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Full Paper

Development of a Method for a Sensitive Simultaneous Determination of Acetaminophen and Tryptophan in Biological Samples

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Abstract- A chemically modified electrode is constructed based on multi-walled carbon nanotube modified glassy carbon electrode (MWCNTs/GCE) for simultaneous determination of acetaminophen (ACT) and tryptophan (TRY). The measurements were carried out by application of differential pulse voltammetry (DPV), cyclic voltammetry (CV) and chronoamperometry (CA) methods. Application of DPV method showed that the linear relationship between oxidation peak current and concentration of ACT and TRY were in the range of 2 μM to 360 μM, and 3 μM to 360 μM, respectively. Under optimal conditions the modified electrode exhibited high sensitivity and stability for both ACT and TRY determination, making it a suitable sensor for the simultaneous submicromolar detection of ACT and TRY in solutions. The analytical performance of modified electrode has been evaluated for detection of ACT and TRY in human serum and human urine with satisfactory results.

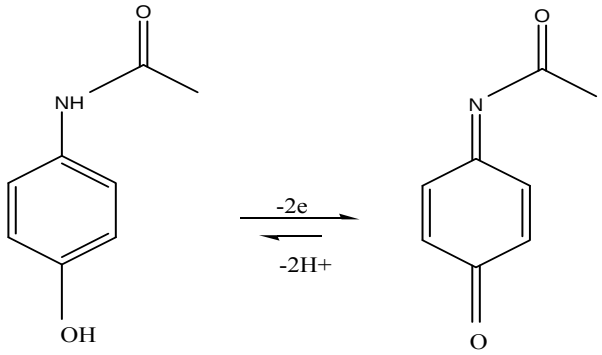
Keywords- Acetaminophen, Tryotophan, Carbon Nanotube, Modified Glassy Carbon Electrode

1. INTRODUCTION

Carbon nanotubes (CNTs) are carbon materials that have a new kind of porous nanostructure, that have been found to possess properties such as high electrical conductivity, high surface area, chemical stability and significant mechanical strength [1,2]. They can be used to promote electron transfer reactions when used as electrode materials in electrochemical devices [3,4].

Acetaminophen (ACT) is an important medicine, also known as paracetamol, is a widely used analgesic anti-pyretic drug. Its action is similar to aspirin and is a suitable alternative for the patients who are sensitive to aspirin. It is also found that overdoses of ACT will damage liver and kidney. Unfortunately, its ready access has resulted in its increased use in effort suicide [5, 6]. Various methods have been reported for the single determination of ACT as spectrophotometric method [7-11], near infrared transmittance spectroscopy [12], Fourier transform infrared spectrophotometer [13], spectrofluorimetry [14,15], chromatography [16,17].

However, the majority of these methods suffer from some disadvantages such as high costs, long analysis times and requirement for sample pretreatment, and in some cases low sensitivity and selectivity that makes them unsuitable for routine analysis. Therefore, development of a simple, inexpensive, sensitive and accurate analytical method for determination of ACT is of great importance. ACT is an electroactive compound which can be oxidized electrochemically (Scheme 1). The development and application of electrochemical sensors for ACT analysis, with respect to its sensitivity, accuracy, and simplicity, has been of greater interest in recent years [18-20].

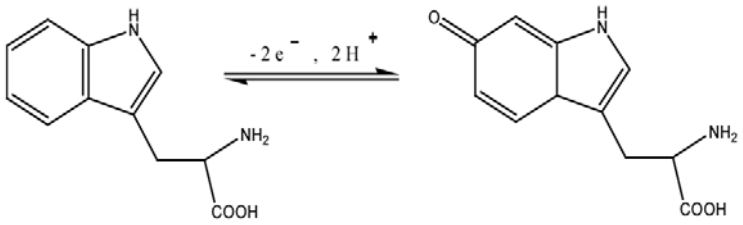


Scheme 1. Oxidation mechanism of ACT

Tryptophan (TRY) is an essential amino acid for human and herbivores but is scarcely present in vegetable products. It is sometimes added to dietary and food products as a food fortifier and to pharmaceutical formulations in order to correct possible dietary deficiencies. The analysis of TRY is of great importance in the biochemical, pharmaceutical and dietetic

fields as they are precursor molecules of hormones, neurotransmitters and other relevant bimolecules [21]. Methods for the determination of TRY are mainly based on HLPC [22,23] and spectrophotometric procedures [24]. Most of the spectrophotometric methods involve laborious and slow procedures with the modification of tryptophan by numerous reagents. Chromatographic separation could be often complex and time-consuming.

