ELECTROANALYSIS OF PATULIN

Maria-Lavinia POPA-COBIANU[1], Raluca-Ioana STEFAN-VAN STADEN[2], Ruxandra-Maria ILIE-MIHAI[3,*]

Patulin is a toxin often found in fruit juices like apple juice. Fast assessment of patulin in fruit juices is needed before its consume. Therefore, a textile- based material filmed with a composite diamond like carbon and Ag nanolayer and modified with ß-cyclodextrin served as material for the design of an electrochemical sensor. Differential puls voltammetry was used for all measurements, when a sensitivity of 493.90 μA mol^{-1}mL was obtained, within a linear concentration range between 1.00×10^{-12} and 1.00×10^{-7}mol L^{-1}. A recovery of 93.45% and a relative standard deviation of 1.30% were obtained when patulin was determined from apple juice.

Keywords: Patulin, electroanalysis, apple juice.

1. Introduction

Patulin is a toxin found in different food products like juices [1-4]. Patulin contamination of fruit products cause a significant risk to human health; the tolerated level of patulin by human body is 50μg kg^{-1} body [2]. Patulin induces apoptosis, pyroptosis, and ferroptosis [3]. Gao et al. [5] proposed an enzymatic detoxification from patulin, of fruits and their juices; this enzymatic biodegradation of patulin has a minimal effect on the quality of fruits and their juices [5,6].

Accordingly, determination of patulin in fruits and their juices is a need in order to avoid health problems. To date, there are the following methods proposed for the assay of patulin in fruits and their juices: (a) chromatographic methods of analysis [7-10] when limits of determinations of 4.0 μg L^{-1} and 39.60 ng L^{-1} where reported when HPLC/UV [8,9] and HPLC/MS [10] where used, and (b) electrochemical methods of analysis [11, 12] when an electrochemical sensor [11], and an aptamer based sensor [12] where used for the analysis of patulin. The lowest limit of determination obtained when the electrochemical sensor was used for the assay of patulin was 0.08 fmol L^{-1} [11].

[1] PhD student, Faculty of Chemical Engineering and Biotechnologies, National University of Science and Technology POLITEHNICA Bucharest, Romania, e-mail: lav_popa@yahoo.com.
[2] CS I, Prof. Dr. habil, Laboratory of Electrochemistry and PATLAB Bucharest, National Institute of Research for Electrochemistry and Condensed Matter, and Faculty of Chemical Engineering and Biotechnologies, National University of Science and Technology POLITEHNICA Bucharest, Romania, e-mail: ralucavanstaden@gmail.com.
[3] CS II, Laboratory of Electrochemistry and PATLAB Bucharest, National Institute of Research for Electrochemistry and Condensed Matter, Bucharest, Romania, e-mail: i.ruxandra04@yahoo.com.

Electrochemical sensors are excellent tools for fast on-site assays of the different substances in juices; no sampling of the juice is needed; high recoveries values are recorded as the measurements can be accurately performed with high selectivities and sensitivities. Moreover, this method proves to be cost-effective due to the electrode's high level of robustness and reliability, as supported by previous studies [12, 13].

The novelty of this paper is the utilization of a textile - based material filmed with a composite diamond like carbon and Ag nanolayer and modified with ß-cyclodextrin as working electrochemical sensor, surrounded by deposition of a silver nanolayer and a platinum nanolayer to give a combined disposable electrochemical sensor that can be used for the assay of patulin in apple juice.

2. Experimental

2.1. Materials and reagents

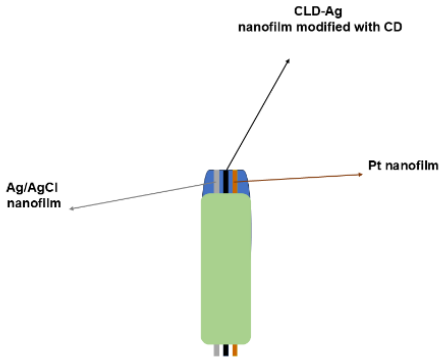
The following substances were bought from Sigma Aldrich: ß-cyclodextrin, patulin. Serial dilution method was used to obtain the series of patulin solutions with concentrations higher than 1.00×10^{-14} mol L^{-1}.

