

VOLTAMMETRIC ANALYSIS OF MELATONIN AND DOPAMINE BY USING GRAPHENE OXIDE/POLYIMIDE BASED BIOSENSOR

Grafen Oksit/Poliiimid Temelli Biyosensör Kullanarak Melatonin ve Dopaminin

Voltammetrik Analizi

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ABSTRACT

In this research, for the simultaneous analysis of melatonin (MET) and dopamine (DP), graphene oxide/polyimide (GO/PI) composite electrode was prepared with the modification of Pt electrode. Firstly, GO/PI composite structure was synthesized from 2,6-diaminopyridine based polyimide and 5% GO. Then, the obtained composite structure as the membrane was coated on the electrode surface. MET and DP responses of the prepared GO/PI composite electrode were investigated by Differential Pulse Voltammetry (DPV) technique. Linearity was obtained over a concentration range of 85-105 μM for MET ($R^2 = 0.9976$). For DP analysis, the linearity was also monitored over a concentration range of 85-105 μM ($R^2=0.9988$). The detection limits of GO/PI modified electrode were approximately 13.45×10^{-5} M and 9.61×10^{-5} M for MET and DP, respectively. The prepared GO/PI composite modified electrode exhibited good repeatability, wide linear range and sensitivity for MET and DP. The obtained results indicated that while uric acid (UA) is present in the medium, GO/PI composite can be used as an excellent membrane in the design of voltammetric sensors to analyze MET and DP simultaneously.

Keywords: Dopamine, Electrochemical biosensor, Graphene oxide/polyimide composite, Melatonin

ÖZ

Bu araştırmada, melatonin (MET) ve dopaminin (DP) eş zamanlı analizi için, Pt elektrodun modifikasyonu ile grafen oksit/poliimid (GO/PI) kompozit elektrot hazırlanmıştır. İlk olarak, GO/PI kompozit yapısı, 2,6-diaminopiridin temelli PI ve % 5 GO'den sentezlenmiştir. Daha sonra membran olarak elde edilen kompozit yapı elektrot yüzeyi üzerine kaplanmıştır. Hazırlanan GO/PI kompozit elektrodunun MET ve DP yanıtları Diferansiyel Puls Voltametri (DPV) tekniği ile araştırılmıştır. MET ölçümleri için doğrusallık 85- 105 μM ($R^2=0.9976$) konsantrasyon aralığında elde edilmiştir. DP analizi için de doğrusallık 85-105 μM konsantrasyon aralığında izlenmiştir ($R^2=0.9988$). GO/PI ile modifiye edilmiş elektrodun tayin limitleri, MET ve DP için sırasıyla yaklaşık 13.45×10^{-5} M ve 9.61×10^{-5} M'di. Hazırlanan GO/PI kompozit modifiye elektrot, MET ve DP için iyi tekrarlanabilirlik, geniş doğrusal aralık ve hassasiyet sergilemiştir. Elde edilen sonuçlar, GO/PI kompozitinin ortamda ürik asit (UA) varlığında, MET ve DP'i eş zamanlı olarak analiz etmek için mükemmel bir membran olarak kullanılabileceğini göstermiştir.

Anahtar kelimeler: Dopamin, Elektrokimyasal biyosensör, Grafen oksit/poliimid kompozit, Melatonin

INTRODUCTION

Dopamine (DP) is a major catecholamine neurotransmitter species, which has considerable importance in the human brain and body. DP acts a prominent role in the mammalian central hormonal and nervous system. Current studies showed that dopamine deficiency can give rise to critical neurological disorders like Parkinson's, Schizophrenia and Alzheimer's (Fang et al., 2007; Reddy, Kumara Swamy & Jayadevappa, 2012; Wang, Sun & Tang, 2002; Wightman, May & Michael, 1988). Melatonin (MET) is also an important hormone secreted naturally by the brain (Kvetnoy et al., 2002; Tarocco et al., 2019). Recent experimental studies have indicated that melatonin deficiency can lead to risks of breast cancer (Sánchez-Barceló et al., 2005), prostate cancer (Siu, Lau, Tam & Shiu, 2002), type 2 diabetes (McMullan, Schernhammer, Rimm, Hu & Forman, 2013), types of dementia (Hardeland, 2012) and some mood disorders (Hardeland, Madrid, Tan & Reiter, 2012).

In recent years, several techniques such as the High Performance Liquid Chromatography (HPLC) (Martins, Khalil & Mainardes, 2017), Chemiluminescence (Lu, Lau, Lee & Kai, 2002), Liquid Chromatography-Mass Spectrometry (LC-MS) (Escriva, Manyes, Barbera, Martinez-Torres & Meca, 2016) and electrochemical detection methods (Kumar, Sharma & Goyal, 2016; Smajdor, Piech, Pięk & Paczosa-Bator, 2017) have been commonly used for the detection of MET. Similarly, several techniques like Fluorescence (Amjadi, Manzoori, Hallaj & Sorouraddin, 2014), HPLC (Ye, Gao & Li, 2014), Spectrophotometry (Khajehsharifi, Pourbasheer, Tavallali, Sarvi & Sadeghi, 2017) and electrochemical detection methods (Liu et al., 2016; Zhang, Li, Ma, Chen & Zhang, 2017) have been used for the analysis of DP. Although these techniques are advantageous in separating the target molecule, novel detection methods with lower cost and faster response times are still being investigated. Especially, electrochemical detection methods have more advantages compared to the other methods because of very fast response time, high sensitivity, extremely cost-effective, simplicity and low detection limit (Soltani, Tavakkoli, Shahdost-Fard, Salavati & Abdoli, 2019). For the purpose, several sensor applications have been reported by modifying electrodes with various materials to analyze MET and DP simultaneously (Bagheri, Afkhami, Hashemi & Ghanei, 2015; Manikandan & Dharuman, 2017; Molaakbari, Mostafavi & Beitollahi, 2015; Soltani et al., 2019). Recently, the polymeric materials have been used as a membrane in the sensor field. Polyimides (PIs) can be given as an example of these polymeric membrane structures. PIs and PI based materials have considerable chemical resistance, good adhesion, superior mechanical strength, ideal thermal and electrical stability, high corrosion resistance, separation

