

Electrochemical Characterization a New Epoxy Graphite Composite Electrode as Transducer for Biosensor

¹ Abel I. Balbín Tamayo, ¹ Laura S. Riso, ² Aurora Pérez Gramatges,

**² Percio Augusto Marini Farías, ^{1*} Ana Margarita Esteva Guas
and ³ Hideko Yamanaka**

¹ Departamento de Química Analítica, Facultad de Química, Universidad de La Habana,
Zapata s/n entre G y Carlitos Aguirre, Vedado, Plaza de la Revolución, CP 10400. La Habana, Cuba

² Departamento de Química, Pontifícia Universidade Católica do Rio de Janeiro,
Rua Marquês de São Vicente, 225 - Gávea, Rio de Janeiro - RJ, 22451-900 Brasil

³ Instituto de Química, Universidade Estadual Paulista – UNESP,
CP 355, 14800-900, Araraquara, SP, Brazil

¹ Tel.: (53-7) 879 6153; 878 1263; 870 3922; 879 4734

* E-mail: anam@fq.uh.cu

Received: 22 June 2016 /Accepted: 22 July 2016 /Published: 29 July 2016

Abstract: A new graphite epoxy composite composition was developed by the manufacturer of transducer for DNA biosensor, which were characterized by cyclic voltammetry, electrochemical impedance spectroscopy and scanning electron microscopy field emission (FESEM). The dependence of peak potential, the anode current relationship, and cathodic current with scan rate was evaluated by voltammetry for the redox system $\text{Fe}(\text{CN})_6^{3-/4-}$. Well-defined voltammograms were obtained using the graphite-epoxy composite 3.3/2.5/1 m/m/v graphite/araldite/hardener (GEC II). The electrochemical characterization showed a quasi-reversible electrochemical behavior under the control of the linear diffusion system for $\text{Fe}(\text{CN})_6^{3-/4-}$. The GEC III was also successfully used in the detection of the signal oxidation guanine monophosphate (GMF) and adenine (AMF) by square wave voltammetry. These results demonstrate the potential of GEC II in the electrochemical detection of DNA. Copyright © 2016 IFSA Publishing, S. L.

Keywords: Graphite-epoxy composite electrode, Voltammetry, Nucleotide.

1. Introduction

Rigid carbon composites are ideal for the construction of electrochemical sensors. Matrices based carbon are the most frequently applied because of its high conductivity, and rigidity, resulting in high mechanical stability [1-5]. In recent years, they have gained great attention using composite graphite, because of its electrical and mechanical properties as

hardness, to develop various electrochemical transducers.

In electrochemistry, composite electrodes are made of a conductive phase (silver, gold, or any carbon allotrope as graphite) and an insulating phase (organic solvents, silicone, polyurethanes or epoxy resin) [6-10]. The ratio of these two phases not only determine the conductivity of the electrode but also the mechanical properties [11]. This type of electrodes can

be constructed in different shapes and sizes which allow its application in different situations. Its surface has low background currents favoring high signal-to-noise and lower detection limits, and may be renewed by a simple polishing process, offering a new active layer after removal of the outer surface, extending the useful life of these electrodes.

In particular, compound graphites have high intrinsic resistance and low relaxation time, when compared to the time scale of electrochemical processes [12, 13]; therefore, parameters and details about the phenomena of percolation through the solid can be neglected.

The voltammetric response of this composite is similar to an array of microelectrodes, effective edge ("edge effects"), high current density and partially blocked areas [14, 15] of. Such electrodes are easily modified, allowing incorporate different modifiers such as, ligands, enzymes, mediators and others.

The objective was to construct and characterize epoxy graphite electrodes to use in the development of sensors in the detection of biologica relevant, such as DNA.

The preparation there must performed by varying the proportion of graphite in the epoxy resin and monitoring the electrochemical response to the redox system $\text{Fe}(\text{CN})_6^{3-/4-}$.

2. Materials and Methods Used

2.1. Procedures

Graphite powder 50 μm particle size, from Merck and an epoxy resin composed of Araldite M and Hardener HR, both from Ciba-Geigy company were used in preparing the epoxy composite graphite [16].

Material composition was improved by varying the percentage of graphite in the mixture to obtain a mixture having a formable, malleable consistency and ensure hardening after 48 hrs at 40 °C. (Table 1).

