Biocatalysis and Agricultural Biotechnology 22 (2019) 101375

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: http://www.elsevier.com/locate/bab

Electrochemical detection of flutamide with gold electrode as an anticancer drug

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ARTICLE INFO

Keywords:
Anticancer drug
Electrochemical sensor
Cyclic voltammetry
Flutamide
Gold electrode
Biotechnology

ABSTRACT

In this research, the Gold electrode was used to investigate the electrochemical behavior of the Flutamide by cyclic voltammetry and differential pulse voltammetry method. In addition, the morphology of the gold electrode surface was demonstrated with field emission scanning electron microscope (FESEM). Some of the effective parameters such as pH, scan rates, and Flutamide concentration were optimized. The pH 7 was selected as optimal pH. The currents of peak according to Flutamide concentration from 6 to 60 μM and 100–600 μM were linear, and the limit of detection was calculated 1.8 μM. This electrochemical sensor could detect Flutamide selectable in the presence of some interferents.

1. Introduction

Flutamide (FLT) is scientifically known as 4-nitro-3-trifluoromethyl-isobutylalanide (Brahman et al., 2017), as shown in Fig. 1. This compound is widely used as a non-steroidal anti-androgenic agent for controlling and treating prostate cancer (Banerjee et al., 2017). Flutamide and some of the anti-androgens cause to stop testosterone secretion as a stimulant for the prostate cancer cells. Cells of prostate cancer are not able to grow without Testosterone. Flutamide does not treat prostate cancer, but it keeps cancer under control for several months or years (Brahman et al., 2012; Ahmadi et al., 2015; Farias et al., 2017; Kamel et al., 2018; Rezaeifar et al., 2018).

Flutamide was first synthesized by Nery et al. (1967) at an American pharmaceutical company called Shiring Plag. Initially, this substance was synthesized as an antibacterial agent, while the nature of the anti-androgenic agent was determined in other studies. Clinical trials on this drug began in 1971 and were commercially distributed in 1983, and are known by other names such as nilutamide, bicalutamide, and yolxin, and its commercial form is in tablet form and capsule (Sufrin and Coffey, 1976; Álvarez-Lueje et al., 1998; Núñez-Vergara et al., 2001).

Each medication may have side effects in addition to expected effects. Some of the most important side effects of Flutamide drug include sleep disorders, skin reactions, liver damage, anemia, headache, feeling of imbalance, blurred vision, depression, nausea, Jaundice, as well as the reduction of white blood cells and blood platelets (Karthik et al., 2017; Johnson and Sonthalia, 2019).

Several analytical methods such as spectrophotometry, liquid chromatography, polarography, liquid chromatography, mass spectrometry, and electrochemical sensors were used to detect and measure Flutamide (Ensafi et al., 2016; Temerk et al., 2016; Švorc et al., 2017; Abdelwahab et al., 2018; Kubendhiran et al., 2018). Among these methods, electrochemical sensors are more appropriate due to high reliability, fast response, and low-cost equipment, ease of use, time-saving, high sensitivity, and good selection. In electrochemical methods, the electrical properties of the analyte solution in the electrochemical cell are important (Pecková et al., 2012; Ezoji and Rahimnejad, 2016, 2018; Zokhtareh and Rahimnejad, 2018; Mutharani et al., 2019; Zabihollahpoor et al., 2019).

Electrochemical sensors are the largest and most powerful group of chemical sensors. Kremer (1906) first constructed the first type of electrochemical sensor. Electrochemical means the interaction between electricity and chemistry, which refers to the transfer of charge from the electrode to a liquid or solid phase. During this process, chemical variations occur at the surface of the electrodes, and the load is guided from the chamber to the sample. The reactions occurred at the electrode surface, and the transfer of charge within the target species and electrode was chemically performed (Brett, 2001; Wilson et al., 2001; Yamazoe, 2005; Ezoji and Rahimnejad, 2016; Maduraiveeran et al., 2018).