characteristics, dimensional stability, biocompatibility, insulating properties, fluorescence properties and low flammability (Cai et al., 2013; Hsiao, Liou, Kung, Pan & Kuo, 2009; Lau, 2014). Therefore, PIs have been commonly used as a membrane in the design of electrochemically various sensors for the analysis of different biological compounds (Chen, 2018; Dai et al., 2014; Ekinçi, Köytepe, Paşahan & Seckin, 2006; Paşahan, Köytepe & Ekinçi, 2011). In addition to these polymers, polymeric composite materials have been designed by adding some conductive additives to the structure. For example, graphene containing materials are commonly employed for improving the features of polymers because of their remarkable thermal and electrical conductivity, good surface area, ideal aspect ratio and low manufacturing costs (Kim et al., 2010; Lee, Wei, Kysar & Hone, 2008). Among these graphene containing materials, graphene oxide (GO) has been employed as electrode material owing to the attractive characteristics like good surface area, exceptional electrical and thermal conductivity (Compton & Nguyen, 2010; Yoonessi et al., 2013). In recent years, GO containing PI composites have been widely used in the development of sensors such as dopamine sensor, hydroquinone and catechol sensor, glucose sensor, wearable sensor and strain sensor (Choi, Kim & Kim, 2016; Jia et al., 2016; Shen, Xia, Du & Wang, 2017; Wang et al., 2019; Zhang, Fan, Huang, Zhang & Liu, 2015). Studies on the sensor applications of these composites are still ongoing.

In the present study, graphene oxide/polyimide (GO/PI) composite was prepared to be used as a modified electrode in the electrochemical sensor. Firstly, the synthesis of PI was performed with 2,6-diaminopyridine (DAP) and pyromellitic dianhydride (PMDA). Subsequently, GO/PI composite containing 5% GO was prepared. The Platinum (Pt) electrode was modified with the prepared GO/PI composite. While uric acid (UA) is present in the medium, 5% GO reinforced PI composite was employed to analyze MET and DP simultaneously. As a result of using this procedure, it was obtained an electrode with wide linear ranges, easy of application, high correlation coefficient and repeatability.

MATERIALS AND METHODS

Materials

Pyromellitic dianhydride (PMDA) was provided by Fluka and 2,6-Diaminopyridine (DAP), graphene oxide (GO), Dopamine (DP), Melatonin (MET), uric acid (UA) were supplied by Sigma-Aldrich Chemical Company. Electrochemical measurements were carried out in phosphate buffer salts (0.1 M PBS buffer; pH 7).

Instrumentation

In the voltammetric measurements of DP and MET, the DPV technique was used within the three-electrode system. While the Pt electrode was used as the working electrode, the Ag/AgCl reference electrode and the Pt wire counter electrode were used. Electrochemical voltammograms were measured with BAS 100W (Bioanalytical Systems, Inc) electrochemical analyzer. DPV measurements were carried out in the range of -0.2 V to 1.1 V at a scanning speed of 10 mV/s.

Preparation of PI and GO/PI composite

The synthesis of PI was performed with the polycondensation reaction of PMDA and DAP as a result of the nucleophilic substitution reaction. PMDA (0.10 mol) was added stepwise under the flow of nitrogen into a stirred solution of DAP (0.10 mol) dissolved in NMP (30 mL) and was stirred at room conditions for about 6 h to form amic acid. The resulting amic acid solution was refluxed by stirring at 120 °C for about 10 hours. Subsequently, the mixture was refluxed again at 180 °C for approximately 18 hours. The resulting product was precipitated with water and filtered. The residue was washed by using methanol to eliminate impurities and kept in a vacuum dryer at about 80 °C.

GO (5% w/w) to PI solutions dissolved in NMP was added for the preparation of PI/GO composite. The mixture was sonicated and stirred to disperse GO homogeneously in the PI solution (Wang et al., 2019). Figure 1 exhibits the synthesis stages of the GO/PI composite structure.

