A new differential pulse voltammetric method for the determination of clozapine in drug dosage forms has been described. In this study, the amount of clozapine in commercial forms has been determined at bismuth modified glassy carbon electrode by taking advantage of the electrochemical oxidation of it. The glassy carbon electrode was modified with BiCl₃ to prepare the bismuth film electrode. The acetate buffer solution at pH = 5.00 was selected as the supporting electrolyte in where the maximum current was observed for clozapine at the bismuth film electrode. The effect of pH on peak current and peak potential of clozapine was investigated by differential pulse voltammetry (DPV), and the effect of scan rate on the peak current was examined by cyclic voltammetry (CV). With this developed voltammetric method, the detection limit for clozapine in the working range of 1 µM and 10 µM was found to be 6.112×10⁻⁹. The amount of clozapine in the drug tablets was stated after the determination of analytical parameters. The recovery study was executed to check the accuracy and precision of the applied method.

Keywords: Clozapine; bismuth film; drug analysis; modified electrode; differential pulse voltammetry

INTRODUCTION

Clozapine, which has a heterocyclic structure is 8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo-diazepine and Fig. 1 shows the chemical structure of this compound [1,2]. Clozapine, which is a strong antipsychotic agent from the dibenzodiazepine group, is called a typical antipsychotic since it has typical pharmacological and clinical features. Many studies comparing clozapine with traditional antipsychotics have shown that clozapine is at least as effective as traditional antipsychotics, even in some cases, this effect has increased [3].

Clozapine has been used in the treatment of patients with schizophrenic resistant to treatment who are caused by neuroleptic drugs that block the typical D2 receptor and develop unsuccessful physiological conditions. Also, clozapine treatment as in conventional antipsychotics stops the abnormal movements of Tardive dyskinesia and clozapine can additionally cure the defect of movement. On the other hand, clozapine is partially active in the treatment of tardive dystonia [4, 5]. Clozapine is also used to treat some schizophrenic patients cannot be treated by traditional neuroleptic drugs. Since the analysis of clozapine in pharmaceutical and chemical liquids is important, different analytical methods such as chromatography [6,7] and spectrophotometry [8] have been used to determine dosage forms. Studies on the therapeutic and toxic effects of drugs have shown that sensitive methods are required to obtain results at the trace level. The sensitivity of the studies with spectroscopic methods is not sufficient. Voltammetric and polarographic studies of the electrochemical behavior of clozapine are available in the literature [9,10]. To date, techniques for the detection of clozapine, including high-performance liquid chromatography, spectrophotometry, chemiluminescence, capillary electrophoresis, and titrimetry, are available [11, 12]. These methods, however, are exhausting, costly, and require the pretreatment of samples. On the other hand, electrochemical techniques such as voltammetry supply a wide linear working range as well as high precision, low cost, and high accuracy. Therefore, studies on the electrochemistry of drugs have increased in recent years [13, 14]. Since clozapine is a good electroactive substance, electroanalytical results of oxidation behavior in different electrodes have been described [15,16].

Electrochemical techniques are strong and multipurpose analytical techniques that can easily solve many pharmaceutical-related problems. Especially vol-
Differential pulse voltammetry conditions were: Pulse amplitude, 50 mV; pulse width 50 ms; scan rate, 20 mV s\(^{-1}\).

**The Procedure of Pharmaceutical Preparations**

The stock solution of 1×10\(^{-2}\) M clozapine was prepared daily in methanol. The standard solutions for the calibration study were prepared by diluting the appropriate volumes of these stock solutions. The measurements for individual concentration was repeated three times. After preparing the calibration graph (10\(^{-5}\) µM - 1 µM), the determination process was started from the drug tablet containing clozapine. Ten drug tablets (each tablet containing 25 mg clozapine according to the label) were weighed and well ground to a fine powder. The stock solution containing 1×10\(^{-2}\) M clozapine in methanol was prepared in a 100 mL volumetric flask.

