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Research Paper

Garniture of graphene oxide with NiO@polypyrrole core-shell nanoparticles for making a novel nanosensors for the determination of piceatannol in human and plant samples

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Abstract

For the first time in the world, we expanded an electrochemical nano sensor for detemination of an anti-cancer drug piceatannol based on a new type of nanocomposite: graphene oxide nano sheets arrayed with core-shell NiO@polypyrrole nanoparticles (NiO@Ppy/GO) blended with nafion and sheded on the surface of glassy carbon electrode. The nanocomposite was specified by transmission electron microscopy (TEM), scanning electron microscopy (SEM), energy dispersive Xray spectroscopy (EDX), X-ray diffraction and Fourier transform infrared spectroscopy (FT-IR) techniques. The square wave voltammetry as a sensitive technique and cyclic voltammetry were selected for the detemination of piceatannol in 0.1M phosphate buffer solution (pH 7.0). Several factors were measured such as pH value, scan rate and supporting electrolyte type for the quantification of piceatannol. Moreover, selectivity and repeatability measurements were also investigated.

Under the optimized factors, linear range and limit of detection were calculated 0.01–10.0 µM and 0.003 µM, respectively. Relative standard deviation for 3.0 µM and 6.5 µM were obtained 3.25% and 1.83%, respectively.

The offered nanosensors was practicaled successfully for the quantification analysis of piceatannol in grape skin essential oil and urine sample with good results

Keywords: Piceatannol determination, NiO@Ppy nanocompositem Cyclic and square wave voltammetry, GO nanosheets.

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Introduction

piceatannol ((E)-4-[2-(3,5Dihydroxyphenyl) ethenyl] 1,2-benzenediol, 3,3′,4,5′-Tetrahydroxy-trans-stilbene), a plant secondary product, is an existing polyphenol in grapes skin, in wine, Japanese knotweed and passion fruit that inhibits protein-tyrosine kinase activity [1, 2]. Also, it can suppress proliferation and the migration of vascular smooth muscle cells (VSMC) treated with *Tumor necrosis factor* (TNF-α) including leukemia, lymphoma, and melanoma. So, piceatannol may be an effective therapeutic approach to treat atherosclerosis [3-5]. Piceatannol is known as a hydroxylated metabolite and an analog of resveratrol (3,5,4'-trihydroxy-*trans*-stilbene) that has higher antioxidant and anticuancer activities than resveratrol [6]. Piceatannol can be affective on human dermal cells with the suppression of melanogenesis and synthesis of collagen [7]. The

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positive effects of piceatannol were represented in health, pharmaceuticals, cosmetics and functional foods [8-10] and also vasorelaxant effects in rat thoracic aorta [11]. So, its measurement is important. Already, several methods were performed for the analysis of piceatannol, such as high performance liquid chromatography (HPLC) [12], HPLC-UV [13, 14] and gas chromatography-mass spectroscopy(GC-Mass) [15], but we used electrochemical method with excellent advantages in comparing the other reports, such as simplicity, sensitivity, rapidly and cheapness [16] Previously, resveratrol was determined with voltammetry method [17, 18] as well as the expressed oxidation reaction of resveratrol to piceatannol [19]. But, for the first time, we directly purchased piceatannol and performed voltammetric method to determine it. However, due to its high catalytic ability, low cost and good stability, graphene oxide (GO) has many advantages over other carbon nanostructures in analyses applications [20] So, considering the properties of all the three materials we prepared a composite nanostructure comprised of decorated GO with NiO@Ppy core-shell nanoparticles. Highly conductive NiO NPs were used as core structure and Ppy as shell was formed via in-situ polymerization on the GO nano sheets. Herein, we studied square wave voltammetry (SWV) as a sensitive technique for the quantification of piceatannol using NiO@Ppy/GO/Nafion on the surface of glassy carbon electrode. Also, the sensor was applied successfully to determine piceatannol in grape skin and urine sample.

Materials and methods

Apparatus

Voltammetric experiments were performed with electrochemical and electroanalytical instruments (SAMA 500, I. R. Iran). A three electrode system was used, the NiO@Ppy/GO/Nafion modified glassy carbon electrode as a working electrode, Ag/AgCl as a reference and platinum wire as an axillary electrode. The pH solutions measurement was performed by a Metrohm 827 pH meter (Herisau, Switzerland). TEM images were recorded with an EM-900 transmission electron microscopy (Zeiss, Germany), Xpert MPD diffractometer (Philips, Netherlands) and Bruker Tensor 270 Fourier-transform infrared spectrometer were employed to record XRD patterns and FTIR spectra, respectively. To record SEM images and EDX analysis, a Mira III scanning electron microscopy (Tescan, Czech Republic) equipped with an energy-dispersive X-ray analyzer was utilized.

