Research Article

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Enhancement of graphene oxide through β-cyclodextrin composite to sensitive analysis of an antidepressant: Sulpiride

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Abstract: In this study, the electrochemical behavior of sulpiride (SUL) was examined deeply by using pyrolytic graphite electrode modified with graphene oxide (GO) and β-cyclodextrin (β-CD). The developed nanosensor indicated considerable impact by significantly increasing the signal of SUL compared with the bare electrode. Cyclic voltammetry (CV), differential pulse voltammetry (DPV), and square wave voltammetry (SWV) methods were utilized to investigate the SUL electrochemical analysis in aqueous solutions. Under optimum experimental conditions, calibration plot for SUL with a limit of detection of 2.83 \times 10^{-9} \text{ M} was obtained at accumulation time of the 30 s using square wave adsorptive stripping voltammetric technique (AdSSWV) in the range of 1.0 \times 10^{-7} to 5.0 \times 10^{-5} \text{ M}. The effects of accumulation potential, accumulation time, pH, scan rate, electrolyte, and interfering agents were studied to obtain the intensive peak signal of the analyte. The presented method is validated and successfully performed for the determination of the SUL tablet and capsule. The fabricated nanosensor was carried out for the detection of SUL in the urine. Excellent recoveries among 104.37, 103.82, and 101.95\% were obtained for tablet and capsule forms and urine analysis.

Keywords: sulpiride, nanosensor, electrochemistry, graphene oxide, pyrolytic graphite electrode

1 Introduction

One of the most common psychiatric disorders among adults is depression. Studies to understand the course and nature of depression have increased significantly in recent years. The prevalence of depression is worrying because depression is associated with high cost and a poor social impact [1,2]. Therefore, it is very important to treat depression. Sulpiride (SUL) is used therapeutically as an antidepressant, antipsychotic, and digestive aid and is a dopamine D2 receptor antagonist, which is a member of a benzamide class of drugs [3,4]. The development of sensitive methods for the determination of SUL is important due to its relatively widespread use (Scheme 1).

Voltammetry is a type of electrochemical technique in which the current of the working electrode is appreciated as a function of the potential carried out to the electrode [5]. Electrochemical techniques have advantages such as cost-effectiveness, ease of usage, and accuracy compared to other detection methods [6,7]. In addition, electrochemical techniques are easy, are less time consuming, and do not require thorough pretreatment of samples [8]. Square wave voltammetry is one of the most used voltammetry techniques that have properties of great sensitivity and speed of analysis.

The use of nanomaterials in electrochemical techniques is an increasing field of research thanks to the unique properties of nanomaterials, such as improved mass transfer, high surface area, and improved signal-to-noise ratio, which improve the performance of the electrodes [9,10]. Graphene has quickly attracted the attention of researchers because of its excellent electrical conductivity and high capacitance [11]. Cyclodextrin (CDs) is a widely known natural host molecule procured from the enzymatic corruption of starch. CDs and their derivatives have drawn a great interest in the pharmaceutical area in recent years, and an increasing number of researches have been devoted to drug analyses [12]. In
prepared using ultrapure water. Supporting electrolytes used in this study were as follows: phosphate buffer (PB) solution at pH 2.0–8.0, acetate buffer solution at pH 3.5–5.5, and Britton–Robinson buffer (BRB) solution at pH 2.00–10.0.

The urine sample for this study was taken from a healthy volunteer (after obtaining volunteer’s written consent). Ultrapure water was used to prepare all aqueous solutions.

2.2 Apparatus

Cyclic voltammetry (CV), differential pulse voltammetry (DPV), adsorptive stripping square wave voltammetry (AdSSWV) were applied by using Palmsens 4 electrochemical analyzer system with software PSTrace 5.4. All the electrochemical studies were performed using a classic three-electrode system consisting of a working electrode, a counter electrode, and a reference electrode. Diamond electrode (DE), glassy carbon electrode (GCE), and pyrolytic graphite electrode (PGE) were tested on all voltammetric methods as working electrodes. Ag/AgCl immersed in saturated KCl solution (3 M) was used as the reference electrode, and a platinum wire was used as the counter electrode. All pH measurements were performed by using a pH meter Model 538 (WTW, Austria). The SW voltammograms were conducted with the following parameters: step potential, 0.005 V; modulation amplitude, 0.025 V; modulation time, 0.05 s; interval time, 0.5 s. For AdSSWV experiments, optimum stripping conditions were as follows: accumulation potential: 0.4 V and accumulation time: 30 s.

