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Melatonin Determination at Trace Levels by ErO_2/MnO_2 Nanocomposite Modified Carbon Paste Electrode

Taher Alizadeh,* Somayeh Amjadi, and Maedeh Akhoundian

Department of Analytical Chemistry, Faculty of Chemistry, University College of Science, University of Tehran, Tehran, Iran P.O. Box 14155-6455, Tehran, Iran

*Corresponding Author, Tel.: +98-21-61112788

E-Mail: talizadeh@ut.ac.ir

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Abstract- A new method based on a carbon paste electrode, modified with ErO_2/MnO_2 nanocomposite was introduced for melatonin determination. The differential Pulse Voltammetry (DPV) method was found to be a suitable analytical technique to acquire an enhanced electro-oxidation signal and thus it was chosen as the signal recording method. The nanocomposite was characterized using Scanning electron microscopy and X-ray diffraction analysis method. The electrocatalytic activity of the carbon paste electrode modified with ErO_2/MnO_2-CPE toward melatonin electro-oxidation was substantiated utilizing DPV and electrochemical impedance spectroscopy ((EIS). The EIS technique gave a clear indication for the intensification the of charge transfer rate by the nanocomposite described. Different parameters, affecting the electrode efficiency were investigated and optimized. Using DPV and ErO_2/MnO_2-CPE, a linear dynamic range of 4.0×10^{-8}-2.0×10^{-5} mol L^{-1} as well as a detection limit of 6.0×10^{-9} mol L^{-1} was obtained for melatonin measurement. The relative standard deviation percentage (RSD%) of four repeated measurements was found to be about 4.08%. The developed method was used for the estimation of melatonin amount in urine samples which led to satisfactory results.

Keywords- Melatonin; Erbium dioxide; Manganese dioxide; Sensor; Differential pulse voltammetry

1. INTRODUCTION

Melatonin (N-acetyl-5-methoxytriptamine), a methoxyindole hormone secreted by the mammalian pineal gland, plays an essential role in regulating the cycle of sleep-wake and the rhythm of biological circadian [1]. MEL is in charge of different physiological and behavioral processes such as psychiatric, reproductive, neurological, and as a neuroprotective agent in Parkinson and Alzheimer diseases [2]. It has a considerable effect on endogenous cycles known as the "internal clock." Moreover, it is exhibited to be a multifunctional molecule, showing anti-inflammatory, immunoregulatory, antitumor, and antioxidant properties [3-5].

MEL is connected to seasonal affective disorder, sleep efficiency, mood regulation, immunological functions, retinal physiology, sexual maturation, and reproduction [6,7]. The release and production of MEL depend on the day-light effect. MEL secretion decreases in light, and increases in dark; it also decreases in old age [8].

As an endocrine substance, MEL can be present in different body fluids and tissues, such as urine, blood, and saliva, and shows oscillating concentrations according to the 24-hour cycle [9]. Thus, the monitoring of melatonin levels is essential in medicine to follow its signaling mechanisms and the way it affects the endocrine system. It can also play a remarkable role in the early diagnosis of disease in clinical and physiological difficulties and the quantification of this marker has the potential [10,11]. Its maximum concentration under normal conditions, in human plasma, is about 50-200 pg/mL (0.22-0.86 nmol/L) at night time and lower than 2.0 pg/mL (8.6 pmol/L) during the daylight [12].

Several analytical methods have been reported for melatonin determination. Enzyme-Linked Immunosorbent Assay (ELISA) [13] and radioimmunoassays (RIA) [14] are among the most widely used methods for the MEL. Although these methods enable for detection of extremely low levels of this hormone in biological samples, they show disadvantages including high cost, time-consuming sample processing, and specialized personnel, materials, and equipment.

Other analytical methodologies which have been described for the quantification of melatonin are including chromatography techniques [15-19], capillary electrophoresis [20], spectrofluorometric method [21-26], mass spectrometry [27], chemiluminescence [28], and electrochemical methods [29-37].

