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Electroanalysis of Amino Acid Tyrosine by an Electrochemical Sensor Modified by Strontium Ferrite Nanostructure

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An electrochemical sensor was fabricated by strontium ferrite (SrFe) nanostructure modified carbon paste electrode (SrFeME). This electrode showed a good catalytic effect on oxidation of amino acid tyrosine (Tyr) in voltammetric studies. It can improve the anodic peak current of Tyr significantly and also decrease the oxidation overpotential of this amino acid. The peak current increased by two times and the overpotential decreased more than 110 mV. The enhanced oxidation signal of Tyr at the modified electrode is attributed to the improved electron transfer rate at these nanoparticles and enhanced active surface area of the modified electrode by the nanoparticles. This electrode was used as an electrochemical sensor for the measurement of Tyr by differential pulse voltammetry. The anodic peak current was linearly correlated to Tyr concentration in the range from 0.8-300.0 μM , and the detection limit calculated was 0.15 μM ($S/N = 3$). The presence of ascorbic acid, dopamine, glucose and a number of ions could not affect the Tyr measurement. Finally, the proposed sensor was successfully used for measurement of Tyr in real samples.

Keywords: Electrode, Electroanalysis, Nanomaterials, Voltammetry

INTRODUCTION

Tyrosine (Tyr) or 4-hydroxyphenylalanine is a non-essential amino acid. In addition to being a proteinogenic amino acid, tyrosine has a special role by virtue of the phenol functionality [1]. Tyrosine is necessary for the manufacture of thyroid hormones, and is a precursor to some neurotransmitters (*e.g.* dopamine) and can participate as phosphotyrosine in proteins that are part of signal transduction processes [2]. In plants, Tyr also plays an important role in photosynthesis and is a precursor to alkaloids, natural phenols and pigments [3]. In humans, Tyr absence could produce albinism, hypochondria, or depression and in contrast, its high concentration in culture medium increases sister chromatid exchange [4].

Determination of biological substances can be performed by different instrumental analytical methods.

Among these, electrochemical methods have some advantages such as simplicity, inexpensively, availability and applicability in different media [5-7]. Tyr as other biological substances with redox activity can be determined by electrochemical methods at the surface of modified electrodes [8-11].

Nanostructured materials with high surface to volume ratio and quantum properties have various applications in different scientific fields [12-14]. Modification of different types of the electrodes with nanostructured materials is a noticeable task for the researchers [15-17]. On the other hand, ferrites are basically a class of materials containing iron oxides with fascinating magnetic and electric properties. Strontium ferrite nanostructure ($\text{SrFe}_{12}\text{O}_{19}$) has attracted lots of attention due to its vast area of applications beside stable characteristics. Crystal structure of M-phase ferrite is complex but can be described as a hexagonal structure with unique axis. $\text{SrFe}_{12}\text{O}_{19}$ possesses 64 ions per unit cell along with 11 different symmetry sites. Oxygen atoms are closely packed at interstitial position with Sr and

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Fe in ten layers along with the C-axis. The iron atoms are positioned at five different crystallographic sites including octahedral site, tetrahedral site and trigonal bipyramid [18]. Structures and materials based on strontium ferrite, due to their specific characteristics, have widespread applications in the electrical and radio, computer and medical industries as well as in the structure of electrodes, fuel cells and second type batteries [19,20].

In this research, due to the importance of Tyr measurement from biological point of view, a novel electrode was developed as a Tyr sensor. This electrode was modified by strontium ferrite nanoparticles for the first time. It can measure Tyr in a simple, sensitive, accurate, and inexpensive pathway. Also, the selectivity of the electrode for Tyr was investigated rather than some inorganic ions and biological compounds and acceptable results were obtained.

EXPERIMENTAL

Apparatus and Chemicals

Electrochemical measurements were performed by a potentiostat/galvanostat (SAMA 500, electroanalyzer system, I.R. Iran). All electrochemical measurements were carried out in a three electrode cell including modified or bare CPE, saturated calomel electrode (SCE) and a platinum wire as working, reference and counter electrodes, respectively. The pH value of solutions was adjusted using a Milwaukee pH meter.

All solutions were freshly prepared with deionized water. Phosphate buffers were prepared from orthophosphoric acid and its salts (Merck, Germany). The graphite fine powder and paraffin oil (DC 350, 0.88 g cm^{-3}), both from Merck (Germany), were used as received. Tyr and all other reagents were from Sigma-Aldrich. The strontium ferrite nanopowder with a particle size less than 100 nm (obtained by the BET technique) has a chemical formula $\text{SrFe}_{12}\text{O}_{19}$, and crystalline structure with a hexagonal phase.