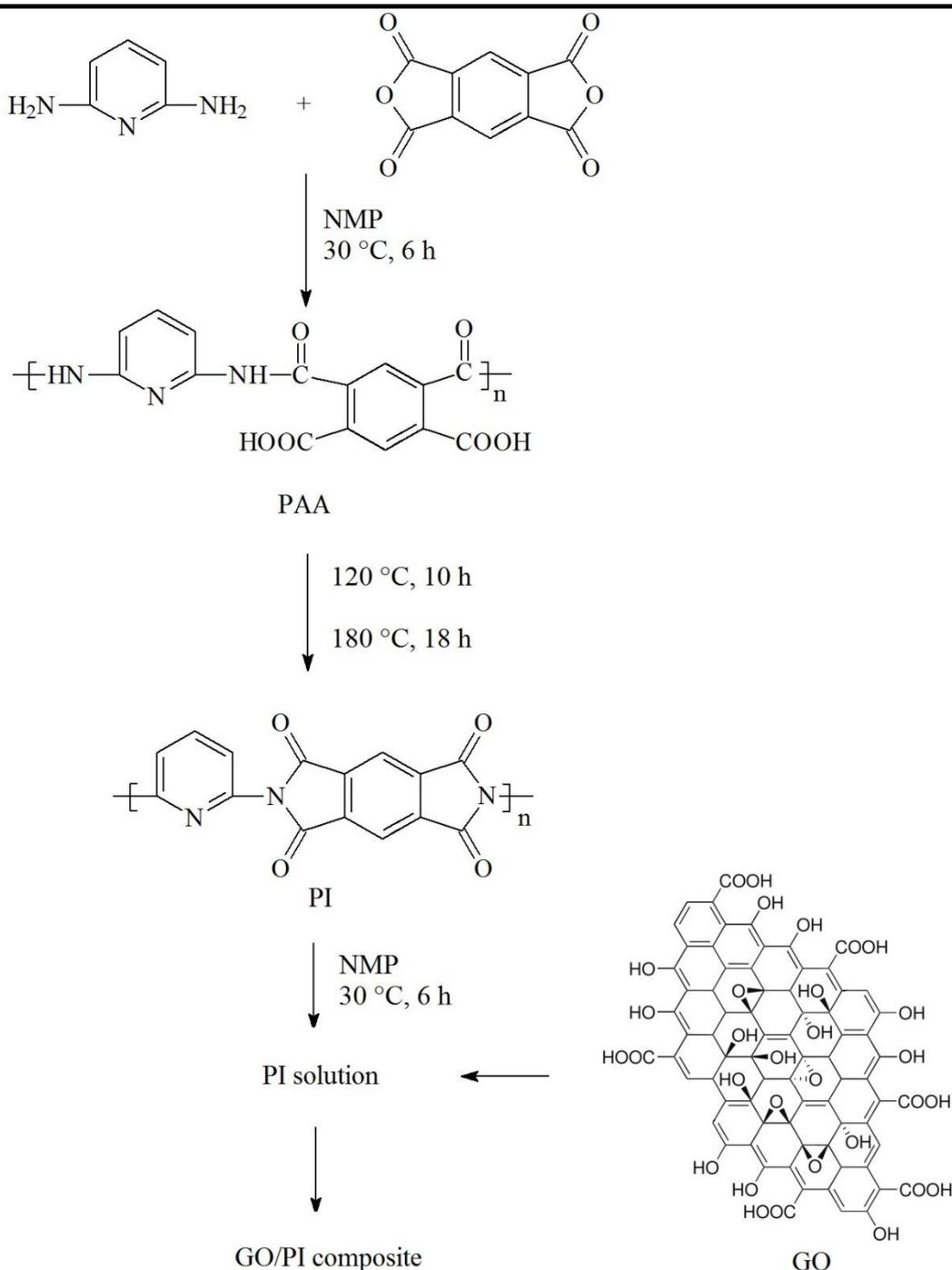


Figure 1. Preparation of the GO/PI Composite Structure.

Fabrication of the GO/PI composite modified electrode (GO-ME)

Prior to modification, the surface of the Pt working electrode was polished with diamond polishing agents and aqueous alumina slurry (Ekinçi et al., 2006). The 5% GO containing PI composite electrode was obtained by adding dropwise onto bare Pt electrode 2 μL of GO containing PI composite and left to dry at room conditions for about 3 days. Figure 2 represents the fabrication scheme of the GO/PI composite modified electrode.

