Multivariate determination of total sugars content and ethanol in bioethanol production using electrodes glassy carbon modified with MWCNT/MeOOH and chemometric data treatment.

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ABSTRACT

This work reports characterization and application of a voltammetric electronic tongue using an array of glassy carbon electrodes modified with multi-walled carbon nanotubes containing metal (Pd, Au, Cu) and oxy-hydroxide nanoparticles (MetalsOOH of Ni, Co) towards the determination of total sugar content in products related with sugarcane-bioethanol production. The Artificial Neural Network (ANN) based prediction model has resulted satisfactory for the carbohydrate sum and the obtained response had shown an adequate accuracy. Voltammetric data was first adapted for the computation using the Fast Fourier transform, and results from the electronic tongue approach were compared with use of different electrodes alone. Final performance was better using uniquely the Ni oxy-hydroxide modified electrode, especially in the quantification of ethanol.

Keywords: Electronic Tongue, Carbohydrates, Artificial Neural Network, Multi-walled carbon nanotubes, Metal nanoparticles, Second generation ethanol.

1. INTRODUCTION

In the production process of fuel ethanol and sugar, both products of sugarcane industries, a factor of great importance is the control of losses in the process attributed to reducing sugars. Reducing sugars refer to the percentage of reducing sugars (glucose and fructose) present in the juice. The control of reducing sugars is associated with the entire industrial process from the payment of the producer to the production of sugar and ethanol [1].

Ethanol assay is very important and necessary in numerous industries and biotechnological processes such as production of wine, beer, foodstuffs, cosmetics and pharmaceutical products [2]. The alcoholic fermentation is a crucial step in such production process, frequent measurements are essential in order to optimized the process and to obtain a high yield of the desired product, in particular, continuous monitoring of sugars and ethanol is very important in the bioprocess processing of sugars into ethanol, should give important information on the progress of the reactions [3-5]. Among the factors that inhibits fermentation the ethanol stress is considered to be the major hindrance to the complete fermentation and higher production of ethanol, as the accumulation of ethanol during the fermentation process results in the inhibition of fermentative microorganisms [6]

Various analytical methods have been reported for the determination of sugars and ethanol such as, high performance liquid chromatography HPLC)/ refractometer detector [7], fluorimetry [8, 9], colorimetry [10], spectrophotometry [11], gas chromatography (GC)/mass spectrometry (MS) [6], capillary electrophoresis [12] and near infrared spectroscopy [13]. Besides that to such many of this equipment are high cost, laboratory conditions and trained personnel, electrochemistry has the characteristic of convenience, low cost, and simplicity [1].

Abundant applications of enzymatic sensors for sugars [14] and ethanol [2] are reported in literature, with the majority dedicated to glucose in contrast to non-enzymatic ones. In their description, there is a balance of advantages and disadvantages of which both are significant. Despite dominating the glucose sensor market, enzymatic systems have a number of critical flaws such as high

oxygen dependency, and can be therefore questioned for maximum reliability. Furthermore, the sensory ability of enzymatic sugar biosensors may be highly impacted by the presence of other electroactive interferences in the sample that are always commonplace in real industrial samples and it is still constrained by usage of mildly enzymic conditions, such as pH ranges of 2-8, temperatures below 44°C, and ambient humidity levels [14, 15].

Sensitive and selective sugar and ethanol sensors are relevant for use in blood sugar monitoring, food industry, bio-processing and in the development of renewable and sustainable fuel cells.

Many efforts have been made to find new electrocatalytic materials for oxidation of fructose, glucose and ethanol such as, cobalt hydroxide nanoparticles electrodeposited on the surface of glassy carbon electrode [16], multi-wall carbon nanotubes containing copper oxide nanoparticles [17], nickel hydroxide modified electrode [5], nickel hydroxide nanoparticles on boron-doped diamond electrodes[18], copper(II) oxide nanorod bundles [19], gold nanoparticle-constituted nanotube array electrode[20], palladium-nickel and palladium coated electrodes prepared by electrodeposition from ionic liquid[21], palladium nanoparticles distributed on surfactant-functionalized multi-wall carbon nanotubes [22], nickel hydroxide deposited indium tin oxide electrodes [23], nanoporous platinum–cobalt alloy electrode [24] and glassy carbon electrode decorated with multi-wall carbon nanotubes with nickel oxy-hydroxide [25].

A new methodology in the sensors field is the use of one sensor with chemometric data treatment or use of sensors in arrays coupled with complex data treatment, that is, the use of electronic tongues; these are versatile sensor systems capable to simultaneously monitor the level of different analytes, or analytes in presence of their interferents, or to resolve mixtures of similar analytes [26-31].

An electronic tongue is a multisensor system, which consists of a number of low-selective sensors and uses advanced mathematical procedures for signal processing based on Pattern Recognition and/or Multivariate data analysis – Artificial Neural Networks (ANNs), Principal Component Analysis (PCA), etc. [32] Therefore, the electronic tongue is an analytical system applied to liquid analysis formed by a sensor array in order to generate

multidimensional information, plus a chemometric processing tool to extract meaning from these complex data [27, 33].

(manel veja se vamos colocar essa parte) From the beginning of this technique, there are electronic tongue devised using potentiometric sensors, but also using of the voltammetric type. In these original works, originated at the laboratories of Prof. Winquist in Linkoping (Sweden), the sensor array was formed by an array of different metallic disc electrodes, and a scanning voltammetric technique was used to generate the analytical information [31]. The voltammetric principle has also been applied to develop electronic tongues since its early years, such as simultaneous identification and quantification of nitro-containing explosives by advanced chemometric data treatment of cyclic voltammetry at screen-printed electrodes [34], instrumental measurement of wine sensory descriptors using a voltammetric electronic tongue [33], evaluation of red wines antioxidant capacity by means of a voltammetric e-tongue with an optimized sensor array[35], voltammetric electronic tongue for the qualitative analysis of beers [27] and cava wine authentication employing a voltammetric electronic tongue [36].

