



Original scientific paper

## Pre-treated pencil graphite modified electrode sensor for melatonin

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### Abstract

Since hormones are essential for controlling a wide range of physiological processes in living organisms, sensitive and precise hormone detection methods are needed. In this work, a pencil graphite electrode was pre-treated in 0.1 M KOH solution over many potential cycles. A potential pretreated pencil graphite modified electrode (PPGME) was developed for detecting bioactive molecules and the hormone melatonin (MEL), using phosphate buffer solution as the supporting electrolyte. Various electrochemical parameters, including pH effects, sweep rates, and studies of different concentrations, were investigated for the determination of melatonin. The PPGME was employed for the simultaneous analysis of melatonin and paracetamol. Interference studies for both analytes showed excellent selectivity and a significant peak current response using the PPGME. The detection limit for MEL was determined to be 0.442  $\mu\text{M}$  and this modified PPGME was also applied for the detection and recovery of MEL in a pharmaceutical sample.

### Keywords

Hormone; paracetamol; voltammetry; electrochemical sensor; pharmaceutical tablets

### Introduction

Hormones are chemical messengers produced by glands that regulate various bodily functions. Imbalances, due to lifestyle, can cause symptoms like fatigue, mood changes, and infertility, and may lead to serious health issues. Monitoring hormone levels is vital for overall health [1,2]. Melatonin is a hormone mainly produced by the pineal gland, with additional production in brain cells [3,4]. It regulates circadian rhythms and supports brain, immune, and cardiovascular health

through antioxidant and anti-inflammatory actions [5,6]. Imbalances in melatonin are linked to insomnia, depression, cancer, and other diseases [7-10]. Its levels follow a daily cycle, peaking at night and dropping during the day, and can be disrupted by light exposure [11-13]. Melatonin supplements are widely used to address sleep issues, such as jet lag, and are regulated by the Food and Drug Administration (FDA). Accurate detection in biological samples is important for diagnosing health conditions and monitoring bodily functions [14,15].

Paracetamol (acetaminophen) is a widely used pain reliever and fever reducer [16-18]. Due to its extensive use, it can accumulate in the body and environment, posing risks to humans, animals, and ecosystems [19-21]. Though mostly metabolized, small amounts are excreted unchanged and can enter water systems [22-24]. Even at low concentrations, such pharmaceuticals affect aquatic life and health. This highlights the need for effective detection methods, with electrochemical sensors offering a promising solution [25-27].

Electrochemical sensors are highly effective for point-of-care melatonin detection due to their sensitivity, speed, low cost, and ease of use. They can detect low melatonin levels in biological samples, making them ideal for quick diagnostics. Pencil graphite electrodes (PGEs) are widely used in electroanalysis due to their affordability, mechanical strength, low background current, and ease of customization. They are disposable, commercially available, and ideal for miniaturized devices. Untreated PGEs are unsuitable for electrochemical use and require surface pre-treatment to enhance conductivity, surface area, and adsorption [28]. The electrochemical behaviour and catalytic activity of electrodes largely depend on surface features like area, morphology, and presence of functional groups. These can be modified through methods such as polishing, cleaning, or heat treatment. Among them, electrochemical pre-treatment stands out for its simplicity and low cost. This method not only cleans the surface but also generates new edge-plane sites rich in oxygenated functional groups, significantly enhancing the electrode's electrochemical performance [29-32]. In this novel study, a PGE was employed for the electrochemical detection of melatonin and paracetamol using a surface pre-treatment method involving potential cycling in a KOH solution. The analysis was conducted through cyclic voltammetry (CV) and differential pulse voltammetry (DPV). The base pre-treatment effectively activated the electrode surface, enhanced its surface area, and consequently, improved the sensitivity of the PGE. The proposed approach is straightforward, economical and effective, offering easy adaptability to electrode preparation systems for the electrochemical detection of melatonin.

## Experimental

### *Reagents and stock solutions*

Melatonin was acquired from Hi-media (molecular mass  $232.27 \text{ g mol}^{-1}$ ). Sodium dihydrogen phosphate ( $\text{NaH}_2\text{PO}_4$ ), disodium hydrogen phosphate ( $\text{Na}_2\text{HPO}_4$ ), potassium ferrocyanide ( $\text{K}_4[\text{Fe}(\text{CN})_6]$ ), potassium chloride (KCl), potassium hydroxide (KOH), and pencil-lead rods (0.7 mm) were obtained from Sigma Aldrich and Camlin, respectively. The stock solution of melatonin (0.25 mM) was prepared in ethanol. The phosphate buffer (PB) of the desired pH was obtained by mixing 0.2 M  $\text{NaH}_2\text{PO}_4$  and 0.2 M  $\text{Na}_2\text{HPO}_4$  in the proper ratio. A solution of potassium hydroxide (KOH) at a concentration of 0.1 M was prepared using double-distilled water.

