Talanta 83 (2010) 651–657

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Modified magnetic nanoparticles in an electrochemical method for the ochratoxin A determination in *Vitis vinifera* red grapes tissues

Martín A. Fernández-Baldo, Franco A. Bertolino, Germán A. Messina, Maria I. Sanz, Julio Raba*

INQUISAL, Departamento de Química, Universidad Nacional de San Luis, CONICET, Chacabuco 917, D5700BWS San Luis, Argentina

ARTICLE INFO

Article history:
Received 7 August 2010
Received in revised form 6 October 2010
Accepted 8 October 2010
Available online 27 October 2010

Keywords:
Modified magnetic nanoparticles
Electrochemical method
Ochratoxin A
Wine grapes
Square-wave voltammetry
ELISA

ABSTRACT

This work described the development and characterization of an electrochemical method using square wave voltammetry (SWV) combined with the use of modified magnetic nanoparticles (MNPs), which had shown a rapid and sensitive determination of ochratoxin A (OTA) in wine grapes (Cabernet Sauvignon, Malbec and Syrah) post-harvest tissues. The wine grapes were inoculated with *Aspergillus ochraceus* to obtain OTA in artificially infected samples. The OTA was directly determined using square-wave voltammetry. The current obtained is directly proportional to the concentration of OTA present in the samples. This method has been used for OTA determination in wine grapes and it was validated against a commercial ELISA test kit. The limits of detection calculated for electrochemical detection and the ELISA were 0.02 and 1.9 $\mu g\,kg^{-1}$, respectively and the coefficients of variation for accuracy and precision dates were below 5.5%. This method promises to be suitable for the detection and quantification of OTA in apparently healthy fruits post-harvest for assuring safety and quality of food as well as consumer's health.

1. Introduction

Ochratoxins are cyclical pentaketides containing a portion of isocoumarin, usually bound to L-phenylalanine for a carboxyl group, through an amide bond. There are five types of ochratoxins, however, the most toxic is the ochratoxin A (OTA). In its structure of 7-carboxyl-5-chloro-8-hydroxyl-3,4-dihydro-3R-methylisocoumarin-7-L-β-phenylalanine (Fig. 1), the portion of isocoumarin is chlorinated and linked to an L-phenylalanine [1,2]. OTA is a naturally occurring mycotoxin produced by several species of *Aspergillus* (e.g. *A. ochraceus*) and *Penicillium* (e.g. *P. verrucosum*) growing in different agricultural commodities in the field or during storage [3]. This mycotoxin has been increasing concern worldwide because of the hazard it poses to human health. Also it exhibits an unusual toxicokinetics with a half-life in blood of 35 days after oral ingestion [4]. The International Agency of Research on Cancer (IARC) has classified OTA as a possible carcinogenic compound (Group 2B, possibly by induction of oxidative DNA damage) for humans (World Health Organization, 1996) since it causes immuno suppression and immuno toxicity [5]. OTA has been found mainly in food derived from plants, such as cereals [6–8], coffee [9], nuts [10], spices [11], and wine [12,13]. It has also been reported to occur in fruits (apples, oranges, plums, grapes and dried fruits) and fruit juices (apple juice, grape juice, and orange juice) [14,15]. Due to these findings many countries have established limits on OTA levels in food, typically between 1 and 10 $\mu g\,kg^{-1}$, depending on the type and quality of the foodstuffs [16]. This is an important parameter that justifies the development of an inexpensive analytical method for a fast and reliable determination of OTA at these concentrations.

Many detection techniques have been used for the determination of OTA in different kind of samples [17]. Traditional methods for OTA analysis are usually performed by thin layer chromatography (TLC), high performance liquid chromatography (HPLC), and gas chromatography (GC), coupled to UV/V, fluorescence (FL) or mass spectrometry (MS) [18–20]. Immunoaffinity columns (IACs) coupled to traditional methods have been widely used as a cleanup tool and their use is highly recommended, allowing the isolation of the analyte from most matrix interferences, due to its specificity, and analyte preconcentration, necessary when low limits of detection are required [21]. However, these techniques require expensive and sophisticated equipment as well as complicated and time-consuming solvent cleanup steps. Enzyme linked immune sorbent assays (ELISAs) have also been proposed for the quantification of OTA [22,6]. Several ELISA strategies were recently investigated for the development of OTA electrochemical immunosensors based on different OTA immobilization procedures [23,24]. Therefore, the ELISA as alternative method for the detection and quantification of OTA, unfortunately, requires a tedious assay time. Nowadays, the electroanalytical methods shows important advantages compared to traditional methods currently in use because no separation procedure is required prior to substrate determination. Furthermore, the required instrumentation is typ-

* Corresponding author. Tel.: +54 2652 42 5385; fax: +54 2652 43 0224.
E-mail address: jraba@unsl.edu.ar (J. Raba).

0039-9140/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.talanta.2010.10.018

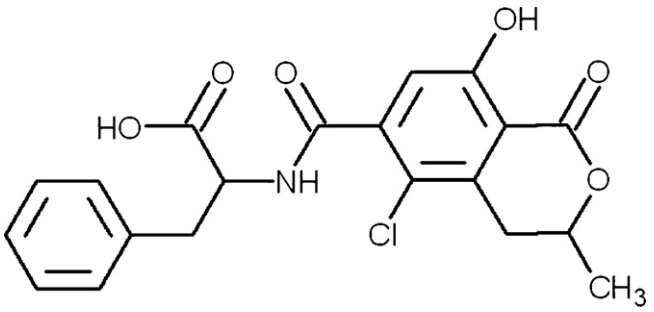


Fig. 1. Chemical structure of ochratoxin A.

ically less expensive, smaller amounts of solvent are necessary and the analysis time is shorter than chromatographic measurements. Among the electroanalytical methods, Osteryoung square wave voltammetry (OSWV), pioneered by Baker and Jenkins [25] in the 1950s, has become a popular technique for analytical work due to its speed and sensitivity [26]. However, the detection limits obtained by this technique are not adequate for OTA determination.

