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MWCNTs modified carbon paste electrode for sensitive determination of antiparkinson drug entacapone in the bulk, pharmaceutical and human biological human samples

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Abstract

In this paper, the voltammetric properties of entacapone, an anti-Parkinson's disease drug, were investigated by cyclic voltammetry and differential pulse voltammetry at MWCNTs modified carbon paste electrode in phosphate buffer in presence of Tween 20 and validated for quantitative determination of entacapone in bulk, pharmaceutical dosage forms and human plasma. Several factors such as pH, type of surfactant and scan rate were investigated in order to study the optimum conditions for determination of entacapone. A good linear relationship was obtained within the concentration range from 50×10^{-9} to 2.4×10^{-3}M. The limits of detection and limit of quantification were found to be $8\times10^{-10}$and 2.4×10^{-9}molL^{-1}, respectively. The proposed method was simple, rapid and economic, so it is suitable for routine analysis of entacapone in pure form and dosage forms and for pharmaco kinetic studies.

Keywords: entacapone, Tween20, carbon paste electrode, multi-walled carbon nanotubes, cyclic voltammetry, differential pulse voltammetry.

1. Introduction

Catechol-O-methyl transferase (COMT) is an important enzyme, it found in nearly all human tissue. COMT play an important role in inactivating (metabolic degradation of) catechol amines of physiological origin (e.g. noradrenaline, adrenaline and dopamine) as well as of environmental origin (e.g. in food and medicines [1,2].

Entacapone (ENT), (E)-2-cyano-3-(3,4-dihydroxy-5-nitrophenyl)-N, N-diethyl prop-2-enamide (Fig. 1A) is an inhibitor of catechol-O- methyl transferase (COMT), used in the treatment of Parkinson's disease as an adjunct to levodopa (Fig. 1B) and carbidopa(Fig. 1C) therapy[3].

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ENT is a member of the class of nitrocathechols [4].The mechanism of action of entacapone is believed to be through its ability to inhibit COMT and alter the plasma pharmaco kinetics of levodopa. When entacapone is given in conjunction with levodopa and an aromatic amino acid decarboxylase inhibitor, such as carbidopa and levodopa, plasma levels of levodopa are greater and more sustained than after administration of levodopa and an aromatic amino acid decarboxylase inhibitor alone. In fact, ENT prevents COMT from metabolizing levodopa into 3-methoxy-4-hydroxy-L-phenylalanine in the periphery, which does not easily cross the blood brain barrier so increased and more sustained plasma levodopa concentrations are reached as compared to the administration of levodopa and decarboxylases inhibitor, Thus ENT is added to extend the duration and effect of levodopa in the brain, and thus allows levodopa to be given less often and in lower doses [5-7]

The presence of nitro group at the ortho position to the hydroxyl group is critical for entacapone's potency and ability to inhibit COMT[8].

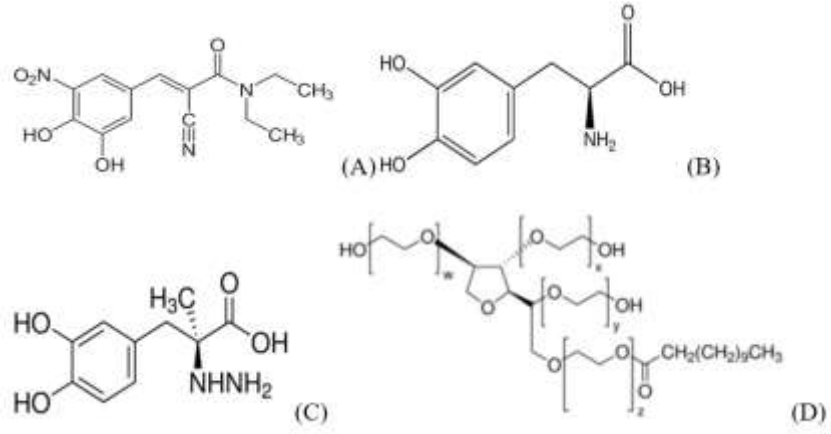

Fig. 1. Chemical structures of entacapone (A), levodopa (B), carbidopa(C), Tween 20(D