2.3 Preparation of the GO/β-CD modified PGE

Graphene oxide was obtained as a solution from Sigma-Aldrich Company. Graphene oxide solution was aqueous, 

Table 1: Comparison of electrochemical studies about sulpiride

| Electrode                        | Method                  | Linear range                      | LOD              | Ref.   |
|----------------------------------|-------------------------|-----------------------------------|------------------|--------|
| Mercury electrode                | Linear sweep stripping voltammetry | 0.1−0.6 µg/mL                    | 0.025 µg/mL      | [21]   |
| Ni(OH)₂-Gr-IL/CPE                | Square wave voltammetry  | 1.0 × 10⁻⁹ to 2.0 × 10⁻⁴ mol L⁻¹ | 2.5 × 10⁻¹⁰ mol L⁻¹ | [20]   |
| Hanging mercury drop electrode   | Linear sweep cathodic stripping voltammetry | 2 × 10⁻⁹ to 5 × 10⁻⁸ M           | 2 × 10⁻¹⁰ M      | [22]   |
| GO/β-CD/PGE                      | AdSSWV                  | 1 × 10⁻⁸ to 1 × 10⁻⁵ M            | 2.83 × 10⁻⁹ M    | This work |
homogeneous brown liquid with a graphene oxide concentration of 4 mg/mL. β-Cyclodextrin is composed of seven alpha-(1 → 4) linked α-glucopyranose units. Before modifying the working electrode, PGE was cleaned by polishing the electrode surface with 0.05 nm alumina slurry. After rinsing the surface thoroughly with deionized water, the polished PGE was dried in air at room temperature. The GO/β-CD/PGE was fabricated by electrooxidation of the PGE in PB modification solution at pH 6.0. The PGE was immersed in the modification solution, and the electrooxidation was carried out by CV with 10 cycles. The applied potential was performed between −2.0 and +2.0 V. At the end of these processes, the electrode surface is coated with the modification agent.

2.4 Sample preparations

A stock solution of 1 mM SUL was prepared using in ultrapure water. Modification solution was prepared by mixing 0.01 M β-CD and 2 mL GO diluted in 10 mL phosphate buffer of pH 6.0 and stirred in the ultrasonic bath for 30 min. Electrochemical experiments were performed in an electrochemical cell containing a total of 10 mL of supporting electrolyte. Ascorbic acid, dopamine, glucose, uric acid, and some inorganic compounds in desired amounts were dissolved in water for interference experiments. Using BRB of pH 10.0 as a supporting electrolyte, all stock solutions were diluted to the required concentration.

For examination of the drug tablets, ten tablets of SUL (Dogmatil® 200 mg) were ground into a fine powder in a mortar. 1 mM SUL solution was prepared by dissolving the required amount of powder in a 25 mL measuring flask using the same solvent used earlier. Then, the solution was completely mixed and ultrasonicated for 30 min and then filtered. Suitable solutions were prepared by taking appropriate portions of this solution and diluting with the BRB electrolyte of pH 10.0. Merasa® capsules containing SUL (50 mg/capsule) were ground to a homogeneous fine powder in a mortar. Then, it was prepared similar to the SUL tablet. To explain whether the excipients that exist in pharmaceutical formulations indicate any interference, recovery experiments were carried out, and so, a certain amount of the pure SUL was added to the tablet and capsule forms of SUL. The acquired solutions were analyzed by AdSSWV.

1 mL of SUL, 5.4 mL of acetonitrile, and 3.6 mL of urine were added to a centrifuge tube for the preparation of the urine standard solution. The mixture was centrifuged for 25 min at 3,000 rpm to allocate the protein residuals, and then, the supernatant was collected. Acetonitrile was used as a precipitating medium for protein residues. The urine standard solution was diluted with BRB (pH 10.0) supporting electrolyte for the recovery experiments. All the measurements were carried out at least three times for calibration and at least five times for recovery and tablet studies.

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Electrochemical behavior of SUL