In recent years, with the rapid development of the nanostructured materials, the magnetic nanoparticles (MNPs) have received considerable attention for their novel properties [27,28]. MNPs are a class of nanoparticles (i.e., engineered particulate materials of <100 nm) that can be manipulated under the influence of an external magnetic field. MNPs are commonly composed of magnetic elements, such as iron, nickel, cobalt and their oxides. Up to date, the application of MNPs have stretched to various areas including magnetic recording, magnetic fluids [29], immobilization of proteins [30], detection of DNA hybridization [31], drug delivery [28], etc. Recently, MNPs have also been studied by electrochemistry [32]. The excellent physical properties of the nanoparticles, such as high surface/volume ratio, unique absorption spectrum, and electric conductivity, present itself a great beneficial and suitable for the applications in immobilization. The use of MNPs as versatile and efficient substrates for the immobilization of antibody or antigen have showed not only an enhance the of the amount of antibodies or antigens immobilized on its surface, but also preserve the activity of the immobilized biomolecules [33,34].

The ability of OTA to persist through the food chain and the observation of both acute and chronic effects in animals and humans have made evident the need to develop reliable, easy, cost-effective and fast analytical methods for its monitoring and control.

In our knowledge, there is not a assay which involves the growth of *Aspergillus ochraceus* and its OTA production in wine grapes (Cabernet Sauvignon, Malbec and Syrah) with modified MNPs and OSWV using glassy carbon as working electrode for OTA determination. Thus, in this report, we present and discuss a simple, accurate, sensitive and selective method for OTA determination in wine grapes. In this method OTA is captured by modified MNPs with antibody anti-OTA. Then the bound OTA was desorbed with an acidic media and it was directly determined using OSWV. The results obtained have very promising analytical applications for direct determination of OTA at trace levels.

2. Materials and methods

2.1. Reagents and solutions

All reagents used were of analytical or biochemical grade. OTA was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and used as received. The mouse monoclonal antibody 3C5 to Ochratoxin A (ab23965) was obtained from Abcam Inc. (Cambridge, England) and used as received. Glutaraldehyde (25% aqueous solution) and $HClO_4$ were purchased from Merck (Darmstadt, Germany) and used without further purification. The MNPs amino functionalized (55 mg mL^{-1}) were purchased from Fluka (Buchs/Schweiz, USA). Methanol (MeOH) 99,98% was purchased by Biopack, Bs As, Argentina. All other reagents employed were of analytical grade and were used without further purifications. All solutions were prepared with ultra-high-quality water obtained from a Barnstead Easy pure RF compact ultra-pure water system.

The AgraQuant® for OTA ELISA kit for the quantitative determination of OTA was purchased from Romer Labs®, Misiones, Argentina and was used in accordance with the manufacturer's instructions [35].

2.2. Apparatus and experimental measurements

Electrochemical experiments were performed in unstirred solutions using a BAS 100B/W electrochemical analyzer (Bioanalytical System, West Lafayette, IN), using positive feedback routine to compensate the ohmic resistance. Cyclic and square wave voltammograms were obtained using a three electrodes system consisted of a glassy carbon (GCE) working electrode model BAS MF-2012, 3.0 mm diameter and 0.071 cm^2 geometrical area. It was polished with wet alumina powder, copiously rinsed with bidistilled water and sonicated in a water bath for 2 min. Then, it was electrochemically activated in aqueous 1 mol L^{-1} KOH by applying a potential of 1.2 V over 5 min, according to the methodology described by Anjo et al. [36]. Finally, it was transferred to a blank solution (aqueous 0.2 mol L^{-1} $HClO_4$) and cycled 10 times between 0 and 1.4 V. When this pre-treatment was applied to the working electrode, the peak currents and peak potentials obtained in the voltammograms were highly reproducible. The reference electrode was an Ag|AgCl|3 mol L^{-1} NaCl BAS MF-2052 and the counter electrode was a large area Pt wire. Before each new solution was studied, the working electrode was carefully polished, PK-4 polishing Kits BAS MF-2060, and rinsed following the general guideline recommended for BAS. To ensure reproducible measurement conditions the samples were deareated by bubbling nitrogen through the solutions for at least 10 min prior to measurement. The temperature for electrochemical experiments was 25 ± 1 °C.

All pH measurements were made with an Orion Expandable Ion Analyzer (Orion Research Inc., Cambridge, MA, USA) Model EA 940 equipped with a glass combination electrode (Orion Research Inc.).

Absorbance was determined with a Bio-Rad Benchmark microplate reader (Japan) and Beckman DU 520 General UV/vis spectrophotometer.

2.3. Organism and culture conditions

A. ochraceus (NRRL) 3174 was used in this study. The fungus was maintained on potato dextrose agar (PDA) at 4 °C. For conidial production, *A. ochraceus* was grown on PDA at 25 ± 2 °C for 7 days. After a week, spores were harvested and suspended in 10 mL of sterile the 0.01 mol L^{-1} phosphate buffer saline (PBS) pH 7.2 containing 0.005% (v/v) Tween 80. The concentration of spore suspension was determined with a Neubauer chamber and adjusted with PBS to 1×10^5 spores mL^{-1}.

2.4. Growth and OTA-production ability in wine grapes

Quality wine grapes (Cabernet Sauvignon, Malbec and Syrah), without any commercial postharvest treatment, were purchased from a local fruit market in Mendoza City, Argentina. Fruits selected were free of wounds and rots and as much as possible homogeneous in maturity and size. The wine grapes were used after a short storage period at 4 °C (no longer than 1 week). Before each experiment the wine grapes were dipped in a NaClO 0.5% solution

for 1 min, rinsed with sterile distilled water, allowed to air dry at room temperature and randomized. The fruits peel was wounded with a sterile steel scalpel by making injuries 1 mm deep by 1 mm wide by 1 mm long. Two wounds were made at the top and bottom and two on the circumference of each fruit. 20 μL of the above spore suspension of *A. ochraceus* (1×10^5 spores mL^{-1}) was applied to each wound. Three wine grapes constituted a negative control. The wine grapes samples (Cabernet Sauvignon, Malbec and Syrah) were incubated at 28 ± 2 °C. After 7 days, for a maximum of 20 days, four pieces (20 g) of tissues containing the wounds were removed with a sterile cork borer (10 mm diameter) from each fruit to determine OTA production. Each experiment was repeated three times.