TRY is an electroactive compound which can be oxidized electrochemically (scheme. 2). Electroanalytical methods, with respect to their sensitivity, accuracy and simplicity, have been more of interest in recent years for TRY analysis [25, 26].



Scheme 2. Oxidation mechanism of TRY

Overdose consumption of ACT can alter TRY metabolism by inhibiting tryptophan 2, 3-dioxygenase activity thus increasing the availability of TRY for the production of serotonin in brain [27]. As a consequence simultaneous determination of ACT and TRY compounds could be of considerable value.

There are few reports of the use of an electrochemical sensor for the simultaneous determination of ACT and TRY compounds [28, 29]. In this work we introduce the simple application of a multi-walled carbon nanotubes modified glassy carbon electrode (MWCNTs/GCE) as a sensitive sensor for this purpose. In addition, the analytical performance of this sensor for determination of ACT and TRY in human serum, human urine and in actual pharmaceutical preparation samples is evaluated.

2. EXPERIMENTAL

2.1. Reagents and solutions

All chemicals were analytical grade and used without further purification. ACT and TRY were obtained from Merck and Sigma chemical companies, respectively. Multi-walled carbon nanotubes (MWCNTs) (>95 wt%, 5-20 nm) were purchased from PlasmaChem GmbH companies, respectively. Stock standard solutions of 10 mM ACT and 10 mM TRY were freshly prepared in 0.1 M phosphate buffers of pH 7. All subsequent ACT and TRY solutions

used were prepared by diluting these standard solutions with 0.1 M phosphate buffer (pH 7). The 0.1 M phosphate buffer solutions (PBS) used was prepared by dissolving appropriate amounts of sodium hydrogen phosphate and sodium dihydrogen phosphate in triply distilled water. Fresh human serum samples were obtained from the Razi Institute of Vaccine and Serum Company (Tehran, Iran). The human serum and urine samples were filtered and diluted 100 times with 0.1 M PBS of pH 7 and checked for the determination and the recovery by spiking appropriate amounts of ACT and TRY to the samples.

2.2. Instrumentation

All voltammetric measurements were carried out using a multi-walled carbon nanotube modified glassy carbon electrode (MWCNTs/GCE) as a working electrode, an Ag/AgCl/3 M KCl as a reference electrode and platinum wire as an auxiliary electrode. DPV, CV and CA experiments were carried out using an Autolab PGSTAT 30 Potentiostat Galvanostat (EcoChemie, The Netherlands) coupled with a 663 VA stand (Metrohm Switzerland). All potentials cited are with respect to the potential of the reference electrode. The pH measurements were made with a Metrohm 744 pH meter using a combination glass electrode. SEM image was obtained using a JEOL 7000F HRSEM with an acceleration voltage of 15 kV.

2.3. Modification of the electrodes

A glassy carbon electrode (GCE, 2 mm diameter, Metrohm) was polished using 0.3 and 0.05 μm aluminum slurries and rinsed thoroughly with triple distilled water. The GCE was cleaned by ultrasonic agitation for 5 min in ethanol and then in the distilled water, individually. A stock solution of 1 mg mL^{-1} MWCNTs–DMF was prepared by dispersing 1 mg of MWNTs in 1 mL DMF. 20 μL of the MWCNTs–DMF solution was coated on GC electrode surface. To obtain MWCNTs/GCE, the electrode was dried at room temperature. The fabricated MWCNTs/GCE was placed in the electrochemical cell containing 0.1 M PBS. In order to obtain stable responses, several cycles in the potential window of -0.1 to 1.0 V were applied using CV method.

2.4. General procedure

A 10 mL solution containing the appropriate amounts of ACT and TRY in 0.1 M PBS at pH 7.0 was transferred into the voltammetric cell. The voltammograms were recorded by applying positive-going potential from 0.2 to 0.8 V. These showed anodic peaks around 0.33 and 0.57 V corresponding to ACT and TRY compounds with heights proportional to their concentrations in solutions. The calibration curves were obtained by plotting anodic peak currents of ACT and TRY versus the corresponding concentrations. All experiments were carried out under open circuit conditions.

