

Full Paper

Carbon Paste Electrode Modified with TiO₂/Fe₃O₄/MWCNT Nanocomposite and Ionic Liquids as a Voltammetric Sensor for Sensitive Ascorbic Acid and Tryptophan Detection

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Abstract- A carbon paste electrode modified with TiO₂/Fe₃O₄/MWCNT nanocomposite and ionic liquids (TFMWCNT/IL/CPE) was employed for the simultaneous determination of ascorbic acid (AA) and tryptophan (TRP). The TFMWCNT/IL/CPE displayed excellent electrochemical catalytic activities toward AA and TRP oxidation compared with bare CPE. In the differential pulse voltammetry technique, both AA and TRP gave sensitive oxidation peaks at 270 and 700 mV respectively. Under the optimized experimental conditions, AA gave linear responses over range of 2.5 to 100.0 μM. The lower detection limit was found to be 1.13 μM for AA. The investigated method showed good stability, reproducibility, and repeatability for determination of AA and TRP in pharmaceutical samples.

Keywords- Ascorbic acid, Tryptophan, TiO₂/Fe₃O₄/MWCNT nanocomposite, Ionic liquids

1. INTRODUCTION

Ascorbic acid (AA), also known as vitamin C, is very popular for its antioxidant property, and present in the human diet as a vital vitamin. It is needed for many functions in the body, including helping the body use carbohydrates, fats, and protein [1-3]. Lack of AA can lead to

a condition called scurvy, which causes muscle weakness, swollen and bleeding gums, loss of teeth, and bleeding under the skin [4]. Moreover, it is also used for the prevention and treatment of senility, the common cold, treating asthma, some mental illness, infertility, allergies, eye ulcers, blood clots, gum disease, pressure sores and AIDS. Also AA is being used to reduce the risk of cardiovascular disease and certain types of cancer (skin cancer, cervical dysplasia and breast cancer) [5-9].

Tryptophan (TRP), as an essential amino acid for the human body and a vital constituent of protein biosynthesis of living organisms is important in nitrogen balance and the maintenance of muscle mass and body weight in humans [10-12]. Also in many biochemical processes, it is a fundamental precursor of hormone for neurotransmitter serotonin and other relevant biomolecules [13-16]. For as much as TRP cannot be synthesized directly in human body and the scarce presence in vegetables, it is commonly added to dietary, food products as a food fortifier and to pharmaceutical formulations. But, when defectively metabolized, a waste product will be produced in the brain to cause hallucinations and delusions [17,18].

It is well known that AA and TRP are usually co-existing substances in biological matrixes and abnormal levels of these species will lead to several diseases and disorders [19]. Therefore, to establish a simple, accurate, rapid and inexpensive method for simultaneous detection of AA and TRP in food, pharmaceutical products and biological fluids is very necessary.

Recently, electrochemical techniques have been received considerable interest for detection and quantification of small biomolecules, owing to their high sensitivity, high accuracy, simple operation mode and low cost [20-28]. However, one major problem is that the oxidation peaks of these electroactive species are too close at a bare electrode, which results in overlapping voltammetric response and making their simultaneous detection highly difficult [29]. To overcome this fault, a variety of materials have been used for the modification of electrode [28,29].

Among conventional electrodes carbon paste electrode (CPE) is one of the most conductive matrixes for preparing chemically modified electrodes (CMEs) [30]. CPE is made from a mixture of conducting graphite powder and a pasting liquid. These electrodes are simple to make and offer an easily renewable surface for electron exchange [31,32].

In the past few decades, nanoparticles of a variety of shapes, sizes and compositions exhibit excellent conductivity and fascinating catalytic properties, which make them suitable for constructing novel electrochemical sensors for simultaneous determination [33-35]. Nanocomposites can significantly improve the electrocatalytic properties of substrates, decrease the overpotential, increase the reaction rate and improve reproducibility of the electrode response in the area of electroanalysis [36]. The results indicated that nanocomposites modified electrodes show an enhanced response for the determination of various important biological and clinical species [37-39].