The present study aimed to evaluate the electrochemical behavior of Flutamide as a chemotherapy drug with a gold electrode as an electrochemical sensor. Some parameters influencing the electrochemical

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https://doi.org/10.1016/j.bcab.2019.101375
Received 2 June 2019; Received in revised form 22 July 2019; Accepted 5 October 2019

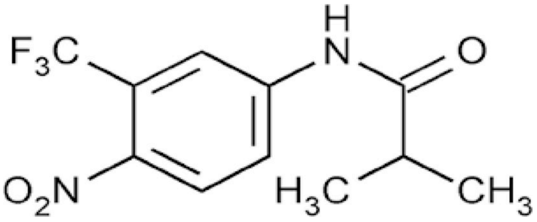

Fig. 1. Flutamide chemical structure.

response of Flutamide were optimized. Given the characteristics of the gold electrode, sensitivity, selectivity, and efficiency of this method should be highlighted.

2. Materials and method

2.1. Reagents and solution

KH_2PO_4 and KH_2PO_4 (Sigma-Aldrich, Darmstadt, Germany) solutions were prepared at the concentrations of 1 M to prepare the 0.1 M phosphate buffer (PB) from pH 2 to 8. A fresh Flutamide 1.0×10^{-3} M stock solution was prepared daily by dissolving a certain amount of pure Flutamide (Euticals, German) into ethanol (Merck, Germany). Then, the solution was kept in a refrigerator at the temperature of 4 °C. All chemicals and reagents were in analytical grades and double distilled water was used to prepare all of the solutions.

2.2. Apparatus

All of the voltammetric and electrochemical assessments were performed by IVIUM (Vertes, Netherland) at the room temperature. Due to the elimination of the loss of ohm, a three-electrode system containing an Ag/AgCl electrode as a reference electrode, a bare gold electrode (AuE) and a platinum as an auxiliary electrode in a 20 ml cell were used to perform all of the electrochemical experiments. In addition, all of the voltammetric experiments were performed at room temperature. Finally, the pH values of the solutions were performed with a pH meter (METTLERTOLEDO, Switzerland).

2.3. Preparation of gold electrode

The surface of the electrode should be completely polished before each test in order to obtain acceptable and repeatable results. First, the Au electrode was washed with distilled water, and then polished with alumina on microcloth. Accordingly, the electrode surface was polished again after adding a few drops of distilled water. Finally, the Au electrode was emerged in distilled water and sulfuric acid, which was ultrasonic for 5 min at the room temperature of 25 °C for final purification, respectively.

2.4. Analytical procedure

Flutamide was measured by the current of oxidation peak at a potential limit from −0.1 V to −0.9 V by using a cyclic voltammetry method. Before each stage, the test was taken in the Blank solution at the same potential limit from −0.1 V to −0.9 V because of minimizing the response errors.

3. Result and discussion

3.1. Electrooxidation of FLT

FESEM technique was used to evaluate the morphology of the gold electrode surface. The electrochemical behavior and properties of the electrode were influenced by surface morphology. FESEM image of Gold electrode shows empty spaces, which increase the absorption rate of the Flutamide at the electrode surface (Fig. 2).

A cyclic voltammetry method was used to evaluate the electrochemical behavior of Flutamide at the surface of the gold electrode. Fig. 3 displays the cyclic voltammogram response of 10^{-5} M Flutamide (curve a) in 0.1 M PB (pH 7.0) and 1 M NaCl at a 50 mV/s scan rate compared to the signals in the blank solution (curve b). Depending on the shape, the electrooxidation of the gold electrode in the blank solution is not active and does not represent any anodic or cathodic peak. As shown in Fig. 3, Flutamide at the gold electrode surface has an oxidation peak at the potential of −0.6 V, with a current peak of 15/4 μA relative to the reference electrode. As a result, no reduction peak was observed, indicating that Flutamide behavior at the electrode surface is irreversible.