After each measurement, the MWCNTs/GCE was regenerated by washing the electrode successively with triple distilled water and 5% sodium hydroxide solution consecutively. Finally the electrode was rinsed carefully with distilled water, 5% sodium hydroxide solution and finally with the water to remove any adsorbate from the electrode surface.

3. RESULT AND DISCUSSION

3.1. Characterizing of the MWCNTs/GCE

Scanning electron microscopy (SEM) was used to observe directly the morphology of MWCNTs/GCE. The SEM images of the MWCNTs/GCE (Fig. 1) showed that the GCE surface was mostly covered with homogenous MWCNTs, which were in the form of small bundles or single tubes.

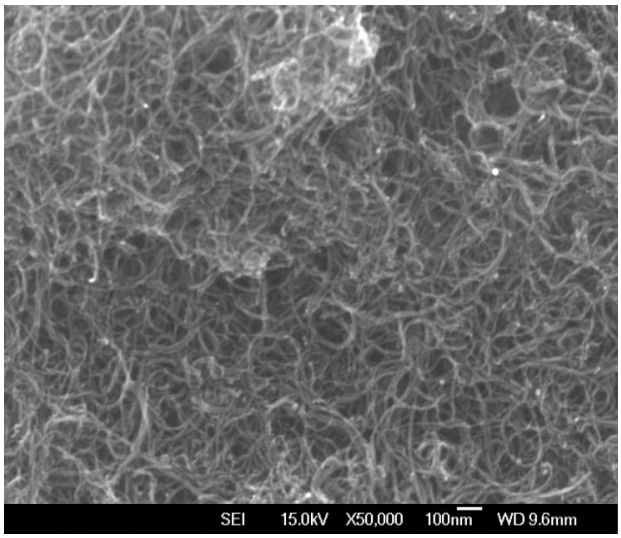


Fig. 1. SEM image of MWCNTs film on glassy carbon

The relative electrochemical surface areas of the modified MWCNTs/GCE and bare GCE were determined by CVs measured between -0.1 and 0.6 V in 4 mM ferricyanide solution (PBS) at several scan rates. The modified MWCNTs/GCE showed a surface area 9.8 times that of GCE.

3.2. Electrochemical characterizations of ACT and TRY on MWCNT/GCE

The cyclic voltammogram recorded for 100 μM ACT and 300 μM TRY using MWCNTs/GCE is shown in Fig. 2. The ACT, unlike the TRY, showed a reversible oxidation that is related to the electrocatalytic behavior of the MWCNTs.

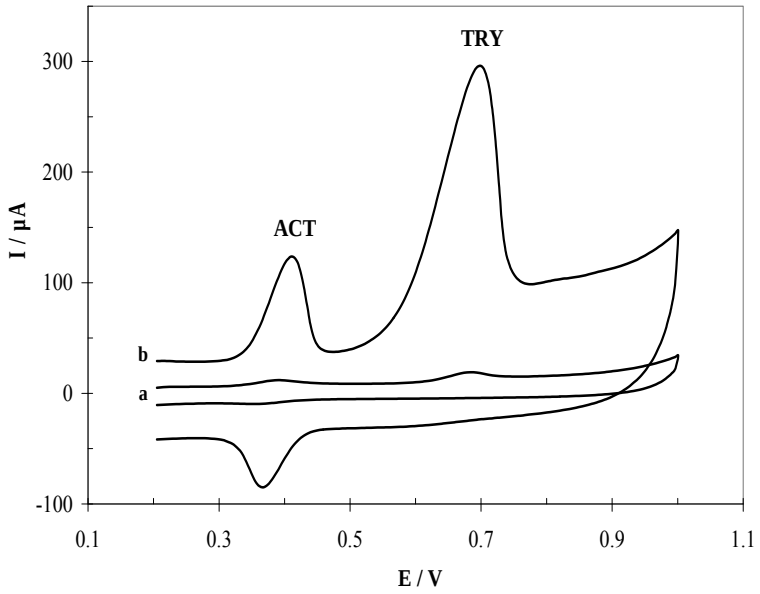


Fig. 2. Cyclic voltammograms of 100 µM of ACT and 300 µM TRY at (**a**) GCE and (**b**) MWCNTs/GCE at scan rate of 50 mV s^{-1} in 0.1 M phosphate buffer solution (pH 7.0)

