

CYCLIC VOLTAMMETRIC STUDY OF FUMONISIN B1 ON SCREEN PRINTED ELECTRODE SENSOR

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ABSTRACT

Fumonisin mycotoxins are secondary metabolites produced by pathogenic fungi, which have severe toxic effects on liver, kidney and intestinal tissues leading to liver and esophagus cancers in humans and deleterious impact on animal health. Classical analysis needs advanced laboratories, expert operators, and challenging sample preparation besides expensive cost. The electrochemical sensors seemed as a strict substitution of classical analysis, in the existing study, cyclic voltammetry (CV) was used for simple detection of fumonisin B1 (FB1) based on screen-printed platinum electrode (SPPE) connected to Metrohm Auto-Lab PGSTAT204 potentiostat. CV measurements were adjusted at scan rate of 50 mV/s three runs from +1 to -1 V. The three electrodes of the electrochemical cell (SPPE, SPGE and SPCE) were covered by drop casting 50 μ L of the sample solution of diluted Fumonisin series (50-400) μ g/L was prepared daily by the working PBS standard solution. The graph belongs to SPPE and SPCE are more responsive than SPGE, SPPE that exhibits cathodic peak at about -0.8 V which more reduction than -0.5 which appeared in SPCE. So, we carried on SPPE to continue our studies. The linear relationship appears between concentrations of FB1 and current in direct relationship with R-square (COD)=0.96249, slope=2.94245E-6, linear range of 50 μ g/L to 400 μ g/L, LOD=84.17 μ g/L, LOQ =280.5 μ g/L. The approach described here complied with the National Authority for Food Safety's advice about chemical pollutants in Egyptian food. Due to the proposed method's minimal instrumentation costs and ease of handling, real samples could be utilized.

Keywords: Fumonisin, electrochemical sensor, cyclic voltammetry, screen printed electrodes, food safety, mycotoxin.

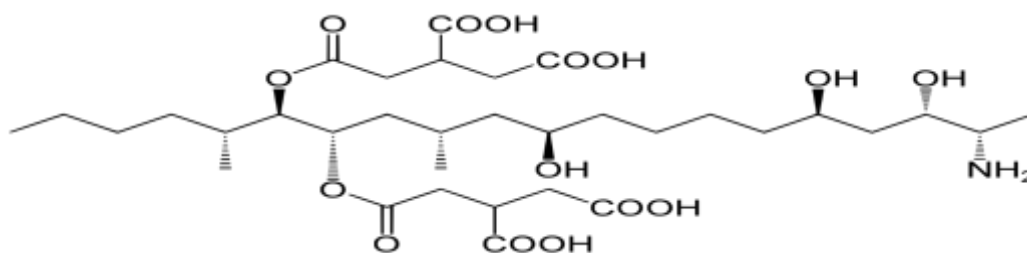
INTRODUCTION

Fumonisin mycotoxins are secondary metabolites produced in cereals, peanut, grape, rice, wheat, barley, rye, oat, millet, maize and maize-based products and grain products (tortillas, corn flask, chips) by pathogenic fungi such as *Fusarium verticillioides*, *Fusarium proliferatum*, and others like *Fusarium* spp, and *Aspergillus nigri* (Rheeder *et al.*, 2002; Astoreca, *et al.*, 2007a and b; Frisvad *et al.*, 2007; Mogensen *et al.*, 2009; Kumar *et al.*, 2017; Dall'Asta & Battilani, 2016; Cendoya *et al.*, 2018). Actually, above twenty-eight fumonisins have been characterized as fumonisin A, B, C, and P (Yazar & Omurtag, 2008; Braun & Wink, 2018; Marasa, 1996). Further among fumonisin B, FB1, FB2, and FB3 are most prominent, FB1 being the most prevalent toxic form that can co-exists with other forms of fumonisin, such as FB2 and FB3 (Damiani *et al.*, 2019). These (FB1, FB2, and FB3) forms are the main food contaminants.

While some toxin synthesis may occur during transportation and storage, predictably, *Fusarium* mycotoxins found in food are produced mainly in the field, where the two main factors impacting on the growth and production of toxins are temperature and water potential temperature (Yazar & Omurtag, 2008). FB1, FB2 and FB3 can cause serious human and animal diseases (Desjardins *et al.*, 2000; Severino *et al.*, 2006). Exposure to fumonisin B1 (FB1) causes pulmonary edema in hogs and leukoencephalomalacia (LEM) in equine. LEM has been reported in countries, as USA, Argentina, Brazil, Egypt, South Africa and China. FB1 can also harm the central nervous system, liver, pancreas, kidney and lungs in a number of animal species. The

relationship between exposure to *F. moniliforme* in maize and the incidence of esophageal cancer was studied in the Transkei from 1976 to 1986 (Rheeder *et al.*, 1992).