The electrochemical behavior of SUL was examined using different electrodes such as PGE, GCE, and DE with CV and SWV methods (Figure 1). At the beginning of this study, the Boron Doped Diamond electrode (BDDE), glassy carbon electrode (GCE), and pyrolytic graphite electrode (PGE) were tested as the working electrode in all voltammetric methods, and the highest peak current and the best peak shape were obtained with PGE. Hence, PGE was used in further experiments. DPV and SWV methods were utilized for detection of SUL. SWV peak currents of SUL showed higher current values than DPV. Operating conditions for SWV were as follows: pulse amplitude, 45 mV; frequency, 20 Hz; potential step, 10 mV. SWV was used for the examination of electrooxidation of SUL in different aqueous solutions. 1.0 × 10−4 M SUL redox behavior determined by CV is shown in Figure 1. The irreversible behavior of SUL was observed in BRB solution (pH 10.0) at the PGE. Anodic responses were achieved at 0 and 1 V for SUL. The cathodic response was not observed on the reverse scan (Figure 1). The irreversible behavior of SUL was observed in BRB solution (pH 10.0) at the PGE. Anodic responses were achieved at 0 and 1 V for SUL. The cathodic response was not observed on the reverse scan (Figure 1). The electrochemical behavior of SUL was examined at modified PGE using different media at different pH values as shown in Figure 2. The first voltammetric peak of SUL was intensive and figured out, and therefore, further experiments were performed based on the first oxidation peak. Figure 3 shows the square wave voltammograms of SUL in BRB of pH 10.0 as a supporting electrolyte at the bare and modified PGE. Compared to the bare electrode, the value of anodic peak current achieved at the modified PGE using adsorptive stripping square wave voltammetry was two times higher, which is a clear proof of enhancement in the electroactive surface area and then improvement in the response of peak current of SUL in BRB of pH
In this study, different types of electrodes and different types of modification agents were used to explore the best nanosensor for SUL determination (Figure 3). Among these modification agents, GO showed a higher peak value than bare PGE. Modified electrodes showed enhancement in the peak values, which infers a faster rate of electron transfer thanks to the synergistic effect of GO and β-CD. Before the determination of SUL via the PGE, the impacts of experimental conditions of the SWV on the peak potential and peak current were examined in the following ranges: square-wave step potential (2–10 mV), frequency (10–100 Hz), and pulse amplitude (10–45 mV) (Figure 4). The parameters of the optimized SWV for the detection of SUL were as follows: step potential, 10 mV; frequency (f), 20 Hz; pulse amplitude, 45 mV. Although the current of SUL increased at higher frequency values, the oxidation potential shifted to higher potentials, which shows difficult oxidation values of SUL.

### 3.2 Optimization of amount of modifier on the PGE

The effect of the amount of modification agent on the peak current of SUL was investigated to find out the best sensor system. In this respect, various ratios of GO and β-CD were immersed on the electrode surface. SWV was performed to examine the optimum amount of modification agent for achieving the most intensive peak current of the SUL. Maximum peak current response of SUL was achieved at a ratio of 2 mL GO:1.0 × 10⁻² M β-CD. Further experiments were applied using modification amount with an optimized ratio of 2 mL GO:1.0 × 10⁻² M.
β-CD. This increased SUL response may be by the increased PGE surface area. This increase in SUL response may be related to the increased PGE surface area.

### 3.3 Effect of pH

Phosphate buffer (PB) solutions, acetate buffer solutions, and Britton–Robinson buffer (BRB) were used as the supporting electrolyte, and all pH values were investigated in detail. According to these results, the highest, reproducible, stable peak current was obtained with BRB. Hence, BRB was used in further analytical experiments. The impact of pH was analyzed to choose the suitable pH medium based on the sensitivity, peak shape, and reproducibility for square wave voltammetric analysis of SUL. Hence, buffer solution with different pH values was used as the supporting electrolyte, and the response of $E_p$ and $I_p$ with pH was examined by the SWV technique (Figure 2). Obtained results indicated that the current peaks of SUL are extremely based on pH values. As shown in Figure 2, the oxidation peak potential of SUL shifts negatively with an increase in the pH of the solution, exhibiting attendance of protons during electrode reaction.

$$E_p = 1.2441 - 0.0727 \text{ pH } (R^2 = 0.99).$$

According to the relation of $E_p$ and pH, the slope of this equation presented that this oxidation reaction was occurred by equal electron and proton numbers.

3.4 Effect of scan rate

For examination of whether the procedure of SUL oxidation at the surface of modified PGE is adsorption or diffusion controlled, scan rate workings were applied. The effect of the scan rate on the electrochemical oxidation of SUL was performed in the range of 5–1,000 mV s$^{-1}$ via GO/β-CD-modified PGE by CV.

As a result of the scan rate experiments, the density of the peak current of SUL increased as the scanning speed increased, and the peak potentials shifted to more positive values. The positive shifting in the peak potentials as a result of the scanning rate equals the irreversible electron transfer processes. The relationship between the logarithm of the peak currents was plotted.
against the scan rate logarithm and confirmed that the process was controlled by adsorption or diffusion.

\[
\log I_p (\mu A) = 1.0496 \log v - 7.5842 \quad (R^2 = 0.99).
\]

The slope value of the plot of the log of peak current versus the log of scan rate close to 1 recommends an adsorption-controlled procedure, whereas close to 0.5 indicates a diffusion-controlled procedure. In the aforementioned equation, the value of the slope is very close to the theoretical value of 1, which indicates that the procedure is adsorption controlled.