The differential pulse voltammograms (DPVs) recorded for ACT and TRY for bare GCE, and modified MWCNTs/GCE are shown in Fig. 3. Voltammograms is that for 250 µM of ACT, and 150 µM of TRY in PBS (pH of 7.0) at GCE. Voltammogram **b** is that of ACT and TRY at MWCNTs/GCE under the same conditions. As can be seen, for the GCE the oxidation peaks for ACT and TRY are very small. However at MWCNTs/GCE surface (voltammogram **b**), both ACT and TRY showed considerable increases in their oxidation peak currents. These phenomena could be attributed to the larger active surface area of the modified electrode and its catalytic effects.

3.3. Effects of potential scan rate

The effect of potential scan rate on the oxidation responses of ACT and TRY were investigated in the 10-110 mV s^{-1} range of scan rate (not shown). A linear relationship between the anodic peak current and scan rate were found for ACT and TRY as follows:

Ipa (µA) = 2.414ν (mV s^{-1}) + 8.7651	(R^2 = 0.993)	**ACT**
Ipa (µA) = 1.412ν (mV s^{-1}) + 8.3537	(R^2= 0.9963)	**TRY**

The linear relationship between peak currents and scan rates, suggests that the redox reactions of ACT and TRY compounds at MWCNTs/GCE, are adsorption-controlled processes.

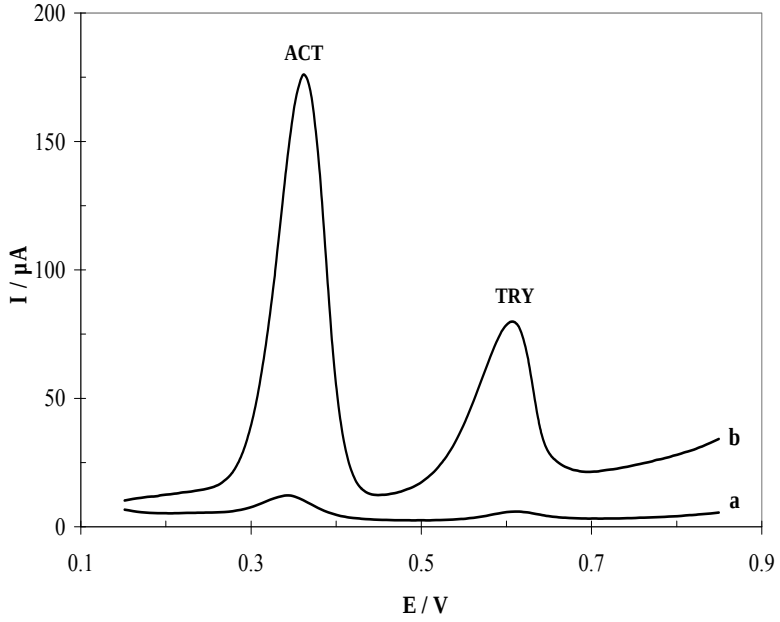


Fig. 3. Differential pulse voltammograms of 250 µM of ACT and 150 µM TRY at (**a**) GCE and (**b**) MWCNTs/GCE in 0.1M PBS (pH 7). Other conditions: Open circuit, t_{acc}=60 s, pulse amplitude=50 mV and scan rate=10 mV s^{-1}, interval time 0.5 s, modulation time=0.2 s and step potential=5 mV

3.4. Effects of operational parameters

3.4.1. Effects of supporting electrolyte and solution pH

The oxidation peak current of ACT and TRY at MWCNTs/GCE in 0.1 M PBS was found to be higher than that in other supporting electrolytes, such as acetate, citrate and ammonia buffer solutions. Therefore a 0.1 M PBS was adopted as the electrolyte for experiments.

The effects of solution pH on the electrochemical responses of the MWCNTs/GCE towards the simultaneous determinations of 30 µM ACT and 15 µM TRY in 0.1 M PBS was investigated using the DPV method. Variations of the observed peak current with electrolyte pH over the range 4 to 10 are shown in Fig. 4. It can be seen that the anodic peak currents of ACT increase with increasing solution pH until it reaches 7. However at higher pH the oxidation peak current starts to diminish. The oxidation peak current for TRY also increases with pH but starts to fall away from pH 6. Therefore, the pH value of 7, which is close to biological pH value, was selected as the optimum pH for further experiments.

