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Development of an electroanalytical method to control quality in fish samples based on an edge plane pyrolytic graphite electrode. Simultaneous determination of hypoxanthine, xanthine and uric acid☆

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ABSTRACT

A simple, rapid and non-expensive method is proposed to determine hypoxanthine (Hx), xanthine (Xa) and uric acid (UA) in untreated fish samples based on an edge plane pyrolytic graphite electrode (EPPGE). The square wave voltammetry was the electrochemical technique used. In addition, the experimental design and multi-response assays were used to optimize the pretreatment of the working electrode. Therefore, the EPPGE is used in the simultaneous determination of these analytes for the first time. The optimal accumulation conditions of the analytes on the surface of the working electrode were obtained through a composite central design. The linear range was from 0.1 to 50 μM for Hx and Xa, and from 0.1 to 25.0 μM for UA. The limits of detection (LOD) were 0.08, 0.06 and 0.03 μM for Hx, Xa and UA, respectively. Recovery assays were made in order to validate the proposed method. Recovery percentages were between 90 and 110%. Therefore, the method is a good, simple and fast option for the simultaneous determination of Hx, Xa and UA in fish samples, allowing to perform a control quality of samples.

1. Introduction

Hypoxanthine (Hx; 1H-purin-6(9H)-one) and xanthine (Xa; 3,7-dihydropurine-2,6-dione) are intermediates, while uric acid (UA; 7,9-dihydro-1H-purine-2,6,8(3H)-trione) is the end product in the purine metabolism of plants and animals [1]. This mechanism is well known and it starts with the degradation of adenosine triphosphate (ATP) to adenosine diphosphate (ADP). Then, ADP is degraded to adenosine monophosphate (AMP), which is converted to inosine monophosphate (IMP). IMP is transformed to inosine (IN), which is converted to Hx. Hx is then transformed to Xa, and finally Xa is converted to UA [2]. Thus, this mechanism is a typical process of decomposition of ATP in fish meat after its death [3].

Based on the mechanism previously described, Hx, Xa and UA play an important role in fish quality control. Hx content is used to estimate the freshness of fish [4]. There are several numerical indexes to determine the freshness of fish meat. It has been discovered that the concentration of Hx gives a good idea of the time elapsed since the death of the fish [5,6]. Thus, the Hx content in the fish sample is taken into account to estimate or calculate such indexes. On the other hand, the determination of Xa in tissue sample is essential for the diagnosis of different diseases, such as gout, hyperuricemia, xanthinuria, and renal failure. Thus, Xa determination is of clinical and industrial importance, and has great relevance in the quality control of fish samples [7,8]. In addition, the control of UA in fish tissue is important because brings in relevant information in the clinical control of the sample [9,10].

There are in the literature a large number of methods for the simultaneous determination of Hx, Xa and UA, such as high-performance liquid chromatography (HPLC) [11,12], capillary electrophoresis (CE) [13,14], and electrochemical methods [15–26]. The electrochemical methods have several advantages over the other methods mentioned such as low cost, high accuracy, precision, sensitivity, and selectivity. However, electrochemical methods may present some problems such

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as lack of reproducibility of the electrode surface, passivation of the surface of the working electrode, etc. Therefore, the electrochemical pretreatment of the surface of working electrode is an interesting alternative to solve these problems. Several papers have been published related to this subject [27–29]. It is very important, before beginning the process of pretreatment of the working electrode surface, to define which are the most important variables that must be taken into account. Thus, the pH and the composition of the reaction medium, the potential and time applied at the working electrode, are parameters which must be taken into account [30–32].

The use of chemometric tools is very important in the area of electroanalysis, mainly the experimental design. The application of this methodology has great benefits such as the reduction in the number of experiments, and as a consequence a reduction also in the use of reagents. In addition, several parameters may be simultaneously optimized, and the possible interaction between then can also be studied, which it is not possible when the univariate optimization is applied [33,34]. The use of multi-responses methodology is an interesting alternative to optimize simultaneously different parameters in the same experiment. It has been applied in different electroanalytical methods [34–36].

In this work we developed an electroanalytical method to determine the Hx, Xa and UA content in Argentinian fish samples, which can give information about of the quality of the treated samples. Therefore, we used the anodic stripping voltammetry applied to a preanodizated edge plane pyrolytic graphite electrode (EPPGE) to perform these studies.