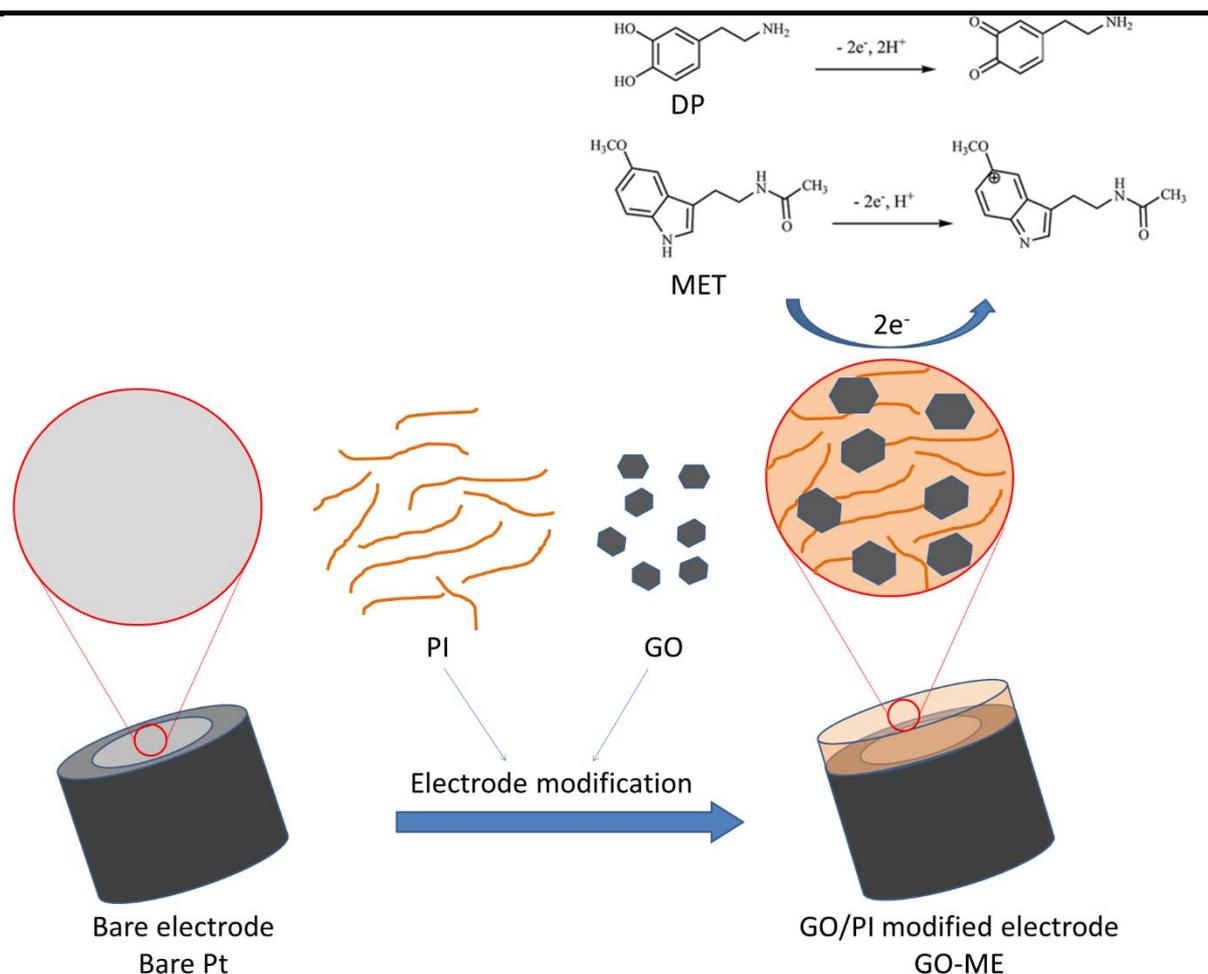


Figure 2. Fabrication of the GO/PI Composite Modified Electrode (GO-ME).

RESULTS AND DISCUSSION

Simultaneous analysis of MET and DP is very important since both of them coexist in biological fluids. However, one of the major obstacles for the determination of MET and DP in biological liquids is the coexistence of various interfering species (Bagheri et al., 2015). While the high concentration of UA is present in the medium, the electrochemical analysis for simultaneous detection of MET and DP on traditional electrodes usually is difficult.

Therefore, the oxidation signals of MET, DP and UA were investigated by the DPV technique. Figure 3 represents DPV curves of 60 μM UA in PBS buffer (pH 7.0) at the bare Pt electrode and the electrode which was modified with 10 μL of 5% GO/PI composite.

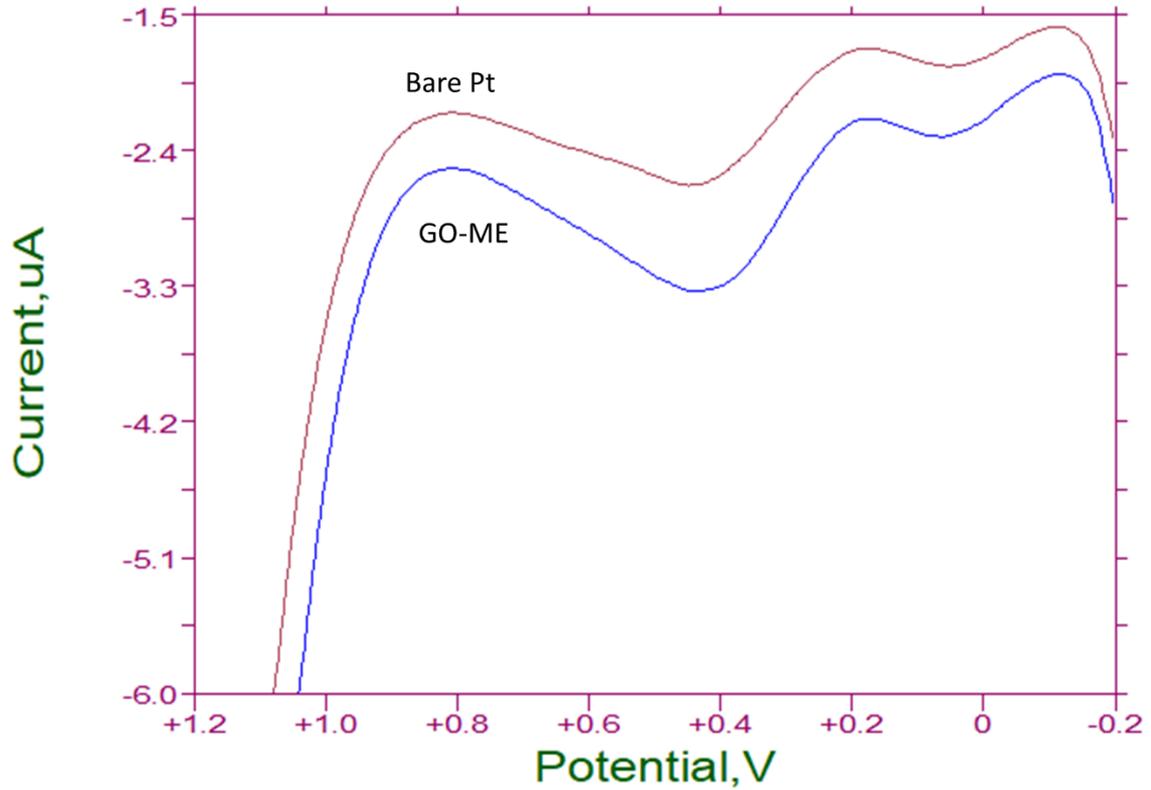


Figure 3. DPV Curves of 60 μM UA in PBS Buffer (pH 7.0) at the Bare Pt Electrode and at the Electrode (GO-ME) Modified with 10 μL of 5% GO/PI Composite.

Figure 4 displays the DPV curves of 60 μM DP in pH 7.0 PBS buffer at the bare Pt electrode and at the electrode which was modified with 10 μL of 5% GO/PI composite. According to the voltammetric measurements, it was found that the 5% GO/PI composite modified electrode had an ideal oxidation signal for DP.