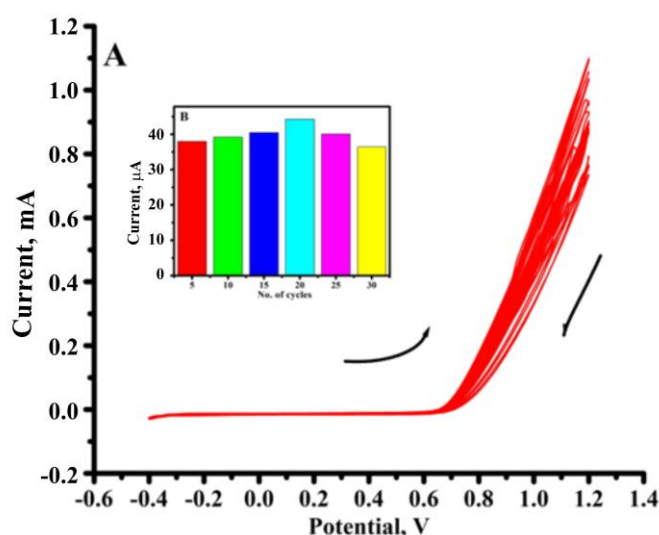
### *Instrumentation*

A CHI-660c potentiostat (CH Instruments electrochemical workstation) was used to perform differential pulse voltammetry (DPV) and cyclic voltammetry (CV) measurements. All electrochemical investigations were implemented in a traditional three-electrode cell system. A pencil

graphite electrode (PGE) having 0.7 mm diameter and 4 cm length was used as the working electrode, which was first treated with KOH solution. A saturated calomel electrode (SCE) was utilized as the reference electrode and the circuit was completed by a bright platinum electrode used as the counter electrode.

#### *Preparation of pretreated pencil graphite modified electrode*

The PPGME was prepared by electrochemical activation of PGE in  $25 \times 10^{-4}$  M KOH over a different number of cycles (5 to 30) at potentials ranging from -0.4 to 1.2 V and a scan rate of  $0.1 \text{ V s}^{-1}$ . Figure 1A presents CVs of 20 potential cycles performed at PGE. The PPGMEs formed by different number of potential cycles were tested using 0.1 mM  $\text{K}_4[\text{Fe}(\text{CN})_6]$  in 1 M KCl solution as the supporting electrolyte. Figure 1B shows that the anodic peak current ( $I_{pa}$ ) increased in the first 5, 10, and 15 cycles. However, at the 20<sup>th</sup> cycle, the electrode exhibited the maximum peak current response, with subsequent cycles showing a decrease in peak current. Consequently, the PPGME that had been pretreated with 20 potential cycles was selected for further assessment.



**Figure 1.** (A) Cyclic voltammograms of bare graphite electrode (PGE) in potassium hydroxide (0.1 M KOH) at a sweep rate of  $0.1 \text{ V s}^{-1}$  for 20 cycles; (B) bar graph of  $I_{pa}$  of  $1\text{mM K}_4[\text{Fe}(\text{CN})_6] \cdot 3\text{H}_2\text{O}$  in 1 M KCl versus number of potential cycles used for formation of PPGME

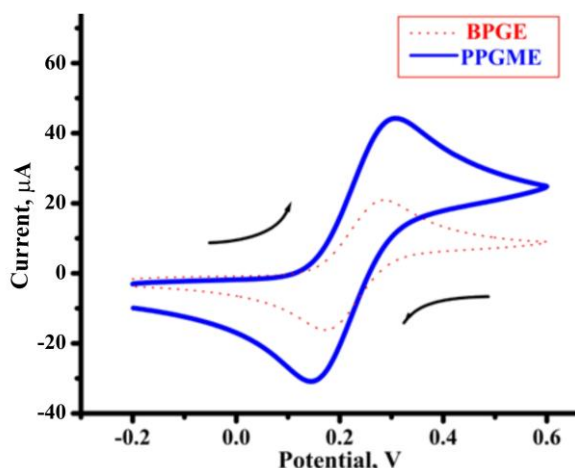
## Results and discussion

### *Electrochemical characteristics of PPGME*

The electrochemical characterization of the PPGME was further analysed using a 0.1 mM  $\text{K}_4[\text{Fe}(\text{CN})_6]$  in 1 M KCl solution as the supporting electrolyte, employing a sweep rate ( $\nu$ ) of  $50 \text{ mV s}^{-1}$ . Figure 2 compares CVs of PPGME (20 cycles) and bare PGE (BPGE), showing significantly higher redox peak currents for PPGME. Also, the anodic to cathodic peak separation ( $\Delta E_p$ ) decreased significantly, accompanied by a stable enhancement of the redox peak current at PPGME. An approach to determine the total electroactive surface area accessible for electron transfer processes is given by Randles-Ševičik's Equation (1):

$$I_p = (2.69 \times 10^5) n^{3/2} A D^{1/2} C_0 \nu^{1/2} \quad (1)$$

where  $I_p / A$  represents the peak current,  $A / \text{cm}^2$  denotes the electroactive surface area,  $C_0$  stands for the concentration of the electroactive species,  $n$  signifies the number of exchanged electrons,  $D / \text{cm}^2 \text{ s}^{-1}$  denotes the diffusion coefficient of analyte and  $\nu / \text{V s}^{-1}$  denotes the scan rate. It was determined that the electrode surface area for PPGME was  $0.054 \text{ cm}^2$ .