Recently, room temperature ionic liquids (RTILs) which are compounds that consist only of ions, are liquids at around room temperature have been developed and received much attention in many areas of chemistry and electrochemical industry [40,41]. The acknowledged advantages of these RTILs include no measurable vapor pressure, good thermal and chemical stability, high conductivity, and low toxicity. However, these binders may create a new opportunity to challenge traditionally non-conductive pasting liquids used in CPE [42].

The main target of this work was to develop a simple but sensitive electrochemical sensor for the simultaneous determination of AA in the presence of TRP. Based on the effective electrochemical activity of the carbon paste electrode modified with $\text{TiO}_2/\text{Fe}_3\text{O}_4/\text{MWCNT}$ nanocomposite and ionic liquids TFMWCNT/IL/CPE toward these species, a very sensitive and selective electrochemical method has been proposed for the simultaneous determination of AA and TRP in pharmaceutical samples.

2. EXPERIMENTAL

2.1. Apparatus and chemicals

The electrochemical measurements were performed with an Autolab potentiostat/galvanostat (PGSTAT 302N, Eco Chemie, the Netherlands). The experimental conditions were controlled with General Purpose Electrochemical System (GPES) software. A conventional three electrode cell was used at 25 ± 1 °C. An Ag/AgCl/KCl (3.0 M) electrode, a platinum wire, and TFMWCNT/IL/CPE were used as the reference, auxiliary and working electrodes, respectively. A Metrohm 710 pH meter was used for pH measurements. AA, TRP and all of the other reagents were of analytical grade and were obtained from Merck (Darmstadt, Germany). The buffer solutions were prepared from orthophosphoric acid and its salts in the pH range of 2.0-9.0. Ionic liquids (n-hexyl-3-methylimidazolium hexafluoro phosphate) was purchased from Sigma Aldrich Co. $\text{TiO}_2/\text{Fe}_3\text{O}_4/\text{MWCNT}$ was synthesis by reported method [43].

2.2. Preparation of the electrode

TFMWCNT/IL/CPE were prepared by mixing 0.04 g of $\text{TiO}_2/\text{Fe}_3\text{O}_4/\text{MWCNT}$ nanocomposite with 0.96 g graphite powder and approximately, ~0.8 mL of ionic liquids with a mortar and pestle. The paste was then packed into the end of a glass tube (ca. 3.4 mm i.d. and 15 cm long). A copper wire inserted into the carbon paste provided the electrical contact. For comparison, ionic liquids/carbon paste electrode (IL/CPE) in the absence of $\text{TiO}_2/\text{Fe}_3\text{O}_4/\text{MWCNT}$ nanocomposite, (TFMWCNT/CPE) consistent of $\text{TiO}_2/\text{Fe}_3\text{O}_4/\text{MWCNT}$ nanocomposite powder and paraffin oil, and bare carbon paste electrode (CPE) consisting of graphite powder and paraffin oil were also prepared in the same way.

2.3. Preparation of real samples

One milliliter of a Vitamin C ampoule (Darou Pakhsh Company, Iran, contained 1 mg/mL of AA) was diluted to 10 mL with 0.1 M PBS (pH 7.0); then, different volume of the diluted solution was transferred into each of a series of 25 mL volumetric flasks and diluted to the mark with PBS. The AA content was analyzed by the proposed method using the standard addition method.

Five Vitamin C tablets (labeled 250 mg per tablet, Osvah Pharmaceutical Company, Iran) were grinding. Then, the tablet solution was prepared by dissolving 400 mg of the powder in 25 mL water by ultrasonication. Then, different volume of the diluted solution was transferred into a 25 mL volumetric flask and diluted to the mark with PBS (pH 7.0). The AA content was analyzed by the proposed method using the standard addition method.