The present work reports the application of a sensor using glassy carbon electrode modified with multi-wall carbon nanotubes decorated with Nickel oxy-hydroxide nanoparticles (GCE/MWCNT/NiOOH) towards the analysis of total sugars (fructose and glucose) and ethanol using chemometric data treatment. The analytical response of the GCE/MWCNT/NiOOH sensor was compared with electronic tongue formed by glassy carbon electrode modified with multi-wall carbon nanotubes decorated with metal (Paladium, Gold, Copper, Cobalt and Nickel) oxy-hydroxide nanoparticles (GCE/MWCNT/MetalsOOH). As such, it combines the responses from GCE/MWCNT/NiOOH sensor and voltammetric electronic tongue formed by GCE/MWCNT/MetalsOOH modified electrodes, plus an advanced response model employing a specifically trained Artificial Neural Networks (ANNs), with pretreatment of the data was compressed using the Fast Fourier transform (FFT). This preprocessing is needed given the high dimensionality of the considered data.

2. MATERIALS AND METHODS

2.1. Reagent and Instruments

Purified multiwalled carbon nanotubes (MWCNTs) with an outer diameter of 30 nm were purchased from SES Research (Houston, Texas, USA). Cobalt (II) chloride hexahydrate ($Cl_2Co.6H_2O$), nickel(II) sulfate hexahydrate ($NiSO_4.6H_2O$), palladium chloride ($PdCl_2$), ammonium fluoride (NH_4F), boric acid (H_3BO_3), sodium borohydride ($NaBH_4$), N-N-Dimethylformamide 99.8% (C_3H_7NO), D-Glucose, D-Fructose and sodium hydroxide (NaOH) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ethanol 96% v/v was purchased from Scharlau (Spain). Sulphuric acid 96% (H_2SO_4), nitric acid 69%(HNO_3) and ammonia 32% (NH_3) were purchased from Merck (Darmstad, Germany). All solutions were made using MilliQ water from MilliQ System (Millipore, Billerica, MA, USA). The buffer used for formation of nickel and cobalt nanoparticles was phosphate buffer (0.1 mol L^{-1} Na_2HPO_4 and 0.1 mol L^{-1} mono-hydrated NaH_2PO_4 pH 7.0).

2.2. Measuring procedure

The amperometric measurement cell was formed by the 5 (five) GCE/MWCNT/MetalsOOH modified electrodes plus a reference double junction Ag/AgCl electrode (Thermo Orion 900200, Beverly, MA, USA) and a commercial platinum counter electrode (Model 52-67, Crison Instruments, Barcelona, Spain). Cyclic voltammetry measurements were taken using a 6-channel AUTOLAB PGSTAT20 potentiostat (Ecochemie, Netherlands), in multichannel configuration, using the GPES Multichannel 4.7 software package. For this, potentials were cycled between -0.7 V and +0.8 V vs. Ag/AgCl, with a scan rate of 50 mV s^{-1}. Electroanalytical experiments were carried out at room temperature (25 °C) under quiescent conditions. Briefly, samples were measured as described with the GCE/MWCNT/MetalsOOH sensor voltammetric array, and afterwards obtained responses were analysed by means of the different chemometric tools described below.

2.3. Experimental design for the quantification model

In order to prove the capabilities of the GCE/MWCNT/NiOOH sensor and Electronic Tongue (ET), the simultaneous quantification of different sugars and ethanol was attempted. To this aim, a total set of 24 samples were manually prepared with a concentration range of 0.5 to 5.0 mM for each compound. The set of samples was divided into two data subsets: a training subset formed by 16 samples (66.6%) which were distributed based on a 42 factorial design, with 2 factors and 4 levels, and used to build the response model, plus 8 additional samples (33.3%) for the testing subset, distributed randomly along the experimental domain and used to evaluate the model predictive ability.

2.4. Data processing

In order to reduce the large dataset generated for each sample (5 sensors x 336 current values at different potential) a preprocessing stage was necessary to compress the original data. The objective of this step was to reduce the complexity of the input data while preserving the relevant information, the compression of the data allows also to reduce the training time, to avoid redundancy in input data and to obtain a model with better generalization ability.

The data was compressed using the Fast Fourier transform (FFT) [Ref]. In this manner, the original data was reduced to 32 coefficients for each voltammogram without any loss of relevant information. With this, the 1680 inputs per sample were reduced down to 160 coefficients, achieving a total compression ratio up to 90.48%.

2.5. Preparation of electrodes for electronic tongue

2.5.1. Decoration of MWCNTs with Pd, CuO and Au nanoparticles

Before the modification with metal nanoparticles MWCNTs were purified with an acidic pre-treatment. Briefly, the MWCNTs were dispersed in a 3:1 mixture of concentrated H_2SO_4/HNO_3 acids using an ultrasonic bath for 90 min [37]. Purified MWCNTs were then used for further modification with different metal nanoparticles.

2.5.2. Palladium decorated multi-wall carbon nanotubes (MWCNT/Pd)

This modification was performed following the protocol reported by Cipri et al. [37]. Briefly, a flask was prepared with a solution consisting of $PdCl_2$ (14.0×10^{-3} mol L^{-1}), H_3BO_3 (1.57×10^{-1} mol L^{-1}) and NH_4F (6.67×10^{-2} mol L^{-1}). Then, purified MWCNTs (100 mg) were dispersed in the above solution via ultrasonication for approximately 1 h and the pH was adjusted to 8–9. A solution of $NaBH_4$ (17 mL) was added dropwise under vigorous stirring and followed by another 8h of stirring to complete the reaction. The MWCNT/Pd were filtered, washed and then dried under vacuum at 50°C.