On the other hand, the contamination of breast milk by fumonisins has been reported in several studies (Polychronaki *et al.*, 2007; Mahdavi *et al.*, 2010; Mogoha *et al.*, 2014). Current studies have exposed the relationship between contact to FBs and growth ailment in children (Kimanya *et al.*, 2010; Shirima *et al.*, 2014; Chen *et al.*, 2018). According to Shirima *et al.* (2014), fumonisins exposure negatively obstructed child growth among children in Tanzania, which was assured by urinary biomarker levels of Fumonisin (UFB1). Fumonisin has been categorized by International Agency for Research on Cancer (IARC) (Kushiro *et al.*, 2008) and the U.S. Environmental Protection Agency (EPA) (Kadir & Tohill, 2010) as 2B carcinogens (possible human carcinogen). Therefore, in 2001, the Joint FAO/WHO expert committee on food additives and contaminants (JECFA) established a provisional maximum tolerable daily intake (PMTDI) for Fumonisin (the sum of FB1, FB2, and FB3) of 2 µg kg⁻¹ of body weight per day followed by that of the U.S. Food and Drug Administration (FDA). Their chemical formula of fumonisin B1 is diesters of propane-1,2,3-tricarboxylic acid and either 2-(acetylamino)- or 2-amino-12,16-dimethyl-3,5,10,14,15-pentahydroxycosane (Thakur & Smith, 1996). As shown in (Figure1) C₃₄H₅₉N₁O₁₅



Figure(1): Molecular formula of fumonisin B1 (FB1)

Fumonisin has been usually analyzed by chromatographic methods, such as thin layer chromatography (TLC), liquid chromatography (LC), gas chromatography-mass spectrometry (GC-MS) and high-performance liquid chromatography (HPLC), which are costly and exhausting time methods and, in spite of their higher specificity and sensitivity, require appropriate instrumentation and trained personnel. As a less expensive substitute, the ELISA method requirements include a stable source of antibodies and incubation time for enzyme-substrate reactions, showing inconvenient for use in the field and, it may produce false positive signal (Wang *et al.* 2006; Shi *et al.*, 2015; Rhouatia *et al.*, 2013).

All this led to prefer electrochemical sensors, which are high sensitivity, fast response, low-cost, easy to use and suitability to miniaturization (Lin, 2005). Nowadays, cyclic voltammetry (CV) is perhaps the most adaptable electroanalytical technique for the research of chemical reactions of electroactive species. CV has been widely used in the fields of physical, organic, and inorganic chemistry as well as biochemistry (Wang *et al.*, 2015).

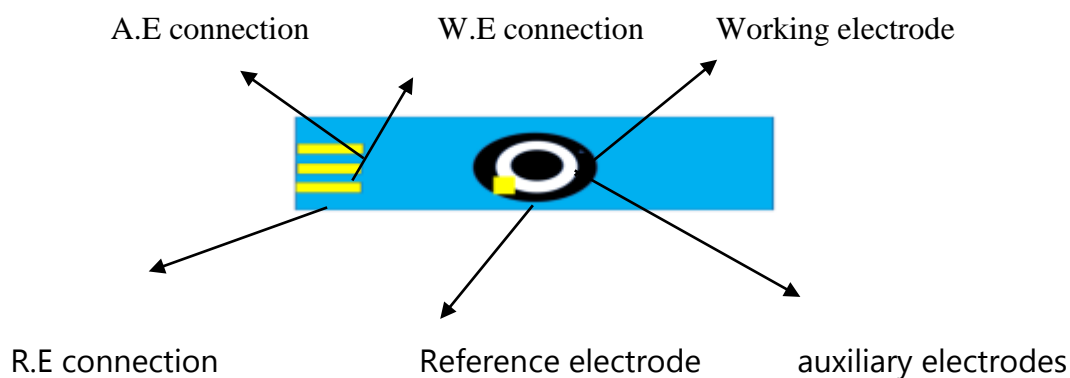
In the present study, we applied Screen-Printed Platinum Electrode (SPPE) for detection of Fumonisin by using cyclic voltammetry.

