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Original scientific paper

Glutathione detection at carbon paste electrode modified with ethyl 2-(4-ferrocenyl-[1,2,3]triazol-1-yl)acetate, $ZnFe_2O_4$ nanoparticles and ionic liquid

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Abstract

The purpose of the present study was to introduce a newly designed approach for determination of glutathione using modified carbon paste electrode with $ZnFe_2O_4$ nanoparticles, ionic liquid (1-butyl-3-methylimidazolium hexafluorophosphate) and ethyl-2-(4-ferrocenyl--[1,2,3]triazol-1-yl)acetate (EFTA/ZFO/IL/CPE). According to the results from the electrochemical experiments, oxidation current of glutathione on the modified electrode surface was incremented and its oxidation potential was decreased compared to bare CPE. A linear response was observed for the electrode at different glutathione concentrations (0.2 to 300.0 μM).

Keywords

Chemically modified electrode, cyclic voltammetry, differential pulse voltammetry, real sample analysis

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Introduction

Glutathione (GSH,γ-glutamyl-L-cysteinyl-glycine) is an important tripeptide thiol within a eukaryotic and mammalian cell and contributes to several biological functionalities (e.g., expressing gene, synthesizing DNA and protein, producing cytokine, and toxin metabolism). Studies found that glutathione exists in the tissues of mammals and plants at concentrations of about 1 to 10 mM. Unusual levels of glutathione are one of the indexes of different illnesses, including Alzheimer's disease, diabetes, Parkinson diseases (PD), AIDS, arthritis, atherosclerosis, and several kinds of cancers. Therefore, it is of high importance to develop a fast and efficient analytical method for glutathione determination for early medical diagnoses and the prevention of initial outset of side effects [1-3].

There are numerous potential analytical methods for glutathione determination such as spectrophotometry, titrimetry, mass spectrometry, high-performance liquid chromatography, and spectrofluorimetry [4-8]. However, a majority of the mentioned techniques generally have a lot of shortcomings, including complex and costly devices, laborious processes, and the requirements of the trained staff. Therefore, developing of a simple, fast, inexpensive, and accurate technique should be of key importance in this case [9-23]. Electrochemical methods are methods of choice due to their inherent properties, such as cost-effectiveness, easy operation, rapid response, sensitivity and selectivity [24-39].

Due to the inherent sluggishness of heterogenous charge transfer of glutathione at carbon and metallic electrodes, its electrochemical oxidation/reduction is usually performed at modified electrodes coated with the substances which serve as electron transfer mediators, increasing the charge transfer rate. Modifiers are usually prepared in the form of nanomaterials [40-50] which, apart from charge transfer mediation properties, take advantage of their very high surface area, increasing the selectivity and the sensitivity of the method [51-54].

Various nanoparticles are currently being applied in sensors' construction as modifiers. Among them, zinc ferrite nanoparticles caught significant attention in nano-medicine because of low Zn^{2+} toxicity. This is especially desirable for biocompatible MRI contrast agents in medicine due to the high toxicity of existing contrast agents. On the other hand, permissible dosages for $ZnFe_2O_4$ nanoparticles are 18 and 15 mg/day for Fe and Zn, respectively, making them a suitable option for MRI contrast agents. This is considerably higher than other biocompatible materials [55].

Efficient mediator needs to have a low relative molar mass while being reversible, fast reacting, regenerated at low potentials, pH independent, stable in both oxidized and reduced forms, unreactive with oxygen and nontoxic. Among the most successful mediators are those based on ferrocene and its derivatives that meet the above criteria [56].

Ionic liquids (ILs) have been generating increasing interest over the last decade. Ionic liquids have a great potential for possible electrochemical applications due to the high thermal stability, no volatility, high polarity, large viscosity, high intrinsic conductivity, and wide electrochemical window [56].

In this study we focused on the electrochemical oxidation of glutathione using ethyl-2-(4-ferrocenyl-[1,2,3]triazol-1-yl)acetate (EFTA) as a mediator in a composite electrode consisting of EFTA/$ZnFe_2O_4$ NPs/ionic liquid modified carbon paste electrodes surfaces (EFTA/ZFO/IL/CPE).