Literature survey simply reveals that different analytical techniques have been applied for determination of ENT, including liquid chromatography [9-17], spectrophotometry [3, 18-25], capillary electrophoresis [26] and electrochemical methods [1, 27-30]

Although, the other chromatographic methods are sensitive enough for assay of ENT in biological fluids, they are complicated, expensive, require sample pretreatment and time-consuming (which are hazardous for column efficiency and need prolonged time for column saturation and washing). Consequently, electrochemical methods have been employed because of their inherent specificity, rapid response, high sensitivity, low cost, simplicity and relatively short analysis time. However, in the electrochemical methods, the ENT based sensors have some common drawbacks such as poor stability, reusability and slow electron transfer or some of them just for detection and determination ENT in pharmaceutical formulation or had very narrow linear range and they were not fully validated. Therefore, there is a demand for the development of a sensor that is comparatively more stable, reproducible and sensitive for ENT determination in real samples.

The integration of nanotechnology and electrochemistry is expected to produce major advances in the field of electrochemical sensors. During recent years, nanomaterials, such as carbon nanotubes (CNTs) and transition metallic nanoparticles (NPs), have been widely applied in sensors.

CNTs are receiving more and more research interests since its discovery by Iijima [31]. MWCNTs consisting of several layers of graphene [32]has also been widely used in the study of electrochemical sensors due to its wide potential window, interesting electrochemical properties, and good compatibility with biological samples [32].

Carbon nanotubes can be used to promote electron transfer reactions when used as electrode material in electrochemical devices, electrocatalysis and electroanalysis processes due to their significant mechanical strength, high electrical conductivity, high surface area, good chemical stability, as well as relative chemical inertness in most electrolyte solutions and a wide operation potential window[33-40] .

A surface active agent (surfactant) tends to adsorb at the interface between bulk phases, such as air and water, oil and water or electrode and solution. Surfactants naturally have a very large impact on chemistry of current interest, their behavior in solution involves aggregation or adsorption and organization on the electrode surface and it influences the electrode processes and the rate of electron transfer[34] .The role of surfactants to improve the sensitivity and selectivity in electrochemistry is well documented [35-37]. Tween20 (T20) (Fig. 1D) is a polylobate-type nonionic surfactant formed by the ethoxylation of sorbitan before the addition of lauric acid C58H114O26.

The objective of this investigation is the determination of ENT in bulk powder, tablets and human serum through the electrochemical oxidation of ENT using cyclic voltammetry (CV) and differential pulse voltammetry (DPV) at MWCNTs modified carbon paste (MWCNTs-CPE) based on the enhancement effect of T20.

2. Experimental

2.1. Materials and reagents

Entacapone was purchased from Ramopharmin (IRAN). Graphite fine powder, paraffin oil and reagents were analytical grade from Merck. Multi-walled carbon nanotubes, trimethyl ammonium bromide(CTAB), cetyltrimethyl ammonium chloride(CTAC), sodium dodecyl sulfate (SDS) and tween 20(T20) were supplied from Sigma–Aldrich. All of the other reagents used for experiments and analysis were of analytical grade, used without further purification and purchased from Merck. The phosphate buffer solution (PBS) was prepared by mixing appropriate NaCl and NaH_2PO_4-Na_2HPO_4 Britton-Robinson (B-R) buffer solutions with various pH values were used as the supporting electrolytes.

Commercial pharmaceutical samples of ENT (Entadorm 100 in tables from Ramopharmin Company; mixture of carbidopa, levodopa and ENT) was purchased in a local drugstore and subjected to a simple sample preparation step.

2.2. Apparatus and instrumentations

Electrochemical measurements were performed with a Metrohm 757 va computrace electrochemical workstation. A conventional three-electrode system was used for all electrochemical experiments, which consisted of an Ag/AgCl (saturated KCl) as the reference electrode, a platinum wire as the auxiliary electrode, MWCNTs-CPE as working electrode. The morphology of the MWCNTs was observed using scanning electron microscopes (SEM) from MIRA3 TESCAN. All pH measurements were performed using a 86505 AZ digital pH meter. An ultrasonic homogenizer bath Sono Swiss (SW3) was used for preparation of sample solution.