3.2. Effect of pH

The pH values of the solution are considered as one of the factors affected on the reaction between the electrode and Flutamide. Cyclic voltammetry method was used to evaluate the effect of pH value on the electrochemical oxidation of 10^{-5} M Flutamide in 0.1 M PB and 1 M NaCl from $8 \geq pH \geq 2$ (Fig. 4A). As shown, the pH of the solution could influence potential and current peaks, respectively. As illustrated in Fig. 4B, the oxidation peak of Flutamide in the gold electrode surface increases to pH 7 and then decreases. As a result, 7 pH = is selected as optimal pH. Further, a linear relationship was observed between potential peak and solution pH by plotting the changes in the potential of oxidation peak in the Flutamide drug in terms of pH changes:

$$Ep\ (V) = -\ 0.025\ pH – 0.5226,\ R^2 = 0.9717$$

Furthermore, by increasing pH value, the potential of the oxidation peak was displaced by negative values (Fig. 4C).

3.3. Effect of scan rate

A cyclic voltammetric method was used to examine the influence of the scan rate on the current peak (Ip) and potential peak (Ep) of Flutamide at the gold electrode surface. Fig. 5A illustrates the record of cyclic voltammograms of 10^{-5} Flutamide in 0.1 M PB (pH. 7) and 1 M NaCl at different scanning speeds (20–200 mV/s). As shown, the potentials and

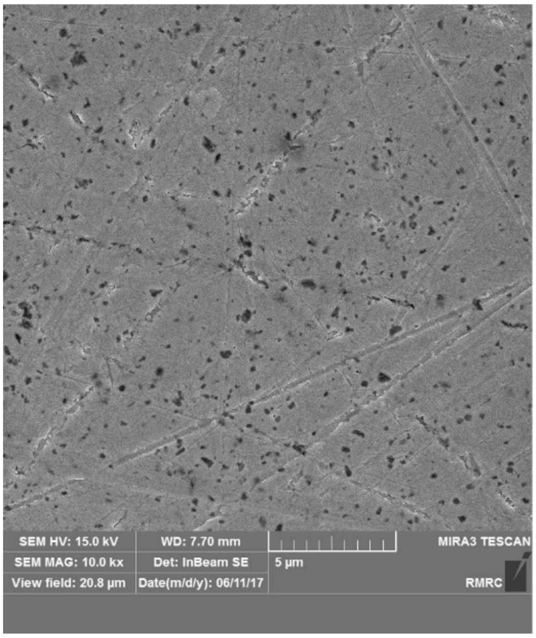

Fig. 2. FESEM image of the bare gold electrode

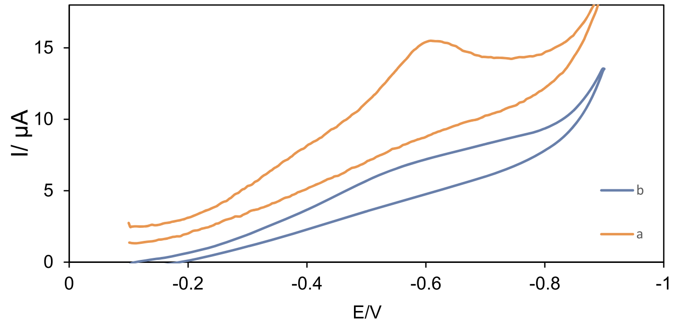

Fig. 3. The CV of 1.0×10^{-5} M FLT in the presence of 0.1 mol L^{-1} PB (pH 7) at the bare AuE (a) cyclic voltammogram of the blank solution on the AuE (b) at 0.05 V s^{-1} scan rate.

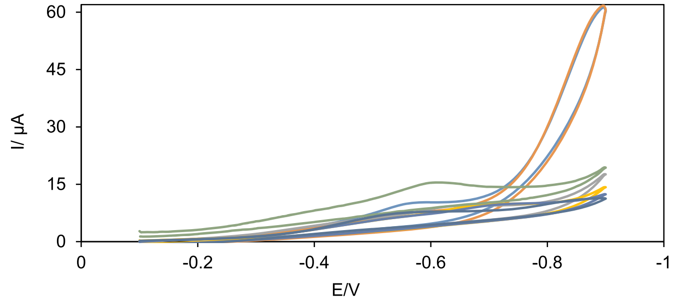

Fig. 4A. Effect of the pH value on the shape of the oxidation peak at pH2.0–8.0.