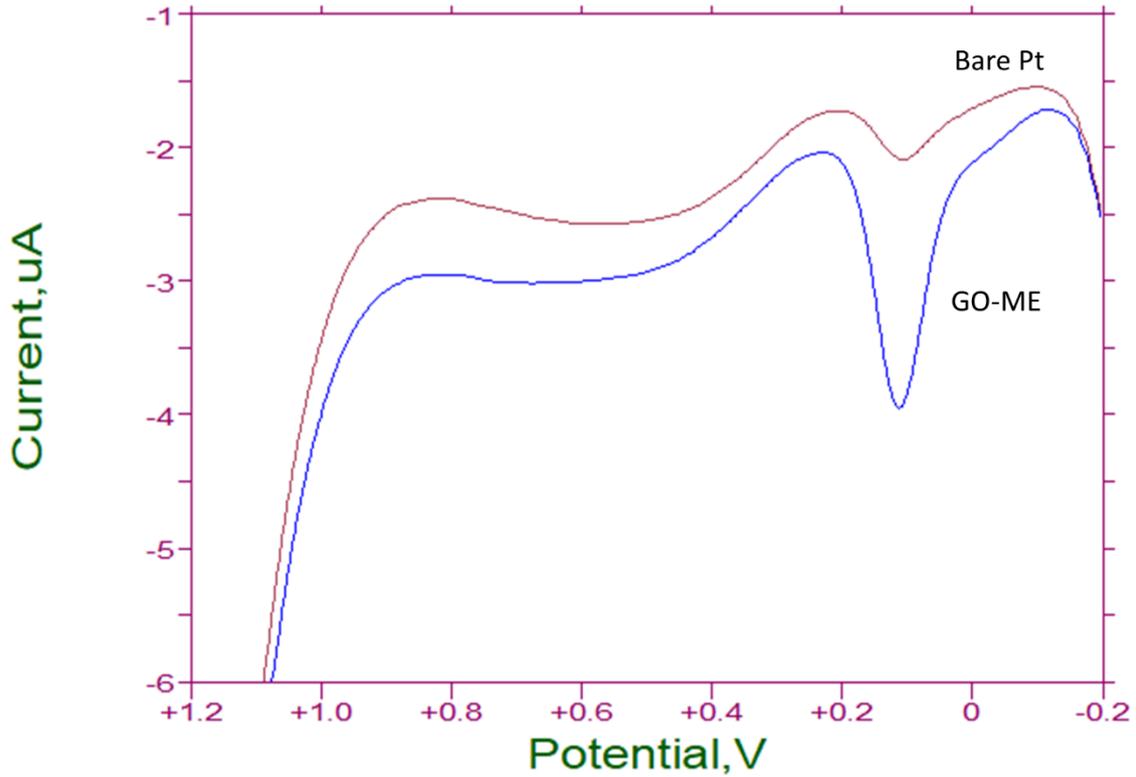


Figure 4. DPV Curves of 60 μM DP in PBS Buffer (pH 7.0) at the Bare Pt Electrode and at the Electrode (GO-ME) Modified with 10 μL of 5% GO/PI Composite.

Figure 5 displays the DPV curves of 60 μM MET in pH 7.0 PBS buffer at the bare Pt electrode and at the electrode which was modified with 10 μL of 5% GO/PI composite. As a result of DPV curves, it was found that the 5% GO/PI composite modified electrode had an ideal oxidation signal for MET.

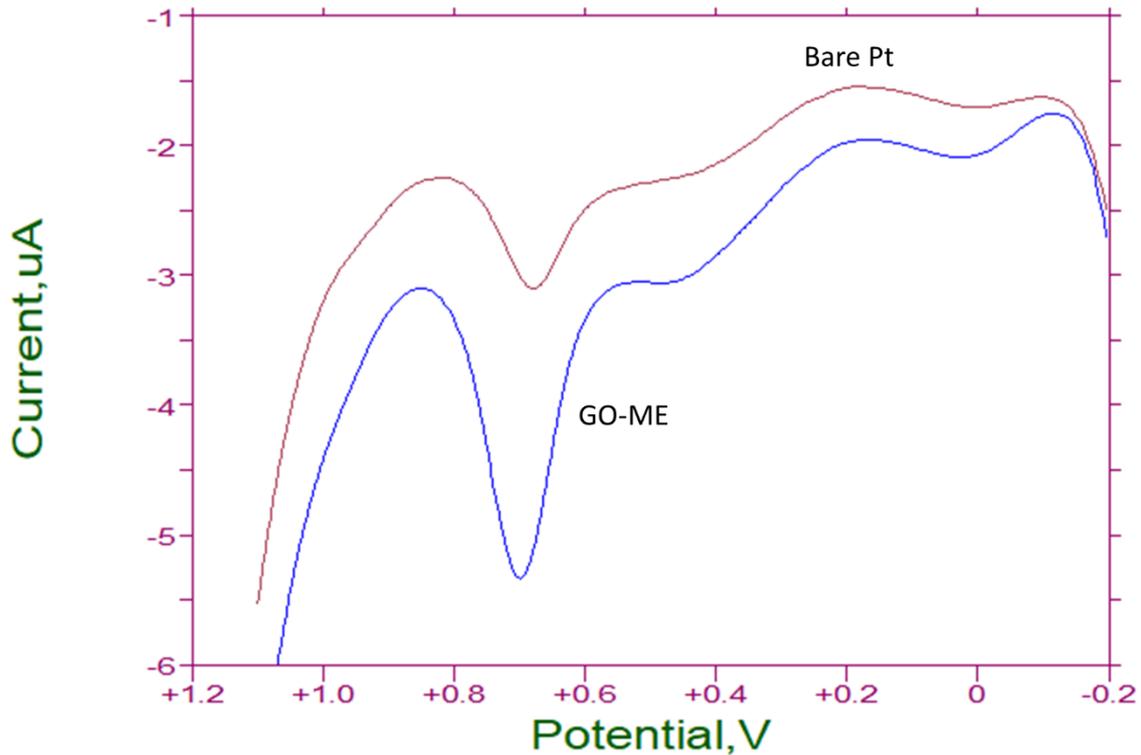


Figure 5. DPV Curves of 60 μM of MET in PBS Buffer (pH 7.0) at the bare Pt Electrode and at the Electrode (GO-ME) Modified with 10 μL of 5% GO/PI Composite.