Table 1. Different ratio (m/v/v) graphite, Araldita M & Hardener HR, used in the manufacture of composites.

	Proportion X:Y:Z		
	GEC I	GEC II	GEC III
Graphite (x)	1.3	3.3	5
Araldita M (y)	2.5	2.5	2.5
Hardener HR (z)	1	1	1

The electrode consists of a copper wire immersed in a capillary internal diameter of 1mm, which previously was introduced into a graphite composite epoxy, to complete a depth of 100 mm (Fig. 1). Then it was placed in an oven at 40 °C for 48 hours. The polished surface of the extra fine sandpaper (P4000) and alumina and before each measurement to obtain a reproducible cyclic voltammograms surface was carried out in NaOH 0.1 molL⁻¹ in a range of -1 V

potential to 1 V 100mVs⁻¹. This surface was characterized by cyclic voltammetry, electrochemical impedance spectroscopy and scanning electron microscopy field emission (Field Emission Scanning Electron Microscope, FESEM, JSM-6701F).

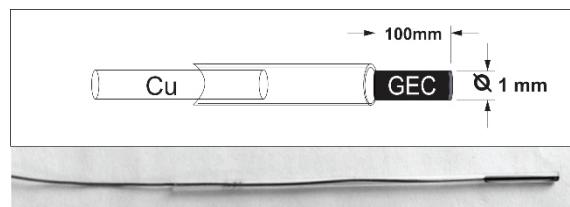


Fig. 1. Construction of voltammetric transducer. A: copper wire, B: glass capillary, C: graphite-epoxy composite.

2.3. Electrochemical Characterization

The cyclic voltammetry for the redox system $\text{Fe}(\text{CN})_6^{3-/4-}$, using a potentiostat BAS CV, the electrochemical cell consisting of a platinum electrode against a reference electrode of Ag/AgCl and composite graphite working electrode was used with geometric area of 0.0078 cm². Sweeps were conducted from 0.600V to -0.200 V potential, varying sweep rate from 10 to 100 mV s⁻¹. The probe used to monitor the response of the electrode was equimolar solution of $\text{K}_3[\text{Fe}(\text{CN})_6]/\text{K}_4[\text{Fe}(\text{CN})_6]$ in PBS pH 6.9 KCl 0.1 mol L⁻¹.

Measures impedance spectroscopy were performed on a potentiostat/galvanostat EG & G Princeton Applied Research 263 coupled to a frequency response analyzer & G Princeton Applied EG Research 1025. The electron transfer reaction of redox system $\text{Fe}(\text{CN})_6^{3-/4-}$, was used for impedance measurements PBS phosphate buffer solution pH 6.9 KCl 0.1 mol L⁻¹.

The measurements were performed applying a potential perturbation amplitude of 10 mV, using the open circuit potential recorded 10 point / decade in the frequency range $0.5 \text{ mHz} \leq f \leq 100 \text{ Hz}$. In each half stabilized potential 300 s an open circuit value, a value that was imposed by 300 s. The experiments were repeated 3 times. The values obtained were adjusted using ZView2 (Scribner Associates Inc.).

3. Results and Discussion

Cyclic voltammetry is one of the most versatile electroanalytical techniques used in the study systems, because it provides information on the reactivity of the species and the mass transport process [17]. This being the reason why it is also used for the characterization of electrochemical reactions, kinetic studies and determination of possible intermediate species. The cyclic voltammetry performed in this work, with the epoxy graphite electrodes in the redox system $\text{Fe}(\text{CN})_6^{3-/4-}$ as a model, it was used to compare the electrochemical response of these electrodes.

3.1. Electrochemical Behavior of Redox System $\text{Fe}(\text{CN})_6^{3-/4-}$.

The electrochemical response of graphite electrodes epoxy analyzed through redox response system $\text{Fe}(\text{CN})_6^{3-/4-}$, measuring the potential difference peak obtained from cyclic voltamperograms (Fig. 2) of the Fe solution $\text{Fe}(\text{CN})_6^{3-/4-}$ concentration 5 mmol L⁻¹. The charge transfer process in this system depends on the material of the working electrode and the form of preparation [18].

