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Cyclic Voltammetric Study of Aflatoxin G1 at the Mercury Electrode

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Abstract : A cyclic voltammetry (CV) study of aflatoxin G1 (AFG1) in Britton-Robinson buffer (BRB) using a control growth mercury electrode (CGME) is described. CV was carried out by anodic and cathodic potential scan over the range of 0 to -1500 mV with no accumulation time. The effect of the different scan rates and pH of BRB on the peak height and peak potential of the analyte were also studied. The results from this study showed that the reduction process on the hanging mercury electrode gives a single characteristic cathodic peak at -1184 to -1246 mV (versus Ag/AgCl) in BRB pH of 3.0 to 10.0. The BRB pH of 9.0 was noted as the best condition for the detection of AFG1 as the peak gives a maximum peak current. Effect of the scan rate and pH of BRB on both responses has shown that the reduction of AFG1 is irreversible, pH dependent and the limiting current is adsorption controlled.

Keywords: aflatoxin G1 compound, cyclic voltammetry, Britton-Robinson buffer and control growth mercury electrode (CGME)

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Introduction

Aflatoxin G1 (3,4,7a,10a-tetrahydro-5-methoxy-1H, 12H furo[3',2':4,5]furo[2,3-h]pyrano[3,4-c][1]-benzopyran-1,12-dione), structural formula shown in Figure 1, is a type of aflatoxin that occurs naturally (1). It is one of the compounds in mycotoxin group that is produced by *Aspergillus flavus* and *Aspergillus parasticus* fungi (2,3). It is found in various contaminated food such as peanuts and peanut products, barley, maize, cottonseed, coffee beans and others (4). It appears green under ultraviolet light (5,6). In health aspect, it is considered a human carcinogen by the International Agency for Research on Cancer (IARC) as reported in the World Health Organisation (WHO)'s monograph (7)

Several analytical methods for aflatoxin determination have been developed and reported in the literature. Most are based on thin layer chromatography (TLC) and high performance liquid chromatography (HPLC) with UV-absorption, fluorescence, mass spectrometry or amperometric detection (8, 9,10, 11).

This paper describes the cyclic voltammetric (CV) study of AFG1 using CGME as the working electrode. CV is the most widely used technique for acquiring qualitative information about electrochemical reactions because of its ability to rapidly provide considerable information about the kinetic of the system, associated chemical reaction, number of electron transferred, reversibility of a system, adsorption and diffusion characteristics. Using this technique, the solution is never stirred hence mass transport is diffusion controlled (12). It is often the first electrochemical experiment performed in an electrochemical study especially for any new analyte since it offers a rapid location of redox potentials of the electroactive species.

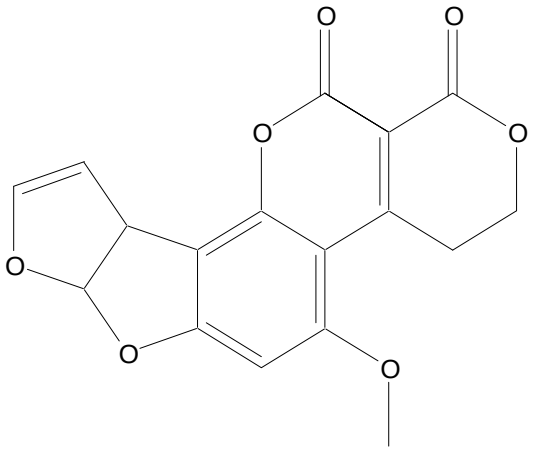


Figure 1 : Chemical structure of AFG1

Materials and Methods

All reagents employed were of analytical grade. Water purified from a Nano Pure Ultrapure water system (Barnstead / Thermolyne) was used for all dilution and sample preparation. AFG1 was purchased from SIGMA: 1 mg was dissolved in 100 ml of hexane: acetonitrile (98:2) to produce a 10 ppm concentration. A stock of Britton-Robinson Buffer (BRB) solution 0.04 M was prepared as follows: 2.47 g boric acid (Fluka), 2.30 mL acetic acid (MERCK) and 2.70 mL orthoposphoric acid (Ashland Chemical) were diluted to 1 L with deionised water. The pH of the solution was adjusted to the desired value by adding 1 M sodium hyroxide (BDH) or 1 M hydrochloric acid (MERCK). Hexadistilled mercury, grade 9 N, used by the controlled growth mercury electrode (CGME) stand was purchased from MERCK.

