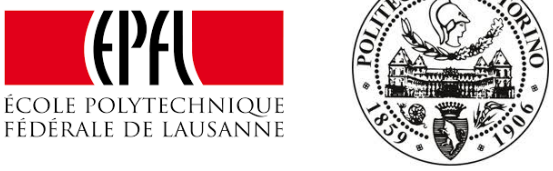

POLITECNICO DI TORINO

NANOTECHNOLOGIES FOR ICTs

DEPARTMENT OF ELECTRONICS AND TELECOMMUNICATIONS

Advanced Materials for Sub-second Sensing of Dopamine, Serotonin and Adenosine

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Abstract

Carbon microelectrodes and carbon nanostructured electrodes have been tested for sub-second detection of dopamine with Fast scan cyclic voltammetry (FSCV). Dopamine is a neurotransmitter that plays a critical role in numerous neurodegenerative and mood disorders such as Parkinson's disease, addiction, depression and schizophrenia. Dopamine releases takes place with millisecond temporal and nanometer spatial resolution. Novel micro and nanoscale electrodes are needed with superior sensitivity and improved spatial resolution to gain improved understanding of dopamine dysregulation. In the first part of the work, improved thin film polymeric coatings for carbon fiber electrodes have been achieved by electro - polymerizing Nafion perfluorinated resin and 3,4 - ethylenedioxythiophene (ED OT) with two different surfactants. Increased sensitivity and good selectivity for sensing sub-second release of dopamine (DA), serotonin (5-HT) and adenosine in the presence of large concentrations of ascorbic acid (AA) and dihydroxyphenylacetic acid (DOPAC) was demonstrated. The influence of different surfactants on the carbon fiber microelectrode (CFE) coatings has been characterized for neurotransmitters detection with fast scan cyclic voltammetry (FSCV). Two different surfactants have been analyzed: sodium dodecyl sulfate (SDS) and sodium dodecyl benzene sulfonate (SDB S). The dopamine signal is increased by 4X-9X, while the serotonin signal is increased by 4X, and the adenosine signal is increased by 3X compared to bare carbon with these improved coatings. We report one of the highest sensitivity values ever reported for dopamine, serotonin and for adenosine. In the second part of the work, carbon nano-structured electrodes obtained from pyrolysis of a photo-resist film (PPF) have been tested for dopamine detection with the same method (FSCV). The nanorods were fabricated by pyrolysis of a lithographically defined polymeric structure. This methodology enables integration of nanoscale electrodes with integrated circuits and traditional electronics.
A 5X higher signal with respect to bare carbon fibers have been achieved with an actual electrode area of 4 times smaller dimension, having also one of the highest sensitivity value ever reported for PPF electrodes.

Sommario

Microelettrodi di carbonio e elettrodi nanostrutturati di carbonio sono stati testati per la rilevazione di dopamina con il metodo "Fast Scan Cyclic Voltammetry" (FSCV). La dopamina è un neurotrasmettitore che svolge un ruolo fondamentale in numerosi disturbi neurodegenerativi e dell'umore come il Parkinson, la dipendenza, la depressione e la schizofrenia. Il rilascio della dopamina avviene con risoluzione temporale del millisecondo e risoluzione spaziale nanometrica. Nuovi elettrodi alla micro e nanoscala sono necessari con una maggiore sensitività e una migliore risoluzione spaziale per ottenere una maggiore comprensione della disregolazione della dopamina. Nella prima parte del lavoro sono stati ottenuti sottili rivestimenti polimerici per elettrodi in fibra di carbonio mediante elettro polimerizzazione della resina Nafion e del monomero EDOT, con due tensioattivi diversi. È stata dimostrata una maggiore sensitività e una buona selettività per il rilevamento della dopamina (DA), della serotonina (5-HT) e dell'adenosina in presenza di grandi concentrazioni di acido ascorbico (AA) e acido diidroxyfenilacetico (DOPAC). L'influenza dei diversi tensioattivi sui rivestimenti microelettrodi (CFE) in fibra di carbonio è stata caratterizzata per il rilevamento dei neurotrasmettitori con FSCV. Sono stati analizzati due diversi tensioattivi: dodecil solfato di sodio (SDS) e dodecilbenzene solfonato sodico (SDBS). Il segnale della dopamina è aumentato di 4X-9X, mentre il segnale di serotonina è aumentato di 4 volte e il segnale di adenosina è aumentato di 3 volte rispetto al solo carbonio con questi nuovi rivestimenti. Riportiamo qui uno dei valori di sensitività più alti mai riportati per la dopamina, la serotonina e per l'adenosina.
Nella seconda parte del lavoro, gli elettrodi nano-strutturati di carbonio ottenuti dalla pirolisi di un film foto-resist (PPF) sono stati testati per la rilevazione della dopamina con lo stesso metodo (FSCV). Le nanostrutture sono state fabbricate dalla pirolisi di una struttura polimerica litograficamente definita. Questa metodologia permette l'integrazione di elettrodi a nanoscala con circuiti integrati e elettronica tradizionale.
Un segnale 5X superiore rispetto alle sole fibre di carbonio è stato raggiunto con un'area di elettrodo effettiva di dimensione 4 volte più piccola, avendo anche uno dei valori di sensitività più alti mai riportati per elettrodi PPF.

Résumé

Microélectrodes de carbone et électrodes à nanostructures de carbone ont été testées pour la détection de signaux en millisecondes de la dopamine avec "Fast Scan Cyclic Voltammetry" (FSCV). La dopamine est un neurotransmetteur qui joue un rôle vital dans de nombreux troubles de l'humeur et neurodégénératives telles que la maladie de Parkinson, la toxicomanie, la dépression et la schizophrénie. La libération de dopamine a lieu avec une résolution temporelle du milliseconde et spatiale du nanomètre. De nouvelles électrodes micro et nanométriques sont nécessaires avec une plus grande sensibilité et une meilleure résolution spatiale pour mieux comprendre le dérèglement de la dopamine.
Durant la première partie du travail ont été obtenus de minces revêtements polymères pour les électrodes en fibres de carbone par électro polymérisation de la résine Nafion et du monomère EDOT, avec deux agents tensioactifs différents. Une plus grande sensibilité et une bonne sélectivité pour la détection de la dopamine (DA), de la sérotonine (5-HT) et de l'adénosine en présence de fortes concentrations d'acide ascorbique (AA) et d'acide diidroxyfenilacetico (DOPAC) ont été démontrées. L'influence des différents agents tensio-actifs sur les revêtements de microélectrodes (CFE) de la fibre de carbone a été caractérisée pour la détection de neurotransmetteurs avec FSCV.
Deux tensioactifs différents ont été analysés: le dodécylsulfate de sodium (SDS) et le dodécylbenzène sulfonate de sodium (SDBS). Le signal de la dopamine a augmenté par 4X-9X, tandis que le signal de la sérotonine a augmenté de 4 fois et le signal d'adénosine a augmenté de 3 fois par rapport au carbone seul avec ces nouveaux revêtements. Voici l'une des valeurs de sensibilité les plus élevées jamais rapportées pour la dopamine, la sérotonine et l'adénosine. Dans la deuxième partie de l'ouvrage, les électrodes à nanostructures de carbone obtenues à partir de la pyrolyse d'un film de résine photosensible (PPF) ont été testées pour la détection de la dopamine avec la même méthode (FSCV). Les nanostructures ont été fabriquées par la pyrolyse d'une structure polymère lithographiquement définie. Cette méthode permet l'intégration d'électrodes nanométriques avec des circuits intégrés et l'électronique traditionnelle. Un signal 5X supérieur par rapport au soleil de fibres de carbone a été réalisé avec la zone d'électrode effective de taille 4 fois plus petite, ayant également l'une des plus hautes valeurs de sensibilité jamais rapportées pour les électrodes PPF.

