Voltammetric detection of Domoic Acid at a multiwalled carbon-nanotube electrode

F. Bento[a], D. Geraldo[a], R. Oliveira[a], C. Teixeira[a], E. González-Romero[b]

[a]*Departamento de Química, Universidade do Minho, 4710 Campus de Gualtar, Braga, Portugal,*
[b]*Departamento de Química Analítica y Alimentaria, Faculdade de Química, Universidad de Vigo, 36310 Vigo, Spain,*

Introduction

Domoic acid (DA) is a heterocyclic amino acid and a structural analogue of kainic acid and proline (figure 1). In mammals, including humans, DA acts as a neurotoxin, causing loss of short-term memory, brain damage and, in severe cases, death. DA enters in the food chain via phytoplankton, which serves as food to marine organisms as crustaceans, anchovies and sardines. DA concentrations at the tissues of these marine species can reach high values as a result of bioaccumulation [1]. To protect consumers from amnesic shellfish poisoning (ASP), most countries have defined a regulatory limit for shellfish of 20 mg/g (20 ppm) in accordance with the recommendations of Inverson and Farah [1].

The standard AOAC method, based on high performance liquid chromatography (HPLC), provides good reproducibility, high precision and the analysis of its isomers, although it is rather time-consuming [2, 3]. Thus, there is a need for a fast, selective and sensitive method suitable for a rapid screening of DA. The enzyme-linked immunosorbent assay (ELISA) is the only one that can be used for this aim [3].

Following an affinity recognition process, an electrochemical immunosensor has been developed based on screen-printed electrodes (SPEs). The detection is performed by means of the alkaline phosphatase activity in competitive assays [4, 5]. The use of this immunossensor did not become widespread, probably due to the intrinsic drawbacks that are typical of immunoassays, namely the long incubation times, high costs of the antibodies and the difficulties associated to their regeneration.

The direct electrochemical detection of DA is not reported in literature, probably, because it is not electroactive on the most common electrode materials, such as carbon, gold or platinum.

Experimental

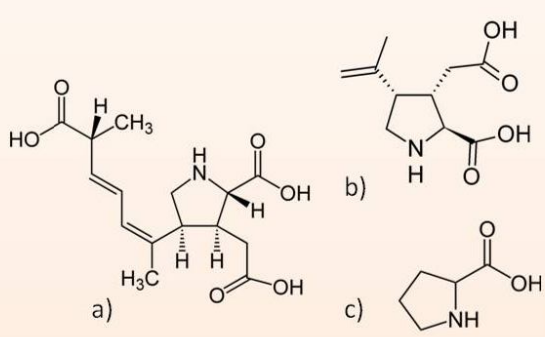

Fig. 1- a) domoic acid, b) kainic acid and c) proline

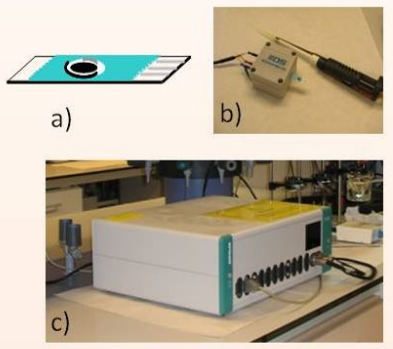

Fig. 2- a) Screen-Printed Carbon Electrodes modified with Multiwalled Carbon Nanotubes (CNTs) from DropSens, b) electrode connector c) Autolab Potenciost / galvanostat PGSTAT30

The electrochemical behavior of DA was investigated at SPE/CNTs using multiple scan cyclic voltammetry, recorded in 0.1 M phosphate buffer solutions of different pHs.

A drop of 2 µl of DA solution (water : methanol, 50 : 50) was placed on the working electrode. The solution was left for several minutes to allow the adsorption of DA at open circuit before the addition of 50 µl of phosphate buffer.

The effect of accumulation time on the peak current was studied using 0, 15, 30, 60, 120 and 180 s. The peaks current showed that a significant increase within the first 15 s and then stabilized until 120 s. Further increase in the accumulation time causes a decrease of peak currents. Taking account of sensitivity and also response repeatability, the accumulation time was set at 120 s.