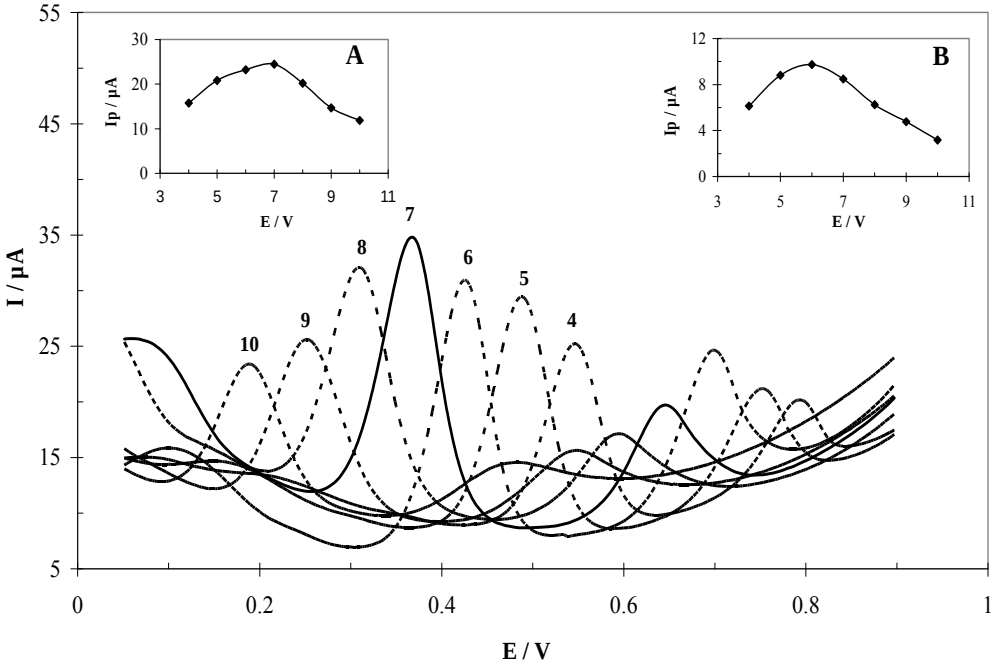


Fig. 4. Effects of pH on the differential pulse voltammogram peak currents of oxidations of 30 µM ACT and 15 µM TRY compounds at MWCNTs/GCE in phosphate buffer solutions. Insets: Plots of anodic peakes currents (I_{pa}) as a function of pH of buffer solutions. Other conditions as shown in Fig. 3

3.4.2. Effects of accumulation time

Fig. 5 shows how the anodic peak currents, obtained from DPV experiments, vary with accumulation time for 200 µM ACT and 200 µM TRY. Initially, peak currents for these compounds increase with accumulation time up to 60 s. However after 60 s the rate of increase falls away and they eventually plateau. An accumulation time of 60 s was chosen for further experiments.

3.5. Linear dynamic range and detection limit of the method

The electrochemical responses for simultaneous additions of ACT and TRY to a 0.1 M PBS of pH 7 using MWCNTs/GCE are depicted in Figures 6 and 7. Fig. 6 shows differential pulse voltammograms together with the corresponding calibration curves obtained using a MWCNTs/GCE for various concentrations of ACT and TRY. For ACT we obtained, over a linear dynamic range of 2 µM to 360 µM, a calibration equation of $I_p(\mu A)=0.661c\ (\mu M)+9.6531$ ($R^2=0.999$) A detection limit of 0.062 µM (S/N=3) was observed. A linear relationship was also observed for TRY over a range of 3 to 360 µM. The calibration equation in this case was $I_p(\mu A)=0.401c\ (\mu M)+1.3031$ ($R^2=0.999$), and the detection limit was 0.182 µM.