Keywords

Biosensors, carbon fiber, thin film polymers, dopamine, serotonin, adenosine, carbon nanorods, 3D nanostructure, polymer pyrolysis

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Research group

This work has been done in IBM TJ Watson Research Center in Yorktown Heights (NY). The expertise of the research group where I carried out my work was related to bio nanosensors and neural probes, as well as electrochemical measurements.

Publications

This work has been published in two research journals:

- S. Demuru, H. Deligianni "Surface PEDOT:Nafion Coatings for Enhanced Dopamine, Serotonin and Adenosine Sensing", J. Electrochem. Soc. (2017) 164, 14, G129-G138, doi: 10.1149/2.1461714

- S. Demuru,L. Nela, N. Marchack, S. J. Holmes, D. Farmer,G. Tulevski, Q. Lin, H. Deligianni."Scalable Nanostructured Carbon Electrode Arrays for Enhanced Dopamine Detection" (2017). ACS Sens. (2018), doi: 10.1021/acssensors.8b00043

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Chapter 1

Introduction

Neurotransmitters are molecules that allow neurons to send signals to target cells to communicate, influencing behaviors like reward and motivational habits. [1, 2, 3]. Dopamine (DA) is a neurotransmitter of the central nervous system with a key role in Parkinson disease.[4] Since dopamine is electro-active, it can be easily oxidized at an electrode surface with electrochemical methods, for example using Fast Scan Cyclic Voltammetry (FSCV). [5] Other molecules such as serotonin (5-HT), norepinephrine and adenosine are electro-active neurotransmitters that can be measured electrochemically. [6, 7] The problem is that these molecules, except for adenosine that is oxidized at higher potentials, [7, 8] have all very similar oxidation and reduction potentials and it is difficult to distinguish them in vivo. [1] As a matter of fact, ascorbic acid(AA) and 3,4-dihydroxyphenylacetic acid (DOP AC) may completely mask dopamine detection in the brain. DOPAC is a metabolite of dopamine and ascorbic acid is an antioxidant, both present at high concentration in the brain and may interfere with dopamine. [9] Therefore, it is very important to develop electrode materials with sufficient sensitivity and selectivity for in-vivo detection of dopamine, serotonin and adenosine. Carbon has been shown to be one of the best materials to perform such measurements in vivo. [3, 10, 11] Previous studies have been done to increase the sensitivity and selectivity of bare carbon fiber electrodes. Conductive polymers show good results increasing the surface area of the electrode and enhancing the sensitivity. Among the various conductive polymers, poly(3,4-ethylenedioxythiophene) (PEDOT) is a very promising material for biomedical applications due to its biocompatibility and chronic in-vivo stability. [12, 13] Starting from the monomer 3,4-ethylenedioxythiophene (EDOT), through electro-polymerization, it is

possible to incorporate dopants in the polymer matrix such as graphene oxide (GO) [13], PSS [14], carbon nanotubes (CNTs) [15, 16] and Nafion. [17, 18] Nafion has been widely used due to its biocompatibility and small response to anionic metabolites such as AA and DOPAC, and its high selectivity for cationic species such as DA, adenosine and serotonin. [18, 19] One problem of the EDOT monomer is its limited solubility in water, so it was thought that adding a surfactant will improve its solubility. [20] Some attempts to incorporate surfactants, such as sodium dodecyl sulfate (SDS), in a polymer matrix have been reported earlier. [21] SDS has been added also in positively charged substrates as a self-assembled monolayer, showing interesting orientation of its anionic part to the subtrate. [22] These earlier studies examined the effect of SDS on the resulting properties of the polymer and did not specifically address the effect of different coatings on neurotransmitter measurements. An EDOT:Nafion solution in acetonitrile has been proposed earlier and optimized for deposition on a carbon fiber, resulting in a coating with good biocompatibility, sensitivity and selectivity for dopamine. [11] In this work, we demonstrate a novel EDOT:Nafion aqueous solution that provides improved thin film polymeric coatings for the carbon fibers. We have analyzed the influence of two different surfactants on the coatings, sodium dodecyl sulfate (SDS) and sodium dodecyl benzene sulfonate (SDBS), for the detection of neurotransmitters with fast scan cyclic voltammetry. The sensitivity and selectivity for dopamine and serotonin, against interferents such as ascorbic acid and DOPAC were evaluated. Furthermore, adenosine measurements with these improved coatings are reported for the first time.

In the second part of the work, glassy a carbon nanorods electrode has been tested for dopamine detection.
Glassy carbon (GC) is a very promising material for electrochemical measurements due to its good electrical properties and excellent mechanical and chemical stability. Moreover, GC can be obtained by the pyrolysis of photoresist films (PPF), allowing the use of conventional photolithography to pattern complex shapes.[23, 24] Small electrodes offer several advantages for in vivo measurements of neurotransmitters, including reduced tissue damage, enhanced sensitivity and potential for large-scale integrated microelectrode arrays.[18, 25] The electrochemical detection of dopamine and other positively charged neurotransmitters relies on their adsorption at carbon surfaces, and high surface area electrodes are expected to show higher sensitivity.[26] Nanostructures could drastically increase the active surface area, both minimizing tissue damage and enhancing the sensitiv-

ity of neurotransmitter detection. As previously mentioned, glassy carbon microfibers are the most commonly used electrodes for dopamine detection with fast scan cyclic voltammetry.[3, 9] While carbon fibers cannot be easily integrated for multichannel detection, integrated arrays with several electrode sites can be easily obtained with photoresist films (PPF). This would allow the detection of multiple neurotransmitters in different locations along with simultaneous electrophysiology measurements[27, 28], increasing our basic knowledge of the human brain and allowing for correlations with behavior. Previous works have reported the patterning of micrometer-sized pillars from pyrolyzed carbon.Another approach is to use oxygen plasma treatment of PPF to create random nanofibers and pores in the carbon film. [15] Carbon nanotubes grown on fiber microelectrodes have also been used for dopamine detection. [16] A single novel nanocarbon fiber electrode (CFNE) has been previously demonstrated to monitor individual exocytotic events within a synapse in real time. [6] Nanostructured electrodes thus offer immense potential to enrich this research field by enabling electrochemical measurements with great sensitivity and extremely high spatial and temporal resolution. [29] In this work, using advances in nanolithography we present for the first time a reproducible glassy carbon nano-structured microelectrode which can be used to detect low concentrations of dopamine with fast scan cyclic voltammetry (FSCV). The novel patterned PPF material has been characterized with scanning electron microscopy (SEM), X-ray photoelectron spectroscopy (XPS), Raman spectroscopy, resistivity and contact angle measurements and fast scan cyclic voltammetry.

Chapter 2

Fast scan cyclic voltammetry

Cyclic voltammetry (CV) is a very popular technique for electrochemical studies, obtaining information of complex reactions at the electrode-electrolyte interface. Though this technique, the electrochemical behavior of a system can be easily studied using steps at different potentials, while measuring the current produced. The potential is generally varied linearly with time using scan rates raging from 10 mV/s to 1000 V/s.[30] The advantages of using high scan rates are increased sensitivity and temporal resolution in the detection. In fact, Fast Scan Cyclic Voltammetry (FSCV) allows the detection of millisecond release of dopamine and other neurotransmitters with limit of detection in the nM range, being one of the most used technique in vivo. [1]
The use of micro and nano electrodes, in combination with fast scans, allows to obtain both high spatial and temporal resolution. [30] A typical voltammogram can be seen in **Figure 2.1**. The main problem of this technique is the large background current arising from the charge of the solution, making it difficult to distinguish the signal of interest from the background. This problem has been solved with the Analog Background Subtraction (ABS) feature. A typical example before and after ABS can be seen on **Figure 2.2**. The signal before the subtraction is due to the charging of the buffer solution at the electrode interface. After the subtraction, the signal is almost equal to zero, so that we can easily see any small variation of current due to the redox of the desired analyte.