Results

Effect of pH on cyclic voltammograms of DA

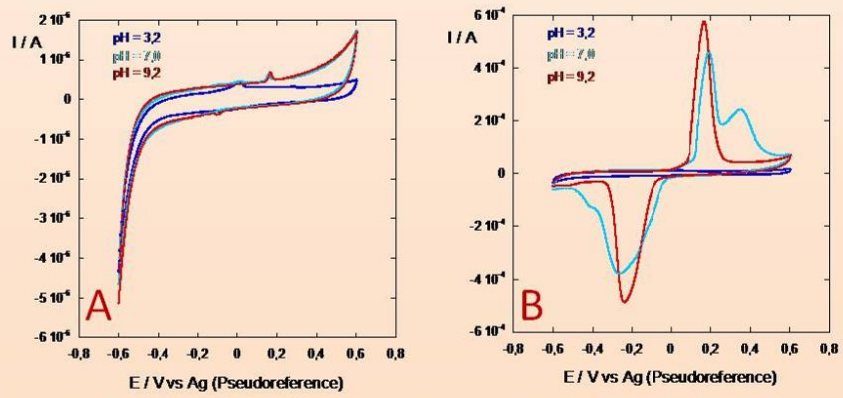

Fig.3- Cyclic voltammograms at SPE/CNTs of A) 0,1 M phosphate buffer B) 0,32 pM DA in 0,1 M phosphate buffer. Scan rate 100 mV s^{-1}.

Effect of anodic and cathodic potentials on cyclic voltammograms of DA

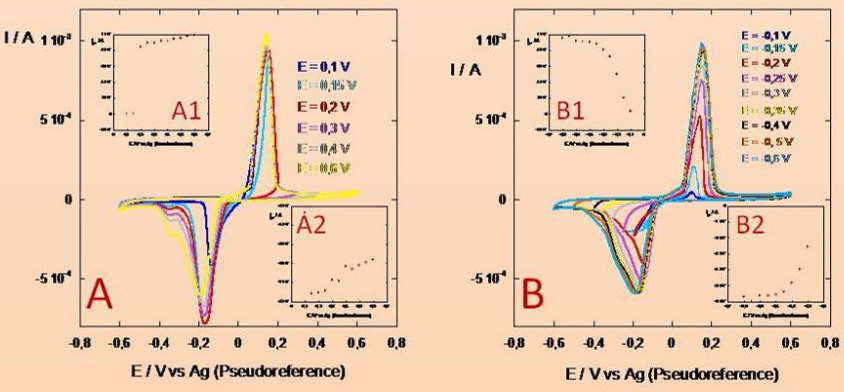

Fig. 4 - Cyclic voltammograms scans at 100 mV s^{-1} on SPE/CNTs of DA 0.32 pM in phosphate buffer 0.1 M pH=7.0: A) Effect of the anodic potential limit: E_i = -0.25 V, E_{anodic} = 0.1; 0.15; 0.2; 0.3; 0.4 or 0.6 V and E_f = 0.6 V; B) Effect of the cathodic potential limit: E_i = 0 V to, $E_{cathodic}$ =-0.1; -0.15; -0.2; -0.25; - 0.3; -0.35; -0.4; -0.5 or -0.6 V and E_f = 0.6 V; Insets: A1: Ipa vs Ea; A2: Ipc vs Ea; B1: Ipa vs Ec; B2: Ipc vs Ec

