REVISTA MATÉRIA
V.26 N.01
ISSN 1517-7076 articles e12946, 2021

Study of the interaction of the bioactive compound saponin from *Glycyrrhiza glabra* with a carbon nanotube matrix

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ABSTRACT

Saponins are bioactive compounds belonging to the secondary metabolism of plants widely used for their beneficial actions to human health. In this work the association of the saponin from *Glycyrrhiza glabra* with a matrix of multi-walled carbon nanotubes was promoted to obtain a composite material with improved functional characteristics. For this investigation, chemically modified electrodes (CME) based on carbon paste were developed. Firstly, a carbon paste electrode (CPE) modified with the addition of saponin (SAP) was developed. For the electrochemical optimization of this system, CPE/SAP, studies were carried out using cyclic voltammetry. The determination of parameters such as formal potential (E^o) and potential separation (ΔE) indicated that the saponin used as a carbon paste modifying agent generated a matrix that favors the transfer of electrons even at low applied potentials. The second step was performed by preparing the modified carbon paste electrode with the carbon nanotube matrix (CPE/CNT). This material, surprisingly, showed a redox pair probably due to the presence of iron atoms from the preparation method, something very beneficial for the purposes of this work. Next the saponin was associated to the carbon nanotube matrix in order to investigate the behavior of the hybrid material formed (CPE/SAP-CNT), which evidenced a significant improvement in the electron transfer process when the saponin interacts with the carbon nanotube matrix, increasing the anodic peak current by more than 3.6 times in relation to the CPE/SAP and 2.1 times when compared to the CPE/CNT. Another important issue concerns the stability of the systems, with the saponin associated with the carbon nanotube matrix presenting significantly improved stability, being able to be used for more than 8 hours or 200 voltammetric cycles with loss of signal of the order of only 2%, while the CPE/SAP showed a 60% loss of signal under the same conditions of use.

Keywords: Saponin, muti-walled carbon nanotubes, modified electrodes, carbon paste.

RESUMO

As saponinas são compostos bioativos pertencentes ao metabolismo secundário das plantas amplamente utilizadas por suas ações benéficas à saúde humana. Neste trabalho promoveu-se a associação da saponina de *Glycyrrhiza glabra* com uma matriz de nanotubos de carbono multiparedes para obter um material compósito com características funcionais melhoradas, para essa investigação foram desenvolvidos eletrodos quimicamente modificados (EQM's) à base de pasta de carbono. Primeiramente foi desenvolvido o eletrodo a base de pasta de carbono (EPC) modificado com a adição da saponina (SAP), para a otimização eletroquímica deste sistema, EPC/SAP, realizou-se estudos empregando-se a voltametria cíclica. Os experimentos possibilitaram a observação de um par redox com boa corrente de picos anódico e catódico, a determinação de parâmetros como potencial formal (E^o) e separação de potencial (ΔE) indicou que a saponina empregada como agente modificador da pasta de carbono gerou uma matriz que favorece a transferência de elétrons mesmo em baixos potenciais aplicados. A segunda etapa foi realizada mediante a

Corresponding Author: Calline Pereira dos Santos Received on: 04/10/2020 Accepted on: 09/11/2020

10.1590/S1517-707620210001.1246

preparação do eletrodo de pasta de carbono modificado com a matriz de nanotubos de carbono (EPC/NTC). Este material, surpreendentemente, apresentou par redox provavelmente devido a presença de átomos de ferro proveniente do método de preparação, algo muito benéfico para os propósitos deste trabalho. Posteriormente a saponina foi associada à matriz de nanotubos de carbono com intuito de verificar o comportamento do material híbrido formado (EPC/SAP-NTC), esse estudo evidenciou uma melhoria significativa na transferência de elétrons quando a saponina interage com a matriz de nanotubos de carbono, aumentando a corrente de pico anódico em mais de 3,6 vezes em relação ao EPC/SAP e 2,1 vezes me relação ao EPC/NTC. Outro aspecto importante diz respeito à estabilidade dos diferentes sistemas, com a saponina associada à matriz de nanotubo de carbono apresentando estabilidade significativamente melhor, podendo ser usada por mais de 8 horas ou 200 ciclos voltamétricos com perda de sinal da ordem de apenas 2%, enquanto o EPC/SAP apresentou perda de sinal de 60 % nas mesmas condições de uso.

Palavras-chave: saponina, nanotubos de carbono multiparede, eletrodos modificados, pasta de carbono.

1.INTRODUCTION

The study of the electrochemical behavior of bioactive compounds has grown significantly in recent years, especially as a modifying agent in working electrodes, so-called chemically modified electrodes (CME) often associated with matrices of materials such as carbon nanotubes and other nanomaterials used in the development of amperometric sensors or biosensors. A biosensor is an analytical device that combines a biological component with a detection component, *i.e.*, a transducer element that ensures rapid and accurate conversion into measurable signals [1-3].

The development of CME, especially those based on the carbon paste, consists of joining the matrix of the base electrode with the chemical modifying agent, implementing changes that make it possible to control the physicochemical properties of the surface of the generated electrode, emphasizing that the manipulation of the physical-chemical nature of the electrode can result in significant improvements in the system, such as: reducing interference phenomena, electrode fouling problems, unwanted precipitation or adsorption, increased sensitivity, increased stability, biocompatibility, among other performance gains of the electrode\resulting sensor [4, 5]

The great effort that is made in the search for better electrochemical sensors occurs, since they are devices with peculiar characteristics, which present themselves with increasing use, with data in experimental conditions easily obtained, with good precision and accuracy, possibilities of miniaturization and, consequently, allow obtaining important information in different systems, increasing its applicability ranging from the detection of gas molecule to the study of the kinetics of chemical signals from a biological cell to implanted sensors [4, 6, 7].

