

Study of the Electrochemical Behavior of Melatonin on Different Electrodes in Aqueous Solution

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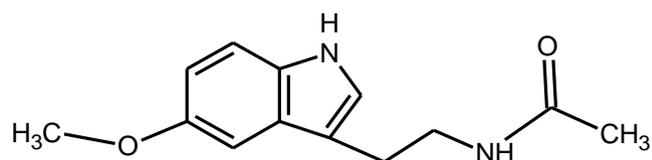
The electrochemical investigation of melatonin was researched utilizing cyclic, linear sweep voltammetry at a glassy carbon (GC), gold (Au) and platinum (Pt) terminals. The aim of this work was to study the behavior of melatonin at different electrodes. The cyclic voltammetry (CVs) of melatonin (1.0×10^{-4} mol L⁻¹) were critically investigated at various sweep rate (10-1000 mV/s) at GCE, AuE and PtE in B-R buffer solution of pH 7. The repeatability, reproducibility and selectivity of all electrodes were investigated. These electrodes were successfully applied for the analysis of melatonin pharmaceutical dosage forms without electroactive interferences from the tablet excipients

Keywords: Cyclic voltammetry, Glassy carbon electrode, Gold electrode, Melatonin, Platinum

1. INTRODUCTION

Melatonin (MT) chemically is an N – [2-(5methoxy-1H-indol-3-yl) ethyl] acetamide (Scheme 1) (Merck index), is a hormone delivered by the pineal organ situated in the accurate focal point of the brain at night, then decreasing to lower level during the day [1-3]. It is clinically used as endogenous antioxidant [2, 4-11] in the treatment of cancer, immune disorder [12-15], mental disorders and depression [16,17]. The significant work for most scientists is the quantification of melatonin in pharmaceutical formulations and biologic fluids. So, a lot of MT determination methods were developed. Some techniques of chromatography combined with various detectors such as mass spectrometry [18–20], UV–VIS [21,22] or diode array detector [23]. Other specific methods of quantification of melatonin include capillary electrophoresis with electrochemical detection [24], an immunosorbent enzyme-linked assay (ELISA) [25]. The solid electrodes and the hanging mercury drop electrode are used for the determination of melatonin among the large difference of working electrodes used in voltammetry [26].

In modern electrochemistry, solid electrodes have become more popular, due to the possibility of different modifications. Boron doped diamond electrode [27,28], carbon paste electrode [29,30] and glassy carbon electrode [22,30,31] were used for the determination of melatonin according to literature. To obtain high sensitivity of the measurements performed, different electrodes are now being produced, such as multi-walled carbon nanotubes [32,33], palladium nanoparticles [34], ZnO₂ nanorods [35], or graphene [36–38]. In the branch of electrochemistry, especially carbon nanomaterials such as graphene, carbon nanotubes, and carbon black [39,40] are of great interest. The proposed method showed that the repeatability, reproducibility and selectivity of all electrodes were successfully applied for the analysis of melatonin pharmaceutical dosage forms without electroactive interferences from the tablet excipients.



Scheme 1. Chemical structure of melatonin

2. EXPERIMENTAL

2.1. Reagents and materials

Analytical chemicals-grade of reagents (A.R) have been used as obtained. 50% HCl (Analar) and HNO₃ (2.0 mol L⁻¹) were used to clean bottles and glassware's and then rinsed with water. A stock solution of melatonin (1 × 10⁻³ mol L⁻¹) was prepared in ethanol. A series of Britton–Robinson (B–R) buffers (pH 2–11) were prepared by mixing the same amount of BDH acetic (4 × 10⁻² mol L⁻¹), phosphoric (4 × 10⁻² mol L⁻¹) and boric (4 × 10⁻² mol L⁻¹) acids in ultra-pure water and by adding different amounts of NaOH (2 × 10⁻¹ mol L⁻¹) solution the pH of the solutions were then changed to the necessary pH [41]. Melatonin was purchased from Sigma (Saint Louis, MO, USA). A stock solution of melatonin (1 × 10⁻³ mol L⁻¹) was prepared in methanol and stored at 4 ° C in appropriate vials shielding the solution from sunlight. Regular diluting of the stock solution provided fresh working standard solutions. Britton–Robinson buffer and 30 per cent ethanol were used for the electrolyte.

2.2. Instrumentation

The cyclic voltammetry measurements were performed on VSP-300 multi-channel potentiostat (Bio-logic Science, France). The working electrodes such a glassy carbon electrode (3mm), a gold electrode, and a platinum working electrode, were used, respectively. A saturated calomel electrode (SCE) was used as a reference electrode whereas pt wire was used as counter electrode, respectively. A 10–100 μL (Volac) and Martini pH-meter (model Mi 150) digital micro-pipette was used to prepare

standard and test solutions, and pH measurements. Ultrapure water was collected from the Milli-Q Plus (Millipore, Bedford, MA, USA) method and used to prepare all solutions.

2.3. Preparation of bare glassy carbon electrode (GCE)

The GCE was polished using a polishing cloth to reflect smoothness with 0.05 μm of alumina slurry, rinsed with 1:1 $\text{HNO}_3\text{-H}_2\text{O}$ (v / v). The electrode was, rinsed with ethanol and deionized water, and finally dried.

2.4. Preparation of gold electrode (AuE)

The surface of the gold electrode was cleaned by polishing with alumina (0.3 μm , Metrohm, England), immersion in hydrogen peroxide for 30 min, thoroughly rinsing with Milli-Q water and drying with a nitrogen gas stream.

2.5. General recommended CV procedures

The electrochemical cell was pre-cleaned and washed with nitric acid (10% v / v) and deionized water. The suggested general CV procedures was performed using AuE, GCE and PE as follows: an appropriate volume (10 mL) of an aqueous solution containing BRB (pH 7) as the electrolyte support and the required volume (1 mL) of melatonin ($1.0 \times 10^{-3} \text{ mol L}^{-1}$) was transferred to the voltammetric cell. Stirred and purged the sample solution was done by using N_2 gas for 30 minutes. The stirrer was then stopped and the background voltammogram of the support using pt electrode was then reported by applying a potential scan of -1 to 0.2 V vs. SCE

Similarly, the influence of the sweep rate ($v = 10 - 1000 \text{ mV s}^{-1}$) on the CVs of melatonine ($1.0 \times 10^{-4} \text{ mol L}^{-1}$) at the optimized pH 7 was also carried out using gold electrode by applying a potential from -0.2-1.5 V vs. SCE. Also, the CVs was recorded by GCE from -1V to 1.5V.