2.3. Procedures

2.3.1. Generation of oxygen functionalities on MWCNTs

The oxygen functionalities on the surface of MWCNTs can improve their electrocatalytic performance, and long MWCNTs (several microns in length) present diffusion. These functional groups can interact with various nano-scale materials to construct nanostructures with appropriate distribution. These mass transport restrictions can be minimized with short MWCNTs. The most popular reported method to cut MWCNTs is acid oxidation[38] As-received MWCNTs were first oxidized in acid solution of HNO_3 and H_2SO_4 (1: 3 by volume) and stirring for 24 h to remove impurities and to generate surface functional groups, then washed thoroughly DIW(pH=7) and dried at 120℃ in a vacuum oven for 24 h .

2.3.2. Preparation of the electrode

Modified carbon nanotube paste electrodes were prepared by mixing graphite powder (0.5 g), MWCNTs (0.1g) with paraffin oil (nearly 0.3 mL) in a mortar for 20 min until a uniformly-wetted paste was obtained. The carbon paste was packed into the hole of the electrode body and smoothed on a filter paper until its shiny appearance. Electrical contact was made by inserting a copper wire into the glass tube at the back of the mixture. When necessary, a new surface was obtained by pushing an excess of paste out of the tube and polishing it on a filter paper.

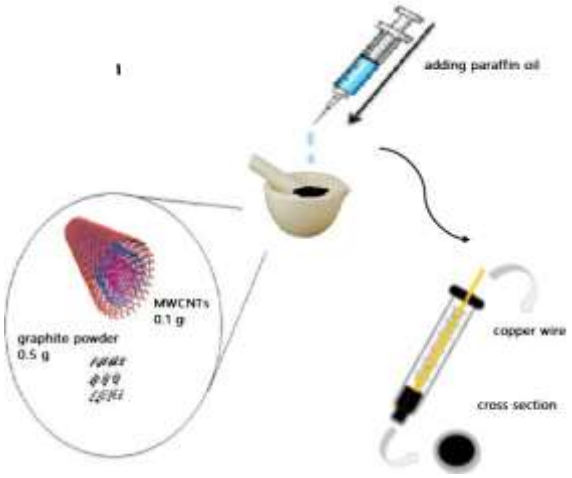

Fig.2. schematic representation of the steps preparation of MWCNTs-CPE

2.3.3. Preparation of pharmaceutical formulations

For preparation of pharmaceutical samples, ten tablets of ENT (Entadorm 100) were weighted and finely powdered in a mortar with pestle. An adequate amount of the obtained fine powder dissolved in methanol. The flask was sonicated for 25 min to mix well and filtered to separate out the insoluble excipients. The filtrate was further diluted with the same solvent for covering the working concentration range. An aliquot of this solution was then analyzed according to the proposed voltammetric procedure based on standard addition method.

3. Results and discussion

3.1. Surface morphological characterization

SEM of MWCNTs is shown in Fig. 3. In the figure MWCNTs could be seen in the form of tubes some of which twisted together. The modification of CPE using MWCNTs not only enlarges the ratio surface area of the electrode surface but also improves the electron transfer rate between the electrode surface and the bulk solution.

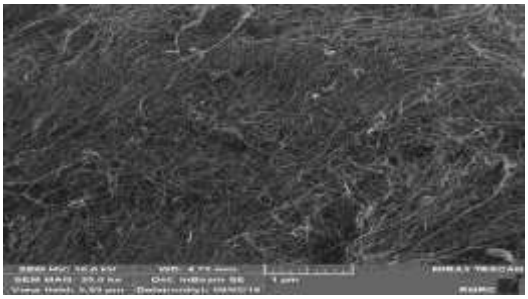

Fig.3. SEM of MWCNTs

3.2. Electrochemical oxidation of ENT

The cyclic voltammetry technique was employed for investigation the reversibility of the oxidation process of ENT at the CPE(A) and MWCNTs (B). The CV voltammogram of ENT (Fig. 4) displayed only one well cathodic peak and no anodic peak in the reverse scan was recorded, which means that the oxidation of ENT is irreversible. The results indicated that MWCNTs-CPE was good electrode for this investigation.

