Full Paper

Novel Studies about the Electrooxidation of a Deoxynivalenol (DON) Mycotoxin Reduction Product Adsorbed on Glassy Carbon and Carbon Paste Electrodes

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Abstract
A novel and fast square-wave adsorptive anodic stripping voltammetric procedure on glassy carbon and carbon paste electrodes is described for the indirect trace quantitation of deoxynivalenol (DON) mycotoxin that frequently contaminates soya and foodstuff. The in situ homogeneous reduction of the toxin by product/s of dissolved oxygen electroreduction in pH 8 buffer solution makes the quantitation of DON possible through a simple electroanalytical technique. Moreover, its potential use for the analysis of real samples was demonstrated using a soya flour sample spiked with DON without previous separating procedures. The DON detection limits were 3.6 and about 6 ppb from standard solutions and soya flour matrix, respectively.

Keywords: Deoxynivalenol (DON), Mycotoxins, Adsorptive accumulation, Glassy carbon electrodes, Carbon paste electrodes, Square-wave voltammetry, Cyclic voltammetry

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1. Introduction

Deoxynivalenol (DON), which chemical structure is shown in Figure 1, is a mycotoxin that belongs to type B trichothecenes group and is produced by several *Fusarium* species [1].

DON is a worldwide contaminant of foods and feeds [2, 3]. *Fusarium* head blight (FHB) is an important disease of barley in several countries. In South America, particularly in Argentina, Brazil, Paraguay, and Uruguay, it constitutes an increasing problem [4, 5]. FHB takes place both in the field and during storage, producing mycotoxins in moldy corn and wheat that are toxic to animals and humans [6–8]. Studies suggest that DON may also produce chronic effects in humans [9], and causes a variety of toxic effects by inhibiting the synthesis of DNA, RNA and proteins [10]. Consumption of feed contaminated with DON has been reported to lead to vomiting, feed refusal, weight loss, and diarrhea in both experimental animal and livestock [11].

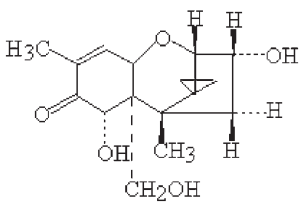

Fig. 1. Chemical structure of deoxinivalenol mycotoxin.

Because of the potential dangers due to contamination of foods or feeds with DON, there is a need to develop rapid and highly sensitive methods for easy identification and quantification of this mycotoxin.

Several analytical methods have been developed to detect DON. The most commonly used methods include GC/ECD [12], TLC/fluorescence [13], HPLC/UV [14], HPLC/MS [15, 16], HPLC/ immunoaffinity columns [17, 18]. Enzyme-linked immunosorbent assay (ELISA) has also been proposed [19, 20]. These methods usually require significant amount of time associated with labor-intensive cleanup, sophisticated instrumentation and skilled operators.

Electrochemical methods can be ideal for such tasks since their selectivity often requires less intensive cleanup, in turn allowing rapid and inexpensive detection. As far as we know, only two studies have used electrochemical detectors combined with HPLC separation for detection of trichothecenes [21, 22]. Extreme potentials such as -1.4 V and $+1.0$ V, respectively, in basic media, were applied in such studies. Besides, there are scarce reports which show some attempts to study the electrochemistry of trichothecenes [23, 24]. In those reports, it was demonstrated that DON could be reduced at potentials of about -1.4 V vs. SCE on Hg electrodes in basic solution. At such rather extreme potentials, however, electrochemical selectivity is compromised and extensive deoxygenation is required. More recently DON was indirectly detected by electrochemical techniques [25]. The mycotoxin was hydrolyzed in basic

conditions at 80 °C for 1 h and then its products were oxidized at 0.6 V vs. ECS. Although the reported method is relatively simple and inexpensive its detection limit is above the recommended one by present regulations for processed cereal-based food and baby foods for infants and young children [26].

In this paper, a novel square-wave adsorptive anodic stripping voltammetric procedure is described for the indirect trace quantitation of DON in standard solutions as well as in soya flour extract samples. We describe the adsorptive accumulation of DON on glassy carbon (GC) and carbon paste electrodes (CP) from stirred solutions of pure commercial reagent by using cyclic (CV) and square-wave (SWV) voltammetries. The adsorption isotherm model which better describes the experimental results is also discussed. The proposed methodology has the particular advantages of being faster than regular ones for the determination of DON and that separating procedures in the real matrix are not necessary.