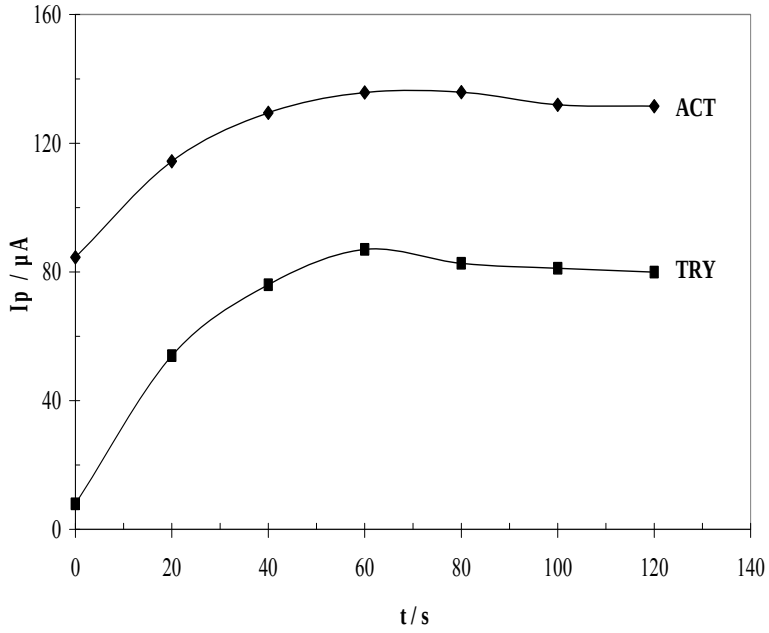


Fig. 5. Effect of accumulation time on the differential pulse voltammogram peak currents of 200 µM ACT and 200 µM TRY in phosphate buffer (pH 7) solution

Table 1. Maximum tolerable concentrations for common interfering species

Interfering species	**TRY** C_{int}[a]/µM	**ACT** C_{int}[a]/µM
L dopa	900	650
dopamin	800	600
L analin	1600	1500
L glotamic acid	1550	1100
uric acid	550	250
ascorbic acid	750	550
aspartic acid	3500	3000

[a] C_{int} refers to interfering compound concentration

Fig. 7 displays hydrodynamic chronoamperogram response of the rotated modified electrode (2500 rpm) with successive injection of ACT and TRY at an applied potential of 0.8 V in PBS (pH 7). For ACT, a linear dynamic range was from 6 µM to 500 µM. A

calibration equation of $I_p(\mu A) = 0.2895c\ (\mu M)+3.3927$ (R^2=0.9978) (Inset of Fig. 6A) and a detection limit of 0.808 μM (S/N=3) were obtained. For TRY, a linear relationship was in the range of 6 to 450 μM. A calibration equation of $I_p(\mu A)=0.3115c\ (\mu M)+4.4502$ (R^2=0.9914) (Inset of Fig. 6B) and a detection limit of 0.713 μM were obtained.

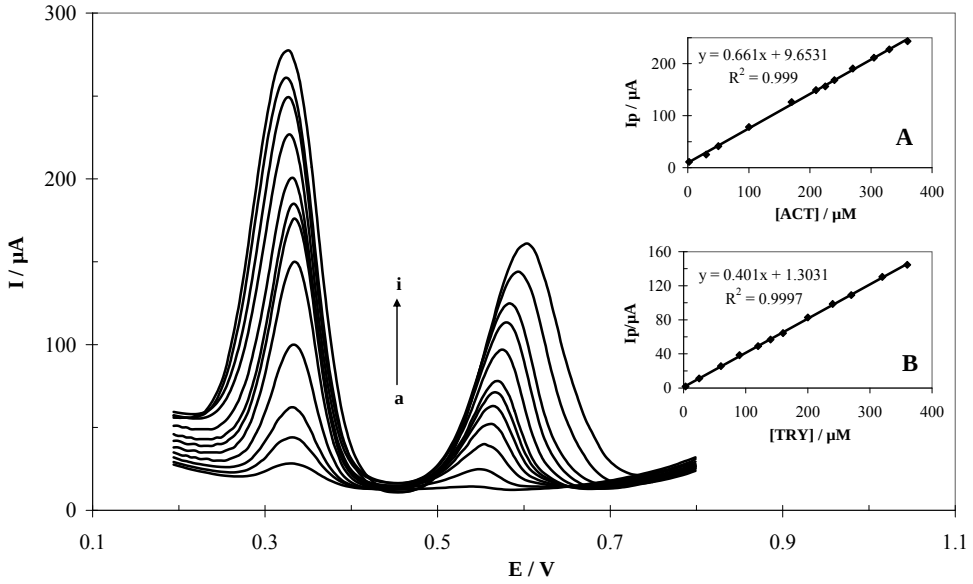


Fig. 6. Differential pulse voltammograms for different concentrations of ACT and TRY mixture as (a) 2+3, (b) 30+25, (c) 50+60, (d) 100+90, (e) 170+120, (f) 210+140, (g) 225+160, (h) 240+200, (i) 270+240, (j) 305+270 and (k) 330+320 , (l) 360+360 respectively, in which the first value is the concentration of ACT in μM and the second value is the concentration of TRY in μM. Insets: (A) Plot of peak currents as a function of ACT concentration. (B) Plot of the peak currents as a function of TRY concentration