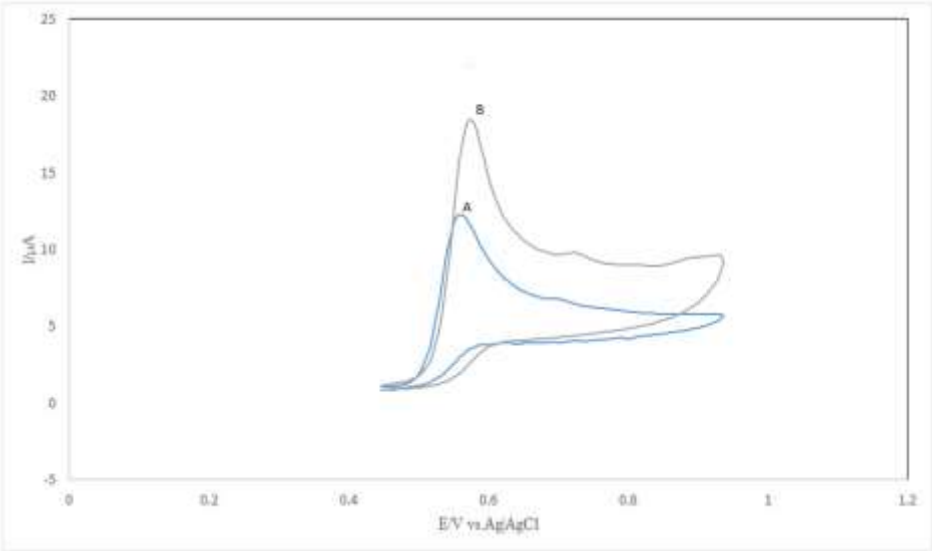

Fig. 4. Cyclic voltammogram of ENT in BR buffer of pH 2.0 at CPE(A) and MWCNTs-CPE(B)

The proposed reaction pathway could be represented as shown in (Fig. 5).

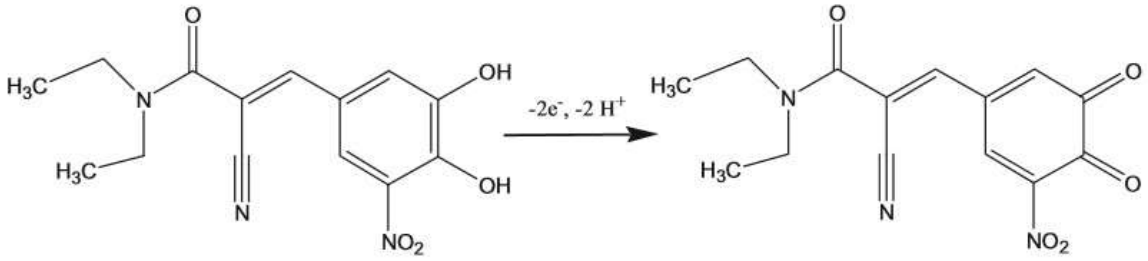

Fig. 5. The oxidation mechanism of ENT.

3.3. Optimization of experimental conditions

3.3.1. Effect of pH

The effect of pH upon the oxidation of ENT was investigated by using cyclic voltammetry (CV) at MWCNTs-CPE electrode and scan rate of 100 mVs^{-1}. The experiment was repeated by using buffer solutions of different pH values to obtain the optimum pH. The oxidation peak potential of ENT shifts to more negative values with the increase of pH of the medium (over the pH range from 2.0 to 8.0), denoting that protons are involved in the electrode reaction process. In acidic media of pH from 2 to 6, a well defined oxidation peak is obtained which may be due to the oxidation of hydroxyl groups, as shown in Fig.6. Two broad peaks are obtained at pH values from 6.0 to 8.0, which may be attributed to the oxidation of hydroxyl groups and tertiary amine group for the first peak and second peak, respectively. The number of protons participated in the rate determination was estimated equal 1 and the number of electrons transferred was 2. The higher

current value was obtained at pH 2.0. Hence, pH 2.0 is chosen to be the optimum pH in this study.