Finally, the aim of this study is to demonstrate that DON can be quantified electrochemically at trace levels in order to assess possible analytical applications.

2. Experimental

2.1. Chemicals and Solutions

DON was obtained from Sigma Chemical Company and used as received. Water, acetonitrile and hexane were Sintorgan, HPLC grade. NaCl and pH 8 buffer solution (BS, boric acid + sodium hydroxide + hydrogen chloride) were Merck p. a. and were used as received.

Working solutions of DON were prepared daily by adding aliquots of stock solution to aqueous pH 8 BS.

2.2. Extraction of DON from Soya Flour Samples

DON was extracted from spiked (1 ppm) soya flour sample [27] using a method developed for cereals [28] and then adapted for soya flour [29]. An amount of 0.94 ppm was determined for DON in the sample by HPLC method [29].

A 10.4954 g portion of the spiked soya flour sample was taken from milled flour and placed in a 100 mL erlenmeyer flask containing 50 mL acetonitrile-water (9:1), 20 mL hexane and 2 g of NaCl. Mixture was shaken during 30 min in an oscillating shaker and then quantitatively filtered. An adequate aliquot of this solution was taken to prepare a stock solution (A) of concentration 7.0×10^{-7} mol dm^{-3}. Therefore, the standard addition method [30] was used to perform the DON electrochemical quantification in the spiked soya flour sample. Thus, different aliquots of 2.5×10^{-6} mol dm^{-3} standard DON pH 8 BS (B) were added to the electrochemical cell containing solution (C). Solution (C) was prepared by adding 300 μL of DON solution (A) to pH 8 BS up to a final volume of 10.30 mL, rendering a DON concentration of $(2.04 \pm 0.08) \times 10^{-8}$ mol dm^{-3}.

2.3. Electrodes and Instrumentation

Electrochemical measurements were performed in a three compartments Pyrex cell. Working electrodes (WE) were GC and CP disks. Before each experimental measurements series, GC electrodes were polished successively with wet alumina powder (0.3 and 0.05 μm from Fischer), rinsed copiously with distilled water and sonicated in a water bath for 2 min. The electrochemical area (0.069 cm^2) was determined as reported previously [31]. The CP electrode (Bioanalytical Systems Inc., 1.6 mm i.d., 3 mm o.d.) was pretreated by restoring a new thin layer of carbon paste prior to each experiment and then polishing with a soft glass paper. It was composed of uniform graphite particles mixed with paraffin binder (Bioanalytical Systems, Inc.). The counter electrode (CE) was a platinum foil of large area (approx. 2 cm^2). The reference electrode (RE) was an aqueous saturated calomel electrode (SCE) separated from working solution through a fine Luggin capillary. Experiments were performed in aerated solutions under reproducible convective conditions using a magnetic stirrer and a stirring bar, unless otherwise indicated, in which cases solutions were deaerated by bubbling pure nitrogen.

CV and SWV experiments were performed with an Autolab PGSTAT 30 potentiostat/galvanostat controlled by GPES 4.9 electrochemical analysis software, both Eco-Chemie, Utrecht, The Netherlands. In most of experiments, characteristic parameters used to obtain square-wave voltammograms were as follows: square-wave amplitude (ΔE_{sw}) was 50 mV, staircase step height (ΔE_s) was 10 mV, and frequency (f) was 10 Hz.

In all these experiments, the mycotoxin accumulation step was accomplished in stirred solutions. Manipulation of all laboratory material was done using thin plastic gloves for safety purposes. Experimental measurements were performed at 20 °C. In every case, determinations were performed, at least, by duplicate and averages are the values shown or plotted.

3. Results and Discussion

Cyclic voltammograms of DON recorded in an exhaustive deaerated pH 8 BS at GC and CP electrodes showed a reduction peak centered at $E \approx -1.45$ V (Fig. 2a) in agreement with the result previously reported for the same system on mercury electrodes [23].