MATERIALS AND METHODS

Instruments: The electrochemical measurements were recorded using PGSTAT204 includes a base potentiostat/ galvanostat from Metrohm made in Netherlands running on powerful NOVA software it can be used for most of the standard electrochemical technique connected at room temperature.

The potentiostat can be expanded with a miniatures Screen- Printed Electrodes consisting of ceramic substrate L33 x W10 x H0.5 mm and silver electric contacts SPE which based on

carbon, gold, platinum, silver or carbon nanotubes inks. 1) Screen-Printed Carbon Electrodes (SPCE), Working and auxiliary electrodes are made of carbon, while reference electrode is available in silver (110).



Figure(2): shows general structure of Screen – Printed Electrode

2) Screen-Printed Gold Electrodes, (220AT) (SPGE): The cell consists of: Working and Auxiliary electrode are Gold, Reference electrode: Silver. 3) Screen-Printed Platinum electrodes (550) (SPPE): The electrochemical cell consists on: Working and Auxiliary electrodes made from Platinum, Reference electrode: Silver.

They should be stored at room temperature, protected from light in a dry place. pH measurements were recorded by using a Thermo-Orion pH/mV meter (model SA 750, Thermo Scientific, USA) at $25 \pm 1^\circ\text{C}$, equipped with a Rose Sure-Flow combination pH electrode (model 8172BNWP, Thermo-Orion, USA).

Reagents: Fumonisin in methanol from Rida screen further diluted with purification-phosphate buffer solution (PBS 0.1M) which work as supporting electrolyte were used for 6000 $\mu\text{g/L}$ stock solution of Fumonisin (Which stored in dark bottle in the refrigerator).

Hydrochloric acid (HCL) solvent, Sodium hydroxide (NaOH) from Merck. Different pH Fumonisin 50 µg/L solution were prepared, Diverse scan rate for 100 µg/L Fumonisin, and diluted Fumonisin series (50-400) µg/L was daily prepared PBS standard solution.

All reagents and chemicals were analytical grade and solutions were prepared using deionized water.

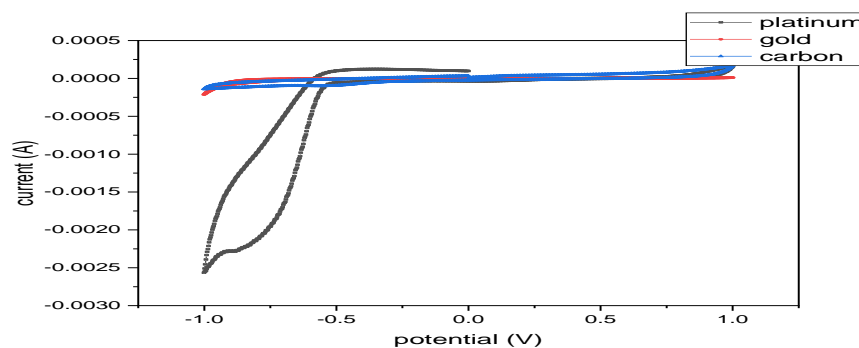
Procedures: Voltammetric measurements were conducted using Autolab **PGSTAT204** electrochemical analyzer controlled by NOVA 1.11 software at room temperature by using a three-electrode configuration via specific connectors that act as interface between them. CV measurements were adjusted at scan rate of 50 mV/s three runs from +1 to -1 V. The three electrodes of the electrochemical cell were covered by drop casting 50 µL of the sample solution. The result is a (CV), in the form of a cycle between current and potential, where potential is plotted on the x-axis, and current is plotted on the y-axis. The CV spectra were plotted in the form of Nernst equation.

$$E = E^{\circ} - \frac{RT}{nF} \ln Q$$

E: Cell potential, E° : standard potential, R: universal gas constant (8.314 J K⁻¹ mol⁻¹), n: the number of transferred electron, F: Faraday's constant (96485 C/mol), the exact temperature, T (K), Q: reaction quotient