pH effect on the voltammetric response of the adsorbed DA

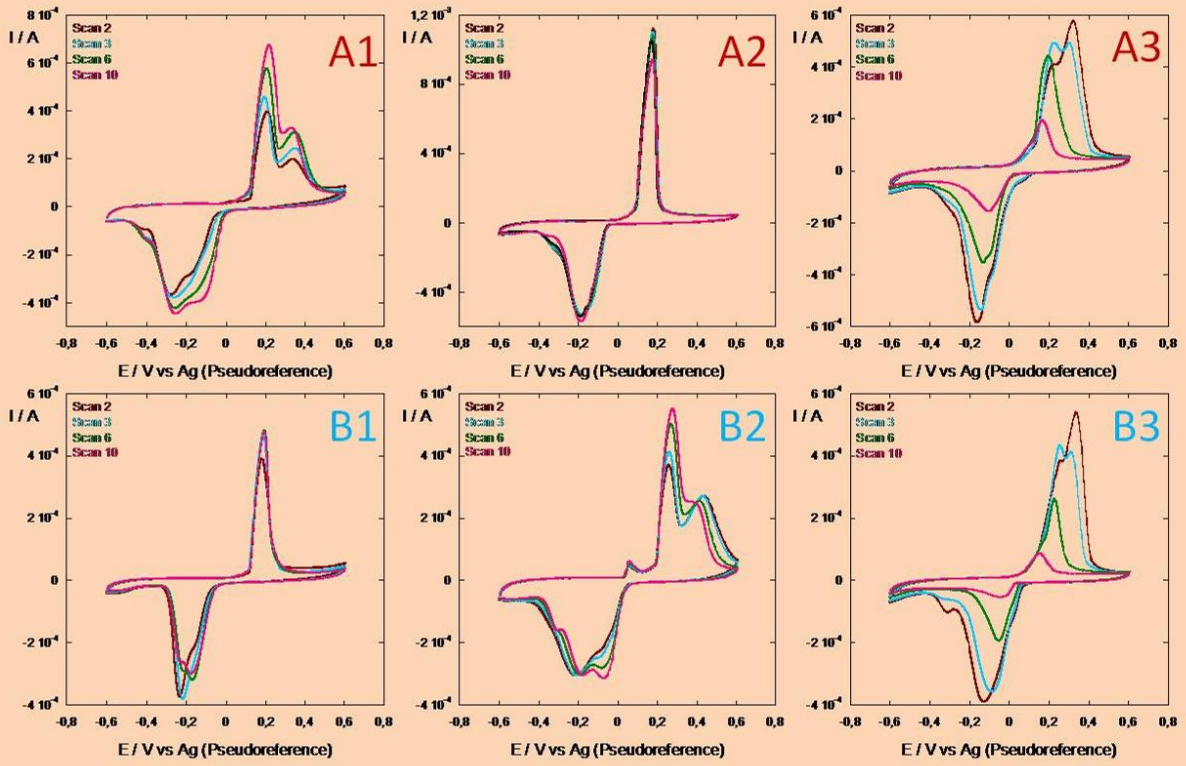

Fig. 5- Cyclic voltammograms recorded at 100 mV s^{-1} on SPE/CNTs of DA 32 pM in 0.1 M phosphate buffer solutions of different pH: A1) DA adsorption at pH 7.0; A2) Solution pH9.2 after A1) and A3) Solution pH3.2 after A2); B1) DA adsorption at pH 9.2; B2) Solution pH7.0 after B1) and B3) Solution pH3.2 after B2).

Effect of scan rate on cyclic voltamogramms of DA

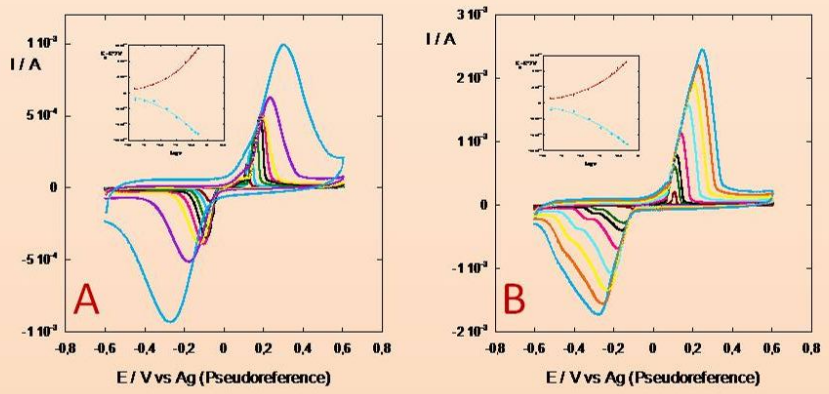

Fig. 6- Cyclic voltammograms of DA 0.32 pM at SPE/CNTs using different scan rates: 5; 10; 20; 50; 100; 200; 300; 400 and 500 mV s^{-1} in 0.1 M phosphate buffer solutions: A) pH 9.2 and B) pH 7.0. Inset: Laviron plots [6].

Conclusions

The voltammetric response of DA at SPE/CNTs is characterized by:

- pH 9.2: one well-defined anodic and one cathodic peak
- pH 7.0: two anodic and three cathodic peaks
- pH 3.2: one anodic and one cathodic peaks of lower intensities
- The current intensity of the voltammograms tend to increase by voltammetric cycling until about 12 scans
- Peaks current increase with scan rates (5 - 500 mV s^{-1}), although the variation was linear only for the lower values
- Epc - Epa tend to increase with scan rate
- Based on Laviron´s model values of 0.73 for αn and 0.65 for $(1-\alpha)n$ were obtained from the cathodic and anodic peak data, respectively, for experiments conducted at pH 7.0.

Maximum values of Ipc and Ipa were attained for:

- a cathodic potential of-0.35 V
- an anodic potential of 0.2 V

References

1. Inverson, F., Truelove, J., Nat Toxins 1994, 2, 334–339.
2. Pineiro, N., Vaquero, E., Leao, J. M., Gago-Martinez, A., Vazquez, J. A. Rodriguez, Chromatographia 2001, 53, S231-S235.
3. Kawatsu, K., Hamano, Y., Noguchi, T.,Toxin 1999, 37, 1579–1589.
4. Micheli, L., Radoi, A., Guarrina, R., Massaud, R., Bala, C., Moscone, D., Palleschi, G., Biosens Bioelectron 2004, 20 (2), 190-196.
5. Kreuzer, M. P., Pravda, M., O'Sullivan, C. K., Guilbault, G. G., Toxicon 2002, 40 (9), 1267-1274.
6. Laviron, E., J. Electroanal. Chem. 1979, 101, 19-28.

Acknowledgements: The authors thank to Ana Gago Team (UVigo) for their kind help and DA gift