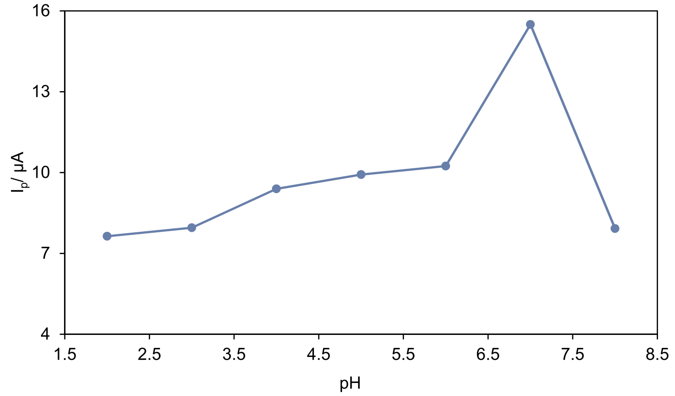

Fig. 4B. Peak current variation with pH for 1.0×10^{-5} M FLT.

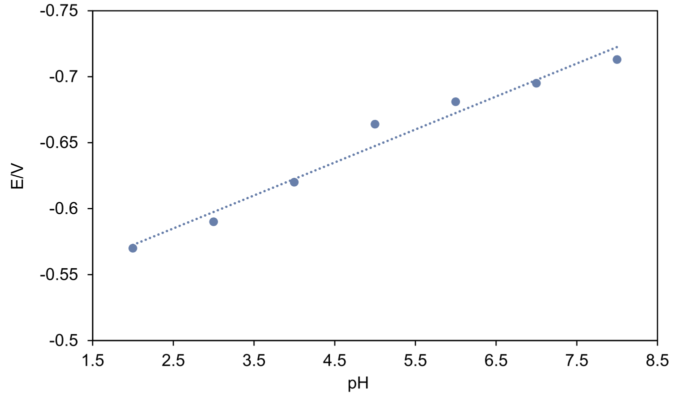

Fig. 4C. Potential of oxidation peak variation with pH for 1.0×10^{-5} M FLT.

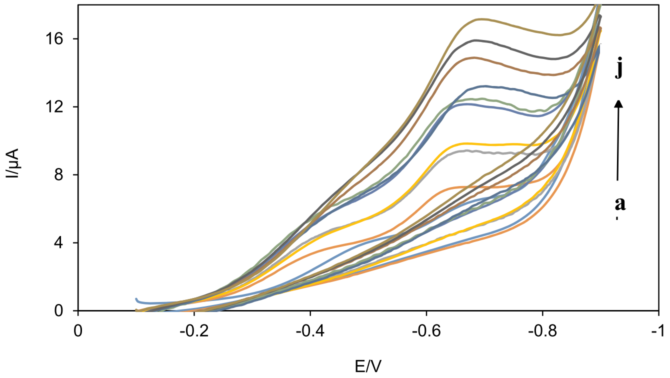

Fig. 5A. CV of the 1.0×10^{-5} M FLT in PB at pH 7.0 at scan rate of (a) 0.02, (b) 0.04, (c) 0.06, (d) 0.08, (e) 0.10, (f) 0.12, (g) 0.14, (h) 0.16, (i) 0.18, (j) 0.20 V s^{-1}.

currents of the oxidation peak of the Flutamide at the gold electrode surface are influenced by the potential of the scan rate. An increase in the scan rate leads to an increase in the current of oxidation peak. In addition, the potential of the oxidation peak increases to the negative values indicating the presence of kinetic limitations during the load transfer process.

As shown in Fig. 5B, a variation of the Flutamide oxidation in the current peak is linear based on the scan rate. Thus, the electrochemical measurements of the Flutamide at the gold electrode surface are controlled by surface absorption as follows:

$$Ip\ (\mu A) = 0.0598\ \nu\ (mV\ /s) + 5.2669,\ R^2 = 0.986$$

Further, the potential changes in oxidation peak are linear in terms of the log in the scan rate potential (Fig. 5C). Thus, we have the following equation based on Laviron equation:

$$E_{pa} = E^0 - \{RT\ /\ [(1\text{-}\alpha)\ nF]\}\ \ln\ \{RTk_0\ /\ [(1\text{-}\alpha)\ nF]\} + \{RT\ /\ (1\text{-}\alpha)\ nF]\}\ \ln\ \nu$$

The value of α as a transfer coefficient is 0.9, and the value of n as the number of electron transfer is 2. Furthermore, K_0 is equal to 0.78 based on the equation.