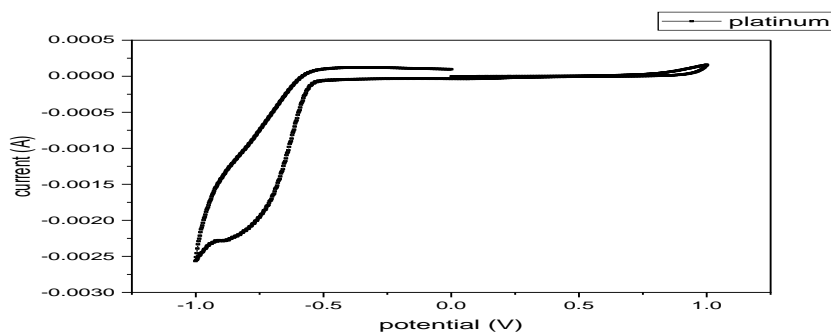
RESULTS AND DISCUSSION

Study of the effect of different screen-printed electrodes

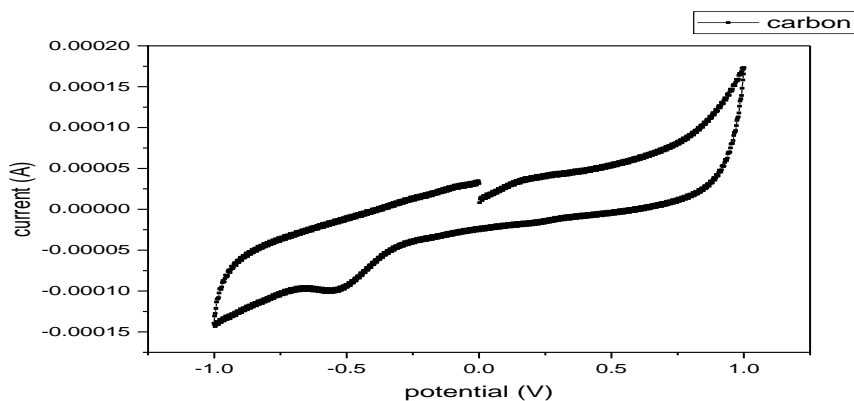


Figure(3): Cyclic voltammetry application running into three Screen-printed Electrodes (Carbon, Gold and Platinum) 50 $\mu\text{g/L}$ Fumonisin

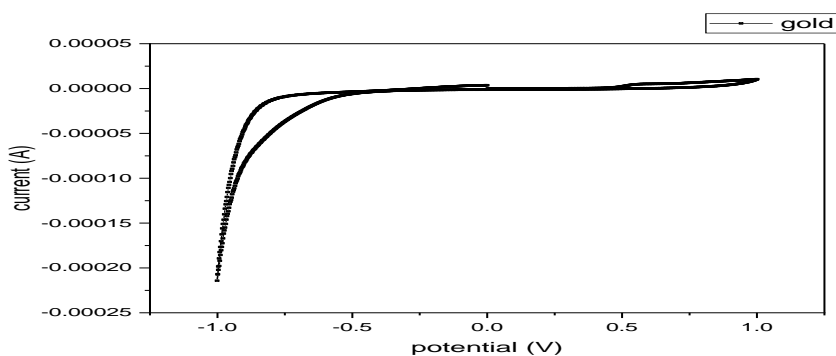
Figure 3 combines three graphs of three different kinds of microchips, black color for SPPE, red for gold and blue one for carbon. To clarify each one, we must separate it into three graphs. Which are 3a,3b, and 3c.



Figure(3a): CV applied on SPGE with 50 $\mu\text{g/L}$ Fumonisin



Figure(3b): CV applied on SPCE with 50 µg/L Fumonisin

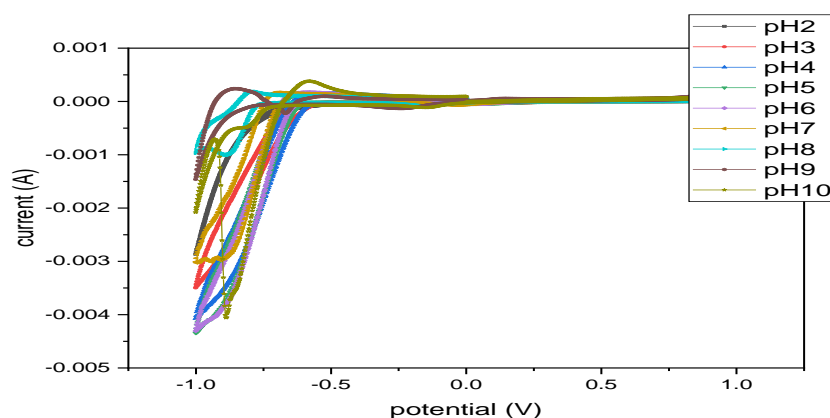


Figure(3c): CV applied on SPGE with 50 µg/L Fumonisin

It is noticeable from Figure 3 that the graph belongs to SPPE and SPCE are more response than SPGE due to appearance of cathodic peak at about -0.8 V and -0.5 V respectively; but in Figure 3c it is showed that no cathodic or anodic peak so, this graph was excluded from study. At graph 3a and 3b notes that potential of fumonisins on SPPE is more electrochemical reduction than happen on SPCE, that mean FB is reduced via electron transfer from a working platinum electrode stronger than carbon working electrode.