Figure 6 shows while 80 μM DP and 80 μM UA are present in the medium, the DPV results obtained for the different concentrations (80; 90; 100; 110; 120; 130 μM) of MET for 5% GO/PI composite electrode. From DPV results, it was found that the voltammetric current signal of MET increased linearly with increasing MET concentration in the range of 80 to 130 μM at the 5% GO/PI composite electrode in the presence of UA interferent, while the oxidation current signals of DP has not shifted.

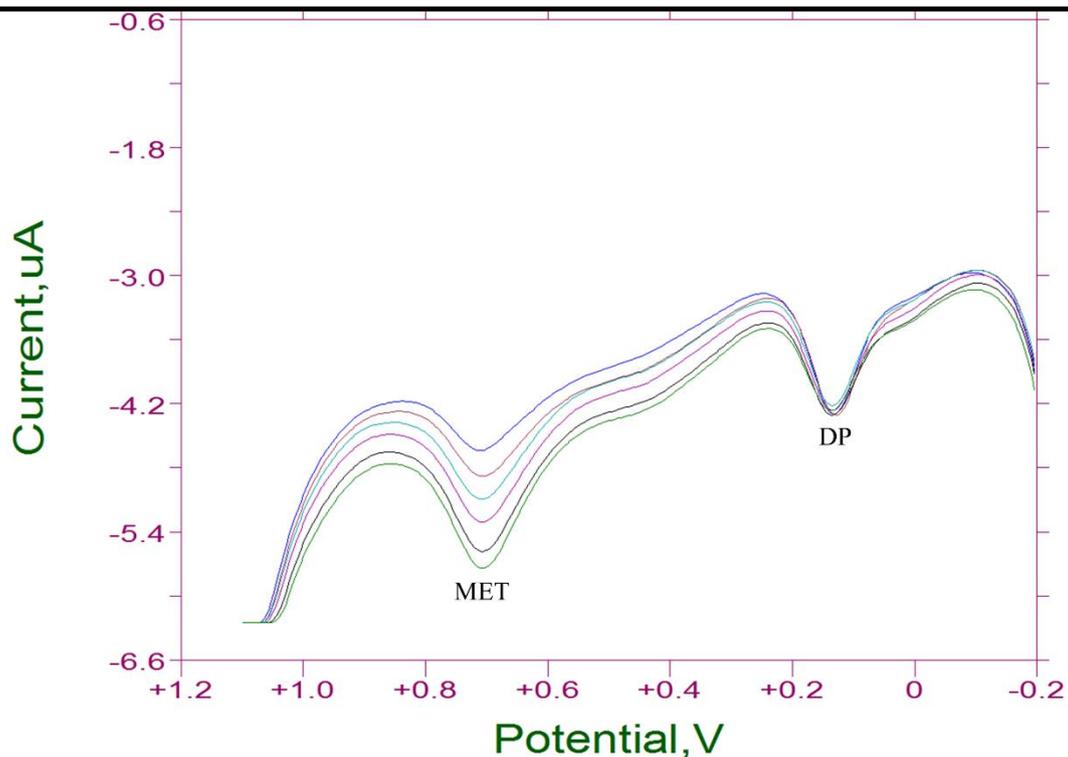


Figure 6. DPV Curves of 5% GO/PI Composite Electrode for Various Concentrations of MET in the Presence of 80 μM UA and 80 μM DP. (Melatonin: 80; 90; 100; 110; 120; 130 μM).

Figure 7 represents the calibration curve plotted with the current signals in Figure 6 against various MET concentrations. According to DPV results of 5% GO/PI composite electrode, the oxidation current signals were linearly increased with the concentrations of MET in the range of 80 to 130 μM with a high correlation coefficient ($R^2 = 0.9976$).

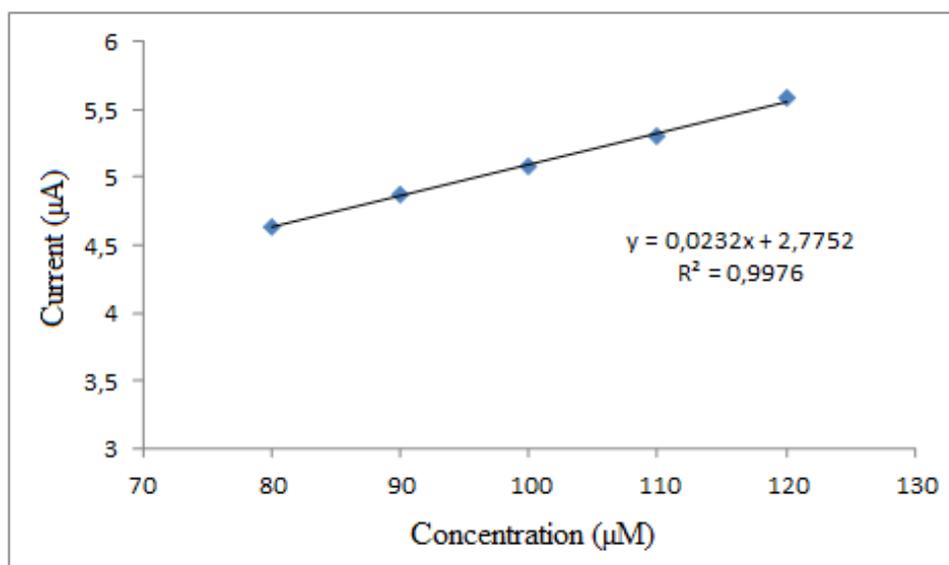


Figure 7. Calibration Curve of 5% GO/PI Composite Electrode for MET Response.

Figure 8 displays the DPV results obtained at the 5% GO/PI composite electrode for varying concentrations of MET and DP in pH 7.0 PBS buffer. As a result of the increase in concentrations in the range of 80 to 105 μM of them, the oxidation current signals of MET and DP also increased (Figure 8).

