Universidad de Valladolid

ESCUELA DE INGENIERÍAS INDUSTRIALES

UNIVERSIDAD DE VALLADOLID

ESCUELA DE INGENIERIAS INDUSTRIALES

Grado en Ingeniería en Tecnologías Industriales

Desarrollo de sensores basados en polipirrol para la detección de aminoácidos

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Universitatea Dunarea de Jos din Galati

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TFG REALIZADO EN PROGRAMA DE INTERCAMBIO

TÍTULO: **Development of sensors based on polypyrrole for the detection of amino acids**

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CENTRO: **Facultad de ciencias**

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Cinco palabras claves que describen el TFG:

Electroquímica, Voltametria ciclica, Sensores, Polipirrol, Aminoacidos

Resumen en español:

En este proyecto han sido realizado estudios de electroquímica con el objetivo de desarrollar sensores para la detección de aminoácidos. Los sensores han sido modificados por dos métodos de electropolimerización, voltametria cíclica y cronoamperometria, en tres diferentes disoluciones de pirrol con diferentes agentes dopantes: ferrocianuro de potasio, nitroprusiato de sodio y ácido sulfúrico. Una vez modificados, dichos sensores fueron estudiados con voltametria cíclica en disoluciones de KCl donde fueron comparados y estudiada su estabilización y cinética. Los sensores cuya electropolimerizacion hayan obtenido mejor rendimiento fueron ultilizados para los estudios de aminoacidos. Fueron realizados los mismos estudios de voltametria cíclica en las tres disoluciones de KCl con los aminoácidos: triptófano, valina y fenilalanina y posteriormente comparados con los resultados obtenidos con la primera disolución de KCl. Finalmente fue realizado un análisis PCA con los resultados obtenidos con el fin de saber si los aminoácidos pueden ser discriminados.

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SPECIALIZAREA CHIMIE

Lucrare de licență

Coordonator științific,
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Galați
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Realizarea unor senzori pe bază de polipirol pentru detecţia aminoacizilor

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"DUNĂREA DE JOS" UNIVERSITY OF GALAȚI
FACULTY OF SCIENCES AND ENVIRONMENT
SPECIALIZATION CHEMISTRY

Development of sensors based on polypyrrole for the detection of amino acids

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INDEX

CHAPTER 1

1.1 OBJECTIVES

The purpose of this project is the developing and the characterization of different sensors based on polypyrrole doped with different dopping agents. The comparison of the results obtained with sensors developed by different techniques will define the role of doping agents in the sensors behaviour. The best sensors will be used in the experiments related to the detection of amino acids in aqueous solutions.

1.2 DESCRIPTION

In this project, it will work with pyrrole solutions to develop sensors by electrosynthesis. In order to do this task, it will be selected two methods of electropolymerization, cyclic voltametry and chronoamperometry, and it will be used three solutions with different doping agents. Once finished the fabrication process, the sensors will be immersed in KCl solutions, where it will study the stabilization of the electrochemical signal and the kinetics of the electrochemical processes. Finally, the sensors will be used in the detection of three amino acids in aqueous solutions.

THEORICAL SECTION

CHAPTER 2

2.1. SENSORS

This part describes the functioning of the sensors focusing in the behaviour of an electrochemical sensor. A sensor is a device that detects physical or chemical variations and transforms them in the electrical signals.

An electrochemical sensor is electrical conductor that is immersed in the fluid, where there is a charge transference between the substances from the solution and the sensor, allowing the free energy in the interface that is captured by the sensor and it is transformed in electrical signal.

A chemical sensor has two different parts:

- Receptor: it interacts selectively with the analyte.
- Transductor: it transforms the signal generated in the recognition step in electrical signal. The magnitude must be related to the concentration or activity of the recognized species in the previous step. The transductor is connected directly to the associated with electronics components or signal processors, not belonging to the sensor, but they are essential for using the sensor properly.

2.2. VOLTAMPEROMETRY

Voltamperometry includes different electrochemical techniques that are based on measuring the current in function of applied potential. Electrochemical techniques are used to detect some electroactive substances in solution.

For the electrochemical cell three electrodes are used, the working electrode, the reference electrode and the counter electrode.

The working electrode makes contact with the desired analyte applying the potential in a controlled manner that facilitate electron transfer from the electrode to the analyte. The reference electrode act as a reference in measuring and controlling the potential of the working electrodes, but in any moment, any current pass through it. By the counter

electrode, all necessary current to balance the current observed on the working electrode passes. To achieve this current, the assistant potential often oscillates at the ends on the edges of the window of solvent, where it is oxidized or reduced the solvent or electrolyte support.

2.3 CYCLIC VOLTAMMETRY

The cyclic voltammetry works varying the potential of an electrode immersed in a solution, measuring the obtained current. The variation of potential is limited by a maximum and a minimum (usually, in the function of the solvent) and it has a constant scan rate. The cycle starts in initial potential 0 V and the potential increases until reaching the first limit V1, and once reaching it, it decreases until the second limit V2 and finally it finishes in the initial point.

In the cyclic voltammetry can appear peaks, cathodic peaks show with negative currents while those anodic peaks appear with positive currents. Peaks means that it is taking place an oxidation process for anodic peaks and one reduction process for cathodic peaks. When there are peaks, these maximum and minimum peaks currents. The maximum peak means the entire reactant species on the electrode surface have been oxidized, and in case of minimum peak means they have been reduced.

A redox process is reversible if a redox system can keep the balance when is subjected to a potential sweep. That means, the concentration values in the surfaces of oxidation and reduction follow the Nernst equation.

The reversibility of the process is because electron transfer is faster than other processes, such as diffusion. The difference between potential peaks has to satisfy the following equation:

$$\Delta E = |Epa - Epc| = 2{,}303\frac{RT}{nF}$$

E_{pa} = anodic peak potential, V

E_{pc} = cathodic peak potential, V

T= Absolute temperature, K

R= gas constant, 8.314 J/mol K

F= Faraday constant

n= number of electrons involved in the electrochemical process

If it is bigger the gap between anodic and cathodic peaks, the process is more irreversible. There is a hysteresis associated to an overpotential polarization that arises from a combination of diffusion rates of analyte and an activation barrier of the transfer of electrons from the electrode to the analyte.

In addition, in a reversible process, the current of the anodic and cathodic peaks have the same magnitude. In an irreversible reaction, anodic and cathodic have different current magnitude.

EXPERIMENTAL SECTION

CHAPTER 3. MATERIALS AND METHODS

3.1 ELECTRODES

It has been used several DRP-150 sensors and they have been chemically modified through a electropolymerization process with polypyrrole doped with three different doping agents, obtaining three types of sensors. These Screen-Printed Carbon Electrodes have been designed for working in microvolumes or to develop specific sensors.

Ceramic substrate: L33 x W10 x H0.5 mm

Electric contacts: Silver

The electrochemical cell consists of:

Working electrode: Carbon (4 mm diameter)

Auxiliary electrode: Carbon

Reference electrode: Silver

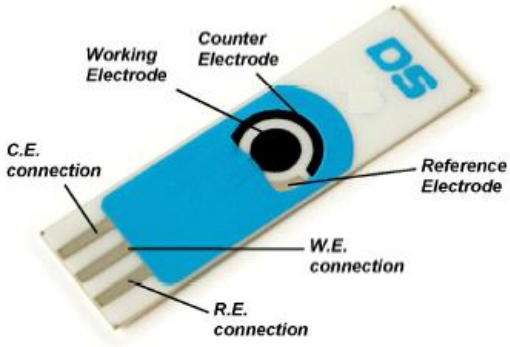


Figure 1. Parts of a sensor

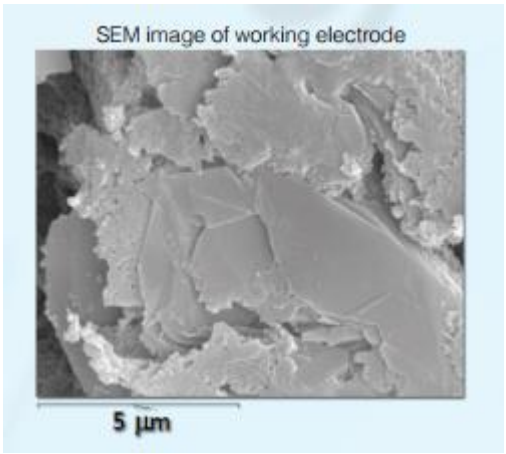


Figure 2. DRP-150 sensor

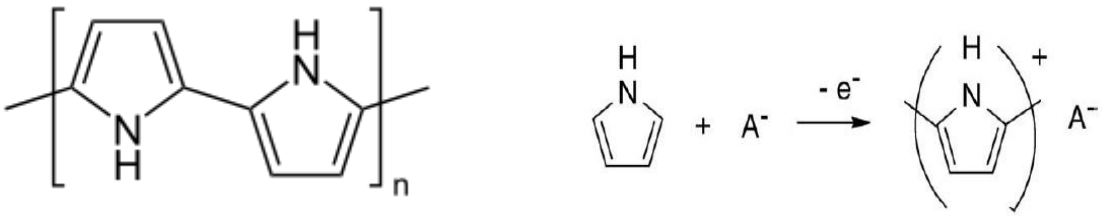
3.2 POLYPYRROLE ELECTROSYNTHESIS

3.2.1 Polypyrrole

Polypyrrole is an electroactive polymer formed through polymerization of pyrrole, whose molecular formula is C_4H_5N.

Figure 3. Chemical structure of pyrrole

Polypyrrole is amorphous material with weak diffraction and it is brittle. It is stable in normal conditions. Pyrrole is non-conducting but their oxidized products are good conductors. This conductivity depends on conditions and reactives used in the oxidation. Pyrrole has a band structure like non-conducting or semiconducting materials. To change this structure, it is used doped. In order to do this, it is used an electrode in a pyrrole solution and it is applied a current or potential. The result is that the polymer loses electrons and obtains positive charge. Polypyrrole is used to develop chemical sensors for different applications.



a) b)

Figure 4. a) Chemical structure of polypyrrole b) The scheme of electrochemical or chemical process for polypyrrole obtaining

3.2.2 Potassium Ferrocyanide

Potassium ferrocyanide is a salt formed with the anion ferrocyanide and the cation potassium. Its chemical formula is $K_4[Fe(CN)_6]$.

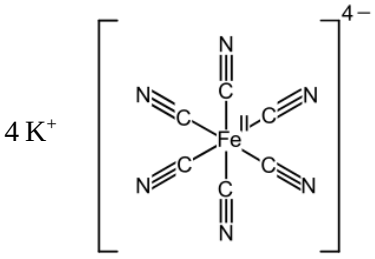


Figure 5. Chemical structure of Potassium Ferrocyanide

3.2.3 Sodium Nitroprusside

Sodium Nitroprusside is formed by an anion, octahedral iron centre with five cyanide ligands and a nitric oxide ligand. Its chemical formula is C_5N_6OFe.

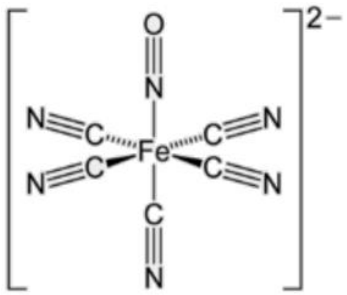


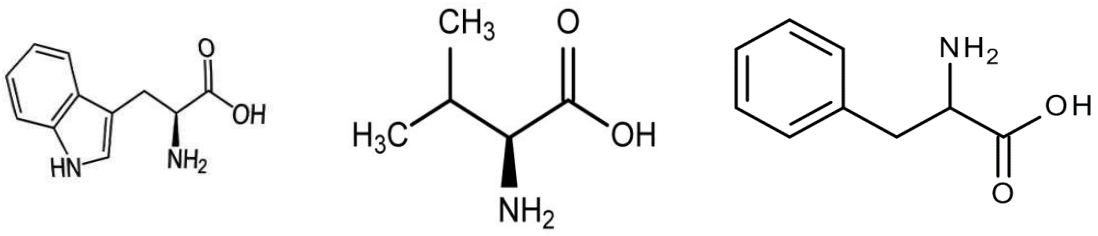
Figure 6. Chemical structure of nitroprusside anion

3.2.4 Sulphuric Acid

Sulphuric acid is formed by a pyramidal structure with a sulphur atom in the centre surrounded by oxygen atoms. The oxygen atoms without double ligand are connected with hydrogen atoms. Its chemical formula is H_2SO_4.