3.6. Repeatability and long-term stability of the electrode

Thus, the repeatability of the analytical signal has been studied. Indeed, the relative standard deviations (RSD) of 1.32% and 1.46% for 100 μM ACT and 100 μM TRY respectively over ten consecutive determinations has been obtained. The low values of RSD suggest good repeatability of the modified electrode for determination of ACT and TRY. Stability of the proposed electrode was tested by measuring the decrease in voltammetric current during repetitive DPV measurements of ACT and TRY solutions with MWCNTs-CHT/GCE stored in solution or air for certain period of time. For example, in the determination of 150 μM ACT and 100 μM TRY in 0.1 M PBS (pH 7), subjecting the modified electrode to eight measurements , led to a less than 9 and 11% decrease in the voltammetric currents after 24 h, respectively. When the electrode was stored in the

atmosphere for 7 days, the corresponding current responses fell less than 12.5% and 15.5% in a solution containing 150 µM ACT and 100 µM TRY, respectively.

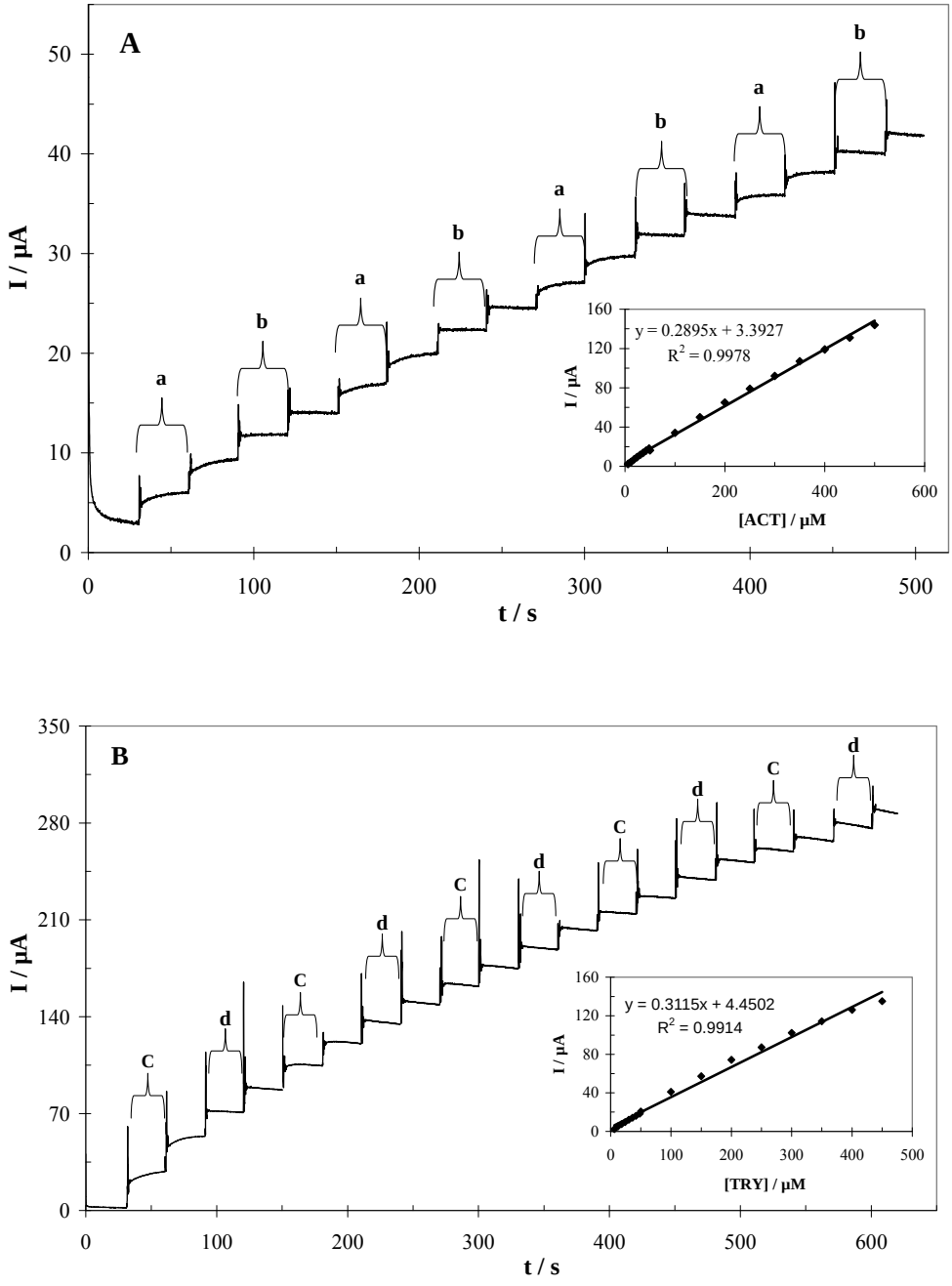


Fig. 7. Hydrodynamic Amperometric response at rotating MWCNTs/GCE (rotating speed 2500 rpm) held at 0.8 V in PBS (pH 7) for simultaneous determination of ACT and TRY by successive additions of (A) (a) 6µM TRY and (b) 6 µM ACT (Inset: corresponding calibration curve for ACT), and (B) (c) 50 µM TRY, (d) 50 µM ACT (Inset: Corresponding calibration curve for TRY)

3.7. Interference studies

The effect of common interfering species in solutions of 100 μM ACT and 100 μM TRY under our optimum conditions were investigated. The results are summarized in Table 1. The tolerance limit listed is the concentration of the interfering species that still gives an error of ≤ 10% in the determination of ACT and TRY. The results confirm that the proposed method is free from interference from the most common interferants.

3.8. Analytical applications