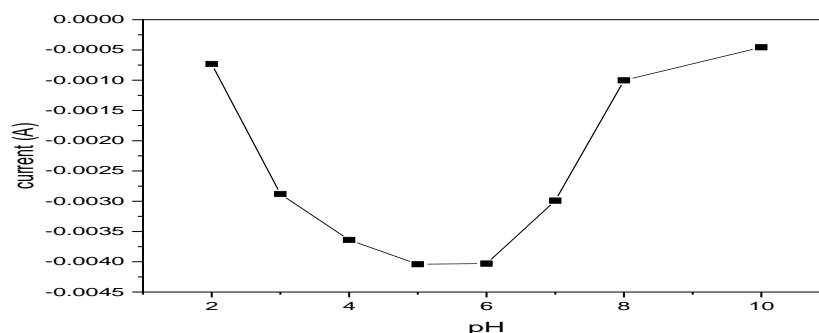
Overtime, Figures 3a and 3b proved reduction reaction of fumonisin in two Screen-Printed Electrodes (carbon and platinum) that mean increased SPPE and SPCE activity in electrochemically active area, and favorable surface functional group orientation towards Fumonisins. Since the SPPE is more response and active than carbon, we selected SPPE, to complete the rest of the studies on this microchip. Obviously, CV is perhaps the most versatile electroanalytical technique for the study of chemical reactions of electroactive species used for redox processes, in the analyses of electrochemical reactions between ions and surface atoms of electrodes (Wang, 2000; Nicholson & Shain, 1964).

Effect of the pH



Figure(4a): Potential V vs current on a CV with varying fumonisins pH values from pH 2-pH 10.

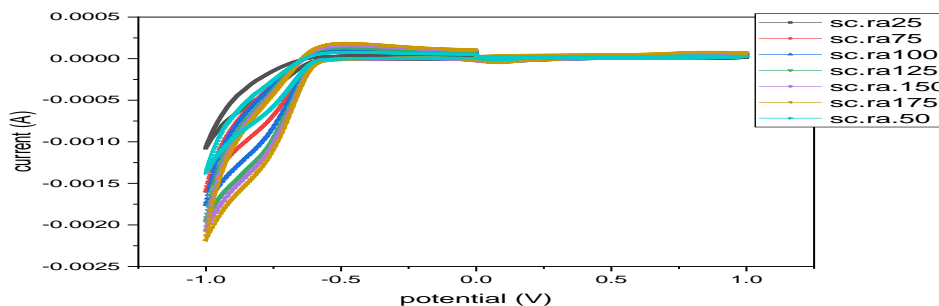
In order to optimize the response of SPPE towards fumonisins reduction, the effect of pH on the cathodic reduction of 50 $\mu\text{g/L}$ Fumonisins was explored by cyclic voltammetry (Figure 4a).



Figure(4b): Bell shape graph plotted pH in x-axis against potential in y-axis

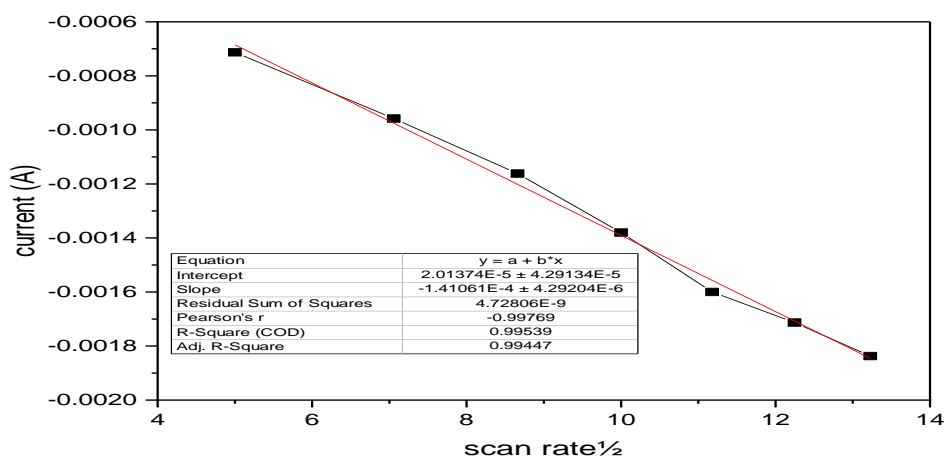
Low Hydrogen ion raises current value that mean more alkaline solution facilitate the chemical reaction. In order to optimize the response to SPPE towards fumonisins reduction increase cathodic potential peak shown in CV of Figure 4a. Otherwise, graph of figure 4b shows direct proportion from pH 6 to pH 10 that mean as pH increase current increase in direct relationship, but pH 2 to pH 5 that as pH increase current decrease inverse relationship that mean in acidic solution current decrease as pH increase to pH 5, Constant current from pH5 till pH 6 therefore, the is optimal region is from pH 6 until pH 10 in alkaline solution.