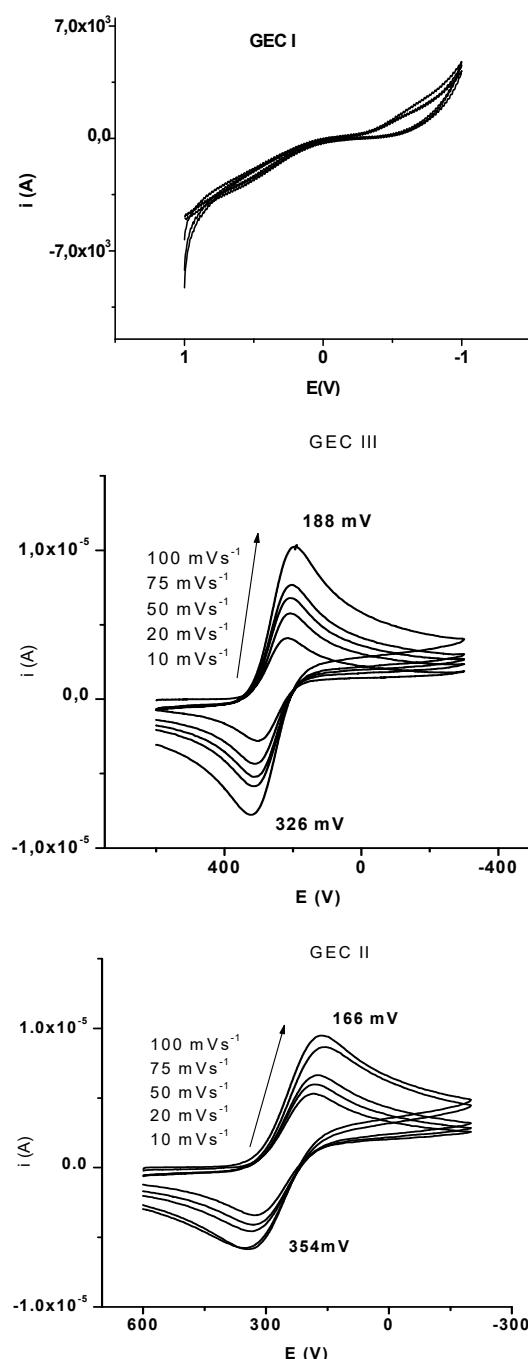


Fig. 2. Cyclic voltammograms (CV) of $\text{Fe}(\text{CN})_6^{3-/4-}$ epoxy using graphite electrodes: GEC I, GEC II and GEC III. Concentration $\text{Fe}(\text{CN})_6^{3-/4-}$ 5 mmol L⁻¹ PBS pH 6.9 KCl 0.1 mol L⁻¹ Scanning Speed: 10 to 100 mV s⁻¹.

The voltamperograms show different behaviors for composites evaluated. All composites showed good conductivity, although the composite (GEC I) with less graphite showed no signal for the system studied.

The redox system present a potential difference peak (193 ± 10) mV and (115 ± 8) mV on the electrodes GEC II and GEC III respectively, were outside the range of practical criteria $\Delta E_p = (59 \pm 20)$ mV [19], for which the process is considered reversible redox, behaving like a quasireversible system.

It was evaluated the influence of the sweep speed on the potential difference (Fig. 3), as another criterion of reversibility of the system.

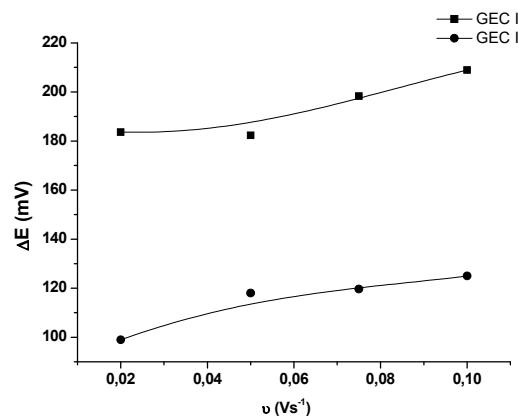


Fig. 3. Potential difference vs. ΔE_p scan speed (v) cyclic voltammogram for a solution of $\text{Fe}(\text{CN})_6^{3-/4-}$ (5 mmol L⁻¹) PBS pH 6.9 KCl 0.1 mol L⁻¹, using GEC II (■) and GEC III (●) as working electrodes.

That despite the difference of 80mV between ΔE_p between the responses of the electrodes; there is an increase of the potential difference with the speed of typical sweep cuasireversibles systems, in which the shape of the curve and the potential difference depend on the scanning speed transfer coefficient load (α) and the constant heterogeneous charge transfer [20].