2.5.3. Gold decorated multiwalled carbon nanotubes (MWCNT/Au)

The decoration with Gold was performed following and slightly modifying the procedure reported by Shi et al. [38]. Briefly, a flask was prepared with a solution of 0.5 mL of $HAuCl_4 x 3H_2O$ (1.0×10^{-2} mol L^{-1}), 0.5 mL of an aqueous solution of trisodium citrate (1.0×10^{-2} mol L^{-1}) and 18.4 mL of double distilled water. Purified CNTs (20 mg for a Au loading ratio of 10% wt%) were added to the above solution and then 10-12 mL of ethanol were immediately introduced under vigorous stirring and the mixture was ultrasonicated for 10 min. Ice-cold freshly prepared $NaBH_4$ aqueous solution (0.6 mL, 0.1 mol L^{-1}) was then added to the above mixture while stirring and leaving it overnight. The MWCNT/Au were separated by centrifuging, washed with double distilled water for several cycles and then dried under vacuum overnight at 50 °C.

2.5.4. Carbon nanotubes integrated with copper oxide nanoleaves (MWCNT/CuO)

The modification with copper was performed by modifying the procedure reported by Yang et al. [39]. Briefly, in a flask sodium dodecyl sulfate (SDS) was dissolved (0.1 mol L^{-1}) in 300.0 mL of distilled water stirring at 60°C; Then 50 mg of purified MWCNTs were ultrasonically dispersed in 25.0 mL of DMF and then added to the SDS solution and ultrasonicated for 10 min, then $CuSO_4 x 5H_2O$ (2.5 g) was added to the above solution and ultrasonicated for 10 min. The solution of 100 mL of NaOH (0.5 mol L^{-1}) was slowly dropped into the

above solution and then stirred for 10.0 min. The resulting solution was then centrifuged, washed with doubly distilled water for few cycles and then dried at 60°C.

2.5.5. Glassy carbon surface modification GCE/MWCNT/PdOOH

All surfaces of the GC electrodes were polished with 0.3 µm alumina powder (Merck) and cleaned in ethanol and Milli-Q water.

2.0 mg MWCNT/Pd were dispersed in 1.0 mL N,N-dimethylformamide (DMF) with a ultrasonic bath to give a 2.0 mg mL^{-1} black solution. 3 steps of 10 µL of the black solution were dropped on the GC electrode surface to prepare the GCE/MWCNT/Pd electrode surface at the end of each step, the electrode was allowed to dry at 50 °C for 3.0 hours. After modification, the glassy carbon electrode modified with carbon nanotubes containing palladium nanoparticles (GCE/MWCNT/Pd) was passivated to form palladium oxy-hydroxide using a solution of NaOH 0.1 mol L^{-1} in the potential range of -0.8 to 0.8 V at a scan rate of 50 mV.s^{-1} for 20 cycles in cyclic voltammetry. Then, the GCE/MWCNT/PdOOH was washed thoroughly with deionized water and dried. The electrochemical behavior of the GCE/MWCNT/PdOOH electrode was evaluated in aqueous solutions with supporting electrolyte NaOH 0.1 mol L^{-1}.

2.5.6. GCE/MWCNT/AuO

2.0 mg MWCNT/Au were dispersed in 1.0 mL N,N-dimethylformamide (DMF) with a ultrasonic bath to give a 2.0 mg mL^{-1} black solution. 3 steps of 10 µL of the black solution were dropped on the GC electrode surface to prepare the GCE/MWCNT/Au electrode surface. At the end of each step the electrode was allowed to dry at 50°C for 3.0 hours. After each modification the glassy carbon electrode modified with carbon nanotubes containing gold nanoparticles (GCE/MWCNT/Au) was passivated to form gold oxide using a solution of NaOH 0.1 mol L^{-1} in the potential range of -0.4 to 0.5 V at a scan rate of 50 mV s^{-1} for 20 cycles in cyclic voltammetry. Then, the GCE/MWCNT/AuO was washed thoroughly with deionized water and dried. The electrochemical behavior of the GCE/MWCNT/AuO electrode was evaluated in aqueous solutions with supporting electrolyte NaOH 0.1 mol L^{-1}.

2.5.7. GCE/MWCNT/CuOOH

2.0 mg MWCNT/Cu was dispersed in 20.0 mL N,N-dimethylformamide (DMF) with a ultrasonic bath to give a 0.1 mg mL^{-1} black solution. 2 steps of 10 µL of the black solution were dropped at the GC electrode surface to prepare the GCE/MWCNT/Cu electrode surface at the end of each step, the electrode was dried at 50°C for 3.0 hours. After each modification the glassy carbon electrode modified with carbon nanotubes containing copper nanoparticles (GCE/MWCNT/Cu) was passivated to form copper oxy-hydroxide with a solution of NaOH 0.1 mol L^{-1} in the potential range of -0.5 to 0.3 V at a scan rate of 50 mV s^{-1} for 20 cycles in cyclic voltammetry. Then, the GCE/MWCNT/CuOOH was washed thoroughly with deionized water and dried. The electrochemical behavior of the GCE/MWCNT/CuOOH electrode was evaluated in aqueous solutions with supporting electrolyte NaOH 0.1 mol L^{-1}.

2.5.8. GCE/MWCNT/CoOOH

1.0 mg MWCNT was dispersed in 10.0 mL N,N-dimethylformamide (DMF) with a ultrasonic bath to give a 0.1 mg mL^{-1} black solution. 5.0 µL of the black solution was deposited at the GC electrode surface to prepare the GCE/MWCNT electrode surface. Immediately after the glassy carbon electrode was modified with carbon nanotubes (GCE/MWCNT) cobalt nanoparticles were electrodeposited using 1.0×10^{-3} mol L^{-1} of CoCl$_2$ in phosphate buffer 0.1 mol L^{-1} (pH 6.5). The electrodeposition was carried out by cyclic voltammetry in a potential range of -1.1 to 1.2 V at a rate of 100 mV.s^{-1} for 30 cycles [40]. To complete the modification, the glassy carbon electrode modified with carbon nanotubes containing cobalt nanoparticles (GCE/MWCNT/Co) was passivated to form cobalt oxy-hydroxide at NaOH 0.1 mol L^{-1} in the potential range of -0.3 to 0.7 V at a scan rate of 50 mV s^{-1} for 45 cycles in cyclic voltammetry. Then, the GCE/MWCNT/CoOOH was washed thoroughly with deionized water and dried. The electrochemical behavior of the GC/MWCNT/CoOOH electrode was evaluated in aqueous solutions with supporting electrolyte NaOH 0.1 mol L^{-1}.