Effects of the scan rate



Figure(5a): CV of 100 µg/L Fumonisin effected with different scan rate from (25-175) mV/S.

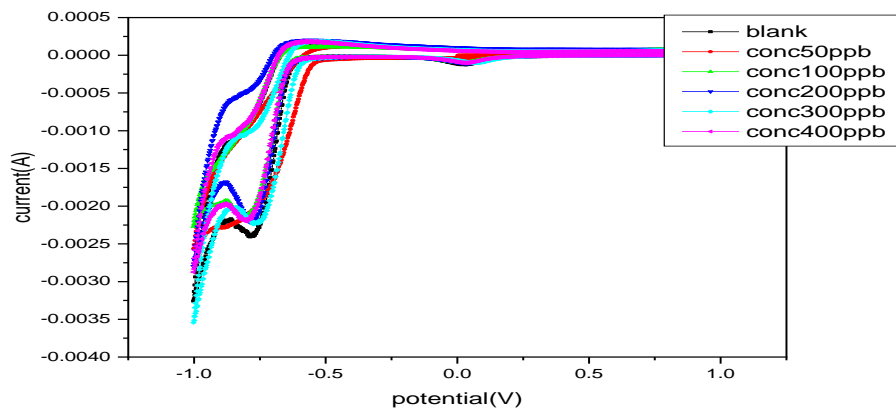
The scan rate determines the length of a cyclic voltammetry experiment, hence increasing the scan rate reduces the length of the experiment. Potential scan rate ($v=E/t$), often known as the rate of potential changes, is modifiable. The scan rates employed in conventional voltammetric investigations range from a few millivolts per second to several volts per second. Figure 5 demonstrates how the cathodic peak current (I_{pc}) of fumonisins and scan rates were both changed.



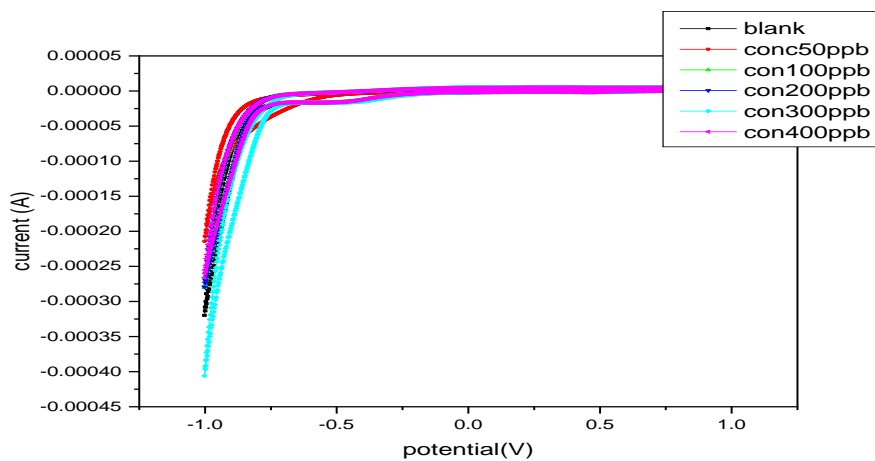
Figure(5b): inverse relationship between square root of scan rate and current for 100 µg/L Fumonisin.

Equation $y = a + b \cdot x$ shows a strong linear relationship between the I_{Pc} values and the square root of scanning rates. R-square COD is 0.99539, and the slope is $-1.4106E-4$. The results suggested that diffusion was responsible for regulation the electrochemical process at the SPPE contact.