3.3 AMINO ACIDS

Amino acids are organic compounds that have amine and carboxyl groups. The amino acids used in this study are L-Tryptophan, L-Valine and L-Phenylalanine. They are essential amino acids in humans, apolar and hydrophobe. L-Tryptophan and L-Phenylalanine are aromatic but L-Valine is aliphatic.



a) b) c)

Figure 7. Chemical structure of L-Tryptophan a), L-Valine b) and L-Phenylalanine c)

3.4 ELECTROCHEMICAL CELL

Inside the electrochemical cell, it occurs the transformation of an electric current in a chemical oxidation-reduction. In order to do this, it is used the different working electrodes, Platinum counter electrode and Ag/AgCl reference electrode. The cell has 50 mL of volume.

3.5 POTENTIOSTAT

The electrodes are connected to a potentiostat that check and control the potential in the process varying the current. The pontentiostat keep constant the potential in the working electrode comparing it with the reference electrode. In the potentiostat it can check if the electrodes are working correctly and if the process is stable.

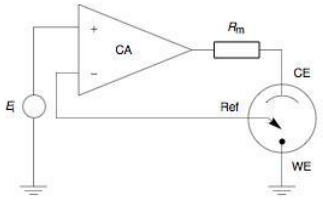


Figure 8. Potentiostat scheme

3.6 SOFTWARE

In this Project, it has been used the software Echem. With Echem it has been able to control the cyclic voltametry experiments carried out in the electrochemical cell from the computer choosing the settings features, potential, time, velocity, limits, filters and electrodes.

3.7 SOLUTIONS

The solutions used in this project are summarized in the Table:

Table 1. The solutions used in this study

	Component 1	Amount 1	Component 2	Amount 2
1	Pyrrole	335 μL/0.1M	Potassium Ferrocyanide	0.1M
2	Pyrrole	335 μL/0.1M	Sodium Nitroprusside	.0.1M
3	Pyrrole	335 μL/0.1M	Sulphuric Acid	0.1M
4	KCl	0.7456 g/ 0.1 M	-	-
5	KCl	0.7456 g/ 0.1 M	Tryptophan	0.0204 g/ 0.001 M
6	KCl	0.7456 g/ 0.1 M	Valine	0.0118 g/ 0.001 M
7	KCl	0.7456 g/ 0.1 M	Phenylalanine	0.0166 g/ 0.001 M

CHAPTER 4. RESULTS

4.1 SENSOR PREPARATION

It has been used several DRP-150 sensors and they have been modified through a electropolymerization process from three pyrrole solutions containing three different amino acids. Three doping agents with different characteristics and properties have been used: potassium ferrocyanide, sodium nitroprusside and sulphuric acid. The electropolymerization has been accomplished by chronoamperometry or cyclic voltammetric. Pyrrole and doping agent concentrations were 0.1 M in all the cases.

4.1.1 Chronoamperometry

The settings used in the electropolymerization process by means of chronoamperometry have been the same for all three solutions. The selected potential was 0.9 V and the applied time was 120 s.

The chronoamperograms obtained in the case of each doping agent are presented in the Figure 9.

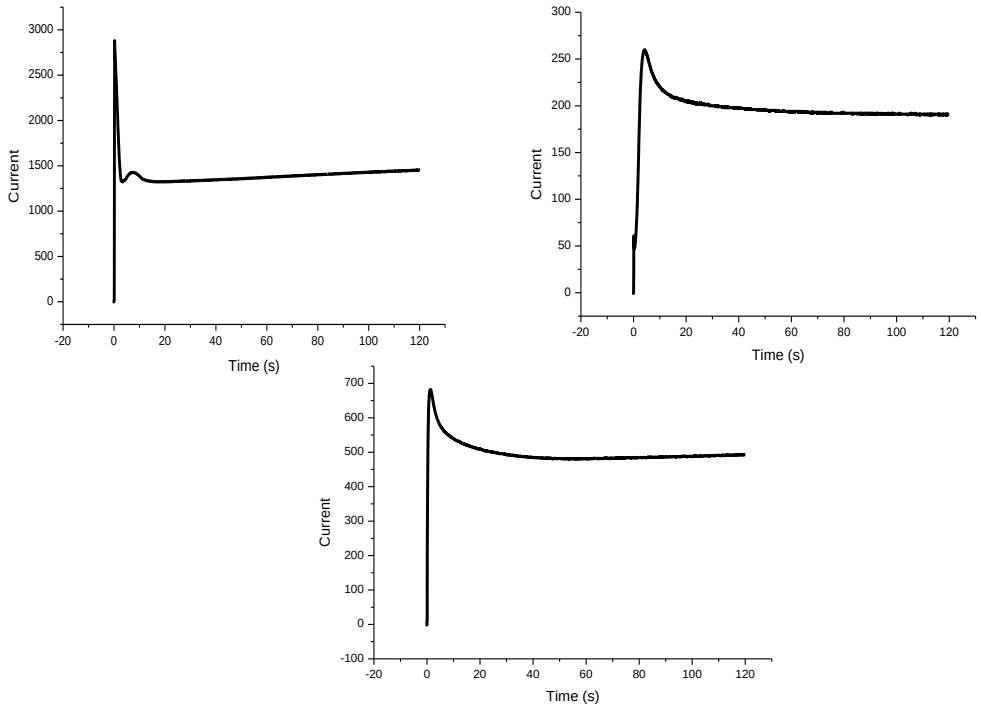


Figure 9. The chronoamperograms of popypyrrole electrosynthesis in μA using as doping agents a) potassium ferrocyanide, b) nitroprusside and c) sulphuric acid

For a better visualization of the differences between the chronoamperograms these are presented in the same Figure.

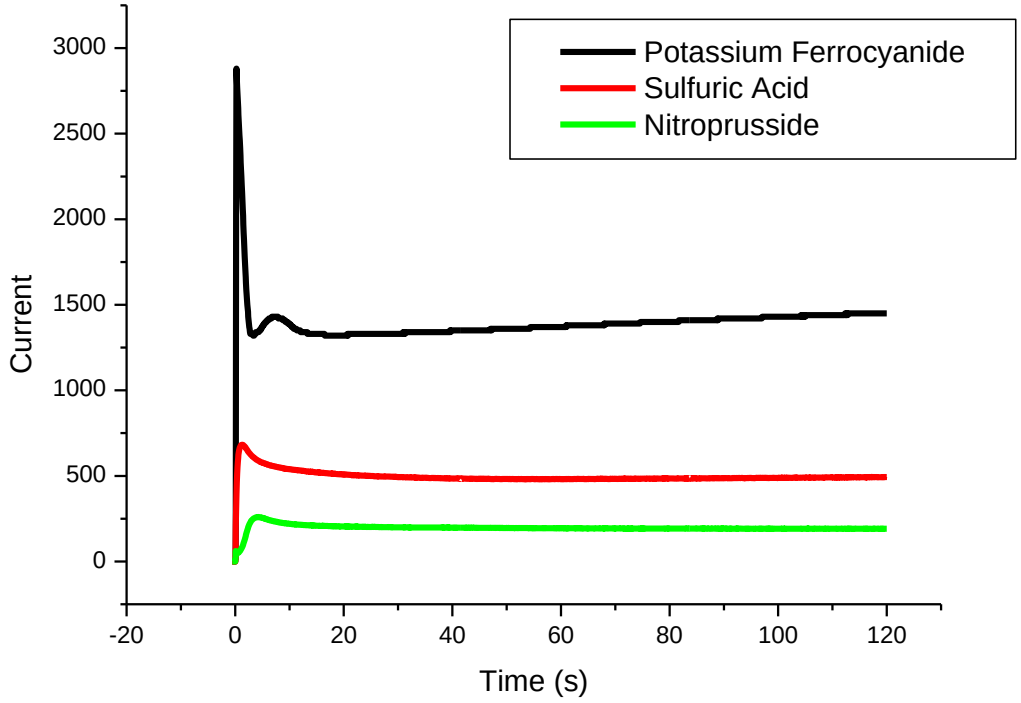


Figure 10. The chronoamperograms of popypyrrole electrosynthesis in μA using as doping agents, potassium ferrocyanide, sulphuric acid and sodium nitroprusside

In this figure, we can see the difference among the currents employed for the polypyrrole electrosynthesis in presence of several doping agents. The highest current obtained was in the case of potassium ferrocyanide with a current between 1250 and 1500 μA. The other currents obtained were much lower. In the case of sulphuric acid, the current was 500 μA and in the case of sodium nitroprusside was 200 μA.

$$I(C_5N_6OFe) < I(H_2SO_4) < I(K_4[Fe(CN)_6])$$

The thickness of the polymer obtained by the electropolymerization process depends on the current applied or generated in the system. The thickness will be higher if the current is higher. The electropolymerization process is more effective, the yield of the process is higher and the quantity of polymer on the sensor is greater.

$$\text{Thickness } C_5N_6OFe < \text{Thickness } H_2SO_4 < \text{Thickness } K_4[Fe(CN)_6]$$

In conclusion, the sensor doped with potassium ferrocyanide will have more thickness. In the opposite case is the polypyrrole doped with sodium Nitroprusside which have the smallest thickness. However, the sensitive performance depends not only on thickness and also in the morphology of the surface, which is related with electroactive area.

4.1.2 Cyclic Voltammetry

In order to carry out the electropolymerization of polypyrrole by cyclic voltammetry, it has been selected 10 cycles with a velocity of 100 mV/s. In addition, the potential limits will be between -1V and 1V.

- Potassium Ferrocyanide as doping agent

In the next Figure is presented the cyclic voltammograms of polypyrrole generation in the presence of potassium ferrocyanide.

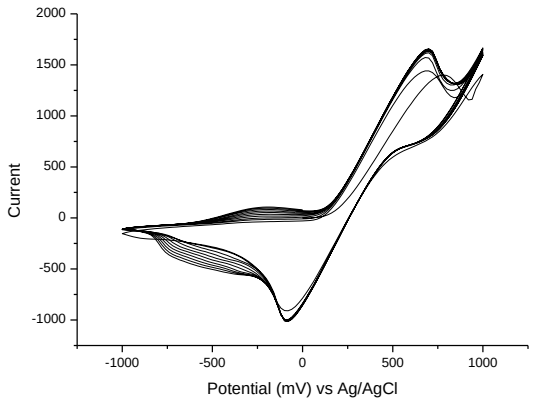


Figure 11. The cyclic voltammograms of polypyrrole generation in the presence of potassium ferrocyanide

In these cyclic voltammograms, it can be seen that the current vary in every cycle, it increases and this means that the thickness increase. These increases appear between -650 and -100 μA and -100 and 150 μA.

- Sodium Nitroprusside as doping agent

In the Figure 12 are presented the cyclic voltammograms registered during the electrodeposition of polypyrrole in the presence of sodium nitroprusside.

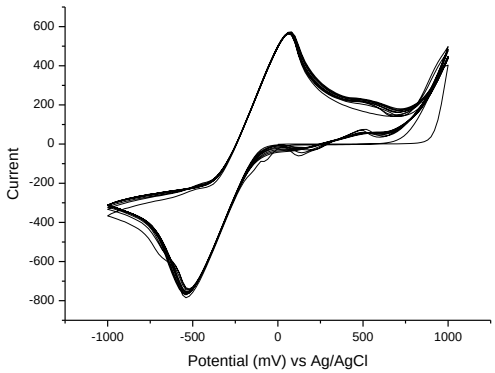


Figure 12. The cyclic voltammograms registered during the electrodeposition of polypyrrole in the presence of sodium nitroprusside

As can be seen in the Figure, the variation of currents with the increasing cycles is smaller as in the case of ferrocyanide, so the thickness of the polymer deposited is smaller.

- Sulphuric Acid as doping agent

  In the Figure 13 are presented the cyclic voltammograms registered during the electropolymerization of pyrrole in the presence of sulphuric acid.

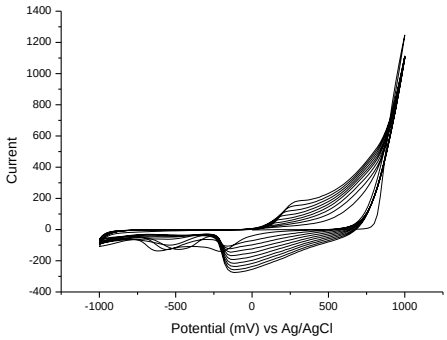


Figure 13. The cyclic voltammograms registered during the electropolymerization of pyrrole in the presence of sulphuric acid

In this last case, the thickness increases rapid with the number of cycles, as it can be seen, between -300 and 50 μA and 0 and 600 μA both in the anodic and cathodic branches, but the variation of the current is much lower than the others doping agents where the Cyclic Voltammetry is used as electrochemical technique for the deposition of polypyrrole.

For the comparison of the results, in the Figure 14 are presented the cyclic voltammograms of all doping agents in the overlapping way.