2.5.9. GCE/MWCNT/NiOOH

1.0 mg MWCNT was dispersed in 10.0 mL N,N-dimethylformamide (DMF) with a ultrasonic bath to give a 0.1 mg mL^{-1} black solution. 5.0 µL of the black solution was deposited at the GC electrode surface to prepare the GC/MWCNT electrode surface. Immediately after the glassy carbon electrode was modified with carbon nanotubes (GCE/MWCNT) a solution of NiSO$_4$ (5.0×10^{-3} mol L^{-1}), in phosphate buffer 0.1 mol L^{-1} (pH 6.5), was used for the electrodeposition of nickel nanoparticles. The electrodeposition was carried out by cronoamperometry at a potential of -1.3 V for 30 seconds. To complete the modification, the glassy carbon electrode modified with carbon nanotubes containing nickel nanoparticles (GCE/MWCNT/Ni) was passivated to form nickel oxy-hydroxide at NaOH 0.5 mol L^{-1} in the potential range of -0.5 to 1.0 V at a scan rate of 100 mV s^{-1} for 30 cycles in cyclic voltammetry [41]. Then, the GCE/MWCNT/NiOOH was washed thoroughly with deionized water and dried. The electrochemical behavior of the GCE/MWCNT/NiOOH electrode was evaluated in aqueous solutions with supporting electrolyte NaOH 0.1 mol L^{-1}.

3. RESULTS AND DISCUSSION

3.1. GCE/MWCNT/Metals oxy-hydroxide modified electrodes response

The voltammetric response for each of the electrodes towards individual compounds was the first response feature checked. That is, to ensure that enough differentiated signals were observed for the different electrodes, generating rich data that might be a useful departure point for the multivariate calibration model.

To this aim and under the described conditions in Section 2.5, individual standard solutions of total sugars (fructose and glucose) and ethanol were analyzed and their voltammograms inspected Figure 1 displays the voltammetric behavior using the electronic tongue formed by GCE/MWCNT/MetalsOOH modified electrodes for the electrooxidation study of total sugars and ethanol.

In Figure 1 (A) is shown the voltammogram of GCE/MWCNT/PdOOH total sugars and ethanol electrode during electro-oxidation of total sugars that occurs at potentials around -0.1 V vs. Ag/AgCl and ethanol that occur at potential around -0.3 vs. Ag/AgCl, associated with an anodic peak current and decrease in cathodic peak current, the results suggest that Pd(II)/Pd(I) redox couple in form of PdOOH [42] can catalyze the electro-oxidation of total sugars and ethanol.

In Figure 1 (B) is shown the voltammogram of GCE/MWCNT/AuO electrode during electro-oxidation of total sugars that occurs at potential around 0.25 V vs. Ag/AgCl, with the appearance of the anodic peak current and cathodic peak current of reoxidation at potentials -0.07 V vs. Ag/AgCl typical behavior of gold electrodes, electrocatalytic activity which can be rationalized by the incipient hydrous oxide/adatom model can catalyzes the oxidation of sugars [20, 43, 44], however, it is not possible to observe the oxidation peak for ethanol at voltammogram, indicating that the GCE/MWCNT/AuO electrode does not cause electrooxidation for ethanol.

The voltammogram of GCE/MWCNT/CuOOH shown in Figure 1(C) in electro-oxidation of sugars occurs at potential around 0.60 V vs. Ag/AgCl, with the appearance of the anodic wave. During the positive scan, the Cu nanoparticles can be oxidized to CuOOH. The Cu(III)/Cu(II) redox couple can catalyzed the oxidation of carbohydrates [45-47], however, it is not possible to observe the oxidation peak for ethanol at voltammogram, indicating that the GCE/MWCNT/CuOOH electrode does not cause electro-oxidation for ethanol.

The electro-oxidation of total sugars and ethanol on GC/MWCNT/NiOOH electrode as shown in the Figure 1 (D) occurred at potential around 0.55 V vs. Ag/AgCl associated with increased anodic peak current and decrease in cathodic peak current and the electro-oxidation of ethanol occurred at potential around 0.52 V vs. Ag/AgCl . The results suggest that Ni(III)/Ni(II) redox couple can catalyze the electro-oxidation of compounds [25].

The voltammogram of GCE/MWCNT/CoOOH shown in Figure 1(E) in electro-oxidation of total sugars occurs at potential around 0.65 V vs. Ag/AgCl, with the appearance of the anodic wave. This result indicates that sugars are oxidized by CoOOH species through Co(IV)/Co(III) redox couple moiety and through a cyclic mediation redox process [16, 48], however, it is not possible to

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observe the oxidation peak for ethanol at voltammogram, indicating that the GCE/MWCNT/CoOOH electrode does not cause electrooxidation for ethanol.

The results suggest that the GCE/MWCNT/MetalsOOH modified electrodes can catalyze the electro-oxidation of glucose to ketone forming gluconolactone [21]. In fructose oxidation procedure, glucose is generated through fructose enolization reaction [49]. Besides, clearly differentiated curves are obtained for each modified electrode, giving the desirable condition for an Electronic Tongue (ET) study.

This cross-response nature of the voltammograms can be summarized when plotting max currents (sensitivities) and oxidation peak potential observed for the 5 MWCNT/Metals Nanoparticle modified electrodes. This representation is shown in Figure 2, where can be observed that total sugars and ethanol anodic peak current (Ipa) is different according to each metal nano-composite of the ET formed by GCE/MWCNT/Metals oxy-hydroxides modified electrodes and was also observed that the oxidation potentials (Epa) showed different behaviors according to each metal, this is a desirable condition for any ET study.