All voltammograms were recorded with a BAS CV-50W Voltammetric Analyser in connection with a Control Growth Mercury Electrode (CGME) stand equipped with a three-electrode system consisting of an Ag/AgCl reference electrode, a platinum wire counter electrode and the CGME as working electrode. A 20 mL capacity BAS MR-1208 cell was used. In all voltammetric analysis, 10 mL supporting electrolyte solution was deaerated by a stream of nitrogen for at least 20 min. A pH meter Cyber-scan model equipped with a glass electrode combined with an Ag/AgCl reference electrode, was employed for the pH measurements.

Cyclic voltammograms of AFG1 were obtained after purging the test solution at least 5 min with oxygen free nitrogen. It was carried out in 10 mL BRB. The AFG1 was scanned from 0 to -1500 mV and back with a scan rate of 200 mV s^{-1}. Other parameters were initial potential (E_i) = 0 mV, high potential (E_h) = 0 mV, low potential (E_l) = -1500 mV, quiet time = 2 s and sensitivity = 1 µm/V

Results and Discussion

Cathodic cyclic voltammogram obtained at CGME in 0.04 M BRB solution at pH 9.0 with a scan rate of 200 mV s^{-1} is shown in Fig 2. A well-defined reduction peak appears at -1245 mV and no oxidation peak is observed in the anodic branch, which shows that AFG1 reduction is irreversible. This was confirmed by anodic cyclic voltammogram which gave the same results. Suggested mechanism for the irreversible reduction of AFG1 at the mercury electrode surface is shown in Figure 3.

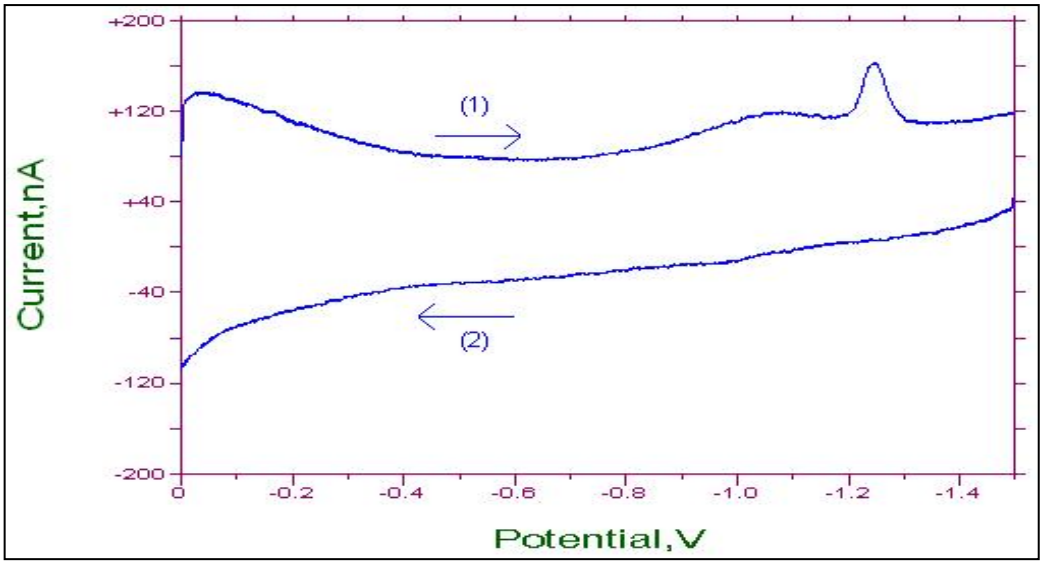


Figure 2 : Cathodic voltammograms of AFG1 in BRB pH = 9.0. [AFG1] = 1.5 µM. Condition; E_i = 0 mV, E_h = 0 mV, E_l = -1500 mV, scan rate = 200 mVs^{-1}, quiet time = 2 s and sensitivity = 1 µm/V