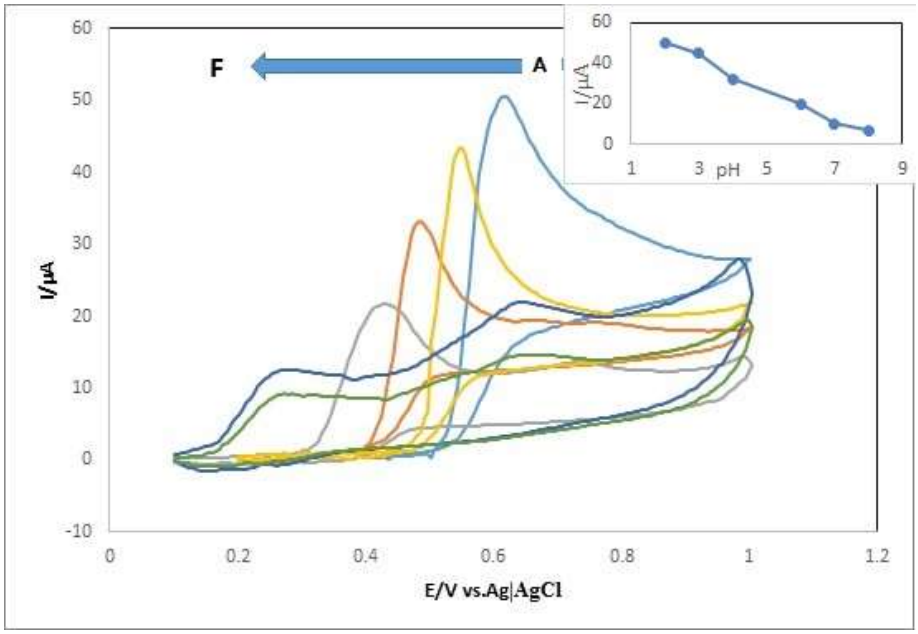

Fig.6. Effect of solution pH on the oxidation of 1000 µM ENT at MWCNTs-CPE; scan rate 100 mV/s. pH=A) 2.0, B)3, C)4, D)6, E)7 and F)8. The inset: the linear relationship between pH and I.

3.3.2. Effect of presence of various surfactant

Figure.7 shows the comparison among CTAB, CTAC, SDS and T20 at the same concentration towards the oxidation of ENT. The oxidation peak current of ENT was 15.23µA at CPE without addition of any surfactant as shown in Fig. 4, while the peak current values were 11.1, 10.8, 19.3 and 21.8 µA in case of CTAB, CTAC, SDS and T20, respectively. The nonionic surfactant T 20 was shown an enhanced effect on the oxidation peak current of ENT, while the peak current decreases by addition of cationic surfactant CTAB, CTAC. Because ENT has no charge in the aqueous media, thus T20 is the best surfactant for the determination of ENT at MWCNTs-CPE.

