1.5. *pH effect*

The solution's pH has an important influence on the electrochemical reaction. SWV was carried out to determine the effect of pH on the electrochemical behavior of RESV with the poly(L-phenylalanine) gold electrode. The peak potential shifted positively upon decreasing the pH, from 0.7 V for pH 1.2 to 0.5 V for pH 5 (Fig. 7A). From Figure 7B, we observe that the peak current increased gradually and reached a maximum at pH 1.2. Thus, pH 1.2 was chosen as the optimum pH value in the electrochemical detection of RESV. To adjust the pH of the phosphate buffer, HCl and NaOH were used.

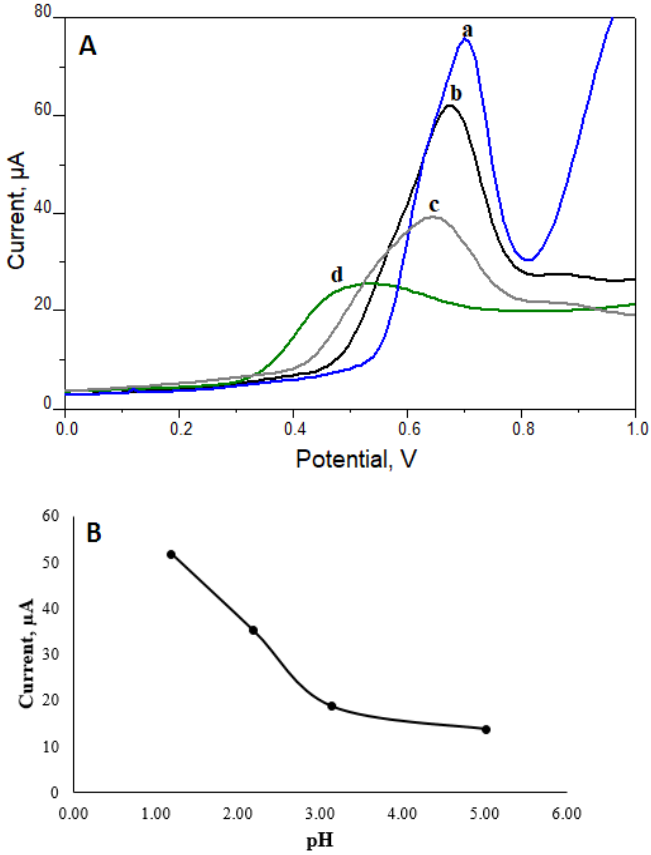


Fig. 7. (A) SWV voltammograms of 1 mM RESV in 0.1 M buffer solution at different pH values (**a**: 1.2, **b**: 2.0, **c**: 3.0, **d**: 5.0) *vs.* Ag/AgCl/(sat. NaCl). **(B)** Relationship between the anodic peak current and pH values. (Experimental conditions; SW amplitude 25 mV, step potential E step 10 mV, accumulation time 60 s, and accumulation potential 0 V)

3.1.6. *Influence of SWV parameters*

The effect of SWV parameters was experimentally studied. The influence of SWV conditions was presented in literature and demonstrates that SWV parameters can affect the electrode response [25–27]. SWVs were recorded for a solution containing 1 mM of RESV using the selected conditions. Figure 8 summarizes the variation of the signal with the amplitude (A), accumulation potential (E_{acc}; B), and accumulation time (t_{acc}; C) in 1 mM RESV and PBS solution (0.1 M, pH = 1.2). The amplitude's effect was studied from 10 to 100 mV, and the highest peak current was observed at

50 mV, as shown in Figure 8A. The deposition potential was optimized in the range of –0.7 to 0.7 V. The highest stripping signal was observed at E_{acc}= –0.5 V and t_{acc} = 60 s (Figs. 8B and 8C).