3.4. Limit of detection and calibration curve

A differential pulsed voltammetry method was used to determine the relationship between the different concentrations of FLT and the currents of electrochemical oxidation in the FLT at the gold electrode.

The differential pulse voltammograms were recorded in the solutions with different concentrations of FLT (6–600 µM) at the gold electrode surface with 0.1 M PB and 1 M NaCl at a 50 mV/s scan rate (Fig. 6A). As shown, an increase in the concentration of FLT leads to an increase in the amount of oxidation peak.

As illustrated in Fig. 6B, a linear calibration curve was observed for

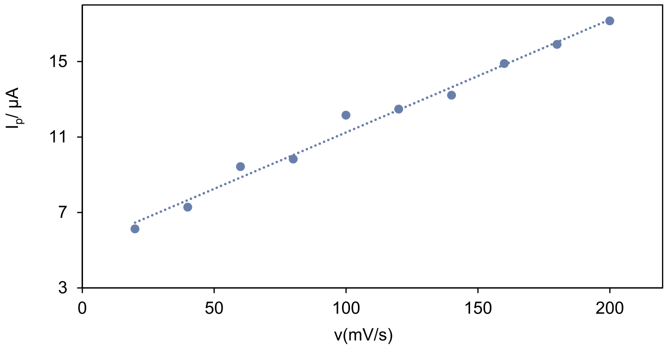

Fig. 5B. Effect of scan rate on the peak currents.

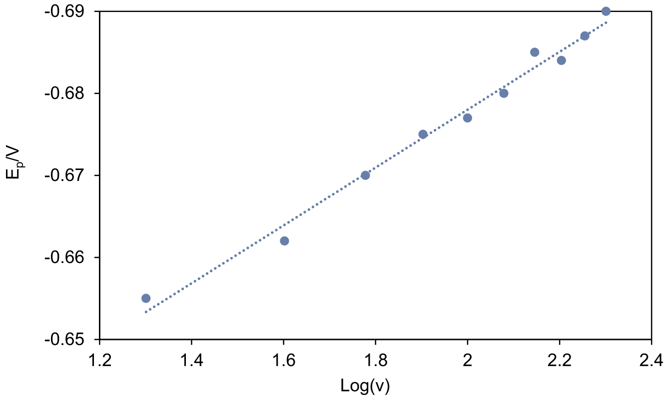

Fig. 5C. The relationship between potential f peaks and logarithm of scan rate.

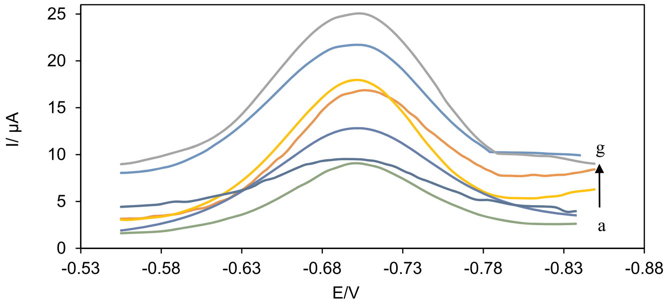

Fig. 6A. DPV of FLT at AuE at various concentration: (a) 6, (b) 10, (c) 30, (d) 60, (e) 100, (f) 300, (g) 600 µM.

FLT under optimal conditions. As shown, the current is linearly available in two concentration ranges including 6–60 µM, Ip = 0.145 C_{FLT} + 8.207 with the correlation coefficient of 0.999 and 100–600 µM, Ip = 0.014 C_{FLT} + 16.941 with the correlation coefficient of 0.997.

To ensure the results, each test was repeated three times for each concentration. Finally, based on the obtained results, limit of detection (LOD) is calculated as follows:

LOD = 3(SD/m), where m indicates the slop of diagrams and, SD shows the response standard deviation.

LOD is considered as the lowest concentration of Flutamide which can be detected, which was calculated as 1.8 µM.

