ELECTROANALYSIS OF DEOXYNIVALENOL IN MILK

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This article proposed the assay of deoxynivalenol in milk using an 2D electrochemical sensor based on single walled carbon nanotubes modified with [N-(pyridin-4-yl-methyl)] octadec-9-enamide. Differential pulse voltammetry was employed for all measurements. The working concentration range was between 8.00×10^{-12} and 8.00×10^{-6} g mL^{-1} with a sensitivity of 4.44 μA g^{-1} mL. Recoveries higher than 94.00% were obtained, when the electrochemical sensor was used for the assay of deoxynivalenol in milk.

Keywords: deoxynivalenol, electrochemical sensor, [N-(pyridin-4-yl-methyl)] octadec-9-enamide, milk

1. Introduction

Deoxynivalenol (DON) is one of the most common mycotoxins from the *Fusarium* mycotoxins' group found worldwide in grains and derivative products, including cow milk and vegetarian milk prepared from different types of grains (vegetal milk) [1-3]. DON is a type-B trichothecenes produced by *Fusarium* fungi [3]. Its toxicity was demonstrated, as DON produced a lot of adverse effects on animals, plants, and humans. The main effects produced in humans are classified as hepatotoxicity, intestinal toxicity, nephrotoxicity, and reproduction toxicity [3]. Studies of DON contamination mechanisms have shown that DON activates hypoxia-inducible factor-1α, which regulates reactive oxygen species production and cancer cell apoptosis [4]. Decontamination of food from DON became one of the most important issues, as DON was often found in corn, wheat, oats, and rice. Accordingly, there have been developed to date physical, chemical, and enzymatic

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decontamination methods that have been able to considerably reduce the levels of DON in food.

The DON assay proved to be necessary at very low levels, with high sensitivity, in both grains and derivative foods, as well as in biological samples from animals and humans in order to prevent the illnesses that may occur due to its high toxicity. To date, there were developed methods based on liquid chromatography [5-7] and electroanalysis [8-12]. Different types of immunosensors: optical immunosensors, electrochemical immunosensors, piezoelectric immunosensors, and aptasensors [8] were able to determine the DON up to pg mL^{-1} levels. A chip based dual-mode sensing chip based on photoelectrochemical and electrochromic assays of DON was developed to be used up to fg mL^{-1} levels.

Due to the fact that fast, reliable assay of DON in food is a need, and taking into account that the electrochemical sensors can be used without any sample processing or with a minimum of processing of the sample, this article proposes a novel 2D electrochemical sensor that can be reliable used on site for the screening test of milk for DON.

The novelty is given by utilization of a screen-printed electrode based on single-walled carbon nanotubes deposited as nanolayers on a plastic material in a dot shape (serving as the working electrode) with a diameter of 4 mm, surrounded by two semi-cercles of Ag/AgCl and Ag lines (1 mm in width) serving as reference and auxiliary electrodes. The working electrode was further modified with a solution of oleamide: [N-(pyridin-4-yl-methyl)] octadec-9-enamide.

2. Experimental

2.1 Materials and reagents

Single-walled carbon nanotubes, DON, were bought from Sigma Aldrich. [N-(pyridin-4-yl-methyl)] octadec-9-enamide was synthesized in house by Cioates Negut accordingly with the procedure published earlier [13]. Phosphate buffer solution pH 7.40 was purchased from Merk.

2.2. Apparatus

A Mini Potentiostat "EmSTAT Pico" (PsTrace 5.8 PalmSens software, Houten, Netherlands) connected to a laptop was used for the differential pulse voltammetry (DPV).

2.3. Design of the 2D electrochemical sensor

Single-walled carbon nanotubes (SWCNT) were deposited as nanolayers on a plastic material in a dot shape (serving as the working electrode) with a diameter of 4 mm, surrounded by two semi-cercles of Ag/AgCl and Ag lines (1 mm in width)

serving as reference and auxiliary electrodes. The SWCNT dot was modified using a 10^{-3} solution of [N-(pyridin-4-yl-methyl)] octadec-9-enamide: a drop of the [N-(pyridin-4-yl-methyl)] octadec-9-enamide solution was added to the top of the dot, and it was let dry for 12 h. The 2D electrochemical sensor was stored at room temperature.

2.4. Recommended procedure

The following parameters were applied for the differential pulse voltammetry (DPV): scan rate 0.15 V/s, scanning potential range: -0.1 to 1.8 V, 25°C. A standard solution with concentrations between 10^{-2} and 10^{-16} g mL^{-1} was used to characterize the 2D electrochemical sensor. A calibration equation based on the correlation of the intensity of the peak with the concentration of DON was obtained after applying the linear regression method. Unknown concentrations of DON in milk samples were calculated from the equation of calibration.