Five Vitamin C effervescent tablets (labeled 500 mg per tablet, Hakim Pharmaceutical Company, Iran) were grinding. Then, the tablet solution was prepared by dissolving 1.07 g of the powder in 25 mL water by ultrasonication. Then, different volume of the diluted solution was transferred into a 25 mL volumetric flask and diluted to the mark with PBS (pH 7.0). The Vitamin C content was analyzed by the proposed method using the standard addition method.

3. RESULT AND DISCUSSION

3.1. Electrochemical behavior of AA at the surface of various electrodes

Fig. 1 displays cyclic voltammetric responses from the electrochemical oxidation of 70.0 μM AA at the surface of TFMWCNT/IL/CPE (curve d), IL/CPE (curve c), TFMWCNT/CPE (curve b) and bare CPE (curve a). The results showed that the oxidation of AA is very weak at the surface of the bare CPE, but the presence of ILs in CPE could enhance the peak current and decrease the oxidation potential (decreasing the overpotential). A substantial negative shift of the currents starting from oxidation potential for AA and dramatic increase of the current indicates the electrochemical ability of TFMWCNT/IL/CPE (curve d) and IL/CPE (curve c) to AA oxidation. The results showed that the combination of TFMWCNT and the ionic liquids (curve d) definitely improved the characteristics of AA oxidation. However, TFMWCNT/IL/CPE shows much higher anodic peak current for the oxidation of AA compared to ILCPE, indicating that the combination of TFMWCNT and IL has significantly improved the performance of the electrode toward AA oxidation.

3.2. Effect of scan rate

The effect of potential scan rates on the oxidation current of AA has been studied (Fig. 2). The results showed that increasing in the potential scan rate induced an increase in the peak current. In addition, the oxidation process is diffusion controlled as deduced from the linear

dependence of the anodic peak current (I_p) on the square root of the potential scan rate ($v^{1/2}$) over a wide range from 20 to 500 mV s^{-1} .

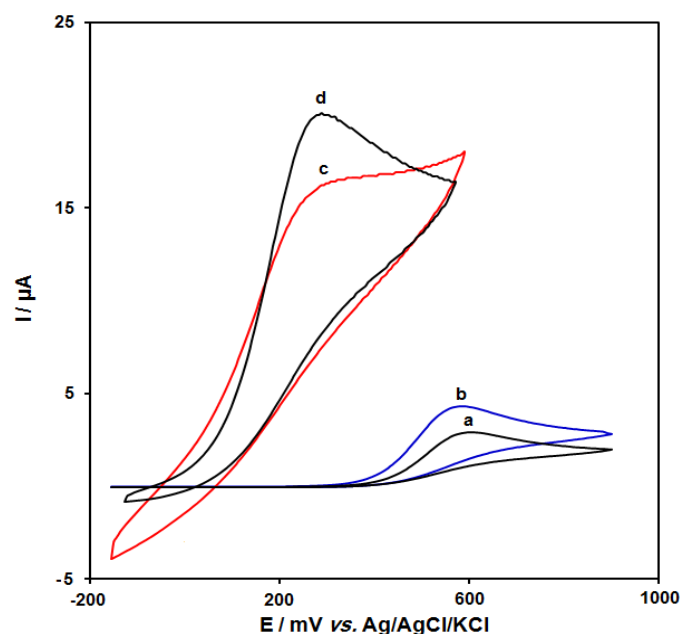


Fig. 1. CVs of a) CPE, b) TFMWCNT/CPE, c) IL/CPE and d) TFMWCNT/IL/CPE in the presence of $70.0 \mu\text{M}$ AA at a pH 7.0, respectively. In all cases the scan rate was 50 mV s^{-1}

