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**ESCUELA DE INGENIERÍAS  
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**ESCUELA DE INGENIERIAS INDUSTRIALES**

**GRADO EN INGENIERÍA EN TECNOLOGÍAS INDUSTRIALES**

# **Sensores electroquímicos basados en nanomateriales de carbono**

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**TFG REALIZADO EN PROGRAMA DE INTERCAMBIO**

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**TÍTULO: Electrochemical sensor based on carbonaceous nanomaterials**

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**Resumen:**

Caracterización de tres sensores basados en materiales de nanocarbono mediante el análisis de diferentes compuestos. El objetivo del proyecto ha sido analizar que sensor es el más sensible para detectar los compuestos electroactivos en soluciones. Se han utilizado tres tipos diferentes de sensores: Electrodo de carbono modificado con nanotubos de carbono (CNT), Electrodo de carbono modificado con Nanofibras de carbono grafitizadas (CNF) y Electrodo de carbono modificado con grafeno (GPH). El comportamiento de estos sensores se ha analizado estudiando inicialmente en soluciones de Cloruro de Potasio, Ferrocianuro de Potasio y Catecol. El sensor más sensible ha sido utilizado para la detección de Dopamina en solución acuosa.

**Palabras Claves:**

Carbon, Nanomateriales, Sensor, Voltamperometría, Dopamina

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# 1.THEORETICAL PART

## 1.1 CARBON BASED MATERIALS

Carbon is one of the most plentiful elements that exists in nature. It can be chemical combined with through covalent bond. Carbon materials have very different properties due to the wide variety of structures that it has.<sup>1</sup>

Carbon nanofibers, carbon nanotubes and graphene have been used during the project as sensitive materials in the electrochemical detection of electroactive compounds. The structure of such materials are presented in Figure 1.



*Figure 1. Structure of carbon nanofibers, carbon nanotubes and graphene.<sup>2</sup>*

Graphene is formed with small particles of carbon grouped very dense in sheets and in hexagonal cells. It has few defects, exceptional rigidity and the possibility of expanding much more than any other glass material.<sup>3</sup>

Carbon nanotubes have a cylindrical structure which has extraordinary properties such a good thermal conductivity, mechanical and electrical.

Carbon nanofibers are structures with cylindrical shape with graphene located as cones or cups. They have extraordinary mechanical and electrical properties and easy made, therefore are low cost.<sup>4</sup>

## 1.2 CHEMICAL SENSORS

It is a self-contained device capable of supplying real time analytic sample information. The sample is called analyte or determined.

Two functions are carried out when chemical sensor is operating: recognition and transduction. Firstly, the analyte interacts with recognition receptors. Recognition receptors are compounds, which are able to interact with the analyte.

This interaction between analyte and interactive element from the chemical sensor, changes the analyte concentration. This variation allows transduction element to change this information into a measurable physical value.<sup>5</sup> An allegory of chemical sensor is presented in Figure 2.

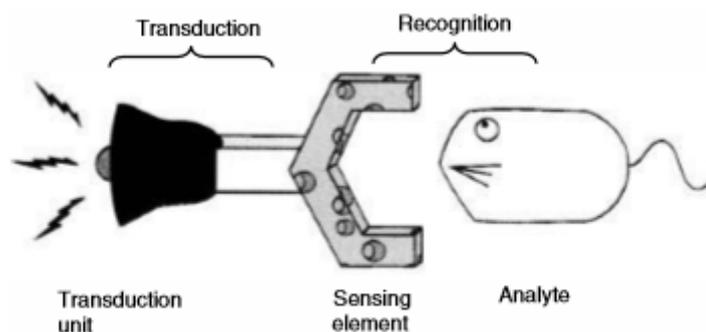


Figure 2. Allegory of a chemical sensor.<sup>5</sup>

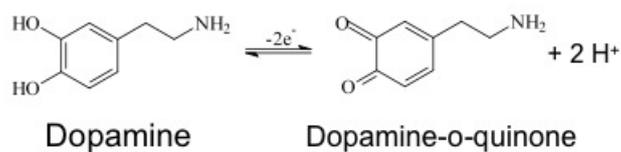
Voltammetric sensors based on modified electrodes have been used in the project. Measurements by means of these sensors are based on the electric current reading between the working and reference electrode in an electrochemical cell as a function of analyte concentration.

## 1.3 IMPORTANCE OF DOPAMINE

Dopamine is a substance which formula is  $C_6H_3(OH)_2 - CH_2-CH_2-NH_2$ . It is a neurotransmitter that has important roles in hormonal and renal systems. Its level alterations can cause important neurologic sickness such as senile dementia, HIV infection, schizophrenia and Parkinsonism.<sup>6</sup>

Dopamine has many functions in the brain, including important roles in behavior and cognition, motor activity, motivation and reward, regulation of milk production, sleep, mood, attention, and learning.

Therefore it is important to measure properly the dopamine level which is one of the project aim. There are numerous studies to find a rapid, low cost, sensitive and accurate quantitative method for detection and quantification of dopamine.<sup>7</sup> The electrochemical oxidation of dopamine is presented in Figure 3.



*Figure 3. Electrochemical oxidation of dopamine*

As can be seen the dopamine can be electrochemically oxidized, and therefore the voltammetric sensors can be used for this purpose.

## 2. EXPERIMENTAL PART

### 2.1 MATERIALS AND METHODS

#### 2.2.1. Sensors

Three different screen printed carbon electrodes have been used in all the electrochemical experiments. Commercial electrodes from Dropsens Ltd. (Spain) have been employed. Electrochemical nanomaterials have been used to modify the screen-printed carbon electrodes, in order to increase the sensitivity.

All commercial devices have included the working electrode, the reference electrode (silver) and the counter electrode (carbon) in the same alumina plate (Figure 4).

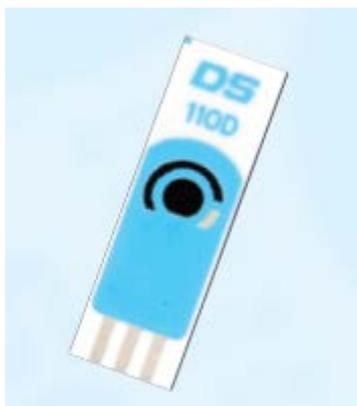


Figure 4. Image of Dropsens sensor<sup>8</sup>

#### 1. Carbon Nanofibers Modified Screen-printed Electrodes:

Carbon Nanofibers Modified Screen-printed Electrodes are designed to develop sensors and biosensors with an improved electrochemical active area. Its ceramic substrate measure is L33 x W10 x H0.5 mm. This electrodes consist on working electrode made of carbon nanofibers, auxiliary electrode made of carbon and reference electrode made of silver.<sup>8</sup>

#### 2. Carbon electrode modified with carbon nanotubes:

This carbon electrode has the same ceramic substrate measure as CNF-SPE from the same brand. Working electrode is made of Carbon nanotubes, auxiliary electrode is made of carbon and reference electrode is made of silver.<sup>8</sup>

### 3. Carbon electrode modified with Graphene

This electrode modified with graphene as a carbon nanomaterial are designed for the sensors and biosensors development. Its working electrode is made of GPH/Carbon, meanwhile auxiliary electrode is made of carbon and reference electrode is made of silver.<sup>8</sup>

#### 2.2.2 Electrochemical Cell

In the electrochemical cell is where the non-spontaneous chemical reaction happens, due to the electric current that is provided. In each electrode part of the redox reaction takes place. The loss of electrons (oxidation) happens at the anode, meanwhile the electron gain (reduction) takes place at the cathode.<sup>9</sup>

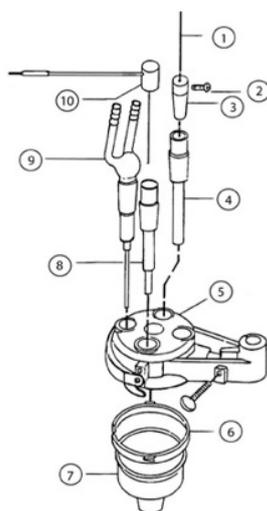


Figure 5. Scheme of the electrochemical cell.<sup>10</sup>

The electrochemical cell that has been used in the project is from AMATEKSI, model RDE0018.<sup>10</sup>