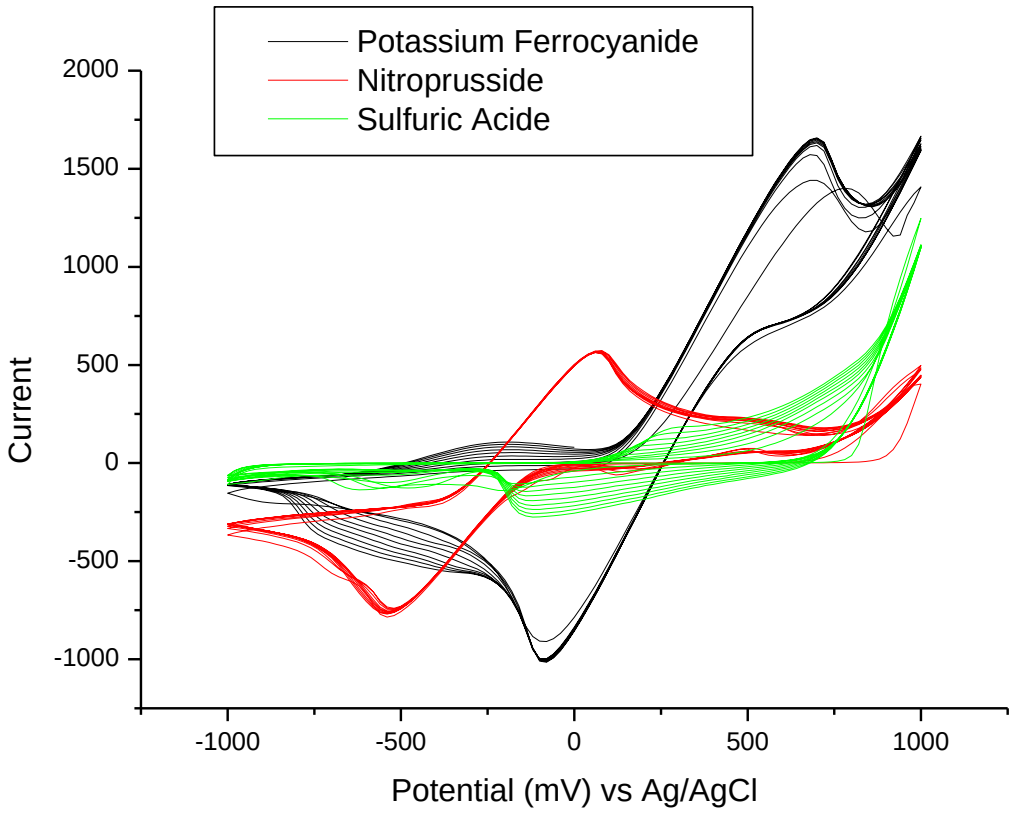


Figure 14. The cyclic voltammograms of polypyrrole electrodeposition by cyclic voltammetry in μA in the presence of ferrocyanide, nitroprusside and sulphuric acid

As can be seen in the Figure, when the doping agent is potassium ferrocyanide the current has the highest values, such as it was happened in chronoamperometry. But in this case of nitroprusside, the cyclic voltammograms has greater current comparing with with sulphuric acid.

$$I(H_2SO_4) < I(C_5N_6OFe) < I(K_4[Fe(CN)_6])$$

The thickness increase with the number of cycles in the cases of potassium ferrocyanide and sulphuric acid. The thickness barely changes in the cycles in nitroprusside solution. However, nitroprusside solution passes more current than in the sulphuric acid solution.

$$\text{Thickness } C_5N_6OFe < \text{Thickness } H_2SO_4 < \text{Thickness } K_4[Fe(CN)_6]$$

In conclusion, the sensor modified with the doping agent potassium ferrocyanide will work better, being more sensitive. But there are different results if compare the chronoamperometry with cyclic voltammetry registered in the presence of nitroprusside and sulphuric acid. In this case, polypyrrole doped with nitroprusside has increased performances comparing with polypyrrole doped with sulphuric acid.

4.2 SENSORS WORKING IN KCl SOLUTIONS

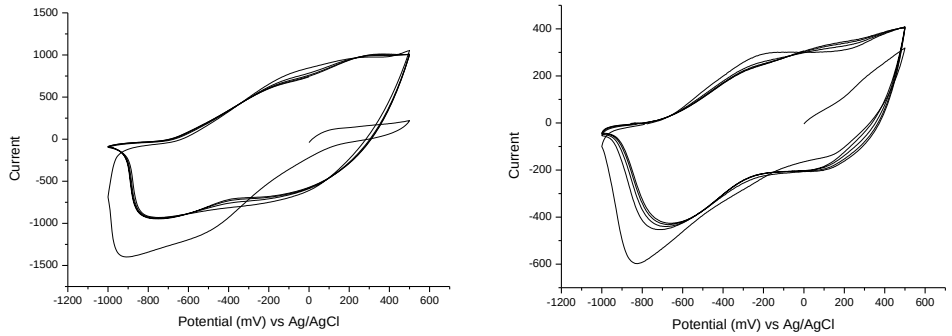
When it was finished the electropolymerization process, it can start to work with the sensors and evaluate the sensitive results of the cyclic voltammetry. In order to do this, the sensors were used in a KCl solution 0.1 M. To prepare this solution needs 0.7456 g of KCl in 100 mL of purified water.

4.2.1 Stabilization

The first cyclic voltammograms were registered in order to evaluate the stabilization of the electrochemical signal. In this part it will be study the stabilization of signals after some cycles and the peaks observed. For this purpose, it was chosen 5 cycles and 100 mV/s of scan rate.

- Potassium Ferrocyanide

In the Figure 15 are presented the cyclic voltammograms of polypyrrole based sensors doped with potassium ferrocyanide obtained by cyclic voltammetry or chronoamperometry.



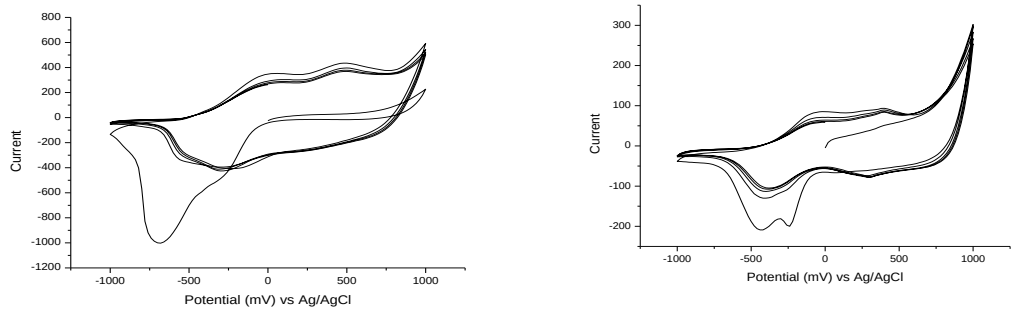
a) *b)*

Figure 15. The cyclic voltammograms of polypyrrole based sensors doped with potassium ferrocyanide in μA obtained by chronoamperometry a) and chronoamperometry b)

The potentials are from -1 V and 0.5 V. The sensors obtained by cyclic voltammetry need more cycles for stabilization. Both cyclic voltammograms present two anodic peaks and two cathodic peaks. The anodic and cathodic peaks from the left part of the curve are related to polypyrrole oxido-reduction processes, while the peaks on the right part of the cyclic voltammograms are related to potassium ferrocyanide oxido-reduction captured in the polymeric matrices of polypyrrole.

- Sodium Nitroprusside

In the Figure 16 are presented the cyclic voltammograms of polypyrrole based sensors doped with sodium nitroprusside obtained by cyclic voltammetry or chronoamperometry.



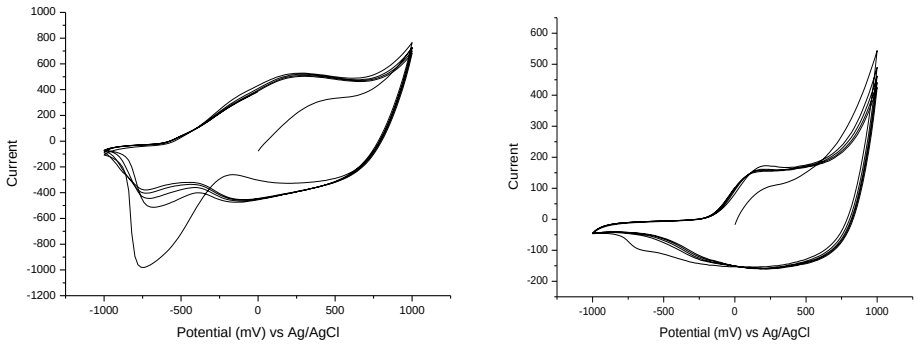
a) *b)*

Figure 16. The cyclic voltammograms of polypyrrole based sensors doped with sodium nitroprusside in μA obtained by chronoamperometry a) and cyclic voltammetry b)

In this case the cyclic voltammograms are registered between -1 V and 1V. These sensors need more cycles for the stabilization and the first cycles are very different to the subsequent ones. It can be seen two cathodic peaks and two anodic peaks. In the figure b) the cathodic peak and the anodic peak are caused by the doping agent. However, in the figure a) both cathodic peaks are related to the oxido-reduction processes of polypyrrole.

- Sulphuric Acid

In the Figure 17 are presented the cyclic voltammograms of polypyrrole based sensors doped with sulphuric acid obtained by cyclic voltammetry or chronoamperometry.



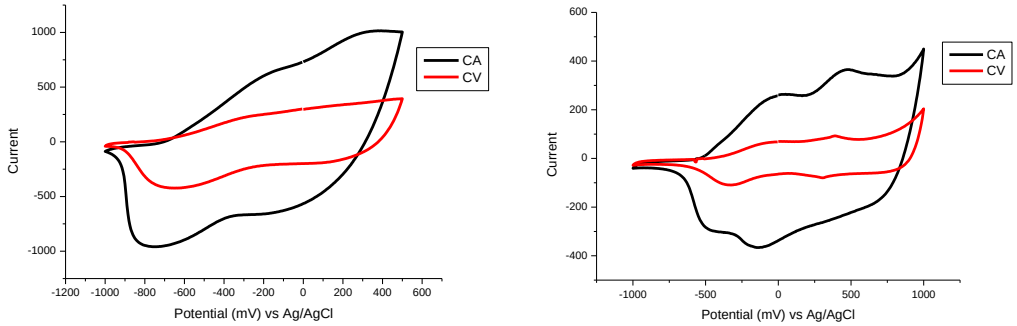
a) *b)*

Figure 17. The cyclic voltammograms of polypyrrole based sensors doped with sulphuric acid in μA obtained by chronoamperometry a) and cyclic voltammetry b)

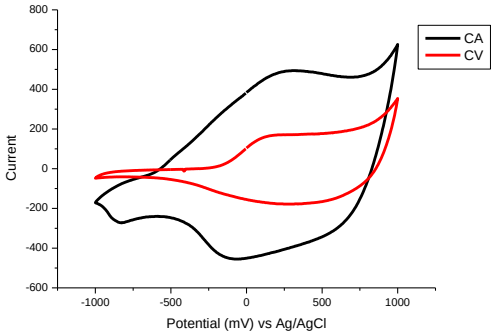
4.2.2 Comparison of the Electropolymerization methods.

It is going to compare the responses of sensors obtained by electropolymerization by chronoamperometry and cyclic voltammetry.

In the Figures 18 are presented the cyclic voltammograms of sensors obtained by chronoamperometry and cyclic voltammetry immersed in 0.1M KCl solution.



a) b)



c)

Figure 18. The cyclic voltammograms of sensors obtained in μA by Chronoamperometry and Cyclic Voltammetry immersed in 0.1M KCl solution. a) Potassium ferrocyanide b) Sodium nitroprusside c) Sulphuric acid.

As can be seen, there is a big difference between the two kinds of sensors based on polypyrrole electropolymerized by aplying different techniques. Both present the same peaks and the same shape but the sensors based on polypyrrole obtained by CA have greather currents than sensor based on polypyrrole obtained by CV. Therefore, the sensors based on polypyrrole obtained by CA exhibits more sensitivity.

$$I(CV) < I(CA)$$

In addition, it can be seen that the sensors based on polypyrrole doped with potassium ferrocyanide have the highest range of the currents and the sensors based on polypyrrole doped with nitroprusside the lowest currents.