Among the many bioactive compounds, saponins (SAP) are noteworthy for their versatility, since they have both bioactive action and surfactant properties, which allow their use in several situations such as: applications in the food and cosmetic and health industry [8]. The saponic compounds are widely distributed in nature and are present in plant species belonging to secondary metabolites, with potential biological activities such as: antioxidant, anti-inflammatory, expectorant, antimicrobial and other therapeutic activities [9]. In terms of chemical structure they are described as a group of triterpenic or steroidal glycosides predominantly found in the plant kingdom, in different parts of plants, including roots, shoots, flowers, seeds and leaves and to a lesser extent in marine animals [10-13].

The stabilization of biomolecules on the surface of most of the systems used in the preparation of CME falls short, besides problems in the process of electron transfer occurs. On the other hand, carbon nanotubes exhibit unique properties for the development of sensors, such as: large surface area, high mechanical, chemical and thermal resistance besides the excellent conductivity. All these characteristics make the carbon nanotube (CNT) much needed by the scientific community in several areas of knowledge [14-16].

Thus, the association of the individual properties of saponin with CNT can lead to the formation of a nanocomposite with great possibilities of application, as already seen in similar systems describing the association of biomolecules with nanomaterials [17-20]. In the case of the use of CNT in the development of CME, its potential is clearly realized by the generation of high-performance sensors and biosensors, especially when associated with powder graphite, in the development of carbon paste-based sensors [21-23]. Thus, the present work aimed at the study of the electrochemical behavior of saponin from *Glycyrrhiza glabra* in the development of CME based on carbon paste, as well as the system formed from the association of both saponin and CNT.

2. MATERIALS AND METHODS

2.1 Materials

The following materials were used: Saponin from *Glycyrrhiza glabra* (Sigma Aldrich), Potassium Chloride (Sigma Aldrich), Citric Acid Monohydrate (Nuclear), Potassium Phosphate Monobasic (Nuclear), Boric Acid (Synth), Mineral oil (Schering-Plough), Graphite powder ~~99.9%~~ (Synth). CNT were obtained from the pyrolysis of a mixture of 84% camphor and 16% ferrocene at 850 °C. First, the mixture was evaporated at 200 °C, and then transported into the reaction zone within the quartz tube inside an oven by an inert gas (N_2) at a flow of 200 sccm. The ratio of 16% ferrocene provided a high conversion of the mixture to CNT, and the film grew at a rate around 15 µm / min [24]. To weigh the masses of the solutes it was used a scale analytical (Shimadzu model AUW220D with five decimal places). To measure the pH, a JENWAY (3305) pH Meter was used. All reagents used were of analytical grade. Ultra pure water, resistivity 18.22 MΩcm was used in sample preparation All experiments were performed at room temperature, 22 ± 1 ºC.

2.2 Development of chemically modified electrodes (CME) based on carbon paste

The chemically modified electrodes based on carbon paste used as working electrode were developed in our laboratory [25]. Initially the working electrode is presented as a glass tube which coats a copper wire connected in a gold disc base forming a lower cavity of 4.0 mm of inner diameter and 1.5 mm in depth for the insertion of the carbon paste. This working electrode is then used in an electrochemical cell of three electrodes according to as shown in Figure 1. For the execution of this research, three carbon-based work electrodes were developed, namely:

- CME (Carbon Paste Electrode- CPE/Saponin-SAP), 2.0 mg of saponin, 30.0 mg of graphite powder and 20 µL of mineral oil were mixed until complete of the resulting paste. Subsequently, the paste was placed in the lower cavity of the working electrode, taking care to not leave voids or deformation on the surface of this electrode.

- CME (CPE/CNT), 5.0 mg of CNT, 30.0 mg of graphite powder and 20 µL of mineral oil were blended until complete homogenization of the resulting paste. Subsequently, the same procedure as in the previous CME was followed.

- CME (CPE/SAP-CNT), 2.0 mg of saponin, 5.0 mg of CNT, 30.0 mg of graphite powder and 20 µL of mineral oil were mixed until complete homogenization of the resulting paste. Subsequently, the same procedure as in the previous CME was followed.

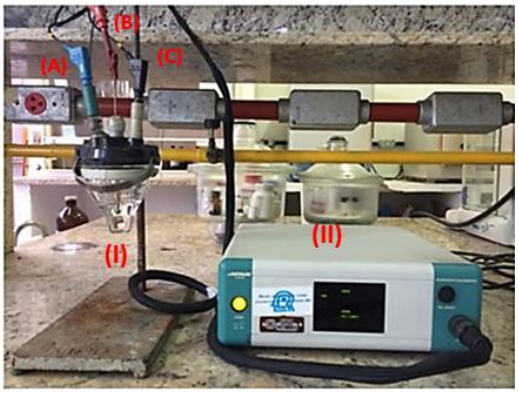

Figure 1: The left (I) represents the electrochemical cell with three electrodes. (A) Reference electrode, (B). Working electrode and (C) Counter electrode. On the right (II), potentiostat equipment.