2.5. Samples

Four different milk samples were used; all of them were bought from the supermarket. Sample no 1 contains cow's milk, sample no 2 coconut milk, sample no 3 soy milk, and sample no 4 almond milk. All milk samples were buffered in a ratio of 1:1 (milk:buffer, v:v) before DON determination.

2.6. Selectivity studies

Other mycotoxins, such as aflatoxin B1 (AFB1), ochratoxin A (OTA), and patulin (PAT), were checked as possible interferents. The mixed solution method was used to determine the amperometric selectivity coefficients. The mixed solutions contain the DON and the other mycotoxin in a ratio of 1:10 (mol:mol).

The equation (1) used for the calculation of the amperometric selectivity coefficients was [14]:

$$Ki,j(amp) = \left(\frac{It}{Ii} - 1\right) * \frac{ci}{cj} \quad (1)$$

where $K_{i,j}$ *(amp)* is the amperometric selectivity coefficient; I_t is the height of the peak recorded for the mixed solution; I_i is the height of the peak obtained for the DON solution having the same concentration as in the mixed solution; and c_i and c_j are the concentrations of the DON and of the other mycotoxin.

3. Results and discussions

3.1. Parameters obtained for the 2D electrochemical sensor

Figure 1 shows the voltammograms used for the calibration of the proposed 2D electrochemical sensor when used for the assay of DON.

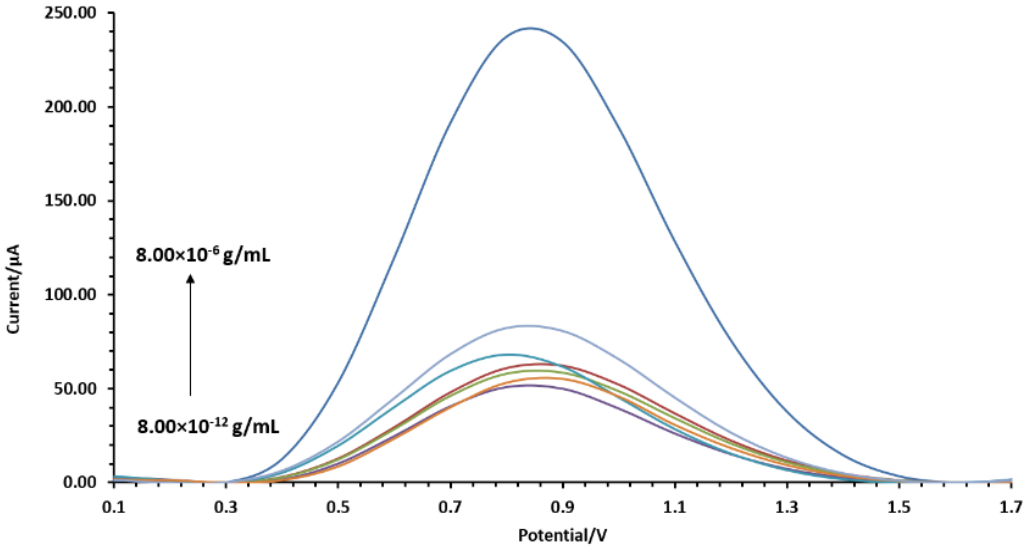

Fig. 1. Differential pulse voltammograms obtained for different concentrations of DON, when a scan rate of 0.15 V s^{-1} was used

Figure 2 displays the calibration graph.

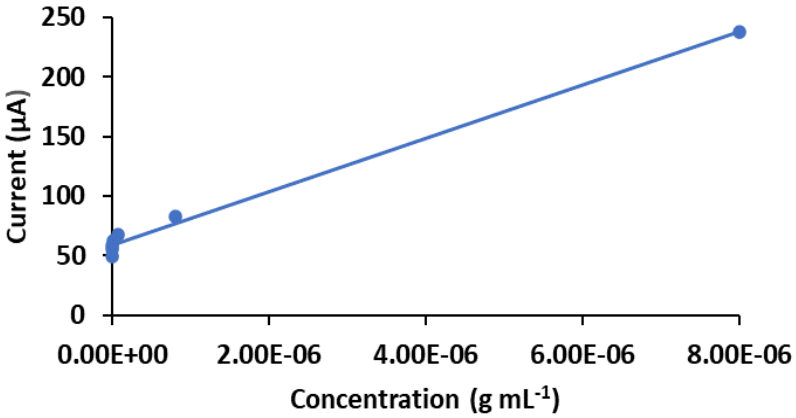

Fig. 2. Calibration graph obtained for DON using the proposed 2D electrochemical sensor

The parameters of the proposed 2D electrochemical sensors are shown in Table 1.