For each test, the three electrode device was placed in a blank solution, and the cyclic voltammetry was successively tested for electrode renewal twenty times.

3. RESULT AND DISCUSSION

3.1. Cyclic Voltammetry by GCE

The CVs of melatonin ($1.0 \times 10^{-4} \text{ mol L}^{-1}$) were critically investigated at various sweep rate (10-1000 mV/s) at GCE in B-R buffer solution of pH 7. The CVs were recorded on freshly polished GCE before each sweep rate vs. SCE. Melatonin compound showed two well-defined oxidation peak at 370 mV and 940 mV coupled with one well defined reduction peak at 310 mV in the investigated potential range from - 1.0 to 1.5 V vs. SCE reference electrode as shown in (Fig.1). The oxidation peak

corresponds to oxidation of (-NH-) to radical cation ((-N⁺·)) is most likely proceed via losing one electron at 370mV (I) (scheme 2). This peak was further reduced to (-NH) group at 310 mV (II). The (NH⁺) radical group is irreversibly oxidised to (-N-) with another oxidation peak at 940 mV by losing one electron (III).

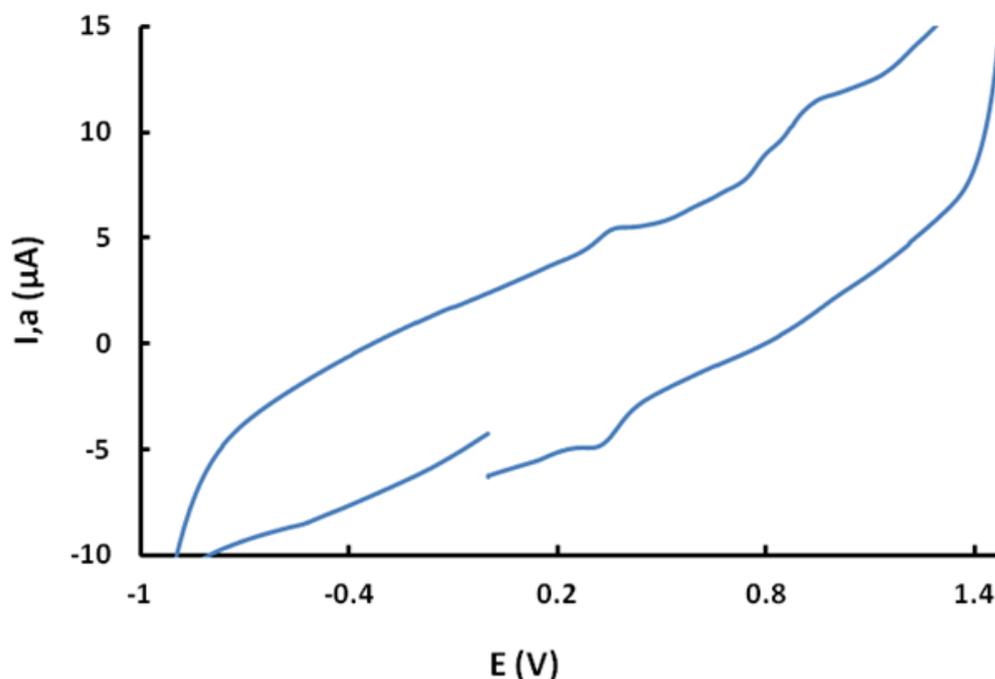
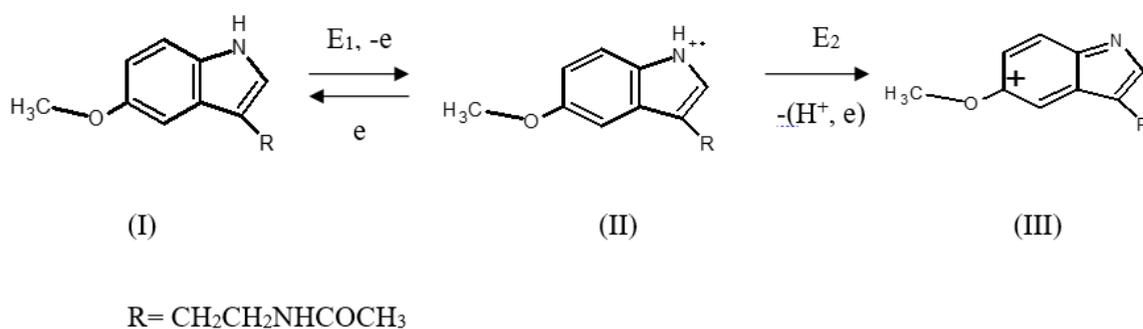


Figure 1. Cyclic voltammograms of 1×10^{-4} mol L⁻¹ melatonin at GCE and B-R buffer (pH=7) at 100 mVs⁻¹ scan rate and 60 s accumulation time.



Scheme 2. Mechanism of oxidation of melatonin on GCE

When the sweep rate was increased, the $E_{p, a}$ was anodically shifted to Confirm that the electrochemical oxidation process is irreversible [42-44]. On the other hand, by increasing the sweep frequency (Fig.2), the anodic peak current was steadily increased, indicating the occurrence of sluggish chemical reactions and a small mass transfer following the electrochemical process [44, 45]. The anodic peak current decreased significantly on continuous scanning and the signal from the baseline is hardly discernible. This behavior is most likely due to the potential fouling of the GCE electrode by the melatonin oxidation products produced under the experimental conditions used. The measured peak-

peak potential difference value ($\Delta E_p = E_{p,a} - E_{p,c}$) was > 59 mV, confirming the irreversible existence of the observed electrochemical reduction mechanism in the potential range used (-1.0 – 1.5 V).