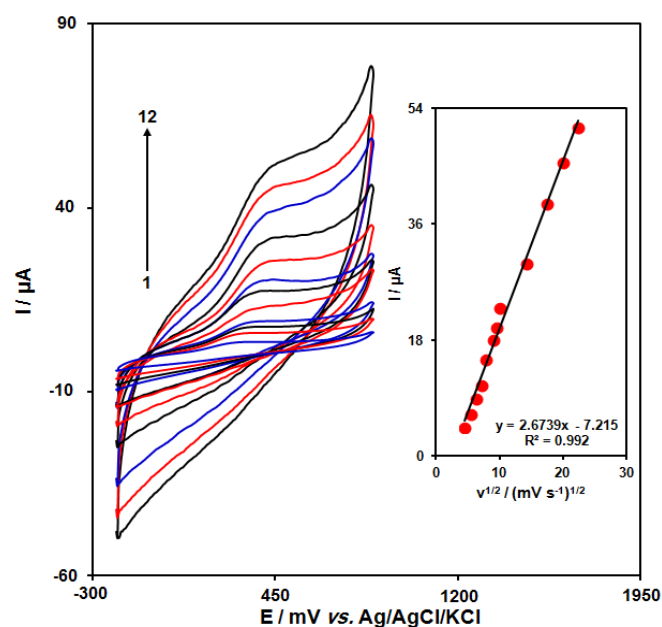


Fig. 2. CVs of TFMWCNT/IL/CPE in 0.1 M PBS (pH 7.0) containing $2.5 \mu\text{M}$ AA at various scan rates; numbers 1-12 correspond to 20, 30, 40, 50, 60, 80, 90, 100, 200, 300, 400 and 500 mV s^{-1} , respectively. Inset: Variation of anodic peak current vs. square root of scan rate

3.3. Chronoamperometric measurements

Chronoamperometric measurements of AA at TFMWCNT/IL/CPE were carried out by setting the working electrode potential at 0.4 V for the various concentration of AA in PBS (pH 7.0) (Fig. 3). For an electroactive material (AA in this case) with a diffusion coefficient of D , the current observed for the electrochemical reaction at the mass transport limited condition is described by the Cottrell equation [44].

$$I = nFAD^{1/2}C_b\pi^{-1/2}t^{-1/2} \quad (1)$$

Where D and C_b are the diffusion coefficient ($\text{cm}^2 \text{s}^{-1}$) and the bulk concentration (mol cm^{-3}), respectively. Experimental plots of I vs. $t^{-1/2}$ were employed, with the best fits for different concentrations of AA (Fig. 3A). The slopes of the resulting straight lines were then plotted vs. AA concentration (Fig. 3B). From the resulting slope and Cottrell equation the mean value of the D was found to be $9.42 \times 10^{-6} \text{ cm}^2/\text{s}$.

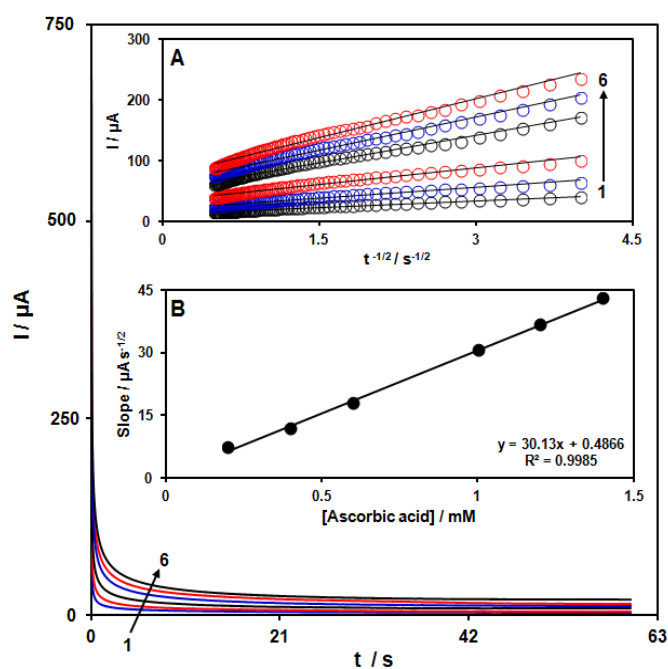


Fig. 3. Chronoamperograms obtained at TFMWCNT/IL/CPE in 0.1 M PBS (pH 7.0) for different concentration of AA. The numbers 1–6 correspond to 0.2, 0.4, 0.6, 1, 1.2 and 1.4 mM of AA. Insets: (A) Plots of I vs. $t^{-1/2}$ obtained from chronoamperograms 1–6. (B) Plot of the slope of the straight lines against AA concentration