Reagents and solutions

Pyrrole monomer (% 98), nickel (II) nitrate hexahydrate ($Ni(NO_3)_2.6H_2O$), sodium dodecyl sulfate (SDS), graphite powder (< 300 µm, 99.99%), piceatannol and ethanol were purchased from Sigma Aldrich (Steinheim, Germany). Sodium Hydroxide (NaOH), cetyltrimethylammonium bromide (CTAB), iron (III) chloride ($FeCl_3$), sulfuric acid (H_2SO_4, 98%), copper (II) sulfate ($CuSO_4$), potassium permanganate ($KMnO_4$), hydrogen peroxide (H_2O_2), hydrochloric acid (HCl), sodium nitrate ($NaNO_3$), sodium dihydrogen phosphate (NaH_2PO_4), disodium hydrogen phosphate (Na_2HPO_4) and phosphoric acid (H_3PO_4) were from Merck (Darmstadt, Germany). All these reagents were used without further purification. The 5.0 mM stock solution of piceatannol was prepared by dissolving it in ethanol each day. The solution was stored in a well closed container to protect from air and light.

0.1 M phosphate buffer solution (PBS) at pH 7.0 was prepared and used as a supporting electrolyte. 3 µm

alumina was utilized for polishing glassy carbon electrode on a smooth polishing cloth and washed with distilled water and ethanol prior to each voltammetric analysis.

The synthesis of NiO NPs

NiO nanoparticles were synthesized by chemical precipitation. First, 9.1 g $Ni(NO_3)_2.6H_2O$ was dissolved in 50 mL deionized water, 1 g CTAB was added to the solution as surfactant and pH was adjusted to 12 using NaOH solution. This solution was stirred for 2 h at room temperature. The light-green precipitate obtained by centrifugation was washed with deionized water and ethanol three times, dried at 70 °C for 24 h and finally, calcined at 600 °C for 2 h. The resultant product was analyzed by SEM, XRD and FT-IR techniques.

The preparation of NiO@Ppy/GO nanocomposite

GO was prepared according to the modified Hummer's method. Briefly, a mixture of 4 g graphite, 100 mL H_2SO4 (98%) and 4 g $NaNO_3$ was prepared, stirred for 2 h in an ice bath and then 12 g $KMnO_4$ was added to the mixture. After 6 h stirring at 35 ˚C, the mixture was cooled to the room temperature and 10 mL 30% H_2O_2 was added to it. The obtained product was centrifuged, washed with 30% HCl and deionized water three times and dried in an oven at 60˚C for 24 h. The obtained product was investigated by SEM.

The synthesis of Ppy-coated NiO nanocomposite was performed by the in-situ polymerization of pyrrole on the GO sheets. For preparation of NiO@Ppy/GO nanocomposite, its composition was optimized using experimental design technique which can simultaneously appraise the effects of

many variables affecting the composite properties. We used Central Composite Design (CCD), the most favorable program, to design the experimental system. So, a mixture containing 3 g NiO NPs and 50 mL 0.6 M aqueous solution of $FeCl_3$ was prepared and stirred to disperse NPs. 0.2 g GO was added to it and ultrasonicated for 30 min. Afterwards, 0.5 mL pyrrole was added dropwise to the mentioned mixture and the reaction was allowed to proceed for 12 h under continuous stirring at room temperature. The produced precipitate was separated by centrifugation, washed with distilled water and ethanol and dried in vacuum at 50 °C for 24 h. The obtained powder was studied by SEM, EDX.