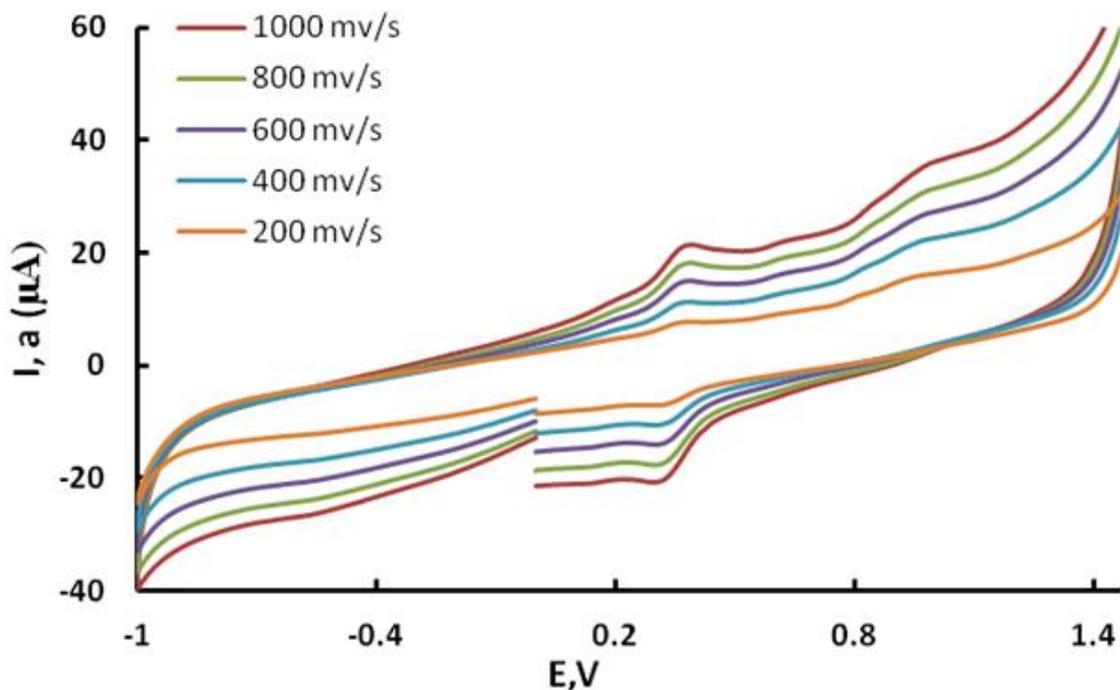


Figure 2. CVs of melatonin (1.0×10^{-4} mol L⁻¹) at pH 7. at various sweep rates (100-1000 mVs⁻¹) at GCE vs. SCE

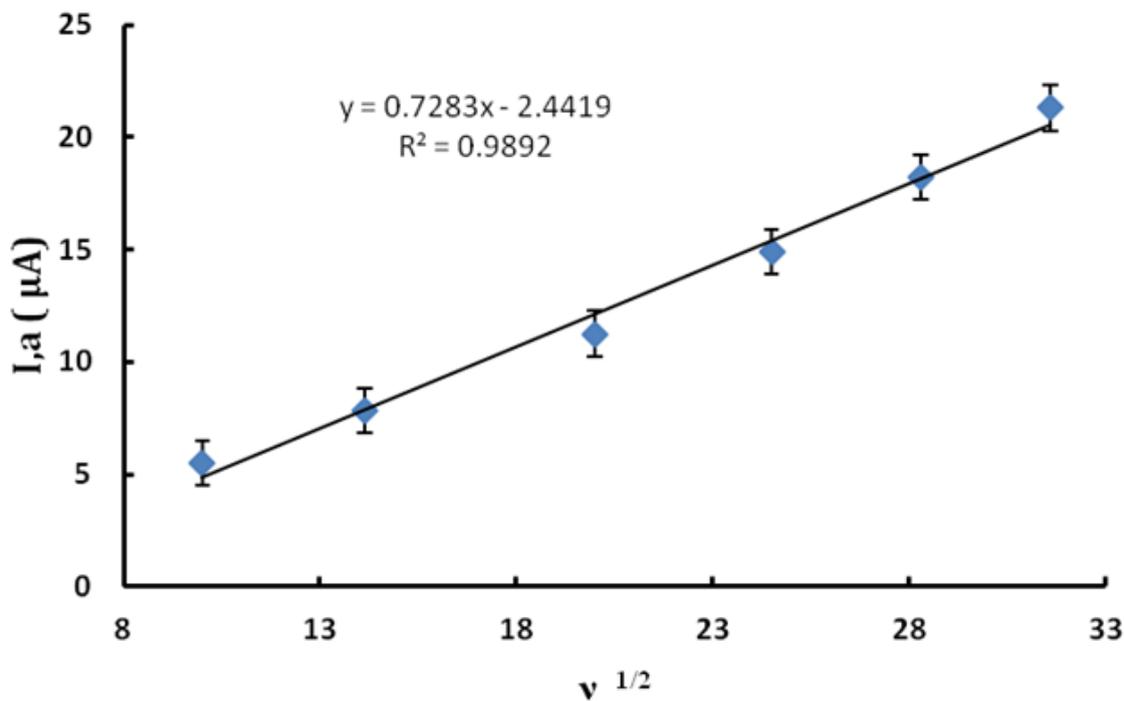


Figure 3. The plot of $I_{p,a}$ vs. square root of the sweep rate (v) of melatonin at pH 7.