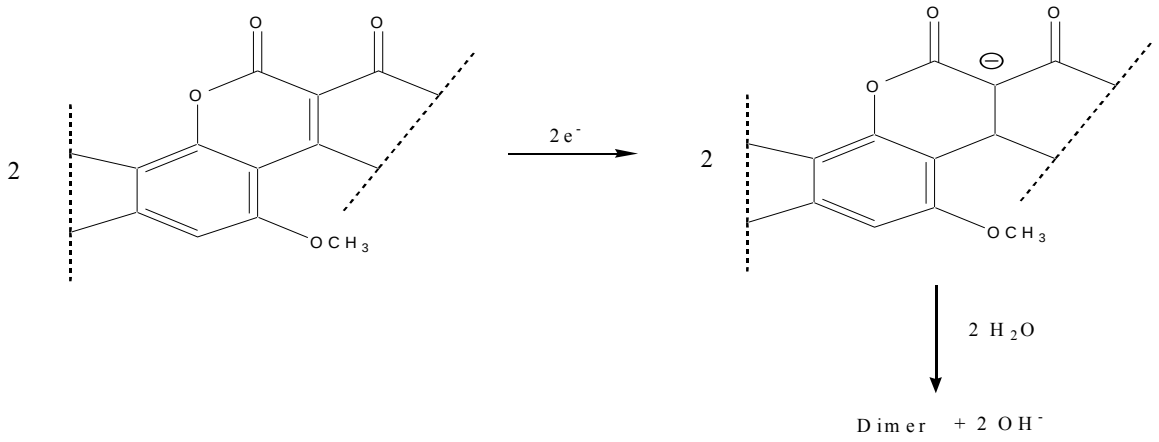


Figure 3 : Suggested mechanism for the irreversible reduction of AFG1 in BRB at pH 9.0 at the mercury electrode surface

The effect of AFG1 concentration was investigated and the results show that the peak potential (E_p) of AFG1 at -1245 mV shows no significant change in position of the peak and the peak current (I_p) of AFG1 increases with increasing concentration of AFG1 as shown in Fig 4. The corresponding equation of this dependence of I_p on the concentration of AFG1 at cathodic peak at pH 9.0 is

I_p (nA) = 24.756 x (µM) + 13.072 (R^2 = 0.9812, n = 9)

The regression equation shows that the I_p of AFG1 does not vary linearly with the concentration of AFG1 suggesting formation of a compact film on the mercury electrode surface (13)

The second and successive scans show substantially higher peak that indicated the electrochemical reduction process of adsorption of AFG1 takes place at the mercury electrode surface as shown in Fig 5a.

