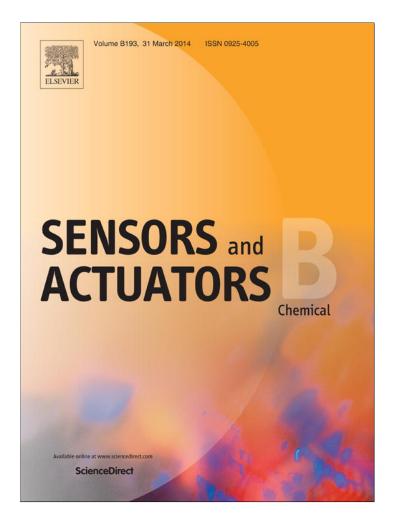
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Non-enzymatic sensing of carbohydrates using a nickel–chromium alloy electrode



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D.E. Pissinis^a, L.E. Sereno^b, J.M. Marioli^{b,*}

^a Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, Avenida Haya de la Torre y Medina Allende, 5000 Córdoba, Argentina ^b Departamento de Química, Universidad Nacional de Río Cuarto, X5804BYA Río Cuarto, Argentina

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ABSTRACT

A highly sensitive non-enzymatic carbohydrates sensor based on a nickel hydroxide film on a nickel alloy electrode was developed. The electro-catalytic film is formed by using a potential scan program developed in our research group. This program facilitated the formation of stable proportions of Ni(II)/Ni(III) oxy-hydroxides which are necessary for the electro-catalysis. The structural morphology and roughness of the oxy-hydroxides were characterized by using scanning electron microscopy and atomic force microscopy. The electrochemical behavior of the electrodes in 0.10 M NaOH and in the presence of carbohydrates was characterized by cyclic voltammetry and chronoamperometry, showing good catalytic activity for the anodic oxidation of carbohydrates. Fast and significant amperometric responses were obtained with the renewable nickel hydroxide films, which were easily constructed on the electrodes' surfaces by potential cycling. The good analytical performance, low cost and straightforward preparation method make this alloy a promising electrode for the development of effective non-enzymatic carbohydrates sensor.

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1. Introduction

The determination and quantification of sugars has a great significance in many fields such as food industries, biotechnology, medical applications, wastewater treatment, and many others [1-4]. Enzymatic sensors based on the immobilization of enzymes on various substrates are the topics of most of the previous studies on this subject [5,6]. However, the greatest problem is naturally insufficient stability and loss of enzyme activity during immobilization process which affects sensitivity and reproducibility of sensor.

The glucose detection industry is dominated by use of enzymatic electrodes, in which the enzyme glucose oxidase is predominantly used. It is a highly selective enzyme, and works at a wide range of pHs relative to other enzymes [7]. Enzyme modified electrodes, however, suffer from limited stability and variable sensitivity, requiring increasingly elaborate fabrication and immobilization methods to overcome these boundaries [8].

Non-enzymatic amperometric sensor for direct determination of glucose is an attractive alternative technique to solve the disadvantages of enzymatic biosensors. A number of metal electrode materials, namely copper [9–11], gold [12,13], platinum [14,15], and nickel [16,17], have been explored extensively in their application to catalytic oxidation of carbohydrates. Nickel alloys have also been frequently used in non-enzymatic glucose detection, with alloys such as Ni–Cu [18,19], Ni–Ti [20,21] and Ni–Cr [19,22]. Particularly, the Ni–Cr (80:20) alloy was proposed as an excellent electrode material for the amperometric detection of carbohydrates after anion-exchange high performance liquid chromatographic separations [19,23]. Moreover, this alloy showed enhanced sensitivity and signal stability when compared with pure nickel electrodes [24].

Nickel and nickel oxy-hydroxide electrodes are good materials for the oxidation of organic compounds in alkaline solutions [25–27]. Various authors have demonstrated that nickel oxy-hydroxide has good electro-catalytic properties for the oxidation of different molecules. For example, aspirin [28], methane [29], acetylcholine [30], and glucose [31,18] were easily oxidized on nickel oxy-hydroxide-modified nickel electrodes. A catalytic reaction mechanism [32] has been generally used to explain the results obtained in the electro-oxidation of several organic molecules at oxy-hydroxide-modified metal electrodes (see for example [33,34] and references therein). According to this mechanism, Ni(OH)₂ is first oxidized to NiOOH, which then reacts with the organic molecule to regenerate the starting material.