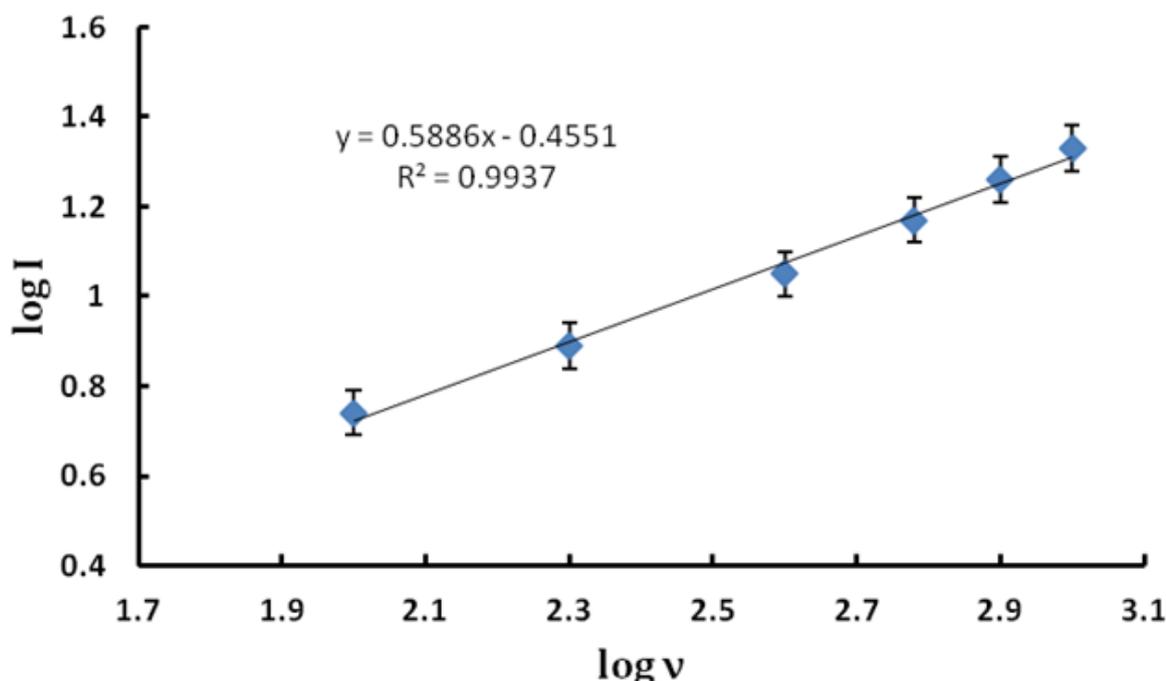


Figure 4. The plot of $\log I_{a}$ vs. $\log v$ of melatonin at pH 7.

Fig. 3 has shown the impact of sweeping rate on CV anodic peak current. In the CVs, the $i_{p,a}$ increased linearly on raising the sweep rate ($v^{1/2}$) suggests that, the electrochemical oxidation process is an electrochemical process regulated by diffusion [45, 46]. Moreover, the plot of $\log I_{a}$ vs. $\log v$ at pH 7 at GCE vs. SCE was linear as shown in Fig. 4 and the following regression equation:

$$\log I_{a} = 0.5886 \log v - 0.4551 \quad (r^2 = 0.9937) \quad (1)$$

was achieved with a slope > 0.5 ($r^2 = 0.9937$). Such data gave further evidence to the oxidation peak's irreversible existence. The slope value (0.5886) was not close to the expected theoretical value (1.0) expected when there is an adsorption process occur on the surface of the GCE [43-45].

3.2. Cyclic voltammetry by Au electrode

The CVs of melatonin ($1.0 \times 10^{-4} \text{ mol L}^{-1}$) were critically investigated at various sweep rate (10-1000 mV/s) at Au electrode in B-R buffer solution of pH 7.0. The CVs were recorded on freshly polished AuE before each sweep rate vs. SCE. Fig.5 revealed two anodic peaks and two peaks of reduction showing up in the reverse direction. At 620 mV and 1100 mV, respectively, the oxidation peaks appeared. The reduction peaks appeared respectively at 650 mV and 950 mV. The peak to peak separation difference (AE) for a completely reversible redox device that transfers one electron was greater than the estimated theoretical value. In the B-R buffer pH 7.0 at different scanning rates (from 100 mV s⁻¹ to 1000 V s⁻¹), the cyclic voltam -mograms of $1.0 \times 10^{-4} \text{ mol L}^{-1}$ melatonin were recorded

(fig.6). The peak currents of oxidation peaks are proportional to the scan frequency along the scanning range examined, suggesting a surface-controlled process.

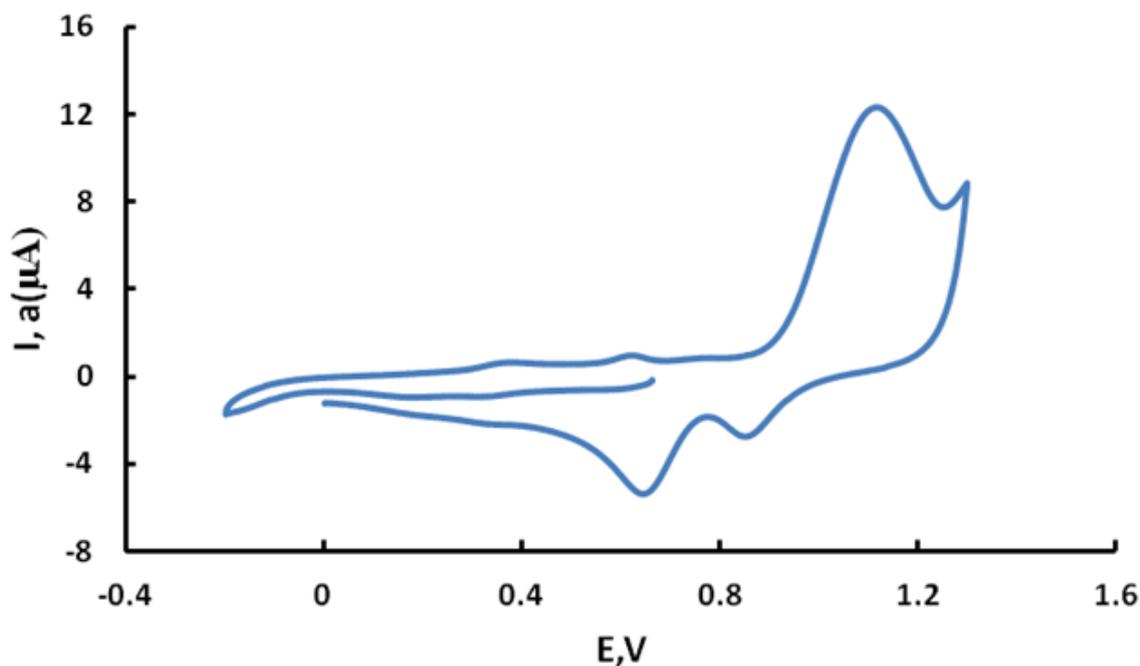


Figure 5. Cyclic voltammograms of 1×10^{-4} mol L⁻¹ melatonin at Au electrode and pH=7 ($v=100$ mV s⁻¹).