Some advantages of EPPGE respect to other electrodes were previously explored by other research groups [37–39]. The electrode preanodization was studied in three different media (sulfuric acid, phosphate buffer and sodium hydroxide solutions), and experiments for this preanodization were carried out using a composite central design: 2^2 + star. The desirability function (multiresponse optimization) was used to obtain the preanodization conditions which give the best signals for each analyte. In addition, the accumulation potential and accumulation time were also optimized using the same experimental design previously mentioned before performing the anodic stripping voltammetry measurements. Finally, the method was successfully tested using fish samples, and it was validated through recovery assays.

2. Experimental

2.1. Fish samples

Fish samples were obtained from commercial markets of Río Cuarto, Córdoba, Argentina. They were stored in a freezer at −18 °C before the analysis. Approximately 5 g of fresh fish meat were weighted and homogenized with 10 mL of 0.1 M phosphate buffer solution (PBS) pH 7.00 with the help of a mortar and a pestle. The supernatant solution was filtered and brought to 25 mL volume with 0.1 M PBS pH 7.00 [9]. An aliquot of this solution was used to determine the Hx, Xa and UA content using the standard addition method. We used four fish samples, two from seawater and two from river water. The two seawater samples were Tuna (*Gasterochisma melampus* M1) and Hake (*Merluccius hubbsi* M2), and the two river water samples were Pacu (*Myleus pacu* M3) and Silverside (*Odontesthes nigricans* M4) [40].

2.2. Reagents and solutions

All reagents were of analytical grade. Ultrapure water (ρ = 18 MΩ cm^{-1}) was obtained from a Millipore-Milli Q system. Hx, Xa and UA were purchased from Sigma–Aldrich. H_2SO_4, NaOH, K_2HPO_4 and KH_2PO_4 were Merck p.a. Stock solutions of Hx, Xa and UA were prepared in PBS, protected from light, and kept in the refrigerator. Working solutions were prepared daily by adding different aliquots of stock solutions to 0.1 M PBS pH 7.00.

2.3. Instrumentation

Voltammetric measurements were performed with an Epsilon potentiostat (BASi-Bioanalytical System, USA) and run with an electrochemical analysis software. A C3 cell stand (BASi-Bioanalytical System, USA) was used for all experiments.

The electrodes were inserted into the cell through holes in its Teflon cover. The working electrodes were a glassy carbon electrode (GCE) (area = 0.071 cm^2) and an EPPGE (area = 0.283 cm^2). A platinum wire and Ag/AgCl, 3 M NaCl (BAS, RE-5B) were used as counter and reference electrodes, respectively.

2.4. Pretreatment of working electrodes

Before each measurement, both EPPGE and GCE were polished manually with alumina 0.05 μm for 120 s, and later sonicated in bi distillated water for the same time. After that, EPPGE and GCE were immersed in PBS and six cyclic voltammograms were recorded in the potential range between −0.20 V and 1.40 V at a scan rate of (v) 0.100 V s^{-1} in PBS to stabilize the electrode surface before performing the anodization process. The anodization potential (E_{an}), and the anodization time (t_{an}) were 2.21 V and 3 s, respectively. These parameters were optimized, using the composite central design: 2^2 + star. In the optimization process, we use 0.5 mM for the concentration of each analyte and three different supporting electrolytes 0.1 M NaOH, 0.1 M PBS pH 7.00, and 0.1 M H_2SO_4. The levels studied for each factor are shown in Table 1.

The desirability function (D) was used to obtain the "maximum response" for Hx, Xa and UA. The general approach of desirability function is to transform each response into an individual desirability function (d_i) that varies from 0 to 1 (lowest to highest desirability) (Eq. (1)) [41]. This equation is used to maximize the overall desirability (D), and values L and H are the lower and higher observed responses for each independent variable, and y_i is the individual value of the response. Then, each value of d (corresponding to an analytical signal of interest) is linearly combined to obtain the overall desirability (D) (Eq. (2)).

$$di = \frac{yi - L}{H - L} \tag{1}$$

$$D = \sqrt[k]{(d1\ \ d2\ \ d3\ldots \cdot dk)} \tag{2}$$

Table 1
Factors, their levels and experiments corresponding to the anodization process.

Factors			Levels	
			Low (−)	High (+)
Anodizing time (t_{an})/s			5	25
Anodizing potential (E_{an})/V			1.0	2.0
Run	E_{an}/V	t_{an}/s		
1	2.2	15		
2	1.5	29		
3	1.0	25		
4	1.5	15		
5	1.5	15		
6	2.0	5		
7	1.5	15		
8	2.0	25		
9	1.0	5		
10	1.5	15		
11	1.5	1		
12	0.8	15		
13	1.5	15		