Among these, electroanalytical methods are known for their significant sensitivity, the possibility of miniaturization, requiring the low volume sample, short-time analysis, low cost, and no sample treatment. Different kinds of electrochemical transducers have been used in electrochemical methods [38-42]. Among those, carbon paste electrode (CPE) has been applied as a favorable electrochemical transducer regarding its specific characteristics including the simplicity of its preparation, the wide potential window on both cathode and anode region, simple modification of them, and the low cost [43-46].

In the present work, a new nanocomposite of ErO_2/MnO_2 was synthesized and applied for the modification of the carbon paste electrode. The electrode prepared was checked for the electrooxidation of melatonin. It was demonstrated that the DPV signal of melatonin was outstandingly increased at the ErO_2/MnO_2-CPE compared to the unmodified electrode. Such improvement in the melatonin signal was correlated to the charge transfer resistance decrement at the modified electrode surface based on the results obtained by electrochemical impedance spectroscopy. However, it seems that the electrode surface is also increased by introducing the ErO_2/MnO_2 nanocomposite in the electrode which can increase the signal of the modified electrode to melatonin. The developed electrode was applied successfully for melatonin assay in some real samples.

2. EXPERIMENTAL SECTION

2.1. Instruments and reagents

Electrochemical data were obtained with a three-electrode system using a potentiostat/galvanostat model PGSTAT302, Metrohm. ErO_2/MnO_2-CPE was used as a working electrode and an Ag/AgCl electrode and a platinum wire were used as the reference and counter electrodes respectively. Electrochemical impedance spectroscopy (EIS) measurements were conducted via AUTOLAB PGSTAT302 electrochemical analysis system and GPES 4.9 software. A three-electrode system including a reference electrode (Ag/AgCl, KCl (sat.)), the counter electrode (platinum), and the working electrode (modified CP electrode) was also applied in the EIS experiments. EIS analysis was performed at the frequency range of 0.1 Hz - 1 MHz, ΔE_{ac} = 50 mV, and dc potential of 0.5 V, in the presence of melatonin.

Tryptophan, melatonin, glucose, phenylalanine, histidine, metonine, dopamine, erbium(III) nitrate pentahydrate, potassium permanganate, and n-eicosane were purchased from Sigma-Aldrich (Munich, Germany). Graphite powder was obtained from Fluka (Buchs, Switzerland). Uric acid, Ascorbic acid, boric acid, acetic acid, phosphoric acid, and other chemicals were of analytical grade and were acquired from Merck (Darmstadt, Germany). The pharmaceutical samples were bought from the pharmacy including Armonia® Fast (Manufactured by Company of Nathura S.p.A, Italy) and melatonin pure (Manufactured by Company of ESI, Italy). For prepare of stock solutions of melatonin, the powder of melatonin was solved by methanol (0.5 ml) and then the desired concentration was obtained by adding water solvent. Once prepared, is stored at 4 °C protected from light.

2.2. Preparation of nano-composites of erbium dioxide and manganese dioxide

The method of co-precipitation was applied to prepare the composite of erbium dioxide and-manganese dioxide. For this purpose, the aqueous solutions of erbium (III) nitrate (0.05

M) and potassium permanganate (0.05 M) were prepared. Then, the potassium permanganate solution was added to the erbium (III) nitrate solution under stirring conditions. After completion of the reaction, the solution pH was adjusted to 7 by NaOH (0.1 M). The obtained dark green sediment was separated from the solvent by centrifugation and washed several times with DI water and ethanol.

Then it was dried in an oven (at 80 ° C) for 2 hours and finally, the obtained product was analyzed by the methods of XRD, FTIR, and SEM.