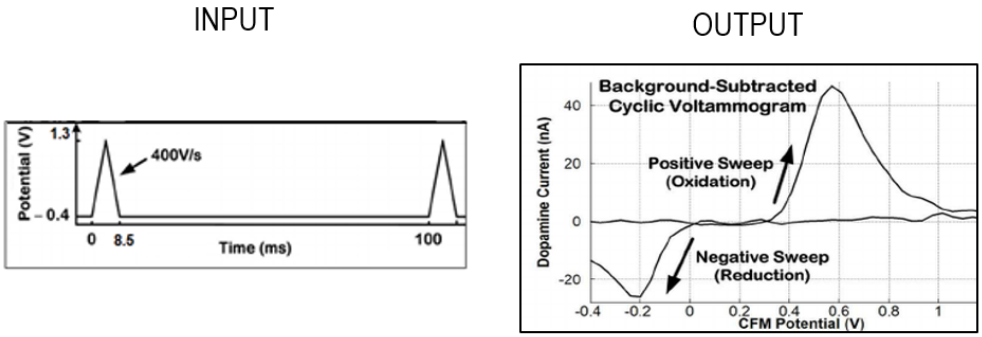

Figure 2.1: *Triangular waveforms in fast scan cyclic voltammetry on the left (input). A background subtracted voltammogram for dopamine detection on the right (output). (Image from: B. Bozorgzadeh, D. P. Covey, C. D. Howard, P. A. Garris, and P. Mohseni, IEEE Journal of Solid State Circuits 2014, 49, 4)*

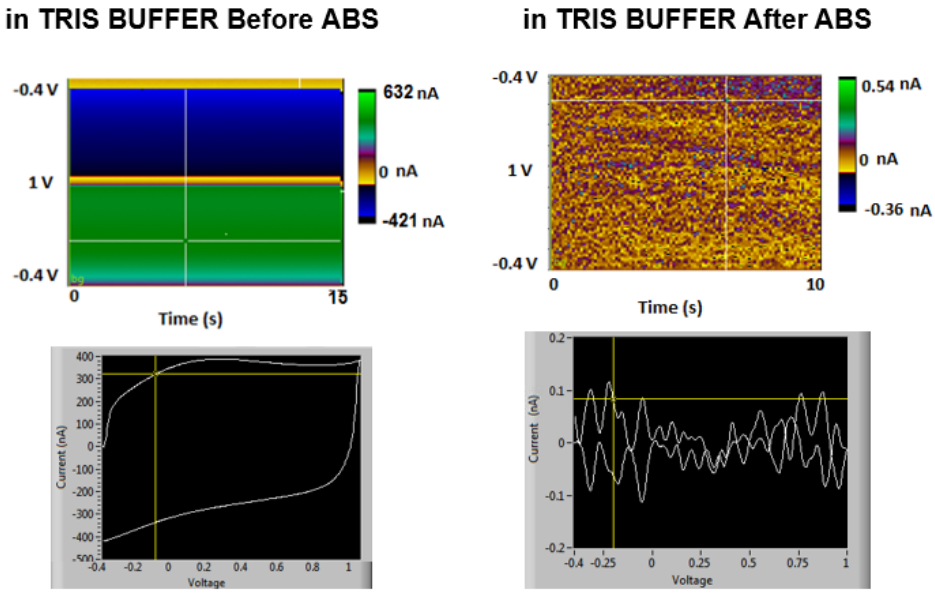

Figure 2.2: *Color plot and Current vs Voltage before (on the left) and after (on the right) analog background subtraction. The color plot represent also the signal variation in time. As can be seen on the left, the background equal to hundreds of nA, is then reduced close to zero in order to make a clear detection of small current variations due to the oxidation and the reduction of the analyte of interest.*

Chapter 3

Experimental method

3.1 Flow injection system

The apparatus consists of dual syringe pumps by New Era PumpSystem Inc. (Farmingdale, NY) that supply a continuous TRIS buffer flow at 1 mL/min towards the carbon electrode and the reference electrode, placed in the flow cell.
This buffer is an ionic solution very similar to the in vivo cerebral-spinal environment (see chemical composition below in the paragraph 3.3).
A bolus of the analyte (dopamine or other electro-active compounds) at the desired concentration is injected through a six-port switching valve by INDEX health and science (Bristol, CT). All experiments were performed in a grounded Faraday Cage (CH Instruments, Austin, TX) to reduce noise. A schematic of the setup is presented in **Figure 3.1**.

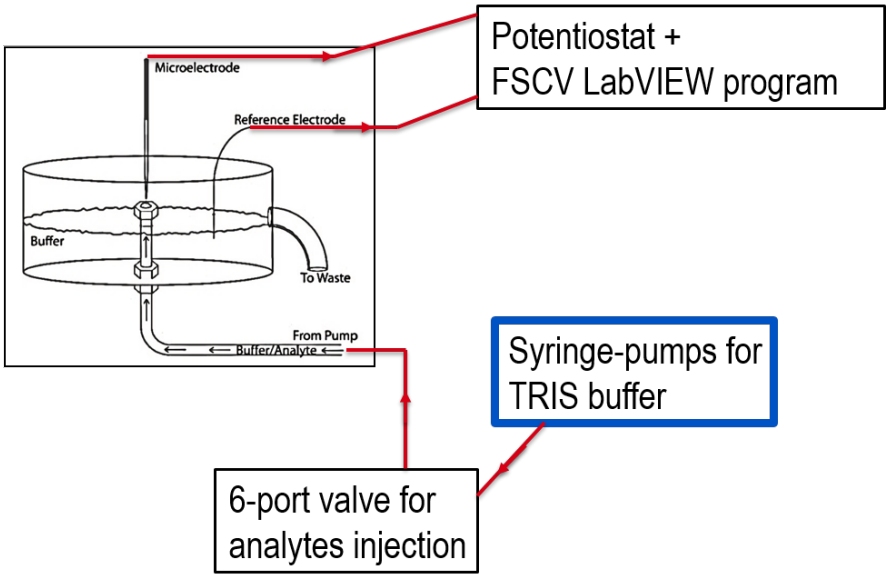

Figure 3.1: *Scheme of the setup for the electrochemical experiments. (Flow cell image from: M. Strand and B. J. Venton, "Flame Etching Enhances the Sensitivity of Carbon-Fiber Microelectrodes," Anal. Chem. 2008, 80, 3708–3715)*

3.2 Data acquisition system

The fast-scan data acquisition system used was developed at the University of North Carolina, at Chapel Hill. The scanrate used for the measurements is in the range of 100-400 V/s. For the detection of dopamine, ascorbic acid, DOPAC and serotonin, carbon fiber electrodes were scanned from -0.4 to 1.3 V (vs Ag/AgCl) with a scan rate of 400 V/s and a repetition rate of 10 Hz for the triangular waveforms.

Then, for the detection of dopamine with carbon nanorods electrodes, cyclic voltammograms were scanned from -0.4 to 1.3 V (vs Ag/AgCl) with a scan rate of 100 V/s and a repetition rate of 10 Hz. A sampling rate of 100,000 Hz has been used. The gain of the amplifier is 200 nA/V. For the detection of adenosine with carbon fibers, a triangular waveform from -0.4 to 1.5 V (vs Ag/AgCl) was applied, due to the higher potential needed for adenosine oxidation. [31] Voltammograms were filtered with a low-pass 4th order Bessel filter at low cutoff frequency of 2,000 Hz and high cutoff frequency of 100,000 Hz. An average of 5 voltammograms were acquired and subtracted by the program to obtain the background-subtracted cyclic voltammograms. Statistical analysis was performed using Microsoft Excel. The values reported are average values $\pm$ standard deviations. All signals

were collected after allowing the carbon fiber background to stabilize for at least 15 minutes at 60 Hz or 30 minutes at 10 Hz prior to experiments, before and after electro-polymerization.