### 3.5 Effect of accumulation parameters

Considering SUL could be adsorbed at the electrode surface, the effect of accumulation time and potential on the peak current of SUL was studied. The accumulation step is widely used in the electrochemical analysis to accumulate analyte and increase sensitivity. The variation of the current of the peaks of the SUL against the accumulation potential was investigated by SWV in the range of 0–1.0 V as shown in Figure 4a. The optimum potential was selected at 0.4 V. Peak currents increased progressively as the accumulation time enhanced up to 30 s. For more than 30 s, the peak current almost unchanged and decreased (see Figure 4b). Therefore, optimum accumulation time was chosen as 30 s.

### 3.6 Analytical performance

DPV and SWV techniques were applied for the simultaneous detection of SUL in BRB (pH 10). In the SWV method, the peak currents were observed to be higher than the peak currents in the DPV method. As a result, optimized conditions of the AdSSWV method were used for the detection of SUL. Under optimum conditions, a linear relationship of SUL changed from \( 1 \times 10^{-8} \) to \( 1 \times 10^{-5} \) M (Figure 5a). The regression equation was \( I_p (\mu A) = 2E + 06C + 5.5863 \) \( (R^2 = 0.999) \), and the slopes of the calibration curve were utilized to estimate the limit of detection (LOD) and the limit of quantification (LOQ) [28].

The LOD and LOQ were \( 2.83 \times 10^{-9} \) and \( 9.42 \times 10^{-9} \) M in BRB at pH 10, respectively. SUL was also determined in urine, and linear calibration curves were achieved in the range of \( 1.0 \times 10^{-7} \) to \( 5.0 \times 10^{-5} \) M (Figure 5b). The LOD and LOQ were \( 1.99 \times 10^{-8} \) and \( 6.65 \times 10^{-8} \) M, respectively, in the urine.

To test the relative standard deviation (RSD%) value of reproducibility of the created technique of the electrode manufacturing, five different electrodes were produced and utilized for the analysis of 10 mM SUL on the same day. Furthermore, the reproducibility of the fabricated sensor between days was also checked, and their RSD% results are presented in Table 2.

The RSD values of less than 2% in BRB (pH 10) and less than 5% in urine samples indicate acceptable repeatability of the proposed method. The intraday and interday repeatability of the fabricated electrode was also examined.

![Figure 5: The obtained AdSSWVs at the GO/β-CD modified PGE for different concentrations of SUL (a) in BRB solution (pH 10.0) and (b) in urine sample.](image)
with 2.5 μM SUL concentration. The results of these studies are presented in Table 2. To test the stability of the method, the electrode was applied in the same SUL concentration after 10 days. As a result of the SWV measurements, no significant changes were observed in the peak current. The RSD value was found to be less than 2%, and these results indicate the excellent stability and repeatability of the proposed nanosensor.

### 3.7 Interference study

The proposed sensor was tested to evaluate SUL response in the presence of matrix effects such as dopamine (DA), ascorbic acid (AA), uric acid (UA), sodium chloride (NaCl), sodium nitrate (NaNO₃), potassium chloride (KCl), and glucose with AdSSWV. Different ratios of interferents were as follows: 1:1, 1:10, 1:100, 1:1,000. The interferents were separately added into the 0.1 mM SUL in the presence of BRB solution (pH 10.0). The effects of each substance are presented in Table 3.

### 3.8 Application of methodology

To appreciate the accuracy, validity, and applicability of the fabricated nanosensor, the standard addition technique was used to analyze SUL in Dogmatil® tablet and Meresa® capsule dosage conditions using the acquired calibration equation. Table 4 shows that the substance values measured by the created sensor for the tablet and capsule dosage forms are near to the requested amount. To control the reliability of the developed sensor and method for applicability, recovery studies were applied by the standard addition method using a modified