2.2. Equipment

The stochastic measurements were performed using an EmStat Pico mini potentiostat (PalmSens BV, Houten, The Netherlands) controlled by a laptop (PStouch mobile v 2.7 operation system).

2.3. Preparation of the electrochemical sensor

The textile material was coated with a combined diamond like carbon (DLC) - Ag nanolayer using the Thermoionic Vacuum Arc (TVA) plasma in vacuum, using procedure and parameters described earlier [15,16]. On the active side of the 2D sensor there were dropped 10µL of 10^{-3}mol L^{-1} solution of ß-CD, to obtain the 2D disposable textile sensor (Scheme 1).



Scheme 1. Design of the combined disposable 2D electrochemical sensor.

2.4. Procedure

Differential pulse voltammetry (DPV) was used for the assay of patulin. The scanning range was between -1V and +1V, with a scan rate of 80 mV s^{-1}. The $E_{1/2}$ was 451 mV. Linear regression method was used to determine the parameters of the equation of calibration. The value of the intensity of current obtained after the measurement of the apple juice was introduced into the equation of calibration to obtain the concentration of patulin.

2.5. Samples

Apple juice was bought from the supermarket, and it was used without any preparation for the assay of patulin.

3. Results and discussion

3.1. Response characteristics of the disposable 2D combined electrochemical sensor used for the assay of patulin

The response characteristics were determined using the DPV, at 25°C. A working concentration range between 0.01 pmol L^{-1} and 0.1 nmol L^{-1}, with a limit of determination of 0.01 pmol L^{-1} and a limit of detection of 2.02 amol L^{-1} were obtained for the assay of patulin. Equation obtained for the calibration of the 2D combined electrochemical sensor (Figs. 1 and 2) is:

$$I = 1 \times 10^{-6}(\pm 6.18 \times 10^{-7}) + 493.90\ (\pm 3.06) C_{Patulin} \quad (1)$$

where I is the intensity of the current (<I> = μA) and $C_{Patulin}$ is the concentration of patulin (<$C_{Patulin}$> = mol L^{-1}).

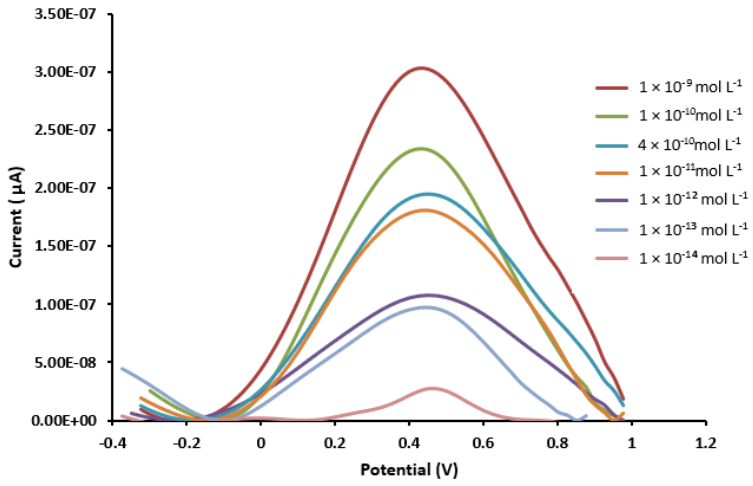


Fig. 1. Differential pulse voltammogram obtained for the assay of patulin at different concentrations.

The correlation coefficient was 0.9976. The determination coefficient regarding the equation obtained from the calibration curve is R^2=0.9953. For the relative standard deviation (RSD) values, calculations were made for the slope and intercept from the equation of calibration. The result obtained were RSD of the slope 3.06% and RSD of the intercept 6.18×10^{-7}%.

The working concentration range made possible the assay of patulin from low to higher concentrations in the apple juice.