General procedure

For cyclic and square wave voltammetric measurements, glassy carbon electrode was polished with alumina and washed with distilled water and ethanol. Therefore, 0.002 g NiO@Ppy/GO nanocomposite was mixed with 2 µL nafion as a binder and casted on the surface of glassy carbon electrode using micropipette. Then, an opportunity was given to dry at room temperature for 2 h. A 10 mL volume of 0.1 M phosphate buffer solution at pH 7.0 was placed in the electrochemical cell. Then, piceatannol in the range of 0.01-**10.0 µM was a**dded into the supporting electrolyte with micropipette. The solution was continuously stirred for 10 min. The modified electrode was put in the cell and the electrochemical procedure was performed. Finally, the proposed electrode signal was recorded in the potential range of 70-2**50 mV, the scan rate of 100 mV s^{-1}, the pulse amplitude of 25 mV, the step** potential of 4 mV and the frequency of 15 Hz using SW voltammetry technique.

Results and discussion.

The characterization of NiO@Ppy/GO nanocomposite

The shape and morphological characteristic of NiO NPs were investigated by SEM and its image is presented in Fig. 1a. It can be clearly seen that NiO NPs are spherical, monodispersed with an average diameter of 27 nm. Fig. 1b shows the SEM image of the prepared GO nano sheets and indicates that layered, thin and partly aggregated sheets with smooth surface have been successfully formed in this study. The SEM image of the NiO@Ppy/GO nanocomposite is shown in Fig. 1c. The nanospheres of NiO@Ppy are well dispersed on the GO surface, the middle size of nano spheres is about 40 nm which confirms Ppy layer formation atop the NiO NPs. Probably, electrovalent interaction between NiO NPs and Ppy molecules has been led to the formation of NiO@Ppy nanocomposite. Moreover, the EDX analysis of NiO@Ppy/GO nanocomposite (Fig. 1e) confirms the presence of C, O, N and Ni elements that are agree with sample composition.