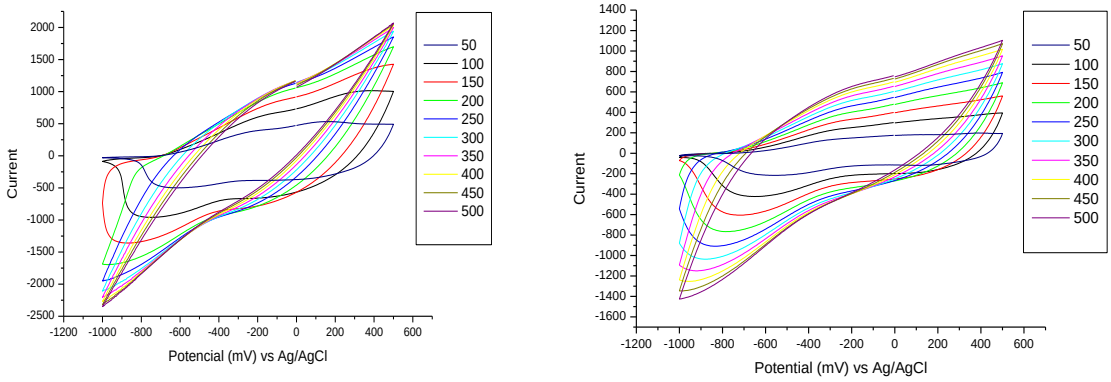
$$I(C_5N_6OFe) < I(H_2SO_4) < I(K_4[Fe(CN)_6])$$

In conclusion, the sensors based on polypyrrole electropolymerized by CA have better performance characteristics in the terms of mechanical stability and sensitivity. The best sensor is the one doped with potassium ferrocyanide.

4.2.3 Kinetics of the electrochemical processes

Next step was to performs the kinetic studies. Cyclic voltammetry responses were registered at different scan rates (from 100mV/s to 1000mV/s). The peaks observed in these Cyclic Voltammetry experiments will be studied in two kinds of graphics: I vs. v and I vs, $v^{1/2}$ and it will be compared. In the figures 19, 21 and 23 are presented the cyclic voltammograms obtained with each sensor at different scan rates and the dependences between the highest peak currents, both anodic and cathodic. In the Figures 20, 22 and 24 are presented the dependences between the anodic and cathodic peak, respectively in relation with scan rate or square root of scan rates.

- Potassium Ferrocyanide



a) b)

Figure 19. The cyclic voltammograms of sensors based on polypyrrole doped with potassium ferrocyanide obtained in μA by a) CA and b) CV immersed in 0.1 M KCl solution registered with different scan rates ranging from 50 to 500 mV/s

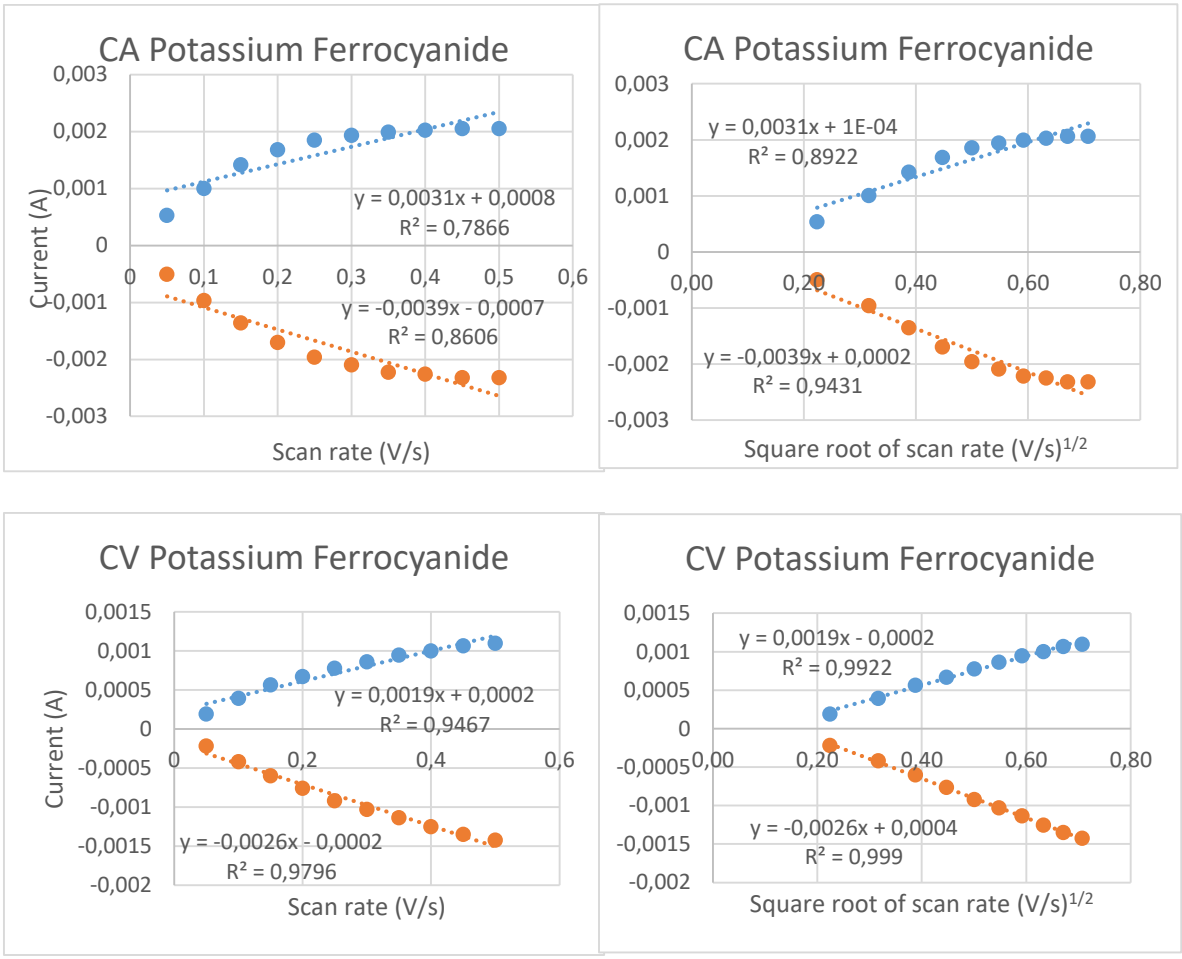
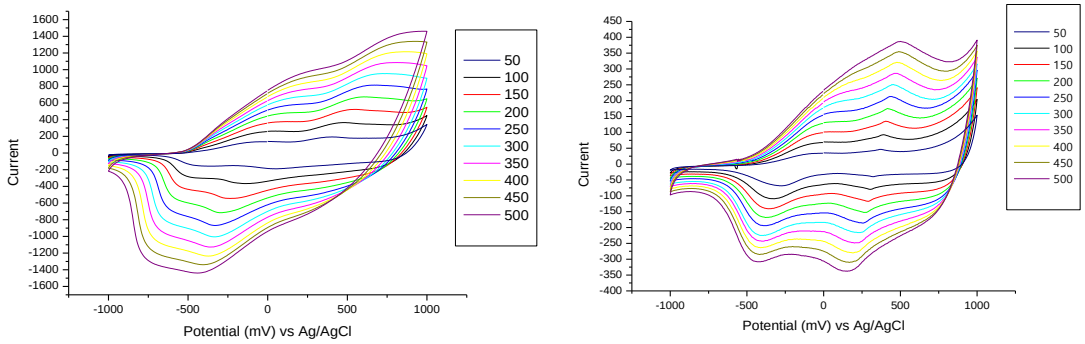


Figure 20. Dependences between peak currents and scan rate or square root of scan rates for the sensors based on polypyrrole doped with potassium ferrocyanide obtained by CA or CV

- Sodium Nitroprusside



a) b)

Figure 21. The cyclic voltammograms of sensors based on polypyrrole doped with sodium nitroprusside obtained in μA by a) CA and b) CV immersed in 0.1 M KCl solution registered with different scan rates ranging from 50 to 500 mV/s

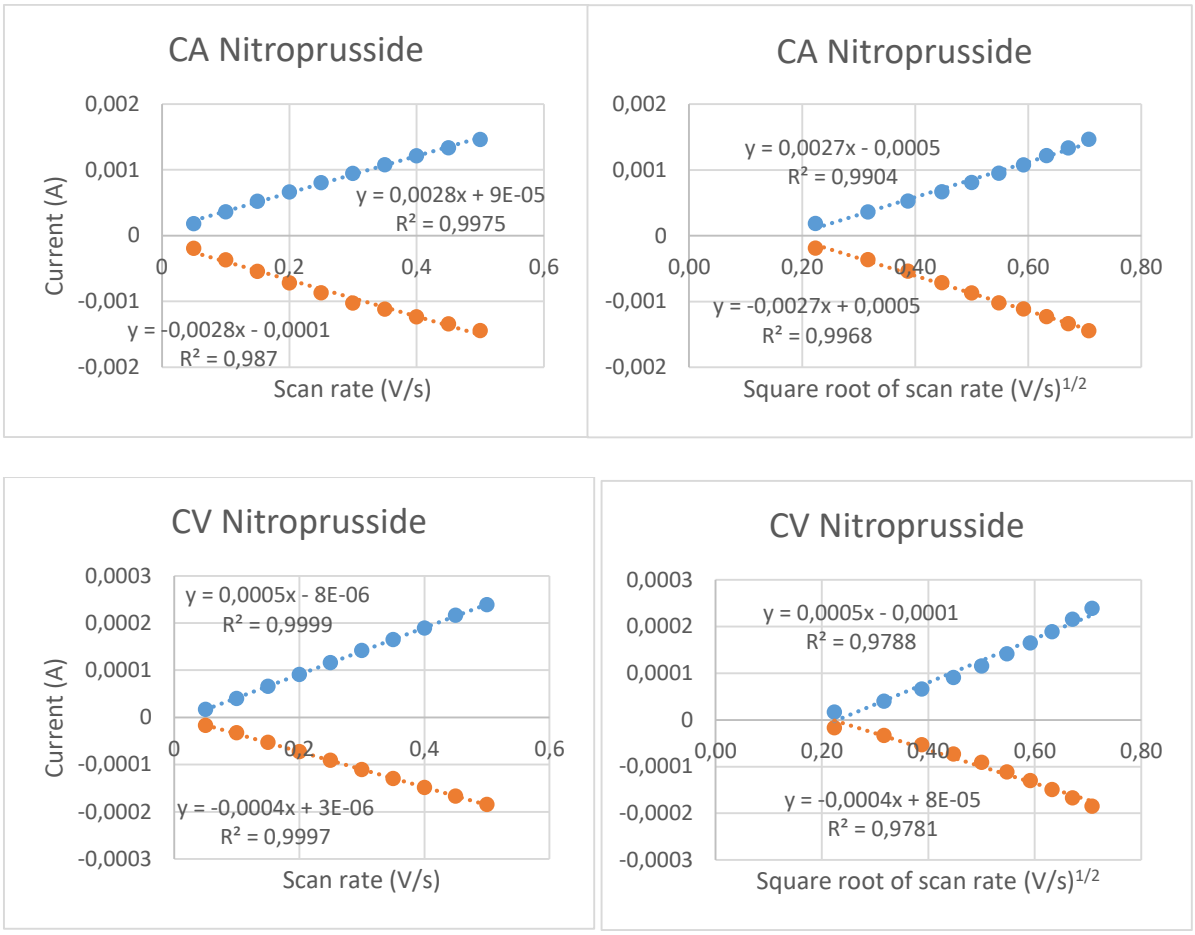
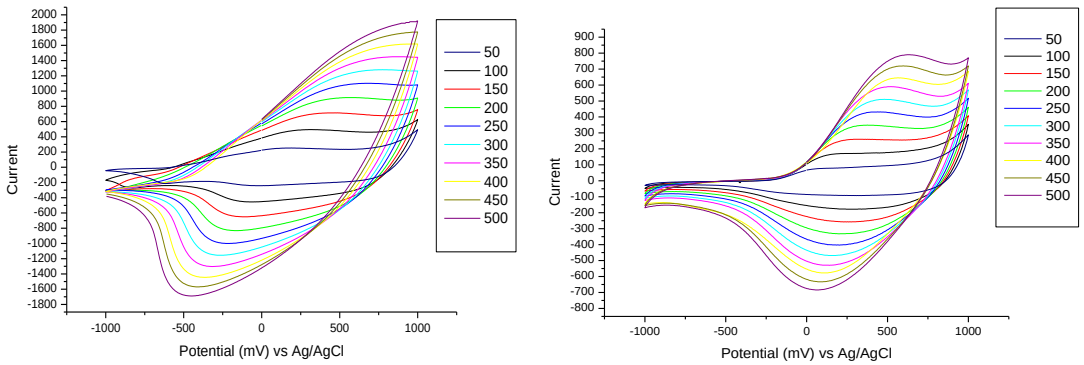


Figure 22: Dependences between peak currents and scan rate or square root of scan rates for the sensors based on polypyrrole doped with sodium nitroprusside obtained by CA or CV.