Fabrication of the Modified Electrode

The strontium ferrite modified electrode (abbreviated as SrFeME) was prepared by mixing 0.05 g of strontium ferrite nanoparticles, 0.95 g of graphite powder and appropriate

amount of paraffin oil with a mortar and pestle until a uniform paste was obtained. The paste was then packed into the end of a plastic tube (3.5 mm i.d. and 10 cm long). The electrical contact was provided by inserting a copper wire into the carbon paste. The unmodified carbon paste electrode was prepared in the same way without adding strontium ferrite nanoparticles.

RESULTS AND DISCUSSION

At first, the electrochemical behavior of Tyr and its electrochemical determination at the surface of the proposed electrode; *i.e.*, SrFeME, are investigated. Then, the calibration curve and analytical parameters of this method as well as the ability to measure the real samples are investigated.

Electrochemistry of Tyr at the Modified Electrode

The electrochemical behavior of Tyr at SrFeME was investigated by cyclic voltammetry technique. Figure 1 shows the cyclic voltammograms of Tyr at modified and unmodified electrodes at scan rate of 50 mV s^{-1} in phosphate buffer solution pH = 7.0.

As can be seen, the peak potential of the Tyr oxidation at SrFeME is about 740 mV (curve a in Fig. 1), while its oxidation at the unmodified electrode surface is greater than 850 mV (Curve b in Fig. 1). Curve (c) shows the response of the SrFeME in the buffer solution without Tyr, which demonstrates this electrode is not electroactive in this pH. Also, Tyr begins to oxidize on SrFeME at potential of 500 mV, while its starting potential for oxidation at the bare electrode is greater than 600 mV. The electrocatalytic role of SrFe nanoparticles in decreasing the oxidation potential of Tyr is related to the improving and increasing the electron transfer rate at these nanoparticles.

In addition to decrease in the Tyr oxidation potential at the modified electrode, a significant increase in the anodic current (peak height and area of the sub-peak) is observed at SrFeME. This observation is related to increase in active surface area of the modified electrode by SrFe nanoparticles. The active surface area of different electrodes was calculated using the slope of the Randles-Seveik equations obtained by the cyclic voltammograms of potassium hexacyanoferrate(II) at different potential scan

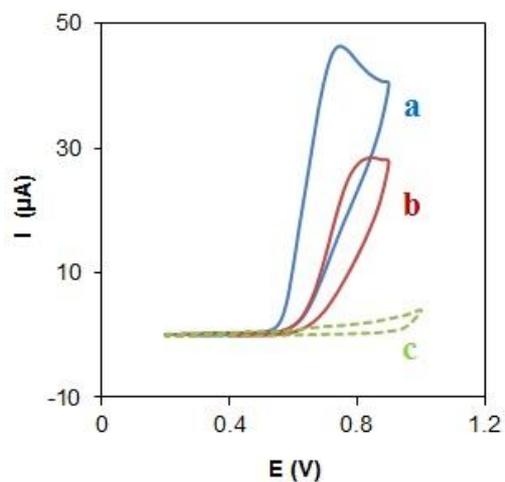


Fig. 1. Cyclic voltammograms of Tyr (with concentration of 0.1 mM in a 0.1 M phosphate buffer solution with pH = 7.0) at the surface of (a) SrFeME and (b) unmodified electrode; (c) shows the response of the modified electrode in the buffer solution.