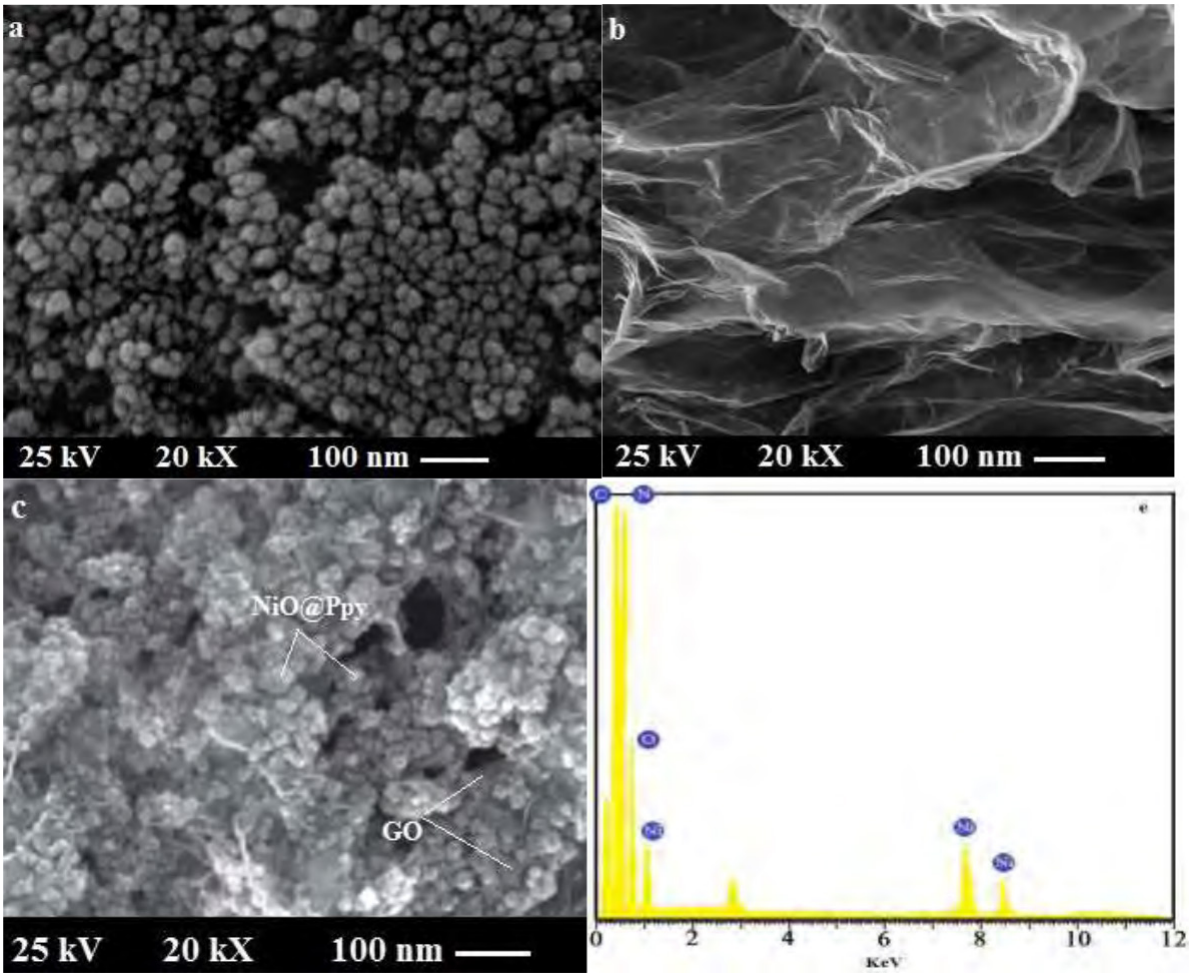

Fig 1: a) The SEM image of NiO NPs, b) The SEM image of GO, c) The SEM image of NiO@Ppy/GO nanocomposite, e) EDS spectrum of NiO@Ppy/GO nanocomposite.

The electrochemical behavior of the modified electrode in determination of piceatannol

The electrochemical oxidation of 10.0 µM piceatannol in 0.1 M PBS (pH 7.0) at GCE, NiO@Ppy/Nafion/GCE and NiO@Ppy/GO/Nafion/GCE were studied by square wave voltammograms at the scan rate of 100 mV s^{-1}. The results have been indicated in Fig. 2. As can be seen, the bare GCE (purple peak) exhibits a low sensitivity of piceatannol. Therefore, in order to increase the sensitivity and reduce the overpotential of piceatannol oxidation, using sensing material for modification of electrodes is necessary. By applying the NiO@Ppy/Nafion/GCE (red peak), the anodic peak current of piceatannol was increased and the peak potential was shifted towards negative values suggesting that Ppy can enhance the electrochemical oxidation of piceatannol and NiO accelerate the electron transfer kinetics. In contrast, NiO@Ppy/GO/Nafion/GCE (blue peak) showed high and sharp oxidation peak response, electrocatalytic activity and reduced overpotential comparing with other electrodes due to GO existence. Therefore, NiO@Ppy/GO/Nafion/GCE showed the excellent effects, such as high electrical conductivity, an enhanced electron transfer and high electrocatalytic activity.

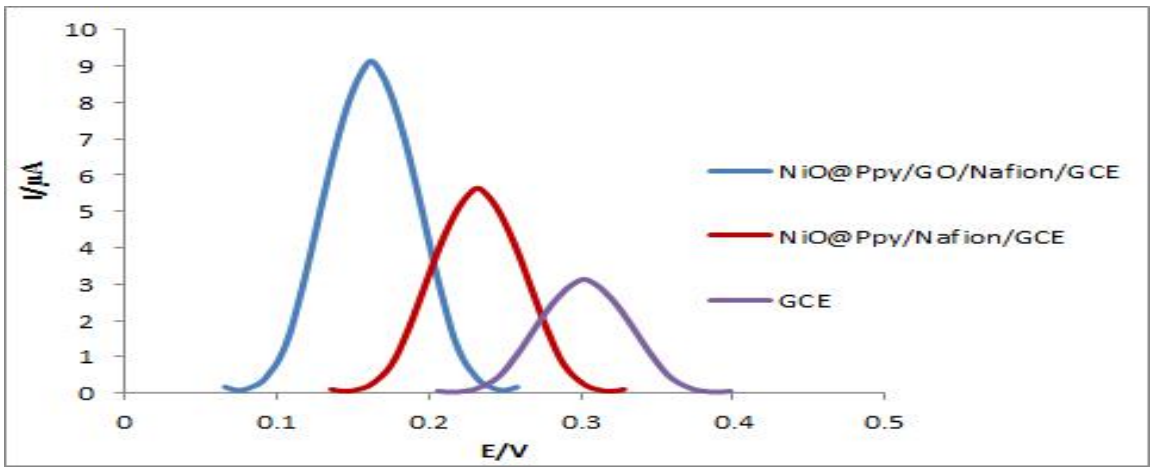

Fig 2: The SW voltammograms of the bare (GCE) and modified electrodes in the presence of 10 μM piceatannol in 0.1 M PBS (pH 7.0) at the scan rate of 100 mVs^{-1}