In previous studies we developed potential scan programs for cyclic voltammetry that allowed us to obtain stable proportions of nickel α - and β -oxy-hydroxides on the electrode surfaces [35].

^{*} Corresponding author at: Departamento de Química, Universidad Nacional de Río Cuarto, ruta 36, km 601, X5804BYA Río Cuarto, Argentina. Tel.: +54 358 4676523; fax: +54 358 4676233.

E-mail addresses: jmarioli@exa.unrc.edu.ar, juanmmarioli@gmail.com (J.M. Marioli).

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We also proposed an electro-catalytic mechanism based on the cyclic voltammetric (CV) response of glucose at Ni and Ni–Cr alloys (namely 90:10 and 80:20 Ni–Cr alloys) [22]. The proposed mechanism helped us to explain the curved nature of the calibration plots, that is, the electro-catalytic peak current as a function of glucose concentration.

In this work we report an alternative route to the enzymatic detection of carbohydrates by applying the electro-catalytic properties of a nickel oxy-hydroxide film on a Ni–Cr (80:20) alloy electrode for the electro-oxidation of glucose, fructose, lactose and sucrose in alkaline solution. The electro-catalytic properties for the oxidation of those carbohydrates were evaluated by chronoamperomety, and the proposed electrode showed an excellent catalytic activity. The developed surface can be used directly as non-enzymatic carbohydrate sensor.

2. Materials and methods

Working electrodes were made of Ni–Cr (80:20) (Goodfellow, England, catalog number NI055150) wires of 0.10 cm diameter embedded in Teflon shrinkable tubes. They were polished with sand paper and then with fine alumina down to 0.05 μ m, rinsed with triply distilled water, ultrasonicated during 5 min, rinsed again with triply distilled water and immersed in the electrochemical cell. The exposed circular area obtained after polishing was ca. 7.8×10^{-3} cm². In order to minimize oxides formation – 1.00 V was applied to the working electrodes for about 60 s before CV experiments. A platinum foil of a larger area (ca. 0.8 cm²) was used as the counter electrode. All potentials are referred to the saturated calomel electrode (SCE). This electrode was placed in a Luggin capillary in the electrochemical cell.

For the formation of the oxy-hydroxides film on the electrode's surface, the potential of the working electrode was swept from 0.00 V to 0.60 V during 5 CV cycles, then a smaller potential range from 0.10 V to 0.60 V was swept during 10 CV cycles. Finally, an even smaller potential range (from 0.20 V to 0.60 V) was swept during the remaining CV cycles (a total of 600 CV cycles) at a potential sweep rate of 0.05 V/s in 0.10 M NaOH. This program facilitated the development of approximately equal quantities of α -Ni(OH)₂ [35].

The electrochemical cell was a conventional two compartments glass cell. Autolab model PGSTAT-30 or PGSTSAT-12 potentiostats were used to control the potential between the working and reference electrodes. The potentiostats were controlled by PC compatible computers using the PGSTAT software version 4.9006. Graphics were obtained with a conventional printer. The temperature of the electrochemical cell was kept at 25.0 ± 0.2 °C.

Atomic force microscopy (AFM) images were performed in tapping mode with a NanoScope V scanning probe microscope, silicon tip, spring constant: 42 N/m (Bruker) operating at ambient conditions. The analyses were made at the Instituto de Investigaciones Fisicoquímicas Teóricas y Aplicadas (INIFTA), Universidad Nacional de la Plata (Argentina).

The elemental analyses of the electrode's surfaces were performed by using an EDAX Genesis 2000 energy dispersive spectroscopy system coupled with a LEO1450VP scanning electron microscope (SEM). The analyses were made at the Electron Microscopy Laboratory and Microanalysis (LabMEM) of the National University of San Luis (Argentina).

Sodium hydroxide solutions were prepared with triply distilled water and analytical grade reagents (Merck, Argentina). The solutions were thoroughly degassed by bubbling high purity N_2 prior to electrochemical experiments. Contact with atmospheric CO₂ was minimized by maintaining N_2 over the solutions during the electrochemical experiments.

The fitting of the voltammetric traces by hyperbolic functions was performed by using the software Origin 8.0.