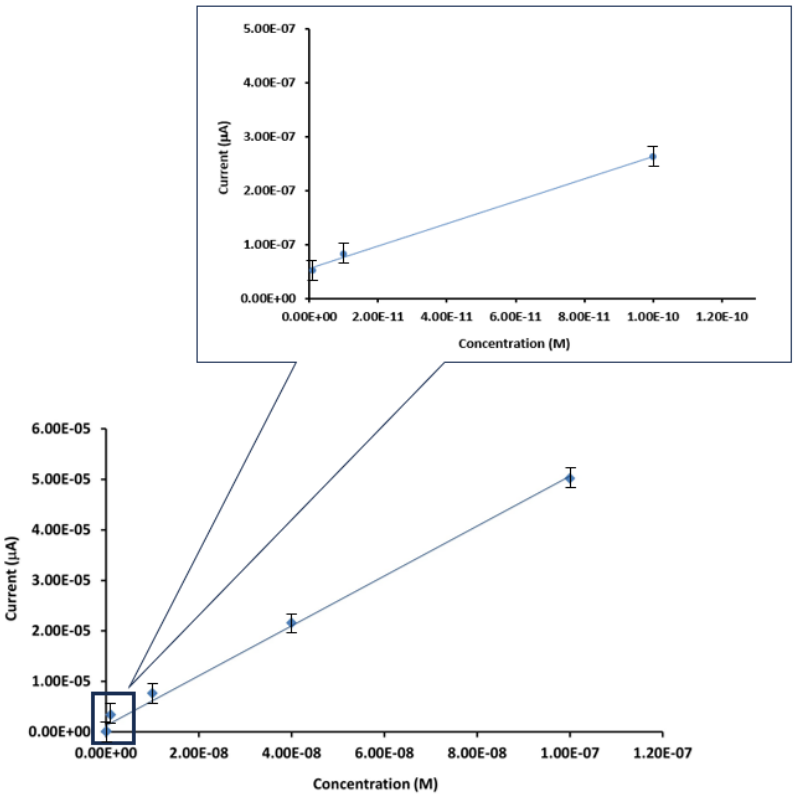


Fig. 2. Calibration of the 2D disposable combined electrochemical sensor used for the assay of patulin. Inset: Calibration curve obtained from 1.0×10^{-14} mol L^{-1} to 1.0×10^{-12} mol L^{-1}.

3.2. The selectivity of the 2D disposable combined electrochemical sensor.

Aflatoxin B1, aflatoxin M1, and ochratoxin A were considered possible interference. Mixed solution method was used; the same solution contains both patulin and the supposed interferent, in a ratio of 1:10 (mol/mol) between patulin and the possible interferent. The values recorded for the amperometric selectivity coefficients are: 2.6×10^{-4} for aflatoxin B1, 3.1×10^{-4} for aflatoxin M1, and 3.0 ×

10^{-4} for ochratoxin A. As all these values are lower than 1×10^{-3}, the aflatoxin B1, aflatoxin M1, and ochratoxin A did not interfere in the assay of patulin. Also, it can be seen from Fig. 3 that aflatoxin B1, aflatoxin M1, and ochratoxin A have different peak potentials from patulin, therefore reinforcing the idea that they didn't interfere with the patulin assay.

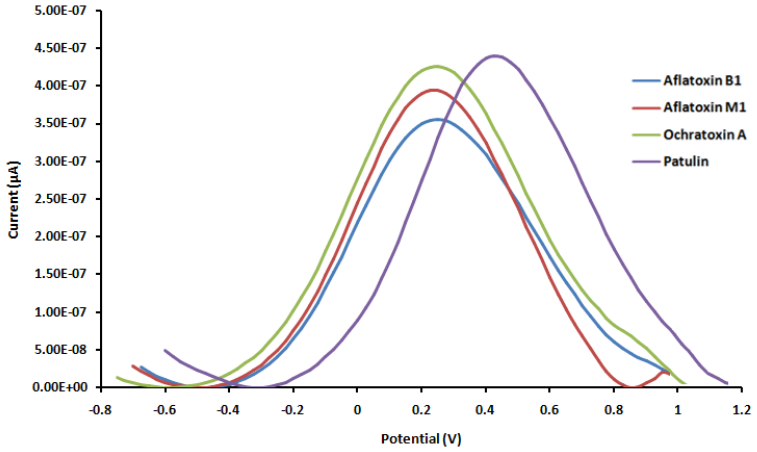


Fig. 3. Differential pulse voltammograms obtained for the selectivity of patulin

3.3. Electroanalysis of patulin in apple juice

Apple juice was used for the assay of patulin directly from the bottle. Fig. 4 shown one of the voltammogram obtained when patulin was determined in the apple juice.