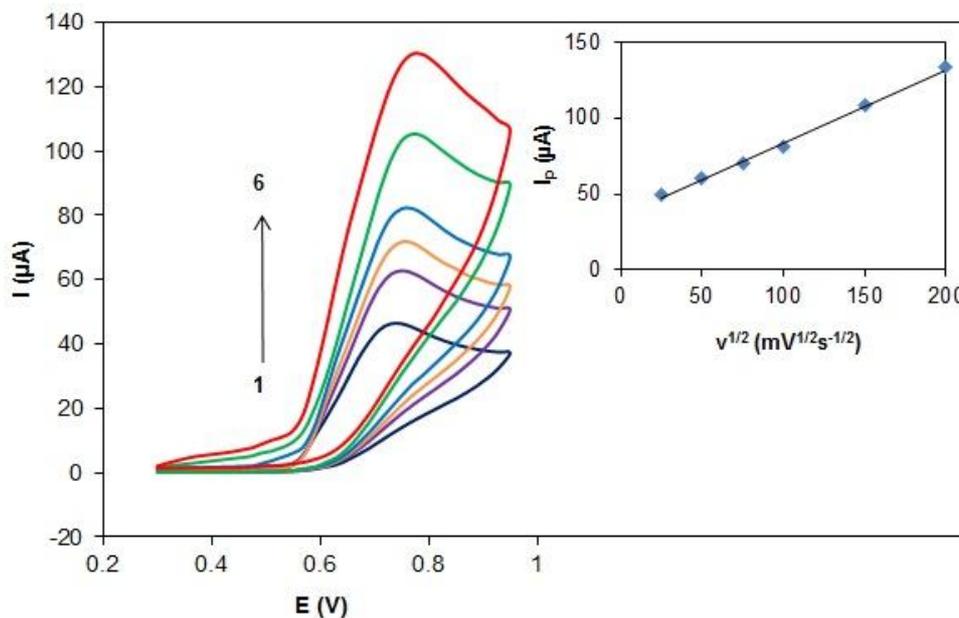


Fig. 2. Cyclic voltammograms for SrFeME in phosphate buffer solution with pH of 7, containing 0.1 mM Try at various potential scan rates. The numbers 1 to 6 correspond to 25, 50, 75, 100, 150 and 200 mV s^{-1} ;

rates. The active surface area was obtained as 0.19 cm^2 for SrFeME, which is almost twice the amount of unmodified electrodes (*i.e.*, 0.1 cm^2). It should be noted that the geometric surface area of both electrodes is approximately

0.09 cm^2 .

Reducing the potential and increasing the current indicate that the modified carbon paste electrode with strontium ferrite nanoparticles enhances the electrochemical

oxidation signal of Tyr. The increased current enhances the sensitivity of the measurement and decreasing the potential improves the selectivity in electroanalysis.

The Effect of the Potential Scan Rate on Try Oxidation

Figure 2 shows the cyclic voltammograms of SrFeME in phosphate buffer solution containing 0.1 mM Tyr at different potential scan rates. The attached diagram of this figure shows that the peak current of the Tyr oxidation (I_p) is linearly proportional to the square root of the potential scan rate ($v^{1/2}$).

Based on the Randles-Seveik equation and the diagram attachment on the Fig. 2, it can be concluded that the electrocatalytic oxidation process of Tyr at the SrFeME is controlled by mass transfer and the species must be diffuse to the electrode surface for the oxidation [21].

Tyr Oxidation Study Using Chronoamperometry

Electrocatalytic oxidation of Tyr at the surface of SrFeME was also studied by chronoamperometric technique. Figure 3 shows the chronoamperograms of SrFeME and unmodified electrode (conventional carbon paste) at potential step of 0.7 V in the presence of Tyr. As can be seen, the modified electrode has a higher steady state current than the bare electrode at the same potential step over 10 s measurements. Therefore, Tyr is more easily oxidized in the presence of SrFe nanoparticles at the modified electrode [5]. Thus, the electrocatalytic effect of the strontium ferrite nanoparticles is confirmed once again for Tyr oxidation.

The diffusion coefficient of Tyr was obtained by chronoamperometry technique. The chronoamperograms of different concentrations of Tyr and the plots of the $I-t^{1/2}$ extracted from them are presented at Figure 4 and its inset A. The slopes of the resulting straight lines are plotted *versus* the Tyr concentrations (Fig. 4B). The slope of this plot and Cottrell equation give the diffusion coefficient of Tyr as $3.53 \times 10^{-5} \text{ cm}^2 \text{ s}^{-1}$ [20]. The obtained diffusion coefficient for Tyr is in a close agreement with the coefficient reported by others [8].

The Effect of the Solution pH on Try Oxidation

The influence of the solution pH on the current and

potential of the Tyr at SrFeME were investigated in the pH range from 2.0-12.0 by differential pulse voltammetry (DPV). As shown in Fig. 5, by increasing pH, the oxidation potential of Tyr becomes more negative. The inset of Fig. 5 shows the linear relationship between the potentials and solution pH with the Nernstian slope of 0.058 V, indicating an electrochemical reaction with the same number of protons and electrons. Also, the effect of solution pH on the peak current of Tyr was investigated. As shown in Fig. 5 and its inset, the maximum oxidation peak current of Tyr oxidation occurs in pH 7.0 at SrFeME. Therefore, the near biological pH value of 7.0 was selected as an optimum solution pH.