For the calibration curves experiment, amperometric measurements were carried out at 0.5 V while adding from $60 \,\mu\text{L}$ to $200 \,\mu\text{L}$ of 10 mM glucose into $60 \,\text{mL}$ of 0.10 M NaOH solution under a magnetically stirred condition to obtain a step-by-step increase of the final concentration, that is, from approximately $10 \,\mu\text{M}$ to $600 \,\mu\text{M}$.

3. Results and discussion

3.1. Morphology of Ni and Ni-Cr (80:20) alloy electrode

The morphology of the oxy-hydroxide film formed on the nickel-chromium alloy electrodes in sodium hydroxide solution was examined by AFM and SEM [36]. The electrodes' surfaces were prepared by voltammetric cycling between 0.20 V and 0.60 V during 600 cycles. Fig. 1A and B shows representative photomicrographs of the electrodes' surfaces after the voltammetric treatment. A greater roughness at the Ni–Cr(80:20) alloy electrode was observed in the morphology analysis of the oxy-hydroxide films [22]. The increase in roughness was confirmed by the root mean square (RSM) roughness values of 3.0 and 4.3 nm obtained from the area (500 × 500 nm) of the nickel oxy-hydroxide film on the surface of the electrodes [37,38]. The forming a rougher nickel oxy-hydroxide film in the presence of chromium indicates that a larger surface area is exposed in the electro-catalytic behavior.

The chemical contrast image indicates that chromium is homogeneously distributed in the nickel matrix at the Ni–Cr (80:20) alloy electrode, Fig. 1C. The results of chemical composition analysis obtained by EDAX revealed that the alloy contains 80% Ni and 20% Cr, Fig. 1D.

3.2. Catalytic oxidation of carbohydrates

The Ni–Cr (80:20) alloy electrode showed good catalytic activity for the anodic oxidation of carbohydrates in 0.10 M NaOH. Fig. 2 shows the cyclic voltammetric responses in the absence and in the presence of different concentration of glucose, Fig. 2A and of sucrose, Fig. 2B.

An oxidative current peak, at approximately 0.45 V on the positive going potential scan, is observed in the cyclic voltammetric current density vs. potential (j/E) profiles obtained with the working electrode Ni–Cr (80:20) in the absence of carbohydrates. The bell shaped peak is indicative of a surface confined electrochemical reaction. This oxidative current peak has been ascribed to the oxidation of Ni(II) to Ni(III), as shown in Eq. (1)

$$Ni(OH)_2 + OH^- \rightleftharpoons NiOOH + H_2O + e$$
(1)

A pair of reductive current peaks is observed during the negative going potential scan. The reductive current peak observed at approximately 0.38 V has been ascribed to the reduction of β -NiOOH to β -Ni(OH)₂, while that observed at approximately 0.30 V has been ascribed to the reduction of γ -NiOOH to α -Ni(OH)₂ [35].

An increment of the peak current and oxidative charge under the peak observed at 0.45 V is observed when glucose is added to the electrolyte solution. A decrease of peak currents and reductive charges is observed in the reductive waves obtained during the negative going potential scan. This behavior is characteristic of electro-catalytic mechanisms [32]. Equivalent behaviors are observed when different carbohydrates are added to the electrolyte solution. Fig. 2B shows the *j/E* behavior of the nickel oxy-hydroxide film on a Ni–Cr (80:20) alloy electrode when different concentrations of sucrose were added to the electrolyte solution.

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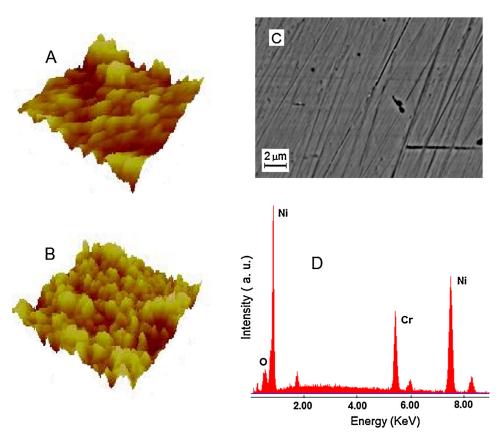


Fig. 1. (A) Three dimensions AFM image of nickel oxy-hydroxide film formed on the surface of the Ni electrode (x = 500 nm, y = 500 nm, z = 24 nm), (B) AFM image of nickel oxy-hydroxide film formed on the surface of the Ni–Cr (80:20) alloy electrode (x = 500 nm, y = 500 nm, z = 30 nm), (C) SEM microphotograph of nickel oxy-hydroxide film formed on the surface of the Ni–Cr (80:20) alloy electrode and (D) EDAX spectrum of the composition of Ni–Cr (80:20) alloy at the surface.