Experimental

Chemicals and instruments

A potentiostat/galvanostat Autolab PGSTAT 302N instrument equipped with a general-purpose electrochemical system (GPES) software has been utilized to measure electrochemical parameters. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and EFTA/ZFO/IL/CPE were used as the reference, auxiliary and working electrodes, respectively. A digital pH-meter (Metrohm 710) was employed for measuring the pH values.

The chemicals and solvents were used without further purifications from Aldrich. The buffer solutions of different pH values (between 2.0 and 9.0) were prepared from orthophosphoric acid and its salts.

Preparation of modified electrode

EFTA (0.01 g) was mixed manually with graphite powder (0.95 g) and $ZnFe_2O_4$ nanoparticles (0.04 g). Then certain levels of ionic liquid and liquid paraffin were added and blended for 20 minutes. The uniform wet paste was appended into the bottom of a tube made up of glass of 15 cm length and 3.4 mm in diameter. Electrical contacts were created by positioning copper wires inside the carbon paste. Additionally, extra paste was inserted into the tube and polished by a weighing paper to establish a fresh surface.

Results and discussion

Electrocatalytic oxidation of glutathione at EFTA/ZFO/IL/CPE

Figure 1 shows cyclic voltammograms of the EFTA/ZFO/IL/CPE with (trace a) and without (trace b) added glutathione in the solution. For comparison, the response of unmodified CPE in the presence of glutathione is also shown (trace c). The redox reaction shows one reversible redox peak pair at the potentials of 330 mV. According to the literature the peaks correspond to one-electron reduction/oxidation of ferrocene moiety. By adding the glutathione into the solution, anodic current is significantly increased while cathodic current disappeared. Such behavior is a characteristic of a mediated electron transfer confirming the hypothesis of a mediated oxidation reaction of glutathione by EFTA at modified CPE. The reaction mechanism of glutathione at EFTA mediated electrode is described by Scheme I.

pH dependence of the glutathione oxidation at modified electrode shows mediated current maximum in neutral solution so the medium of pH 7.0 was used for further study.

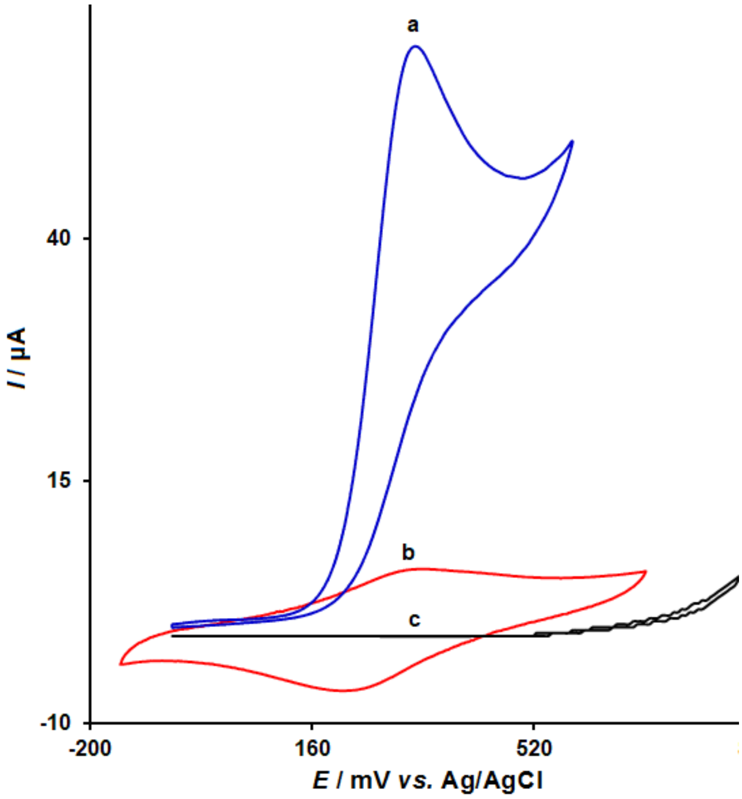
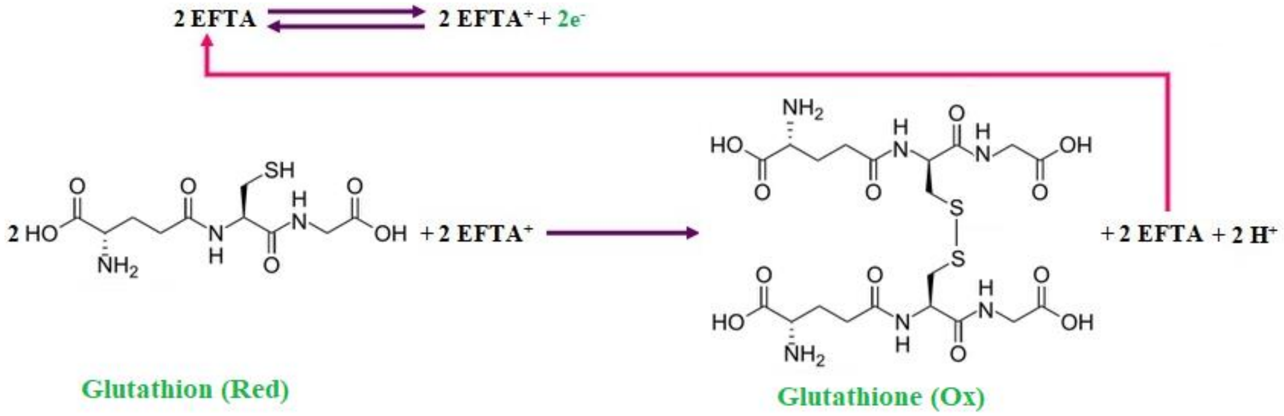