3.3 Chemicals

All chemicals were purchased from Sigma-Aldrich (St. Luis, MO) unless otherwise stated. Dopamine hydrochloride, serotonin, adenosine, L-ascorbic acid, 3,4-Dihydroxyphenyl-acetic acid (DOPAC) stock solutions were prepared daily in water and diluted with Tris buffer at the desired concentration for testing. The Tris buffer solution (1 L) consists of 3.25 mM KCl, 1.2 mM $MgCl_2x6H_2O$, 2.0 mM Na_2SO_4, 1.25 mM $NaH_2PO_4x2H_2O$, 140 mM NaCl, 15 mM Trizma HCl, 1.2 mM $CaCl_2x2H_2O$, adjusted to pH 7.4 daily with the addition of hydrochloric acid and sodium hydroxide. Potassium Chloride was purchased from J. T. Baker Inc. (Phillipsburg, NY) and Na_2SO_4 from Alfa Catalog Chemicals (Danvers, MA). The electrodeposition solution was prepared by dissolving 200 or 400 μM (0.007 g or 0.014 g of EDOT in 200 mL water) 97% pure solid 3,4-ethylenedioxythiophene (EDOT) (molecular weight 174.31 g/mol) in deionized water. 1% volume Nafion perfuorinated resin (5 wt %) solution was added to the aqueous EDOT solution (2 mL Nafion solution in 200 mL water). Also, 70mM (4 g of SDS and 4.8 g of SDBS) of Sodium Dodecyl Sulfate (SDS) or Sodium Dodecyl Benzene Sulfonate (SDBS), were added to the solution.

A scheme of all the chemicals used for the first part of the work with carbon fibers can be seen in **Figure 3.2**.

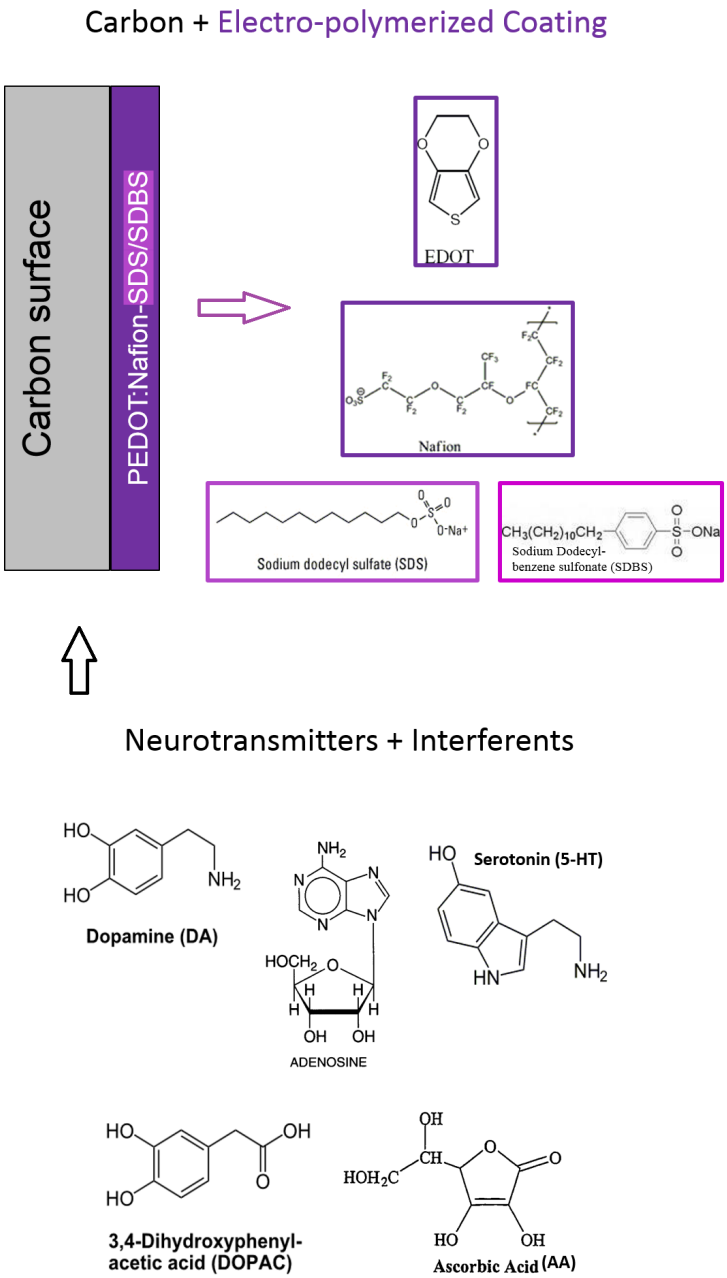

Figure 3.2: *Summary of all the chemicals used during the experiment of electropolymerization and electrochemical characterization with carbon fiber microelectrodes.*

3.4 Electro-polymerization

Electrodeposition was carried out in an aqueous solution of EDOT and Nafion. Carbon fibers 7 μm x 100 μm (World Precision Instruments, Sarasota, FL) were used to test the different coatings containing PEDOT:Nafion with and without the addition of SDS or SDBS. The EDOT:Nafion solution had a pH of 3.3. When SDS was added, the pH of the solution was raised to 3.5. In contrast, the EDOT:Nafion-SDBS solution had initially a neutral pH of 7, which was adjusted to pH 3.5 with the addition of hydrochloric acid. The pH of the electrodeposition solution was adjusted to acidic pH because the protonation of conductive polymers is thought to be enhanced in acidic media. [17] Solutions were stirred overnight at room temperature prior to deposition. Electro-polymerization was performed in a three-electrode set-up using a carbon fiber working electrode, a Ag/AgCl reference electrode (BASi Inc.West Lafayette, Inc.) and a platinum mesh as a counter electrode. The potential for electro-polymerization was controlled using a CH Instruments potentiostat (Austin, TX). Cyclic Voltammetry with scans from 1.5 V to -0.8V and back, at 100 mV/s for 15 cycles were performed for electro-polymerization.

Chapter 4

Carbon fiber micro-electrodes

4.1 Bare carbon fibers

The current response of bare carbon microelectrodes with different dopamine concentrations was first analyzed. The calibration has been performed with FSCV at 400 V/s averaging the values for 5-7 fibers. The dopamine concentrations prepared were 0.25 - 0.5- 1μM (low concentrations) and 50 - 100 μ M (high concentrations). The dopamine oxidation current peak (Ipa), the ratio between the oxidation and the reduction dopamine current peak (Ipa/Ipc) and the voltage distance between the oxidation and the reduction peaks (ΔEp) are presented in 4.1.