No significant complementary peak was observed on the reversal sweep in all the potential range at scan rates used, putting clearly in evidence a complex electroreduction mechanism. On the other hand, cyclic voltammograms recorded in aerated solution (see experimental section), show that the classical reduction of oxygen is verified at about −0.9 to −1.0 V vs. SCE [32]. Under this condition, anodic sweeps show a novel anodic peak (Fig. 2b), corresponding to a surface process, centered at about 0.10 (CP) – 0.15 (GC) V, which peak current depends on the time elapsed at −1.0 V. This anodic peak is absent in solutions

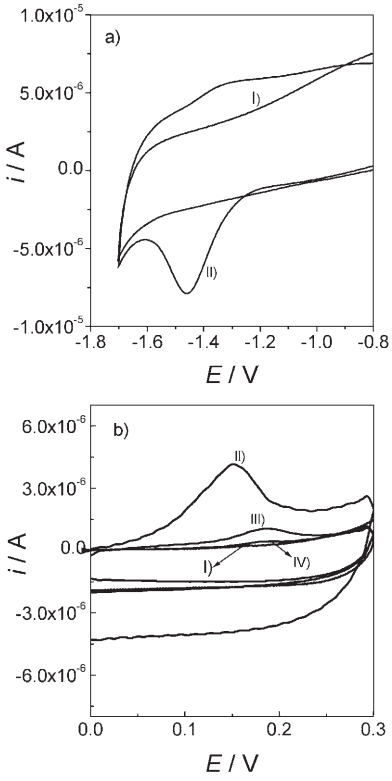

Fig. 2. a) Cyclic voltammograms in the absence (I) and in the presence (II) of DON in deaerated pH 8 buffer; $c_{DON}^* = 1.0 \times 10^{-5}$ mol dm^{-3}. b) Cyclic voltammograms in (I) DON free aerated pH 8 buffer; (II) Aerated solution; (III) N_2 bubbling time: 20 min; (IV) N_2 bubbling time: 60 min. (II) – (IV) $c_{DON}^* = 1.3 \times 10^{-6}$ mol dm^{-3}, $E_{acc} = -1.0$ V, $t_{acc} = 300$ s. Working electrode: GC disk ($A = 0.069$ cm^2). Reference electrode: SCE, $\nu = 0.050$ V s^{-1}.

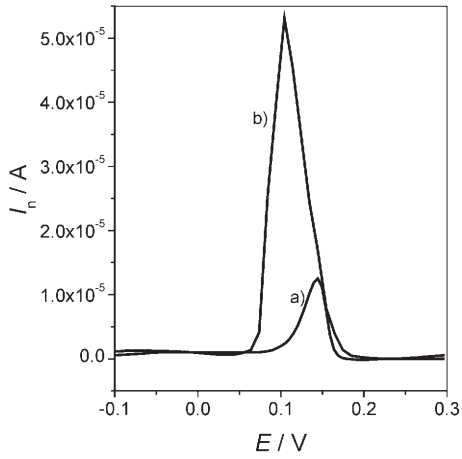

Fig. 3. Net peak current ($I_{p,n}$) from square-wave voltammograms for the electrooxidation of DON adsorbed product in pH 8 BS. a) Working electrode: GC disk, $c_{DON}^* = 5.0 \times 10^{-6}$ mol dm^{-3}. b) Working electrode: CP disk, $c_{DON}^* = 1.67 \times 10^{-7}$ mol dm^{-3}. Reference electrode: SCE. $\Delta E_{sw} = 50$ mV, $\Delta E_s = 10$ mV, $f = 10$ Hz. $E_{acc} = -1.0$ V, $t_{acc} = 300$ s. Stirred solutions at 20 °C.

free of DON. Clearly, the appearance of this anodic peak is related to the electrooxidation of a product of the reaction between DON and the electroreduction product of oxygen at −1.0 V. The nature of the species responsible for this peak is still unknown although a possible explanation could arise by taking into account the possible reduction of DON by the product of oxygen reduction in this basic medium, i. e. peroxide anion [32]. Thus, DON could be reduced by peroxide giving an easily oxidizable product which adsorbs on the electrode surface. Products of the hydrolysis of DON could be oxidized at +0.6 V in acidic medium by a diffusion controlled process [25] and could be disregarded as responsible for adsorption peak at about 0.10 – 0.15 V found in this work, although a quinone type species that are products of ring opening reaction have been suggested to give peaks at about 0.2 V [25].

Experiments performed by SWV by using different accumulation times (t_{acc}) at GC and CP electrodes showed an increase in the net anodic peak currents ($I_{p,n}$), which clearly indicates the surface nature of this electrochemical signal.