2.3 Electrochemical Measurements

The electrochemical measurements were performed in a potentiostat model micro-autolab III of Metrohm pensalab with the use of an electrochemical cell composed of 3 electrodes, silver/silver chloride as the reference electrode, a platinum wire as an auxiliary electrode and chemically modified electrodes CPE/SAP or CPE/SAP-CNT as working electrodes, Figure 1. All the electrodes were submitted to the voltammetric

tests obtaining voltamograms with variation of different scanning speeds of the potential, different electrolytes support in different concentrations, and different concentrations of hydrogen, in order to know the best working conditions. It is important to point out that based on these results, we sought to highlight characteristics such as the formal potential, the potential of peak separation, anode and cathodic peak current, the relationship between currents, system reversibility, electrochemical and chemical stability, characteristics of its electrons transfer, etc., assuring the electrochemical optimization of the developed electrodes. Cyclic voltammetry studies were conducted with the General Purpose Eletrochemical System (GPES) and their treatment was done by the OriginPro version 8 software [26].

3. RESULTS AND DISCUSSION

3.1. Electrochemical response of saponin modified carbon paste electrode (CPE/SAP)

Saponins are known to be glycosides of steroids or polycyclic terpenes, their structure has amphiphilic character, part of it with lipophilic (triterpene or steroid) and other hydrophilic (sugars). At the root of licorice is glycyrrhizinic acid (GL), called glycyrrhizin, a bidesmosidic triterpene saponin. Figure 2 shows the chemical structure of glycyrrhizin, whose hydrophilic moiety (glycone) is represented by two glycuronic acid molecules linked to the glycyrrinic acid chain~~25~~.

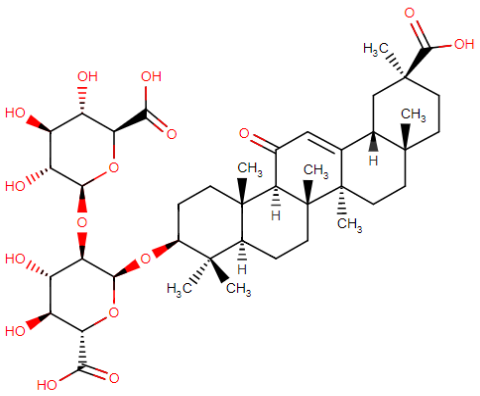

Figure 2: Chemical structure of saponin from *Glycyrrhiza glabra*. (source: drugbank, https://www.drugbank.ca/drugs/DB13751)

With such a versatile structure, saponin begins to arouse interest in the scientific community, mainly in nanotechnology, in the sense of exploring its use as part of the development of new materials. In a recent study, De Oliveira *et al.* [27] investigated the interaction between the triblock copolymer Pluronic F127 and a natural bioactive amphiphilic saponin extracted from *Glycyrrhiza glabra* roots. Combined, these results indicate the formation of micelle-like complexes, with structure dependent on the SAP concentration, as highly promising agents in nanobiotechnology applications. In another study, Yu *et al*. [28] checked synergy between poly(vinylpyrrolidone) (PVP)-coated silica nanoparticles (CPs) and saponin β-escin was exploited to develop long-lasting foams using low component concentrations. The successful synergy of CPs and saponin β-escin was shown to provide excellent foamability and foam stability, with no direct interaction between the two components. Such studies expand the alternatives of application of this type of saponin and allow the studies to open new perspectives, since saponin has been shown to be a multipurpose bioactive compound. Thus, saponin was used as a carbon paste modifying agent.

This first stage of the study was based on the electrochemical behavior of the CPE/SAP electrode, aiming to establish the best conditions regarding the support electrolyte concentration, the sweep rate effect, as well as the pH effect of the electrolyte solution used in the electrochemical cell. Figure 3 (A) containing the carbon paste electrode without the modifying agent shows no peak currents, *i.e.* no redox pair, as for the

voltammogram of Figure 3 (B) containing the modified carbon paste electrode with the saponin, there is a peak current, which shows the positive voltammetric response of electron transfer with the appearance of a redox pair, which made it possible to calculate the formal potential E^0 = (Epa + Epc)/2) of 65 mV vs Ag/AgCl, with Epa being the anodic peak potential and Epc being the cathodic peak potential. In this voltammogram was also calculated the peak separation ΔE = (Epa-Epc) finding the value of 58 mV. The low formal potential value and low peak separation indicate that this system operates at low energy to promote the electron transfer process on the electrode surface and does so with good reversibility indicating that saponin appears to fit well into this carbon matrix [29].

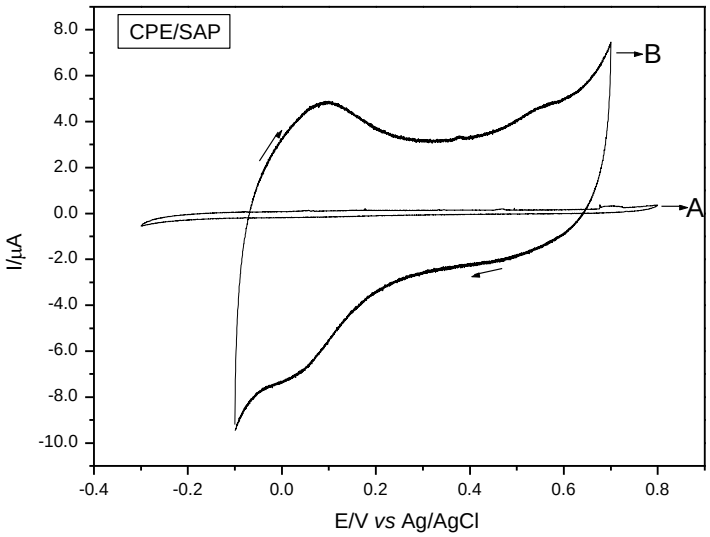

Figure 3: Cyclic voltammograms obtained in 0.25 mol L^{-1} sodium phosphate solution, pH 6.5 and potential scanning speed of 10 mV s-1. (A) CPE, (B) CPE/SAP.