3.2. Evaluation of Electron Transfer Process

By analyzing the influence of scan rate (v) over the anodic peak current (i_{pa}), satisfy the equation (1):

$$i_{pa} = K\vartheta^{1/2}, \quad (1)$$

where: i_{pa} is the anodic peak intensity; v is the sweep speed; and K is the Randles-Sevcik constant.

Were obtained as shown in Fig. 4, corresponding to anodic processes GEC II equations (2):

$$i_{pa} = 0.0022\vartheta^{1/2} \quad (2)$$

for $R^2 .96156$, while for the GEC III was (3):

$$i_{pa} = 0.0084\vartheta^{1/2} \quad (3)$$

with R^2 0.91578; adjustments in both linear intercept values were considered not significant because the associated errors associated with the same were greater. Demonstrating in both cases a linear correlation between current and square root of the scan rate, indicating kinetics redox processes governed by diffusion [20].

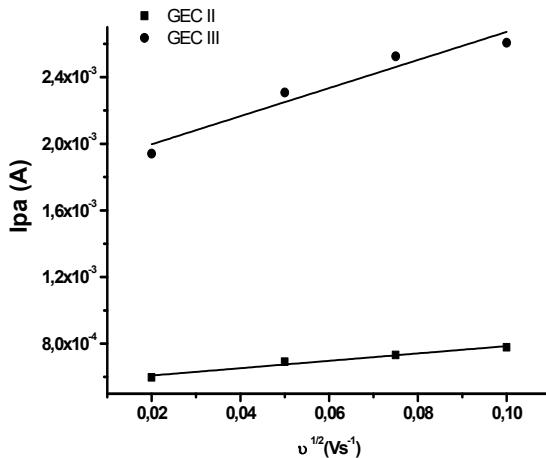


Fig. 4. Effect of scan speed (v) on the anode current (ipa) System for $\text{Fe}(\text{CN})_6^{3-/4-}$ using GEC II (●) and GEC III (■) as working electrodes ($\text{Fe}(\text{CN})_6^{3-/4-}$ 5 mmol L $^{-1}$ PBS pH 6.9 KCl 0.1 mol L $^{-1}$. (—) linear adjustment.

The ratio of ipa/ipc at different scanning speeds, presents values close to unity, criteria accepted as no kinetic processes associated with electronic transfer. Table 2.

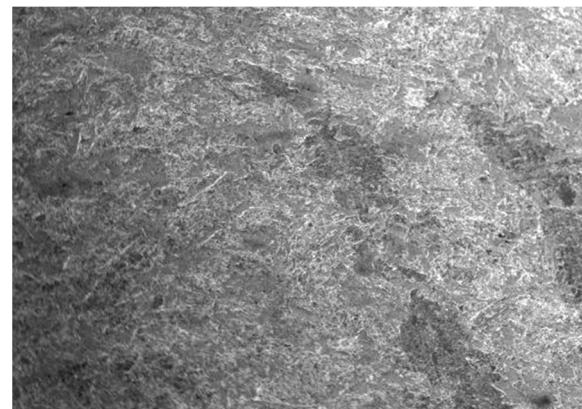
Table 2. Influence of the scanning speed ratio in the anodic and cathodic current (ipa / ipc).

v (mV/s)	(ipa / ipc).	
	GEC 2:1	GEC 3:1
20	0.96 ± 0.04	1.08 ± 0.02
50	1.02 ± 0.01	1.09 ± 0.04
75	1.12 ± 0.07	1.15 ± 0.06
100	1.19 ± 0.09	1.09 ± 0.03