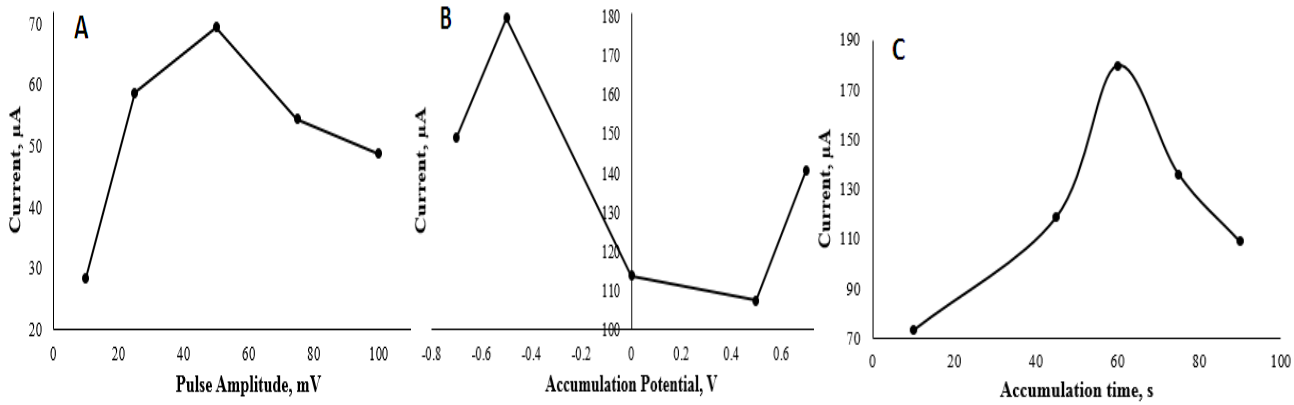


Fig. 8. SWV parameter effects: amplitude **(A)**, accumulation potential (E_{acc}; **B**), and accumulation time (t_{acc}; **C**)

3.2. *Resveratrol detection*

In order to check the affinity of the poly(L-phenylalanine) gold electrode toward RESV, SWV measurements were employed in 0.1 M PBS buffer at pH = 1.2.

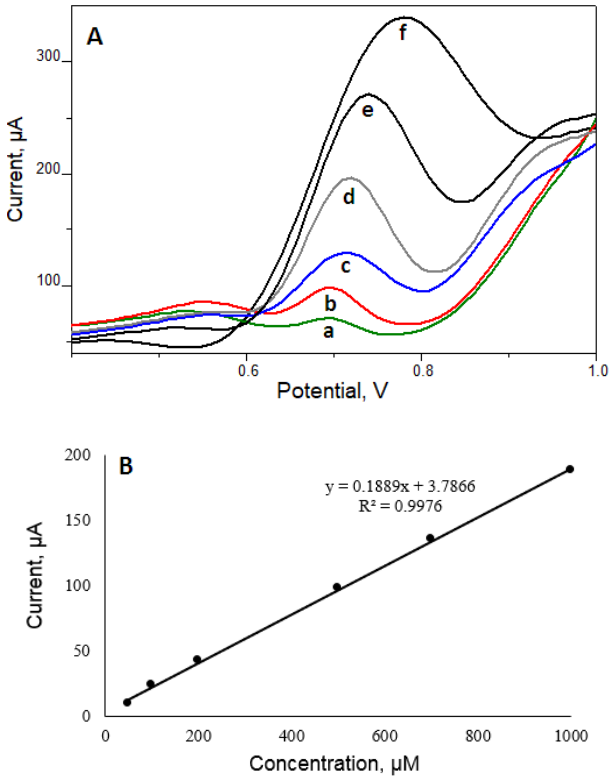


Fig. 9. Anodic SWVs **(A)** and calibration graph **(B)** of RESV (**a:** 50, **b:** 100, **c:** 200, **d:** 500, **e:** 700, **f:** 1000 µM) in PBS (0.1 M), pH = 1.2. Accumulation time 60 s, accumulation potential -0.5 V, amplitude 50 mV, and frequency 70 Hz.

SWVs were registered at different RESV solution concentrations under the optimum conditions studied above. An increase in current peak intensity was noticed with increasing RESV concentration (Fig. 9A). This is explained by the oxidation of RESV under the modified electrode. To evaluate the selectivity of the modified electrode, we plotted the variation of current (µA) as a function of RESV concentration (Fig. 9B).

The features of the modified gold electrode, as well as linear ranges, detection limit, and quantitation limit are summarized in Table 1. A linear dependence of the current response is obtained (R^2 = 0.9976) in a concentration range of 50 to 1000 µM with the modified electrode with a sensitivity of 0.2 µA/µM.

T a b l e 1

Analytical performance of the modified electrode obtained by SWV

Matrix	Poly(L-phenylalanine)/Gold E
Sensibility (µA/µM)	0.2
Domain of linearity (µM)	50–1000
Detection limit (µM)	35.16

The reproducibility of measurements using the poly(L-phenylalanine) gold electrode under the same conditions and same RESV concentration was studied. Repeated measurements demonstrated similar voltammograms, and it proved the reproducibility of our modified electrode.