- Sulphuric acid



a) b)

Figure 23. The cyclic voltammograms of sensors based on polypyrrole doped with sulphuric acid obtained in μA by a) CA and b) CV immersed in 0.1 M KCl solution registered with different scan rates ranging from 50 to 500 mV/s

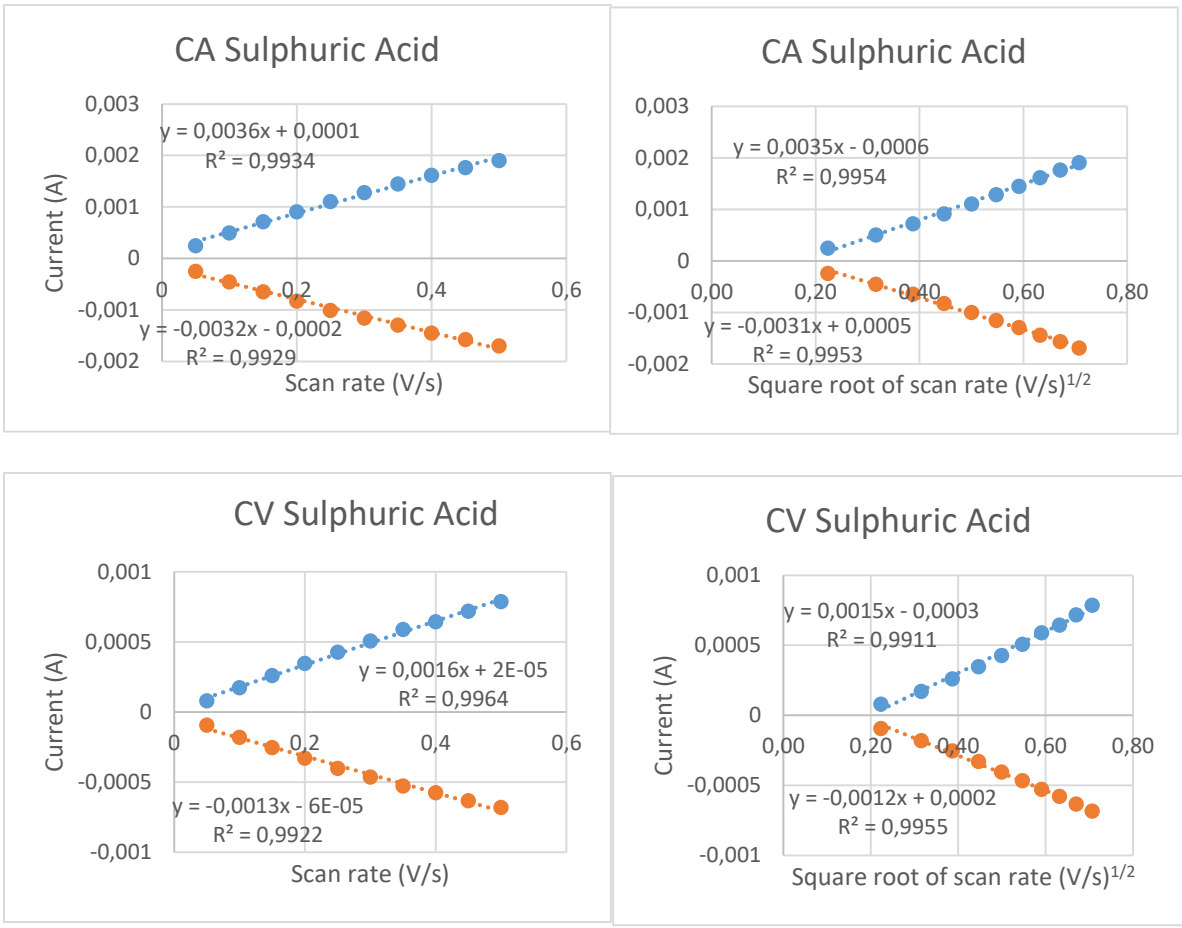


Figure 24. Dependences between peak currents and scan rate or square root of scan rates for the sensors based on polypyrrole doped with sodium nitroprusside obtained by CA or CV

In the Table 2 are summarized the kinetics results with scan rate obtained for all the sensors developed in this study.

Table 2. The regression equations and the coefficients of determination obtained by the linear fitting between peaks currents and the scan rate

Type	Regression equation I_{pa} vs. v (Anodic)	Regression equation I_{pc} vs. v (Cathodic)	Correlation R^2 (Anodic)	Correlation R^2 (Cathodic)
CA $K_4[Fe(CN)_6]$	y = 0.0031x + 0.0008	y = -0.0039x - 0.0007	0.7866	0.8606
CV $K_4[Fe(CN)_6]$	y = 0.0019x + 0.0002	y = -0.0026x - 0.0002	0.9467	0.9796
CA C_5N_6OFe	y = 0.0028x + 9E-05	y = -0.0028x - 0.0001	0.9975	0.987
CV C_5N_6OFe	y = 0.0008x + 2E-05	y = -0.0007x - 2E-05	0.9974	0.9973
CA H_2SO_4	y = 0.0036x + 0.0001	y = -0.0032x - 0.0002	0.9934	0.9929
CV H_2SO_4	y = 0.0016x + 2E-05	y = -0.0013x - 6E-05	0.9964	0.9922

In the Table 3 are summarized the kinetics results with square root of scan rate obtained for all the sensors developed in this study.

Table 3. The regression equations and the coefficients of determination obtained by the linear fitting between peaks currents and the square root of scan rates

Type	Regression equation I_{pa} vs. $v^{1/2}$ (Anodic)	Regression equation I_{pc} vs. $v^{1/2}$ (Cathodic)	Correlation R^2 (Anodic)	Correlation R^2 (Cathodic)
CA $K_4[Fe(CN)_6]$	y = 0.0031x + 1E-04	y = -0.0039x + 0.0002	0.8922	0.9431
CV $K_4[Fe(CN)_6]$	y = 0.0019x - 0.0002	y = -0.0026x + 0.0004	0.9922	0.999
CA C_5N_6OFe	y = 0.0027x - 0.0005	y = -0.0027x + 0.0005	0.9904	0.9968
CV C_5N_6OFe	y = 0.0007x - 0.0001	y = -0.0006x + 0.0001	0.99	0.9904
CA H_2SO_4	y = 0.0035x - 0.0006	y = -0.0031x + 0.0005	0.9954	0.9953
CV H_2SO_4	y = 0.0015x - 0.0003	y = -0.0012x + 0.0002	0.9911	0.9955

Studying the correlation results, in the case of sensor based on polypyrrole doped with potassium ferrocyanide has a higher correlation in every case when I vs $v^{1/2}$ is fitted, so that this process is controlled by diffusion. In the case of sensors electropolymerized in presence of sulphuric acid and sodium nitroprusside have different results. In the first case there are more I vs $v^{1/2}$ fittings with higher determination coefficients, while in the second case there are more linear fittings of I vs v. In this case the processes are controlled by electron transfer.

We have obtained different results, the electrochemical processes in the case of doping agents potassium ferrocyanide and Sulphuric Acid are controlled by diffusion while the electrochemical process with sodium nitroprusside is controlled by the electron transfer

In conclusion, the sensor electropolymerized by CA has better performances and the doping agent with better results is potassium ferrocyanide.

4.3 AMINO ACIDS STUDY

In this part the sensors will be immersed in 0.1M KCl solutions and amino acids: Tryptophan, Valine and Phenylalanine with the concentration of 10^{-3} M in order to determine if the sensors are able to detect the presence of the amino acids in the solution. The responses in 0.1M solutions were used for the comparing purposes. The solutions have been prepared in a balloon of 50 mL using ultrapure water and 0.3728 g of KCl and 0.0102 g of tryptophan 0.0059 g of valine or 0.0083 g of phenylalanine.

4.3.1 Stabilization of the electrochemical response

First of all, it will be study the stabilization of the CV, when we will see the effects of the amino acids in the positions of peaks (potential) and the influence on the responsive currents. In order to do this, task it will be used 5 cycles at a scan rate of 100 mV/s.

- Potassium Ferrocyanide

In the Figure 25 are presented the responses of sensors based on polypyrrole doped with potassium ferrocyanide immersed in solutions of KCl and amino acids.

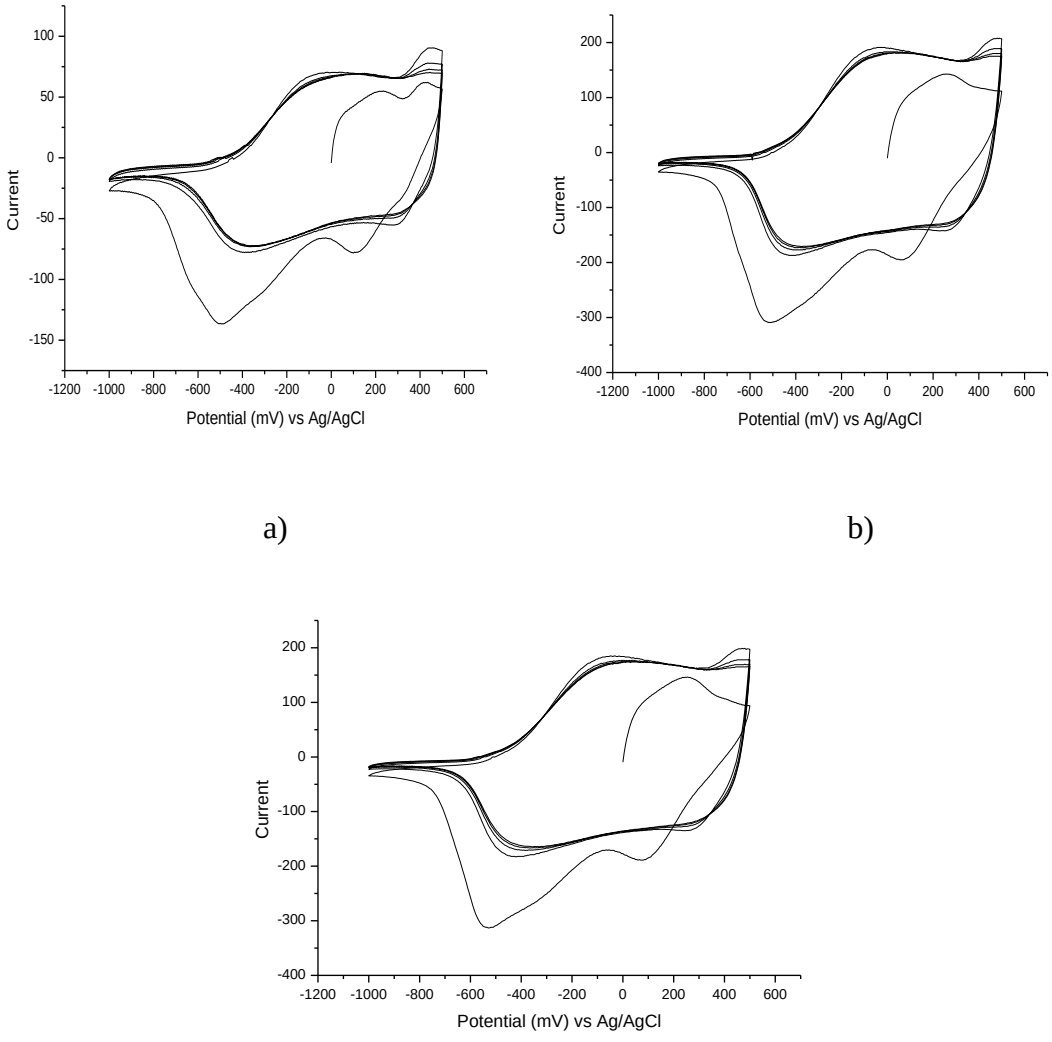


Figure 25. The stabilization of the CVs for the sensors based on polypyrrole doped with potassium ferrocyanide. a) Tryptophan, b) valine and c) phenylalanine

The amino acids determine the shift of the peaks to the right (positive potential). With the amino acids the peaks are easier to be seen, being better defined. The current of entire CV has decreased significantly. The solutions containing valine and phenylalanine determine similar results. These solutions have a range of current from -200 to 200 μA while the solution of tryptophan determine a range of current at the half values.

- Sodium Nitroprusside

In the Figure 26 are presented the responses of sensors based on polypyrrole doped with sodium nitroprusside immersed in solutions of KCl and amino acids.