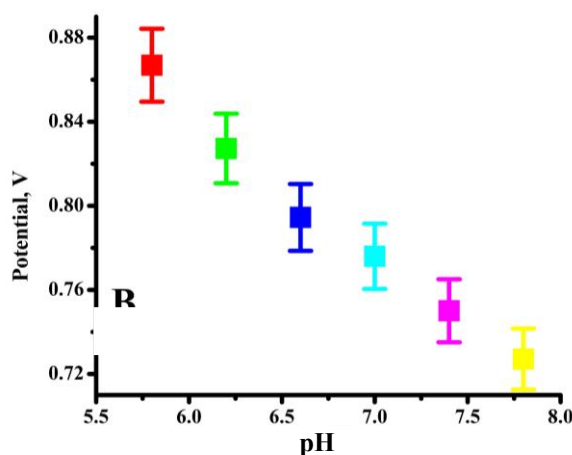
**Figure 2.** Cyclic voltammograms of 1 mM  $K_4[Fe(CN)_6] \cdot 3H_2O$  in 1 M KCl for bare PGE and PPGME at a sweep rate of  $0.05 \text{ V s}^{-1}$

### Significance of pH on the redox peak current of MEL at PPGME

Electrochemical reactions can be studied using solutions of varying pH levels. Melatonin (MEL) is an electroactive hormone that undergoes oxidation readily. The voltammograms were recorded over a potential range from 0 to 1 V and the cyclic voltammograms (CVs) of  $10 \mu\text{M}$  MEL at PPGME in 0.2 M PB at different pH ranging from 5.8 to 7.8, using a sweep rate of  $50 \text{ mV s}^{-1}$ . The most intense and sharper peak current response of MEL was observed at pH 7.4. Above pH 7.4, a significant decrease in the anodic peak current ( $I_{pa}$ ) was observed and the pH dependence of anodic peak potential ( $E_{pa}$ ) is defined by Equation (2):

$$E_{pa} / \text{V} = (-0.0592 m/n) \text{pH} \quad (2)$$

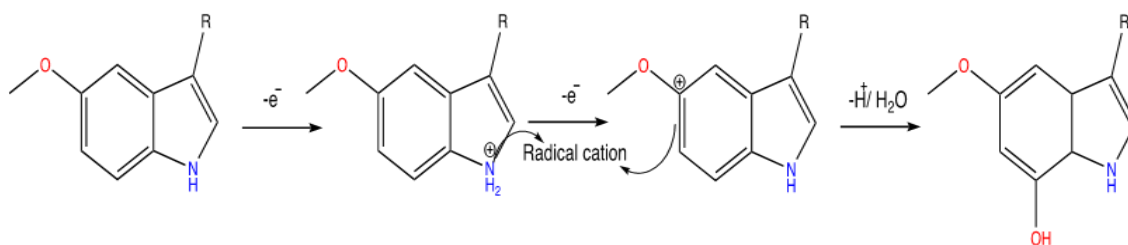
Here,  $m$  denotes the number of protons, while  $n$  denotes the number of electrons transferred during the electrochemical process. Figure 3 shows the linear equation:  $E_{pa} / \text{V} = -0.0677 \text{pH} + 1.2513$ , with correlation coefficient ( $R^2$ ) = 0.9846. Protons significantly influence the electron exchange process at the electrode.



**Figure 3.** Plot of  $E_{pa}$  versus pH for  $10 \mu\text{M}$  MEL at PPGME in 0.2 M PB

The slope value is very close to the Nernstian value, so it can be concluded that there is an equal number of exchanges between protons and electrons based on the correlation of the slope.

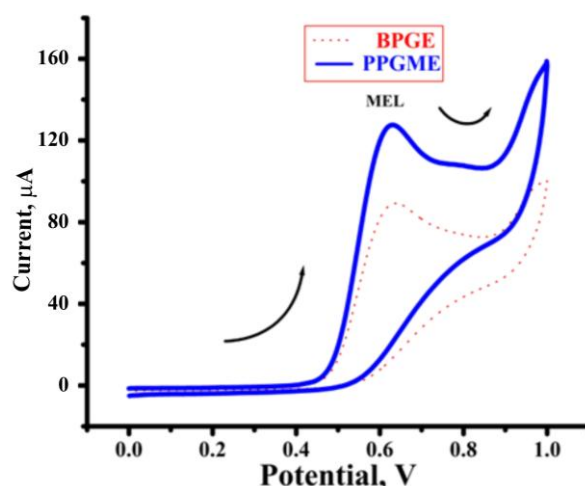
The research indicates that the electrochemical oxidation of MEL on PPGME depends on pH. It involves a two-electron process accompanied by the transfer of two protons, resulting in the formation of MEL and the oxidation scheme is denoted in Scheme 1. PPGME demonstrates a significant enhancement of redox peak current at pH 7.4, effectively reducing overpotential.