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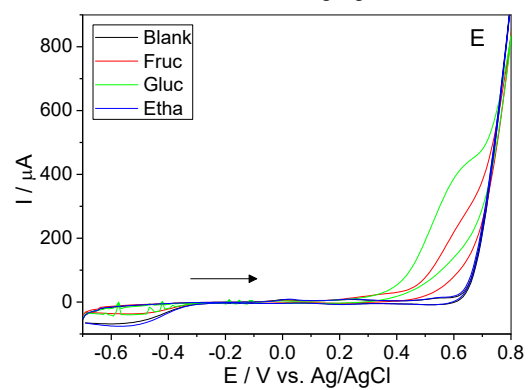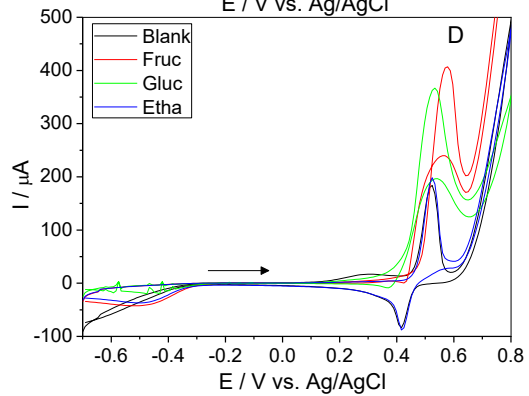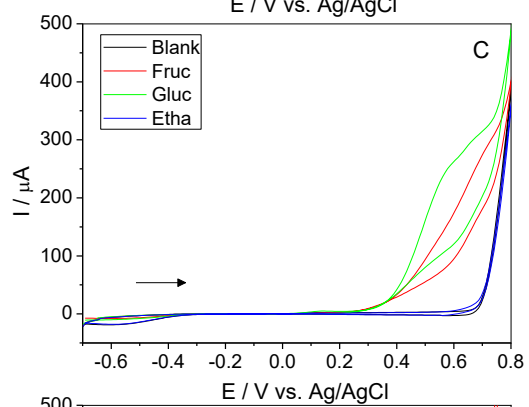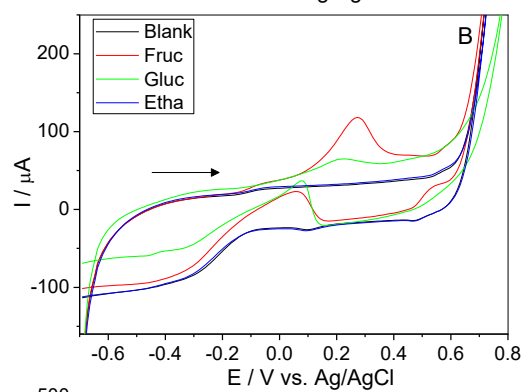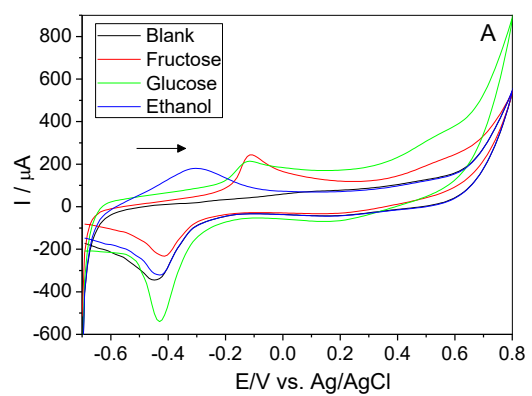
Figure 1: Example of the different voltammograms obtained with (A) GCE/MWCNT/PdOOH, (B) GCE/MWCNT/AuO, (C) GCE/MWCNT/CuOOH, (D) GCE/MWCNT/NiOOH and (E) GCE/MWCNT/CoOOH for 0.1 mol L^{-1} NaOH and stock solutions 5.0×10^{-3} mol L^{-1} of fructose, glucose and ethanol. (blank) Absence, (Fruc) Fructose, (glu) Glucose and (Etha).
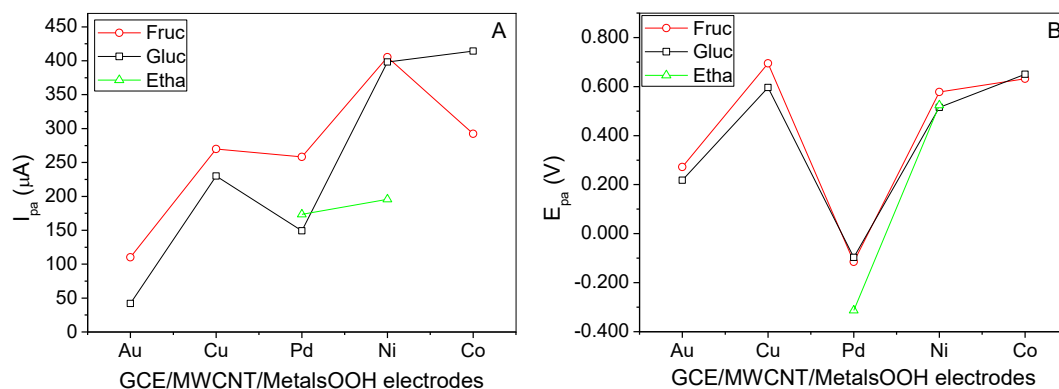
Figure 2: Studies of response (A) anodic peak current (Ipa) and (B) potential oxidation (Epa) for 5.0 mM of each of the two sugars (fructose and glucose) and ethanol for electronic tongue formed by GCE/MWCNT/Metals oxy-hydroxide nanoparticle modified electrodes (metals = gold, palladium, copper, nickel and cobalt).