The human serum and urine samples were filtered and diluted 100 times with 0.1 M PBS of pH 7 and checked for the determination and the recovery of ACT and TRY to the samples. The applicability of the method used for the determination of ACT and TRY in human serum and human urine was studied by spiking samples of these with known amounts of ACT and TRY, obtaining differential pulse voltammograms and determining the concentrations using standard addition method. The results are summarized in Table 2. They confirm that the proposed methods could be efficiently used for the determination of trace amounts of these compounds in typical biological systems.

Table 2. Determination of ACT and TRY in human serum and urine with MWCNTs/GCE

Analyte	Added (μM)		Found[a] (μM)		R.S.D. (%)		Recovery (%)	
	ACT	TRY	ACT	TRY	ACT	TRY	ACT	TRY
	0.0	0.0	0.0	0.0	-	-	-	-
Human serum	10.0	10.0	9.86	9.82	2.1	1.4	98.6	98.2
	20.0	20.0	19.85	20.3	2.8	2.1	99.2	101.5
	0.0	0.0	0.0	0.0	-	-	-	-
Human urine	10.0	10.0	9.91	9.85	201	2.1	99.1	98.5
	20.0	20.0	20.4	19.65	2.9	2.3	102	98.2

[a] Average of five determinations at optimum conditions

4. CONCLUSION

In this paper we introduced a new application of multi-walled carbon nanotube modified glassy carbon electrode for simultaneous determination of ACT and TRY. We have shown that the application of MWCNTs can increase anodic peak currents for ACT and TRY

compounds on the electrode surface. The results indicated that the use of a MWCNTs/GCE allows the simultaneous determination of ACT and TRY with good sensitivity and selectivity. The electrode showed high stability in repetitive experiments due to high water stability and high mechanical strength of MWCNTs. The effects of potential interferants were studied, and were found to be insignificant for most common ones. When the procedure was used for the determination of ACT and TRY in biological samples of human serum and urine, satisfactory results were obtained without the necessity of sample pretreatments and time-consuming pretreatments. The simple fabrication procedure, high speed, reproducibility, high stability, wide linear dynamic range, low detection limit and high sensitivity, all suggest that the proposed sensor is an attractive candidate for practical applications and automated analysis.

Acknowledgments

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