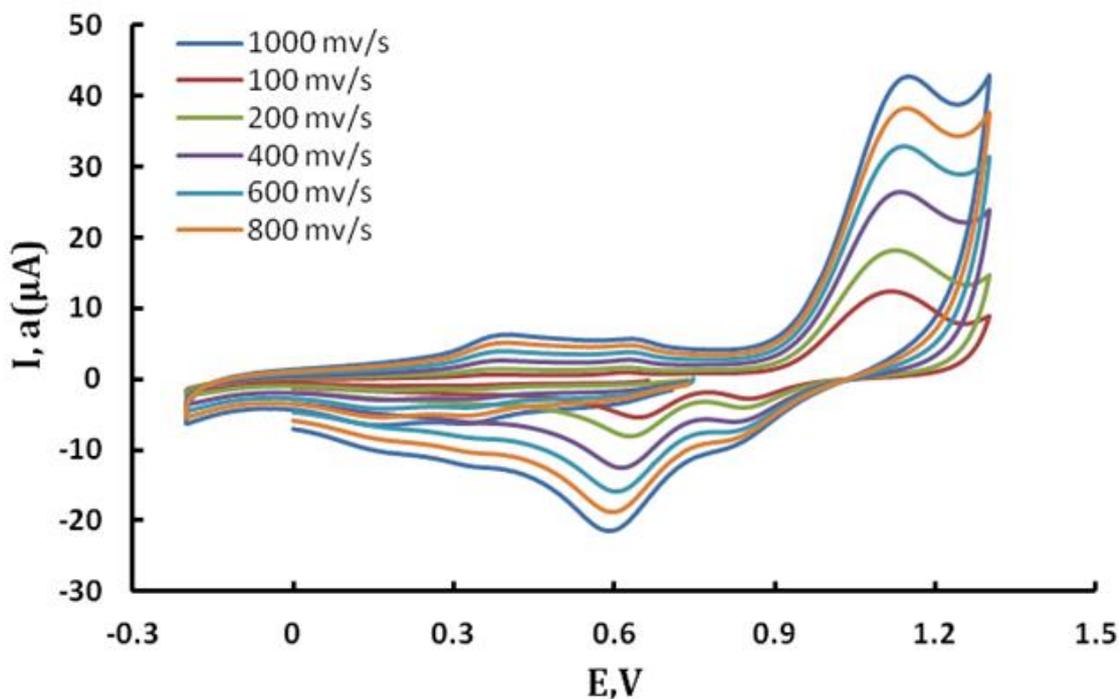


Figure 6. CVs of melatonin (1.0×10^{-4} mol L⁻¹) at pH 7 and various sweep rates (100-1000 mV/s) at Au electrode vs. SCE .

The $E_{p,a}$ was shifted anodically by increasing the sweep frequency, confirming the irreversible existence of the electrochemical oxidation process [42-44]. On the other hand, by increasing the sweep speed, the anodic peak current was steadily increased, indicating slower chemical reactions and a small mass transfer following the electrochemical process [44,45].

Fig 7 has shown the effect of the sweep rate on the anodic peak current of the CVs.. In the CVs, the $I_{p,a}$ increased linearly on raising the sweep rate ($v^{1/2}$) suggests that the electrochemical oxidation process is an electrochemical process operated by diffusion [45,46]. Moreover, the plot of $\log I_a$ vs. $\log v$ at pH 7 at Au electrode vs. SCE was linear as shown in Fig. 8 and the following regression equation:

$$\log I_{a} = 0.5248 \log v + 0.0478 \quad (r^2 = 0.9996) \quad (2)$$

was achieved with a slope > 0.5 ($r^2 = 0.9996$). The irreversible existence of the oxidation peak was further verified by these results. The slope value (0.5248) was not close to the expected theoretical value (1.0) when an adsorption process occurs on the Au electrode surface [43- 45].

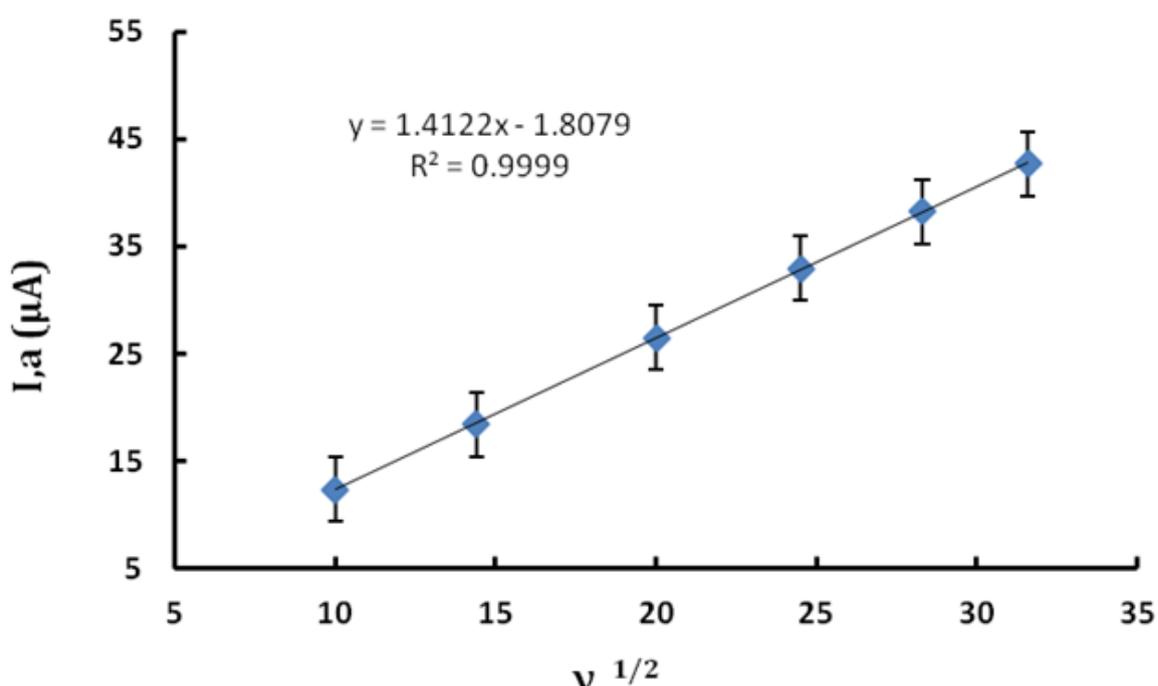


Figure 7. The plot of $I_{p,a}$ vs. square root of the sweep rate (v) of melatonin at pH 7.