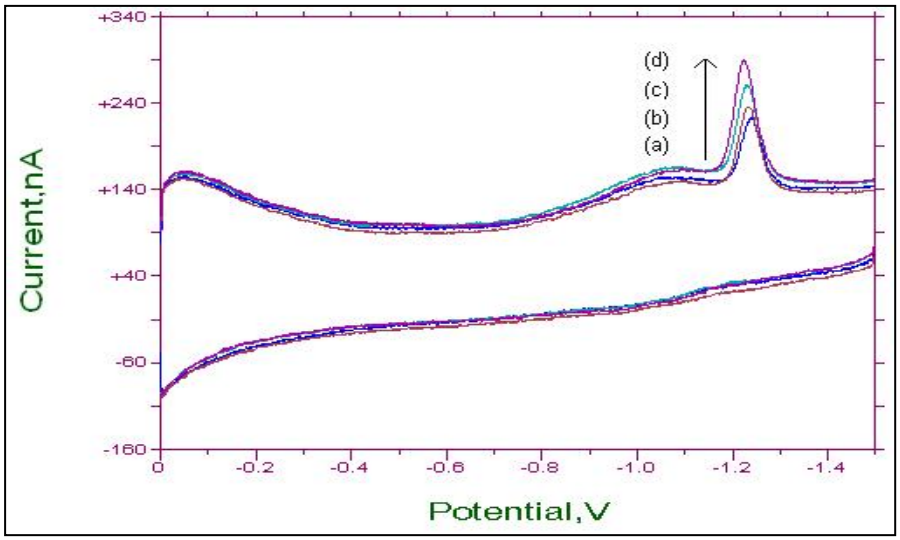
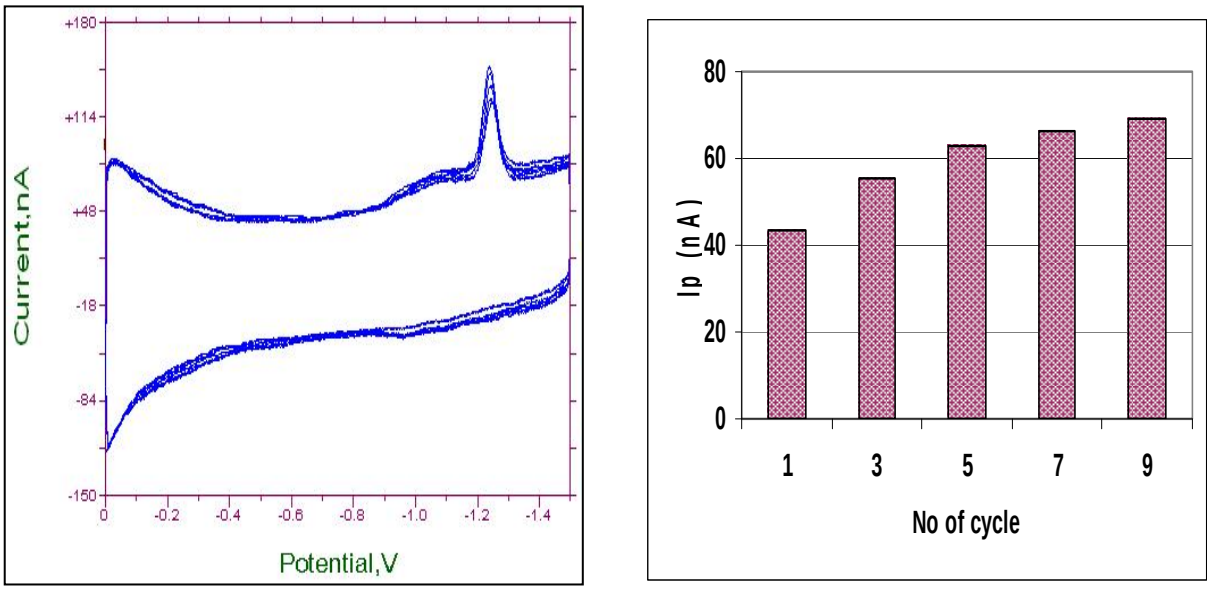


Figure 4 : Cathodic voltammograms of AFG1 in BRB pH = 9.0 with increasing concentration of AFG1. [AFG1] = 1.5 µM (a), 2.25 µM (b), 3.0 µM (c), 3.75 µM (d) and 4.25 µM (e). Condition: E_i = 0 mV, E_h = 0 mV, E_l = -1500 mV, scan rate = 200 mVs^{-1}, quiet time = 2 s and sensitivity = 1 µm/V