3.2. Influence of the support electrolyte and its concentration on the electrochemical response of the CPE/SAP electrode

To verify the influence of the different support electrolytes on the electrochemical behavior of the CPE/SAP system, voltammetric experiments were performed. The values of formal potential, peak separation and anodic peak current were found (Table 1). Through the data it is possible to notice that all the electrolytes employed promote good conditions of operation of the electrochemical cell with very interesting parameters, that is, low peak separation and near zero formal potential. What differentiates the electrolytes is the peak current level. In this case the cell being operated in phosphate solution presents a current level well above the others. The concentration also did not significantly influence the operation of the electrochemical cell suggesting good ion mobility while maintaining the electroneutrality on the electrode surface. This performance can be explained either by the characteristic of the carbon paste in adapting to changes near its surface, or by the properties of the system keeping the reduced and oxidized forms of CPE/SAP constant, even when subjected to changes in the support electrolyte concentration [30].

Table 1: CPE/SAP electrochemical response as a function of the support electrolyte employed and the variation of its concentration. Experiment conducted at pH 6.50, sweep speed of 10 mV s^{-1} and potential range from -0.2 to 0.6 V.

Electrolyte	ΔE (mV)	E^0 (mV)	I_{Pa} (μA)
KCl (0.05 mol L^{-1})	70	65	4.0
KCl (0.25 mol L^{-1})	70	65	4.5
KCl (0.50 mol L^{-1})	70	65	4.5
Fosfato (0.05 mol L^{-1})	60	60	9.0
Fosfato (0.25 mol L^{-1})	60	60	9.8
Fosfato (0.50 mol L^{-1})	60	60	9.8
PBC (0.05 mol L^{-1})	90	75	5.0
PBC (0.25 mol L^{-1})	90	75	5.1
PBC (0.50 mol L^{-1})	90	75	5.1

3.3. Study of the electrochemical response using CPE/SAP as a function of the pH of the support electrolyte solution

The electrochemical behavior study of CPE/SAP from the pH variation of the support electrolyte solution (phosphate buffer) was performed in the range of 5.0 to 7.5, setting the potential scanning speed at 10 mV s^{-1}. According to the values of the electrochemical parameters presented in Table 2, potential shape, peak separation potential and peak current, it was noticed that the change in the pH of the support electrolyte solution in the worked range causes few changes in these investigated parameters. Moreover, as it was a moderate variation in the investigated parameters, it suggests that the carbon paste creates a protective effect on the system in order to soften the damage caused by the variation of the H^+ concentration in the solution and also the saponin itself with its bioactive and surfactant properties can soften the influence of the H^+ concentration change in the solution in order to keep the electrochemical parameters close. According to Kim et al. (2006) the CPE has exerting protection to the variation of the pH, and increases system stability regardless of the eletrochemical environment [31].

Table 2: Influence of pH variation of the support electrolyte solution on the electrochemical response of the CPE/SAP system at 10 mV s^{-1} sweep rate in 0.25 mol L^{-1} phosphate buffer.

pH of support electrolyte solution	Δ E_p/mV	E^0/mV	I_{pa} (μA)
5.0	65	100	9.8
6.5	55	65	9.8
7.5	50	55	9.2

3.4. Influence of the sweep rate variation of the applied potential on the electrochemical response of the CPE/SAP system

Investigating the potential sweep rate variation is an important mechanism for understanding the electrochemical behavior of the CPE/SAP system. As can be seen from Figure 4, even with increasing sweep velocity there is no large variation in the displayed cyclic voltammograms, the formal potential and peak separation hardly change, which implies that the saponin-containing system can be considered quite stable when

supported on the carbon paste matrix, as there are no significant deformations in the voltammograms.

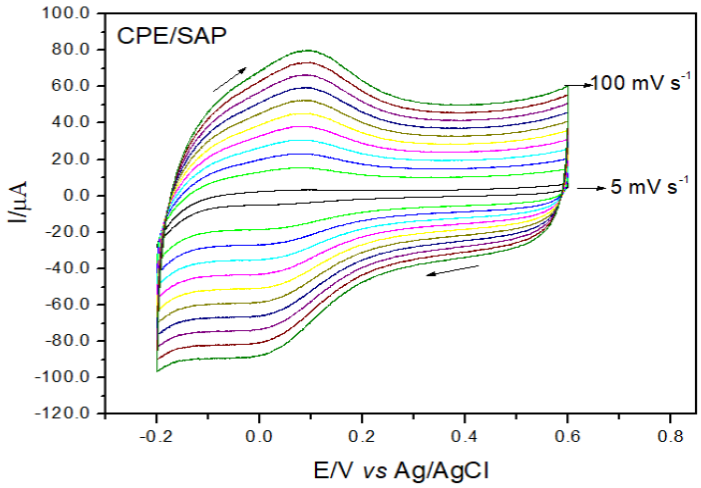

Figure 4: Voltage behavior of CPE/SAP electrode at different potential scanning speeds (10-110 mVs^{-1}). Experiment conducted in 0.25 mol L^{-1} sodium phosphate solution, pH 6.5 and potential range from -0.2 to 6.0 V.

Continuing to evaluate the influence of the sweep rate variation of the applied potential, the graph of Figure 5 shows the correlation of the anode peak current (I_{Pa}) versus to the sweep rate, obtaining a characteristic linearity of systems governed by adsorption, according to Laviron [32]. This behavior is indicative that the adsorption and desorption process of the oxidized and reduced saponin species on the carbon paste electrode surface governs electron transfer to this system.