3.3. Cyclic voltammetry by Pt electrode

The CVs of melatonin ($1.0 \times 10^{-4} \text{ mol L}^{-1}$) were critically investigated at various sweep rate (10-1000 mV/s) at Pt electrode in B-R buffer solution of pH 7.0. The CVs were recorded on freshly polished PtE before each sweep rate vs. SCE. One anodic peak was observed and one reduction peak appeared on the reverse scan (fig .9). The oxidation peak appeared at -125 mV. The peaks of reduction appeared at -840 mV. The peak-to-peak separation difference (AE) was larger than the calculated theoretical value for a fully reversible redox system that transfers one electron.

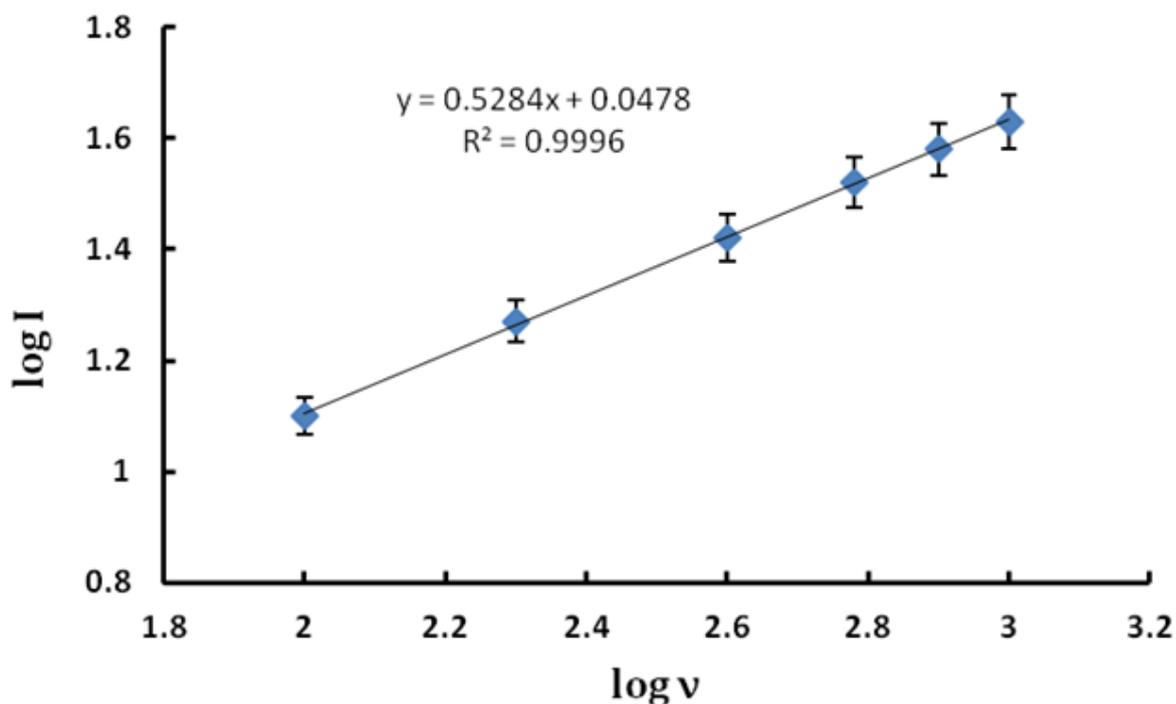


Figure 8. The plot of $\log I_a$ vs. $\log v$ of melatonin at pH 7.