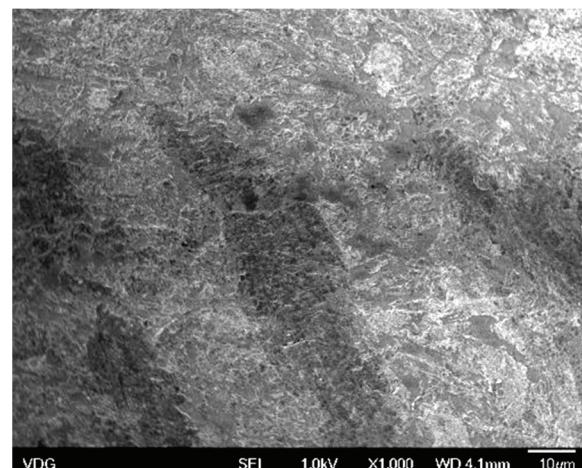
3.3. Surface Characterization

Field Emission Scanning Electron Microscopy was used to characterize the surfaces of the graphite-epoxy electrodes prepared in this work (Fig. 5). At both surfaces, it is possible to observe clusters of material gathered at random areas over the surface. The light points are associated with the epoxy resin (insulator) and the dark areas correspond to the graphite particles (conductor) in the blend. The graphite-epoxy electrode surface appears to be relatively rough, not showing significant differences between the electrodes GEC II and GEC III. These images are similar to those

reported for a graphite-epoxy electrode used as conducting carbon composite in bioaffinity platforms [21, 13].



(a)



(b)

Fig. 5. FESEM micrographs of the polished surfaces of graphite-epoxy composite electrodes: A) GEC II, B) (GEC-III). (Acceleration voltage = 1.0 kV; resolution = 10 μm).

This type composite electrode, comprising graphite particles randomly distributed in epoxy resin, has been reported to behave like a macroelectrode formed by an array of microelectrodes. The answer is greater, because a current major product of the sum of the individual currents of microelectrodes, with the advantage of a lower signal/noise ratio characteristic of the microelectrodes is obtained. It is which has to allow voltammetry at high speeds, minimizing scan times, plus an efficient mass transport of electrochemical species, which allows the use of graphite electrodes epoxy development of various sensors these properties.

3.3.1. Impedance Electrochemical Spectroscopy

Impedance spectroscopy by the properties of the electrode surface was examined using the system $\text{Fe}(\text{CN})_6^{3-/4-}$ as redox probe. This technique is very effective for the characterizations of the surface of the electrodes by electron transfer resistance and capacitance. Since allows differentiation between the capacitive component and the faradaic current by applying a small potential difference to measure the next system to equilibrium conditions.

The electronic transfer to the system $\text{Fe}(\text{CN})_6^{3-/4-}$, reflected in the charge transfer resistance which is directly related to the diameter of the semicircle in the Nyquist plot. This depends on the ion concentration, the type of electrode, the area and the resistance of the composite. This parameter is inversely proportional to the heterogeneous electron transfer constant, both selectivity and response time of the electrode [20]. Low resistance values charge transfer enable greater electroanalytical applicability of the electrodes.

Experimental data for GEC II and GEC III for the system $\text{Fe}(\text{CN})_6^{3-/4-}$, in PBS pH 6.9, and the adjustment shown in Fig. 6. Low values of chi square and coefficients of variation for each parameter used in the fit was obtained, indicating that the experimental data is well explained by the proposed equivalent circuit.

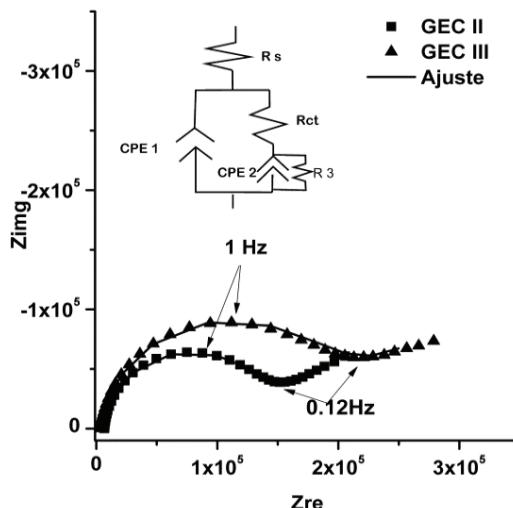


Fig. 6. Diagram of Nyquist. Equivalent circuit (inside), experimental data normalized by geometric area for GEC II (■), GECIII (▲) and fit (—).