The bare fibers response has been compared with values reported in the literature (Table 4.2). The signal considered for the comparison is the oxidation current of dopamine. Also, the carbon fiber dimensions are considered to calculate the current density. In this work, we have an oxidation current

[DA](μM)	n	Ipa(nA)	Ipa/Ipc	ΔEp (V)
0.25	7	4.7$\pm$2	1.8$\pm$0.3	0.8
0.5	7	6$\pm$3	2$\pm$0.3	0.8
1	7	10$\pm$4	2.2$\pm$0.6	0.8
50	5	265$\pm$128	2.2$\pm$0.2	0.9
100	5	304$\pm$148	2.2$\pm$0.2	0.9

Table 4.1: *Calibration values for different dopamine concentrations on bare carbon electrodes.*

Electrode	Signal (nA/μM)	Current density (pA/μm^2)
CF 7 x 100 [15]	6	2.7
CF 7 x 75 [11]	13 ± 2	7.7 ± 1.2
CF 7 x 100(this work)	7 ± 3	3.1 ± 1.3

Table 4.2: *Dopamine measurements on bare carbon fibers.*

signal for the bare carbon electrode of 7 ± 3 nA in 1 μM dopamine, corresponding to a current density of 3.1 ± 1.3 pA/ μm^2. The area of the carbon fibers has been calculated treating the fibers as cylinders with surface area equal to, $2\ \pi$ rl $+\ \pi$ r^2, where r is the radius and l is the length of the carbon fiber. [18] For all the cases triangular scans used were from -0.4 V to 1.3 V.

4.2 Coated carbon fibers

The previously characterized bare carbon fibers were used with different thin film polymeric coatings. The carbon surface has been coated with poly(3,4- ethylenedioxythiophene) (PEDOT) in the presence of Nafion with and without the first surfactant sodium dodecyl sulfate (SDS) or the second surfactant sodium dodecyl benzene sulfonate (SDBS). The different coatings have been characterized with fast scan cyclic voltammetry for 5 different electro-active compounds: dopamine, serotonin, adenosine, ascorbic acid and DOPAC. The polymerization responses obtained with 15 cycles at 100 mV/s in the 3-electropolymerization aqueous solutions prepared are presented in **Figure 4.1**.

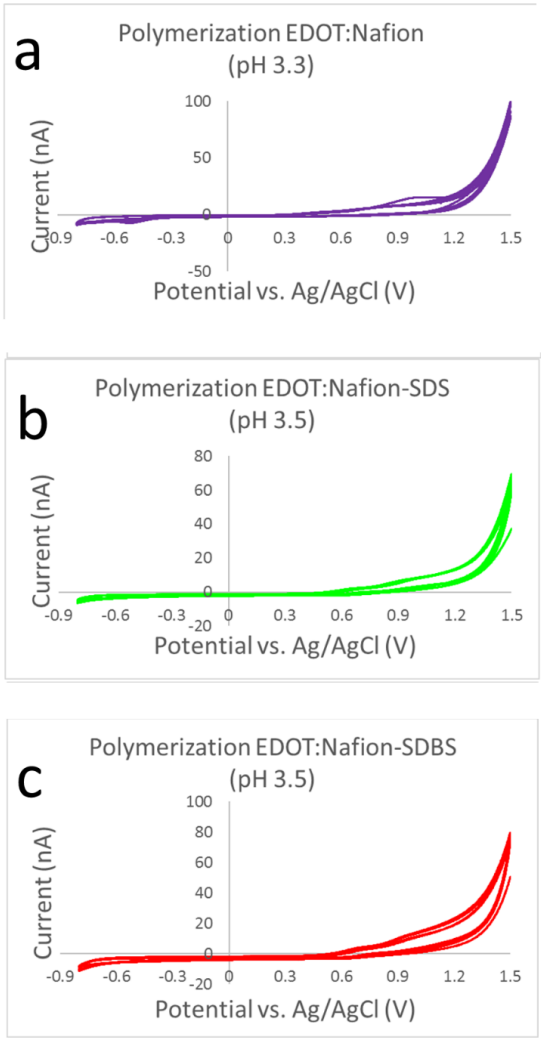

Figure 4.1: *Polymerization curves in aqueous solution containing 200 μM EDOT:Nafion for PEDOT:Nafion (a), PEDOT:Nafion-SDS (b), PEDOT:Nafion-SDBS (c) respectively.*

Figure 4.1a shows the polymerization response of the aqueous solution containing 200 μM EDOT and Nafion at pH 3.3, **Figure 4.1**b the response of the aqueous solution containing 200 μM EDOT, Nafion and sodium dodecyl sulfate (SDS) at pH 3.5, Figure **Figure 4.1**c the response for 200 μM EDOT, Nafion and sodium dodecyl benzene sulfonate (SDBS) adjusted to pH 3.5. In the anodic limit, the current produced, is from the oxidation of EDOT. We observed an increase in capacitance on the cyclic voltammetric curves with the addition of SDS and or SDBS in the EDOT:Nafion solution. The surfactants increase the solution conductivity and decrease the surface tension at the electrolyte electrode interface. Since EDOT is posi-

tively charged, Nafion / Nafion-SDS / Nafion-SDBS are the counter ions necessary for the EDOT polymerization. [11] When scanning the potential toward the anodic values (+1.5 V vs Ag/AgCl) for electro-polymerization, the surfactants and the Nafion molecules become electronegative. [19] In this way, the surfactant and the Nafion ions can be embedded in the positively charged polymer matrix, creating the composite PEDOT film. The electro-polymerization response is similar at higher EDOT concentration (**Figure 4.2**).

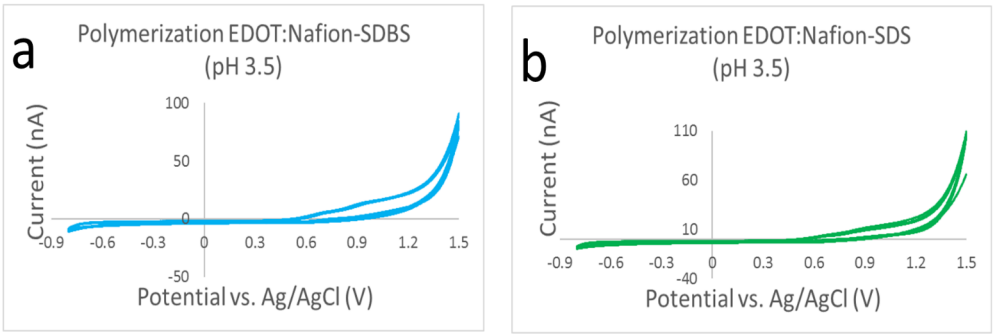

Figure 4.2: *Polymerization curves in water solution for PEDOT:Nafion-SDBS (a) and PEDOT:Nafion-SDS (b) both with 400 µM EDOT.*

In (**Figure 4.3**), the SEM images for the bare carbon surface (**Figure 4.3**a), the coated carbon surfaces with PEDOT:Nafion (**Figure 4.3**b), PEDOT: Nafion-SDS (**Figure 4.3**c), PEDOT:Nafion-SDBS (**Figure 4.3**d) are shown. The coatings shown have been obtained from a 200 μM EDOT solution.

It can be noticed that for PEDOT:Nafion the polymeric coating is nonuniform, with polymeric clusters on the surface. For the PEDOT:Nafion-SDBS we can clearly see a non-homogeneous film with particles incorporated in the polymer or absorbed on the surface. On the other hand, the PEDOT: Nafion-SDS coating seems very uniform, without the typical striations of the bare carbon surface. The characterization for these different coatings has been performed with fast scan cyclic voltammetry (FSCV) for dopamine (DA) detection and, also, for two interferents: 200 μM ascorbic acid (AA) and 10 μM dihydroxyphenyl acetic acid (DOPAC). The response for dopamine, ascorbic acid and DOPAC is presented before and after the thin film coating for each case. The experiments are repeated 2 times for

each coating (6 coated electrodes) with 200 μM EDOT. In addition, detection of serotonin and adenosine were also performed. A higher concentration of EDOT equal to 400 μM has been tested with the 2 surfactants, resulting in testing of 8 different coated carbon fiber electrodes.