2.3. Preparation of the sensor

For the sensor (ErO_2-MnO_2/CPE) construction, 0.0825 g of graphite was homogenized (for 10 min) in a mortar with 0.0075 g of TiO_2-MnO_2 nanocomposite powder. Subsequently, n-eicosane, 0.01 g was melted in a dish, and heated at 45–50 ˚C. Then the melted n-eicosane was added to the graphite/ ErO_2-MnO_2 nanocomposite and mixed with a stainless steel spatula. The final paste was applied to fill a hole at the end of an electrode body (2.00 mm in diameter, 3mm in depth). Finally, with the aid of sandpaper, the excess solidified material was removed. This sensor can be reused by moving the electrode surface on a paper after each experiment, in order to rub out a thin layer of its surface.

2.4. Melatonin measurement in pharmaceutical Samples

Six tablets were weighed and pulverized gently to have a soft powder. An accurate weight of this powder containing about 1.0 mg of melatonin was dissolved in 10 ml methanol in an ultrasonic bath (37 °C) for 15 min. The mixture was then centrifuged at 6000 rpm for 15 min. The collected supernatant was condensed up to 1 ml. The sample solution (having the concentration lied in the calibration curve of the method) was prepared from the stock sample solution by dilution of the required volume of buffer solution and was tested under optimum conditions.

3. RESULTS AND DISCUSSION

3.1. Characterization of nano-composite of manganese dioxide and erbium dioxide

Scanning electron microscopy (SEM) image of the synthesized material is illustrated in Figure 1. According to the image shown semispherical particles have been created via the synthesis protocol described in the experimental section. The particle size was estimated to be about 40 nm.

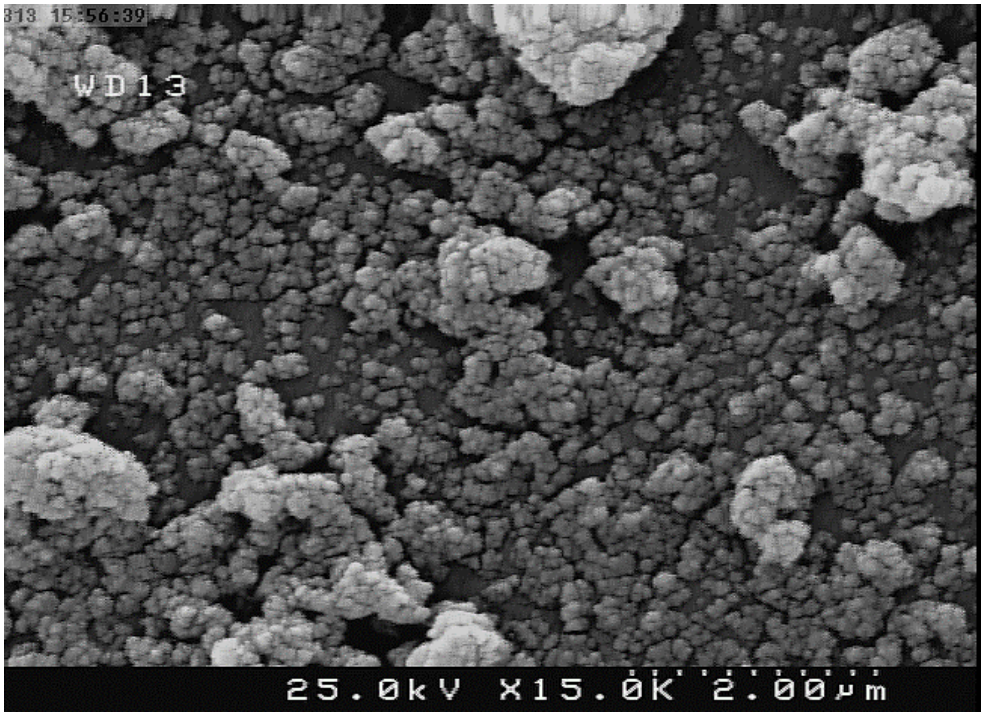

Figure 1. scanning electron microscopy image of ErO_2/MnO_2 nanocomposite

Figure 2 represents the XRD pattern of this material. As can be seen, the pattern exhibits no crystalline structure for the synthesized material. According to the XRD pattern the amorphous structure is assigned for the ErO_2/MnO_2 nanocomposite.