**Scheme 1.** Oxidation scheme of melatonin

#### Electrochemical behaviour of MEL on the surface of PPGME

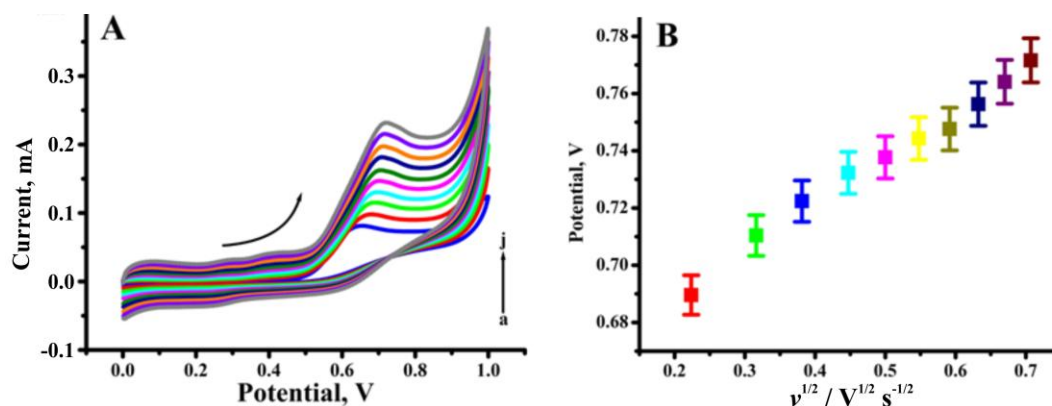
Figure 4 illustrates cyclic voltammograms (CVs) of PPGME obtained for 10  $\mu\text{M}$  MEL in 0.2 M PB at pH 7.4, using a sweep rate of 50  $\text{mV s}^{-1}$ . The potential window ranged from 0 to 1 V. In comparison with bare pencil graphite electrode (BPGE), a markedly increased redox peak current with minimal variation in redox peak potential, illustrating excellent electrochemical properties for detecting MEL, is observed for PPGME.



**Figure 4.** Cyclic voltammograms of 10  $\mu\text{M}$  MEL in 0.2 M PB (pH 7.4) and  $\nu = 0.05 \text{ V s}^{-1}$  for BPGE and PPGME

#### Effect of sweep rate on the redox peak current of MEL at PPGME

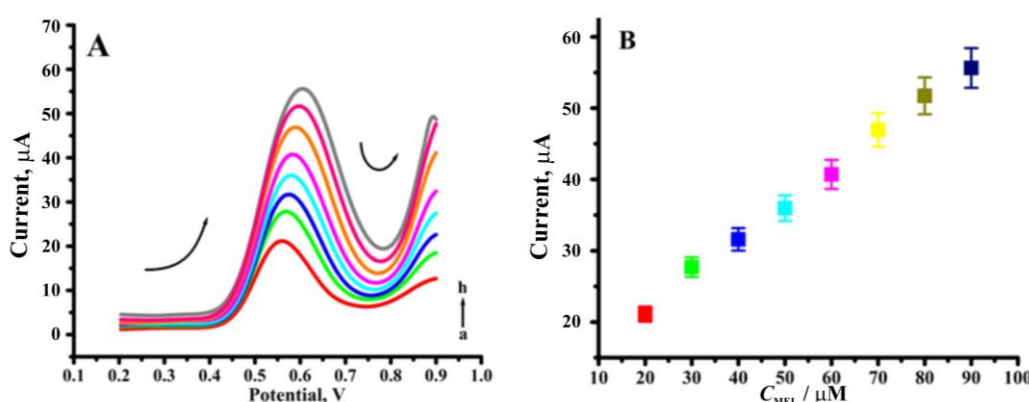
The electrochemical redox performance of 20  $\mu\text{M}$  MEL in 0.2 M PB was studied at different scan rates using the CV technique on PPGME. Figure 5A illustrates that the intensity of the redox peak current of MEL gradually increased and shifted to the positive potential side as the applied sweep rate was raised from 50 to 500  $\text{mV s}^{-1}$ . Figure 5B shows the plot of the anodic peak potential of MEL against the square root of the scan rate at PPGME. The graph indicates a linear relationship and  $R^2 = 0.99277$  and it shows that the process of the electrode was an adsorption-controlled phenomenon.



**Figure 5.** (A) Cyclic voltammograms of 20  $\mu\text{M}$  MEL at PPGME in 0.2 M PBS (pH 7.4) at scan rates of: (a - j) 0.05 to 0.5  $\text{V s}^{-1}$ ; (B)  $E_{pa}$  versus square root of scan rate

### Concentration effect of MEL at PPGME

Figure 6A displays the cyclic voltammograms (CVs) of PPGME obtained at various concentrations of MEL. The concentrations of MEL ranged from 20 to 90 μM in 0.2 M PB at pH 7.4, using a sweep rate of 50 mV s<sup>-1</sup>.