Determination of Tyr and Calibration Plot

The calibration plot of SrFeME for Tyr was obtained, firstly. The technique of DPV was used to obtain the calibration plot. The DP voltammograms of different concentrations of Tyr are shown at Fig. 6A. The results indicate that the DPV peak currents of Tyr were linearly dependent on the concentration of Tyr in two concentration ranges. The first range is 0.8-8.0 μM (Fig. 6B) and the second range is 8.0-300.0 μM (Fig. 6C). The equations of these calibration plots respectively are:

$$I(\mu\text{A}) = 0.411C_{\text{Tyr}}(\mu\text{M}) + 2.6 \quad (1)$$

$$I(\mu\text{A}) = 0.167C_{\text{Tyr}}(\mu\text{M}) + 5.3 \quad (2)$$

where the I is the anodic peak current and the C_{Tyr} is the concentration of Tyr. The decrease of the slope for second range is related to the kinetic limitations at high concentrations. The detection limit was calculated as 0.15 μM by the slope of the first calibration plot at 3σ level.

The ability of the proposed sensor was compared with some other sensors proposed for determination of Tyr. The results of the comparison are indicated in Table 1. As can be seen, the detection limit of the proposed electrode is better than that of two other works (references [11] and [22]). The DL is similar to references [10] and upper than references [8], but these works is done in pH 3.0 and not in natural pH. The reference [23], which is a molecular imprinted polymer, shows the lowest DL for Tyr. Although this electrode can be used in natural pH, it is not able to work at high

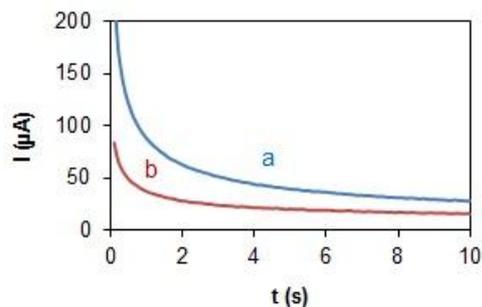


Fig. 3. Chronoamperometric measurements at the potential step of 0.7 V for 1.0 mM Tyr at SrFeME (curve a) and bare CPE (curve b).

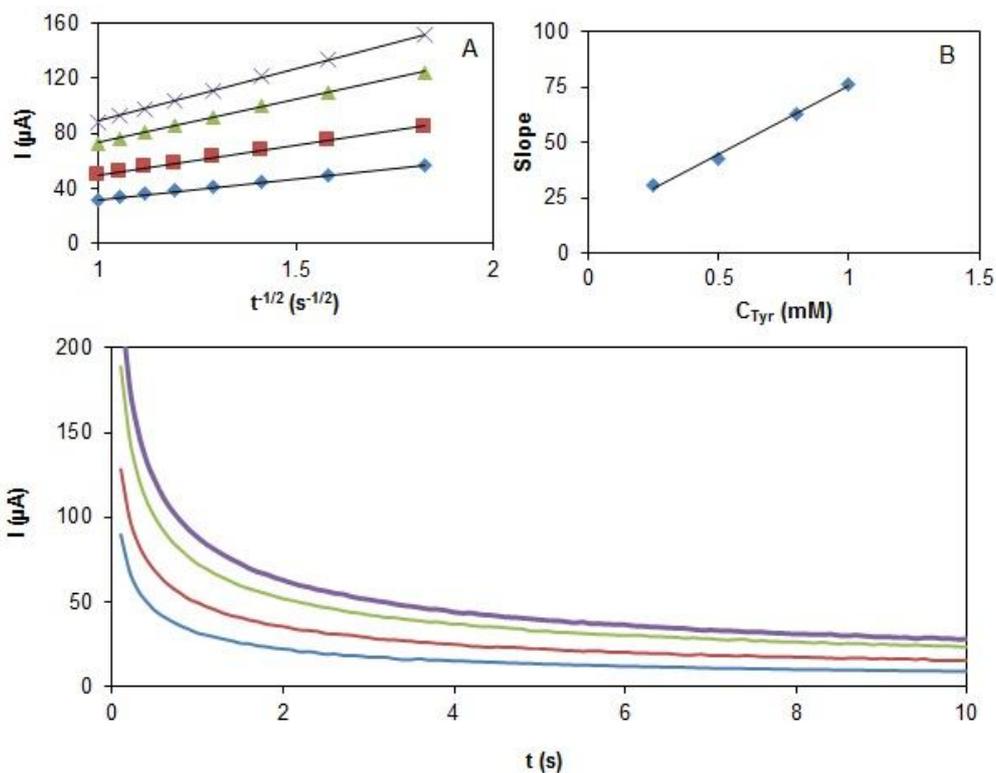


Fig. 4. Chronoamperograms obtained at SrFeME for different concentrations of Tyr from bottom to top: 0.25, 0.5, 0.8 and 1.0 mM; A) plots of I vs. $t^{1/2}$ obtained from chronoamperograms; B) the plot of the slopes of straight lines against Tyr concentration.

concentrations and also its construction is not easy.