**RESULTS AND DISCUSSION**

**Preparation of Working Electrode**

Bismuth-coated glassy carbon electrode is used as a working electrode. The first step of the modification process is polishing a commercial glassy carbon electrode (GC). Therefore, the GC (CHI 104, 0.071 cm\(^2\) area) was polished successively in alumina slurries in which 1-, 0.3-, and 0.05 µm alumina particles, respectively, as described previously [17]. The coating solution was 100 ppm BiCl\(_3\) solution in 0.5 M KCl supporting electrolyte. The aqueous medium was buffered with acetic acid/sodium acetate buffer solution. The coating step was performed to dip the GC in the coating solution and then apply the amperometric technique at -1 V potential. The electrochemical characterization of the bismuth coated GC electrode was achieved by using 1 mM dopamine and ferrocene solutions. The voltammograms for dopamine and ferrocene were demonstrated in Fig. 2 and Fig. 3, respectively.

When we compare the voltammetric results from both the bare GC and bismuth coated GC, it is distinguished that the electrode was successfully coated with bismuth. At the bare GC, there is only one anodic peak observed at about 0.5 V potential with an accompanying cathodic peak. These peaks belong to electrochemical oxidation and reduction of clozapine.
dopamine. On the other hand, one different anodic peak additionally shows up at about -0.1 V potential, which is more negative than that of dopamine. This new narrow peak was attributed to the oxidation of bismuth on the GC surface (Fig. 2).

In addition to the dopamine test, the electrochemical behavior of ferrocene also supported the coating of GC with bismuth. Fig. 3 shows the cyclic voltammograms of 1 mM ferrocene solutions. The first one belongs to the electrochemical oxidation of ferrocene at bare GC electrode. On the other hand, the second one represents the same reaction but at the bismuth coated GC electrode. As shown from the results, there are some differences between the two voltammograms. The oxidation potential shifted to a more negative value because of the surface change. This behavior gave a hint about the potential catalytic effect of bismuth. Besides, a new oxidation peak showed up at around -0.2 V in the case of bismuth coated GC electrode. This potential is very near to the oxidation of bismuth on the surface. These results confirmed the modification of the GC electrode with bismuth and preparation of bismuth coated GC electrode.

The Electrooxidation of Clozapine at Bismuth Modified GC Electrode

The electrooxidation of clozapine was investigated using two different electrodes. When the peak currents of clozapine at bare GC and bismuth coated GC electrodes were compared, the calculation showed that the peak current for the modified electrode (bismuth coated GC) is approximately 75 times higher than that of bare GC electrode. This result demonstrated that the new modified electrode was more sensitive than the commercial GC electrode (Fig. 4).

Determination of Optimum Conditions for Oxidation of Clozapine

The effects of pH and supporting electrolyte on the oxidation potential and peak current were monitoring various conditions. First, the conditions for which the highest current was observed for the oxidation of clozapine were determined. Using differential pulse voltammetry (DPV) technique, the dependence of peak current and potential on pH was investigated in both acetic acid/acetate and Britton Robinson buffer solutions as a supporting electrolyte. The results are tabulated in Table 1 for acetate and BR buffer solutions.

According to the results given in Table 1 and the behaviors observed in Fig. 5 and Fig. 6, the maximum peak current was 0.5958 µA and obtained in an acetate buffer solution with a pH of 5. Fig. 5 shows the effect of pH on the oxidation of clozapine. As seen from the figure, anodic peak potentials away from positive values as the pH values increase. This change occurred as the potential value decreased from 384 mV to 216 mV as the pH increased from 3 to pH 8. This behavior shows that protons are involved in the electrode process.
The Variation of Current with The Scan Rate

The oxidation of clozapine (2 µM) has been investigated in acetate buffer solution (pH=5.0) by using cyclic voltammetry (CV). The voltammograms obtained from nine different scan rates between 50 mVs\(^{-1}\) and 450 mVs\(^{-1}\) were given in Fig. 7. In this range of scan rate values, there is a linear relationship between the square root of scan rate (\(v^{1/2}\)) and current function (\(I_p/Cv^{1/2}\)). This behavior can be seen from Fig. 8 and shows that this is an adsorption controlled current. Furthermore, when the logarithm of the peak current and the scan rate is examined (Fig. 8), the slope is found to be 1.1834, which is another criterion that the current is adsorption controlled [18].