These findings show a selective interaction of DON reduction product with the carbon surface. Similar results were obtained recently for the surface behavior of mycotoxins altertoxin-I [33] and cercosporin [34] on carbon electrodes.

Square-wave voltammograms at different electrode materials are shown in Figure 3.

These results clearly suggest that the adsorption of a DON related compound at the CP electrode surface showed the best analytical response. Thus, although the redox species responsible for the anodic peak were not characterized, its electrooxidation current was employed as the analytical signal for the quantification of DON. Studies were conducted to find the optimum accumulation potential (E_{acc}) as well as the most favorable t_{acc} for performing the preconcentration step at the electrode surface at both electrodes. Studies varying E_{acc} have shown that the current increases as the E_{acc} is shifted to more negative values. It was found that the best E_{acc} vs. SCE was −1 V. On the other hand, stationary currents could be obtained for $t_{acc} \geq 250$ s. Thus, a $t_{acc} = 300$ s under stirring for both, CP and GC electrodes was chosen to perform experiments.

3.1. Adsorption Isotherm

The adsorption isotherm was derived from the dependence between the fractional coverage of the electrode surface (θ) and the concentration of DON (c_{DON}^*). The surface coverage was defined as $\theta = I_{p,n}/I_{p,n,max}$ [34], where $I_{p,n}$ is the net peak current from square-wave voltammograms obtained at $t_{acc} = 300$ s and $E_{acc} = -1$ V vs. ECS, for different c_{DON}^* and $I_{p,n,max}$ is the maximum value of $I_{p,n}$ obtained at the same t_{acc} and E_{acc} for the highest c_{DON}^* studied. The saturation coverage ($\theta = 1$) of the electrode surface was reached at $c_{DON}^* \geq 4 \times 10^{-7}$ M and 1×10^{-5} M at CP and GC electrodes, respectively. The isotherm model which best fitted experimental data was the Frumkin isotherm [35]. Adsorption

Table 1. Adsorption parameters for DON on GC and CP electrodes in pH 8 BS. g: lateral interaction parameter; β: adsorption coefficient; $\Delta G_{ads}^{\circ} = -RT \ln \beta$; χ^2: square chi function.

Electrode	g	$10^{-4}\,\beta$ (mol^{-1} dm^3)	$-\Delta G_{ads}^{\circ}$ (kJ mol^{-1})	χ^2
GC	1.57	8.84	27.7	6.3×10^{-14}
CP	2.07	357.1	36.7	2.0×10^{-17}

data for CP and GC electrodes are shown in Figure 4 along with results of fitting with Frumkin isotherm. Frumkin isotherm is expressed as [35]:

$$\beta c^* = \frac{\theta}{1-\theta} \exp(-g\theta) \quad (1)$$

where β is the adsorption coefficient, g is the parameter characterizing the interaction between the adsorbed species and c^* is the bulk concentration of reactant. Attractive interactions are indicated by $g > 0$ while repulsive ones for $g < 0$.

Figure 4 shows that the optimized Frumkin isotherm (solid line) provides a satisfactory fit to the experimental surface coverage's for monolayers adsorbed on both CP and GC electrodes. In contrast, the optimized data for other adsorption isotherm models do not adequately fit to experimental data. The adsorption parameters are summarized in Table 1.

On the other hand, the best fit is obtained for adsorption on CP, according to statistical parameters.

The positive g values indicate that the apparent Gibbs energy of adsorption increases accordingly with the surface coverage, thus causing further adsorption to be energetically more difficult. Adsorption energy depending on coverage is ascribed to lateral interaction and/or heterogeneity on the surface. The magnitude of β (and thus of the ΔG_{ads}° through $\Delta G_{ads}^{\circ} = -RT \ln \beta$) obtained above confirms a strong adsorption of the redox species on carbon surfaces. The higher adsorption coefficient found for CP suggests that the adsorption process in this material is stronger than on GC. Results found agree satisfactorily with those of similar systems [36].