Selectivity

Selectivity for each analytical method is defined as the

ability of each method to measure the desired species in the presence of interferences. The selective modified electrode (or sensor) can measure the concentration of the target species in the presence of high concentrations of

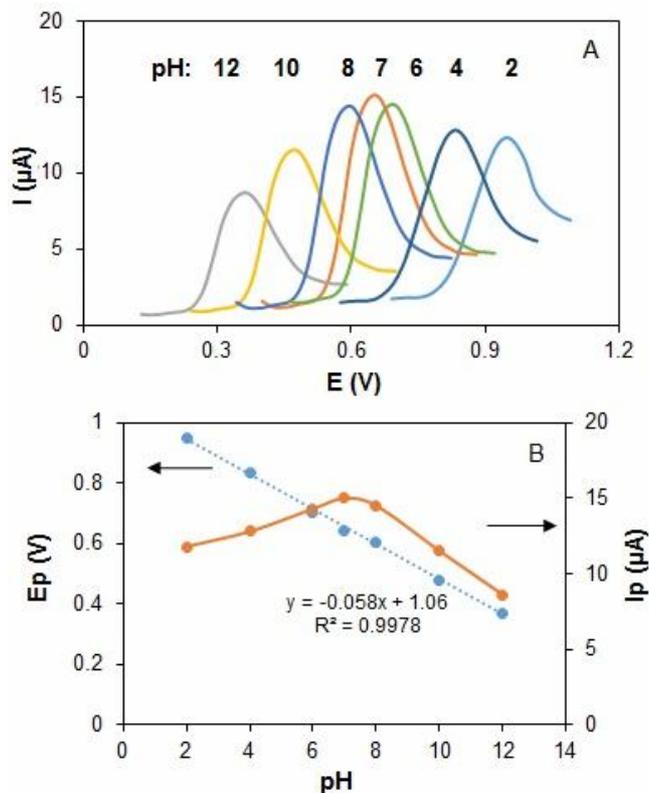


Fig. 5. A) DPVs at SrFeME in different pH values (2.0, 4.0, 6.0, 7.0, 8.0, 10.0 and 12.0) containing 50 μM Tyr, B) the plot of peak potential (E_p) and peak current (I_p) vs. pH.

Table 1. The Comparison of some Electrochemical Sensors in the Electroanalysis of Tyr

Electrode	Modifier	pH	Linear range (μM)	DL (μM)	Ref.
Glassy carbon	Gold nanoparticles/ multiwalled carbon nanotube	6.0	0.4-80	0.21	[11]
Glassy carbon	Graphene nanosheets	3.0	5.0-120.0	0.02	[8]
Carbon paste	Mesoporous silica nanoparticles	3.0	0.5-600	0.15	[10]
Glassy carbon	Zinc Oxide/Graphene	7.0	1.0-800	0.5	[22]
Glassy carbon	MIP/cAuNPs/2-AETGO ^a	7.0	0.001-0.02	0.00015	[23]
Carbon paste	SrFe nanoparticles	7.0	0.8-300	0.15	This work

^aTyrosine imprinted polymer on glassy carbon electrode modified by cubic gold nanoparticles/ 2-aminoethanethiol functionalized graphene oxide.