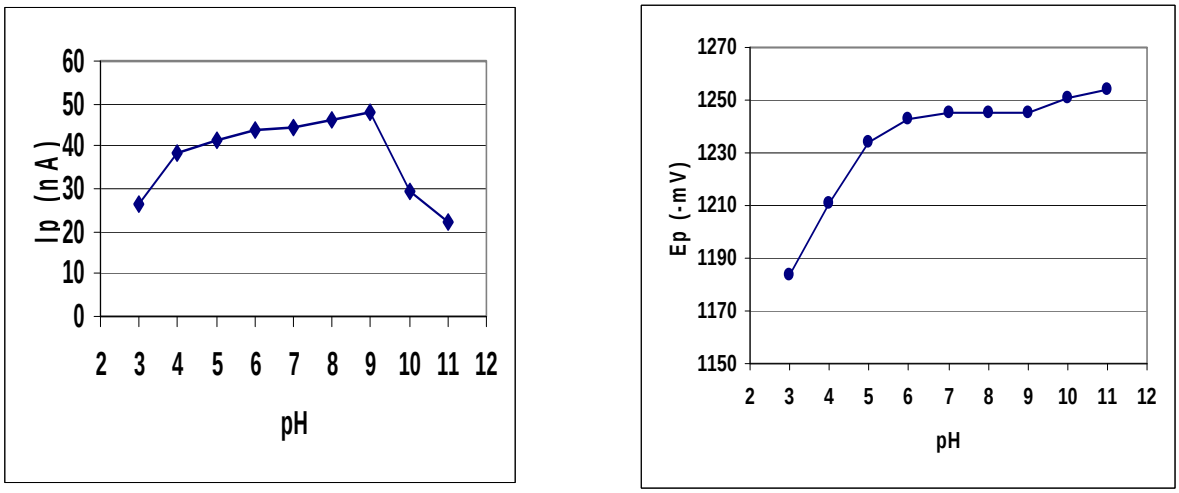
(a) (b)

Figure 5 : Repeatitive cathodic cyclic voltammogram of AFG1 in BRB solution at pH of 9.0 (a) and its respective peak currents (b)

No extra peak neither anodic nor cathodic peak was observed due to adsorption when multiple scans were conducted. The increase in I_p of AFG1 cathodic peak with repeatition of cathodic cyclic voltammetric for AFG1 standard solution is shown in figure 5 b.

The adsorption of AFG1 on the electrode surface is expected since AFG1 consists of functional groups such as ketones, ester and ether groups. The presence of these groups leads to an increase in polarity and enhanced adsorption at the electrode surface (14). The peak current, I_p increases with this repetition due to the adsorption of AFG1 on the electrode surface and with a longer time, the rate of increase of I_p becomes slower because of the formation of double layer at the mercury electrode surface (15).

Effect of pH of BRB was studied from pH of 3.0 to 12.0. The results show that the E_p of AFG1 was shifted toward less negative direction indicating that the reduction of AFG1 was pH dependent. At pH 9.0, maximum peak height of 47.5 nA was observed (Fig 6) hence pH of 9.0 was used throughout this experiment



(a) (b)

Figure 6 : Effect of difference pH of BRB on peak height (a) and peak potential (b) of AFG1

Effect of increasing scan rate, υ (from 20 to 500 mV s^{-}) to the E_p and I_p of AFG1 cathodic peak were observed under the same experimental condition. Linear relationship was observed between log I_p versus log υ which give a slope of 0.5623 (R^2 = 0.9919, n = 9) as shown in Fig 7. The slope of more than 0.5 indicates the diffusion current is influenced by an adsorption on the electrochemical process at the mercury electrode surface (16, 17).