The effect of scan rate

For investigating the nature of electrochemical process on anodic peak current and the peak potential of 5.0 μM piceatannol, cyclic voltammograms (Fig. 3) for the modified glassy carbon electrode were studied in the range of **2-100 mVs^{-1}. It is found that the oxidation peak current was increased with increasing scan rates without** changing peak potential shifts toward positive values. The insets of Fig. 3 shows the square root of scan rate (ν ½) and scan rates (ν) versus the anodic peak currents. The results indicate that there is a linear relation between the piceatannol anodic peak current and the square root of scan rates, while increasing scan rate peak currents resulted in getting out of line. So, the modified electrode process was controlled by diffusion.

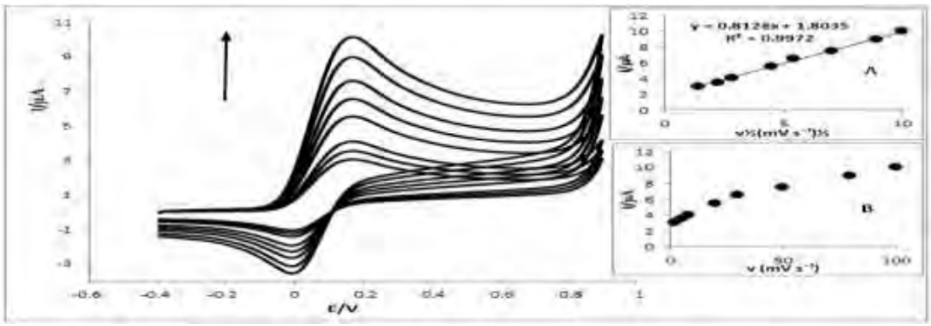

Fig 3: The cyclic voltammograms of piceatannol on the modified GCE at different scan rates (2, 5, 8, 20, 30, 50, 80 and 100 mV s^{-1}) in 0.1 M PBS (pH 7.0). Inset: (A) The relationships of the anodic peak current with square root of scan rate (ν½), (B) scan rates (ν).

The analytical performance of the proposed method

Under the optimized conditions, the figures of merit including linear range, limit of quantification (LOQ), limit of detection (LOD), relative standard deviation (RSD) and also stability were evaluated. Fig. 4 shows the SW voltammograms of the different concentrations of piceatannol obtained by NiO@Ppy/GO/Nafion modified glassy carbon electrode in 0.1 M phosphate buffer solution at biological pH (pH 7.0). Based on the results, by increasing concentration values from 0.01 to 10.0 μM, the anodic peak current was gradually increased. The inset of Fig. 4 confirms the linear relationship between the various concentrations of piceatannol and oxidation peak current with the correlation coefficient (R^2) of 0.9974. The LOQ is expressed with $10S_b/m$ where S_b is the standard deviation of blank sample and m is the slop of calibration plot. So, LOQ was obtained 0.0099 μM. The LOD according to the equation of $3S_b/m$ was calculated 0.003 μM. For investigating the repeatability of method, the RSDs for six replicate measurements at 3.0 μM and 6.5 μM piceatannol were obtained to be 3.25% and 1.83%, respectively. The stability of the modified glassy carbon electrode was evaluated by multi-scan cyclic voltammetry.