Effect of Fumonisin concentrations on Screen-Printed Electrode



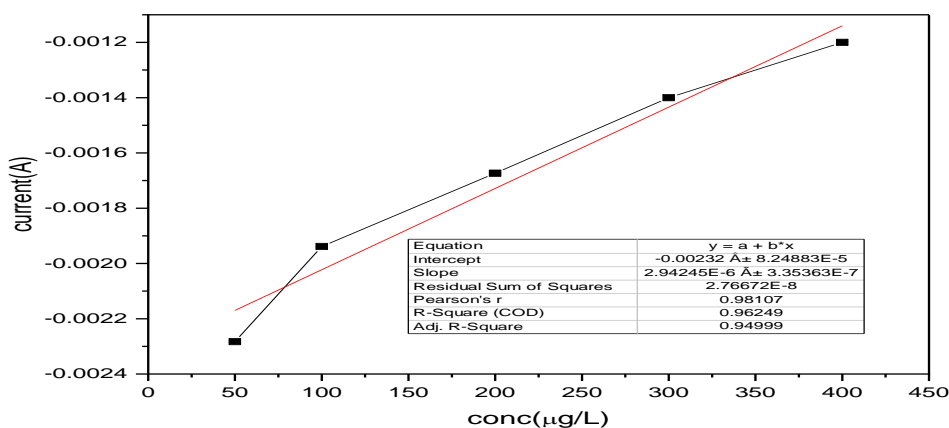
Figure(6a): Different Fumonisin concentrations effect on Screen-Printed platinum electrode



Figure(6b): CV graph of different concentration from concentration (50-400) $\mu\text{g/L}$ Fumonisin on screen printed gold electrode

Figure 6a explain that appearance of peak clearly than figure 6b, that prove complete study with SPPE conveniently.

Calibration curve of Fumonisin concentration in part per billion



Figure(6C): Direct relationship between different concentrations of Fumonisin (50-400) ($\mu\text{g/L}$) at pH 7.4 and current (A) appear in calibration curve.

Fumonisin concentration and current show a linear relationship with R-square (COD)=0.96249, slope=2.94245E-6, linear range of 50 $\mu\text{g/L}$ to 400 $\mu\text{g/L}$, LOD=84.17 $\mu\text{g/L}$, and LOQ =280.5 $\mu\text{g/L}$. This implies that the current increases directly in proportion to the concentration of Fumonisin, as shown by the equation $y = a + b \cdot x$

We succeeded in providing this perfect solution, due to their accuracy and sensitivity, beside it gives smart solutions of expensive devices. For more clarification and explanation, Table 1 Shows maximum level of toxin as Unprocessed maize 4000 $\mu\text{g/L}$, processed maize-based foods and baby foods for infants 200 $\mu\text{g/L}$ and so on, which supports our technique. Therefore, we

arranged our concentration solvents of fumnoisin between linear range 50 µg/L to 400 µg/L, that because lowest permissible maximum of fumnoisin in baby and infant food made from corn is 200 ppb, that our chose is based on Egyptian techniques specification to achieve the required Table 1.

Table(1): The binding technical rule for the maximum permissible limits for chemical contaminants in Egyptian food from the National Authority for Food Safety, which classified mycotoxin as Biological Toxins. Source: Mansour, H. 2022.

Maximum permissible limits of Fumonisin (Sum of B1 and B2) on µg/L	Product name
4000	Unprocessed maize except unprocessed maize intended to be processed by wet milling
1000	Maize intended for direct human consumption, maize-based foods for direct human consumption except foodstuffs used in breakfast foods, snacks, and baby foods
2000	Maize flour and maize meal.
800	Maize-based breakfast cereals and maize based snacks
1400	Milling fractions of maize with particle size more than 500 micron falling with particle within used for direct human consumption particle size more than 500 micron not used for direct human consumption
2000	Milling fractions of maize and other maize milling products with particle size less than or equal to 500 microns.
200	Processed maize- based food and baby foods for infants and young children

Table(2): Comparison of voltammetric studies for Fumonisin with other detection methods