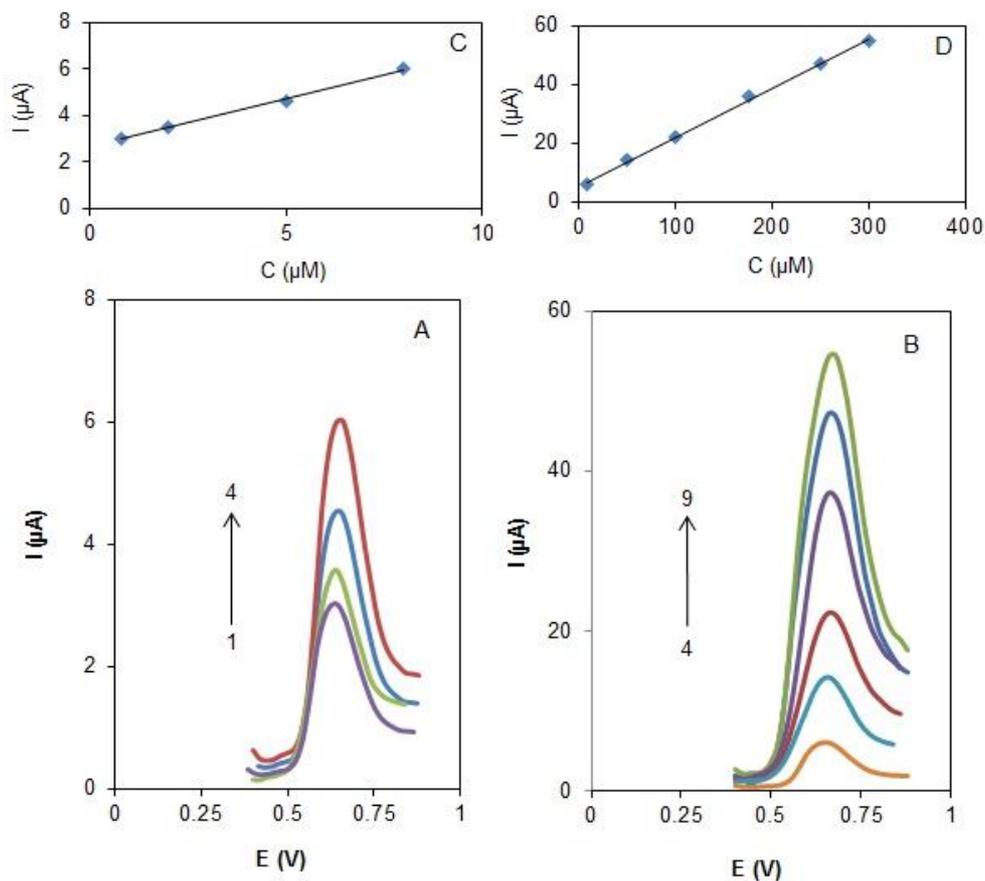


Fig. 6. A) and B) Differential pulse voltammograms of SrFeME in phosphate buffer solution (pH 7.0) containing different concentrations of Tyr, the numbers 1 to 9 corresponding to 0.8, 2.0, 5.0, 8.0, 50.0, 100.0, 175.0, 250.0, 300.0 in μM ; C) and D) are the calibration plots of Tyr.

Table 2. The results of Tyr Measurements in Water and Plasma Samples by SrFeME

Matrix	Sample No.	Tyr found in sample (μM)	Tyr added (μM)	Mean recovery% (n = 5)	RSD (n = 5)
Water	1	N.D. ^a	20.0	100.5	3.1
	2	N.D.	50.0	102.6	2.8
	3	N.D.	100.0	99.0	3.0
Blood plasma	1	91.1	10.0	99.8	3.5
	2	78.5	20.0	105.0	3.2
	3	177.0	30.0	103.5	3.6

^aNot detected.