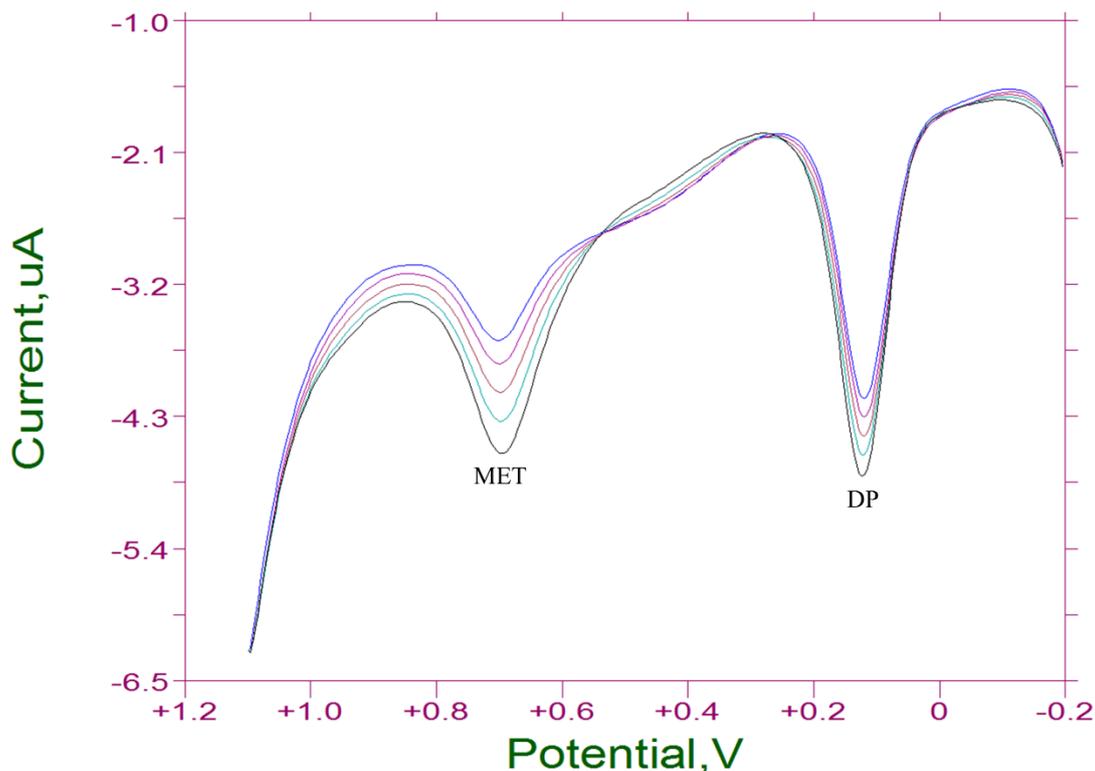


Figure 8. DPV Curves of the 5% GO/PI Composite Electrode in Various Concentrations of DP (80; 85; 90; 95; 100; 105 μM) and MET (80; 85; 90; 95; 100; 105 μM).

Calibration curves of 5% GO/PI composite electrode for MET and DP were plotted by the voltammetric peak current observed in Figure 8. ($R^2=0.9976$ for MET and $R^2=0.9988$ for DP (Figure 9). The electrochemical performance and selectivity of the present modified electrode provided a sensitive and selective determination of MET and DP in the wide linear range of 85-105 μM with 1.345 μM LOD; 0.961 μM LOD.

On the other hand, the limit of detection for MET determination using a graphene-based sensor in the pharmaceutical products was found in the range of 0.87 μM by Apetrei (Apetrei & Apetrei, 2016). Limit of detection for DP determination was found by Kim et al. in the range of 2.64 μM using the graphene electrode (Kim et al., 2010).

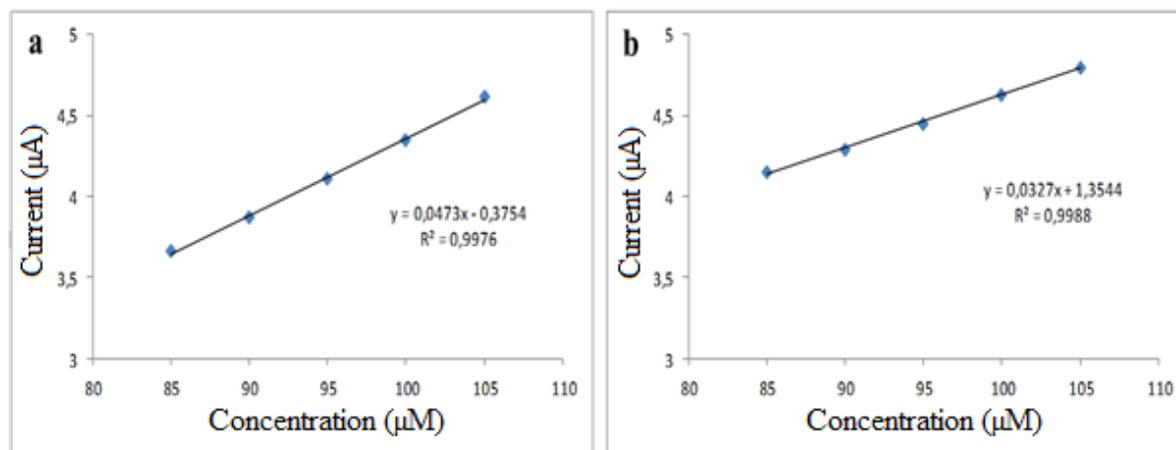


Figure 9. Calibration Curves of 5% GO/PI Composite Electrode a) for MET and b) for DP Response.

While 80 μM UA is present in the medium, the repeatability of the current responses for 5% GO/PI composite sensor was examined for 130 μM MET and 80 μM DP (Figure 10). The voltammetric responses from the consecutive measurements (15 runs) indicated that the 5% GO/PI composite electrode exhibited ideal reproducible responses for recurrent DPVs.