Fig. 6 shows the experimental and simulated imaginary (Z_{img}) vs. real (Z_{re}) impedance plots for $\text{Fe}(\text{CN})_6^{3-/4-}$ species on for GEC II and GECIII, based on the geometric area. The semicircle located at high frequencies corresponds to the charge-transfer reaction, and is followed by a linear region of the $-Z_{\text{img}}$ vs. Z_{re} plot at low frequencies, up to 0.12 Hz. In this frequency region, the impedance response is dominated by the mass transfer of the redox species to and from the electrode surface. The experimental

impedance data for the GECs were well described by the Randles circuit shown in Fig. 6 (inside). In this circuit, R_s is the uncompensated solution resistance (20 mmolL^{-1} phosphate buffer solution, pH 6.9). R_{ct} the charge-transfer resistance corresponding to the oxidation and reduction of the $\text{Fe}(\text{CN})_6^{3-/4-}$ redox species. CPE1 is a constant phase element and corresponds to the capacitance of the double layer or the total capacitance in series of the coated electrode/solution interface, and the diffusion impedance, here better represented by a combination of CPE2 and R_3 . In this circuit, the ideal capacitor was substituted by a constant phase element (CPE) due to the heterogeneous current distribution on the electrode surface, a consequence of the surface roughness, surface defects and inhomogeneities. Table 3 gives the parameters that best fitted the experimental data. The circuit is a porous surface partially blocked. Which is consistent with the surface is conductive and insulating regions as microscopy performed.

Comparing the values of charge transfer (diameter of cycle) to GEC II and III the lower value was obtained for GEC II, although the GEC III contains a higher percentage of graphite (Table 3, Fig. 6). The exact reason for the increase in the value of charge transfer by increasing the percentage of graphite in the composite is not entirely explicable. The composite resin more exposed surface may be subject to greater percolation after hydration of the polymer, resulting in an increase of the largest area in the graphite composite more exposed.

Table 3. Parameters adjusted to the experimental values of impedance for GECII and GEC III electrodes, normalized by the geometric area.

	GEC II	CV %	GEC III	CV %
$R (R_s)$ Ωcm^2	11.3 ± 0.3	2	10.3 ± 0.2	2
R_{ct} Ωcm^2	213 ± 10	4	441 ± 30	6
CPE 1 T F/cm^2	$9.7 \pm 0.2 \text{e-}4$	3	$4.4 \pm 0.1 \text{e-}4$	7
α 1	0.96 ± 0.02	1	0.93 ± 0.01	1
CPE 2 T F/cm^2	$1.9 \pm 0.2 \text{e-}3$	8	$9 \pm 1 \text{e-}3$	1
α 2	0.5	-	0.5	-
R_3	$1 \text{e}6$	-	$1 \text{e}6$	
γ_2	$5 \text{e-}5$		$4 \text{e-}4$	
$\sum \gamma_2$	$1 \text{e-}3$		$9 \text{e-}3$	

A decrease of electron transfer with increasing percentage of graphite was described for composite electrodes by Wang in 2001 [22] considered is related to the complexity of composite electrodes and influenced by the random surface distribution of the insulating regions.

Coefficient α related to the angle of rotation in the complex plane capacitance product. Values very close to 1 for α_1 by both electrodes, product the electric

capacitance double layer, corresponding to the similar surface roughness of both electrodes were obtained. These values are characteristic of a capacitor, and describes the contribution to the current phase of the composite insulator (epoxy resin) [23, 24].

To achieve a description of infinite diffusion a value of 0.5 of α_2 was fix. Since values close to 0.5 α is interpreted as Warburg (infinite diffusion) occurring when probe element diffuses through the material [25].

There are differences in the values of CPE T 1 related to the phase of the composite insulator for both electrodes, which is related to the higher proportion of polymer in the GEC II. While for the phase constant element CPE 2 a higher value for the GEC III is appreciated. Increasing the proportion graphite can result in a different distribution surface roughness and porosity of greater residual current accordingly. Which reduces the signal/noise ratio and therefore increases the detection limit.

According to the results of impedance and taking into account the properties required for an electrode for electroanalytical purposes, such as fast response time and low detection limit and high sensitivity, composites GEC III meets these requirements.

4. Oxidation of Nucleotide

Nucleobases guanine and adenine are reported as electroactive DNA indicators, and therefore, the electrochemical processes involving oxidation of these bases are crucial for electrochemical DNA hybridization in the development of biosensors. Therefore, the electrochemical response of these nucleotide nitrogenous bases, in the electrodes developed in this work to evaluate their potential in the development of DNA biosensors was tested.