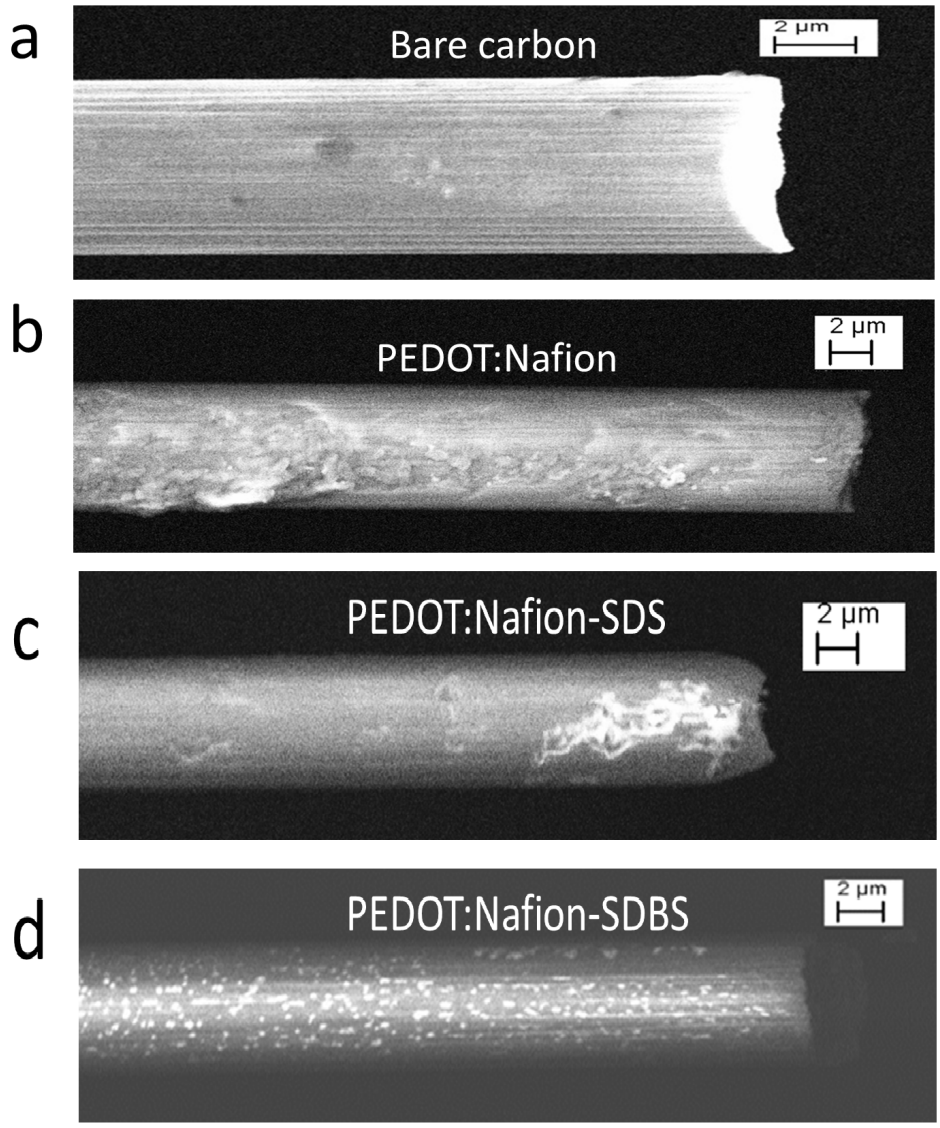

Figure 4.3: *SEM images carbon fiber 7 µm x 100 µm for Bare carbon (a), PEDOT:Nafion (b), PEDOT:Nafion-SDS (c), PEDOT:Nafion-SDBS (d). The PEDOT:Nafion coatings were deposited from a solution containing 200 µM EDOT.*

4.2.1 Fast Scan Cyclic Voltammetry at PEDOT:Nafion carbon fibers

The current response for dopamine, ascorbic acid and DOPAC without any surfactant as dopant was then analyzed. The dopamine oxidation current in **Figure 4.4**a, increases from 10 $\pm 4nA$(1μM dopamine, n=7 electrodes) for bare carbon to 32 $\pm$ 12 nA (1μM dopamine, n=2 electrodes) for PEDOT:Nafion with 200 μM EDOT in the solution, and it is comparable with the result obtained previously with PEDOT:Nafion deposited from an acetonitrile solution containing 200 μM EDOT. The reported signal from the PEDOT:Nafion in acetonitrile was 26 $\pm$ 6 nA/μM. In our case and considering the dopamine calibration curve (**Figure 4.10**a), we calculated a sensitivity of 23.6$\pm$4 nA/μM.
Moreover, the dopamine reaction with PEDOT:Nafion coating is very irreversible, as can be seen by the absence of the reduction peak in **Figure 4.4**a. The serotonin and adenosine signals in **Figure 4.4**b,c will be discussed later. The Ipa/Ipc current ratio for this coated fiber has been calculated to be equal to 12 $\pm$ 7 for 1 μM dopamine, meaning that the reduction peak is very little compared to the oxidation peak. For bare carbon, we had a current ratio of 2.2 $\pm$ 0.6 for 1 μM DA, as can be seen in Table 4.1.

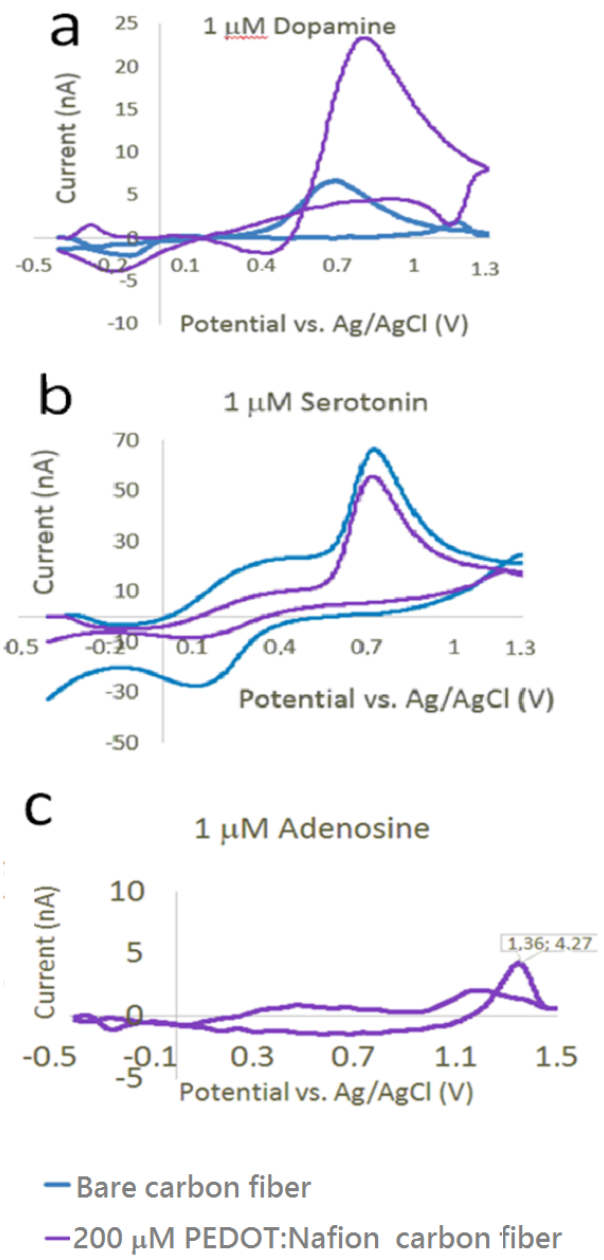

Figure 4.4: *Responses for 1 µM dopamine (a), 1 µM serotonin (b) and 1 µM adenosine with 200 µM EDOT with Nafion and without surfactants.*

The energy dispersive x-ray spectroscopy (EDX) in **Figure 4.5**b shows

evidence of Nafion incorporation on the PEDOT polymer matrix, compared to **Figure 4.5**a of bare carbon without the peak. As it can be seen in **Figure 4.7**a, the oxidation current for 200 μM ascorbic acid (AA) decreases 3 times with the coating PEDOT:Nafion (18 nA for a coated PEDOT:Nafion fiber compared to 58 $\pm$ 1.5 nA for a bare fiber), while the 10 μM DOPAC has almost the same signal (11 nA for a coated fiber and 10 $\pm$ 0.4 nA for a bare fiber). From the above we conclude that the Nafion incorporation in an aqueous solution is less effective to reduce all the anionic components than the PEDOT:Nafion coating obtained by electro-polymerization in acetonitrile.