#### 2.2.3. Reference Electrode (Ag/AgCl)

The reference electrode is an electrode having a stable and known equilibrium potential. It is used to measure the potential of the working electrodes in an electrochemical cell. Silver /silver chloride electrode has been used in this study. It is made up by an Ag wire, in which AgCl is deposited in a KCl solution. This solution is where Ag wire acts as anode.<sup>11</sup> The brand that has been used is AMETEKSI model K0265 (Figure 6).<sup>12</sup>

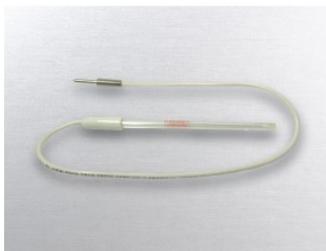


Figure 6. Reference electrode model K0265 from AMETEKSI.<sup>12</sup>

#### 2.2.4. Auxiliary Electrode

Auxiliary electrode is used to make the connection with the electrolyte, so that a current can be applied to the present electrode. The auxiliary electrode is usually made of an inert material, such as a noble metal or graphite. Platine wire has been used in this project.

#### 2.2.5. Chemical Compounds

##### 1. *Ultrapurified Water*

Purified water has been used in the laboratory, due to the many contaminated substances that tap water contains. Minimize substance content improves analysis and experiment laboratory projects because they interact with other substances, that could not be under analysis.<sup>13</sup> It has been used for carrying out all the solutions and also for cleaning all the materials used in the project. The brand that has been used is Water System, Ultrapure, Millipore Simplicity.<sup>14</sup>

##### 2. *Potassium Chloride*

This substance that is composed of chloride and potassium, is a metal halide. Its appearance is white or crystalline and it has not odor. This substance is soluble in polar solvents and it is a “standard solution” in electrical conductivity of solutions. This means that it does not produce redox peaks. It is completely ionized into solvated  $K^+$  and  $Cl^-$  ions.<sup>15</sup>

##### 3. *Potassium Ferrocyanide*

It is a substance whose formula is  $K_4[Fe(CN)_6]$ . It is an inorganic compound. It forms monoclinic lime yellow crystals at room temperature, and it decomposes at its boiling point. Potassium ferrocyanide has a ionic structure, which consist of crosslinked octahedral centers formed by  $[Fe(CN)_6]^{4-}$  with  $K^+$  ions (Figure 7).

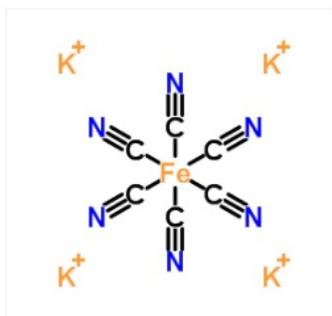


Figure 7. Chemical structure of potassium ferrocyanide. <sup>16</sup>

It is not a standard solution, so it turns into  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  during the oxidation and the opposite behavior during reduction reaction. It is used as typical inorganic compounds in the electrochemical studies and characterization of electrodes.<sup>17</sup>

#### 4. Catechol

Catechol is an organic substance which molecular formula is  $\text{C}_6\text{H}_4(\text{OH})_2$ . Its appearance is colorless crystal and it has phenol odor at ambient temperature. This compound is soluble in water. Its structure is ortho isomer of the three isomeric benzenediols (Figure 8).<sup>18</sup>

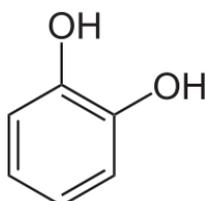


Figure 8. Chemical structure of catechol. <sup>18</sup>

Catechol oxidation is produced by a reversible two electrons and two protons exchange. In electrochemistry appears two well defined peaks, which shows the two oxidation and reduction reactions (Figure 9).<sup>19 20</sup>

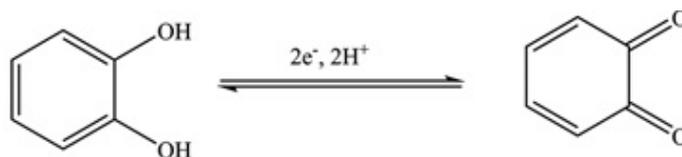


Figure 9. Catechol oxidation process.<sup>18</sup>

## 5. Dopamine

Dopamine is an organic substance from catecholamine and phenethylamine families, which has an important role in brain and body.<sup>21</sup> Its structure consists on a benzene ring with two hydroxyl lateral groups with one amine group joined with an ethyl chain.

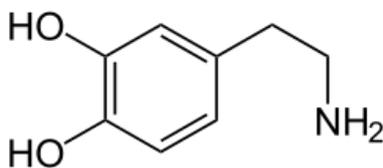


Figure 10. Chemical structure of dopamine.<sup>21</sup>

Dopamine is protonated in acid solution, and in this form is soluble in water and fairly stable, so oxidation reaction does not carry out easily. Furthermore in basic solution, the substance is less soluble in water and more reactive.<sup>22</sup>

The brand that has been used during the project is Sigma-Aldrich.<sup>23</sup>

### 2.2.6. Cyclic Voltammetry

Cyclic voltammetry technique is carried out for analyzing the reaction's mechanism determination and analyzing the electroactive species and surfaces.

Cyclic voltammetric experiment works varying the swept potential linearly with time. Potential starts  $E_i$ , when the chemical specie is not electroreduced, and it goes until  $E_v$ , when the chemical specie is reduced. After this electron transfer, potential is reversed returning to the initial value  $E_i$ . This return gives rise to cause transfer of electrons in the opposed direction.<sup>24</sup> The process is showed in the image below:

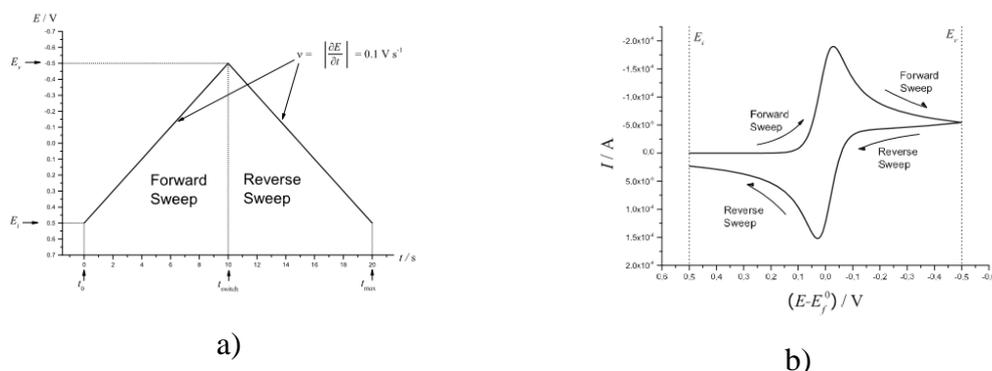


Figure 11a. Waveform of the potential applied during a cyclic voltammetry experiment.<sup>24</sup>

Figure 11b. Cyclic voltammogram produced by the application of the potential waveform in figure 11a.<sup>24</sup>

In this project, potential values has been among -0.4 V and +0.7 V, with the scan rates among 50 mV/s and 1000 mV/s.

Cyclic voltammetry has been used for analyzing the peaks, which are appearing because of the reduction and oxidation process. This peaks give so many information about the chemical species and the electrochemical process, such a reversible or irreversible behavior and sensibility level of low concentration of the analyzed component.

## 2.2 EQUIPMENT

### 2.2.1 Analytical balance

Analytical balance is an industrial equipment designed to measure small quantities. The aim is to obtain accurate results.

The balance is composed of an inner plate covered by a transparent box. This box prevents dust and flow air that could affect the measurement operation.

The brans that has been used is Partner - AS 220.C2.