3.4. Calibration plot and limit of detection

The peak current of AA oxidation at the surface of the modified electrode can be used for determination of AA in solution. Therefore, differential pulse voltammetry (DPV) experiments were done for different concentrations of AA (Fig. 4). The oxidation peak

currents of AA at the surface of a modified electrode were proportional to the concentration of the AA within the ranges 2.5 to 100.0 μM . The detection limit (3σ) of AA was found to be 1.13×10^{-6} M.

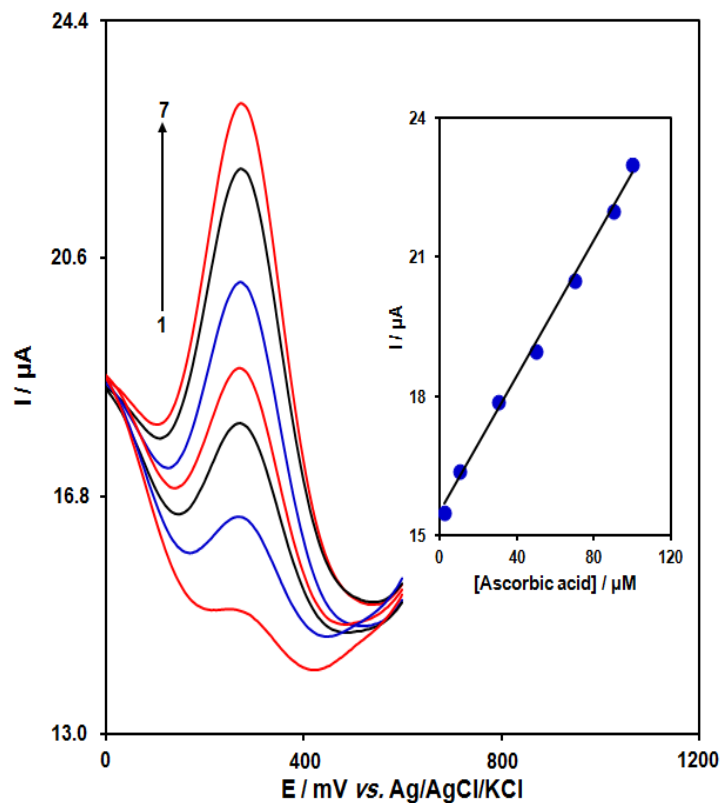


Fig. 4. DPVs of TFMWCNT/IL/CPE in 0.1 M PBS (pH 7.0) containing different concentrations of AA. Numbers 1-7 correspond to 2.5, 10.0, 30.0, 50.0, 70.0, 90.0 and 100.0 μM of AA. Inset: plots of the electrocatalytic peak current as a function of AA concentration in the range of 2.5 to 100.0 μM

3.5. Simultaneous determination of AA and TRP

To our knowledge, no paper has used the TFMWCNT/IL/CPE for simultaneous determination of AA and TRP and this is the first report for simultaneous determination of AA and TRP using TFMWCNT/IL/CPE. Determination of two compounds was performed by simultaneously changing the concentrations of AA and TRP, and recording the DPVs (Fig. 5). The voltammetric results showed well-defined anodic peaks at potentials of 270 and 700 mV, corresponding to the oxidation of AA and TRP, respectively, indicating that simultaneous determination of these compounds is feasible at the TFMWCNT/IL/CPE.