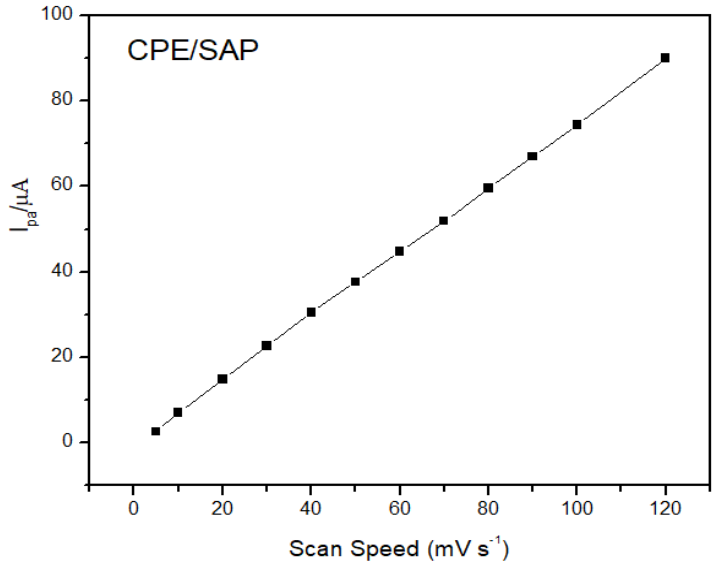

Figure 5. Anode peak current as a function of potential scan speed from 10 to 80 mV s^{-1}. Experiment conducted with CPE/SAP in 0.25 mol L^{-1} phosphate solution and pH 6.5.

3.5. Study of the electrochemical behavior of saponin associated with the CNT matrix

In order to enhance the applicability of saponin, its association with a nanostructured material that could improve its electrochemical performance and stability gains was implemented. So, the results presented below are important in the context of immobilization and stabilization of biomolecules in nanostructured materials for the development of novel electrochemical sensors [33-35].

Unlike other nanostructured materials [18] the carbon paste-associated CNT employed in this work has interesting electrochemical characteristics. As can be seen in Figure 6, this material has a low formal potential redox pair (287 mV s^{-1}), low peak separation (22 mV s^{-1}), Ipa / Ipc ratio close to the unit and negligible distortion of these parameters with the variation of the potential scan speed. This behavior suggests the presence of some element that promotes electron transfer via redox pair on the surface of this material (carbon paste) and this is achieved with good reversibility and stability when associated with graphite powder, and the presence of this well-defined redox pair, unlike other carbon nanotubes and nanostructured compounds, it provides this material to function as an electron mediator in systems that have difficulties in electron transfer, decreasing the overpotential and improving the operational stability of the system, justifying its use in this work employing saponin as a modifying agent of the CPE [25].

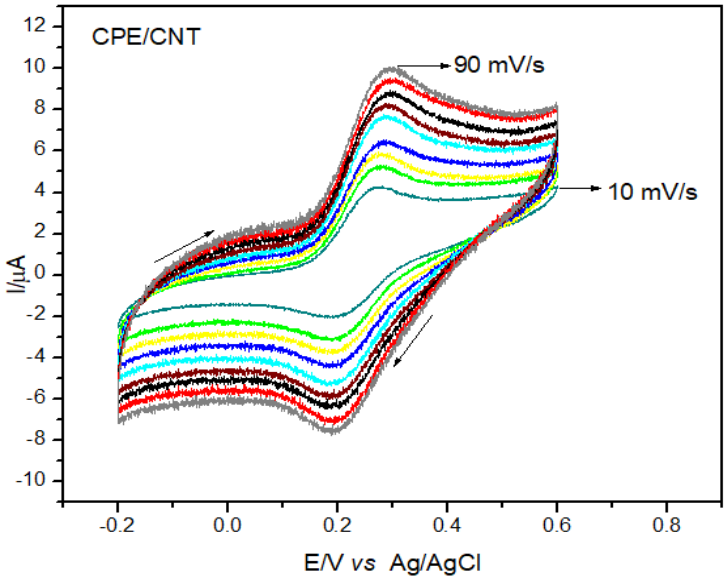

Figure 6: Voltammograms referring to the electrochemical response of the CPE/CNT system as a function of sweep speed variation from 10 to 90 mV s^{-1}. Experiment conducted in 0.25 mol L^{-1} phosphate solution and pH 6.5.

3.6. Study of the electrochemical behavior of the system formed by the association of saponin with the carbon nanotube matrix, CPE/SAP-CNT.

After studying the electrochemical behavior of the CPE/SAP system which proved the saponin's ability to participate in the process of electron transfer on the carbon paste surface in the potential range of -0.2 to 0.6 V *vs* Ag/AgCl, a subsquent study was done in order to verify the electrochemical behavior of the system formed through the interaction of saponin with the carbon nanotube matrix presented in the previous item, developing the chemically modified electrode CPE/SAP-CNT. For comparison purposes in Figure 7 we have the voltammograms of the three worked systems, (A) CPE/SAP, (B) CPE/CNT and (C) CPE/SAP-CNT.

Initially, it was verified that all three cases presented excellent redox process. This figure shows the significant current gain and signal definition presented by the CPE/SAP-CNT system. It is known that this increase in response is verified due to the interaction of saponin with CNT, since in CPE/SAP electrode there is 2 mg of saponin, in CPE/CNT there is 10 mg of CNT and for the CPE/SPA-CNT electrode these same amounts of saponin and CNT were employed, so it is clear that the electrochemical gain is due to the favorable interaction between saponin and CNT. Concerning to CPE/SAP there is a current difference in the order of 3.6 times greater and 2.1 times greater when compared to CPE/CNT. Probably this matrix of carbon nanotubes is acting by mediating the electron transfer between the saponin and the surface of the carbon paste electrode, something that would justify the expressive increase of current of this system [25].