The electrode response to different concentrations of glucose, ribose, maltose and sucrose is shown in Fig. 3. The non-linearity of the electrode's response to the analyte concentrations is one common trend for all the carbohydrates studied. The shape of the

calibration curves was explained [22] by considering an electrocatalytic mechanism with the formation of surface-bound active species between the carbohydrate and specific sites on the oxyhydroxides film at the Ni–Cr (80:20) electrode. Thus, the catalytic

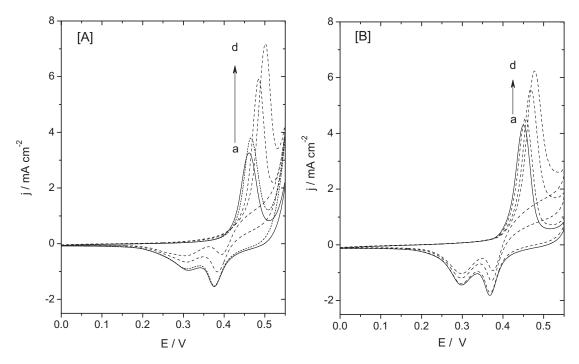


Fig. 2. Cyclic voltammograms at Ni–Cr (80:20) in 0.10 M NaOH. In all cases the electrode surface is obtained after applying 600 CV cycles. (A) Glucose and (B) sucrose. The analyte concentration in cycles "a" through "d" is a = 0.0 M; $b = 1.0 \times 10^{-4}$ M, $c = 1.0 \times 10^{-3}$ M and $d = 2.0 \times 10^{-3}$ M. v = 0.05 V/s.

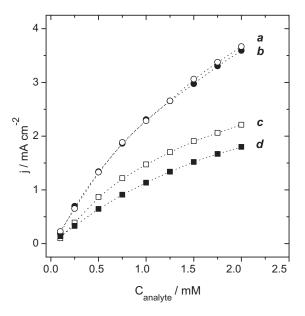


Fig. 3. Glucose calibration curves obtained with Ni–Cr (80:20) working electrodes pretreated with 600 CV cycles. (a) Glucose, (b) ribose, (c) maltose and (d) sucrose. v = 0.05 V/s.

peak current obtained at a given carbohydrate bulk concentration may be expressed as:

$$i_{\max} = \frac{BC_{CH}^*}{K + C_{CH}^*} \tag{2}$$

where *B* and *K* are constants derived from the proposed electro-catalytic mechanism and C_{CH}^* is the carbohydrate bulk concentration [22].

Eq. (2) is an hyperbolic function, with maximum value *B* when $K \ll C_{CH}^*$. *B* represents the maximum current obtainable for a given carbohydrate concentration (any oxidizable analyte in general). This equation was used to fit the carbohydrate calibration curves, and the results are summarized in Table 1. Good correlation coefficients, in the range from 0.995 to 0.999, were obtained in all cases.

The maximum current density, given by $j_{max} = BC_{CH}^*/A(K + C_{CH}^*)$ with *A* being the working electrode geometric area, tends to reach a stable current value given by the parameter $B = nk_3 \varepsilon Q_T$ (the meaning of the symbols are defined elsewhere [22]). The values calculated for *B* shows that monosaccharides are oxidized to a greater degree than disaccharides. The oxidation order of carbohydrates was:

$Glucose \cong Ribose > Maltose > Sucrose$

The difference observed in the *B* values could be attributed to the difficulty of adsorption of the carbohydrate on the oxy-hydroxide sites on the Ni–Cr (80:20) alloy electrode [39]. The oxidation of carbohydrates at the nickel hydroxide surface is mainly performed by primary hydroxyl groups, in which for glucose and ribose these groups are more readily available for oxidation. The analysis of the *K* parameter in Eq. (2) is more complex.

Table 1

Parameters obtained from the hyperbolic fitting of the calibration curves. Ni–Cr (80:20) alloy electrode.