2.5. Sample preparation and extraction

The samples were extracted according to AgraQuant® for OTA ELISA Kit [35]. The mycotoxin production on the wounds was investigated by using the 20 g of peel surface segments removed from each wine grape. OTA was extracted with a mixture 150 mL of 70% MeOH (v/v) and were shacked vigorously for 3 min. Finally the extract was filtered through a Whatman #1 filter paper and 100 mL of the filtrate were collected and used as sample.

2.6. ELISA for determination of OTA

An OTA standard was supplied with the AgraQuant® for OTA ELISA Kit. OTA in samples was determined in duplicate using this commercial ELISA Kit [35]. A standard curve for the spectrophotometric procedure was produced by following the manufacturer's protocol with a range of detection of 0–40 $\mu g\,kg^{-1}$. Concentrations of OTA were detected spectrophotometrically by measuring absorbance changes at 450 nm.

2.7. Immobilization of mouse monoclonal anti-OTA on MNPs amino functionalized

Mouse monoclonal anti-OTA antibody was immobilized on the MNPs amino functionalized in an Eppendorf tube. 100 μL of MNPs amino functionalized were washed with 1.0 mL of PBS pH 7.2 for three times. The pellet was suspended in 1.0 mL of an aqueous solution of 5% (w/w) glutaraldehyde at pH 10.00 (0.20 mol L^{-1} sodium carbonate buffer, pH 10) with continuous mixing for 2 h at room temperature. After three washes with PBS pH 7.2 to remove the excess of glutaraldehyde, 250 μL of antibody preparation (dilution 1:2000 in 0.01 mol L^{-1} PBS, pH 7.2) was coupled to the residual aldehyde groups with continuous mixing for 12 h at 5 °C. The immobilized antibodies preparation was finally washed with PBS pH 7.2 and resuspended in 250 μL of the same buffer at 5 °C. Immobilized antibody preparations were perfectly stable for at least 1 month.

2.8. Square wave voltammetry parameters

In order to establish the optimum conditions for the determinations of OTA by means of OSWV technique, various instrumental variables were studied and the optimum conditions were: step $E = 0.010$ V, S.W. amplitude = 0.050 V, S.W. frequency = 10 Hz, samples per point = 256, studied potential range = 0.7–1.4 V, sensitivity = 1×10^{-5} A V^{-1}.

2.9. Procedure

This method was applied to the determination of OTA in 28 commercials wine grapes (Cabernet Sauvignon, Malbec and Syrah) samples. Initially, the immobilized antibodies preparations on MNPs were conditioned with 1 mL of desorption buffer (0.1 mol L^{-1} citrate buffer pH 2.00), mixing at room temperature for 5 min. After that, they were rinsed with 0.01 mol L^{-1} PBS (pH 7.2) and the nonspecific binding of the immobilized antibodies preparations on MNPs was blocked by 10 min treatment at 37 °C with 3% skim milk in a 0.01 mol L^{-1} PBS (pH 7.2); finally washed three times with 0.01 mol L^{-1} PBS (pH 7.2) and stored in 250 μL of the same buffer.

100 mL of the filtered extract obtained in Section 2.5 containing 0.5–50 $\mu g\,kg^{-1}$ of OTA was put into a 200 mL beaker. Then 30 μL (260 μg) of modified MNPs were added to the solution and they were incubated for 10 min at room temperature in shaking condition. In this step, the OTA present in the samples, reacted immunologically with the anti-OTA antibodies immobilized on MNPs [37,38]. Then the modified MNPs were separated by an external removable magnet and washed three times with 0.01 mol L^{-1} PBS (pH 7.2) to remove the remaining solution, after they were quantitatively transferred into a small volume electrochemical cell using 500 μL of desorption solution (total volume of solution in the electrochemical cell) of 0.2 mol L^{-1} perchloric acid ($HClO_4$). After 1 min, the OTA present in the solution was determined using OSWV. Therefore, the current response was directly proportional to the amount of the OTA present in the samples. The blank solution was prepared in the same way except that 100 mL of bidistilled water was used instead of the sample solution. The difference between the current of the blank and sample solution was then calculated.