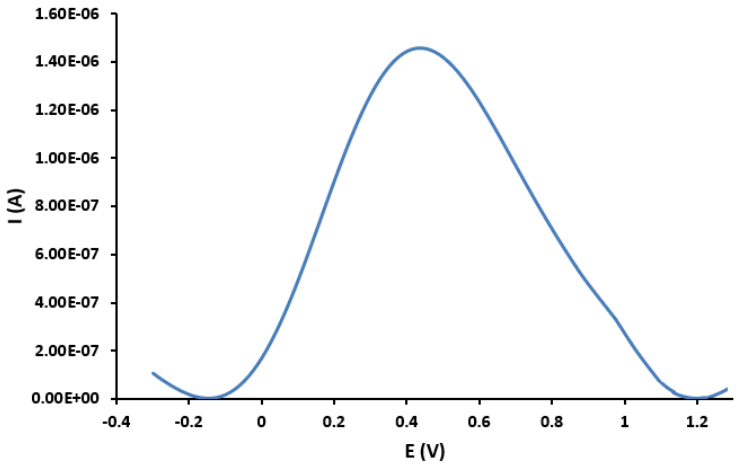


Fig. 4. Voltammogram obtained after the assay of apple juice.

To validate the proposed sensor and the screening method of apple juice, different known amounts of patulin were added to the apple juice. The concentration of patulin was determined before and after the addition of the patulin in the apple juice. The average amount of patulin recovered was 93.45%, with a relative standard deviation of 1.30%, proving a good accuracy and reliability of the proposed sensor and screening method.

Mycotoxins represent a significant concern in food safety owing to their poisonous characteristics and widespread occurrence. They may induce sickness and mortality in people and animals, even at minimal quantities. The identification and surveillance of mycotoxins are essential for safeguarding the safety and integrity of the food supply chain, hence preserving public health.

There are a number of different analytical methods that are currently being utilized for the purpose of detecting patulin. These methods include instrumental techniques such as liquid chromatography-tandem mass spectrometry (LC-MS), gas chromatography-tandem mass spectrometry (GC–MS), high-performance liquid chromatography (HPLC), chemical analysis techniques such as thin-layer chromatography, and immunoassay approaches such as enzyme-linked immunosorbent assay (ELISA).

These technologies are not consistently practical for on-site testing in fields or storage facilities, where prompt decision-making is crucial to avert the spread of contamination. Given these barriers, there is an increasing interest in creating portable, quick, and economical technologies for on-site mycotoxin testing.

Electrochemical sensors are presently used and undergoing ongoing development for diverse applications in food safety. Portable electrochemical sensors provide a viable alternative for the fast, on-site identification of mycotoxins. Recent advancements in analytical technology have included the use of biosensors and nanomaterials for the purpose of enhancing the sensitivity of patulin detection in real-time applications and facilitating its detection more quickly. These developing approaches have the goal of simplifying the testing process and improving accuracy.

These advances minimize the interval between detection and response, facilitating more effective pollution control. With technological advancements, these sensors are anticipated to become more essential to food safety management, greatly enhancing the protection of our food supply and health.

4. Conclusions

The proposed disposable 2D combined electrochemical sensor can be reliable used for the assay of patulin in apple juice. The wide working concentration range and the low limit of detection allow the assay of patulin in apple juice. The main feature of the sensor and screening method is their utilization for the assay of

the quality of apple juice on-site in the supermarket, or wherever is needed. The development of alternative technologies that are capable of detecting patulin in a timely manner while maintaining a high level of sensitivity is thus an important need. Sensor detection techniques have steadily evolved and gained broad attention from researchers working in the area of food safety detection. This is due to the progression of science and technology as well as the development of multidisciplinary integration.

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