The Principal Component Analysis (PCA) is one of the most important methods used in chemometrics and it is the basis for many standards recognition, is a way of reducing a large multivariate data matrix into a matrix with a much smaller number of variables, without losing important information within the data. The principle behind PCA is that the multivariate data can be decomposed by linear projections onto a new co-ordinate system. The new axes, known as principal components (PCs), are orientated so that the first PC captures the largest amount of common variance [50]. PCA can be one way to demonstrate the complementary of the generated information by each electrode, if electrodes are redundant they would appear superimposed, while different response will manifest in their separation. In Figure 3 is shown the PCA for the five GCE/MWCNT/MetalsOOH modified electrodes (metals = gold, palladium, copper, nickel and cobalt); the PCA was prepared by treatment of the sensitivities of total sugars and ethanol, was observed that each sensor

showed performance in different regions this is very positive because that is a desirable condition for any ET study, justifying the inclusion of the five prepared electrodes in the sensor array.
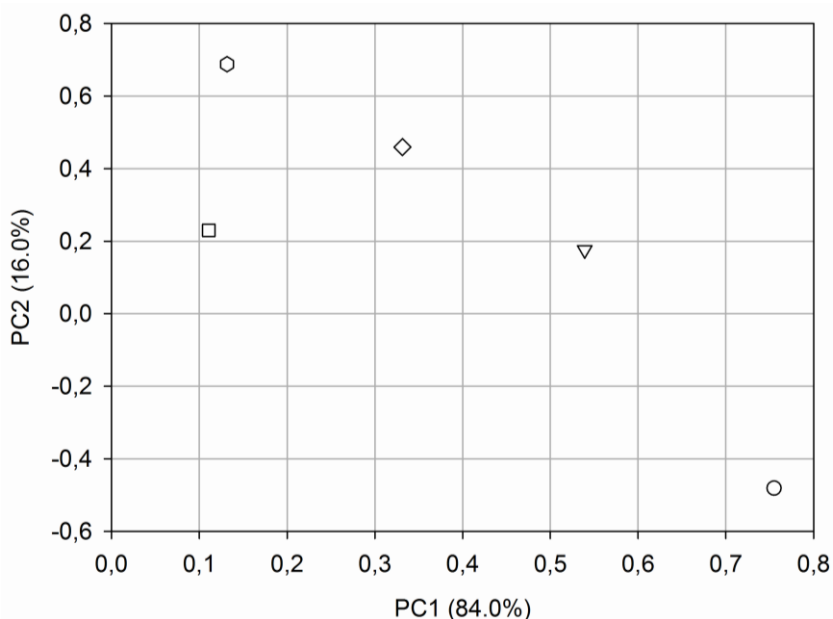
Figure 3: PCA analysis score plot for five GCE/MWCNT/Metals oxy-hydroxide nanoparticle modified electrodes (metals = (□) gold, (▽) palladium, (O) copper, (◇) nickel and (O) cobalt).

The analytical reproducibility (%RSD) for the sensors was estimated using standard solutions of 2×10^{-3} mol L^{-1} glucose measured along 3 different days, obtaining values for GCE/MWCNT/AuO of 3.28%, for GCE/MWCNT/PdO 6.89%, for GCE/MWCNT/CuOOH 3.85%, for GCE/MWCNT/NiOOH 1.73% and for GCE/MWCNT/CoOOH 6.35%. The analytical reproducibilities shown seem reasonable values, because the electrodes are subjected to analysis of many samples thus requiring maintain its reproducibility and stability.

Complete calibrations of considered sugars and ethanol were conducted in 0.1 mol L $^{-1}$ NaOH solution with scan rate of 50 mV s^{-1} in order to fully characterize the used sensors. It was observed in all cases an increase of anodic peak current which was linear with the increase of concentration of sugars and ethanol; Table 1 shows the electrochemical parameters for total sugars and ethanol studied. We observed that the electronic tongue formed by GCE/MWCNT/MetalsOOH modified electrodes has good amperometric sensitivity. The values of limit of detection and quantification are very close,

meaning that the behavior of the different total sugars and ethanol on the electrode is comparable. Therefore we may apply the electronic tongue in the same concentration range for total sugars and ethanol considered.

Table 1. Analytical parameters for fructose, glucose and ethanol at electronic tongue formed by GCE/MWCNT/Metals oxy-hydroxide modified electrodes in 0.1 mol L^{-1} NaOH by Cyclic Voltammetry (v = 50 mV s^{-1}; n = 3).

Compounds	Limit of Detection (mM)	Limit of Quantification (mM)	Sensitivity (µA mM^{-1})	Concentration range (mM)
Palladium				
Fructose	0.31	1.02	55.0	0.4 - 2.0
Glucose	0.32	1.06	25.5	0.4 - 5.0
Ethanol	0.32	1.06	23.7	0.4 - 8.0
Gold				
Fructose	0.20	0.66	16.0	0.4 - 2.0
Glucose	0.64	2.10	7.3	0.8- 5.0
Ethanol	n.d	n.d	n.d	n.d
Copper				
Fructose	0.31	1.02	49.9	0.4 - 5.0
Glucose	0.10	0.33	51.1	0.2 - 5.0
Ethanol	n.d	n.d	n.d	n.d
Nickel				
Fructose	0.15	0.49	47.5	0.2- 5.0
Glucose	0.23	0.76	32.0	0.4 - 5.0
Ethanol	0.33	1.09	6.4	0.4 - 5.0
Cobalt				
Fructose	0.21	0.69	48.3	0.4 - 5.0
Glucose	0.31	1.03	83.6	0.4 - 5.0
Ethanol	n.d	n.d	n.d	n.d

3.3 Building of the ANN Model

For the electronic tongue and GCE/MWCNT/NiOOH sensor studies, a total set of 24 total sugars and ethanol mixtures were manually prepared shown in Figure 4, 16 corresponding to the train subset and 8 to the testing subset with concentrations ranged from 0.5 to 5.0 mM for each compound. These standards were first analyzed and used to build and validate the ANN model under the conditions previously described, the training and testing sets of samples were measured employing the GCE/MWCNT/MetalsOOH modified electrodes array, obtaining a complete voltammogram for each of the electrodes, and each sample. Because of the dimensionality and complexity of descriptive information generated (5 voltammograms per each sample), a compression step with Fast Fourier transform was needed.