| Table 2: Regression data of the calibration curves for the determination of SUL |
|---------------------------------|--------------|--------------|
|                                | SUL in BRB pH 10.0 | SUL in urine |
| Linearity range (M)            | 1 × 10⁻⁸ to 1 × 10⁻⁷ | 1 × 10⁻⁷ to 1 × 10⁻⁶ |
| Slope (μM⁻¹)                   | 2 × 10⁶           | 8.9 × 10⁴     |
| Standard error of slope        | 5.5863           | 0.9374        |
| Intercept (μA)                 | 22272.03         | 65033.12      |
| Standard error of intercept    | 0.0946           | 1.3605        |
| Correlation coefficient        | 0.9991           | 0.9990        |
| LOD (M)                        | 2.83 × 10⁻⁹      | 1.99 × 10⁻⁸   |
| LOQ (M)                        | 9.42 × 10⁻⁸      | 6.65 × 10⁻⁸   |
| Intraday precision (RSD%)⁵     | 1.23             | 1.41          |
| Interday precision (RSD%)⁵     | 1.36             | 1.70          |
| Within-day reproducibilities of nanosensor (RSD%)⁵ | 1.74 | 2.14 |
| Between days reproducibility of nanosensor (RSD%)⁵ | 2.32 | 2.75 |

*Each value is the average of three measurements.

| Table 3: Tolerance of interferences on the determination of 1 × 10⁻⁴ M SUL using GO/β-CD modified PGE |
|---------------------------------|-----------------------------|-------------------|
| AdSSWV                          | Tolerance level ratio (matrix substance: SUL) | ±% error |
| CI⁻                             | 10:1                        | +3.30             |
| NO₃⁻                            | 1,000:1                     | +3.32             |
| K⁺                              | 1:1                         | +1.91             |
| Na⁺                             | 1,000:1                     | +2.62             |
| Dopamine                        | 10:1                        | +5.80             |
| Glucose                         | 1,000:1                     | +1.83             |
| Ascorbic acid                   | 10:1                        | +5.85             |
| Uric acid                       | 10:1                        | +4.06             |

| Table 4: Recovery results achieved from the detection of SUL in urine and pharmaceutical dosage forms |
|---------------------------------|-----------------------------|-------------------|
|                                  | Dogmatil® tablet dosage form | Meresa® capsule dosage form | Urine |
| Labeled claimed (mg)            | 200                         | 50                | —     |
| Amount founda (mg)              | 199.9                       | 49.8              | —     |
| RSD%                            | 0.945                       | 0.981             | —     |
| Bias%                           | 0.5                         | 0.4               | —     |
| Amount added (mg)               | 100                         | 50                | 100   |
| Found (mg)                      | 104.3                       | 51.90             | 101.9 |
| Average recovery%               | 104.37                      | 103.82            | 101.95|
| RSD%                            | 0.79                        | 0.48              | 0.69  |
| Bias%                           | -4.3                        | -3.8              | -1.9  |

*Each value is the main of five experiment.
Electrode in pharmaceutical experiments and urine samples. Excellent recoveries among 104.37, 103.82, and 101.95% show the effectiveness and reliability of the created method for tablet and capsule forms and urine analysis, and the results are presented in Table 4. Obtained results show that the designed method is not influenced by any matrix interference from the excipients available in the tablet and capsule forms and endogenous materials from urine samples. As a result, the amount of SUL can be determined successfully by the fabricated sensor in drug analysis and biological samples.

SUL was detected using mercury electrode, hanging mercury drop electrode, and carbon fiber microelectrodes by different groups in the previous studies [21,22,29]. Mercury electrode has disadvantages such as high mercury consumption and higher charging current. HMDE also has some disadvantages. It is bulky and requires a mercury chamber and regular maintenance of capillaries. Another serious disadvantage is the use of metallic mercury, and hence, there is a potential risk of poisoning, contamination, and disposal. HMDE, unlike solid electrodes, is mechanically unstable and is therefore not particularly suitable for the onsite analysis or flow applications. HMDE is not a suitable substrate for stable modification by chemical reagents or permanent coatings that develop analytical features [30]. In this study, these disadvantages were eliminated using PGE. The proposed method can be successfully and safely used to analyze the drug in tablet and capsule forms, as well as in spiked urine.

4 Conclusion

In this study, square wave voltammetry was applied for the sensitive analysis of antidepressant SUL. Electrochemical method optimization and electrode modification greatly increased the peak current. The limit of detection (LOD) and limit of quantification (LOQ) values were $2.83 \times 10^{-9}$ and $9.42 \times 10^{-9}$ M in BRB at pH 10.0. Under these optimization conditions, application of the capsule and tablet form of the drug was done at the modified electrode, and recovery calculations were completed. Urinary excretion of this drug has been successfully applied. The limit of detection (LOD) value was found as $1.99 \times 10^{-9}$ M in the urine. The advantages of the proposed method are fast, simple, selective, inexpensive, and shorter than compared to other published methods. This study can be helpful in chip technology, clinical studies, and point-of-care testing in laboratories.

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