Figure 1. *CVs of (a) EFTA/ZFO/IL/CPE in 0.1 M PBS (pH 7.0) consisting of 100.0 µM glutathione, (b) EFTA/ZFO/IL/CPE in 0.1 M PBS (pH 7.0), and (c) un-modified CPE in 0.1 M PBS (pH 7.0) with 100.0 µM glutathione*

Linear sweep voltammetry (LSV) method was performed to assess the scan rate effect on electrochemical oxidation of glutathione in 0.1 M phosphate buffer solution (PBS) at a pH of 7.0. As seen in Figure 2 an increase of the scan rate from 5 mV/s to 100 mV/s caused an increase of the oxidation current and a slight positive shift of oxidation peak potential. The plot of the oxidation peak current (I_p) vs. the square root of scan rate ($v^{1/2}$) is linear (Figure 2 inset) indicating a diffusion-controlled oxidation process [57]. In order to determine the diffusion coefficient of glutathione, potential steps from 0 to 380 mV were taken at several concentration of glutathione and current versus time were recorded (Figure 3). From the linear plots of I *vs.* $t^{-1/2}$ (Figure 3 inset), at various concentrations indicated in the figure, the mean diffusion coefficient of 3.0×10^{-6} cm^2/s was calculated.



Scheme 1. *Electrocatalytic oxidation mechanism of glutathione at modified electrode*