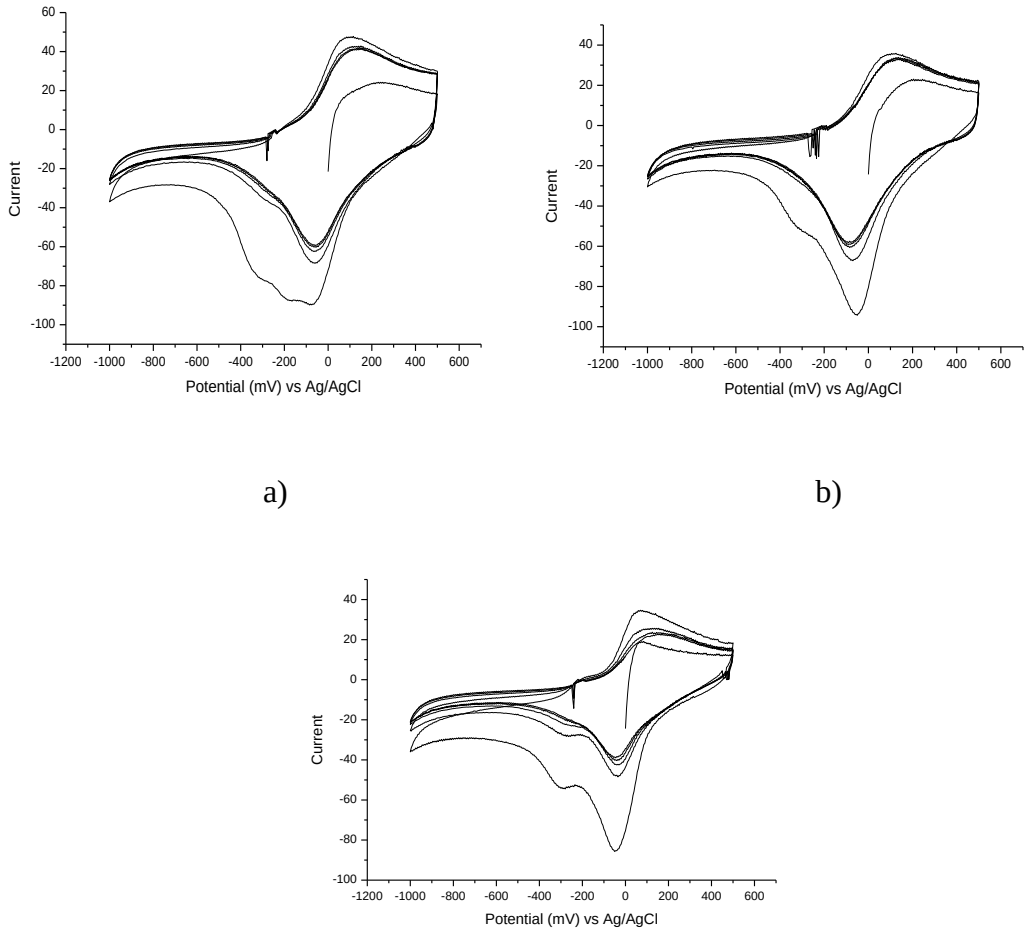


Figure 26: The stabilization of the CVs for the sensors based on polypyrrole doped with sodium nitroprusside. a) Tryptophan, b) valine and c) phenylalanine

In the case of sensors based on polypyrrole electropolymerized with sodium Nitroprusside the range of currents decreases in the presence of amino acids and there are different currents in the peaks potential. The first solution containing tryptophan the current range is (-60, 40) μA, in the second case of solution containing valine is (-60, 35) μA while in the solution containing phenylalanine the range is from -40 to 25 μA. In the cyclic voltammograms of sensors immersed in phenylalanine solution appear a cathodic peak and an anodic peak in -300 and -200 μA range.

- Sulphuric Acid

In the Figure 27 are presented the responses of sensors based on polypyrrole doped with sulphuric acid immersed in solutions of KCl and amino acids.

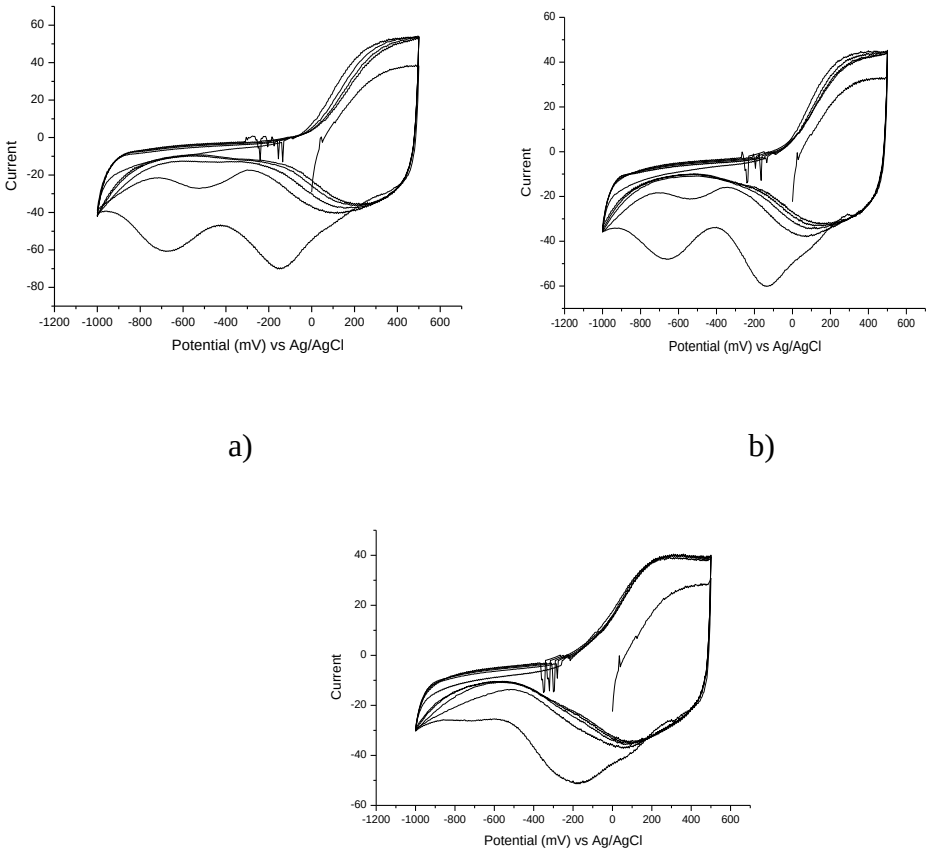


Figure 27: The stabilization of the CVs for the sensors based on polypyrrole doped with sulphuric acid. a) Tryptophan, b) valine and c) phenylalanine

The cyclic voltammograms in all three cases has very similar shapes. The currents in the anodic and cathodic peaks are similar in the three experiments from -35 to 40 μA, although the current in the anodic peak in the case of tryptophan is 55 μA. But we can see the difference between the potentials of the peaks. The peaks in the tryptophan solution have higher potential while the peaks in the phenylalanine have lower potential.

In conclusion, the amino acids have an important effect in the cyclic voltammetry, the range of currents decreases significantly, and also the amino acids have different effects in the position of the peaks.

4.3.2 Kinetics

As it has done in the case of KCl solution, it will be realized a kinetics study of sensors in amino acid solutions, where the peaks will be analyzed and we will see the influence of the amino acids in the dependences of I vs v and I vs $v^{1/2}$. The scan rates are from 50 to 500 mV/s. In the figures 28, 30 and 32 are presented the cyclic voltammograms obtained with each sensor at different scan rates and the dependences between the highest peak currents, both anodic and cathodic. While in the Figures 29, 31 and 33 are presented the dependences between the anodic and cathodic peak, respectively in relation with scan rate or square root of scan rates.

- Potassium Ferrocyanide

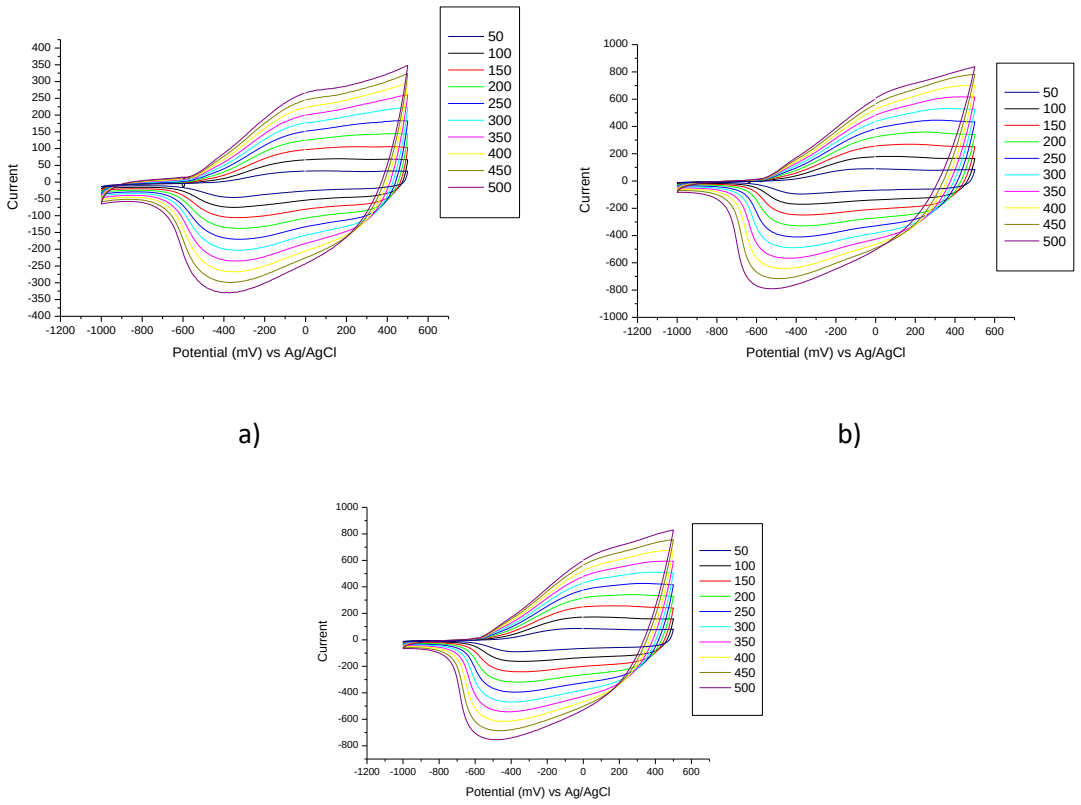
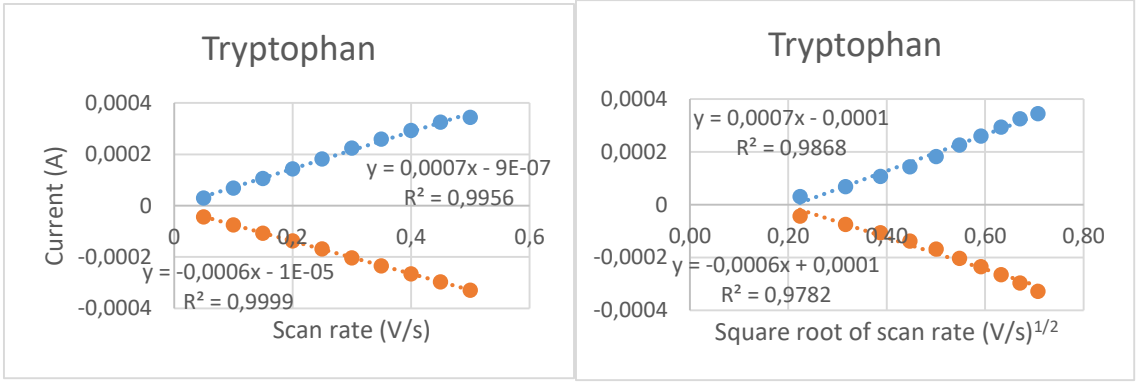


Figure 28: The cyclic voltammograms of sensors based on polypyrrole doped with potassium ferrocyanide obtained in μA immersed in amino acids solutions a) tryptophan, b) valine and c) phenylalanine registered with different scan rates ranging from 50 to 500 mV/s