*Figure 12. Image of Partner – AS 220.C2 balance*

### 2.2.2 Potentiostat

Potentiostat is an electronic device used to control the electrochemical cell and to carry out the electroanalytical experiments.

This electronic hardware keeps the constant potential difference in the working electrodes, taking as reference point the voltage at the reference electrode. Variation corrections are controlled by the auxiliary electrode current.<sup>25</sup>

In the project the EG&G 263A potentiostat has been used, which is shown in the picture below.<sup>26</sup>



Artisan Technology Group

Figure 13. Image of EG&G 263A potentiostat .<sup>26</sup>

### 2.2.3 Software-ECHEM

ECHEM software is designed for the collection, display and analysis of data from electroanalytical voltammetric experiments.<sup>27</sup>

Potentiostat is connected to it, and this software is the control responsible.

Main	Edit	New technique	Get setup	Save setup	Run	Help	setup
CYCLIC VOLTAMMETRY				Setup not saved			
Comment		CO					
Purge Time	PT	240	s	Initial Pot.	IP	0.0000	V
Cond. Time	CT	pass	s	Vertex 1 Pot.	V1	1.300	V
Cond. Pot.	CP	pass	V	Vertex Delay	VD	pass	s
Dept. Time	DT	pass	s	Vertex 2 Pot.	V2	1.000	V
Equil. Time	ET	15	s	Final Pot.	FP	0.0000	V
Scan Rate				Curr. Range			
SR	20.00	mV/s	CR	Auto			
Scan Incr.				No. of Cycles			
SI	2.000	mV	NC	1			
Step Time				Store Cycle			
ST	100.0E-3	s	SC	1			
No. of Points				IR Mode			
NP	1301		IR	none			
Acq. Mode				Uncomp. Res.			
AM	4/4		RU	0.0000	ohms		
Rise Time	RT	high stability	W-User	r	FL	Off	
Working Elec.	WE	Solid	S-SCE	Elec.	RE	User	0.0000 V
Elec. Area	AR	1.000	Z-SSCE	/D	AU	no	
				A-AgCl			
				N-NHE			
				H-Hg/HgSO4			
				C-Cu/CuSO4			
M270				i M273A[14]?			

Figure 14. Screen-print of ECHEM-Software

The project has always been carried out the same procedure managed by the Software. It has started with three cycles at 100 mV/s rate with -0.4 to 0.7 V potential. The aim is to stabilize the electrode. Once that it has been stable, two cycles have been carried out at 100 mV/s rate.

To execute the kinetic study, sweep rate has been increased from 100 mV/s to 1000 mV/s increasing 100 mV/s each time.

#### 2.2.4 Ultrasonic Bath

During the project ultrasonic Elmasonic SH10 bath has been used to facilitate the dissolution of the compounds in ultrapure water. The effect of ultrasonic baths is achieved by high frequency vibrations in water.



Figure 15. Ultrasonic Bath Elmasonic SH10. <sup>28</sup>

#### 2.2.5 Micropipette

It is a laboratory instrument that is managed to suction and transfer small volumes of liquids. Micropipette that has been used in the project can capture values between 20  $\mu\text{L}$  to 2000  $\mu\text{L}$ . This instrument belongs to the brand Eppendorf.



Figure 16. Micropipette. <sup>29</sup>

### **2.2.6 Origin 6**

Origin is an industrial program used to graphing and data analysis software.

This software has been utilized to entering the information from the cyclic voltammetry graphics. The aim has been achieved the slopes or the dependence relationship between graphs to compare information. Although this action has been carried out by Excel.

### **3. PURPOSE OF THE PROJECT**

This project has been carried out in the Universitatea “Dunărea de Jos” din Galați, Laboratory of sensors and biosensors for the analysis of food products – BIOSENS. It has been based on characterization of different sensors based on carbonaceous nanomaterials by analyzing different compounds. The signals of sensors have been registered by cyclic voltammetry in aqueous solutions.

The objective of the project has been to analyze which sensor is the most sensitive to detect the electroactive compounds in solutions. Three different types of sensors have been employed: Carbon electrode modified with carbon nanotubes (CNT), Carbon electrode modified with Graphitized Carbon Nanofibers (CNF) and Carbon electrode modified with Graphene (GPH).

The behavior of these sensors has been studied initially in KCl solutions, and after in potassium ferrocyanide solution and catechol solution, respectively. Once the behavior of each sensor in each solution will be obtained, the results will be analyzed in order to determine the kinetics of the electrochemical reaction and the limiting rate factor.

The sensor which will prove to be the most sensitive will be used to detection of dopamine in aqueous solution, which is very important in medicine and pharmacy.

## 4. RESULTS AND DISCUSSIONS

### 4.1 VOLTAMMETRIC STUDIES IN 0.1 M POTASSIUM CHLORIDE SOLUTION

The voltammetric studies have been carried out in a 0.1M KCl solution using different types of sensors: Carbon electrode modified with carbon nanotubes (CNT), Carbon electrode modified with Carbon Nanofibres (CNF) and Carbon electrode modified with Graphene (GPH).

The voltammetric responses are in the figures presented below.

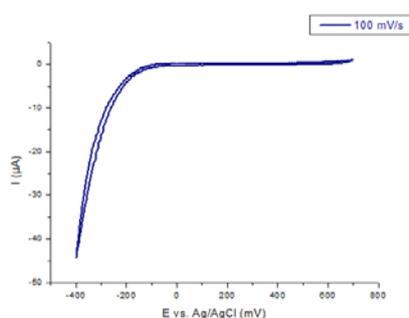


Figure 17. Cyclic voltammogram of CNT-SPE immersed in 0.1M KCl aqueous solution

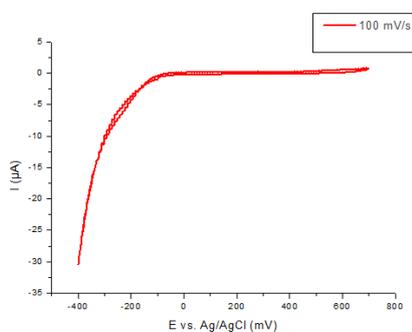


Figure 18. Cyclic voltammogram of CNF-SPE immersed in 0.1M KCl aqueous solution

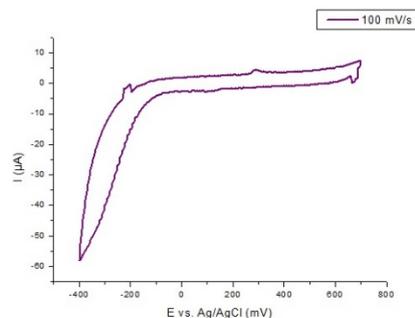


Figure 19. Cyclic voltammogram of GPH-SPE immersed in 0.1M KCl aqueous solution

As it can be observed, no peaks have been showed. KCl solutions are standard solutions in electrochemistry, therefore no reduction and oxidation reactions have occurred in the potential range employed.

With regard to background current, using CNT-SPE is in the range of  $0.2 \mu\text{A}$ , as long as using CNF-SPE is around  $0.15 \mu\text{A}$  and using GPH-SPE is about  $4 \mu\text{A}$ .

Background currents decreases in the order GPH-SPE > CNT-SPE > CNF-SPE.

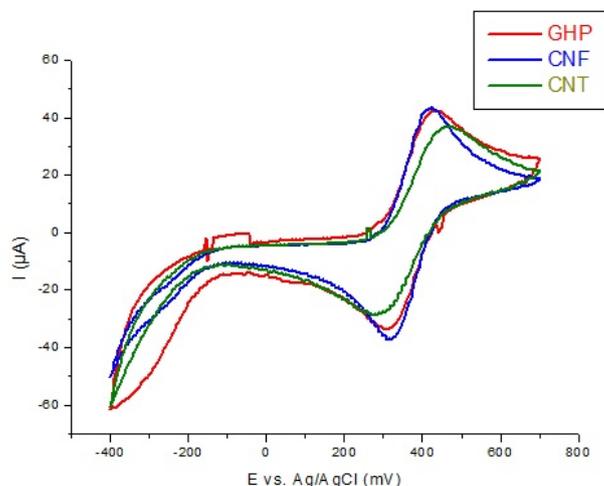
This means that electrode's conductivity is bigger in carbon electrode modified with graphene (GPH), whereas CNF-SPE's conductivity is the lowest.