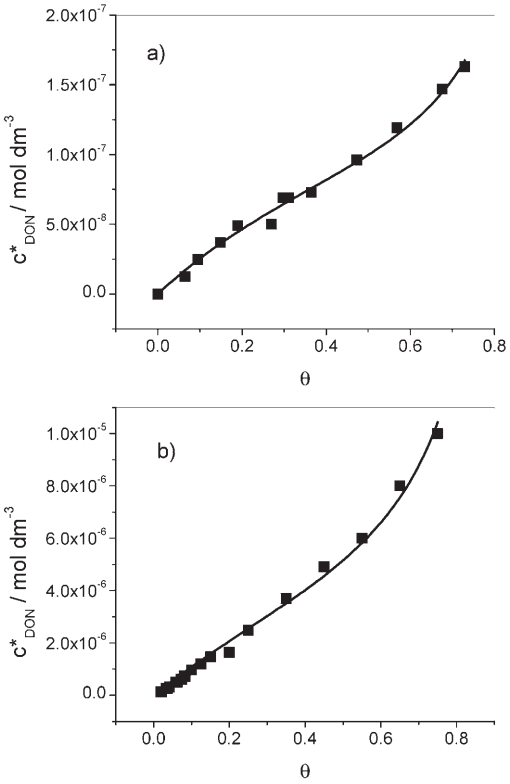

Fig. 4. Adsorption isotherms for DON adsorbed product. a) GC disk; b) CP disk. Reference electrode: SCE. $\Delta E_{sw} = 50$ mV, $\Delta E_s = 10$ mV, $f = 10$ Hz. $E_{acc} = -1.0$ V, $t_{acc} = 300$ s. Stirred pH 8 BS at 20 °C. The solid line is the best-fit curve using the Frumkin isotherm (Eq. 1).

3.2. Quantitative Determination

The net current-potential curve in SWV is the most useful analytical signal [37, 38] which combined with the high sensitivity of adsorptive accumulation methods [39] renders a powerful analytical technique.

Calibration curves for DON were carried out by SWV ($\Delta E_{sw} = 50$ mV, $\Delta E_s = 10$ mV, $f = 10$ Hz) on GC and CP electrodes. Stirred pH 8 BS, $t_{acc} = 300$ s and $E_{acc} = -1$ V were used. Linear relationships between $I_{p,n}$ vs. c_{DON}* were obtained at low concentration ranges (see Fig. 5). The regression parameters, linearity ranges and detection limits (DL) are shown in Table 2.

The detection limits estimated under these conditions for a signal-to-noise ratio of 3:1 were 1.4×10^{-7} mol dm^{-3} (41 ppb) and 1.2×10^{-8} mol dm^{-3} (3.6 ppb) for GC and CP, respectively, which were both quite lower than 200 ppb, the highest value permitted by European Commission for processed cereal-based foods and baby foods for infants and young children [26]. The difference in detection limits

Table 2. Parameters of the calibration plots for the quantification of standard DON on GC and CP electrodes obtained by the proposed stripping adsorptive SWV procedure at $f = 10$ Hz, $\Delta E_{sw} = 50$ mV, $\Delta E_s = 10$ mV, $E_{acc} = -1$ V and $t_{acc} = 300$ s. r: correlation coefficient

Electrode	Regression equations [a]	Linear range (mol dm^{-3})	Detection limit		r
			(mol dm^{-3})	(ppb)	
GC	$I_{p,n} = (2.01 \pm 0.05) \times 10^6\ c_{DON}^* + (0.19 \pm 0.07)$	$1.2 \times 10^{-7} - 3.0 \times 10^{-6}$	1.4×10^{-7}	(41)	0.991
CP	$I_{p,n} = (3.44 \pm 0.07) \times 10^8\ c_{DON}^* + (0.23 \pm 0.06)$	$1.2 \times 10^{-8} - 2.0 \times 10^{-7}$	1.2×10^{-8}	(3.6)	0.996