Determination of Analytical Working Range

The analytical working range was determined according to the measurements obtained by the DPV technique in acetate (pH = 5.00) buffer solution in the concentration range of 1 µM–10 µM (Fig. 10). Voltammograms at different concentrations for clozapine were recorded under 

### Table 1. Voltammetric results for the study of pH and buffer type effect on the electrochemical oxidation of clozapine (2 µM clozapine, 0.2 M acetate buffer, 0.04 M BR buffer)

<table>
<thead>
<tr>
<th>pH</th>
<th>Acetate Buffer</th>
<th></th>
<th>BR Buffer</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Current(µA)</td>
<td>Potential (V)</td>
<td>Current(µA)</td>
<td>Potential (V)</td>
</tr>
<tr>
<td>3.5</td>
<td>0.357</td>
<td>0.444</td>
<td>0.271</td>
<td>0.384</td>
</tr>
<tr>
<td>5.0</td>
<td>0.596</td>
<td>0.320</td>
<td>0.380</td>
<td>0.328</td>
</tr>
<tr>
<td>6.5</td>
<td>0.032</td>
<td>0.204</td>
<td>0.540</td>
<td>0.264</td>
</tr>
<tr>
<td>8.0</td>
<td>0.583</td>
<td>0.080</td>
<td>0.489</td>
<td>0.216</td>
</tr>
</tbody>
</table>

Figure 5. The effect of pH from different buffers on the peak current of 2 µM clozapine solution.

Figure 6. The effect of pH from different buffers on the peak potential of 2 µM clozapine solution.

Figure 7. The cyclic voltammograms of 2 µM clozapine for different scan rates from 10 mVs\(^{-1}\) to 450 mVs\(^{-1}\) at bismuth film electrode in 0.2 M acetate buffer solution (pH=5.00).

Figure 8. The dependence of current function (\(I_p/Cv^{1/2}\)) to the square root of scan rate (\(v^{1/2}\)) from CV study of 2 µM clozapine at bismuth film electrode in 0.2 M acetate buffer solution (pH=5.00).

\[
y = 0.0042x - 0.011 \\
R^2 = 0.9838
\]

Figure 9. The log Ip–log v curve from CV study of 2 µM clozapine at bismuth film electrode in 0.2 M acetate buffer solution (pH=5.00).

\[
y = 1.1871x - 2.6097 \\
R^2 = 0.9896
\]
optimal analytical conditions. For clozapine, a calibration curve with high linearity was obtained in this medium in the range of 1 µM-10 µM (Fig. 11).

According to the equations, LOD=3 s/m and LOQ=10 s/m, the limit of detection and the limit of quantitation were calculated using measured peak currents, respectively. Where “s” is the standard deviation of peak currents (for three measurements) and “m” is the slope of the calibration curve [19]. LOD and LOQ were calculated as 6.112×10⁻⁹ mol/L and 2.0373×10⁻⁸ mol/L for bismuth-modified glassy carbon electrodes, respectively (Table 2).

On the other hand, there are several references in the literature about the determination of CLZ. The results of these methods as well as that of the present study are given in Table 3. Table 3 contains the information about these studies such as working electrodes, the techniques used, the linear ranges, and LOD.

### Analytical Application and Recovery Study

Commercial drug tablets containing 25 mg of clozapine per tablet were exactly weighed and ground to a fine powder. A stock solution of concentration 1×10⁻² mol/L was prepared by weighing the appropriate amount of the powdered solid and dissolving it in methanol. Appropriate solutions were prepared by taking proper volumes of the stock solution and diluting with 0.2 mol/L acetic acid/sodium acetate buffer solution used as supporting electrolyte. The quantity of clozapine in the trading tablet was calculated as 25.3 ± 2.2 mg using the calibration equation.

Besides, a recovery test was performed for the developed method, and the results are given in Table 5. According to the results, the new analytical method has shown that tablets can be applied successfully without any intervention in the determination of clozapine. When the amount of clozapine was added, and the amount of clozapine detected was compared, recovery from the commercial tablets was found to be 97.00%.

### CONCLUSION

In this study, the electrochemical oxidation property of clozapine on the bismuth coated glassy carbon electro-
peaks were obtained by using the DPV technique in different electrolytes selected in acidic and basic regions. The working medium was determined according to the obtained pH-Ip change. When the peak currents of clozapine in the glassy carbon electrode (0.330 μA) and modified electrode (21.69 μA) are compared, the peak current at the modified electrode is about 70 times higher, indicating that the modified electrode is more sensitive than the bare GC electrode. As a result, this study suggests a new method for the determination of CLZ. As seen from Table 3 some different methods and electrodes have been used for the determination of CLZ but it is the first time to use the bismuth modified carbon electrode for this purpose. The linear range and LOD of this method are near the other methods.

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**References**