The maximum and minimum current value in CNT is about  $-45 \mu\text{A}$  and  $2.5 \mu\text{A}$  respectively, in CNF between  $-30 \mu\text{A}$  and  $2.5 \mu\text{A}$  and in the case of GPH is around  $-60 \mu\text{A}$  and  $10 \mu\text{A}$ . These values show that GPH-SPE is the most sensitive to low concentration of  $0.1 \text{ M}$  KCl, followed by CNT-SPE. Meanwhile CNF-SPE is the least sensitive.

## 4.2 VOLTAMMETRIC STUDIES OF SCREEN-PRINTED ELECTRODES IN $10^{-3} \text{ M}$ POTASSIUM FERROCYANIDE + KCl $0.2 \text{ M}$

The cyclic voltammograms have been performed using  $1 \times 10^{-3} \text{ M}$  potassium ferrocyanide in  $0.2 \text{ M}$  KCl solution using different sensors based on different carbon materials: CNT-SPE, CNF-SPE and GHP-SPE.

In the *Figure 2*. is showed the responses of all sensors immersed in the same solution.



*Figure 20. Cyclic voltammograms of screen-printed electrodes immersed in  $10^{-3} \text{ M}$  potassium ferrocyanide + KCl  $0.2 \text{ M}$*

As it has been expected, sharp and well-defined redox peaks have appeared in this case. The peaks are related to the oxidation of  $\text{Fe}^{+2}$  to  $\text{Fe}^{+3}$  during the anodic scan, and reduction of  $\text{Fe}^{+3}$  to  $\text{Fe}^{+2}$  during cathodic scan. That is to say, during reduction it takes an electron, and during oxidation, it loses an electron.

Reversible behavior of the electrochemical processes has been studied. Even though oxidation and reduction peaks have not been set up in the same potential, that is to say that  $\Delta E_p$  (peak-to-peak separation) are not as close as it has been expected for a reversible reaction. Calculating the ratio between cathodic current ( $I_c$ ) and anodic current ( $I_a$ ) has been around one in all the cases. Therefore they can be called quasi-reversible behavior. The main results from cyclic voltammograms have been calculated and are summarized in the following table.

*Table 1. Values of typical parameters calculated from the cyclic voltammograms of all sensors immersed in  $10^{-3}$  M potassium ferrocyanide + KCl 0.2 M.*

	$I_a$ ( $\mu\text{A}$ )	$E_a$ (mV)	$I_c$ ( $\mu\text{A}$ )	$E_c$ (mV)	$I_c/I_a$	$\Delta E_p = E_a - E_c$ (mV)
CNT-SPE	36.76	464.37	-28.43	283.97	0.77	180.40
CNF-SPE	43.11	421.58	-36.69	316.69	0.85	104.89
GHP-SPE	42.39	428.57	-33.87	309.70	0.80	118.87

The CNF-SPE shows the highest reversibility degree. The peak-to-peak separation is the lowest one, and besides  $I_c/I_a$  ratio is the closest to ideal value. On the other hand, CNT-modified screen printed electrode shows the lowest reversibility degree, in which  $\Delta E_p$  is higher compared to the rest and the ratio between redox peaks ( $I_c/I_a$  ratio) is not as close as the others to the ideal value.

As it can be observed in the Figure 20, the value of anodic peak current is higher in CNF, whereas the current peak is the lowest in CNT.

Opposite behavior happens for cathodic peaks, in which higher values are in the following order:  $I(\text{CNT}) > I(\text{GHP}) > I(\text{CNF})$ . Peaks are sharper in CNF-SPE and less sharp for CNT-SPE.

So electrochemical responses of SPE with carbonaceous materials have different features for same compound, being Carbon electrode modified with Carbon Nanofibers (CNF) the most sensitive and CNT the less sensitive one.

## Kinetics Study

In order to calculate the real surface area of SPEs, a kinetics study has been carried out for each sensor using cyclic voltammetry. Modifying the scan rate from 50 mV/s to 500 mV/s, increasing 50 mV/s each time.

The responses of the three different types of carbonaceous electrodes are showing in the following figures.

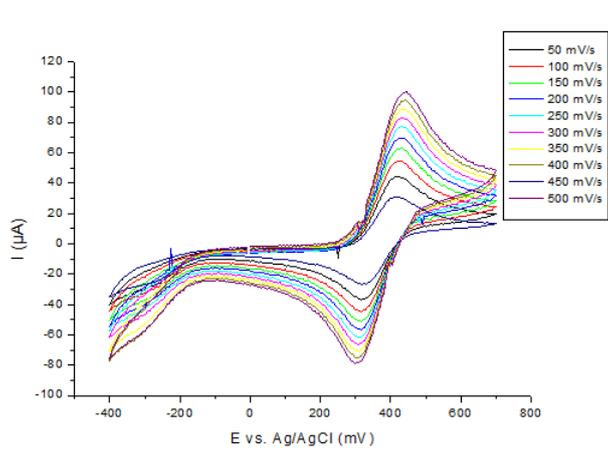


Figure 21. Cyclic voltammogram of CNF-SPE immersed in  $10^{-3}$  M potassium ferrocyanide + KCl 0.2 M registered with different scan rates

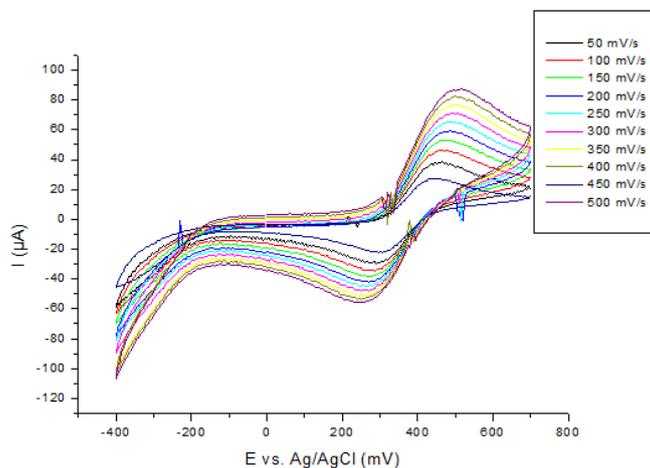


Figure 22. Cyclic voltammograms of CNT-SPE immersed in  $10^{-3}$  M potassium ferrocyanide + KCl 0.2 M registered with different scan rates

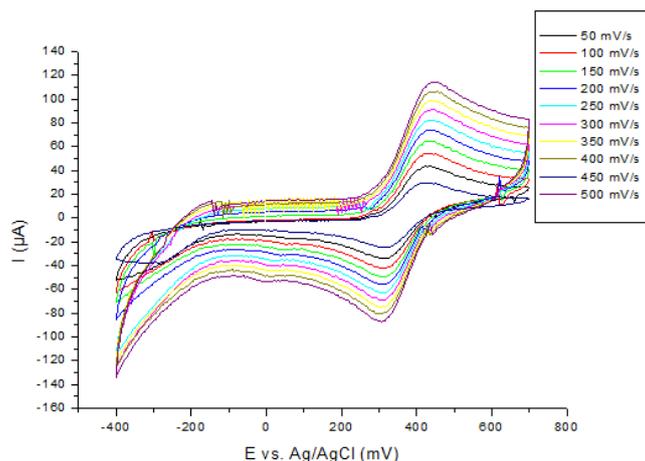


Figure 23. Cyclic voltammograms of GHP-SPE immersed in  $10^{-3}$  M potassium ferrocyanide + KCl 0.2 M registered with different scan rates

Anodic peaks currents data from each scan rate have been achieved. The aim is to find the best linear dependence between the peak current and the scan rate or between the peak current and square root of the scan rate. If the dependence is linear for the first case the redox process is controlled by the electron transfer. If the oxidation process of this compound at the sensor surface is diffusion controlled, the current is directly proportional with the square root of the scan rate.