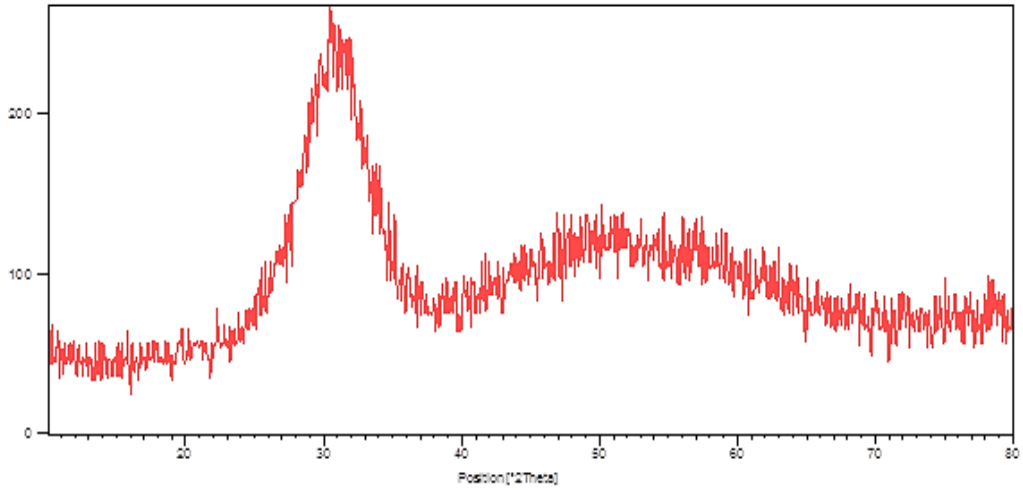

Figure 2. X-ray diffraction of nano-composite of magnesium dioxide and erbium dioxide

3.2. Investigation of electrochemical behavior of melatonin at CPE and ErO_2-MnO_2/CPE

Differential pulse voltammetry responses of melatonin at an unmodified carbon paste electrode (CPE) and that at the CPE electrode impregnated with the ErO_2/MnO_2 nanocomposite are shown in Figure 3 (I). As seen, the loading of nanocomposite of interest in the carbon paste

electrode has enormously increased the electrode response to melatonin. This indicates that the electrode, containing ErO_2/MnO_2 nanocomposite, can lead to a very highly sensitive electrochemical sensor for melatonin determination.

An electrochemical impedance spectroscopy experiment was also applied for further investigation of melatonin electrochemical characteristics at the composite electrode. The obtained results are depicted in Figure 3(II) as the Nyquist plots. The semicircles in the depicted Nyquist plots are attributed to the charge transfer resistance (R_{ct}) in parallel with double-layer capacitance (C_{dl}), which is the electrochemical properties of the double-layer structure, formed at the interface of electrode/solution.

With a comparison of semicircles' diameters in the Nyquist plots, which indicates the charge transfer resistance magnitude, it can be concluded that the charge transfer resistance of melatonin is significantly decreased as a result of the carbon paste electrode's modification with the ErO_2/MnO_2 nanocomposite. The described results indicate that the ErO_2/MnO_2 nanocomposite is acting as an electrocatalyst material, which is capable of facilitating the melatonin's electrochemical oxidation, at the electrode surface.