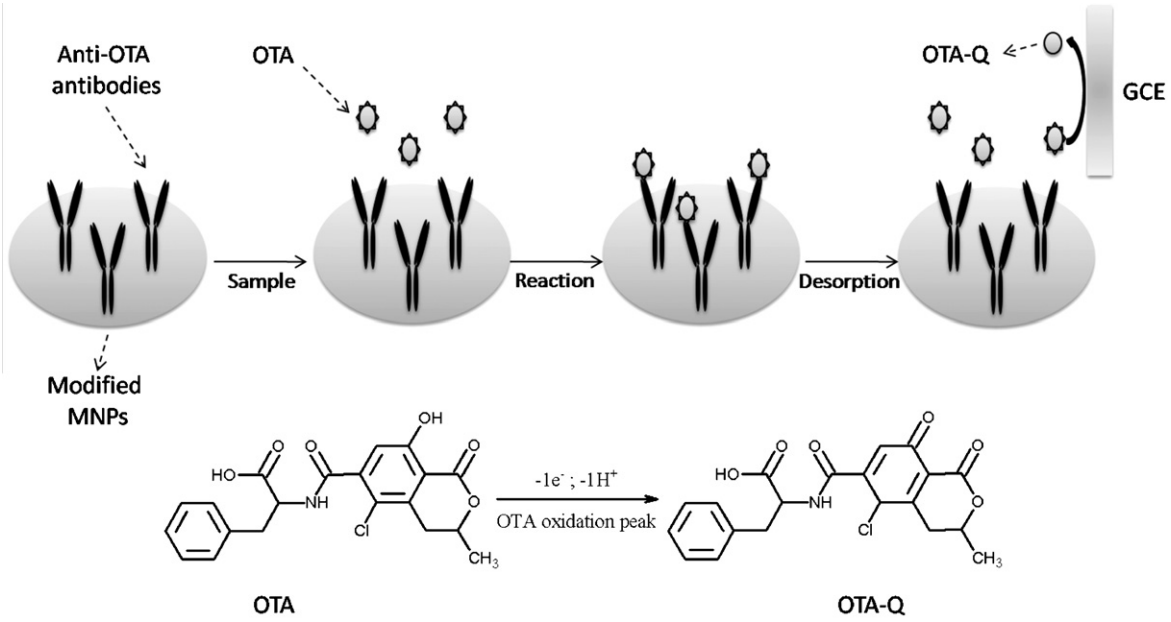
3. Results and discussion

The MNPs have shown to be a powerful and versatile tool in a variety of analytical and biotechnology applications [39–41]. The use of these particles greatly improves the performance of the immunological reaction, due to an increase in the surface area, as well as the faster assay kinetics achieved because the beads are in suspension. One of the most effective technology platforms is the usage of MNPs as substrates for immobilizing bioactive materials, which can be separated by an external magnetic field. Some recent reports demonstrated the successful applications of MNPs in the immobilization of biomolecules. The MNPs modified with amine groups on the surface, provided a plenty anchor sites for covalently binding of antibodies via glutaraldehyde. Therefore, the antibodies for OTA were attached on the surface of MNPs, forming magnetic bionanoparticles via of glutaraldehyde.

3.1. Electrochemical behavior/oxidation

The capture of OTA on modified MNPs modified with antibody-anti-OTA was an efficient method for the preconcentration of very low levels of OTA in real samples. The OTA bound to the nanoparticles could be desorbed and directly measured in an acidic solution by the following reaction (Scheme 1).

The progress of this reaction was monitored measuring the oxidation de OTA, using GCE as working electrode. The increase in the sensitivity depends on the electroanalytical technique to utilize. The advantages of square wave voltammetry (SWV) are the great speed of analysis, the low consumption of the electroactive species in relation with differential pulse voltammetry (DPV), and the reduction of the problems with the poisoning of the electrode surface. A greater advantage of SWV is the possibility to see during only one scan if the electron transfer reaction is reversible or not. Since the current is sampled in both positive and negative-going pulses, the peaks corresponding to the oxidation and reduction of the electroactive species at the electrode surface can be obtained in the same experiment [42]. Also, SWV experiments show a higher



Scheme 1. Schematic representation of the immunological reaction.

sensitivity than DPV since much faster scan rates can be used (100 mV s^{-1} for SWV compared to 5 mV s^{-1} for DPV). Taking into account these facts, the analytical determination of OTA was carried out using SWV by direct measurement of its oxidation peak. The electrochemical behavior of OTA was studied.

A typical cyclic voltammogram of 0.5 mmol L^{-1} OTA in 0.2 mol L^{-1} $HClO_4$ solution as supporting electrolyte (scan rate: 50 mV s^{-1}; $T = 25 \pm 1$ °C) is shown in Fig. 2, and its behavior has been previously studied [43–45]. The cyclic voltammogram of OTA in 0.2 mol L^{-1} $HClO_4$ solution showed a single anodic peak at E_{pa} = +1.25 V for the potential range 0.7–1.4 V, which corresponded to the one-electron transfer of OTA. When the canning was in the negative direction, no reduction peak were observed, showing that the oxidation of OTA in this reaction media is an irreversible process, and also indicating clearly a complicated reaction mechanism with one or more chemical reactions coupled to the initial electron transfer [36,46]. In this irreversible process, there is no peak current ratio (anodic/cathodic), particularly during the repetitive recycling of potential; therefore, it can be considered as criteria for the irreversibility of OTA produced at the surface of electrode under the experimental conditions. Under these observations, we could proposed than measuring the direct electrochemical oxidation of OTA (proposed method) appears to be more sensitive and faster than the measuring the indirect electrochemical oxidation of OTA (reported methods) for its determination, using OSWV as electroanalytical technique. A mechanism for the direct electrochemical oxidation of OTA in pH 2.0 0.2 mol L^{-1} $HClO_4$ was proposed in Scheme 1.

By the other hand, oxidation current was decreasing with a successive scans and it can be explained taking into account that the adsorption of both OTA and OTA oxidation products, at the GCE surface, leads to the blocking of the electrode surface (Fig. 3), because the adsorption of OTA and/or its oxidation product is very strong in acid electrolytes [44].

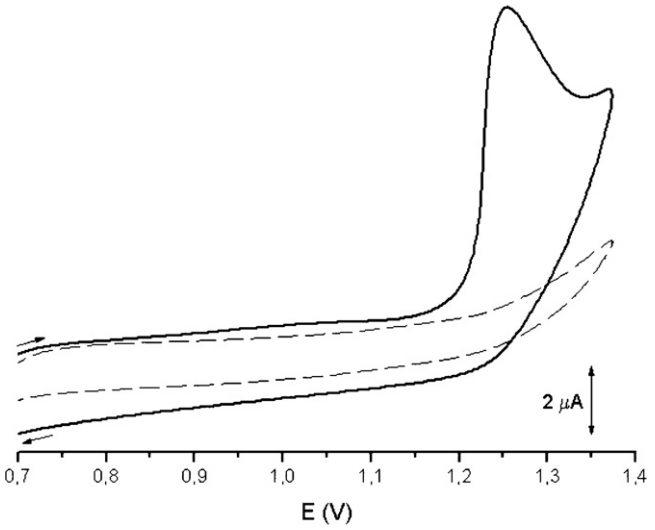


Fig. 2. Cyclic voltammogram of 0.5 mmol L^{-1} OTA recorded in 0.2 mol L^{-1} perchloric acid solution (scan rate: 50 mV s^{-1}; $T = 25 \pm 1$ °C), the working electrode was a GC disk, and the reference electrode was an Ag|AgCl|3 mol L^{-1} NaCl.