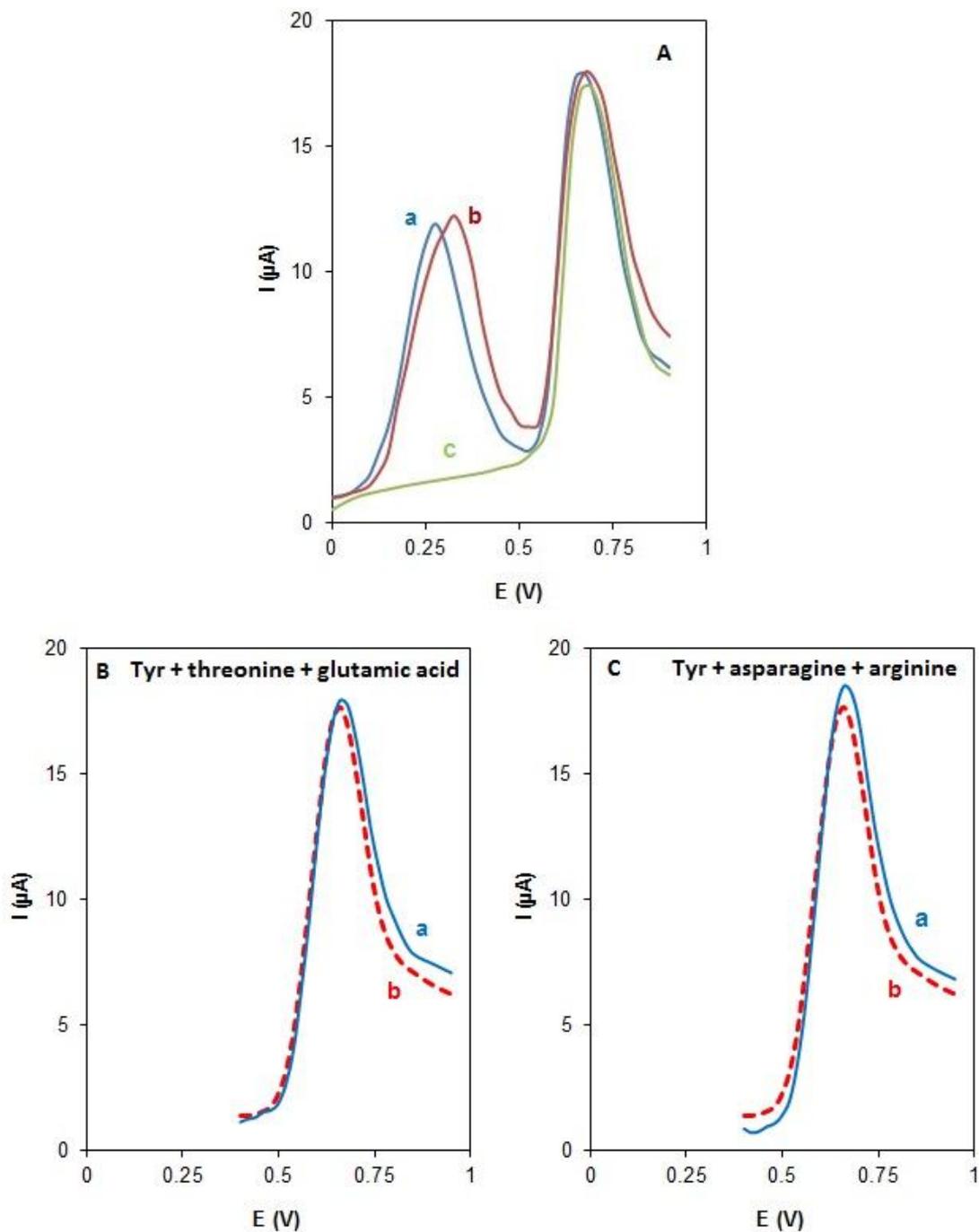


Fig. 7. Differential pulse voltammograms of SrFeME in phosphate buffer solution (pH 7.0) containing A) 70 μM Tyr in the presence of 50 μM ascorbic acid (curve a), dopamine (curve b) and glucose (curve c); B) 50 μM threonine and glutamic acid (curve a); C) 50 μM asparagine and arginine (curve a); in B and C the curves (b) are the DPV of 70 μM Tyr.