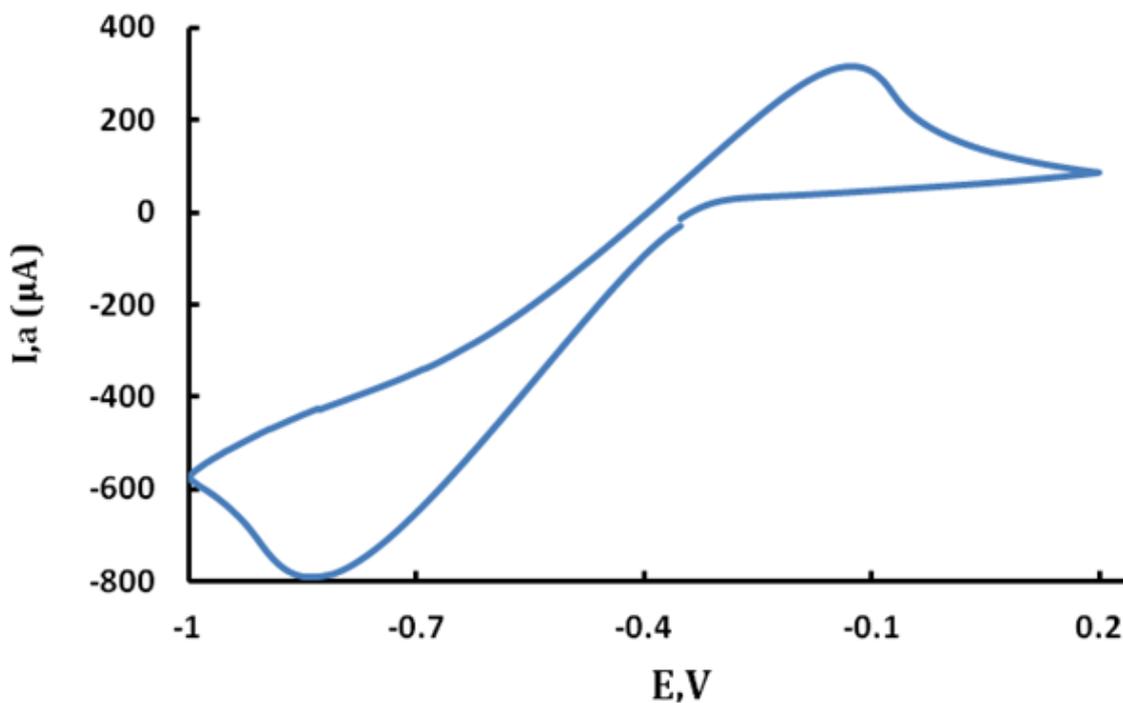


Figure 9. Cyclic voltammograms of $1 \times 10^{-4} \text{ mol L}^{-1}$ melatonin at Pt electrode and pH=7 ($v=100 \text{ mV s}^{-1}$)

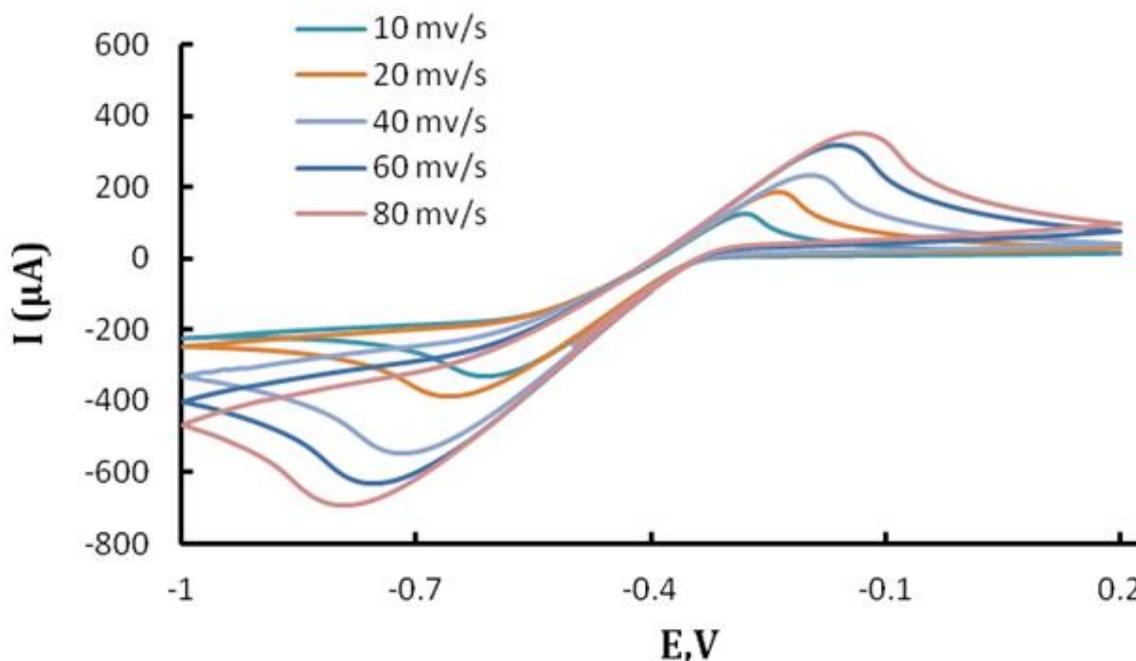


Figure 10. CVs of melatonin ($1.0 \times 10^{-4} \text{ mol L}^{-1}$) at pH 7 and various sweep rates (10-100 mVs^{-1}) at pt electrode vs. SCE .

The cyclic voltammograms of $1.0 \times 10^{-4} \text{ mol L}^{-1}$ melatonin in B-R buffer (pH 7.0) at different scan rates (from 10 mVs^{-1} to 100 mVs^{-1}) were recorded (fig .10). With a slight positive shift in potential, the oxidation peak currents increased as the scan rate increased, a typical feature of irreversible electrochemical reactions. The peak currents of oxidation peaks were proportional to the scan rate along the scanning range examined, suggesting a surface controlled process. Nonetheless, during the successive cyclic waves, the peak current of oxidation decreases significantly and ultimately disappears. This results from the fact that melatonin or its oxidation products block the electrode surface, which reduces the effective reaction sites on the surface of the electrode. It was found that the peak current of oxidation (I_p, a) varies linearly with the square root of scan rate ($v^{1/2}$) fig.11, which is an indication that compound oxidation is primarily a diffusion controlled process. The linear dependence follows the equations as follows:

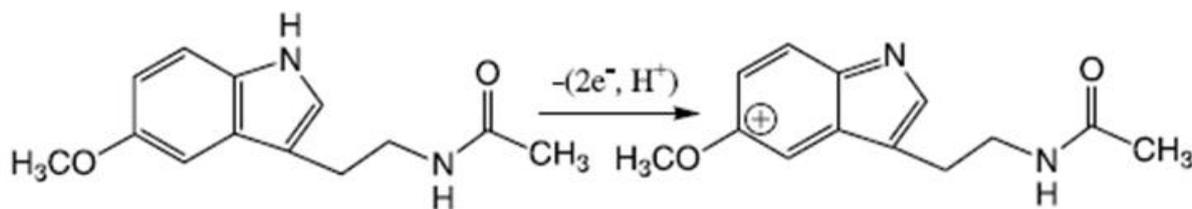
$$I_{p,a} = 39.296 v^{1/2} + 3.8337 \quad (r^2 = 0.9845) \tag{3}$$

Moreover, the plot of $\log I_a$ vs. $\log v$ at pH 7 at pt electrode vs. SCE was linear as shown in Fig. 12 and the following regression equation:

$$\log I_{p,a} = 0.4836 \log v + 1.625 \quad (r^2 = 0.9873) \tag{4}$$

The slope of 0.48 for a diffusion controlled procedure is similar to the hypothetically predicted estimates of 0.5. The quantity of electrons moved (n) in melatonin electrooxidation at pt anode was assessed by the accompanying condition: $\alpha n = 47.7 / (E_p - E_{p/2})$, where α is the transfer coefficient of

charge and n is the quantity of electrons associated with the redox response [42]. The individual cyclic voltammograms at 100 mV s^{-1} (Figure 9) were used for this study. The value of $E_p - E_{p/2}$ was found as 50 mV . Thereafter, it was determined that αn was 0.95 . The value of α for most systems is from 0.3 to 0.7 . Consequently, by accepting $\alpha = 0.5$, which is commonly used for an absolutely irreversible system, the n have been set at 1.91 (~ 2). These outcomes show that the irreversible oxidation of compound includes two electrons for every particle on pt anode. The proposed system for the electrochemical oxidation of melatonin displayed in Scheme 3.