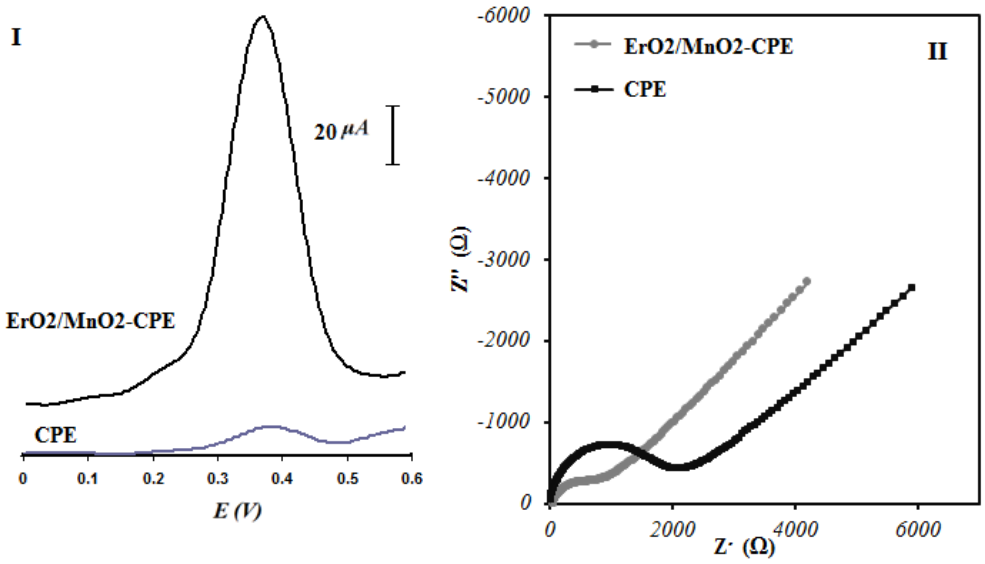

Figure 3. Comparison of differential pulse voltammograms obtained for melatonin (10^{-3} M) on CPE and ErO_2/MnO_2-CPE (Briton-Robinson buffer, pH= 2) (I); Electrochemical impedance spectroscopy (Nyquist diagram) recorded for CPE and ErO_2/MnO_2-CPE in the presence of melatonin

3.3. Optimization of the electrode composition and its working condition

As the most important variable in the modified electrode fabrication, the amount of ErO_2/MnO_2 nanocomposite in the electrode composition was varied and the resulting influence on the electrode signal to a fixed concentration of melatonin was evaluated. As illustrated in Figure 4, the electrode response to melatonin was increased by growing the modifier content

of the electrode up to about 7.5 % (w/w). However, after the optimal content the signal starts to decent slightly. The negative effect of the modifier after its optimal content in the electrode composition, may be assigned to its insulating effect on the electron exchange phenomenon.

The effect of solution pH on the electrode signal was also investigated and it was found that when the solution pH was fixed at pH=2, utilizing a Briton - Robinson buffer, the best response was recorded for melatonin at the ErO_2/MnO_2-CPE.

It was also found that applying a pre-potential to the modified electrode before initiation the DPV scan, led to a magnified electrochemical signal (Figure 5). Therefore, the magnitude of the applied pre-potential as well as its applying time was optimized to obtain the bigger signal for melatonin by the electrode. Based on the mentioned experiment it was found that pre-potential of 0.8 V and potential applying time of 10 s are optimal conditions for achievement the best response to melatonin regarding these variables.

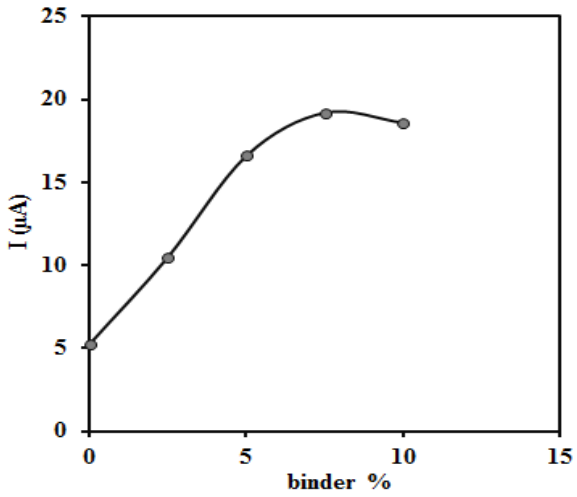

Figure 4. optimization of modifier content in the modified electrode composition ; melatonin concentration 6 µM; pH=2