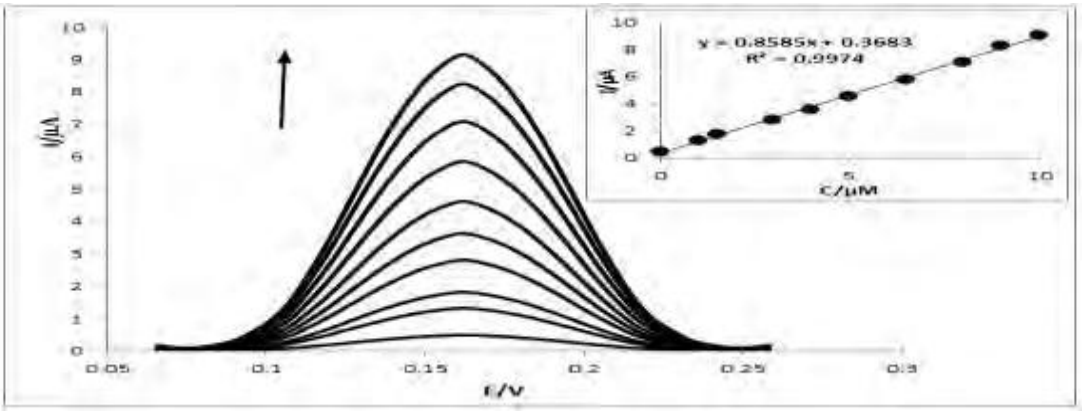

Fig 4: The SW voltammograms response at NiO@Ppy/GO/Nafion/GCE in 0.1 M PBS (pH 7.0) with the different concentrations of piceatannol (down to up: 0.01, 1.0, 1.5, 3.0, 4.0, 5.0, 6.5, 8.0, 9.0 and 10.0 µM). Inset: The linear relationship of anodic peak currents vs. the concentration of piceatannol. Instrumental parameters: scan rate 100 mV s^{-1}, pulse amplitude 25 mV, step potential 4 mV and frequency 15 Hz.

The investigation of interfering compounds

One of the necessary parameters in any analytical method is selectivity. Under the optimal conditions, the influence of some inorganic ions (Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, I^{-}, Cl^{-}) and organic compounds such as glucose, dopamine, fructose, citrate, maltose, uric acid, ascorbic acid, quercetin, resveratrol, piceid, astringin, pterostilbene and proanthocyanidins as co-existing species in the presence of piceatannol were investigated using the proposed method. As can be seen in Table 1, with increasing 1000-fold of inorganic ions and excess of 100-fold of organic compounds to 6.5 µM piceatannol, no significant change in the anodic current of piceatannol was observed and interferences caused an error less than ±5% for the determination of piceatannol.

Table 1: The effect of interferences on the quantification of piceatannol.

Interferences	Molar ratio of the interferences to piceatannol	Recovery (%)
Glucose	350	102.1
Dopamine	700	99.70
Fructose	300	101.2
Citrate	200	99.30
Maltose	400	102.5
Uric acid	650	101.8
Ascorbic acid	550	100.5
Quercetin	250	99.50
Resveratrol	100	101.3
piceid	200	102.0
Astringin	150	101.4
Pterostilbene	200	102.3
Proanthocyanidins	300	101.1
Na^{+}, K^{+}, Mg^{2+}, Ca^{2+}, I^{-}, Cl^{-}	1000	100.2

The determination of piceatannol in real samples

The analytical application of the proposed sensor was performed in two real samples including grape skin essential oil and. So, the essential oil was diluted with analytical grade ethanol prior to each experiment and a certain amount of it was added to the electrochemical cell and used for the voltammetric measurements of piceatannol, demonstrating the good selectivity of our electrochemical sensor in real essential oil grape sample. Fig. 5 shows the SW voltammograms of piceatannol-added essential oil sample in which the concentration of piceatannol is rising. As can be seen, the piceatannol -added essential oil sample illustrated two well peaks at 0.16 V and 0.35 V. The first anodic peak is caused from the piceatannol oxidation and the second peak

depended to the oxidation of the electroactive component of grape skin essential oil. The value of first peak current gradually increased with the continuous addition of piceatannol. So, the results showed that the observed peak at 0.16 V related to the oxidation of piceatannol.

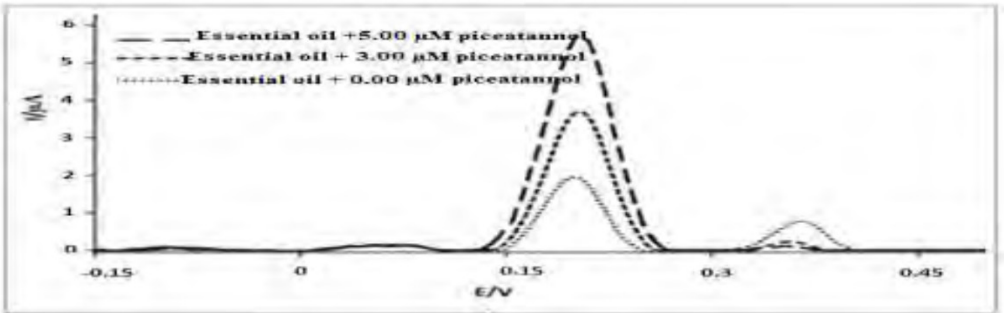

Fig 5: The SWVs of piceatannol-added grape skin essential oil sample with rising piceatannol concentration.

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