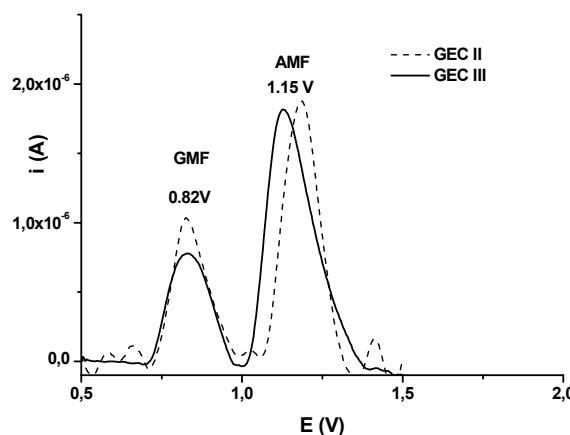


Fig. 6. Signals oxidation guanine monophosphate (GMF) and adenine monophosphate (AMF) 2mmolL⁻¹ in PBS pH 6.7 KCl 0.1molL⁻¹ by square wave voltammetry (- -) GEC II, (—) GEC III.

Signals oxidation of nucleotide guanine monophosphate (GMF) and adenine (AMF)

2 mmol L⁻¹, pH 6.9 in phosphate buffer, have good resolution on the two electrodes. The oxidation potentials for GMF and AMF were on the environment of 0.82 V and 1.1 V, respectively. This values are close to those reported by Erdem [5], which characterizes this values as low compared with the values obtained on other electrodes, indicative of the ease of oxidation of the nitrogenous bases of these transducers, which would allow the electrochemical detection of DNA hybridization without electrochemical markers [26]. Higher oxidation current is observed for guanine monophosphate on the electrode. indicating that this is more sensitive for the detection signal oxidation.

5. Conclusions

A new composition of an epoxy mixture of graphite composites development electrodes was evaluated. The analysis of the potential difference and the peak current of the system $\text{Fe}(\text{CN})_6^{3-/4-}$, for GEC electrodes II and III showed a behavior quasireversible a diffusive process without kinetic complications.

The resistance values of charge transfer capacitance obtained from the results of impedance GEC II present fast response time, low detection limit and high sensitivity suitable for the development of sensors for the detection of biologically relevant molecules, such as GMF and AMF.

Acknowledgements

The CAPES / MES 214/13 and CNPq-Brazil (CNPq No. 009/214) project for financial support.

References

- [1]. Bojorge N., Alhadeff E., Graphite-Composites Alternatives for Electrochemical Biosensor, Metal, Ceramic and Polymeric Composites for Various Uses, John Cuppoletti (Ed.), *InTech*, 2011.
- [2]. Céspedes F., Alegret S., New materials for electrochemical sensing II. Rigid carbon-polymer biocomposites, *TrAC Trends in Analytical Chemistry*, Vol. 19, Issue 4, 200, pp. 276-285.
- [3]. Montes R., Bartrolí J., Baeza M., Céspedes F., Improvement of the detection limit for biosensors: Advances on the optimization of biocomposite composition, *Microchemical Journal*, Vol. 119, 2015, pp. 66-74.
- [4]. Lucarelli F., Marrazza G., Turner APF, Mascini M., Carbon and gold electrodes as electrochemical transducers for DNA hybridisation sensors, *Biosensors and Bioelectronics*, Vol. 19, Issue 6, 2004, pp. 515-530.
- [5]. Erdem A., Pividori M. I., del Valle M., Alegret S., Rigid carbon composites: a new transducing material for label-free electrochemical genosensing, *Journal of Electroanalytical Chemistry*, Vol. 567, Issue 1, 2004, pp. 29-37.
doi:<http://dx.doi.org/10.1016/j.jelechem.2003.10.049>.
- [6]. Apetrei C., Apetrei I. M., Saja J. A. D., Rodriguez-Mendez M. L., Carbon Paste Electrodes Made from

