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Very sensitive electrochemical sensor for moniliformin detection in maize samples

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ABSTRACT

A highly sensitive electrochemical sensor based on self-assembled monolayers (SAMs) of cysteamine on gold electrodes (Au-CA) in pH 4 citrate buffer solutions was developed to quantify the moniliformin (MON) mycotoxin by cyclic voltammetry in contaminated maize samples. Parameters of SAMs modified gold electrodes such as concentration of thiol, modification time and MON accumulation time were adjusted to obtain the best performance of the sensor. The calibration curve was linear in the MON concentration range from 1×10^{-9} to 1×10^{-7} mol L^{-1}. A limit of detection of 8.3×10^{-10} mol L^{-1} (0.1 ppb) was obtained. The standard addition method was used to minimize matrix effects. The concentration of MON determined with the electrochemical sensor was in very good agreement with results found by HPLC UV–vis. The sensor showed a good analytical performance with a relative standard deviation (RDS%) of about 3%.

1. Introduction

Moniliformin (3-hydroxy-3-cyclobutene-1,2-dione, MON, Fig. 1) is a mycotoxin produced by different species of *Fusarium*, mainly *Fusarium proliferatum* [1]. In the nature, MON occurs as the sodium or potassium salt, and presents a pKa less than 1, which makes it soluble in polar solvents [1].

The mechanism of toxicity of MON is not yet well-known, and its toxic-kinetics is still unknown. MON was found in cereals such as maize, rice, barley, wheat, oats, rye, and triticale [2], although the greatest contamination was found in maize samples [3]. The International Agency for Researcher on Cancer (IARC) classifies MON in Group 3, because the agent is not classifiable as carcinogenic to humans [4]. Several studies have confirmed the toxic effect produced by MON in animals [5,6] and its potential responsibility in the Keshan disease that occurred in China [7]. It has been proposed that MON inhibits the oxidation of intermediates in the tricarboxylic acid cycle [8,9], causing respiratory diseases, and myocardial degeneration in animals, and leading, in some cases, to death. It is also proposed that MON competes with pyruvate for active sites of α-ketoglutarate dehydrogenase, pyruvate decarboxylase, and aceto-hydroxy acid synthetase enzymes [10]. Thus, MON was classified into a group of emerging mycotoxins, highlighting its potential toxic effects on both animals and humans [11]. Until now, there are no regulations with respect to the maximum levels of MON allowed in food or grains, or even an official technique for its quantification [2,11]. Thus, the development of relatively fast analytical methods to determine MON in contaminated samples is a major challenge, where sensitivity, selectivity, precision, and accuracy are required. However, several methods have been developed to quantify MON based mostly on chromatography techniques, especially the high performance liquid chromatography (HPLC). All these techniques require a pre-treatment of the samples to achieve a good performance. Often, derivatization reactions or ion-pair agents are necessary to obtain products that can be detected by HPLC UV–vis [12,13], HPLC fluorescence [14], TLC–UV [15], and LC–MS/MS [16]. The technique of capillary zone electrophoresis with diode array detection (CZE–DAD) uses a similar pre-treatment to that of HPLC [17]. The limits of detection (LOD) of these techniques are higher than of 40 ppb. Gilbert et al. [18] have developed a methodology to detect MON by GC–MS and found a LOD of 0.5 ppb.

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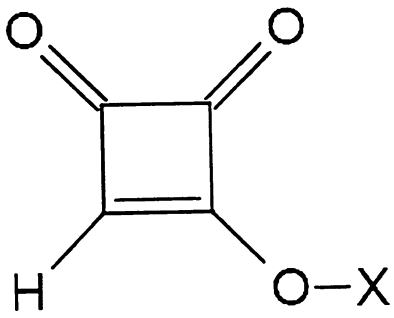

Fig. 1. Chemical structure of moniliformin. X = Na, K or H.

The major problem of this method is the derivatization step of MON with N-methyl-N-(tert-butyldimethylsilyl) trifluroacetamide containing 1% tert-butyldimethylchlorosilane. We have recently determined the thermodynamic and kinetics parameters of MON oxidation at glassy carbon electrodes in acetonitrile solution [19]. We have also proposed an electroanalytical methodology to detect MON in this medium, but the determination of MON in maize samples was not satisfactory.

The use of self-assembled monolayers (SAMs) to functionalize clean metallic surfaces (either obtained by soaking the surfaces in solutions or in vapor phase) has been widely used in the development of electroanalytical techniques to quantify of a large number of analytes [20]. These SAMs are characterized by their high degree of orientation and stability due to the thiol is covalently bonded to the corresponding metallic substrate, particularly, gold, platinum, and mercury [21]. In addition, different interaction forces are present among adsorbed molecules, such as Van der Waals forces, hydrogen bond, and π-interactions [21]. Organosulfur compounds can be aliphatic or aromatic, and have different chain length and variable tail functional groups. Tail groups can be chosen to achieve a better affinity and specificity towards a given analyte, and provide selectivity and sensitivity to the sensor. However, the final response depends on the alkane-thiol chain length and/or the nature of tail functional groups [21,22]. Short alkane-thiol SAMs form thin monolayers with high defect sites generating pinholes [23], where the electrocatalytic activity of different substrates has been observed [24]. The major advantages of SAMs with respect to other surface modification is the well-defined control over the composition, structure, thickness and orientation of the monolayer, which is given by the nature and the alkane-thiol chain length [25,26]. On the other hand, monolayers from organosulfur compounds spontaneously self-assembled onto clean metallic surfaces are easy and simple to form and require not special equipments [21].