Figure 10 shows the reproducibility and stability of SWV responses for 500 µM RESV in 0.1 M PBS, pH 1.2, using 15 different poly(L-phenylalanine)-modified gold electrodes, each performed with three measurements. Using these measurements results, standard deviation was calculated as 0.92, RSD % 0.93, and stability as 99.07.

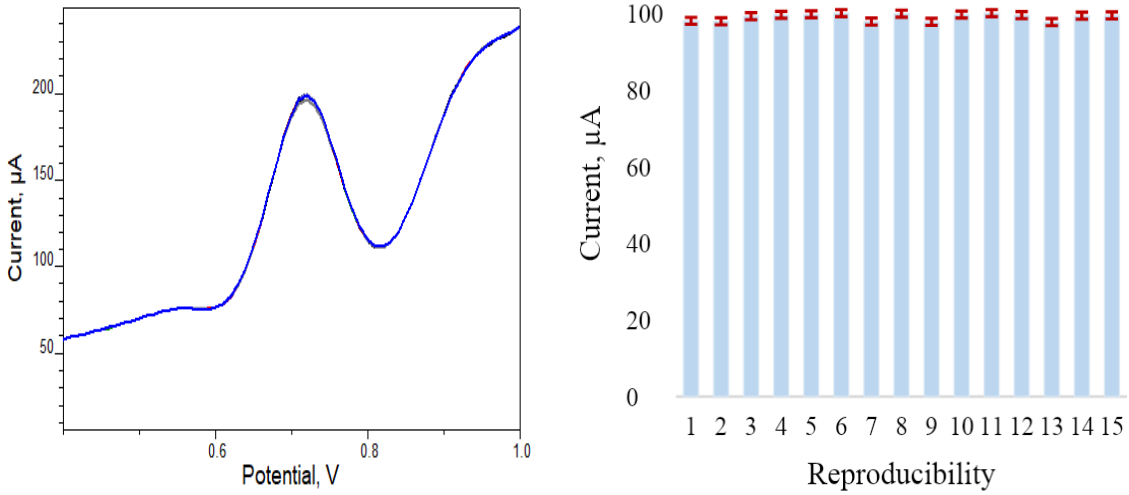


Fig. 10. Reproducibility of the SWV responses and error bars of 500 µM RESV on the poly(L-phenylalanine)-modified gold electrode in 0.1 M PBS, pH = 1.2 (*n* = 15).

3.2.1. *Determination of RESV in a red wine sample*

A red wine sample was used to examine the applicability of the method developed for RESV determination in real samples. The poly(L-phenylalanine) gold electrode was employed for quantification of RESV in a red wine sample. In order to study the recovery, we added a known amount of RESV into the red wine sample, as depicted in Table 2. According to the results obtained in red wine samples, the developed modified electrode clearly could be employed in real sample analysis (Table 2).

T a b l e 2

Determination of RESV in red wine samples

Added	Found (µM)	Recovery %
50 µM	52.75	105.50
100 µM	102.84	102.84

The research method presented here was carried out using simple, selective, sensitive, low cost, and easily-prepared gold working electrodes that require only a short analysis time to determine RESV using poly(L-phenylalanine). There is interest in the deposition of RESV with a fast and easily-prepared poly(L-phenylalanine) gold electrode in PBS buffer (pH = 1.2). The optimum conditions for determination of RESV were as follows: PBS buffer (pH = 1.2), –0.5 V accumulation potential, accumulation time of 60 s, and pulse amplitude of 50 mV. The developed sensor showed, under optimum conditions, sensitivity and selectivity towards RESV with linearity in the range of 50 to 1000 µM. The quantitation and detection limits were 35.15 and 105.5 µM, respectively, after a 60 s accumulation time. Furthermore, the applicability of the proposed electrode in red wine samples gives promising results.

4. CONCLUSIONS

In this study, the electrochemical polymerization of L-phenylalanine in an aqueous solution was performed. The effect of electrochemical parameters on the sensing ability of the electrode towards RESV was studied. The modified electrode exhibited good sensitivity to RESV with a large linear range. The results found with the poly(L-phenylalanine) gold electrode were reproducible with fifteen other measurements, under the same conditions.

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