- Different Carbonaceous Materials: Application in the Study of Antioxidants, *Sensors*, Vol. 11, Issue 2, 2011, pp. 1328-1344.
- [7]. Apetrei C., Saja J., Zurro J., Rodríguez-Méndez M., Advantages of the Biomimetic Nanostructured Films as an Immobilization Method vs. the Carbon Paste Classical Method, *Catalysts*, Vol. 2, Issue 4, 2012, pp. 517-531.
- [8]. Cavalheiro É., Brett C.A., Oliveira-Brett A., Fatibello-Filho O., Bioelectroanalysis of pharmaceutical compounds, *Bioanal Rev*, Vol. 4, Issue 1, 2012, pp. 31-53.
- [9]. Schulein J., Grassl B., Krause J., Schulze C., Kugler C., Muller P., Bertling W. M., Hassmann J., Solid composite electrodes for DNA enrichment and detection, *Talanta*, Vol. 56, Issue 5, 2002, pp. 875-885.
- [10]. Zacco E., Pividori M. I., Llopis X., del Valle M., Alegret S., Renewable Protein A modified graphite-epoxy composite for electrochemical immunoassays, *Journal of Immunological Methods*, Vol. 286, Issue 1-2, 2004, pp. 35-46.
doi:<http://dx.doi.org/10.1016/j.jim.2003.11.014>.
- [11]. Wissler M., Graphite and carbon powders for electrochemical applications, *Journal of Power Sources*, Vol. 156, Issue 2, 2006, pp. 142-150.
- [12]. Bagotsky V. S., ElectrokINETIC Processes, in Fundamentals of Electrochemistry, *John Wiley & Sons, Inc.*, 2005, pp. 595-606.
- [13]. Ramirez-Garcia S., Alegret S., Cespedes F., Forster R. J., Carbon composite electrodes: surface and electrochemical properties, *Analyst*, Vol. 127, Issue 11, 2002, pp. 1512-1519.
- [14]. McCreery R. L., Advanced carbon electrode materials for molecular electrochemistry, *Chemical Reviews*, Vol. 108, Issue 7, 2008, pp. 2646-2687.
- [15]. Kneten K. R., McCreery R. L., Effects of redox system structure on electron-transfer kinetics at ordered graphite and glassy carbon electrodes, *Analytical Chemistry*, Vol. 64, Issue 21, 1992, pp. 2518-2524.
- [16]. Lazo-Fraga A. R., Vasconcelos-Pacheco A., Díaz-García A., Bustamante-Sánchez M., Estévez-Hernández O., Evaluación de diferentes aroiltioureas como ionóforos en sensores de plomo (II), *Revista Cubana de Química*, Vol. 27, 2015, pp. 262-274.
- [17]. Hogan C. F., Bond A. M., Myland J. C., Oldham K. B., Facile Analysis of EC Cyclic Voltammograms, *Analytical Chemistry*, Vol. 76, Issue 8, 2004, pp. 2256-2260.
- [18]. Kiryushov V. N., Skvortsova L. I., Aleksandrova T. P., Electrochemical behavior of the system ferricyanide-ferrocyanide at a -epoxy composite electrode, *Journal Analytical Chemistry*, Vol. 66, Issue 5, 2011, pp. 510-514.
- [19]. Holze R. Piero Zanello, Inorganic electrochemistry: theory, practice and applications, *Journal Solid State Electrochem*, Vol. 10, Issue 7, 2006, pp. 512-513.
- [20]. Bard A. J., Electrochemical Methods Fundamentals and Applications, *John Wiley & Sons, Inc.*, 2001.
- [21]. Pividori M. I., Lermo A., Zacco E., Hernández S., Fabiano S., Alegret S., Bioaffinity platforms based on carbon-polymer biocomposites for electrochemical biosensing, *Thin Solid Films*, Vol. 516, Issue 2-4, 2007, pp. 284-292.
- [22]. Wang J., Anik Kirgöz Ü., Mo J.-W., Lu J., Nasser Kawde A., Muck A., Glassy carbon paste electrodes, *Electrochemistry Communications*, Vol. 3, Issue 4, 2001, pp. 203-208.
- [23]. Jorcin J.-B., Orazem M. E., Pébère N., Tribollet B., CPE analysis by local electrochemical impedance spectroscopy, *Electrochimica Acta*, Vol. 51, Issue 8-9, 2006, pp. 1473-1479.
- [24]. Orazem M. E., Tribollet B., Equivalent Circuit Analogs, in Electrochemical Impedance Spectroscopy, *John Wiley & Sons, Inc.*, 2008, pp. 153-162.
- [25]. Rammelt U., Reinhard G., On the applicability of a constant phase element (CPE) to the estimation of roughness of solid metal electrodes, *Electrochimica Acta*, Vol. 35, Issue 6, 1990, pp. 1045-1049.
- [26]. Paleček E., Nucleic Acids | Electrochemical Methods, in Encyclopedia of Analytical Science (2nd Edition), Poole PWT (Ed.) Elsevier, 2005, pp 399-408.