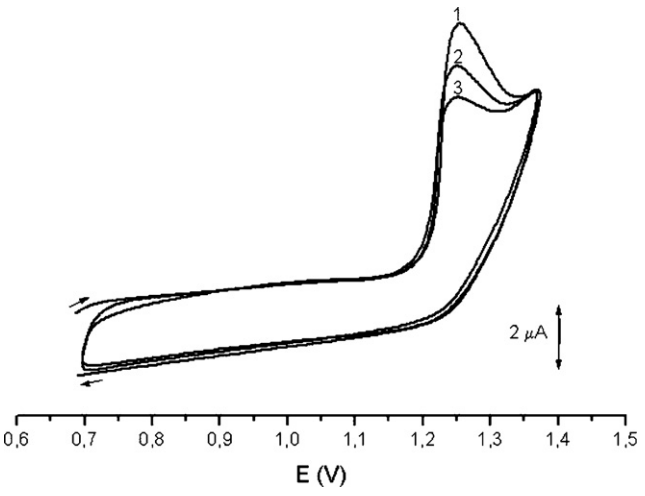


Fig. 3. Consecutive cyclic voltammograms obtained in 0.5 mmol L^{-1} OTA in 0.2 mol L^{-1} perchloric acid solution: (1) first, (2) third and (3) tenth potential scan (scan rate: 50 mV s^{-1}; $T = 25 \pm 1$ °C), the working electrode was a GC disk, and the reference electrode was a Ag|AgCl|3 mol L^{-1} NaCl.

The CVs were also obtained for different scan rates in 0.1 mmol L^{-1} OTA in pH 2.0 0.2 mol L^{-1} $HClO_4$ (data not shown). When the scan rate was increased, the anodic peak current increases too, following a linear relationship with scan rate. It could be explained taking into consideration the adsorption of OTA molecules and/or their oxidation products at the GCE surface. Such a kind of behavior was already described for electrochemical oxidation of OTA at a GCE [44].

On the other hand, the difference between the change of the current for sample reaction (the reaction in the presence of OTA) and blank reaction (the reaction in the absence of OTA) was a linear relationship with the amount of OTA in a defined concentration range. Therefore, this direct electrochemical method was suitable for the determination of OTA in wine grapes after desorption of the modified MNPs.

3.2. Study of variables

In order to optimize the working conditions for the desorption of OTA of the MNPs, its signal was directly monitored by OSWV using GCE as working electrode while pH of the solution was changed. The working pH values were in the 1.0–5.0 range at the 10 μg kg^{-1} OTA level (data not shown). Based on these results, the pH selected was 2.0 (0.06 mL of concentrated $HClO_4$ (72%) in 100 mL of solution containing OTA).

As mentioned before, the OTA bound to the nanoparticles were desorbed in acidic solution. The concentration of acid influenced in desorption of OTA. In order to study the effect of the $HClO_4$ concentration, a known amount of OTA, bound to the modified MNPs, was removed from the MNPs using a 0.5 mL solution of 0.2 mol L^{-1} $HClO_4$. The $HClO_4$ concentration was changed in the range 0.05–0.25 mol L^{-1} (Fig. 4). The difference between the current of blank and sample (ΔI) increased rapidly between 0.10 and 0.18 mol L^{-1} of $HClO_4$ and it remained constant between 0.18 and 0.25 mol L^{-1}. Therefore, a concentration of 0.20 mol L^{-1} was selected as optimum.

The effect of the final volume for the electrochemical determination of OTA was studied using solution volumes of 0.25–2.5 mL containing 0.20 mol L^{-1} $HClO_4$ (data not shown). The results showed that the measured current decreased lineally with solution size up to 2.5 mL. Therefore, a volume of 0.5 mL was selected as optimum.

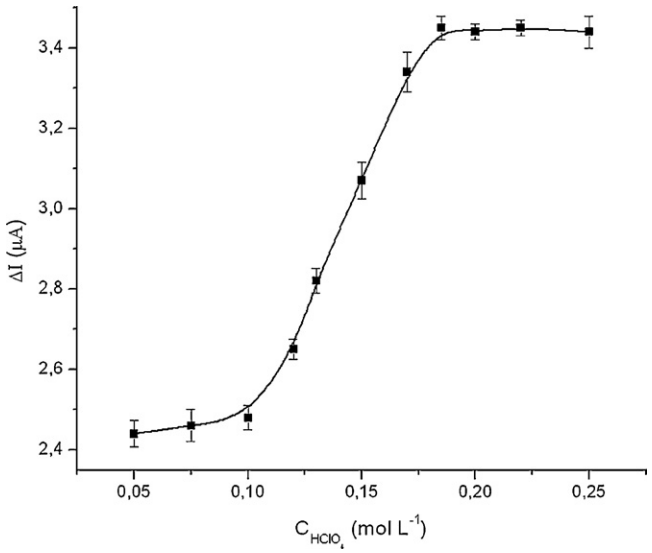


Fig. 4. Study of perchloric acid concentration on the desorption of OTA. C_{OTA}: 10 μg kg^{-1}; C_{MNPs}: 260 μg.

Table 1
Accuracy and precision dates for OTA obtained by the OSWV method.

Added (μg kg^{-1})	Found (μg kg^{-1})	Precision (μg kg^{-1})	Accuracy[b] (%)
OTA			
2.0	1.90	$X^a = 1.90 \pm 0.1$ CV = 5.26%	5.0
5.0	5.10	$X = 5.10 \pm 0.15$ CV = 2.94%	2.0
10.0	10.44	$X = 10.44 \pm 0.22$ CV = 2.11%	4.4
20.0	19.92	$X = 19.82 \pm 0.37$ CV = 1.87%	0.4

[a] X = mean ± S.D.
[b] Accuracy = [(found − added)/added] × 100.

The effect of the sample volume on the antigen antibody reaction was studied for sample volumes of 25–250 mL containing 10 μg kg^{-1} of OTA. The OTA was collected on 260 μg of modified nanoparticles. Variations in sample volume between 25 and 100 mL did not affect the reaction of OTA, but at higher volumes the reaction of OTA decreased. For the determination of lower concentrations of OTA, all experiments were carried out at 100 mL of sample solution (data not shown).

A 500 μL solution containing 10 μg kg^{-1} of OTA were analyzed by the proposed method using 20–400 μg of modified MNPs. The results are shown in Fig. 5. The current was increased in relation with the amount of modified nanoparticles till 220 μg and remained constant at higher values. Therefore, 260 μg modified nanoparticles was used for routine works.