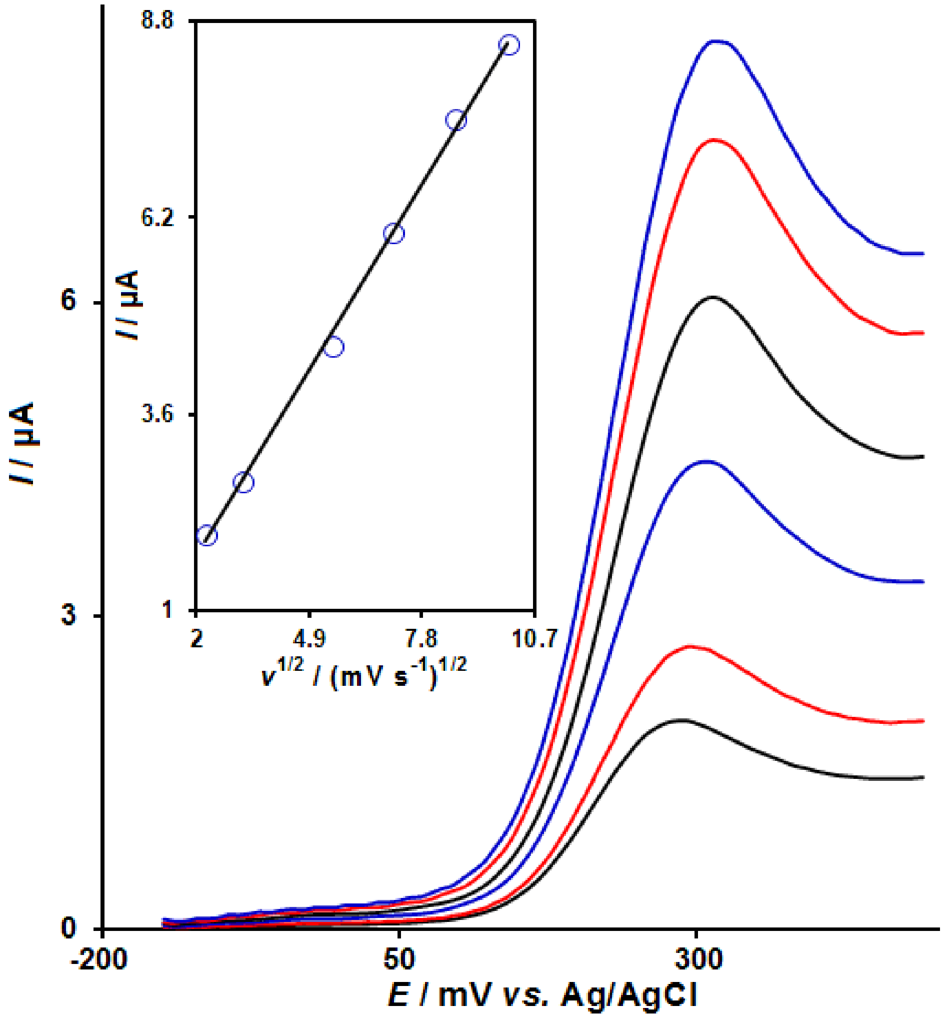


Figure 2. *LSVs of EFTA/ZFO/IL/CPE in 0.1 M PBS (pH 7.0) with 100.0 μM glutathione at different rates of scans. Values 1 to 6 respectively is corresponding to 5, 10, 30, 50, 75 and 100 mV s^{-1}. Inset: Changes in the anodic peak currents against v$^{1/2}$*

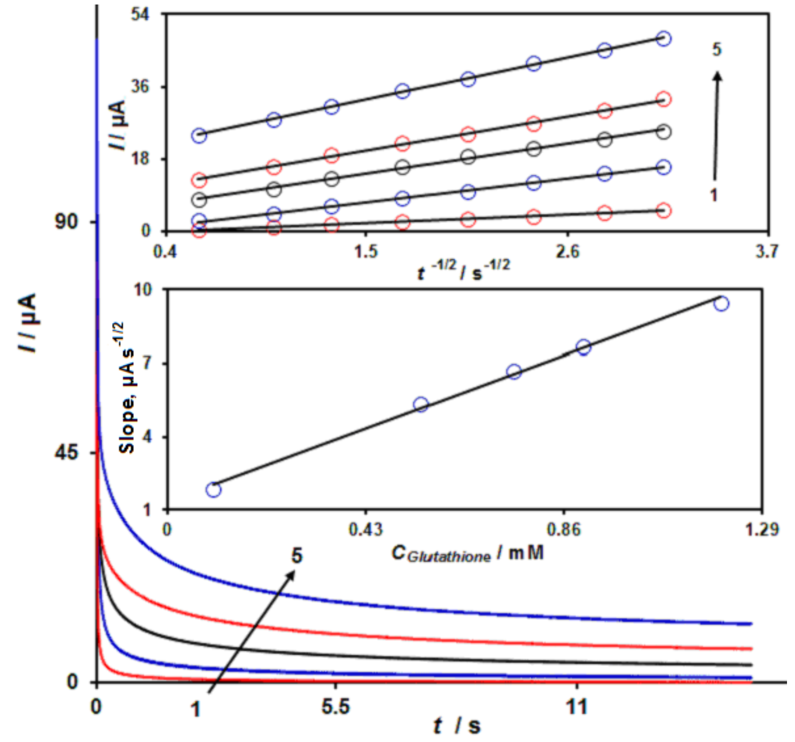


Figure 3. *Chronoamperograms gained at EFTA/ZFO/IL/CPE in 0.1 M PBS (pH 7.0) at various levels of glutathione concentration. 1 to 5 is corresponding to 0.1, 0.55, 0.75, 0.9, and 1.2 mM of glutathione. Insets: up - plots of I versus* $t^{-1/2}$ *achieved by chronoamperegrams 1–5; down - Plot of the straight line slop* vs. *glutathione concentrations*