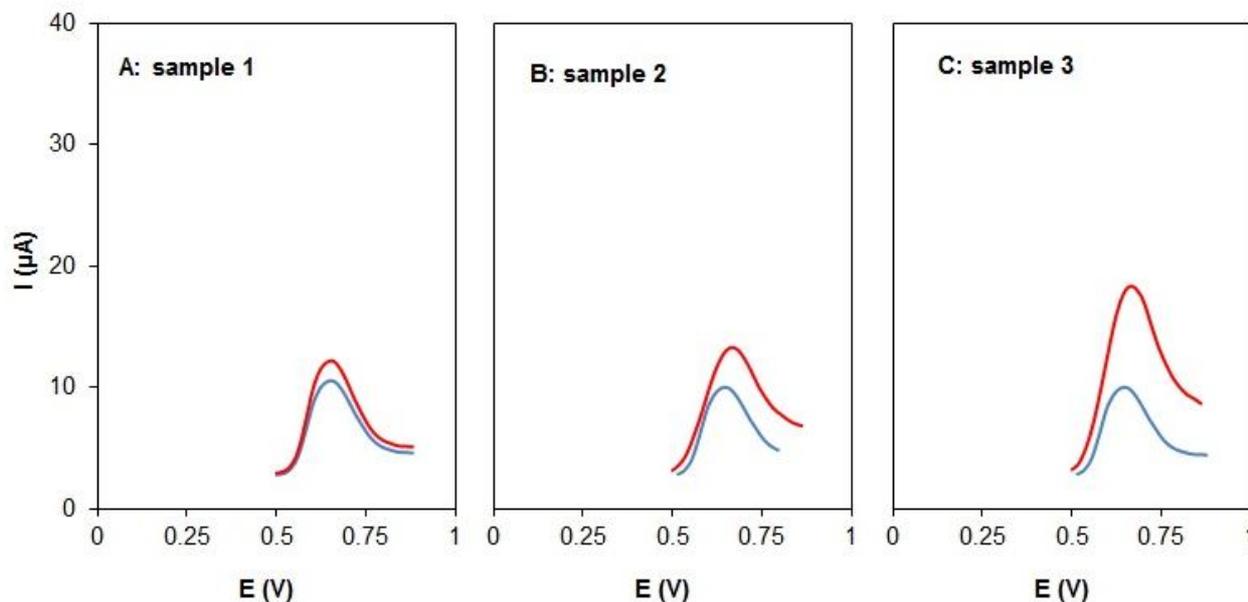


Fig. 8. DP voltammograms of diluted blood serum samples at SrFeME in phosphate buffer solution (below curves with blue colour) and after adding Tyr (upper curves with red colour) with concentration of A) 10, B) 20 and C) 30 μM .

interference substances. This characteristic of the electrode is of great importance in measurement of the species in real samples.

The selectivity of the electrode made in this work was investigated for measuring Tyr relative to the some interference substances. The results showed that ions such as Na^+ , K^+ , Ca^{2+} , Mg^{2+} , NH_4^+ , F^- , Cl^- , CO_3^{2-} , NO_3^- , SO_4^{2-} , PO_4^{3-} and CH_3COO^- at 100-fold concentrations do not have significant effect on Tyr measurements.

Also the presence of some biological substances such as ascorbic acid, catecholamines (*e.g.*, dopamine) and glucose cannot affect the Tyr electrochemical signal. The DP voltammogram of Tyr in the presence of these substances are shown in Fig. 7A. As can be seen, the DPV peaks of the ascorbic acid (curve a) and dopamine (curve b) are separated completely from DPV peak of Tyr. Also, glucose is not oxidized at SrFeME near the Tyr oxidation. The selectivity of the proposed electrode was also examined toward some amino acids. Asparagine, arginine, threonine and glutamic acid, with concentration of 10 times of Tyr, did not have a significant effect on the Tyr electrochemical signal. The DP voltammograms of these amino acids in the

presence of Tyr are shown in Figs. 7B and 7C. However, cysteine and tryptophan make a major disturbance on the Tyr response even at equal concentration with Tyr.

Repeatability, Reproducibility, and Life Time of the Electrode

Repeatability of the measurement was investigated by the proposed electrode. To do this, a solution with a specific concentration of Tyr (50 μM) was tested 10 times by DPV, and peak current was recorded at each time. The relative standard deviation for peak current was calculated as 3.5%.

The reproducibility of the electrode was also evaluated. For this purpose, five different modified electrodes were separately constructed and their peak currents were measured by DPV in a solution containing 50 μM of Tyr. The relative standard deviation for the peak current was obtained by these five electrodes equal to 3.9%.

The lifetime of the electrode was evaluated over a 9-month period. During this period, DPV peak current of Tyr was not significantly different from the initial response. Therefore, the repeatability, reproducibility and lifetime of the proposed modified electrode are appropriate and

acceptable.

Measurement in Real Samples

To evaluate the ability of the electrode in real sample analysis, Tyr was measured in various matrices including three water and three blood plasma samples. For each sample, 2.0 ml of the sample was diluted to 10.0 ml by phosphate buffer solution pH = 7.0 and then it was transferred to the electrochemical cell. The measurement was done by DPV at SrFeME for five times. The results of these measurements are shown in Table 2. As can be seen, Tyr was not detected in water samples, but it existed in plasma samples. Also, different amounts of Tyr were added to each sample and after determination of Tyr, the recovery percentage was obtained. Table 2 shows acceptable results for the recovery percentages. The relative standard deviation (RSD) was also calculated and the low values were obtained (Table 2). DP voltammograms of the diluted solutions of blood serum samples and these solutions after adding certain concentration of Tyr are shown in Fig. 8. Therefore, the modified electrode of strontium ferrite nanoparticles can be used as an electrochemical sensor for the reliable Tyr measurements.

CONCLUSIONS

The results of this study showed that strontium ferrite nanoparticles have electrocatalytic ability for oxidation of amino acid Tyr. The modified carbon paste electrode with these nanoparticles can easily catalyze the Tyr oxidation process. This electrode has a simple and cheap construction. The enhanced oxidation signal of Tyr at modified electrode is attributed to the improved electron transfer rate at these nanoparticles and the enhanced active surface area of the modified electrode by the nanoparticles. The calibration plot of the modified electrode for Tyr showed a linear range of 0.8-300.0 μM and a detection limit of 0.15 μM .

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