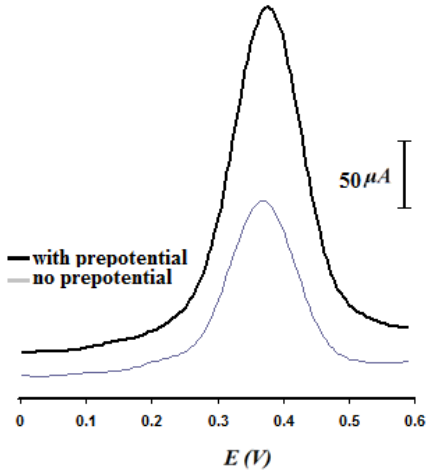

Figure 5. The effect of applied prepotential on the DPV signal of the ErO_2/MnO_2-CPE; melatonin (10^{-3} M)

3.4. The effect of interfering agents

The effect of interfering agents on the electrochemical signal of melatonin at the developed electrode was investigated. For this aim, the DPV signal of melatonin in the presence of various concentrations of some compounds such as tryptophan, uric acid, glucose, histidine, phenylalanine, ascorbic acid, dopamine, and methionine was studied. Figure 6 illustrates the tryptophan interfering effect on melatonin signal as an instance in this case.

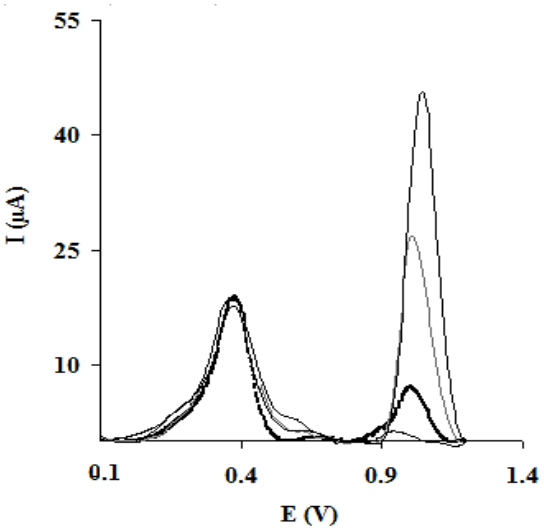

Figure 6. The effect of tryptophan on the electrochemical signal of melatonin (5 µM) at the ErO_2/MnO_2-CPE; (pH = 2); tryptophan concentrations: 25, 50, 150, and 250 µM (from low to high)

Table 1. Interference levels for some tested molecules in the determination of MEL by the sensor

Species	Interference level
glucose	<50
tryptophan	<50
histidine	<100
phenylaniline	<100
dopamine	<15
methionine	<100
Ascorbic acid	<30
Uric acid	<50

It is clear that loading different concentrations of tryptophan in melatonin solution do not affect the melatonin DPV signal in spite of the existence of high similarity between melatonin

and tryptophan chemical structures. The tolerance limit of the tested interfering agents was defined as the maximum concentration of the interfering species that led to an error of ±6% in the determination of melatonin. The results obtained are represented in Table 1. According to the data presented the electrode exhibits good selectivity to melatonin even against structurally similar compounds such as tryptophan.

3.5. Analytical characterization of the method

Figure 7 represents the DPV signals of various concentrations of melatonin, obtained utilizing the ErO_2/MnO_2-CPE. As seen the DPV response increases with increasing melatonin concentration. The calibration curve plotted based on the DPV responses shown, is also depicted in the inset of the figure. According to this plot, the correlation between the melatonin voltammetric signal and its concentration is linear over the concentration range of 0.04-20 µM. The limit of detection (LOD) for the method was obtained to be 6.0 nM (using the $3S_b/m$ formula; S_b: standard deviation of the blank, m: the slope of the calibration curve). The RSD% of four replicated determinations of melatonin with the fabricated sensor was calculated to be 4.08% (n= 3), indicating favorable repeatability for the electrode. It was also found that the signal of ErO_2/MnO_2-CPE was stable even after 8 months.