Table 1

Parameters of the 2D electrochemical sensor

Linear concentration range (g mL^{-1})	8.00×10^{-12} - 8.00×10^{-6}
Calibration equation (<I>=μA, <C_{DON}>=g mL^{-1})	$I = 59.28 + 4.44\times10^{-8} \times C_{DON}$
Correlation coefficient	0.9994
Sensitivity (μA g^{-1} mL)	4.44×10^{-8}
Quantitation limit (g mL^{-1})	8.00×10^{-12}
Detection limit (g mL^{-1})	2.40×10^{-12}
$E_{1/2}$ (mV)	899.34
Number of data points	7

The working concentration range of the 2D electrochemical sensor is wide (6 decades of concentrations). The limits of quantification and detection of DON are far lower than those reported earlier using electrochemical sensors [8-12].

Stability and reliability of the sensor design were determined using ten 2D electrochemical sensors designed accordingly with the protocol described above. The sensitivities recorded for these sensors were compared, and it was determined that a relative standard deviation of 0.24% was recorded, proving the reliability of the design of the 2D electrochemical sensor. Also, for 30 days, the sensitivity of the designed sensors was determined; when measuring the sensitivities, the relative standard deviation was 1.23%, proving the high stability of the sensors over the time.

3.2. Selectivity of the 2D electrochemical sensor

The results obtained for the amperometric selectivity coefficients are shown in Table 2.

Table 2

Amperometric selectivity coefficients

Interferent	K_{sel}^{amp}
AFB1	8.97×10^{-4}
OTA	9.49×10^{-4}
PAT	1.00×10^{-4}

The magnitude order of 10^{-4} obtained for the amperometric selectivity coefficients proved that AFB1, OTA, and PAT do not interfere during the measurements of DON in milk samples.

3.3. Validation of the 2D electrochemical sensor for the determination of deoxynivalenol in milk

Different milk samples obtained from the supermarket were used for the validation of the 2D electrochemical sensor. One sample was cow's milk, and the other three samples were obtained from the processing of the corresponding grains, when coconut milk, soy milk, and almond milk were obtained. The DON was first determined in these samples using the DPV method, and after this step, a known amount of DON was added to each of the samples, and the final concentration of the DON was determined in the milk sample. The found concentration was compared with the expected concentration to determine the recovery of DON in the milk sample. The results are shown in Table 3.

Table 3

Recovery of deoxynivalenol in milk samples using the proposed 2D electrochemical sensor (N=10)

Milk samples	Expected concentration of DON ($g\ mL^{-1}$)	Determined concentration of DON ($g\ mL^{-1}$)	%, Recovery obtained when the electrochemical sensor was used
Cow's milk	5.73×10^{-12}	5.72×10^{-12}	99.83±0.12
Coconut milk	3.47×10^{-11}	3.27×10^{-11}	94.24±0.13
Soy milk	6.34×10^{-12}	6.33×10^{-12}	99.84±0.11
Almond milk	1.05×10^{-10}	1.03×10^{-10}	98.09±0.12

The recovery values obtained for the assay of DON in different types of milk were higher than 94.00%, while the relative standard deviations were lower than 1.00%. The values proved that the proposed 2D electrochemical sensor can be reliably used for the screening of milk samples for the accurate assay of DON. While the milk samples were only buffered before the DON determination using the DPV method, this is an advantage versus previously reported chromatographic and electrochemical methods of analysis [5-12], which, together with the wide linear concentration range and the low limits of detection and determination, made the proposed sensor and method better than those tools and methods proposed to date.

4. Conclusions

The 2D electrochemical sensor based on single-walled carbon nanotubes modified with [N-(pyridin-4-yl-methyl)] octadec-9-enamide was designed and used for the assay of deoxynivalenol in milk samples. The low limits of determination and quantification, as well as the wide linear concentration range and high recoveries in milk samples, facilitate high reliability assay of deoxynivalenol in

milk samples. The features of the proposed 2D electrochemical sensors are connected with their utilization as screening tools for the quality of milk, making possible the signaling of the presence of deoxynivalenol in milk samples and avoiding the development of toxic infections in the population due to the presence of deoxynivalenol in milk samples.

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