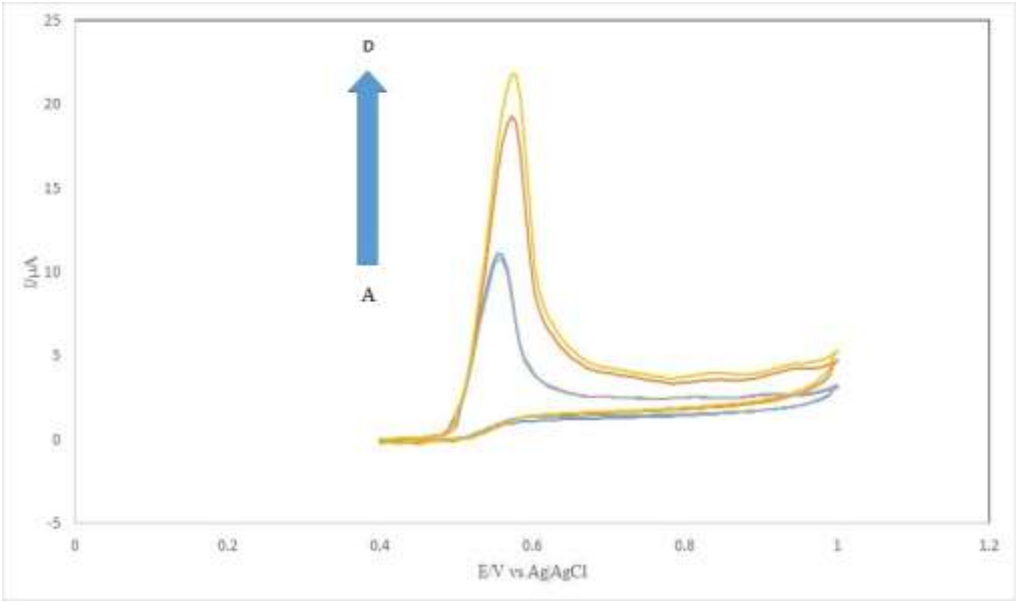

Fig. 7. Cyclic voltammograms of ENT in presence of CTAC (A), CTAB(B), SDS (C) and T20(D) in BR buffer of pH 2.0.

3.3.3. Effect of scan rate

Figure.8 shows the effect of scan rate (v) in the range from 10 to 150 mVS^{-1} on the cyclic voltammetric response of ENT in BR buffer (pH 2.0) in presence of T20. With increasing scan rates, the cathodic peak slightly shifted to the positive potential direction. The peak currents were increased remarkably with increasing scan rates. The variations of peak currents with changing scan rates from 10 to 150 mVS^{-1} were investigated. It was found that the logarithm of oxidation peaks current (logI) is linear to the logarithm of scan rate (log v), with the linear regression equation as logI = 0.9232 log v + 1.3481, r (correlation coefficient) = 0.9978. There was good linear relationship between the peak current intensity and the scan rate (v) indication the process of ENT a modified electrode was controlled by adsorption.

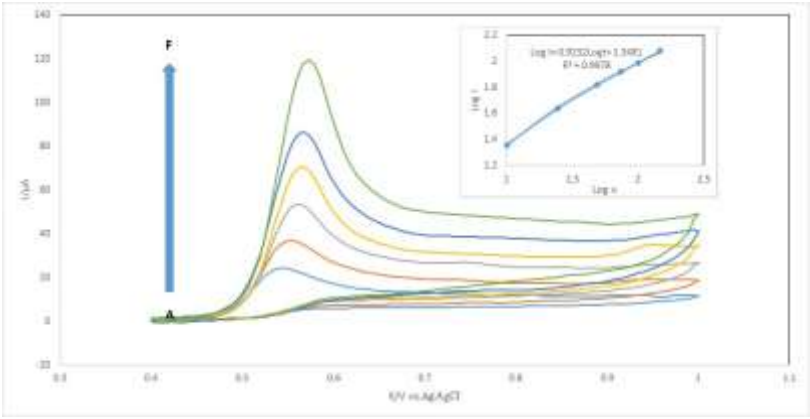

Fig.8. Cyclic voltammograms of ENT as a function of scan rate in presence of T20 at MWCNTs-CPE. The inset: the linear relationship between logI and log v in BR buffer of pH 2.0.

3.3. Method validation

3.3.1. Calibration curve

In order to develop a voltammetric method for determination the drug, differential pulse voltammetry method was selected, because the peaks are better defined at low concentration. Fig.9 indicates the differential pulse voltammograms at various concentration of ENT at MWCNTs-CPE. The concentration of ENT was varied from 50×10^{-9} to 2.4×10^{-3} M.