[a] $I_{p,n}$ (μA) = slope × c_{DON}* (mol dm^{-3}) + intercept

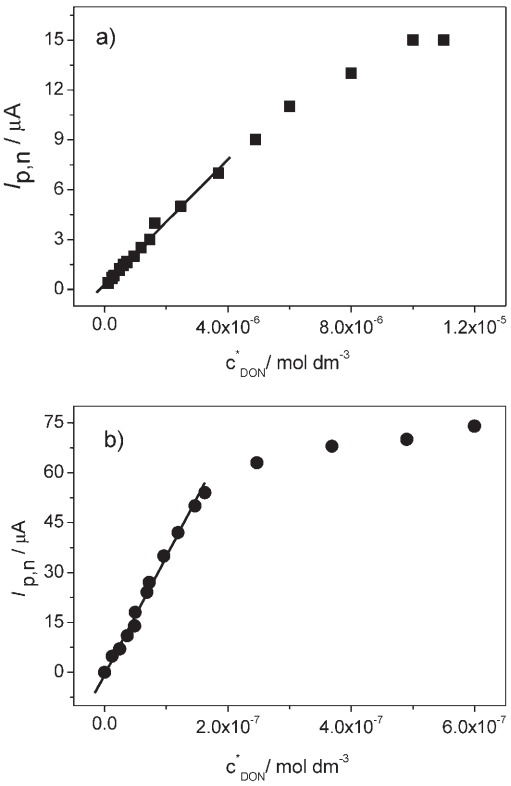

Fig. 5. Calibration curves obtained in pH 8 BS on a) GC disk, b) CP disk. Preconcentration and SWV parameters are the same as those indicated in Figure 4.

between both electrodes is according to values obtained for the adsorption coefficient (β), which predicts stronger adsorption on CP than on GC electrodes.

Additional studies were done to investigate applications of this methodology coupled with standard additional method for the determination of DON on CP electrode in soya flour. DON concentration in extracting solution from soya flour sample spiked with DON (1 ppm) as determinated by HPLC was 0.94 ppm (94% recovery, see experimental section). A 2.04×10^{-8} M working solution was prepared by adequate dilution. Adsorptive stripping SWV coupled with standard addition method was then performed on the working solution. SW voltammograms are shown in the Figure 6.

The linear regression can be expressed by a least squares procedure as (twelve experimental points were taken into account):

$$I_{p,n} = (3.7 \pm 0.1) \times 10^7\ c_{DON}{}^* + (0.72 \pm 0.06)\ (r = 0.991)$$

where $I_{p,n}$ is expressed in microamperes and $c_{DON}{}^*$ in mol dm^{-3}.

DON concentration obtained with standard addition method was 1.94×10^{-8} mol dm^{-3} (95.1% recovery), which agrees quite well with the one determinated by HPLC (1.89×10^{-8} mol dm^{-3}; 92.7% recovery), but closer to the DON concentration in the starting sample (2.04×10^{-8} mol dm^{-3}). Relative standard deviation was 3.5% and the

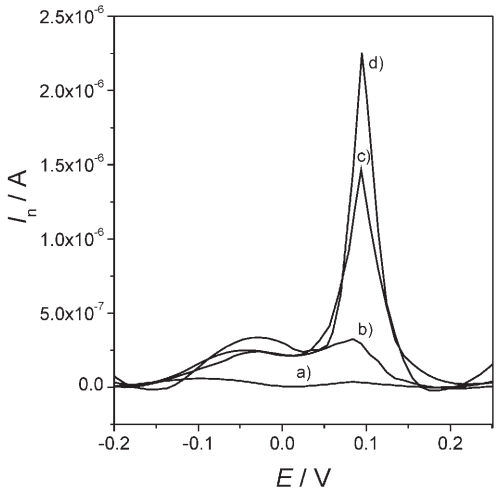

Fig. 6. SW voltammograms for quantitative determination of DON in soya flour sample by standard addition method on CP electrode. a) blank; b) addition of soya flour sample, $c_{DON}{}^* = 2.01 \times 10^{-8}$ mol dm^{-3}; c) addition of 80 μL of 2.5×10^{-6} mol dm^{-3} DON standard solution; d) idem 190 μL. Preconcentration and SWV parameters are the same as those indicated in Figure 4.

detection limit in the real sample was about 6 ppb. The value in slope was about one order of magnitude lower than that showed in Table 2 for commercial pure reactant. This difference probably reflects the effect of the matrix itself. However, the result obtained by the electroanalytical technique is in good agreement with that calculated by HPLC measurement of the same extract and demonstrate the applicability of the proposed method to real fungal contaminated sample containing DON at ppb level. Besides and interestingly, DON could be also detected on soya flour without previous separating method.

4. Conclusions

The application of square-wave voltammetry combined with adsorptive accumulation is studied comparatively for GC and CP electrodes to quantify DON, which frequently contaminates feeds and foodstuff. It has been proved that the proposed methodology is fast and capable of quantifying DON at a very low level (6 ppb) on CP electrode directly in extracting solution from contaminated soya flour without previous separating procedures. This appears as a very promising analytical tool for the determination of DON in real samples.

5. Acknowledgements

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