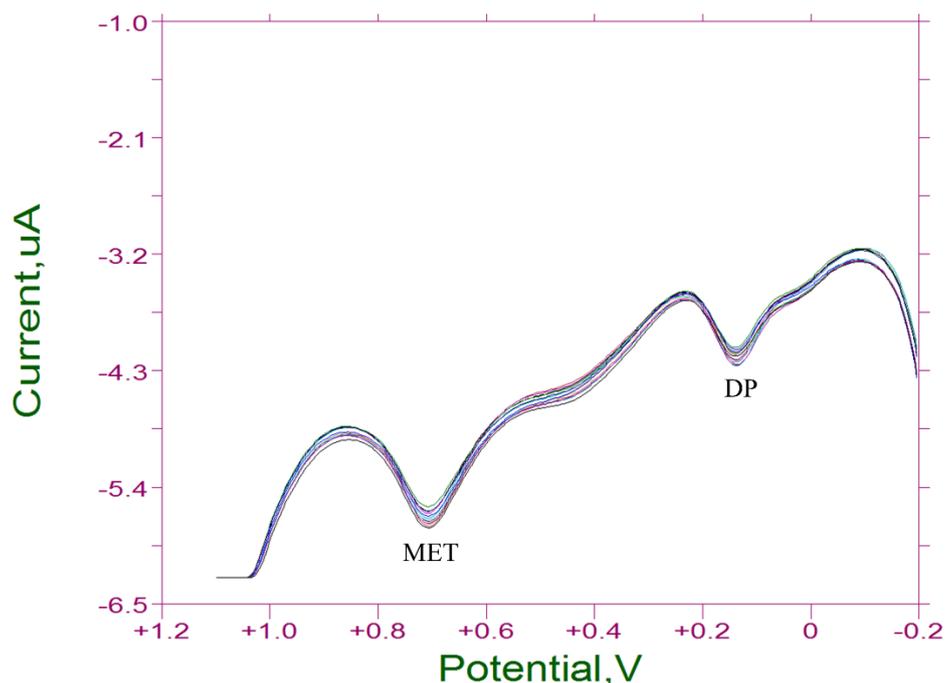


Figure 10. The Repeatability Responses of 5% GO/PI Electrode for 130 μM MET and 80 μM DP in the Presence of 80 μM UA.

In Figure 11, the repeatability results of 5% GO/PI electrode are given. After 15 repeatabilities, % relative standard deviation (RSD) 93.614% peak current was maintained for MET and the measurement was highly reproducible. The standard deviation (SD) and % relative standard deviation (RSD) were obtained 0.064 and 6.386, respectively. In addition, RSD% 92.443% peak current was maintained for DP and the measurement was highly

reproducible. SD and % RSD were obtained 0.076 and 7.557, respectively. On the other hand, the RSD values for MET determination using a graphene-based sensor in the pharmaceutical products were obtained in the range of 1.24% by Apetrei (Apetrei & Apetrei, 2016).

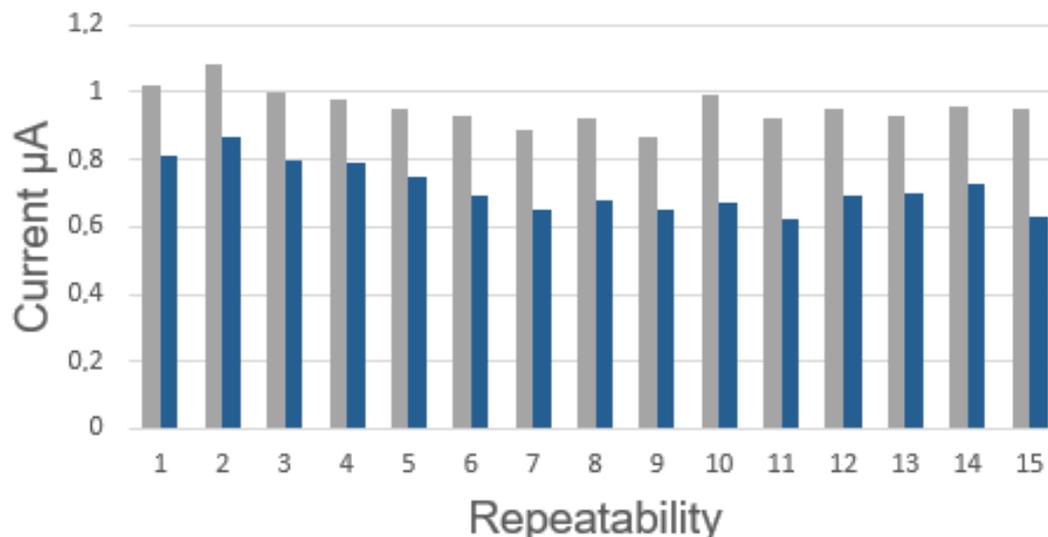


Figure 11. The Repeatability Graphs of 5% GO/PI Composite Modified Electrode (Gray; the Bar Graph of the Figure for MET, Blue; the Bar Graph of the Figure for DP).

CONCLUSION AND RECOMMENDATIONS

In this study, GO-containing PI composite was prepared for use in the design of an electrochemical sensor. The Pt electrode surface was modified with this GO containing PI composite. The prepared 5% GO/PI composite modified electrode was used for the simultaneous analysis of MET and DP. Sensor parameters such as linear range, repeatability and LOD were determined with the 5% GO/PI composite modified electrode by DPV technique in the presence of UA as an interferent. While UA is present in the medium, the 5% GO/PI composite electrode showed excellent reproducibility to analyze MET and DP simultaneously. The DPV results indicated that GO based PI modified sensor had rapid response, sensitivity, ease of application, high reproducibility, low cost and a high correlation coefficient ($R^2=0.9976$ for MET and $R^2=0.9988$ for DP). According to these results, it is suggested that the GO based PI sensor can be used for the simultaneous analysis of MET and DP.

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