Because of its low pK_a, MON is present in its deprotonated form in pH 4 buffer solutions, while cysteamine ($pK_a \cong 7.6$) adsorbed on the gold electrode is mainly protonated at pH 4 [27]. Thus, in principle, the electrostatic interaction between MON and the gold electrode modified with cysteamine SAMs can be used to pre-concentrate MON, and electro-catalyze its electrochemical oxidation.

In this work, we describe the development of a very sensitive electrochemical sensor to determine MON in maize samples, which is based on MON oxidation on cysteamine SAMs modified polycrystalline gold electrodes (Au-CA) in pH 4 citrate buffer solutions (CBS). The electrochemical technique used was cyclic voltammetry (CV). The results obtained by the electrochemical method were compared with those obtained by HPLC, indicating a very good performance of the proposed electrochemical sensor. As far as we know, this is the first electrochemical sensor reported for the detection of MON in real samples.

2. Materials and methods

2.1. Reagents

All reagents were of analytical grade. Sodium salt of MON, 2-aminoethanethiol (cysteamine, CA), 2-(diethylamino) ethanethiol hydrochloride (2-DAET), 8-amino-1-octanethiol hydrochloride (8-AOT), 4-amino-thiophenol (4-ATP) and 4-mercaptopyridine (4-MP) were purchased from Sigma-Aldrich (St. Louis, USA).CBS, ethanol, potassium dihydrogen phosphate (KH_2PO_4), tetrabutylammonium hydroxide (TBAOH), phosphoric acid, sulfuric acid and hydrogen peroxide were purchased from Merck p.a. (Darmstadt, Germany). Acetonitrile and methanol were HPLC grade (Sintorgan, Buenos Aires, Argentina). All reagents were used as received.

A MON stock solution (4.2×10^{-3} mol L^{-1}) was prepared in H_2O (Mili-Q) and stored at 4 °C. Working solutions were prepared daily by adding aliquots of the stock solution to CBS. The final concentration of MON was controlled by UV–vis spectroscopy (see below). Solutions of thiols were prepared daily in ethanol at different concentrations and stored in darkness.

The mobile phase used for HPLC experiments was an ion pair buffer (10 mL of ion pair solution and 50 mL of acetonitrile were diluted with water to 1 L). The ion pair solution was prepared by mixing 50 mL of 20% (w/w) TBAOH in water and 100 mL of 1.1 mol L^{-1} KH_2PO_4. The pH was adjusted to 7.0 by addition of phosphoric acid solution (Sigma-Aldrich, St. Louis, USA).

Both uncontaminated and contaminated with *Fusarium temperatum* RCFT914 strain [28] maize samples were provided by the Departamento de Microbiología e Inmunología, Facultad de Ciencias Exactas, Físico-Químicas y Naturales, Universidad Nacional de Río Cuarto.

2.2. Materials and apparatus

Cyclic voltammetry (CV) measurements were carried out in a conventional three-electrode cell. The working electrode was a polycrystalline gold disk (CH Instrument, Austin, USA) of 2 mm of diameter. Previous to perform the experiments, the gold electrode was successively polished on BAS cloth with wet alumina powder (0.3 and 0.05 μm from Fischer, respectively), washed with water and cleaned in a H_2SO_4 + 30% H_2O_2 (3:1 v/v) solution during 3 min. Then, it was placed in an ultrasonic bath for 5 min. Finally, it was cycled in 0.5 mol L^{-1} H_2SO_4 between 0.2 and 1.7 V until a typical voltammogram of a polycrystalline Au clean surface was obtained [29]. The electrochemical area of the gold electrode was calculated through the charge of the cathodic peak for the reduction of their oxides assuming a charge density of 420 μC cm^{-2}, which corresponds to the reduction of one monolayer of gold oxide for polycrystalline gold [30]. A roughness factor of 5.4 ± 0.7 was calculated from the ratio of electrochemical and geometric surface areas and it was kept constant in all experiments [27]. A similar value of roughness factor was also found by other authors, who used a similar extensive pre-treatment prior to the modification of electrodes [31,32]. An Ag/AgCl (BAS, West Lafayette, USA) electrode and a Pt wire of large area ($A \approx 2$ cm^2) were used as the reference and auxiliary electrodes, respectively. Cyclic voltammograms were performed with a Micro AutoLab PGSTAT 101 potentiostat (Eco-Chemie, Utrecht, The Netherlands) controlled by the NOVA 1.10.2 software. All measurements were carried out at 25.0 ± 0.2 °C.

UV–vis absorption spectra were recorded immediately after the preparation of MON solutions to determine their concentrations. A spectrophotometer Hewlett–Packard model 8452A was used. The UV–vis measurements were performed using silica cells of 1 cm path length. Absorption spectra of MON were recorded at different concentrations in CBS. MON absorption spectra showed two bands, with maxima at λ = 227 nm and at λ = 258 nm,