Carbohydrate	$B (\mathrm{mA} \mathrm{cm}^{-1})$	K(mM)	
Glucose	9.22 ± 0.49	3.03 ± 0.23	
Ribose	8.27 ± 0.58	2.64 ± 0.28	
Maltose	4.83 ± 0.42	2.33 ± 0.32	
Sucrose	4.61 ± 0.24	3.08 ± 0.24	

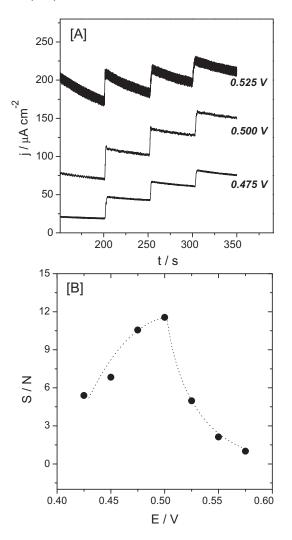


Fig. 4. (A) Amperometric response of Ni–Cr (80:20) alloy electrode after successive additions of $200 \,\mu$ L 10 mM glucose into 60 mL 0.10 M NaOH solution stirred constantly at the indicated applied potentials and (B) signal-to-noise ratio analysis.

3.3. Optimization of the potential for carbohydrate analysis

The potential applied to the working electrode has a key influence on the sensor's response. The dependence of the amperometric sensor response on the applied potential is shown in Fig. 4A, for successive additions of 200 μ L 10 mM glucose into 60 mL 0.10 M NaOH solution constantly stirred, at the indicated applied potentials. The maximum response to carbohydrate additions was obtained at an applied potential of 0.50 V, while noise increases progressively with increasing potential. The best signal-to-noise ratio was obtained at an applied potential of 0.50 V (Fig. 4B). Thus, 0.50 V was used as the best potential for amperometric detection of carbohydrates.

It should be mentioned at this point that all of the carbohydrates in this study behaved similarly with respect to the potential applied to the working electrode. All of them were electro-catalytically oxidized at the potential of the Ni(II)/Ni(III) transition. Thus, the "selectivity" of the nickel oxy-hydroxide modified Ni–Cr (80:20) alloy electrode itself is reduced, and a separation step prior to the analysis would be interesting when analyzing a multi-component sample. This separation can be achieved by using chromatographic equipment, coupled to the simple non-enzymatic detector described in this work.

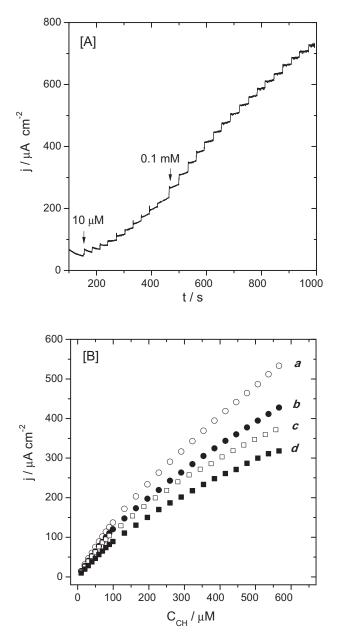


Fig. 5. (A) Amperometric response of the Ni–Cr (80:20) alloy electrode to successive additions of 60–200 μ L 10 mM glucose into 60 mL 0.10 M NaOH solution stirred constantly. Applied potential = 0.5 V. (B) Calibration curves of (a) glucose, (b) ribose, (c) maltose and (d) sucrose.

3.4. Amperometric response of the non-enzymatic sensor for carbohydrates

Fig. 5A illustrates a typical amperometric response for the sensor at an applied potential of 0.50V after the addition of successive aliquots of 0.01 M glucose into 0.10 M NaOH under constant stirring conditions. The oxidation reaction was very fast and the current reached a steady-state current level within 5 s after the additions of glucose. An equivalent behavior was observed for the addition of ribose, sucrose or maltose.

Two linear portions are evident in the amperometric response obtained after carbohydrate additions (Fig. 5B). The first linear portion is observed from approximately $10 \,\mu$ M to nearly $100 \,\mu$ M, while the second portion is observed from approximately $100 \,\mu$ M to nearly $550 \,\mu$ M. The parameters obtained from the linear fitting

Table 2

Parameters obtained from the linear fitting of the calibration curves. Ni–Cr (80:20) alloy electrode, in range 10 μ M–0.10 mM. Fitting equation: y = ax + b.