Depending on the best linear dependence, there are two equations for quasi-reversible electron transfer processes, Laviron equation or Randles-Sevcik equation.

For all sensors, CNT-SPE, CNF-SPE and GHP-SPE, the linear dependences are better between the peak current and the square root of the scan rate.

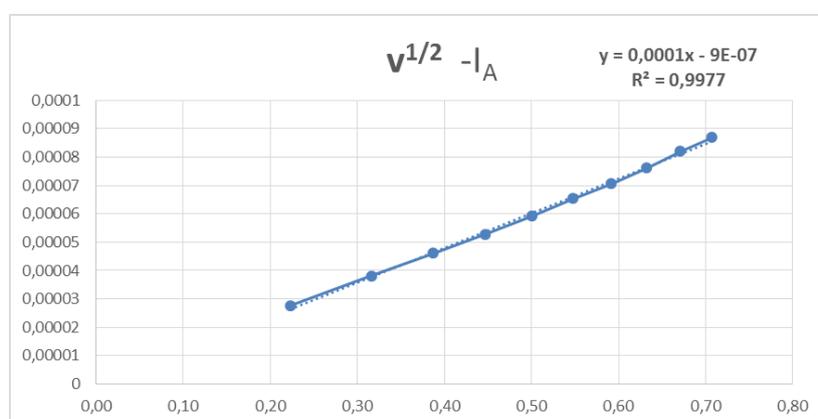


Figure 24. Linear dependence between anodic peak current and the square root of the scan rate in the case of CNT-SPE

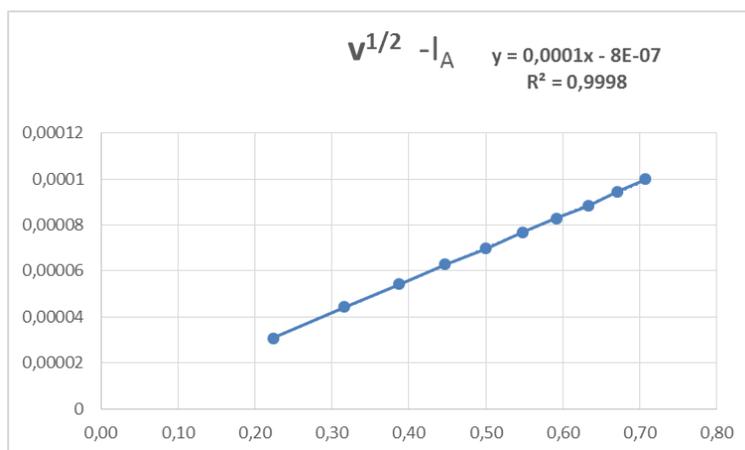


Figure 25. Linear dependence between anodic peak current and the square root of the scan rate in the case of CNF-SPE

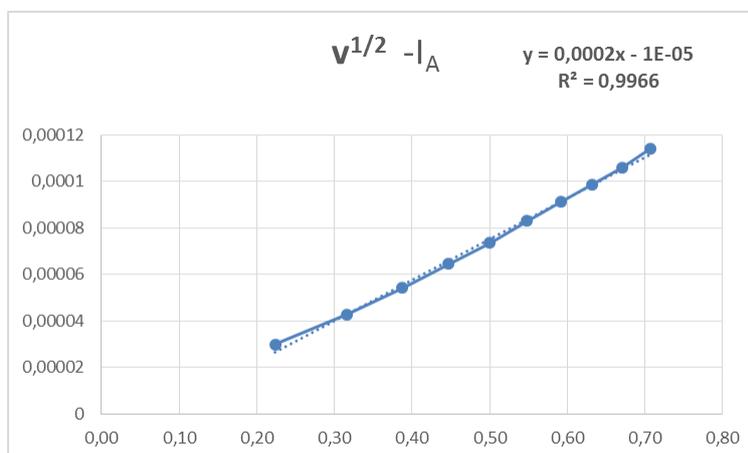


Figure 26. Linear dependence between anodic peak current and the square root of the scan rate in the case of GHP-SPE

For this reason, the electroactive area is determined by using Randles-Sevcik equation:

$$I_P = (2.65 \times 10^5) \cdot n^{1/2} \cdot A \cdot C \cdot D^{1/2} v^{1/2}$$

Where  $n$  takes the value one, since it is the number of electrons exchanging in the redox process, and this substance shows a reversible one electron transfer. Electrode area in  $\text{cm}^2$  corresponds to the letter  $A$ .  $C$  takes the value  $10^{-3} \text{ M}$ , which is the concentration of active molecule ( $\text{mol cm}^{-3}$ ). Furthermore,  $D$  sets the value  $6.057 \times 10^{-6} \text{ cm}^2 \times \text{s}^{-1}$ , which is the diffusion coefficient. And lastly  $v$  is the scan rate in  $\text{V s}^{-1}$ .<sup>30</sup>

This calculations have been carried out for the purpose of working out the roughness factor, which is the ratio between electroactive surface area and geometrical area.

Electroactive surface area is fixed value for all of the electrodes, which has been calculated:

$$A_g = \frac{\pi R^2}{4}$$

R has the value of 2 mm. Therefore the geometrical area of the screen-printed electrode is 0.1256 cm<sup>2</sup>.

The results obtained are showed in the following table:

*Table 2. Results of roughness factor for each screen printed electrode*

Screen-printed electrode	Regression equation I <sub>a</sub> vs. v <sup>1/2</sup>	Electroactive area (cm <sup>2</sup> )	Roughness factor
CNT	y = 0.0001x – 9·10 <sup>-7</sup>	0.1712	1.3630
CNF	y = 0.0001x – 8·10 <sup>-7</sup>	0.1985	1.5809
GPH	y = 0.0002x – 1·10 <sup>-5</sup>	0.2466	1.9637

Surface roughness plays a significant role for assessing material performance. It affects to different properties such a mechanical, physical, electrochemical or optical properties.<sup>31</sup>

Roughness factor is higher than one, being that electroactive area is larger than geometrical area. This is due to the structure of the SPE surface, which has defects such as holes, tucks or steps. This defects increase the real surface area of mass transport.<sup>30</sup>

The highest value is obtained for GHP-SPE, whose background current is the highest as well. This results are in agreement with peaks intensities. GHP Screen Printed Electrode is the most sensitive, while CNT-SPE is the least sensitive.<sup>32</sup>

### **4.3 STUDY OF SCREEN-PRINTED ELECTRODES IN CATECHOL SOLUTION**

Cyclic voltammetry has been performed using 1x10<sup>-3</sup> M Catechol solution in 0.2 M KCl solution using different sensors based on carbon nanomaterials: CNT-SPE, CNF-SPE and GHP-SPE.

Below is showed the responses of each sensor in this solution.

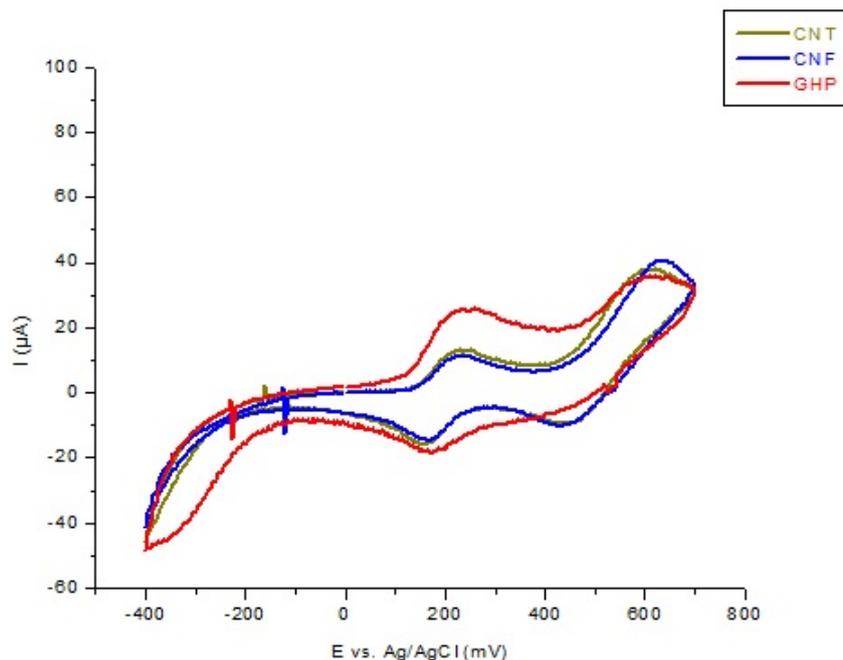


Figure 27. Cyclic voltammogram of all screen printed electrodes immersed in  $10^{-3} M$  catechol +  $KCl 0.2 M$

Two anodic peaks and two cathodic peaks appear in the cyclic voltammograms. As it was expected, oxidation reactions take place. The oxidation process is showed in the below Figure 28.