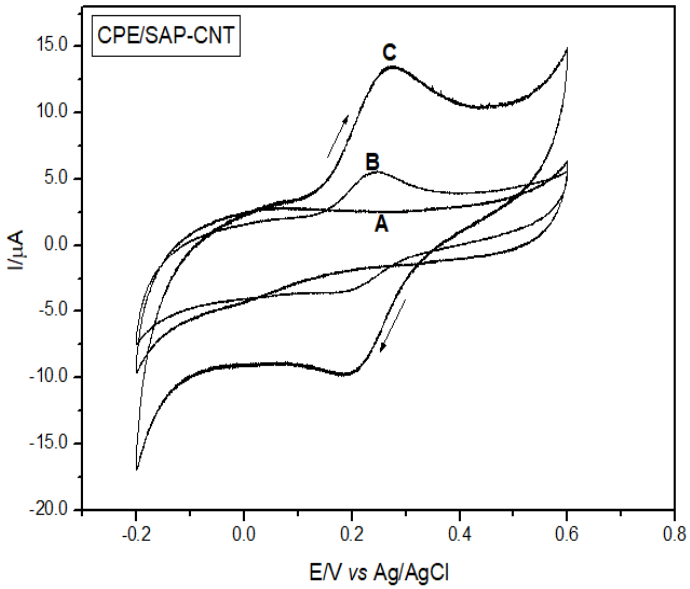

Figure 7. Cyclic voltammograms: (A) CPE/SAP, (B) CPE/CNT, and (C) CPE/SAP-CNT. Experiments performed on 0.25 mol L^{-1} phosphate solution, pH 6.5 and 10 mVs^{-1} sweep rate.

Figure 8 shows the effect of potential scan speed on the electrochemical response of the CPE/SAP-CNT system. Good reversibility is observed in the process, since there is no significant deformation in the voltammogram, that is, the oxirreduction peaks hardly move to either positive or negative points. It is also possible to observe the existence of two redox pairs in the voltammogram, which appears more clearly as the scanning speed increases, normal for a redox process with the presence of a bioactive molecule such as saponin. This is because when it increases at sweep rate the oxidation range can be widened by discharging into two potentials originating from the electrode containing saponin and carbon nanotubes. Thus, they show evidence that there was an effective interaction between saponin and carbon nanotubes in the formation of nanocomposite.

The graph inserted in Figure 8 shows a linear correlation between the peak current intensity and the scanning rate of the chemically modified electrode made of saponin and carbon nanotubes, indicating that the redox process is mainly governed by adsorption phenomena [30].

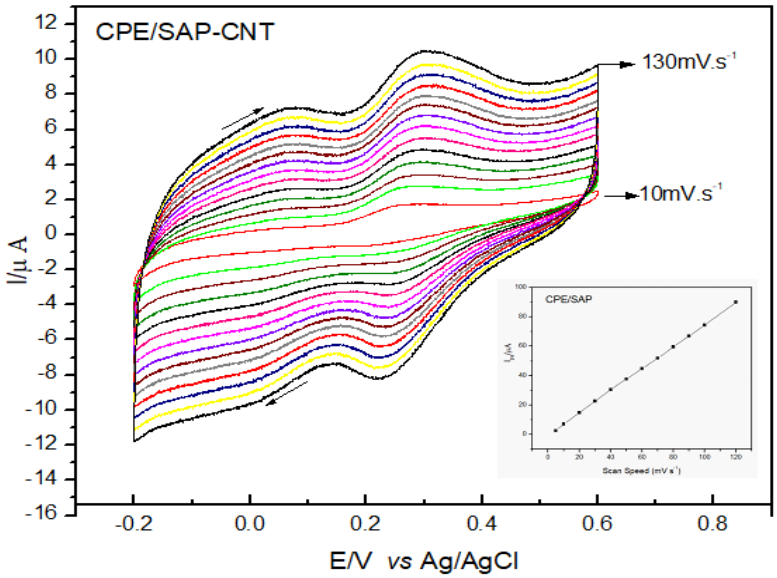

Figure 8. Anode peak current as a function of potential sweep speed. Experiment conducted using CPE/SAP-CNT varying sweep rate from 10 to 130 mV s^{-1} in 0.25 mol L^{-1} phosphate solution, pH 6.5. Inserted figure corresponds to graph of anodic peak current versus potential scan speed.

3.7. Stability test of the carbon paste electrode modified with saponin and carbon nanotubes.

For a clear view of the operational stability of the developed electrodes, the voltammetric experiment was performed by measuring the amperometric current over several cycles and also monitoring the time in hours as shown in Table 3. The CPE/SAP electrode presents significant current, but with Signal decay of more than 60% after 8 hours of use or 200 voltammetric cycles at a speed of 10 mV s^{-1}. Faced with this instability, one can see the importance of a matrix that could contribute to the immobilization of saponin with the carbon paste and do this with gain in stability to the resulting system and that is exactly what happened employing CNT. It can be seen from the CPE/SAP-CNT data that besides the signal magnitude gain there was also a significant stability gain. A condition that clearly justifies the importance of using the carbon nanotube matrix, because there was a very small signal variation, less than 2%, even after 8 hours of use and more than 200 voltammetric cycles at a speed of 10 mV s^{-1}, similar behavior to other recent studies using the same matrix [25, 36].