3.3. Analytical parameters

A calibration curve for the proposed method was made analyzing eight different ($n = 6$) standard solution containing 0.03–100 μg kg^{-1} of OTA in 500 μL of solution.

The calibration curve was plotted using ΔI versus concentration of the standard solutions and it showed a linear range between 0.5 and 50 μg kg^{-1}. The data were analyzed by linear regression least-squares fit method. The calibration graph shows the following equation $\Delta I = 0.127 + 0.332 \times C_{OTA}$ with a correlation coefficient of 0.997. Where ΔI is the difference between current of the blank and sample expressed in μA and C_{OTA} is the concentration of OTA in the sample solution in μg kg^{-1}. The coefficient of variation (CV) for the determination of 10 μg kg^{-1} of OTA solution was below 3% (six replicates). The limit of detection (LOD) (considered as the concentration that gives a signal tree times the standard deviation SD of the blank) was 0.02 μg kg^{-1} with a limit of quantitation (LOQ) (considered as the concentration that gives a signal seven times the SD of the blank) of 0.05 μg kg^{-1}. In order to evaluate the analytical applicability of the proposed method, a recovery study was development. The results are given in Table 1. In this table, to evaluate the accuracy and precision of the method, a series of independent standard samples were used.

The electroanalytical method developed for the determination of OTA using the mouse monoclonal antibody 3C5 to Ochratoxin A (ab23965) is very important because it avoids interferences with many organic compounds such as glucose, fructose or phenolic, tannic, tartaric, malic and ascorbic acids, since all these substances are oxidized at the GCE. As a result of this essay, oxidation current was not observed in the same potential range that OTA (Table 2).

4. Application

In order to evaluate the analytical applicability of the proposed method, it was applied to the determination of OTA in wine grapes

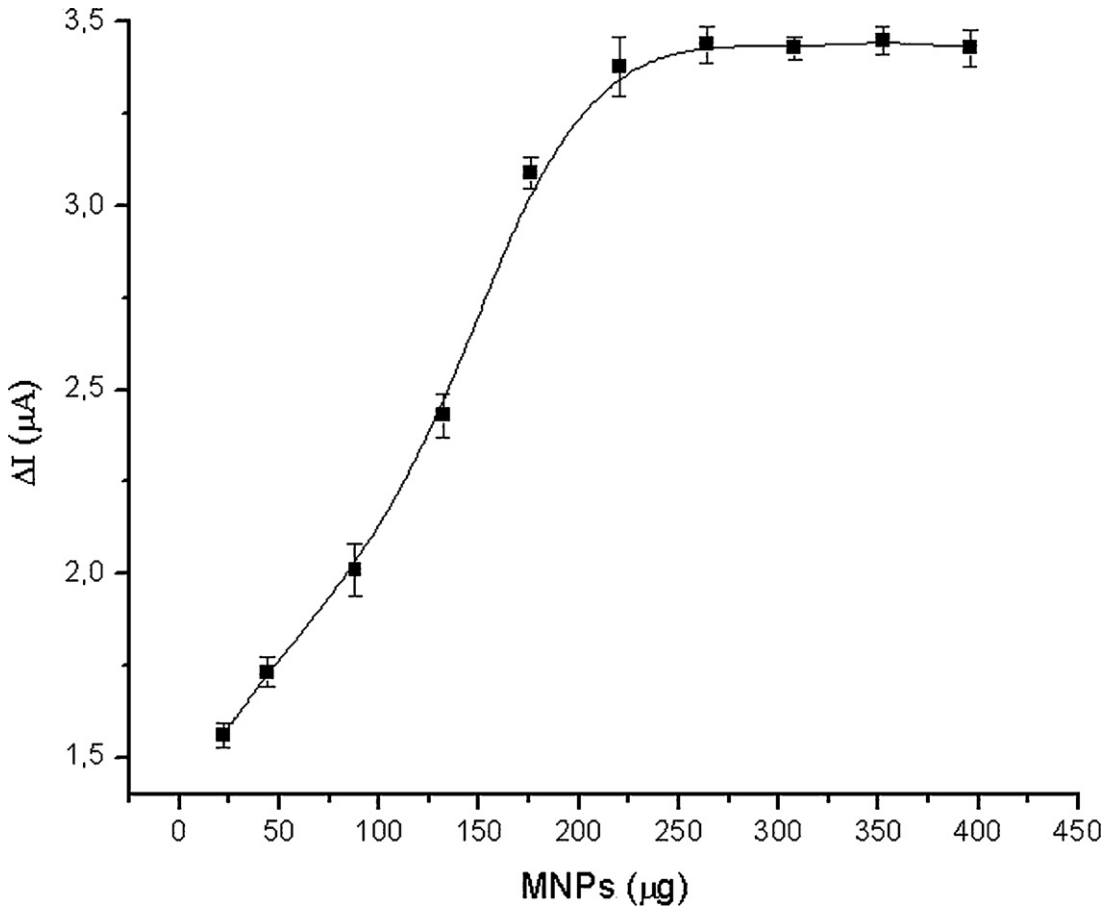


Fig. 5. Study of the amount of modified MNPs on the recovery of OTA. C_{OTA}: 10; $CHClO_4$: 0.2 mol L^{-1}.

samples and PBS doped samples. The wine grapes samples and PBS doped samples were processed following the procedure in Section 2.5 and their OTA contents were measured by the proposed method (Table 2). As this table shows, the results of the recovery of added OTA were satisfactory. On he other hand, it was clearly demonstrated that there were not matrix effects on the OTA bounded on anti-OTA antibody functionalized MNPs, performing analysis of both PBS and extract OTA-added solutions and reading the same OTA contents.

The electrochemical method was compared with a commercial spectrophotometric method [35] for the quantification of OTA in 28 commercials wine grapes tissues samples. The slope obtained was reasonably close to 1, indicating a good correspondence between the two methods (Fig. 6). Compared with the ELISA, our method shows large enhancement in sensitivity and its sensitivity is high enough to determine OTA in unknown samples with very low levels.