Carbohydrate	$a/\text{sensitivity} (\mu A \text{cm}^{-2} \mu M^{-1})$	$b(\mu A \mathrm{cm}^{-2})$	
Glucose	1.37 ± 0.03	4.79 ± 1.63	
Ribose	1.20 ± 0.02	3.98 ± 0.99	
Maltose	0.90 ± 0.01	1.61 ± 0.61	
Sucrose	1.16 ± 0.03	4.09 ± 1.6	

based on the first linear part of the curve of calibration are informed in Table 2.

The pursuit of non-enzymatic glucose sensing is a vigorous and competitive area of research. We used the amperometric response of the nickel oxy-hydroxide film on a Ni–Cr (80:20) alloy electrode to glucose additions and compared its potential as a non-enzymatic sensor with some other glucose sensors already informed in the literature. As shown previously, this response showed two linear ranges, with a correlation coefficient of 0.9966 for that at lower glucose concentrations. The experimental limit of detection, based on a signal-to-noise ratio of 3, was 3.0 μ M with a sensitivity of 1.37 μ A cm⁻² μ M⁻¹ using the calibration graph in the lower concentrations range and sensitivity for glucose amperometric detection at nickel oxy-hydroxide film on a Ni–Cr (80:20) alloy electrode are comparable and even better than those obtained with several glucose sensors reported recently.

3.5. Reproducibility of the response of the nickel oxy-hydroxide film on a Ni–Cr (80:20) alloy electrode to glucose oxidation

The reproducibility of response of the nickel oxy-hydroxide film on a Ni–Cr (80:20) alloy electrode to glucose oxidation was studied by determining its amperometric response to 1.0 mM glucose in 0.10 M NaOH during a period of 7 days. The initial glucose analysis was performed immediately after the formation of the oxyhydroxides layer on the electrode's surface, and the subsequent responses were recorded using the same electrode after storing it in air or in 0.1 M NaOH for the time period indicated. After each period of time, the electrode was washed with distilled water before and after its use. Ten CV cycles, between 0 and 0.55 V vs. SCE in 0.10 M NaOH, were made prior to each amperometric experiment to recondition the oxy-hydroxides layers as well as possible.

The normalized response is defined as the ratio of the amperometric response at any time to that of the first analysis. Fig. 6 shows the normalized current response of the oxy-hydroxides layer over the period of 7 days for the electrode stored in air or in 0.10 M NaOH.

The response of the electrode to glucose oxidation decreased by approximately 40% within 24 h and 80% within one week when the electrode is stored in air after its use (Fig. 6, gray bars). However, the electrode's response to glucose oxidation remains for longer periods of time when the electrode is stored in 0.10 M NaOH, improving greatly its response stability (Fig. 6, black bars). The electrode's response decreases only 1% and 5% at 24 and 48 h, respectively. This results show that the sensitivity of the oxyhydroxides films formed on Ni–Cr (80:20) alloy electrode to glucose oxidation can be greatly improved if the electrode is stored in 0.10 M NaOH between experiments. We believe that the aging and the dehydration of the oxy-hydroxides layer may be the reason for the decrease of the signal when the electrode is stored in air.

These results are in agreement with previous work [24] where the electrodes' response to glucose oxidation diminished only by approximately 40% of the initial response after twenty days of work. The experiments of Ref. [24] were made in a high performance liquid chromatographic system with amperometric detection at constant potential (0.47 V vs. Ag/AgCl) in a continuously flowing

Table 3

Comparison of analytical parameters^a obtained with various non-enzymatic glucose sensors.