Material used	Method applied	LOD ($\mu\text{g/L}$)	Detection range ($\mu\text{g/L}$)	References
Dual-channel ITO-microfluidic electrochemical immunosensor for simultaneous detection of two mycotoxins	DPV (different pulse voltammetry)	0.079	0.3-140	(Lu & Gunasekaran, 2019)
Thionine and 6-(Ferrocenyl) hexanethiol for FB1	DPV	0.0005	0.001-100	(Han <i>et al.</i> , 2020)
PbS QDs	DPV	0.02	0.05-50	(Wang <i>et al.</i> , 2017)
aptasensor based on screen printed carbon electrode modified with gold nanoparticles for the detection of fumonisin B1 in maize flour	CV	0.2	0.0005-0.5	(Naghshbandi <i>et al.</i> , 2022)
Graphene and gold nanocomposites are used to create an aptasensor for detection in maize.	CV	0.1	0.01-100000	(Yating <i>et al.</i> , 2021)
Screen-Printed Platinum electrode	CV	84.17	50-400	This work

This shows that our method is easier than previous methods mention in Table 2, Which takes lot of time and complicated preparation, professional personal, expensive tools and instrument, in the same time our method provides us the required need in our specification. in addition, limit of detection LOD=84.17 $\mu\text{g/L}$ gives specific of this work.

CONCLUSION

In this work, the electrochemical behavior of FB1 at a SPPE was examined by CV. Voltammetry study for Fumonisin based on diffusion and its reduction process at SPPE was developed. Furthermore, a lower detection limit has been achieved. The proposed method has also been successfully applied for determination of Fumonisin in food samples. The method

reported here fulfilled the recommendation of chemical contaminants in Egyptian food from the National Authority for Food Safety. Therefore, the proposed method could be used in real samples due to low cost of instrumentation and ease of handling.

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دراسة الفيومنسرين B1 بالفولتمترى الدوري بناءً على مجس كهرمائي مطبوع على شاشة

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المستخلص

الفيومنسرين هي نواتج أيضية ثانوية تنتجها الفطريات المسببة للأمراض يمكن ان تسبب أمراضا خطيرة للإنسان والحيوان. وتسبب في حدوث وذمة رئوية في الخنازير وتلين الدم الكببي وبيضر الفيومنسرين بي ١ بالجهاز العصبي المركزي لعدد من الحيوانات وسجل في نترانيا سرطان المريء ويعتبر الفيومنسرين بي ١ الاكثر انتشارا وخطورة من باقي انواع الفيومنسرين. بينما يتسبب ظهور الفيومنسرين أثناء النقل والتخزين، وايضا يتم إنتاجه في الغذاء بشكل رئيسي في الحقل، حيث العاملان الرئيسيان اللذان يؤثران على نمو وإنتاج هذه السموم هما درجة الحرارة ودرجة حرارة الماء المحتملة. يحتاج التحليل الكلاسيكي إلى معمل متقدم وتشغيل خبير وصعوبة تحضير العينات بالإضافة إلى تكلفته الباهظة بدت المستشعرات الكهروكيميائية كبديل صامم للتحليل الكلاسيكي، في الدراسة الحالية، تم استخدام قياس الجهد الدوري (CV) للكشف عن الفيومنسرين باستخدام قطب بلاتيني مطبوع على الشاشة (SPPE) متصل ب بوتنشيوميتر، تم تعديل قياسات بمعدل مسح قدره ٥٠ ملي فولت / ثانية ثلاث مرات من +١ إلى -١ فولت. تم تغطية الأقطاب الثلاثة للخلية الكهروكيميائية بواسطة إسقاط ٥٠ ميكرو لتر من محلول العينة من سلسلة الفيومنسرين المخفف (٥٠-٤٠٠) ميكروغرام / لتر. تظهر الرسوم البيانية ذروة كاثودك عند حوالي -٠,٨ فولت.

كما تم تحضير محاليل مختلفة الحمضية من pH (2-10) وايضا scan rate مختلف لتحديد أفضل تكيف لتحليل الكهروكيميائي للفيومنين. تظهر العلاقة الخطية بين تركيزالتيارمن R-square (COD) = 0.96249 ، slope = $2.94245E-6$ ، مستوى حد الكشف = ٨٤,١٧ ميكروغرام / لتر، الحد الأقصى = ٢٨٠,٥ ميكروغرام / لتر. وهذه الطريقة الحالية تتوافق مع توصية الهيئة القومية لسلامة الغذاء بشأن الملوثات في الاغذية المصرية للحد الاقصى لوجود الملوث في الغذاء، ونظرا لسهولة الطريقة وتكلفتها البسيطة وايضا جهاز وادوات صغيرة الحجم يسهل التعامل معها يمكن تطبيقها على الأغذية المتاحة.