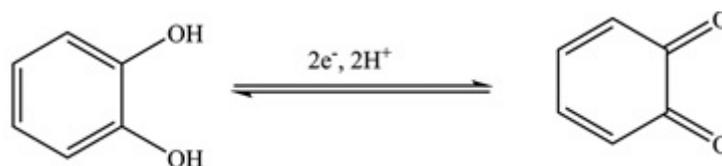


Figure 28. Catechol oxidation process

These two redox peaks are associated with the formation of the o-quinone in a two electron and two proton reversible process.<sup>32</sup>

Same procedure used previously in the case of ferrocyanide has been carried out for catechol reversibility behavior analysis. Calculations are shown in the following table:

Table 3. Values of typical parameters calculated from the cyclic voltammograms of all sensors immersed in  $10^{-3}$  M Catechol + KCl 0.2 M.

$C_6H_6O_2$	$I_a$	$E_a$	$I_c$	$E_c$	$I_c/I_a$	$\Delta E = E_a - E_c$
CNT-SPE	13.15	229.15	-15.75	156.71	1.20	72.44
CNF-SPE	11.29	225.52	-14.45	160.07	1.28	65.45
GHP-SPE	25.57	236.98	-18.71	167.62	0.73	69.36

As it can be observed in the table, CNF-SPE accomplished better reversible behavior than CNT-SPE and GPH-SPE. Peak to peak separation is larger for CNT-SPE, which shows higher irreversibility degree, besides  $I_c/I_a$  ratio is clearly higher than ideal value.

At any rate, all of them have shown quasi-reversible behavior.

Clear and well-defined peaks can be observed in the Figure 27. GPH has shown two well defined anodic peaks and one well defined cathodic peak. Whereas that CNT-SPE and CNF-SPE have shown very similar behavior, in both of them have appeared two well defined cathodic and anodic peaks.

GHP-SP electrode has sharper and intensive peaks. So that GHP is the most sensitive screen-printed electrode in the detection of catechol.

The sensitivity of the electrodes increases in this order:

Sensitivity GHP-SPE > Sensitivity CNF-SPE > Sensitivity CNT-SPE.

### **Kinetics Studies**

Cyclic voltammograms at different scan rates have been carried out using GHP-SPE, CNT-SPE and CNF-SPE, in order to analyze the electrochemical responses. Following the same method as in the ferrocyanide analysis, scan rate was modified between 50mV/s to 500mV/s, increasing 50 mV/s each time.

The Figures are shown below:

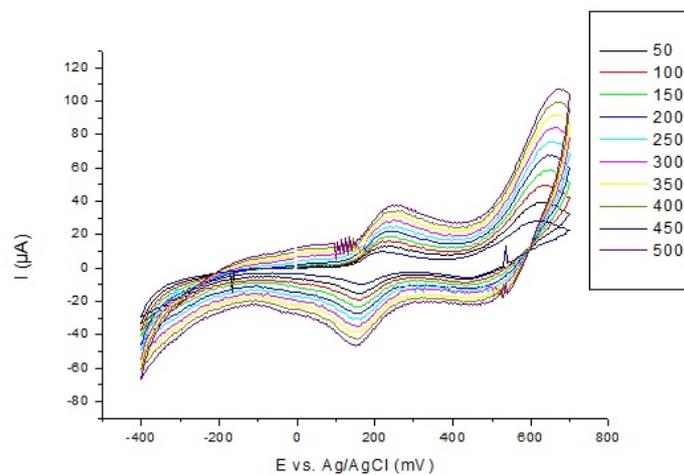


Figure 29. Cyclic voltammograms of CNF-SPE immersed in  $10^{-3}$  M Catechol + KCl 0.2 M registered with different scan rates

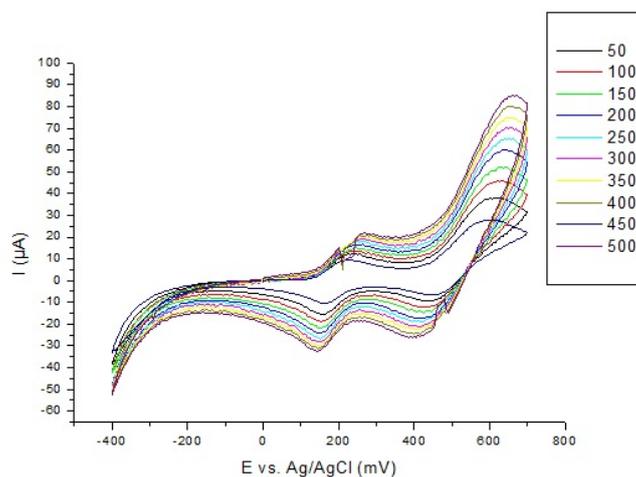


Figure 30. Cyclic voltammograms of CNT-SPE immersed in  $10^{-3}$  M Catechol + KCl 0.2 M registered with different scan rates

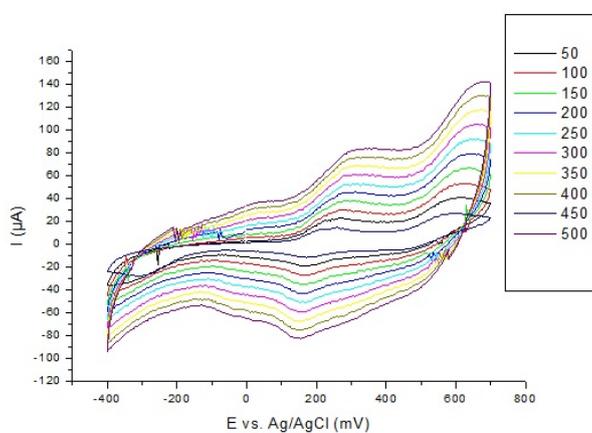


Figure 31. Cyclic voltammograms of GHP-SPE immersed in  $10^{-3}$  M Catechol + KCl 0.2 M registered with different scan rates

Randles-Sevcik or Laviron equations have been used this time with the aim of calculating the diffusion coefficient or the surface coverage of the redox species. In order to apply these equations, the first thing that has been done is to find the better lineal dependence between the peak current and scan rate or between peak current and square root of the scan rate.

In the case of CNT-SPE, lineal dependence is better between peak current and square root of the scan rate, as it is shown in the figure presented below.

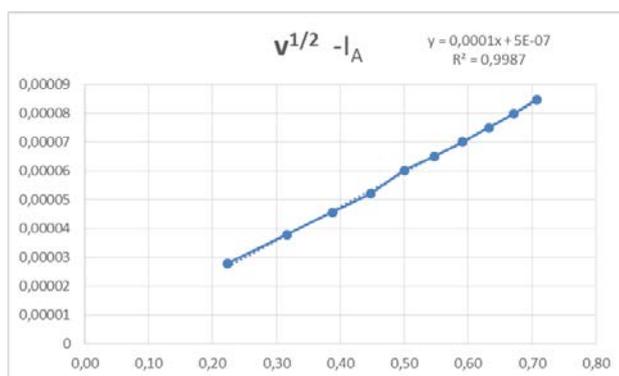


Figure 32. Linear dependence between anodic peak current and the square root of the scan rate in the case of CNT-SPE

Therefore, Randles-Sevcik equation has been applied for the calculation of electroactive area.