The proposed method was compared with the existing methods reported for solid phase extraction and determination of OTA. Table 3 indicates that the results obtained by the proposed method were similar or better than the results obtained by existing methods and provided a wider dynamic range.

Table 2
Analytical applicability of the proposed method. Determination of OTA in wine grapes and PBS doped samples.

Sample	OTA ($\mu g\,kg^{-1}$)	
	Added	Found[a]
Wine grapes (Mendoza, Argentina)	–	–
	2.0	2.03 ± 0.02
	5.0	4.98 ± 0.11
	10.0	9.92 ± 0.07
PBS doped sample	–	–
	2.0	2.04 ± 0.07
	5.0	5.09 ± 0.08
	10.0	9.97 ± 0.04

[a] Mean of six determinations ± S.D.

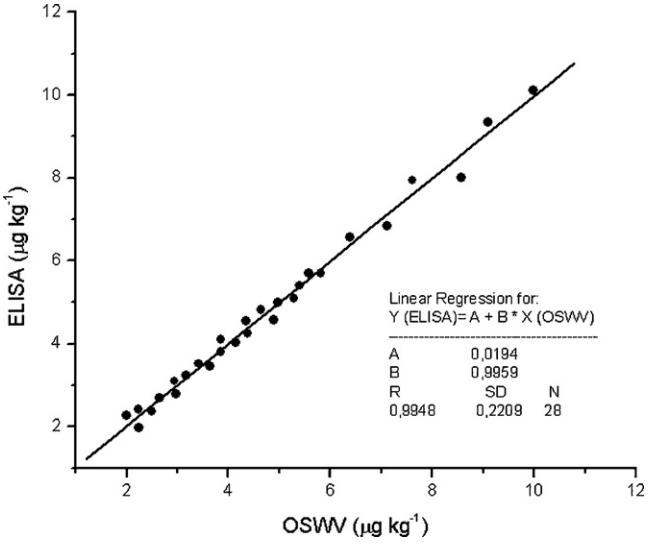


Fig. 6. Correlation between proposed method and spectrophotometric immunoassay.

Table 3
Comparison of the proposed method and other reported methods for the solid phase extraction and determination of OTA.

Samples	Solid-phase	Reported methods	Detection limit[a]	Reference
Wine grapes	Micro-titer plates	ELISA	1.9	[35]
Wine grapes	IAC, C18	HPLC-FLD	1.0	[48]
Table grapes	IAC, C18	HPLC-FLD	0.1	[49]
Table grapes	IAC, C18	Nano-RP-HPLC–ESI-MS	0.001	[50]
Wine grapes	Spherisorb ODS2	SPE-LC/FL	0.004	[51]
Wine grapes	C18 Luna	SPE-LC/FL	0.02	[52]
Wine grapes	MNPs	OSWV	0.02	Proposed method

[a] $\mu g\,kg^{-1}$.

5. Conclusions

This study showed that OTA, a fungal metabolite which presents carcinogenic, teratogenic and nephrotoxic properties, could be monitored using a GCE by OSWV. The development of a voltammetric technique combined with the use of MNPs was suitable for the determination of OTA in wine grapes samples after an specific antigen antibody reaction on modified MNPs. This method showed many advantages like the low cost, wide linear range, reproducibility, accuracy with excellent LOD. The developed procedure was successfully applied to three/several different red grape varieties.

The proposed method could be a very promising analytical tool for the direct determination of OTA in real samples and it could become important if the tolerance levels of OTA in fruits are established in a near future.

6. Safety

OTA is a toxic compound that needs to be manipulated with care and with the appropriate safety precautions. Decontamination procedures for laboratory wastes have been reported by the International Agency on Research on Cancer (IARC) [47] and were employed throughout this experimental work.

Acknowledgements

The authors wish to thank the financial support from the Universidad Nacional de San Luis, the Agencia Nacional de Promoción Científica y Tecnológica, and the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

The authors thank Dr. María de la Luz Zapata and Dr. Juan Carlos Basílico (Universidad Nacional del Litoral) for providing the *A. ochraceus* (NRRL 3174) employed in the present study.