Scheme 3. Mechanism of oxidation of melatonin on pt electrode

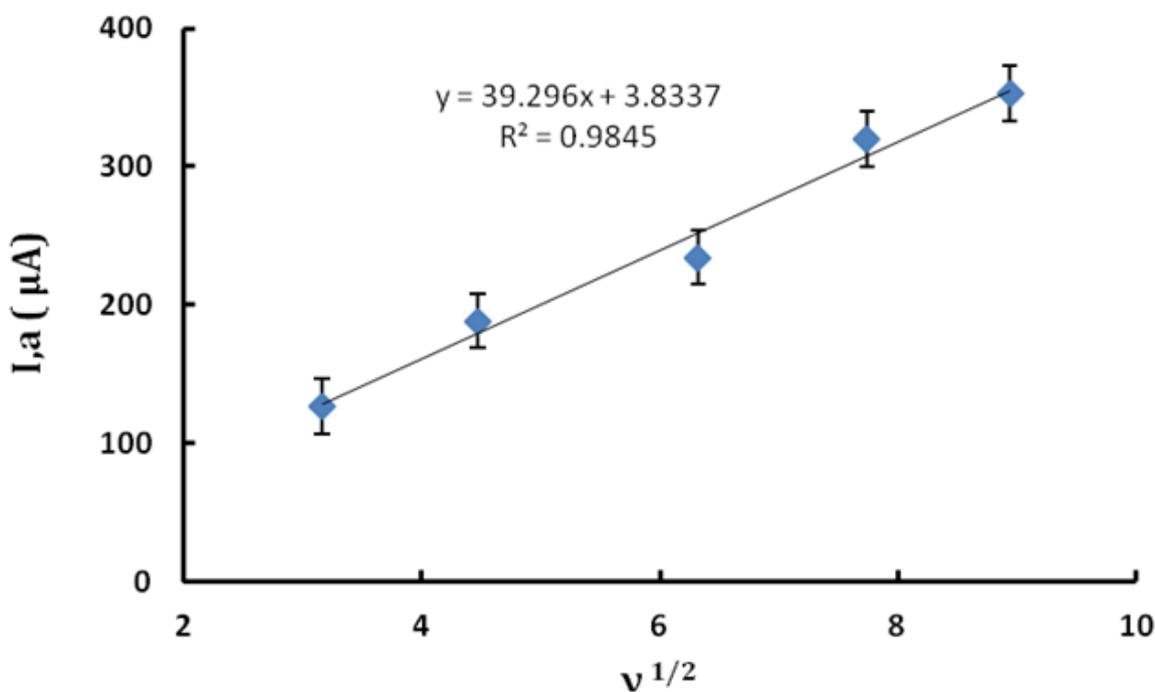


Figure 11. The plot of I_a vs. square root of the sweep rate (v) of melatonin at $\text{pH } 7$.

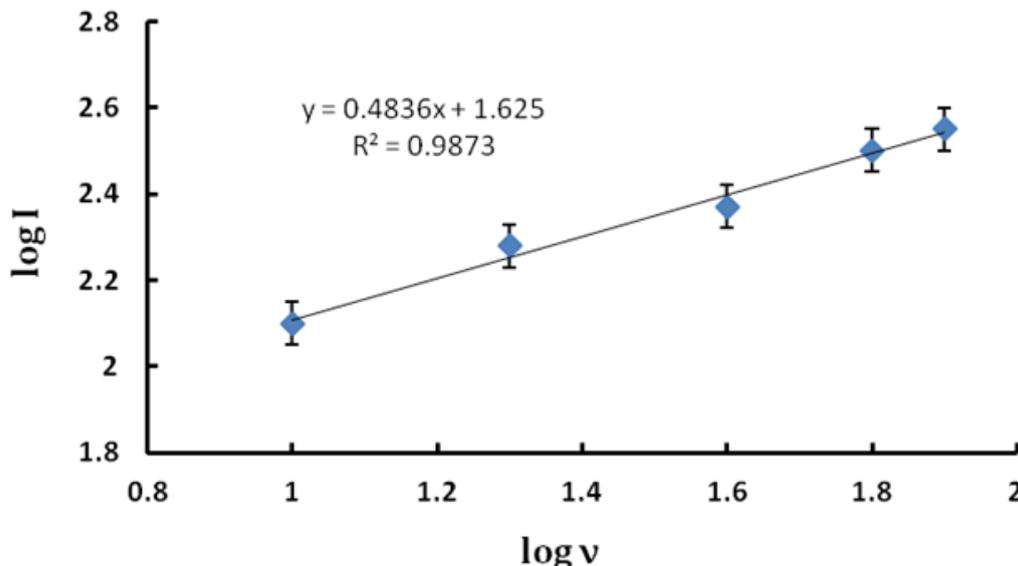


Figure 12. The plot of $\log I_a$ vs. $\log v$ of melatonin at pH 7.

4. CONCLUSION

The presented electrodes appeared that cyclic voltammetry of melatonin ($1.0 \times 10^{-4} \text{ mol L}^{-1}$) at various sweep rate (10-1000 mV/s) in B-R buffer solution of pH 7 successfully applied for the analysis of melatonin pharmaceutical dosage forms without electroactive interferences from the tablet excipients. The repeatability, reproducibility and selectivity of all electrodes are very good. The simple preparation of investigated electrodes enables the application of method to any conditions and provides a reliable signal of melatonin. The data obtained were well in agreement with previous voltammetry measurements.

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