Electroanalysis of glutathione

The peak current of glutathione using EFTA/ZFO/IL/CPE was utilized for quantitative analysis of glutathione. Since DPV has benefits in terms of the greater sensitivity and better informative application features, the adjusted electrode has been employed as the working electrodes in analysing DPV. Regarding the DPV of glutathione using the EFTA/ZFO/IL/CPE, linear response was observed in a range from 2.0×10^{-7} - 3.0×10^{-4} M with the correlation coefficient of 0.9991 (Figure 4). In addition, the related limit of detection of 0.07 µM has been obtained. Table 1. presents a comparison of the analytical figures of merit of the proposed work with other modified electrodes for the detection of glutathione.

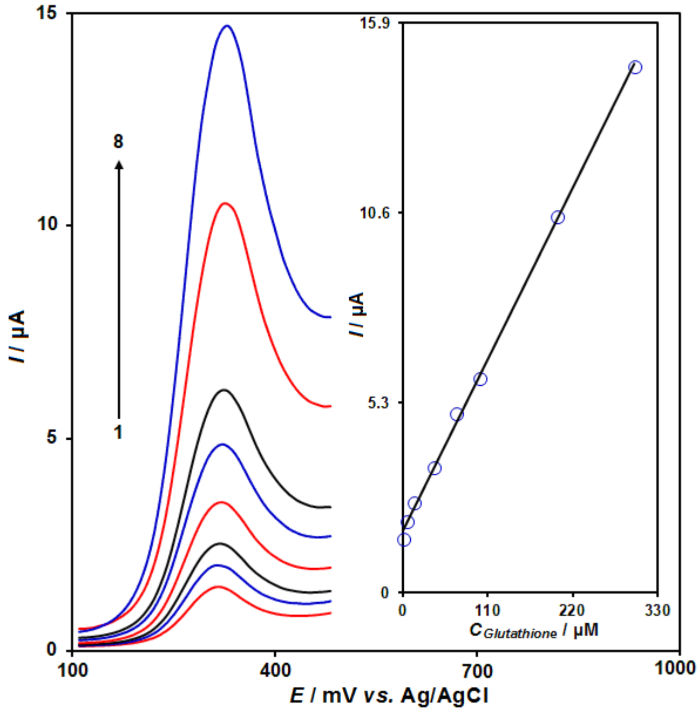


Figure 4. *DPVs of EFTA/ZFO/IL/CPE in 0.1 M (pH 7.0) with various levels of glutathione concentration. 1 to 8 is corresponding to 0.2, 5.0, 15.0, 40.0, 70.0, 100.0, 200.0 and 300.0 µM of glutathione. Inset: DPVs of EFTA/ZFO/IL/CPE in 0.1 M (pH 7.0) with distinct levels of glutathione concentration*

Table 1. *Comparison of the efficiency of some modified carbon paste electrodes used in the electro-oxidation of glutathione, used method - voltammetry*

Modifier	LOD, µM	LDR, µM	Ref.
2CBFAZ	0.02	0.05-200.0	[17]
Ferrocene	2.1	2.2-35.00	[58]
Ferrocene carboxylic acid	0.098	0.1-12.0	[59]
2,7-BFEF	0.5	0.92-11.0	[60]
TTF–TCNQ	0.3	5.0-340.0	[61]
EFTA/$ZnFe_2O_4$ nanoparticles/ionic liquid	0.07	0.2-300.0	This work

Conclusion

This work demonstrates that electrocatalytic activity of EFTA/ZFO/IL/CPE can be successfully utilized for the development for the fast and efficient electroanalytical methodology for glutathione determination. The electrode has linear response over wide concentration range with the limit of detection of 0.07 µM.

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