**Figure 6.** (A) DPV plots of PPGME in 0.2 M PB (pH 7.4) for varying concentration of MEL: (a-h: 20 to 90 μM); (B) calibration plot of  $I_{pa}$  versus concentration of MEL

With increasing concentration of MEL, the anodic peak current ( $I_{pa}$ ) increases in a linear manner. Figure 6B illustrates the calibration graph that correlates the MEL concentration with  $I_{pa}$ . The linear regression equation for MEL at PPGME gives  $I_{pa} = 4.6115 + 1.19027 \times 10^{-4} C_{MEL}$  and  $R^2 = 0.9969$ . The limit of detection (LOD) and limit of quantification (LOQ) were calculated by Equations (3) and (4):

$$LOD = 3 S/M \tag{3}$$

$$LOQ = 10 S/M \tag{4}$$

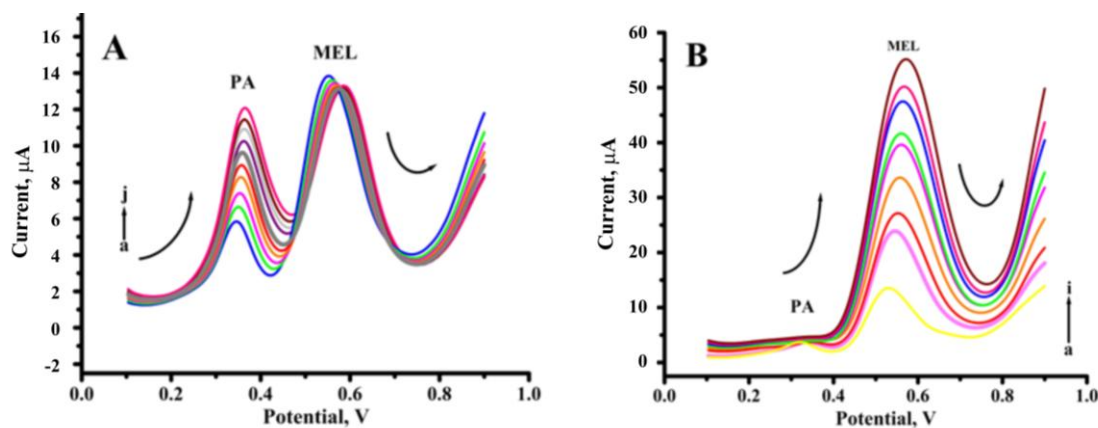
where  $M$  represents the slope of the graph and  $S$  denotes the standard deviation. Using PPGME, the limits of detection (LOD) and quantification (LOQ) were determined to be 0.442 and 1.47 μM, respectively. These results indicate that PPGME exhibits excellent linearity and achieves low LOD and LOQ values when compared with results for other published modified electrodes in Table 1 [33-37].

**Table 1.** LOD of MEL at PPGME compared to results of some other reported electrodes

Electrode	Method	LOD, μM	Reference
B-RGO	CV	0.70	[33]
GPH-CSPE	CV	0.87	[34]
Gr-AV	CV	0.49	[35]
ZnFe <sub>2</sub> O <sub>4</sub> /CPE	DPV	8.0	[36]
CNTs and grapheme based CSPE	CV	1.1	[37]
PPGME	DPV	0.44	Present work

### Interference study of MEL and PA at PPGME

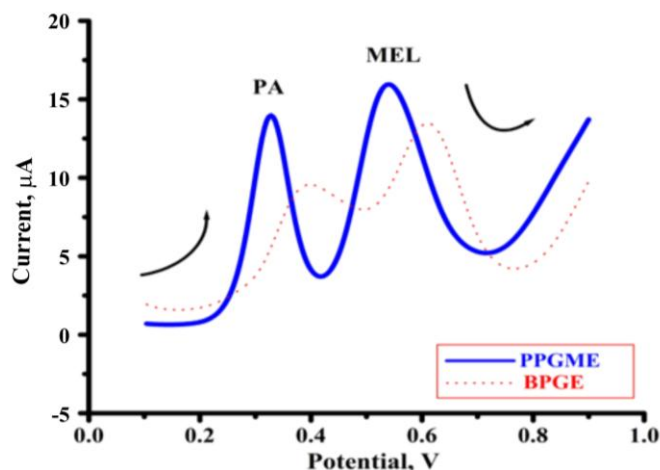
An interference study is crucial for exploring and establishing the practical applicability of a developed electrode material. Here, an interference study was performed using DPV analysis with varying concentrations of MEL, ranging from 10 to 100 μM, in the presence of 10 μM of the electro-active interfering analyte paracetamol (PA). The experiment was carried out at ambient temperature in 0.2 M PBS (pH 7.4), and results are presented in Figure 7A. A significant increase in current density was observed when both MEL and PA were added, showing a distinct signature in the DPV compared to MEL alone and *vice versa*, particularly at PA concentrations ranging from 10 to 90 μM (Figure 7B). This suggests that the PPGME electrode exhibits high selectivity, specifically towards the electro-oxidation of MEL, where the interfering agent PA does not significantly influence it.



**Figure 7.** DPVs of PPGME in 0.2 M PB (pH 7.4) at various concentrations of: (A) PA from 10 to 100  $\mu\text{M}$  in presence of 10  $\mu\text{M}$  MEL; B) MEL from 10 to 90  $\mu\text{M}$  in presence of 10  $\mu\text{M}$  PA

### Simultaneous detection of PA and MEL

The DPV technique was employed to investigate the accuracy of the simultaneous detection of PA and MEL. As seen in Figure 8, at the bare pencil graphite electrode (BPGE), the separation peaks for PA and MEL are not clearly distinguishable and they appear partly merged (indicated by the red dotted line).