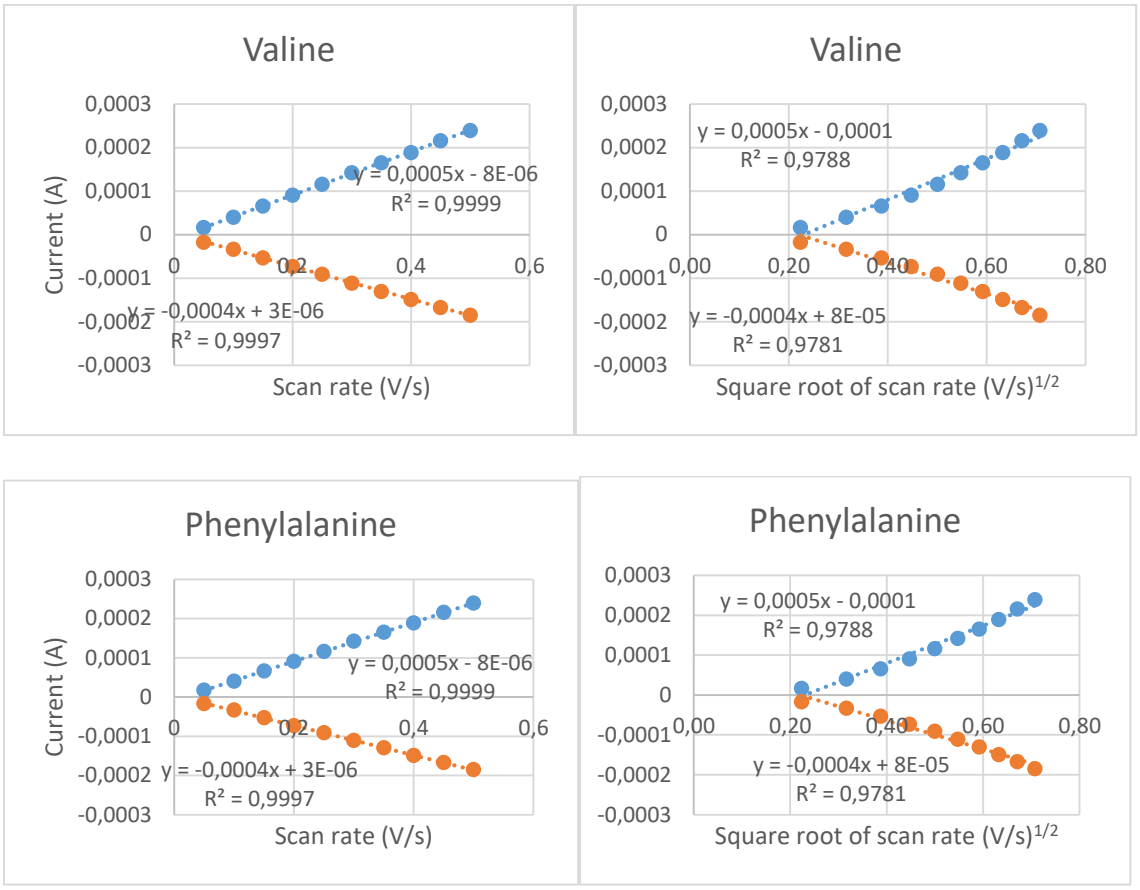


Figure 29: Dependences between peak currents and scan rate or square root of scan rates for the sensors based on polypyrrole doped with potassium ferrocyanide in amino acid solutions

In the Table 4 are summarized the kinetics results obtained for the sensor doped by potassium ferrocyanide.

Table 4. The regression equations and the coefficients of determination obtained for the sensor doped by potassium ferrocyanide

Type	Regression equation I_{pa} vs. $v/v^{1/2}$ (Anodic)	Regression equation I_{pc} vs. $v/v^{1/2}$ (Cathodic)	Correlation R^2 (Anodic)	Correlation R^2 (Cathodic)
Tryptophan v	y = 0.0007x - 9E-07	y = -0.0006x - 1E-05	0.9956	0.9999
Tryptophan $v^{1/2}$	y = 0.0007x - 0.0001	y = -0.0006x + 0.0001	0.9868	0.9782
Valine v	y = 0.0005x - 8E-06	y = -0.0004x + 3E-06	0.9999	0.9997
Valine $v^{1/2}$	y = 0.0005x - 0.0001	y = -0.0004x + 8E-05	0.9788	0.9781
Phenylalanine v	y = 0.0005x - 8E-06	y = -0.0004x + 3E-06	0.9999	0.9997
Phenylalanine $v^{1/2}$	y = 0.0005x - 0.0001	y = -0.0004x + 8E-05	0.9788	0.9781

- Sodium Nitroprusside

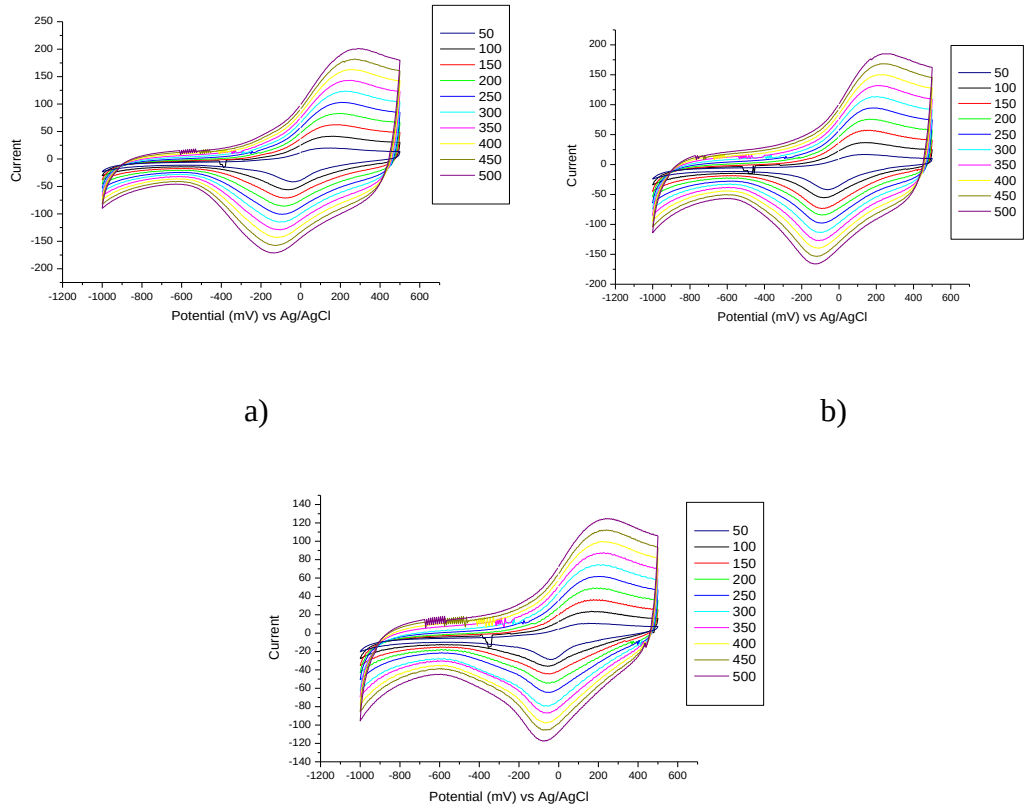
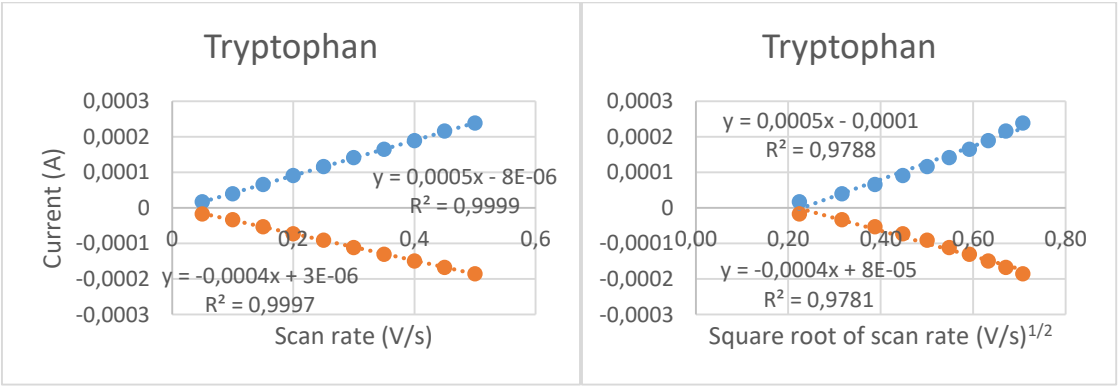


Figure 30. The cyclic voltammograms of sensors based on polypyrrole doped with sodium nitroprusside obtained in μA immersed in amino acids solutions a) tryptophan, b) valine and c) phenylalanine registered with different scan rates ranging from 50 to 500 mV/s



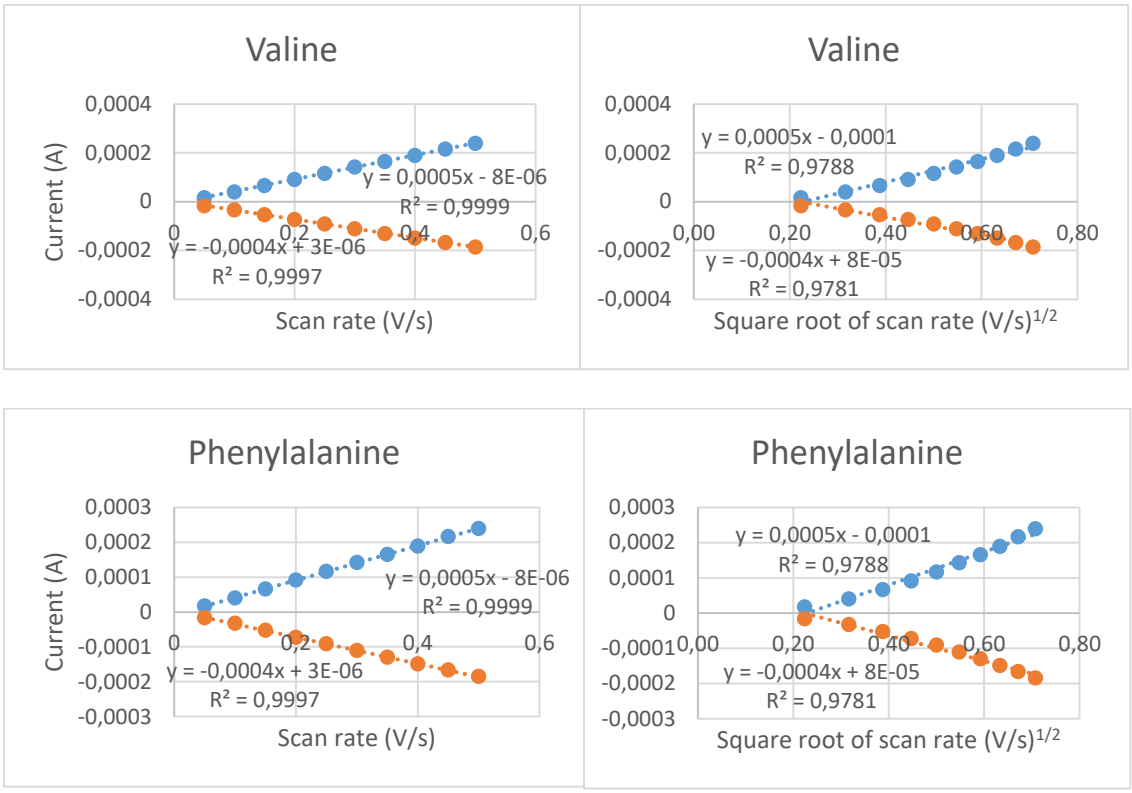


Figure 31. Dependences between peak currents and scan rate or square root of scan rates for the sensors based on polypyrrole doped with sodium nitroprusside in amino acid solutions

In the Table 5 are summarized the kinetics results obtained for the sensor doped by sodium nitroprusside.