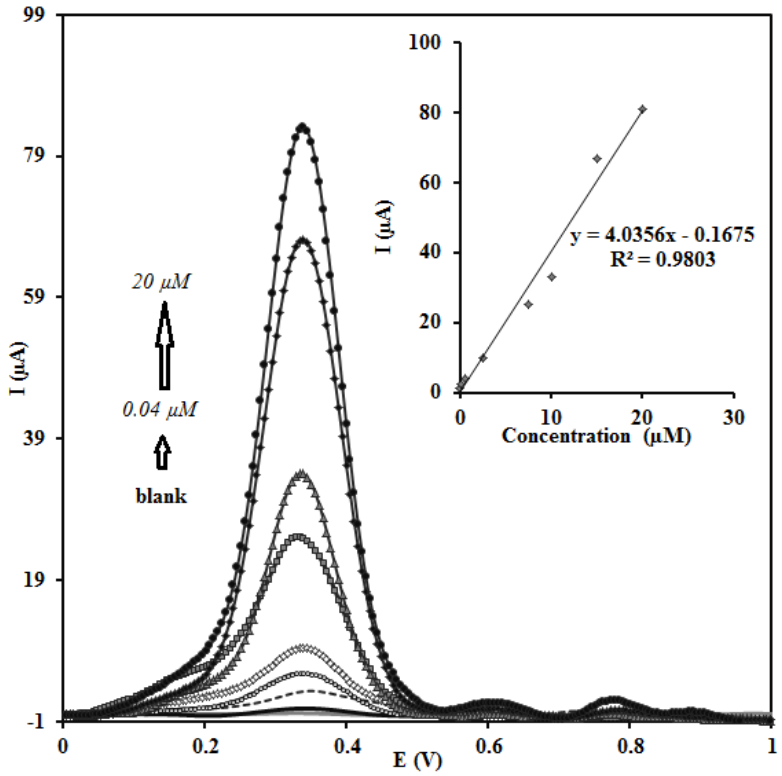

Figure 7. DPV responses for different concentrations of melatonin in the optimal conditions, obtained using the modified electrode (ErO_2/MnO_2-CPE); the calibration curve plotted based on the recorded voltammograms (inset)

3.6. Application of ErO_2/MnO_2-CPE for determination of melatonin in real samples

To evaluate the applicability of the modified electrode in real samples, it was used for the determination of MEL in melatonin pharmaceutical samples. The results obtained are represented in table 2. Both recovery and RDS results depicted indicate that the method has good accuracy and precision for MEL assay in pharmaceutical samples.

Table 2. Determination of melatonin in human plasma, urine, and pharmaceutical samples by ErO_2/MnO_2-CPE (n = 3)

Sample	Labeled value (µM)	Added (µM)	Found (µM)	RSD%	Recovery (%)
Melatonin[a] tablet	1.00	-	1.02	2.2	102.0
	5.00	-	4.84	4.2	96.8
	10.00	-	9.52	3.3	95.2
Melatonin[b] tablet	1.00	-	0.97	5.2	97.0
	5.00	-	4.81	5.2	96.2
	10.00	-	9.42	4.8	94.2

[a] Manufactured by Company of Nathura S.p.A, Italy
[b] Manufactured by Company of ESI, Italy

4. CONCLUSION

In this work, a carbon paste electrode modified with ErO_2/MnO_2 nanocomposite was constructed and utilized for the melatonin assay by differential pulse voltammetry. The electrode represented excellent electrocatalytic characteristics for melatonin electro-oxidation. It was demonstrated that the new melatonin sensing platform is a highly sensitive method for melatonin determination in pharmaceutical samples. Moreover, the proposed method exhibited a very low detection limit as well as high selectivity, which enable us to utilize the sensor for melatonin determination in the presence of some potential interfering agents such as tryptophan.

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