Once the departure data is compressed the ANN architecture was optimized. The parameters that were optimized were the 4 transfer functions employed in the hidden and output layers, the functions evaluated were *tansig, logsig, satlins* and *purelin*, and the number of neurons in the hidden layer, from 1 to 12 neurons. In this manner 192 architectures were evaluated for each case.

In the case of the ET the final architecture of the back-propagation ANN model had 160 input neurons, a hidden layer with 10 neurons and the satlins transfer function, an output layer with 2 neurons and the purelin transfer function. For the case where the Nickel oxy-hydroxide is the only sensor employed the back-propagation ANN had 32 input neurons, a hidden layer with 6 neurons and the purelin transfer function, an output layer with 2 neurons and the satlins transfer function.

The representation of the modeling performance of the system is illustrated in Figure 5 for electronic tongue and in Figure 6 for GCE/MWCNT/NiOOH sensor. These figures shows the comparison graphs of predicted vs. expected concentrations for the ET tongue and the GCE/MWCNT/NiOOH sensor, that were built to check the prediction ability of the two different models.

It may be seen that with the ET a satisfactory trend was obtained for the total sugar as well with the GCE/MWCNT/NiOOH sensor, with the regression line almost indistinguishable from the theoretical one for the training subset.

However, the prediction capabilities for the Ethanol of the two models are very different, it can be seen that the GCE/MWCNT/NiOOH sensor model have far better prediction capabilities. From these data it appears that the sugars and ethanol demonstrate very good correlation coefficients with an R value ≥ 0.99 on the training subset. The model prediction is satisfactory for sugars and the accuracy of the obtained response is adequate, close to the ideal values, with 0 intercept, 1 slope and good correlation coefficients R values ≥ 0.99. However in the case of Ethanol the GCE/MWCNT/NiOOH sensor model has a better correlation coefficient, intercept and slope as can be seen in Table 2.
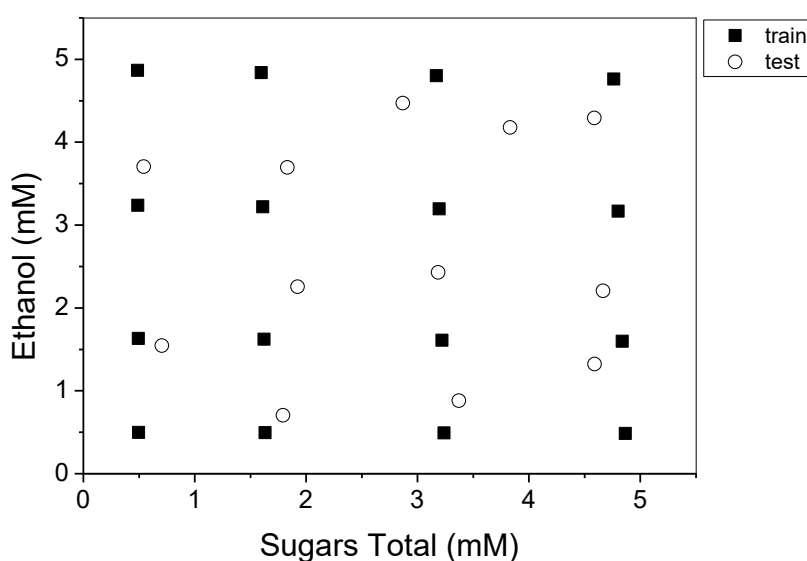
Figure 4: Samples were measured employing the electronic tongue formed by GCE/MWCNT/Metals oxy-hydroxide nanoparticle modified electrodes, obtaining a complete cyclic voltammogram for each of the sensors, and each sample of (▪) Training sets and (∘) testing sets for the mixtures of samples of total sugars and ethanol.
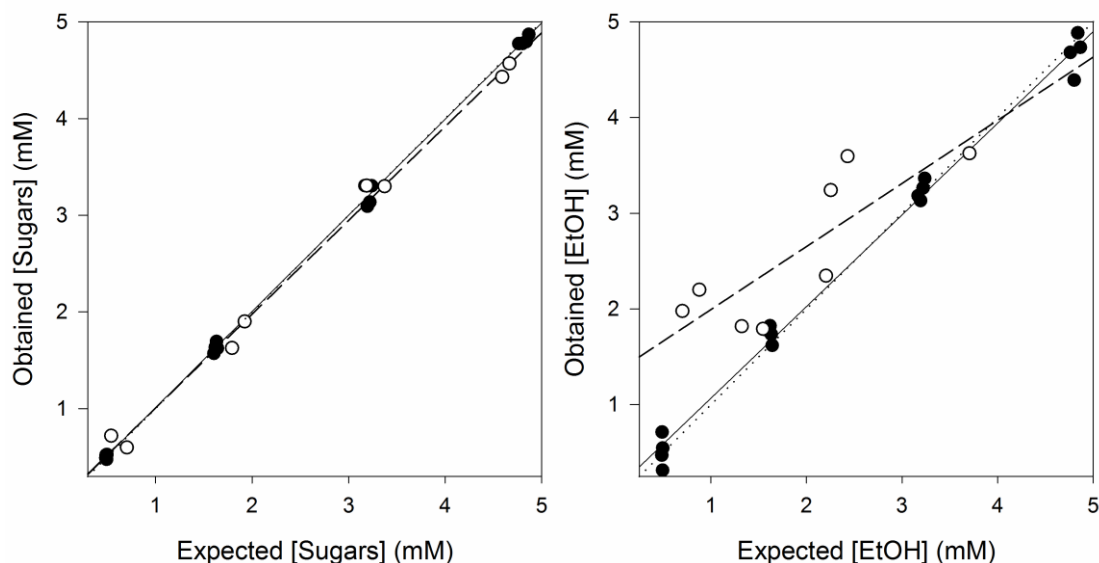
Figure 5: Modelling ability of the optimized electronic tongue formed by GCE/MWCNT/MetalsOOH modified electrodes. Comparison graphs of predicted vs. expected concentrations for (A) Total sugars and (B) Ethanol, both for training (•, solid line) and testing subsets (∘, Dashed line). Dotted line corresponds to theoretical Y=X.
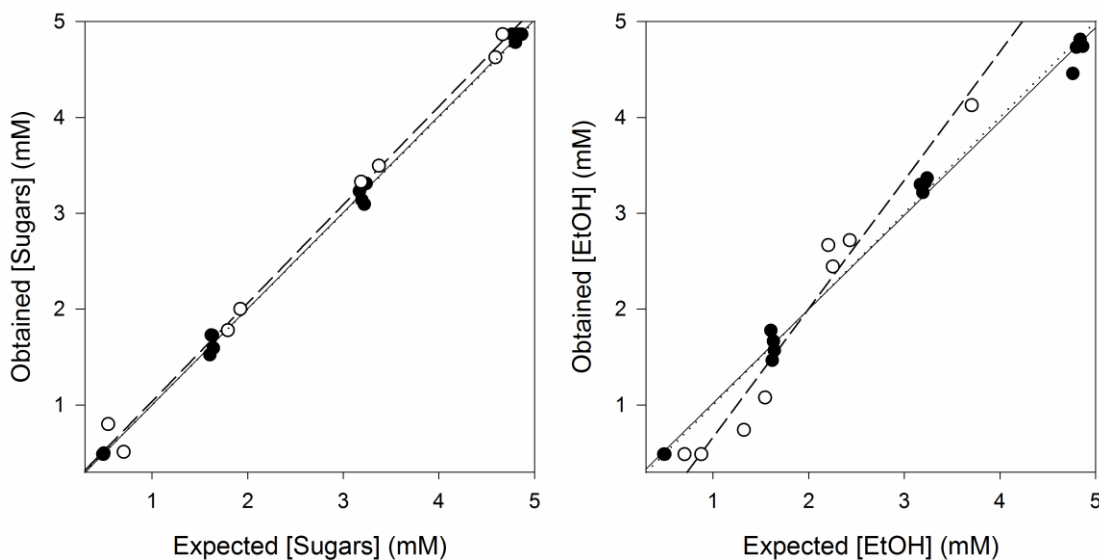
Figure 6: Modelling ability of the optimized electronic tongue formed by GCE/MWCNT/NiOOH sensor. Comparison graphs of predicted vs. expected concentrations for (A) Total sugars and (B) Ethanol, both for training (•, solid line) and testing subsets (∘, Dashed line). Dotted line corresponds to theoretical Y=X.