Table 3. Anode peak current observed for the CPE/SAP-CNT electrode after successive voltammetric cycles in 0.25 mol L^{-1} phosphate solution, pH 6.5, for a sweep rate of 10 mVs^{-1} over the potential range between -0.2 and 0.6 V.

Cycles	1	40	80	120	160	200
I_{pa}(µA) CPE/SAP	4.75	4.45	3.90	3.30	2.60	1.9
I_{pa}(µA) CPE/SAP-CNT	9.80	9.80	9.80	9.75	9.70	9.65

4. CONCLUSION

The electrochemical data proved that the choice of carbon paste electrode was correct due to the overall performance of the developed CME generating current and redox pair on the surface of this material. Saponin,

even being a biomolecule, when modifying the carbon paste, CPE/SAP, showed considerable electrochemical response. This CME has good stability in the face of varying ionic strength and H^+ concentration in the pH range 5.0 to 7.5. The peak separation (ΔE) and the formal potential (E^0) remain almost constant even after 200 voltammetric cycles at 10 mV s^{-1} or 8 working hours, while the anodic peak current showed a 60% decay. Regarding the CME generated from the combination of saponin as the carbon nanotube matrix, CPE/SAP-CNT, it has a remarkable favoritism to the electron transfer on the surface of the resulting material, ie: higher current level, anodic peaks and defined cathodic values, significant increase in operating stability with less than 2% current drop after 200 voltammetric cycles or 8 working hours. So, the hybrid material obtained by the combination of the biomolecule saponin with carbon nanotubes shows a great potential to be employed in the development of novel sensors and biosensors.

5. ACKNOWLEDGMENTS

We thank FAPESB and CNPq for financial support. C.P.S. thanks FAPESB for grant. N.S.M.H. thanks CAPES for grant.

6. REFERENCES

[1] AMBRUSI, R.E., SÁNCHEZ, M.D., GARCÍA, M.D., *et al.*, "Electrocatalytic effect of Ag and Cd bimetallic nanoparticles towards the reduction of nitrate and/or nitrite ions", *Revista Matéria*, v. 23, n. 2, pp. e-12141, 2018.

[2] BARKAUSKAS, J., MIKOLIUNAITE, L., PAKLONSKAITE, I., *et al*., "Single-walled carbon nanotube based coating modified with reduced graphene oxide for the design of amperometric biosensors", *Materials Science & Engineering C*, v. 98, pp. 515-523, 2019.

[3] KANG, B.-C., PARK, B.-S., HA, T.-J., "Highly sensitive wearable glucose sensor systems based on functionalized single-wall carbon nanotubes with glucose oxidase-nafion composites", *Applied Surface Science*, v. 470, pp.13-18, 2019.

[4] MURRAY R.W., EWING A.G., DURST R.A., "Chemically modified electrodes. Molecular design for electroanalysis", *Analytical Chemistry*, v. 59, pp. 379A-390A, 1987.

[5] PEREIRA, A.C., SANTOS, A.S. KUBOTA, L.T. "Tendências em modificação de eletrodos amperométricos para aplicações eletroanalíticas", *Química Nova*, v. 25, n. 6, pp. 1012-1021, 2002.

[6] CORREIA, L.A., MARGARIDO, V.O., SOUZA, A.L., "Electrocatalytic behavior toward nitrite of a hybrid film of fosfomolybdate and organomodified sílica", *Revista Matéria*, v. 24, n. 4, pp. e-12491, 2019.

[7] SOUZA, M.D.F.B., "Eletrodos quimicamente modificados aplicados à eletroanálise: uma breve abordagem", *Química Nova*, v. 20, pp.191-195, 1997.

[8] HOSTETTMANN, K., MARSTON, A., *Saponins: Chemistry Pharmacology of Natural Products*, 1. ed. New York: Cambridge University Press, 1995.

[9] LACAILLE-DUBOIS M.A., WAGNER, H.A., "Review of the biological and pharmacological activities of saponins", *Phytomedicine*, v. 2, n. 4, pp. 363-386, 1996.

[10] NASROLLAHI, V., MIRZAIE-ASL A.S., PIRIA P., *et al*., "The effect of drought stress on the expression of key genes involved in the biosynthesis of triterpenoid saponins in liquorice (Glycyrrhiza glabra)", *Phytochemistry*, v. 103, pp. 32-37, 2014.

[11] VINCKEN, J., HENG, L., GROOT, A., GRUPPEN, H., "Saponins, classification and occurrence in the plant kingdom", *Phytochemistry*, v. 68, pp. 275-297, 2007.

[12] OLESZEK, W.A., *Saponins. In: NAID, A. S. Natural Food Antimicrobial Systems*, CRC Press, Inc., USA, pp. 295-324, 2000.

[13] WEI, J.-H., ZHENG, Y.-F., LI, C.-Y., *et al.*, "Bioactive constituents of oleanane-type triterpene saponins from the roots of *Glycyrrhiza glabra*", *Journal of Asian Natural Products Research*, v. 16, n. 11, pp.