References

[1] P.S. Steyn, in: V. Bettina (Ed.), Mycotoxins: Production, Isolation, Separation and Purification, Elsevier, New York, 1984, p. 183.
[2] European Food Safety Authority (EFSA), EFSA J. 365 (2006) 1.
[3] P. Krogh, Mycotoxins in Food, Academic Press Ltd., London, 1987.
[4] C. Schlatter, J. Studer-Rohr, T. Rásonyi, Food Addit. Contam. 13 (1996) 43.
[5] T.K. Goodman, in: M. Miraglia, H. Van Edmond, C. Brera, J. Gilbert (Eds.), Mycotoxins and Phycotoxins—Developments in Chemistry, Toxicology and Food Safety, Alaken Inc., Fort Collins, USA, 1998, p. 25.
[6] S.H. Alarcón, G. Palleschi, D. Compagnone, M. Pascale, A. Visconti, I. Barna-Vetró, Talanta 69 (2006) 1031.
[7] Y. Sugita-Konishi, T. Tanaka, M. Nakajima, K. Fujita, H. Norizuki, N. Mochizuki, K. Takatori, Talanta 69 (2006) 650.
[8] C. Juan, C.M. Lino, A. Pena, J.C. Moltó, J. Mañes, I. Silveira, Talanta 73 (2007) 246.
[9] M.H. Taniwaki, J.I. Pitt, A.A. Teixeira, B.T. Iamanaka, Int. J. Food Microbiol. 82 (2003) 173.
[10] D.P. Overy, K.A. Seifert, M.E. Savard, J.C. Frisvad, Int. J. Food Microbiol. 88 (2003) 69.
[11] I.Y. Goryacheva, S. De Saeger, I.S. Nesterenko, S.A. Eremin, C. Van Peteghem, Talanta 72 (2007) 1230.
[12] N. Ratola, P. Barros, T. Simoes, A. Cerdeira, A. Venancio, A. Alves, Talanta 70 (2006) 720.
[13] R. Romero-González, A. Garrido Frenich, J.L. Martínez Vidal, M.M. Aguilera-Luiz, Talanta 82 (2010) 171.
[14] M.O. Moss, in: B.M. Lund, A.C. Baird-Parker, G.W. Gould, T.C. Baird-Parker (Eds.), The Microbiological Safety and Quality of Food, Springer, 1999, p. 1490.
[15] A. Marino, A. Nostro, C. Fiorentino, Int. J. Food Microbiol. 132 (2009) 185.
[16] N.W. Turner, E.V. Piletska, K. Karim, M. Whitcombe, M. Malecha, N. Magan, C. Baggiani, S.A. Piletsky, Biosens. Bioelectron. 20 (2004) 1060.
[17] N.W. Turner, S. Subrahmanyam, S.A. Piletsky, Anal. Chim. Acta 632 (2009) 168.
[18] S. Romani, G. Sacchetti, C.C. López, G.G. Pinnavaia, M.D. Rosa, J. Agric. Food Chem. 48 (2000) 3616.
[19] G.S. Shephard, A. Fabiani, S. Stockenström, N. Mshicileli, V. Sewram, J. Agric. Food Chem. 51 (2003) 1102.
[20] J. Blesa, H. Berrada, J.M. Soriano, J.C. Moltó, J. Mañes, J. Chromatogr. A 1046 (2004) 127.
[21] A. Leitner, P. Zöllner, A. Paolillo, J. Stroka, A. Papadopoulou-Bouraoui, S. Jaborek, E. Anklam, W. Lindner, Anal. Chim. Acta 453 (2002) 33.
[22] S.H. Alarcón, L. Micheli, G. Palleschi, D. Compagnone, Anal. Lett. 37 (2004) 1545.
[23] A.E. Radi, X. Muñoz-Berbel, M. Cortina-Puig, J.L. Marty, Electrochim. Acta 54 (2009) 2180.
[24] A.E. Radi, X. Muñoz-Berbel, V. Lates, J.L. Marty, Biosens. Bioelectron. 24 (2009) 1888.
[25] G.C. Baker, I.L. Jenkins, Analyst 77 (1952) 685.
[26] J. Osteryoung, J.J. O'Dea, in: A.J. Bard (Ed.), Electroanalytical Chemistry, vol. 14, Marcel Dekker, New York, 1986, p. 212.
[27] L. Josephson, J.M. Perez, R. Weissleder, Angew. Chem. Int. Ed. 40 (2001) 3204.
[28] S.H. Huang, M.H. Liao, D.H. Chen, Biotechnol. Prog. 19 (2003) 1095.
[29] Z.H. Wang, C.J. Choi, B.K. Kim, J.C. Kim, Z.D. Zhang, Carbon 41 (2003) 1751.
[30] L.M. Rossi, A.D. Quach, Z. Rosenzweig, Anal. Bioanal. Chem. 380 (2004) 606.
[31] M. Pumera, M.T. Castañeda, M.I. Pividori, R. Eritja, A. Merkoçi, S. Alegret, Langmuir 21 (2005) 9625.
[32] G. Zhao, J.J. Feng, Q.L. Zhang, S.P. Li, H.Y. Chen, Chem. Mater. 17 (2005) 3154.
[33] Y. Xiao, H.X. Ju, H.Y. Chen, Anal. Chem. Acta 391 (1999) 73.
[34] C.H. Xu, C.P. He, Y.Z. Fang, J. Electroanal. Chem. 510 (2001) 78.
[35] AgraQuant® for Ochratoxin A Test ELISA Kit, AgraQuant® Misiones/Argentina (Instruction Manual), 2009.
[36] D.M. Anjo, M. Kahr, M.M. Khodabakhsh, S. Nowinski, M. Wanger, Anal. Chem. 61 (1989) 2603.
[37] E. Verpoorte, Lab. Chip 3 (2003) 60.
[38] N. Pamme, Lab. Chip 6 (2006) 24.
[39] J. Richardson, P. Hawkins, R. Luxton, Biosens. Bioelectron. 16 (2001) 989.
[40] T. Matsunaga, Y. Okamura, Int. J. Nanosci. 1 (2002) 383.
[41] M. Seydack, Biosens. Bioelectron. 20 (2005) 2454.
[42] J.G. Osteryoung, R.A. Osteryoung, Anal. Chem. 57 (1985) 101.
[43] M.W. Calcutt, I.G. Gillman, R.E. Noftle, R.A. Manderville, Chem. Res. Toxicol. 14 (2001) 1266.
[44] S.C.B. Oliveira, V.C. Diculescu, G. Palleschi, D. Compagnone, A.M. Oliveira-Brett, Anal. Chim. Acta 588 (2007) 283.
[45] E.A. Ramírez, M.A. Zón, P.A. Jara Ulloa, J.A. Squella, L. Nuñez Vergara, H. Fernández, Electrochim. Acta 55 (2010) 771.
[46] A.J. Bard, L.R. Faulkner, Electrochemical Methods: Fundamentals and Applications, 2nd ed., Marcel Dekker, New York, 2001.
[47] M. Castegnaro, J. Barek, J.M. Fremy, M. Lafontaine, M. Miraglia, E.B. Sansone, G.M. Telling, Laboratory Decontamination and Destruction of Carcinogens in Laboratory Wastes: Some Mycotoxins. IARC Scientific Publication No. 113, International Agency for Research on Cancer, Lyon, 1991.
[48] M.L. Ponsone, M. Combina, A. Dalcero, S. Chulze, Int. J. Food Microbiol. 114 (2007) 131.
[49] M. Solfrizzo, G. Panzarini, A. Visconti, J. Agric. Food Chem. 56 (2008) 11081.
[50] A.M. Timperio, P. Magro, G. Chilosi, L. Zolla, J. Chromatogr. B 832 (2006) 127.
[51] R. Serra, C. Mendonça, L. Abrunhosa, A. Pietri, A. Venâncio, Anal. Chim. Acta 513 (2004) 41.
[52] T. Tonus, A. Dalla Vedova, S. Spadetto, R. Flamini, Rivista di Viticoltura e di Enologia 1 (2005) 3.