Glucose sensor	Linear range	Sensitivity $(\mu A cm^{-2} \mu M^{-1})$	Sensitivity (μΑμΜ ⁻¹)	Detection limit ^b (µM)	References
Copper nanocluster/multi-wall carbon nanotube	0.7-3.5 mM	0.253	-	0.2	[40]
Nanotube-copper nanoparticle hybrid	0.001-2.0 mM	-	1.85×10^{-4}	0.4	[11]
Copper(II) oxide screen printed	50-600 μM	-	$3.2 imes 10^{-4}$	4.0	[10]
Copper(II) oxide nanorod	0–100 µM	2.25	-	1.2	[9]
CuO nanowire	0.4-2 mM	2.45	-	0.05	[41]
Nanoscale nickel hydroxide modified carbon ionic liquid	0.05-23 mM	0.202	-	6.0	[16]
Ni nanoparticle-loaded carbon nanofiber paste	0.002-2.5 mM	0.420	-	1.0	[1]
Nickel(II)/ordered mesoporous carbon	2-1000 µM	0.835	-	0.65	[17]
Nickel modified boron-doped diamond	0.01–10 mM	1.04	-	2.7	[42]
(NiONPs)/GO/Glassy carbon	0.003-3.0 mM	1.087	-	1.0	[43]
Alloy Ni–Cr (80:20)	10-550 μM	1.37	107.5×10^{-4}	3.0	This work
Glucose biosensor	0.01-4.2 mM	0.038		5.0	[44]
Glucose biosensor	1.0-10.0	$1.4 imes 10^{-4}$		160	[45]
Glucose biosensor	2-12.0	0.034			[46]

^a Analytical parameters are obtained from the linear range at lower concentration.

^b Detection limit set at a signal/noise ratio of 3.

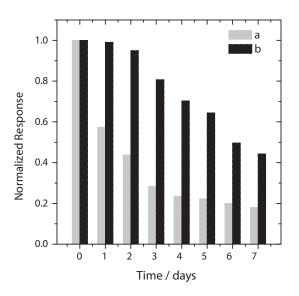


Fig. 6. Plot of the average amperometric current for a 1 mM of glucose solution in 0.10 M NaOH using the Ni–Cr (80:20) alloy electrode over period of 7 days, (a) stored in air and (b) stored in 0.1 M NaOH.

0.10 M NaOH solution. Glucose $(1.0 \times 10^{-5} \text{ M})$ injections were made every half an hour during 15 h each day, and then the electrochemical detector was shut down but the chromatographic pump remained on. Thus, the working electrode was always in contact with 0.10 M NaOH.

4. Conclusions

In this work we report an alternative route to the enzymatic detection of carbohydrates by applying the electro-catalytic properties of a nickel oxy-hydroxide film on a Ni–Cr (80:20) alloy electrode for the electro-oxidation of carbohydrates. The elemental ratio calculated from the results of the SEM–EDAX analysis indicated that the composition of the electrode's surface is very close to those indicated by the manufacturer. The chemical contrast images showed that the alloy is homogeneous and that chromium is homogeneously distributed in the matrix of nickel.

The forming of a rougher nickel hydroxide film in the presence of chromium indicates that a larger surface area is exposed in the electrochemical experiments, suggesting a greater electro-catalytic behavior. Ni–Cr (80:20) alloy electrodes showed good catalytic activity for the anodic oxidation of carbohydrates in 0.10 M NaOH. Two linear ranges were observed when the electrode was used in the non-enzymatic determination of carbohydrates. The experimental limit of detection of glucose, based on a signal-to-noise ratio of 3, was 3.0 μ M with a sensitivity of 1.37 μ A cm⁻² μ M⁻¹ using the calibration graph in the lower concentrations range. It is shown that the detection limit, linear calibration range and sensitivity for glucose amperometric detection at the nickel oxy-hydroxide film on a Ni–Cr (80:20) alloy electrode are comparable and even better than those obtained at several electrodes reported recently. This good analytical performance, the low cost, simple preparation and reusability of the electrode, are features that make the Ni–Cr (80:20) alloy electrode an excellent material for its use as a non-enzymatic carbohydrate sensor.

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Biographies

D.E. Pissinis received his Doctoral degree from Universidad Nacional de Río Cuarto (Argentina) in 2005. He performed postgraduate studies in 2010 at Universidad Nacional de Córdoba, Argentina, in the area of nanostructured platforms for biosensors. Now, his interest includes synthesis of novel nanomaterial and its application in sensor, biosensors and biomaterials.

L.E. Sereno received his Doctoral degree from Universidad Nacional de Córdoba (Argentina) in 1970. He is now an Emeritus Professor at the Chemistry Department in Universidad Nacional de Río Cuarto. His research interests include physical chemistry and electrochemistry.

J.M. Marioli received his Doctoral degree in 1987 from Universidad Nacional de Río Cuarto, Argentina. He was a post-doctoral researcher at Kansas University from 1989 to 1992. He is currently an Associate Professor at Universidad Nacional de Río Cuarto and his research interests include electrochemistry and electroanalytical chemistry.