Table 5. The regression equations and the coefficients of determination obtained for the sensor doped by sodium nitroprusside

Type	Regression equation I_{pa} vs. $v/v^{1/2}$ (Anodic)	Regression equation I_{pc} vs. $v/v^{1/2}$ (Cathodic)	Correlation R^2 (Anodic)	Correlation R^2 (Cathodic)
Tryptophan v	y = 0.0005x - 8E-06	y = -0.0004x + 3E-06	0.9999	0.9997
Tryptophan $v^{1/2}$	y = 0.0005x - 0.0001	y = -0.0004x + 8E-05	0.9788	0.9781
Valine v	y = 0.0005x - 8E-06	y = -0.0004x + 3E-06	0.9999	0.9997
Valine $v^{1/2}$	y = 0.0005x - 0.0001	y = -0.0004x + 8E-05	0.9788	0.9781
Phenylalanine v	y = 0.0005x - 8E-06	y = -0.0004x + 3E-06	0.9999	0.9997
Phenylalanine $v^{1/2}$	y = 0.0005x - 0.0001	y = -0.0004x + 8E-05	0.9788	0.9781

- Sulphuric Acid

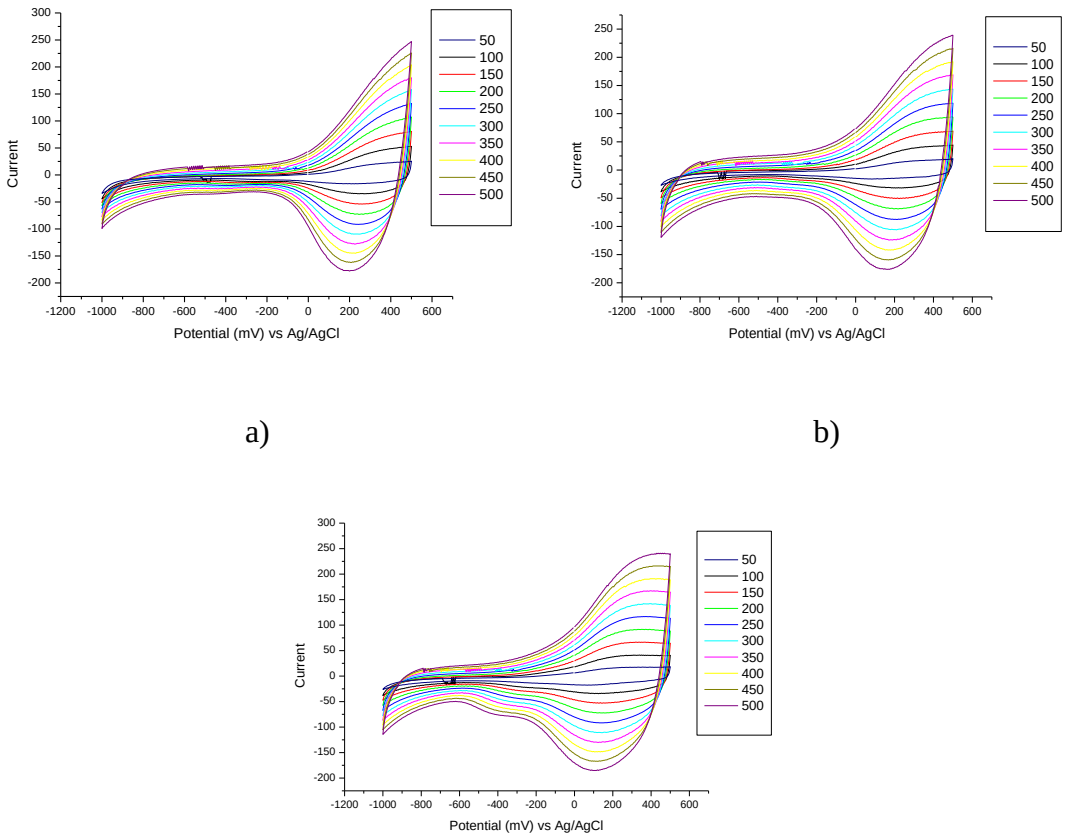
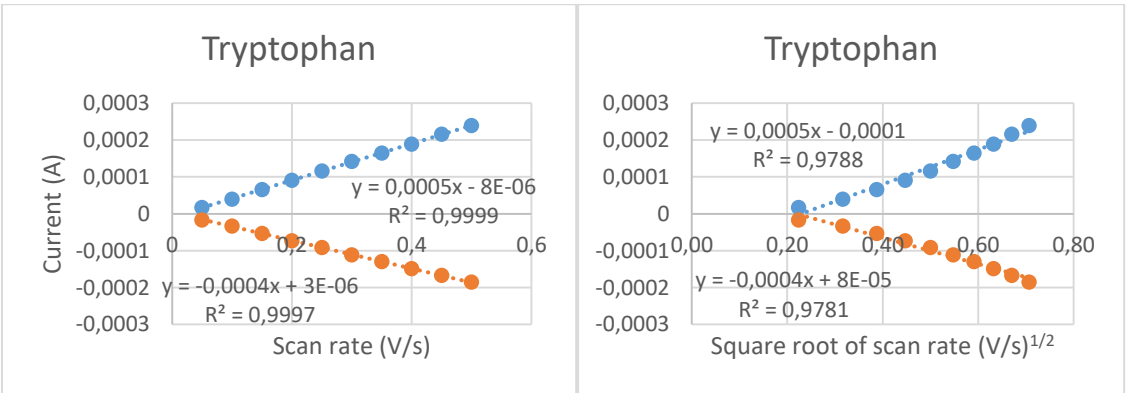


Figure 32: The cyclic voltammograms of sensors based on polypyrrole doped with sulphuric acid obtained in μA immersed in amino acids solutions a) tryptophan, b) valine and c) phenylalanine registered with different scan rates ranging from 50 to 500 mV/s



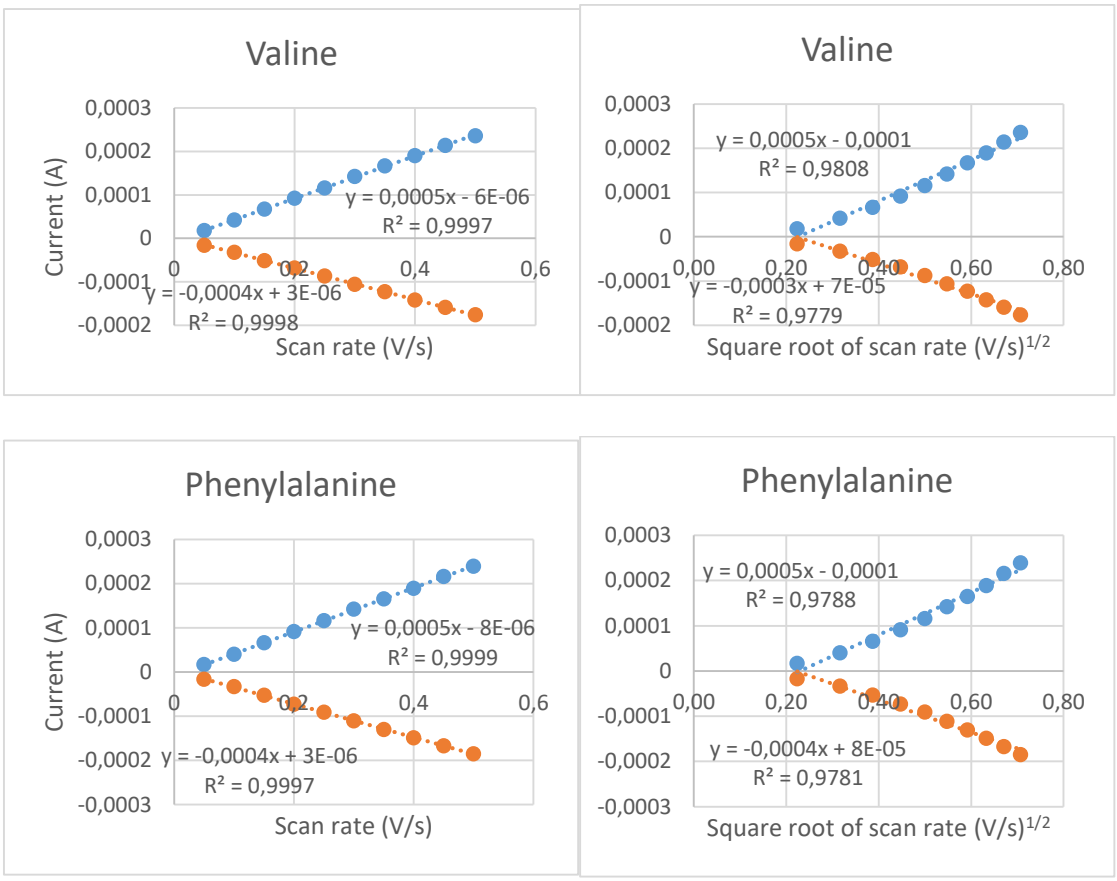


Figure 33. Dependences between peak currents and scan rate or square root of scan rates for the sensors based on polypyrrole doped with sulphuric acid in amino acid solutions

In the Table 6 are summarized the kinetics results obtained for the sensor doped by sulphuric acid.

Table 6. The regression equations and the coefficients of determination obtained for the sensor doped by sulphuric acid

Type	Regression equation I_{pa} vs. $v/v^{1/2}$ (Anodic)	Regression equation I_{pc} vs. $v/v^{1/2}$ (Cathodic)	Correlation R^2 (Anodic)	Correlation R^2 (Cathodic)
Tryptophan v	y = 0.0005x - 8E-06	y = -0.0004x + 3E-06	0.9999	0.9997
Tryptophan $v^{1/2}$	y = 0.0005x - 0.0001	y = -0.0004x + 8E-05	0.9788	0.9781
Valine v	y = 0.0005x - 6E-06	y = -0.0004x + 3E-06	0.9997	0.9998
Valine $v^{1/2}$	y = 0.0005x - 0.0001	y = -0.0003x + 7E-05	0.9808	0.9779
Phenylalanine v	y = 0.0005x - 8E-06	y = -0.0004x + 3E-06	0.9999	0.9997
Phenylalanine $v^{1/2}$	y = 0.0005x - 0.0001	y = -0.0004x + 8E-05	0.9788	0.9781

With these results we can see how it has changed the correlation and the behaviour of sensors in the presence of the amino acids. The correlation in the regression of I vs v is higher and almost 1 in the presence of amino acids while without amino acids have been obtained different results. This means that the amino acids have an important effect in the electrochemical process and it is influenced by electron transfer.

4.3.3 Discrimination among amino acids

In order to determine if the sensors are able to discriminate the amino acids solution, the Principal Component Analysis was performed. The results are presented in the form of scores plot. The input for PCA were the entire voltammograms.

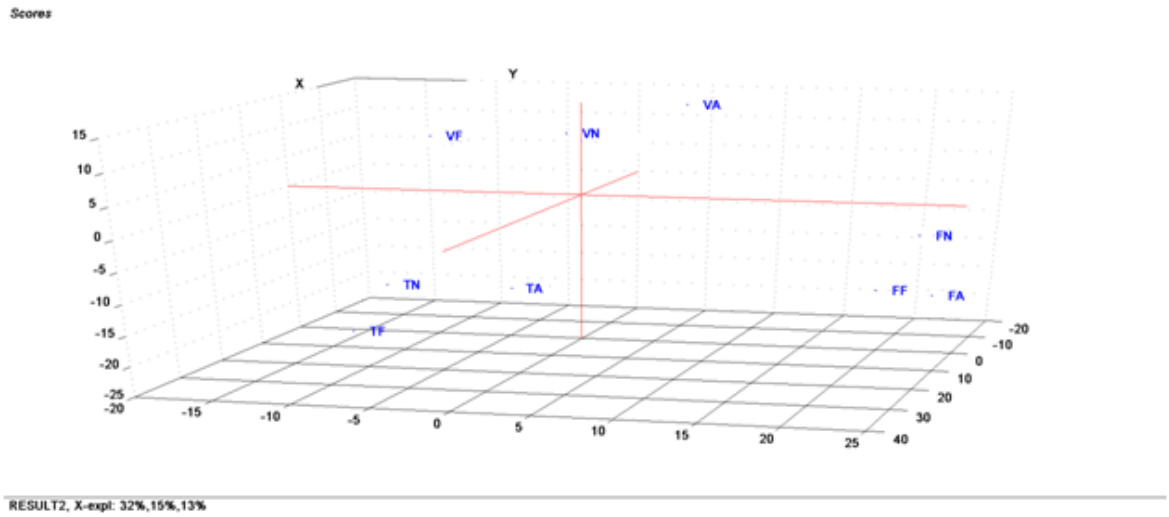


Figure 34. PCA score plot of CVs registered by the sensors based on polypyrrole doped with potassium ferrocyanide, sodium nitroprusside and sulphuric acid obtained by CA

As can be seen in the figure the solutions of amino acids can be easily discriminated one to each other. This results confirm the cross-selectivity of the sensors. Therefore, one sensor provides different signals when different amino acids are analysed. In the same time, different sensors provide different signals when these are immersed in the same amino acid solution.

CONCLUSION

Once the experiments have finished and the different studies have been analysed, it is possible to make a global conclusion. Between the two electropolymerized methods, chronoamperometry and cyclic voltammetry, the first kind of sensors obtained has better responses in KCl solutions with a notably higher range of currents, so that, these sensors will work better as detecting elements. Comparing the dopant agents used, potassium ferrocyanide stands out. With this dopant agent it has been achieved a higher quantity the polymer deposited in the sensor and a higher range of currents in the subsequent cyclic voltammetry in KCl solutions. The sensors doped with the other two dopant agents have obtained worse results. Analyzing the kinetics studies in the KCl solutions have been obtained different results of behaviour and it is not clear to say if those processes are controlled by diffusion or by electron exchange. Working in the amino acids solutions, it can be seen the impact that the amino acids have in the electrochemical process. The cyclic voltammetry has a much lower range of currents and the peaks were moved the potential position, so that, it is possible to see if there are amino acids in the solutions. With the amino acids, the dependences between peak currents and scan rates have been quite better than with square roots of scan rate, this means, in presence of amino acids the process are controlled by electron transfer. Finally, in the scores plot of principal component analysis, it can be observed, that every amino acid solution has obtained different signals, so that, it is possible differentiate them. In this project, the sensors based on polypyrrole with the different dopant agents have obtained with satisfactory results in the detection of amino acids.

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