Table 1 indicates the limit of detection and linear range of FLT detection reported at different electrodes. As shown, the LOD values calculated by the present method are better than those in other reported studies.

3.5. *Effect of interference*

In order to determine the selectivity of the gold electrode, a CV technique was used to demonstrate the ability of the electrochemical sensor to detect FLT in the presence of K^+, Cu^{2+}, Fe^{3+}, glucose, and sucrose. The effect of these interferences on the cyclic voltammograms was performed with the 10^{-5} M FLT and 10^{-3} M of each interferent in cells, respectively.

As shown in Fig. 7, Cu^{2+}, Fe^{3+}, and sucrose are not considered as oxidation or reduction peak is observed on the surface of gold electrode, their presence of which does not influence the detection of FLT. In addition, K^+ and glucose at 0.41451 and 0.436 potential produce an oxidation peak, respectively, but the presence of these two compounds did not interfere in identifying the FLT, and these compounds could be simultaneously measured in a test solution.

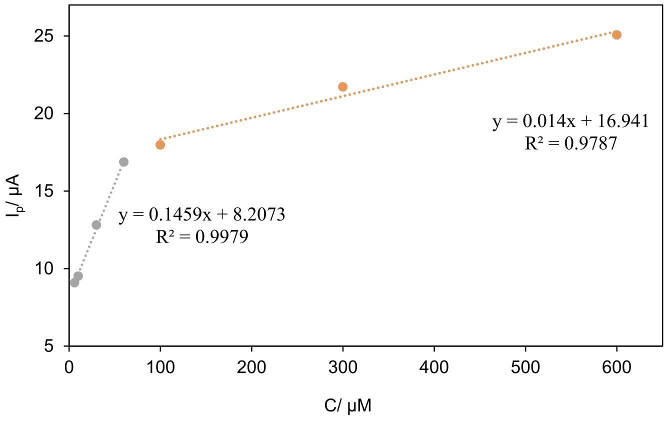

Fig. 6B. Plot for peak current vs. concentrations of FLT.

Table 1
A comparison of linear range and detection limits for FLT in different classical methods.

Numbers	Methods	Linear range (µmol/L)	LOD ()	Reference
1	nano-Ag/ MGCE	100–1000	9.33 µM	Ahmadi et al. (2015)
2	HBP-GO/ PGE	0.1–110	0.029 µM	Rezaeifar et al. (2018)
3	CB/β-CD/ SPCE	0.125–225.8	0.040 µM	Kubendhiran et al. (2018)
4	CS-Au CG/ PBPB	170 to 1245	4.8 nM	Mutharani et al. (2019)
5	AuE	6–600	2 nM	Presented study

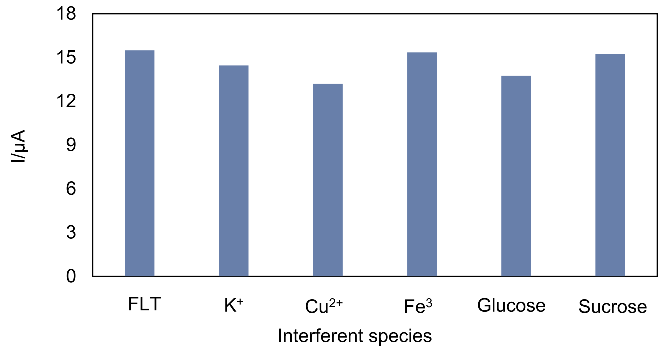

Fig. 7. Influence of interference on the voltammetric response of 1.0×10^{-5} M FLT.

4. Conclusion

In the present study, the gold electrode was prepared as an electrochemical sensor for measuring and detecting the amount of FLT in test solutions. In addition, the effect of experimental solution pH, scan rate, the concentration of FLT, as well as the effect of the interferents on the electrochemical response of the FLT were evaluated. Further, the electron transfer coefficients and the lowest detection limit were calculated. Based on the results, the electrochemical sensor indicated high selectivity and sensitivity, low cost, simplicity, rapid response, and reproducibility. Thus, the gold electrode is suitable for measuring FLT.

Funding

This research was supported by Babol Noshirvani University of technology. The grant number was BNUT/370393/98.

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