**Figure 8.** DPVs recorded for determination of 20  $\mu\text{M}$  MEL and 20  $\mu\text{M}$  PA in 0.2 M PB (pH 7.4) at bare PGE (red dotted line) and PPGME (solid blue line) at scan rate of 0.05  $\text{V s}^{-1}$

The peak potentials ( $E_{\text{pa}}$ ) for MEL and PA are identified at 0.63 and 0.4 V at BPGE. At PPGME, two distinct peaks are clearly observed (shown by the blue-coloured line) as depicted in Figure 8. The peak potentials ( $E_{\text{pa}}$ ) are detected at 0.57 and 0.32 V for MEL and PA, respectively, at PPGME. This confirms the ability to detect MEL in the presence of PA at PPGME.

### Real sample analysis

Here, the pre-treated pencil graphite electrode was now applied for real sample analysis. Melatonin levels in the tablet sample were measured using a pretreated pencil graphite modified electrode. The sample of melatonin (Melonap-5) tablet was acquired from Med Plus Pharmaceuticals Ltd. A specific MEL 5  $\text{mg mL}^{-1}$  content, and the sample was utilized following an appropriate dilution. The tablet sample was diluted using 0.2 M phosphate buffer solution. The outcomes can be found in Table 2. The modified electrode exhibits good selectivity and sensitivity for melatonin. The recovery was within the range of 97.30 to 98.40 % indicating that the suggested method could be effectively used for the detection of melatonin in tablet samples [38,39].

**Table 2.** Determination of melatonin using pharmaceutical sample (n=3)

Added	Amount of MEL, $\mu\text{M}$		Recovery, %
		Found	
10		$9.77 \pm 0.30$	97.70
20		$19.68 \pm 0.4$	98.40
30		$29.20 \pm 0.8$	97.30

## Conclusion

In this research, the melatonin hormone (MEL) was effectively quantified using a surface-activated pencil graphite electrode (PPGME) that employed 0.1 M KOH solution for potential activation. PPGME demonstrated enhanced sensitivity, selectivity, and overall superior performance in the determination of MEL at pH 7.4. The equal number of protons and electrons transferred was determined by determining the pH dependence of the oxidation peak of the MEL. This electrode shows excellent analytical applicability and provides a rapid response for MEL detection. The modified electrode exhibits excellent detection and quantification limits, specifically 0.442 and 1.47  $\mu\text{M}$ , respectively, compared to previously reported modified electrodes. Real sample analysis was also performed, yielding very good recovery results. Moreover, the modified pretreated electrode holds promise for detecting other drugs, hormones, and neurotransmitters in various electrochemical applications.

## References

1. A. M. Hasan-Abad, M. A. Esmaili, A. Ghotaslou, A. Atapour, A. Khoshroo, E. Naghian, Electrochemical Biosensors for Testosterone Detection, *Analytical, and Bioanalytical Electrochemistry* **14** (2022) 1060-1077. <https://www.abechem.com/article.698017>
2. E. B. Bahadır, M. K. Sezgentürk, Electrochemical biosensors for hormone analyses, *Biosensors and Bioelectronics* **68** (2015) 62-71. <https://doi.org/10.1016/j.bios.2014.12.054>
3. J. R. Camargo, I. A. A. Andreotti, C. Kalinke, J. M. Henrique, J. A. Bonacin, B. C. Janegitz, Waterproof paper as a new substrate to construct a disposable sensor for the electrochemical determination of paracetamol and melatonin, *Talanta* **208** (2020) 120458. <https://doi.org/10.1016/j.talanta.2019.120458>
4. G. I. Mohammed, A. L. Saber, Study of the Electrochemical Behavior of Melatonin on Different Electrodes in Aqueous Solution, *International Journal of Electrochemical Science* **15** (2020) 5895-5907. <https://doi.org/10.20964/2020.06.90>
5. E. Molaakbari, A. Mostafavi, H. Beitollahi, Simultaneous electrochemical determination of dopamine, melatonin, methionine, and caffeine, *Sensors and Actuators B* **208** (2015) 195-203. <https://doi.org/10.1016/j.snb.2014.10.130>
6. R. Rahmati, A. Hemmati, R. Mohammadi, A. Hatamie, E. Tamjid, A. Simchi, Sensitive Voltammetric Detection of Melatonin in Pharmaceutical Products by Highly Conductive Porous Graphene-Gold Composites, *ACS Sustainable Chemistry and Engineering* **8** (2020) 18224-18236. <https://doi.org/10.1021/acssuschemeng.0c06675>
7. H. Alesa, J.T. Althakafy, A.L. Saber, Electroanalytical and Spectrophotometric Methods for the Determination of Melatonin-a Review, *International Journal of Electrochemical Science* **15** (2020) 7187-7202. <https://doi.org/10.20964/2020.08.04>
8. L. C. Brazaca, C. B. Bramorski, J. Cancino-Bernardi, S. da Silveira Cruz-Machado, R. P. Markus, B. C. Janegitz, V. Zucolotto, An antibody-based platform for melatonin quantification, *Colloids and Surfaces B: Biointerfaces* **171** (2018) 94-100. <https://doi.org/10.1016/j.colsurfb.2018.07.006>
9. P. Sunon, P. Wongkaew, J. Johns, N. Johns, Characterization of cerium oxide-chitosan nanocomposite-modified screen-printed carbon electrode and application in melatonin