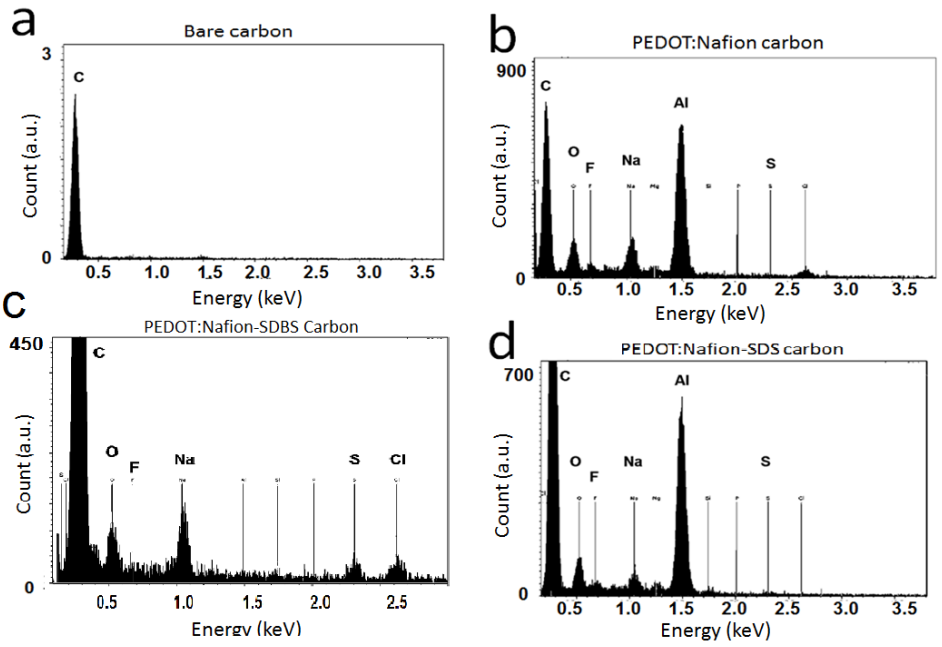

Figure 4.5: *EDX spectroscopy analysis for bare carbon (a), PEDOT:Nafion carbon (b), PEDOT:Nafion-SDBS carbon (c), PEDOT:Nafion-SDS carbon (d).*

4.2.2 Fast Scan Cyclic Voltammetry at PEDOT:Nafion-SDBS carbon fibers

The addition of SDBS in the solution strongly enhanced the dopamine, ascorbic acid and DOPAC signals. The dopamine oxidation current in **Figure 4.6**b increases from 10 $\pm$ 4 nA (1μM dopamine, n=7 electrodes) to 49

$\pm$ 17 nA (1μM dopamine, n=2 electrodes). An increase of the background current can be also seen in **Figure 4.6**a. The Ipa/Ipc ratio for the dopamine signal is equal to 2.4 $\pm$ 0.6. **Figure 4.5** shows the EDX spectrum of the PEDOT:Nafion-SDBS coated fiber, which shows that there is no fluorine incorporation in the polymeric matrix. As expected, in **Figure 4.8**b the ascorbic acid current is not decreased with the PEDOT:Nafion-SDBS coating (56 nA with the coating, while is was 58 $\pm$ 1.5 nA for bare carbon) and that DOPAC is increased by a factor of 3 with respect to bare carbon (30 nA from 10 $\pm$ 0.4 nA for a bare carbon fiber). From these data we hypothesize that the role of SDBS in the polymer is to suppress the Nafion incorporation and to enhance the incorporation of sulfonate groups. The EDS spectrum of the polymeric coating confirms this hypothesis.

Figure 4.5c shows the EDX spectrum of a thin film polymeric coating containing 400 μM EDOT, Nafion and SDBS. In this case, there is no fluorine incorporation in the PEDOT matrix. A sulfur peak is evident, which is indicative of the abundance of sulfonate groups in the polymeric matrix. In this case, a high dopamine signal was obtained, equal to 86nA for 1 μM dopamine concentration (Figure 3.8a). The sensitivity of the probe is 34.4$\pm$14 nA/μM (**Figure 4.10a**). The ascorbic acid signal is almost the same as for the bare carbon, while DOPAC increased up to 71 nA from 10 $\pm$ 0.4 nA (**Figure 4.7**a,b). This strong increase for DOPAC is indicative of the lower selectivity with this coating due to the absence of Nafion. Nafion membranes are strongly dependent on the nature of the solvent in which these are dissolved. [32, 17] With this coating there is a a 5-9 times improvement of the dopamine signal, with SDBS increasing the sulfonate groups in the polymer and the absorption of dopamine on the surface.

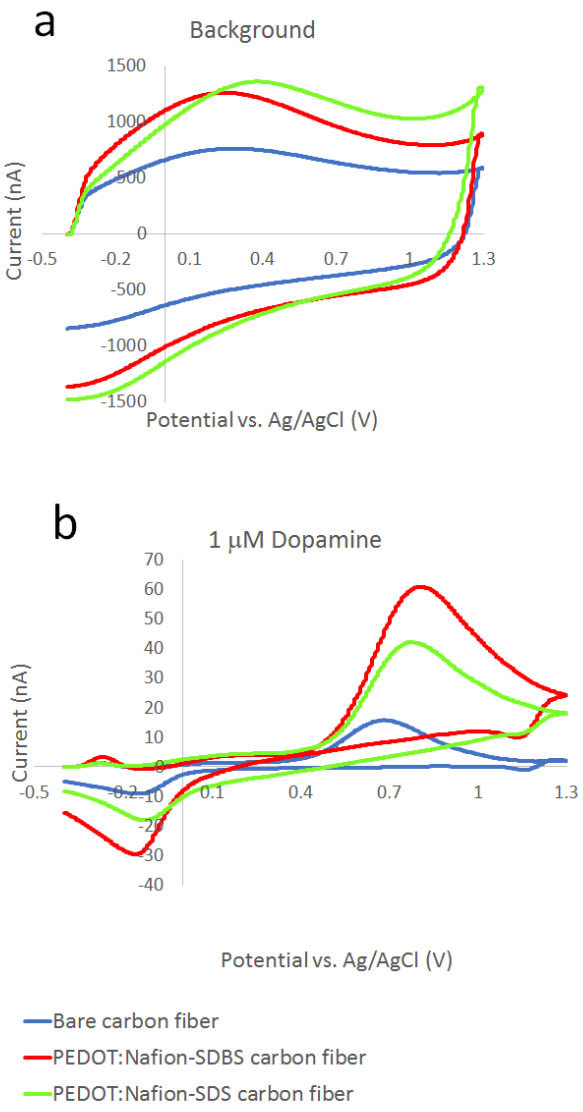

Figure 4.6: *Response of the coated carbon fiber electrodes with PEDOT:Nafion-SDS and PEDOT:Nafion-SDBS in comparison to bare carbon electrodes for the background signal (a) and for Dopamine 1 µM (b) in pH 7.4 TRIS buffer. The PEDOT:Nafion-SDS/SDBS coatings were deposited from a solution containing 200 µM EDOT.*