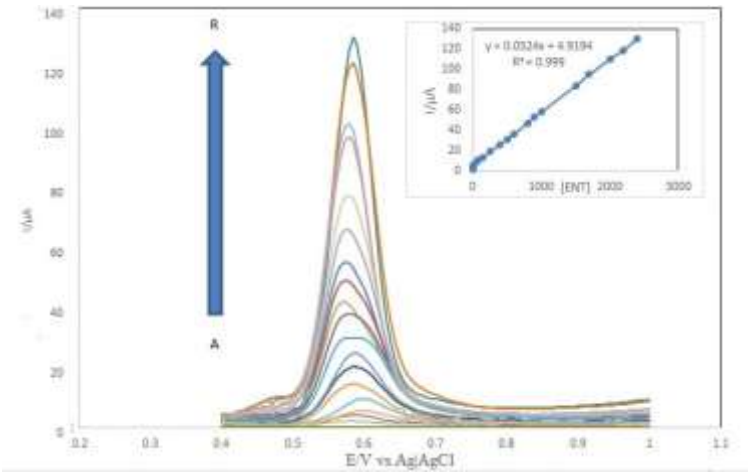

Fig .9. DPVs of ENT at MWCNTs-CPE in 0.1 M PBS.ENT concentration from down to up:0.05,3, 10,50,80,150,250,400,500,600,800,900,1000,1500,170,2000,2200 and 2400 µM .

3.3.2. Detection and quantification limits

The limits of detection and quantification (LOD and LOQ) were found to be 8×10^{-10} and 2.4×10^{-10} molL^{-1}, respectively. The low values of LOD and LOQ reflect that the electrode is very sensitive for ENT determination by the proposed method.

3.3.3. Repeatability of CPE in the optimum condition

The repeatability of the MWCNTs-CPE was investigated by replicate recordings of voltammogram at a fixed ENT concentration of 250 µM. The coefficient of variance (%CV) for the peak currents in CV based on five replicates was 1.35%, indicating an excellent repeatability of the response at CPE. Also, inter-day variation of same con-centration of ENT was analyzed for three consecutive days by performing six measurements on each day. The average %CV value is 1.28%, which demonstrates good repeatability of the method at modified electrode (Table 1).

Table 1. Repeatability experiment at MWCNTs-CPE

Time	Inter-Day Repeatability		Intra-Day Repeatability	
Day	Average Current (I,µA)	Coefficient Of Variance(%CV)	Average Current(I,µA)	Coefficient Of Variance(%CV)
Day 1	9.61[a]	1.30	9.63[a]	1.35
Day2	9.55[a]	1.24	[a]Average of five replicate readings	
Day3	9.59[a]	1.31		
Average	9.58[b]	1.28[b]	[b]Average of three consecutive days readings	

3.4. Applications

3.4.1. Determination of ENT in tablets

Standard addition method was used to quantify ENT in real pharmaceutical formulations such as Entadorm tablets, the results obtained by the proposed method are in good agreement with labeled amounts. The results obtained by the proposed electrode are shown in the Table 2.

Table 2. Results of the ENT detection in pharmaceutical preparation(n=5)

Tablet	Sample	Founding	Recovery(%)	RSD(%)
Entadorm100 Mg	1	105.00±0.05	105	1.2
Entadorm100 Mg	2	99.70±0.05	99.7	1.1
Entadorm100 Mg	3	102.10±0.05	102	2.1
Entadorm100 Mg	4	103.50±0.05	103	1.7

3.4.2. Determination of ENT in human plasma samples

The proposed sensor was applied to determine ENT in human plasma. 1 mL of fresh human plasma was transferred into a 10 mL centrifuge tube containing 2 mL of acetonitrile and spiked with different volumes of standard solution of ENT, then the mixture was centrifuged for 15 min at 5000 rpm in order to eliminate protein residues. Then, ENT was determined in plasma real samples using DPV, and the attained results were listed in Tables 3. The results revealed that the MWCNTs-CPE can be used to determine ENT powerfully in the routine real samples.

Table 3. Determination results of ENT in human plasma

sample	Added(µM)	Found(µM)	Recovery(%)	RSD(%)
Volunteer 1	10	10.70	107	1.2
Volunteer 2	20	21.10	105	2.0
Volunteer 3	30	29.50	98	1.5
Volunteer 3	40	40.50	101	1.3

4. Conclusion

The developed voltammetric method is simple, precise accurate and economical for determination of ENT in bulk drug, tablets and real plasma samples. The proposed method is based on the electrochemical oxidation of ENT at CPE in presence of T20 as a non-ionic surfactant which causes an enhancement in the cathodic peak current. The results of the developed method indicated validity of the method for application in routine quality control of ENT since it is characterized by high sensitivity and simplicity. The limits of detection and quantification (LOD and LOQ) were found to be $8\times10^{-10}$and 2.4×10^{-10} $molL^{-1}$, respectively.

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