Table 3: Results of Electronic tongue formed by GCE/MWCNT/Metals and GCE/MWCNT/NiOOH sensor of the fitted regression lines for the comparison between obtained vs. expected values, both for the training and testing subsets of samples for Sugars and Ethanol and the three considered species (intervals calculated at the 95 % confidence level). RMSE: Root Mean Square Error; Intercept and RMSEvalues are expressed in mM.

	Compound	Correlation	Slope	Intercept (mM)	RMSE (mM)	NRMSE
Electronic tongue formed by GCE/MWCNT/Metals						
Training	**Sugars**	0.999	1.004±0.0396	-0.0115±0.1192	0.0509	0.0106
	Ethanol	0.996	0.9580±0.0968	0.1093±0.2918	0.1358	0.0283
Testing	**Sugars**	0.997	0.9715±0.1464	0.0325±0.4395	0.1289	0.0268
	Ethanol	0.821	0.6600±0.9178	1.3323±1.9194	0.9262	0.193
GCE/MWCNT/NiOOH sensor						
Training	**Sugars**	0.999	1.004±0.04598	-0.0032±0.0063	0.0413	0.0086
	Ethanol	0.998	0.980±0.07811	0.0397±0.2355	0.0752	0.0157
Testing	**Sugars**	0.996	1.026±0.1667	0.0118±0.5004	0.1447	0.0301
	Ethanol	0.980	1.340±0.53735	-0.6773±1.1238	0.3883	0.0809

After optimizing its performance, the electronic tongue and GCE/MWCNT/NiOOH sensor were assessed through studies on synthetic samples of total sugars and ethanol shown in table 3, with three different synthetic samples. It was observed that for analysis of total sugars both as electronic tongue as the GCE/MWCNT/NiOOH sensor was similar values obtained for the concentrations found. However, the values found in the electronic tongue for ethanol are worst when compared with GCE/MWCNT/NiOOH sensor, as it can be seen results found are in good agreement with those expected, it can be seen that the GCE/MWCNT/NiOOH sensor have far better quantification capabilities for ethanol.

Table 3: Results in synthetic samples at electronic tongue formed by GCE/MWCNT/MetalsOOH modified electrodes and GCE/MWCNT/NiOOH sensor for the of total sugars and ethanol

Samples	Total Sugars mM	E.T. Total Sugars Found mM	NiOOH Total Sugars Found mM
1	1.9	1.9	2.0
2	3.2	3.3	3.3
3	1.8	1.6	1.8
	Ethanol mM	Ethanol Found mM	Ethanol Found mM
1	2.2	3.2	2.4
2	2.4	3.6	2.7
3	0.7	2.0	0.5

4.   CONCLUSIONS

In this work, the application of an electronic tongue formed by GCE/MWCNT/MetalsOOH nanoparticle modified electrodes and GCE/MWCNT/NiOOH sensor are described for detection and quantification of total sugars and ethanol in synthetic test samples. The complex responses obtained from the electronic tongue and GCE/MWCNT/NiOOH sensor were successfully processed employing a multilayer ANN and Fast Fourier transform information which proved to be especially suited for building the response model. Therefore this GCE/MWCNT/NiOOH sensor has enormous potential to be applied in hydrolyzed samples from sugarcane at production of ethanol. Merit of the proposed system is to achieve resolution of mixtures of total sugars and ethanol, with performance equivalent to HPLC equipment, without need to use any biosensor component thus permitting more stable responses.

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