- determination, *International Journal of Geomate* **14** (2018) 151-157.  
<https://doi.org/10.21660/2018.42.3559>
10. B. Jung-Hynes, R. J. Reiter, N. Ahmad, Sirtuins, melatonin and circadian rhythms: building a bridge between aging and cancer, *Journal of Pineal Research* **48** (2010) 9-19.  
<https://doi.org/10.1111/j.1600-079X.2009.00729.x>
  11. F. H. Cincotto, D. A. S. Carvalho, T. C. Canevari, H E. Toma, O.Fatibello-Filho, F. C. Moraes, A nano-magnetic electrochemical sensor for the determination of mood disorder related substances, *RSC Advances* **8** (2018) 14040-14047. <https://doi.org/10.1039/C8RA01857J>
  12. D. Duan, Y. Din, L. Li, G. Ma, Rapid quantitative detection of melatonin by electrochemical sensor based on carbon nanofibers embedded with FeCo alloy nanoparticles, *Journal of Electroanalytical Chemistry* **873** (2020) 114422.  
<https://doi.org/10.1016/j.jelechem.2020.114422>
  13. N. Buscemi, B. Vandermeer, N. Hooton, R. Pandya, L. Tjosvold, L. Hartling, S. Vohra, T. P. Klassen, G. Baker, Efficacy and safety of exogenous melatonin for secondary sleep disorders and sleep disorders accompanying sleep restriction, *The BMJ* **6** (2006) 332-335.  
<https://doi.org/10.1136/bmj.38731.532766.F6>
  14. N. Kumar, R. N. Goyal, Simultaneous determination of melatonin and 5-hydroxytryptophan at the disposable poly-(melamine)/poly-(o-aminophenol) composite modified screen-printed sensor, *Journal of Electroanalytical Chemistry* **874** (2020) 114458.  
<https://doi.org/10.1016/j.jelechem.2020.114458>
  15. A. T. Ball, B. A. Patel, Rapid voltammetric monitoring of melatonin in the presence of tablet excipients, *Electrochimica Acta* **83** (2012) 196-201.  
<https://doi.org/10.1016/j.electacta.2012.07.100>
  16. E. Bayram, E. Akyilmaz, Development of a new microbial biosensor based on conductive polymer/multiwalled carbon nanotube and its application to paracetamol determination, *Sensors, and Actuators B: Chemical* **233** (2016) 409-418.  
<https://doi.org/10.1016/j.snb.2016.04.029>
  17. M. Kumar, B. E. Kumara Swamy, S. Reddy, W, Zhao, S. Chetana, V. Gowrav Kumar, ZnO/functionalized MWCNT and Ag/functionalized MWCNT modified carbon paste electrodes for the determination of dopamine, paracetamol and folic acid, *Journal of Electroanalytical Chemistry* **835** (2019) 96-105. <https://doi.org/10.1016/j.jelechem.2019.01.019>
  18. C. M. Kuskur, B. E. K. Swamy, H. Jayadevappa, Poly (Naphthol green B) modified carbon paste electrode for the analysis paracetamol and norepinephrine, *Ionics* **25** (2019) 1845-1855.  
<https://doi.org/10.1007/s11581-018-2606-3>
  19. A. M. Campos, P. A. Raymundo-Pereira, C. D. Mendonça, M. L. Calegari, S. A. Machado, O. N. Oliveira Jr, Size Control of Carbon Spherical Shells for Sensitive Detection of Paracetamol in Sweat, Saliva, and Urine, *ACS Applied Nano Materials* **1** (2018) 654-661.  
<https://doi.org/10.1021/acsnm.7b00139>
  20. B. Avinash, C. R. Ravikumar, M. R. Anil Kumar, H. P. Nagaswarupa, M. S. Santosh, A. S. Bhatt, D. Kuznetsov, Nano CuO Electrochemical sensor for the determination of paracetamol and D-glucose, *Journal of Physics and Chemistry of Solids* **134** (2019) 193-200.  
<https://doi.org/10.1016/j.ipcs.2019.06.012>
  21. K. Annadurai, V. Sudha, G. Murugadoss, R. Thangamuthu, Electrochemical sensor based on hydrothermally prepared nickel oxide for the determination of 4-acetaminophen in paracetamol tablets and human blood serum samples, *Journal of Alloys and Compounds* **852** (2021) 156911. <https://doi.org/10.1016/j.jallcom.2020.156911>
  22. A. Kulo, M. Y. Peeters, K. Allegaert, A. Smits, J. de Hoon, R. Verbesselt, L. Lewi, M. Van De Velde, C.A. Knibbe, Pharmacokinetics of paracetamol and its metabolites in women at delivery