This time,  $n$  takes the value two, because the process involve two electrons transfer. Electroactive area in  $\text{cm}^2$  has been calculated previously, and takes the value  $0.1712 \text{ cm}^2$ .

Table 4. Results of diffusion coefficient of catechol at CNT-SPE.

Screen-printed Electrodes	Regression equation $I_a$ vs. $v^{1/2}$	Electroactive area ( $\text{cm}^2$ )	Diffusion coefficient ( $\text{cm}^2\text{s}^{-1}$ )
CNT-SPE	$y = 0.0001x + 5 \cdot 10^{-7}$	0.1712	$6.0732 \cdot 10^{-7}$

Whereas GHP-SPE and CNF-SPE better lineal dependence are between peak current and the scan rate.

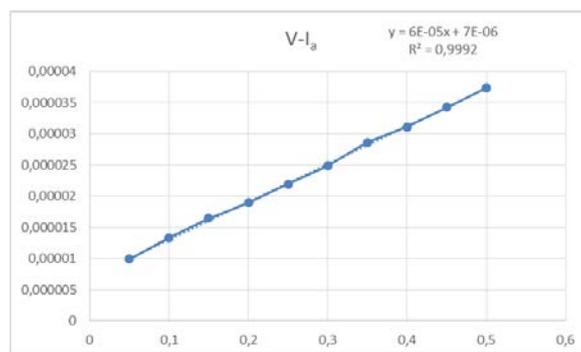


Figure 33. Linear dependence between anodic peak current and the scan rate in the case of CNF-SPE.

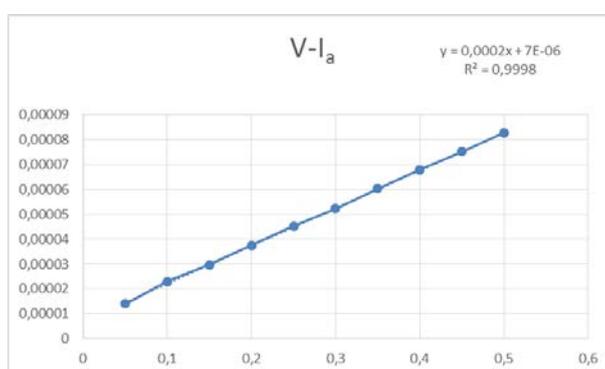


Figure 34. Linear dependence between anodic peak current and the scan rate in the case of GHP-SPE.

Accordingly, the Laviron equation will be used:

$$I_p = \frac{n^2 F^2 v A \Gamma}{4RT}$$

Where  $I_{pc}$  is the cathodic peak current measured in Amperes.  $F$  takes the value 96,485.3365 C/mol which is Faraday constant.  $V$  is the potential scan rate measured in V/s.  $N$  is the number of electrodes that are involved in the redox process. Furthermore  $\Gamma$  is the redox species surface coverage mol/cm<sup>2</sup>.  $R$  sets the value 8.3144621 J/ K×mol, which is the ideal gas constant and  $T$  is the temperatures measured in K. The total surface coverage could be calculated.<sup>33</sup>

*Table 5. Results of surface coverage of the redox species of CNF-SPE and GHP-SPE*

Screen-printed Electrode	Regression equation $I_p$ vs. $v$	Electroactive area (cm <sup>2</sup> )	Surface coverage of the redox species (mol/cm <sup>2</sup> )
CNF-SPE	$y = 6 \cdot 10^{-5}x + 7 \cdot 10^{-6}$	0.1986	$8.0423 \cdot 10^{-11}$
GHP-SPE	$y = 0.0002x + 7 \cdot 10^{-6}$	0.2466	$2.1582 \cdot 10^{-10}$

Carbon electrode modified with Graphene (GPH) has shown the highest surface coverage of the redox species value, so it is more sensitive than CNF-Screen Printed Electrode. The results are in agreement with the results shown by the peak current values. Peak current value has been higher in GPH, therefore GPH is the most sensitive sensor compared to CNT-SPE and CNF-SPE.

#### **4.4 DOPAMINE STUDY AND DETECTION**

Based on the results of the above analysis, CNF-SPE proved to be the most sensitive compared to the other modified screen-printed electrodes. Furthermore, the electrochemical signal is stable, the background current is reduced and the curves are accurate, without electrical noise.

Therefore, analytical procedure for quantitative determination of dopamine has been performed using carbon electrode modified with Carbon Nanofibres (CNF).

As it can be seen in the Figure 35, no redox peaks have been found during the registering of cyclic voltammograms in the blank solution (0.1 M KCl solution).

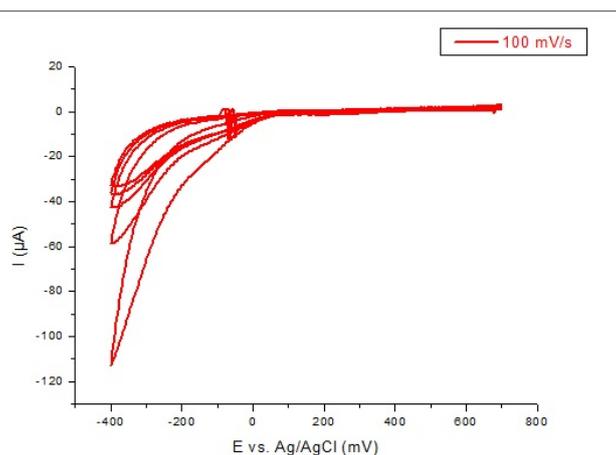


Figure 35. Cyclic voltammogram of screen printed electrodes immersed in Dopamine.

Background current is above 5  $\mu\text{A}$ . Cyclic voltammetric experiment has been carried out by adding different dopamine volumes, from 10  $\mu\text{L}$  to 2160  $\mu\text{L}$ , which correspond to concentrations presented in the table.

Table 6. Volumes of the solution added and the calculus of concentration in the solutions employed in the electrochemical experiments.

V ( $\mu\text{L}$ ) Dopamine	V (L) Dopamine	C Dopamine	V (L) KCl	V (L) Total	C (molar)	C ( $\mu\text{M}$ )
10	0.00001	0.001	0.05	0.05001	$2 \cdot 10^{-7}$	0.20
20	0.00002	0.001	0.05	0.05002	$4 \cdot 10^{-7}$	0.40
40	0.00004	0.001	0.05	0.05004	$7.99 \cdot 10^{-7}$	0.80
60	0.00006	0.001	0.05	0.05006	$1.20 \cdot 10^{-6}$	1.20
110	0.00011	0.001	0.05	0.05011	$2.20 \cdot 10^{-6}$	2.20
160	0.00016	0.001	0.05	0.05016	$3.19 \cdot 10^{-6}$	3.19
210	0.00021	0.001	0.05	0.05021	$4.18 \cdot 10^{-6}$	4.18
260	0.00026	0.001	0.05	0.05026	$5.17 \cdot 10^{-6}$	5.17
360	0.00036	0.001	0.05	0.05036	$7.15 \cdot 10^{-6}$	7.15
460	0.00046	0.001	0.05	0.05046	$9.12 \cdot 10^{-6}$	9.12
560	0.00056	0.001	0.05	0.05056	$1.11 \cdot 10^{-5}$	11.08
660	0.00066	0.001	0.05	0.05066	$1.30 \cdot 10^{-5}$	13.03
1160	0.00116	0.001	0.05	0.05116	$2.27 \cdot 10^{-5}$	22.67
2160	0.00216	0.001	0.05	0.05216	$4.14 \cdot 10^{-5}$	41.41

The results are showed in the following Figure 36:

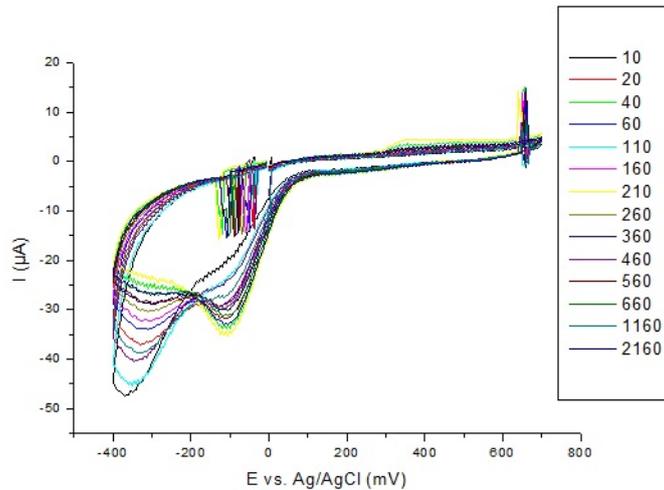


Figure 36.. Cyclic voltammograms registered at CNF-SPE immersed in dopamine solution of different concentrations between 0.2 and 41.41 $\mu$ M (volumes added was between 10  $\mu$ L to 2160  $\mu$ L)

Current peaks are sharper when concentration levels are higher, that is to say that linear relationship between concentration level and current peaks has been found. However, as it can be observed in the below graph, lineal dependence is loosed when concentration is higher. This is due to dopamine adsorption on the modified SPE surface is carried out.<sup>30</sup>

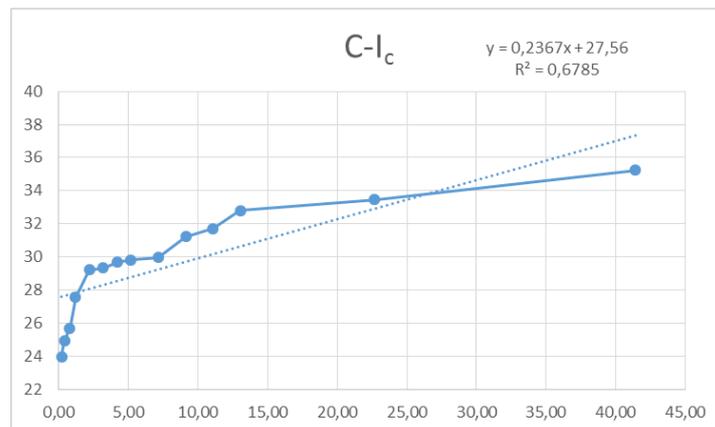
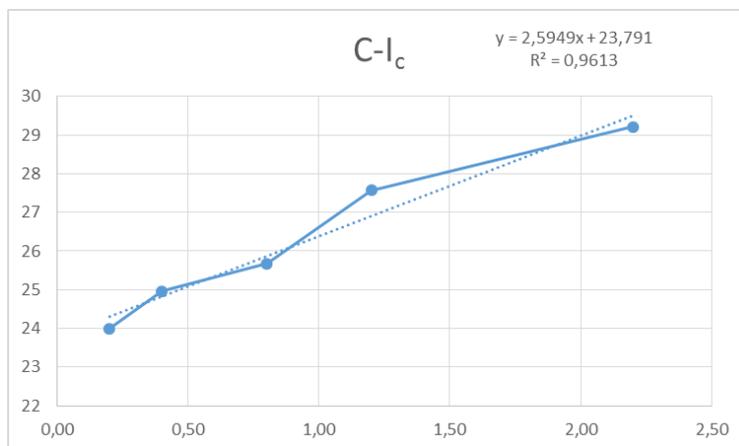


Figure 37. Dependence between cathodic peak current and concentration of dopamine based on voltammetric measurements with CNF-SPE.

Therefore, the calculation of LOD is carried out with the information collected by the first concentration data, where lineal dependence is observed, and the correlation coefficient is closer to one.



*Figure 38. Linear dependence between cathodic peak current and concentration of dopamine based on voltammetric measurements with CNF-SPE (concentration range was 0.2 – 2 μM)*

Detection limit is defined as the minimum quantity or concentration of substance that is detected by an analytical method. This is estimated from the calibration curve (linear range).<sup>34 35</sup>

The limit of detection has been calculated using the equation shown below:

$$LOD = \frac{3\sigma}{m}$$

Where  $\sigma$  is the standard deviation (SD) of the peak current taking seven replicates and  $m$  is the slope of the calibration curve.<sup>30</sup>

The standard deviation has been calculated taking the value 4.1019, whereas slope of the calibration curve ( $m$ ) has been done taking 2.5949  $\mu\text{A}/\mu\text{M}$ .

The LOD value has been calculated to be 4.742  $\mu\text{M}$ . The low results of the limit of detection is due to the fact of the high sensitivity of the sensor developed in this study.

LOD has been compared with other results reported in the literature.

*Table 7. Results of dopamine detection by the use of different tyrosinase biosensors<sup>36</sup> and by the use of different graphene-modified electrodes<sup>37</sup>.*

Electrode	LOD ( $\mu\text{M}$ )	Refs.
Polyacrylamide gel, entrapment of tyrosinase in matrix	5.0	36
Eggshell membrane, glutaraldehyde, entrapment of tyrosinase on surface, physical adsorption	2.5	36
Chitosan, Glassy carbón	1.9	36
Single walled carbon nanotube/polypyrrole, entrapment of tyrosinase on surface, covalent bonding	5.0	36
Graphene/Glassy carbon electrode	2.64	37
Graphene-AuNP/Glassy carbon electrode	1.86	37

As it can be observed in Table 7 the LOD values that have been calculated are similar to those reported in literature, highlighting the great sensitivity of sensors based on carbonaceous nanomaterials.

This results support that the Carbon Nanofiber Screen-Printed-Electrode used is very sensitive for the determination of Dopamine, and it is a sensor highly reliable.

The lower detection limit value, the higher sensor sensitivity. That is, this sensor is capable of detecting a very low amount of analyte.

## 5. CONCLUSIONS

Analyzing the information obtained by cyclic voltammetry for each solution analyzed, it could be concluded such as is described below. The electrodes conductivities of sensors in KCl solution decrease in this order: CNF-SPE > CNT-SPE > GHP-SPE. This information has been obtained by observing the background current. The lowest background current has been obtained by Carbon Nanofibers Screen-Printed-Electrode.

The results obtained in the case of potassium ferrocyanide solution and catechol solution, respectively, regarding reversibility behavior and the sensitivity are discussed below.

As it can be observed, CNF-SPE shows higher reversibility degree in both cases. It has been observed from lower peak to peak separation and  $I_c/I_a$  ratio is closer to ideal value. This means that the separation between the oxidation peaks is smaller for CNF-SPE in potassium ferrocyanide and catechol.

About sensitivity responses, for potassium ferrocyanide response, CNF-SPE has shown the highest sensitivity, meanwhile CNT has shown the lowest sensitivity. For catechol highest sensitivity has shown by GHP-SPE, and lowest by CNT-SPE.

Performing the kinetics studies in potassium ferrocyanide, GHP-SPE has shown higher roughness factor. While CNT has shown the smaller roughness factor value. This means that the active area is higher in GHP-SPE, that is to say that real surface area of mass transport is bigger in this sensor. Therefore it is a more sensitivity sensor. This results agree with the oxidation peaks, which value is so high in GHP-SPE.

For catechol solution, GHP-SPE has shown the bigger surface coverage of the redox species. So GHP-SPE is the most sensitivity sensor which result agree with the most sensitivity sensor.

Taking into account the results, CNF-SPE has shown the best results in the most cases regarding the reversible behavior, the sensitivity and highest active area. That is why it has been decided to use this sensor for dopamine analysis.

Dopamine analysis carried out using CNF-SPE has shown a detection limit of 4.74  $\mu\text{M}$ . Comparing the results with other studies dedicated to detection of dopamine, it has been observed that values are similar. Therefore, CNF-Screen-Printed Electrode is very sensitivity and shows reliable values in the detection of dopamine.

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