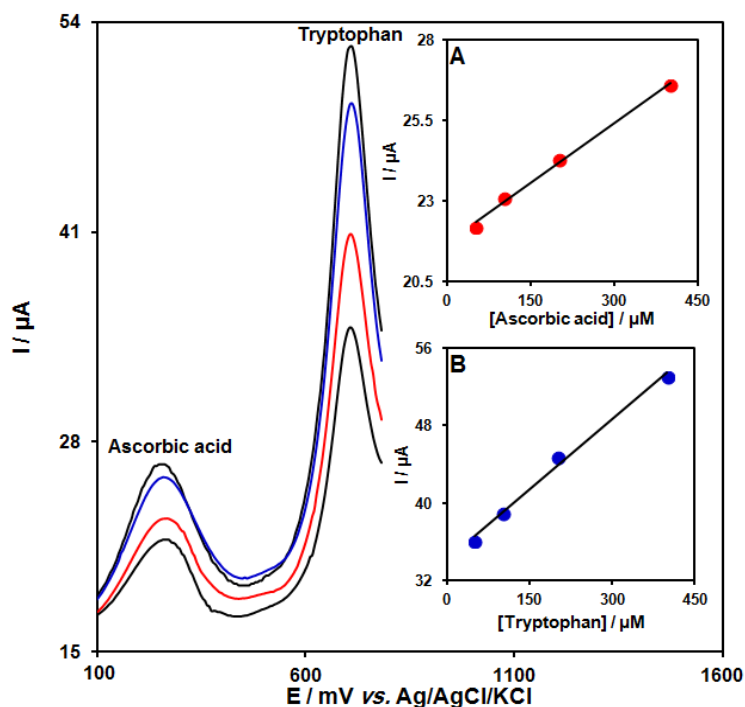


Fig. 5. SWVs of TFMWCNT/IL/CPE in 0.1 M PBS (pH 7.0) containing different concentrations of AA and TRP in μM , from inner to outer: 50.0+50.0, 100.0+100.0, 200.0+200.0 and 400.0+400.0 respectively. Insets (A) plots of I_p vs. AA concentration and (B) plot of I_p vs. TRP concentrations

Table 1. Determination of AA in Vitamin C ampoule, Vitamin C tablet and Vitamin C effervescent tablet. All the concentrations are in μM ($n=5$)

Sample	Spiked	Found	Recovery (%)	R.S.D. (%)
	ascorbic acid	ascorbic acid	ascorbic acid	ascorbic acid
Vitamin C ampoule	0	10.0	-	3.2
	5.0	15.7	104.7	2.7
	10.0	19.6	98.0	2.4
	15.0	25.7	102.8	1.6
Vitamin C tablet	0	5	-	2.6
	10.0	14.7	98.0	1.8
	15.0	20.6	103.0	3.1
	20.0	24.4	97.6	2.5
Vitamin C effervescent tablet	0	5	-	2.4
	10.0	14.8	98.6	3.7
	15.0	19.7	98.5	3.2
	20.0	24.9	99.6	2.6

3.6. Real sample analysis

In order to evaluate the analytical applicability of the proposed method, also it was applied to the determination of AA in Vitamin C ampoule, Vitamin C tablet and Vitamin C effervescent tablet. The results for determination of the three species in real samples are given in Table 1. Satisfactory recovery of the experimental results was found for AA. The reproducibility of the method was demonstrated by the mean relative standard deviation (R.S.D.).

4. CONCLUSION

The results obtained in this work demonstrated the potentiality of the TFMWCNT/IL/CPE for simultaneous determination of AA and TRP. The modified electrode exhibits highly electrochemical activity for the oxidation of AA and TRP associated with negative shifts in anodic peak potentials. Thus, large peak separations obtained with this electrode allow it to simultaneously detect these compounds. Moreover, good sensitivity, high selectivity, low detection limit with the low cost of the sensor, makes this method very suitable for accurate determinations in pharmaceutical and clinical preparations. The proposed method could be applied to the determination of AA and TRP in real samples (commercial drugs) with satisfactory results.

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