- and post-partum, *British Journal of Clinical Pharmacology* **7** (2013) 850-860.  
<https://doi.org/10.1111/j.1365-2125.2012.04402.x>
23. L. S. Manjunatha, B. E. Kumara Swamy, S. C. Sharma, C. Sridhar, M. R. Sanjana, S. Kumar, Iron doped nickel oxide nanoparticle modified carbon paste electrode sensor for paracetamol in presence of ascorbic acid: A voltammetric study, *Materials Chemistry and Physics* **313** (2024) 128682. <https://doi.org/10.1016/j.matchemphys.2023.128682>
  24. M. M. Vinay, Y. Arthoba Nayaka, Iron oxide (Fe<sub>2</sub>O<sub>3</sub>) nano particles modified carbon paste electrode as an advanced material for electrochemical investigation of paracetamol and dopamine, *Journal of Science: Advanced Materials and Devices* **4** (2019) 442-450.  
<https://doi.org/10.1016/j.isamd.2019.07.006>
  25. M. M. Huber, A. Göbel, A. Joss, N. Hermann, D. Löffler, C. S. McArdell, A. Ried, H. Siegrist, T. A. Ternes, U. von Gunten, Oxidation of Pharmaceuticals during Ozonation of Municipal Wastewater Effluents: A Pilot Study, *Environmental Science & Technology* **39** (2005) 4290-4299. <https://doi.org/10.1021/es048396s>
  26. R. R. Sawkar, V. B. Patil, M. M. Shanbhag, N. P. Shetti, S. M. Tuwar, T. M. Aminabhavi, Detection of ketorolac drug using pencil graphite electrode, *Biomedical Engineering Advances* **2** (2021) 100009. <https://doi.org/10.1016/j.bea.2021.100009>
  27. A. B. A. Boxall, C. J. Sinclair, K. Fenner, D. Kolpin, S. J. Maund, When Synthetic Chemicals Degrade in the Environment, *Environmental Science & Technology* **38** (2004) 368A-375A.  
<https://doi.org/10.1021/es040624v>
  28. S. Srinivas, A. Senthil Kumar, Surface-Activated Pencil Graphite Electrode for Dopamine Sensor Applications: A Critical Review, *Biosensors* **13** (2023) 353. <https://doi.org/10.3390/bios13030353>
  29. A. Ozcan, M. T. Garip, Development of a simple and efficient method to prepare a platinum-loaded carbon electrode for methanol electrooxidation, *International Journal of Hydrogen Energy* **45** (2020) 17858-17868. <https://doi.org/10.1016/j.ijhydene.2020.04.230>
  30. A. Ozcan, Y. Sahin, Preparation of selective and sensitive electrochemically treated pencil graphite electrodes for the determination of uric acid in urine and blood serum. *Biosensors and Bioelectronics* **25** (2020) 2497-2502. <https://doi.org/10.1016/j.bios.2010.04.020>
  31. A. Ozcan, Synergistic effect of lithium perchlorate and sodium hydroxide in the preparation of electrochemically treated pencil graphite electrodes for selective and sensitive bisphenol A detection in water samples, *Electroanalysis* **26** (2014) 1631-1639.  
<https://doi.org/10.1002/elan.201400082>
  32. M. Gurbuz, A. A. Ozcan, A. Ozcan, Preparation of activated disposable pencil graphite electrode for the selective and sensitive determination of, a fluoroquinolone antibiotic: Levofloxacin, *Current Pharmaceutical Analysis* **14** (2018) 247-254.  
<https://doi.org/10.2174/1573412913666170317130625>
  33. E. Topçu, K. D. Kırınça, Electrochemical simultaneous sensing of melatonin and ascorbic acid at a novel flexible B-RGO composite paper electrode, *Diamond and Related Materials* **105** (2020) 107811. <https://doi.org/10.1016/j.diamond.2020.107811>
  34. I. M. Apetrei, C. Apetrei, Voltammetric determination of melatonin using a graphene-based sensor in pharmaceutical products, *International Journal of Nanomedicine* **11** (2016) 1859-1866. <https://doi.org/10.2147/IJN.S104941>
  35. R. C. Freitas, L. O. Orzari, L. M. C. Ferreira, T. R. L. C. Paixão, W. K. T. Coltro, F. C. Vicentini, B. C. Janegitz, Electrochemical determination of melatonin using disposable self-adhesive inked paper electrode, *Journal of Electroanalytical Chemistry* **897** (2021) 115550-115557.  
<https://doi.org/10.1016/j.jelechem.2021.115550>
  36. N. Tavakkoli, N. Soltani, F. Shahdost-Fard, M. Ramezani, H. Salavati, M. R. J. M. A. Jalali, Simultaneous voltammetric sensing of acetaminophen, epinephrine and melatonin using a

- carbon paste electrode modified with zinc ferrite nanoparticles, *Microchimica Acta* **185** (2018) 479-498. <https://doi.org/10.1007/s00604-018-3009-x>
37. F.J.V. Gomez, A. Martín, M.F. Silva, A. Escarpa, Screen-printed electrodes modified with carbon nanotubes or graphene for simultaneous determination of melatonin and serotonin, *Microchimica Acta* **182** (2015) 1925-1931. <https://doi.org/10.1007/s00604-015-1520-x>
38. A. Levent, Electrochemical determination of melatonin hormone using a boron-doped diamond electrode, *Diamond and Related Materials* **21** (2012) 114-119. <https://doi.org/10.1016/j.diamond.2011.10.018>
39. X.-P. Wu, L. Zhang, W.-R. Liao, J.-P. Duan, H.-Q. Chen, G.-N. Chen, Study on the electrochemical behavior of melatonin with an activated electrode, *Electroanalysis* **14** (2002) 1654-1660. <https://doi.org/10.1002/elan.200290007>