1044-1053, 2014.
[14] MOLAEI, M.J., "A review on nanostructured carbon quantum dots and their applications in biotechnology, sensors, and chemiluminescence", *Talanta*, v. 196, pp. 456-478, 2019.
[15] PHAM, T.N., PARK, D., LEE, Y., KIM, I. T., *et al*., "Combination-based nanomaterial designs in single and double dimensions for improved electrodes in lithium ion-batteries and faradaic supercapacitors", *Journal of Energy Chemistry*, v. 38, pp. 119-146, 2019.
[16] BALASUBRAMANIANA B., BURGHARD, M., "Biosensors based on carbon nanotubes", *Analytical Bioanalytical Chemistry*, v. 385, pp.452-468, 2006.
[17] MOLAEI, M.J., "A review on nanostructured carbon quantum dots and their applications in biotechnology, sensors, and chemiluminescence", *Talanta*, v. 196, pp. 456-478, 2019.
[18] SANTOS, A.S., COSTA, V.C., FELICIO, R.C., "Comparative Study of Nanostructured Matrices Employed in the Development of Biosensors Based on HRP Enzyme for Determination of Phenolic Compounds", *Electroanalysis*, v. 27, pp. 1572-1578, 2015.
[19] LIU, Y., ZHANG, J., CHENG, Y., JIANG, S.P., "Effect of Carbon Nanotubes on Direct Electron Transfer and Electrocatalytic Activity of Immobilized Glucose Oxidase", *ACS Omega*, v. 3, n.1, pp. 667-676, Jan. 2018.
[20] SONG, H., PARK, T., "Integration of biomolecules and nanomaterials: Towards highly selective and sensitive biosensors", *Biotechnology Journal*, v. 6, n. 11, pp.1310-1316, Nov. 2011.
[21] SANTOS, A. *et. al*., "Determination of Phenolic compounds based on co-immobilization of methylene blue and horseradish peroxidase on multi-wall carbon nanotube", *Electroanalysis*, v. 19, pp. 549-554, 2017.
[22] YANG, N., CHEN X., REN T., *et al*., "Carbon nanotube based biosensors", *Sensors and Actuators B*, v. 207, pp. 690-715, 2015.
[23] ZHOU, J., ZHANG, K., LIU, J., *et al*., "A supersensitive sensor for rutin detection based on multi-walled carbon nanotubes and gold nanoparticles modified carbon paste electrodes", *Analytical Methods*, v. 4, n. 5, pp.1350-1356, 2012.
[24] EDWARDS, E.R., ANTUNES, E.F., BOTELHO, E.C., *et al*., "Evaluation of residual iron in carbon nanotubes purified by acid treatments", *Applied Surface Science*, v. 258, pp. 641-648, 2011.
[25] DE BRITO, A.R., REIS, N.S., OLIVEIRA, P.C., *et. al*., "Development of amperometric biosensor in modified carbon paste with enzymatic preparation based on lactase immobilized on carbon nanotubes", *J. Food Science and Technology*., v. 57, p. 1342-1350, 2020.
[26] SANTOS, A.S., DE JESUS, R.S., SILVA, T.A., COSTA, V.C., "Estudo das Interações entre o Análogo de Fármaco Antipsicótico (Azul de Toluidina) com a Substância Psicoestimulante (Cafeína), Empregando-se Eletrodos Quimicamente Modificados", *Revista Virtual de Quím*ica, v.12, n. 2, pp. 287-297, 2020.
[27] DE OLIVEIRA, R.S.S., HUACHACA, N.S.M., LEMOS, M., *et al*., "Molecular interactions between Pluronic F127 and saponin in aqueous solution", *Colloid and Polymer Science*, v. 298, pp.113-122, 2020.
[28] YU, K., LI, B., WANG, Z., *et al*., "Synergy between Composite Nanoparticles and Saponin β-Escin to Produce Long-Lasting Foams", *Industrial & Engineering Chemistry Research*, v. 59, n.16, pp. 7495-7501, 2020.
[29] SANTOS, A.S., PEREIRA, A.C., KUBOTA, L.T., "Electrochemical and Electrocatalytic Studies of Toluidine Blue Immobilized on a Silica Gel Surface Coated with Niobium Oxide", *Journal of Brazilian Chemical Society*, v. 13, n. 4, pp. 495-501, 2002.
[30] BARD, A. J., FAULKNER, L R., SWAIN, E., ROBEY, C., *Electrochemical Methods: Fundamentals and Applications*, 2. Ed. New York: Jonh willey & Sons, 2001.
[31] KIM, J., GRATE, J.W., WANG, P., "Nanostructures for enzyme stabilization", *Chemical Engineering Sci*ence, v. 61, n. 3, pp.1017-1026, 2006.
[32] LAVIRON, E., in: A. J. BARD (Ed.), *Electroanalytical Chemistry*, v. 12, Dekker, New York, 1982.
[33] CARDOSO, R.M., MONTES, R.H.O., LIMA, A.P. *et al*. "Multiwalled carbon nanotubes: Sized-dependent electrochemistry of phenolic compounds", *Electrochimica Acta*, v. 176, pp. 36-43, 2015.
[34] SANTOS FILHO, S.G., HASENACK, C.M., SALAY, L.C., MERTEN, P., "A less critical cleaning

procedure for silicon wafers using diluted HF dip and boiling in isopropyl alcohol as final steps", *Journal of the Electrochemical Society*, v. 142, pp. 902-907, 1995.
[35] NOSSOL, E., ZARBIN, A., "Carbon paste electrodes made from novel carbonaceous materials: Preparation and electrochemical characterization", *Electrochimica Acta*, v. 54, pp. 582-589, 2008.
[36] DE BRITO, A.R., TAVARES, I.M.C., DE CARVALHO, M.S., *et al*., "Study of the interaction of the lactase enzyme immobilized in a carbon nanotube matrix for the development of the chemically modified carbon paste electrode", *Surfaces and Interfaces*, v. 20, pp. e-100592, 2020.

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