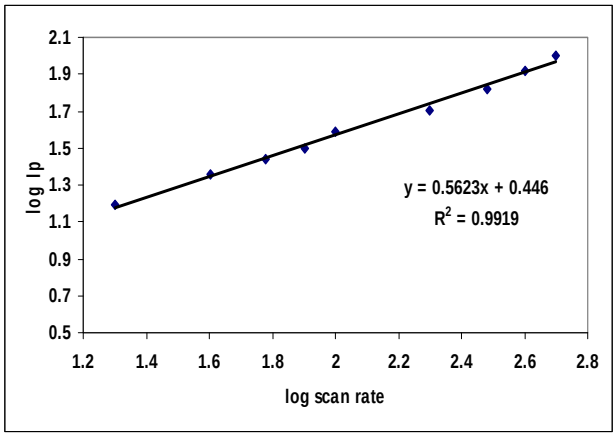


Figure 7 : Plot of log Ip versus log υ for 1.5 µM AFG1 in BRB pH 9.0

The linear plot of E_p versus log υ as shown in Fig 8 (R^2 = 0.9978, n = 9) confirmed that the reduction of AFG1 on the electrode surface is totally irreversible. This irreversible reaction also confirmed by observation of the shifted of E_p towards more negative direction when scan rate is increased, according to the equation;

$$E_p\,(-\text{mV}) = 48.484 \log \upsilon + 1134.8$$
$$(R^2 = 0.9978,\ n = 9)$$

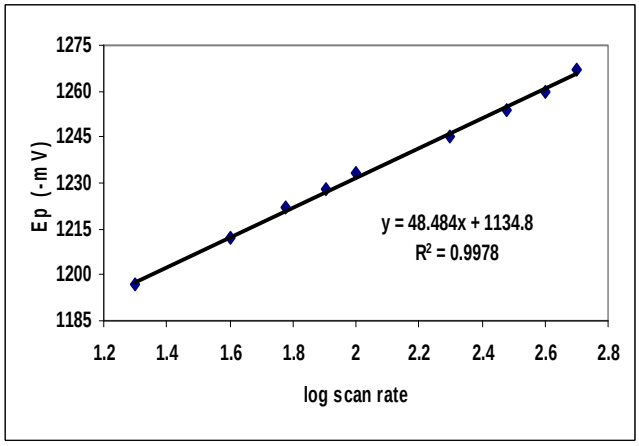


Figure 8 : Plot of E_p versus log υ for 0.15µM AFG1 in BRB pH 9.0

Linear plot of I_p versus υ as shown in Figure 9 indicated AFG1 adsorbed on the electrode surface (18). From the graph, two linear intervals were observed which depend on the scan rate. In the first region which is for the slow scan rate (20 to 80 mV s^{-1}) the linear equation is

$$I_p = 0.2298\,\upsilon + 14.164$$
$$(R^2 = 0.9757,\ n = 5)$$

and at the second region where the scan rate is faster (100 to 500 mV s^{-1}) the linear equation is;

$$I_p = 0.1598\,\upsilon + 19.18$$
$$(R^2 = 0.9892,\ n = 5)$$

which indicates that the reduction and adsorption process of AFG1 at the CGME is governed by the speed of reaction which preferred slow reaction.

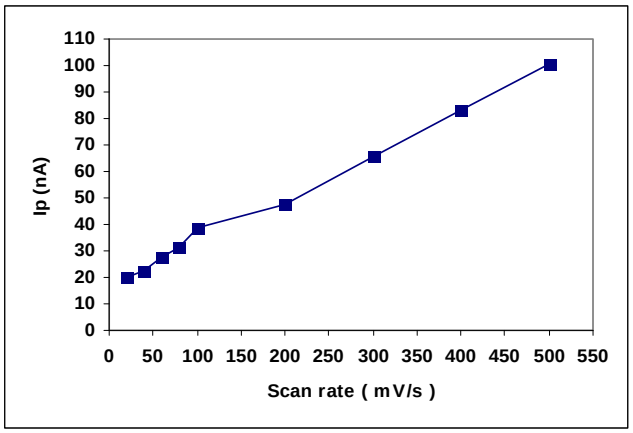


Figure 9 : Plot of Ip versus υ for 0.15 µM AFG1 in BRB pH 9.0

Conclusion

From CV study of AFG1, it is concluded that AFG1 is reducible at mercury electrode with irreversible reduction. The reaction process is adsorption controlled. BRB at pH 9.0 is the most suitable medium for the detection of this reaction to take place. Further experiment will be conducted to develope voltammetric technique such as differential pulse cathodic stripping for determination of AFG1.

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