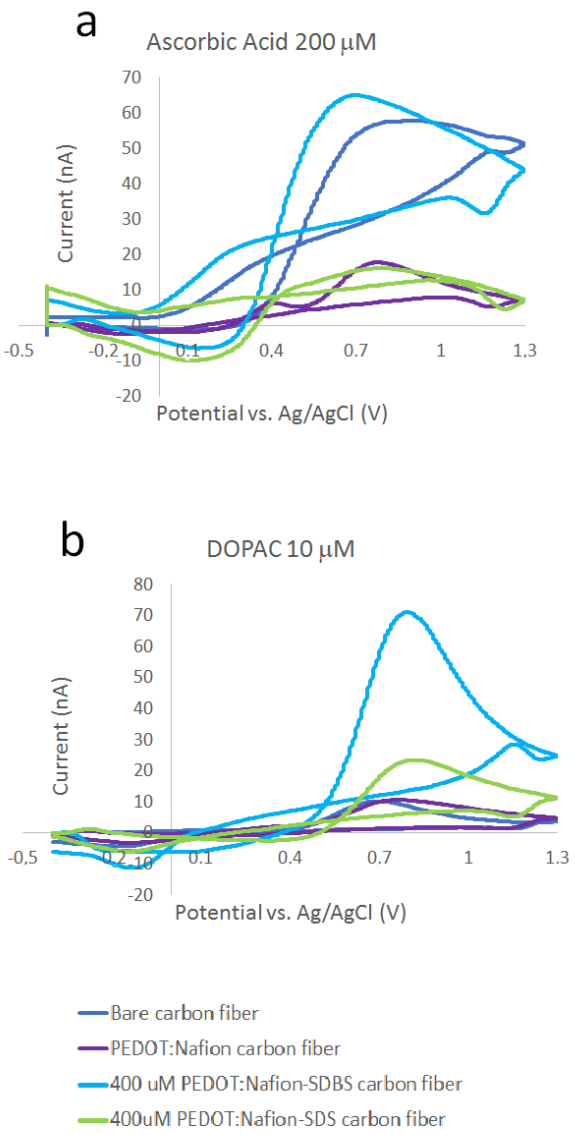

Figure 4.7: *Response of the coated carbon fiber electrodes in comparison of a bare carbon electrode for Ascorbic Acid 200 µM and DOPAC 10 µM in pH 7.4 TRIS buffer. All the coatings are with 400 µM EDOT. The surfactant used is SDBS (a) and SDS (b).*

4.2.3 Fast Scan Cyclic Voltammetry at PEDOT:Nafion-SDS carbon fibers

SDS added as counter ion strongly influences the behavior of dopamine oxidation, of ascorbic acid and of DOPAC. The presence of Nafion is demon-

strated by a decrease of ascorbic acid current by a factor of 3 shown in **Figure 4.8**a. **Figure 4.5**d clearly demonstrates the addition of Nafion in the polymeric matrix, with the appearance of fluorine peak in the EDX spectra. The signal for ascorbic acid is decreased to 17.7 nA compared to a bare carbon signal of 58 $\pm$ 1.5 nA (**Figure 8a**). In contrast, with PEDOT:Nafion-SDS we have that DOPAC signal is increased to 19 nA from 10 $\pm$ 0.4 nA. Dopamine oxidation current increases from 10 $\pm$ 4 nA (1μM dopamine, n=7 electrodes) to 39.6 $\pm$ 4 nA (1μM dopamine, n=2 electrodes) (**Figure 4.6**b). The sensitivity of the probe is 23.7$\pm$1 nA/μM (**Figure 4.10**a). In this case, due to the more homogeneous coating obtained with SDS, we have a calibration signal with a smaller error compared to the PEDOT:Nafion coating and the PEDOT:Nafion-SDBS coating. It can be noticed that SDS ions are interacting with DOPAC, since we have an increase of signal for this interferent.

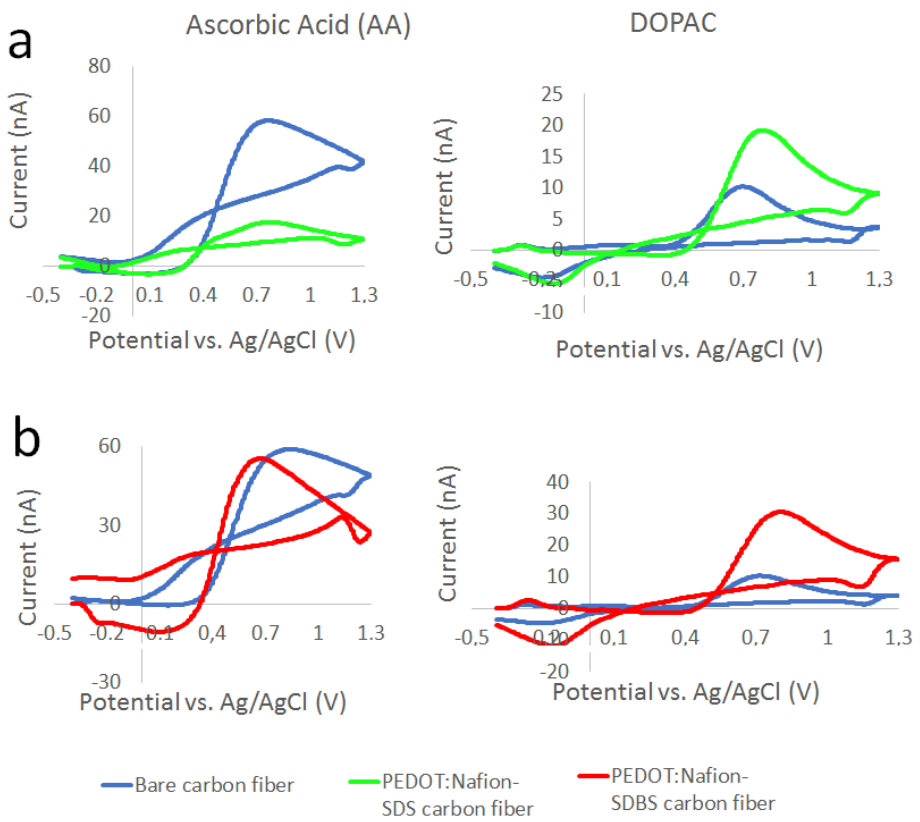

Figure 4.8: *Response of the coated carbon fiber electrodes in comparison to bare carbon electrodes for Ascorbic Acid 200 µM and DOPAC 10 µM in pH 7.4 TRIS buffer. All the coatings were deposited in a solution containing 200 µM EDOT.*

The DOPAC signal is increasing by a factor of 2 (**Figure 8a**), while for dopamine is increasing by a factor of 4 from the bare carbon. The oxidation response for high concentration of DOPAC (10 μM) is smaller than the oxidation response for low dopamine, as a result we can obtain selectivity. Another important change with SDS is that the Ipa/Ipc is now equal to 2.7 $\pm$ 0.5, meaning that the reaction is more reversible than with PEDOT:Nafion. In addition, we analyzed the signal response at 400 μM EDOT, Nafion and SDS. Dopamine oxidation current is equal to 66 nA at 1 μM DA, compared to 40 nA with the lower EDOT concentration (**Figure 4.9**a). Also for higher concentration of EDOT in the solution, ascorbic acid signal was decreased 3 times (**Figure 7a**). On the other hand, DOPAC increased up to 23 nA from 10 $\pm$ 0.4 nA, slightly higher increase than with low EDOT concentration (**Figure 7b**). A summary with 1 μM Dopamine and all the different coat-

ings that have been tested can be seen in **Figure 4.9**a.

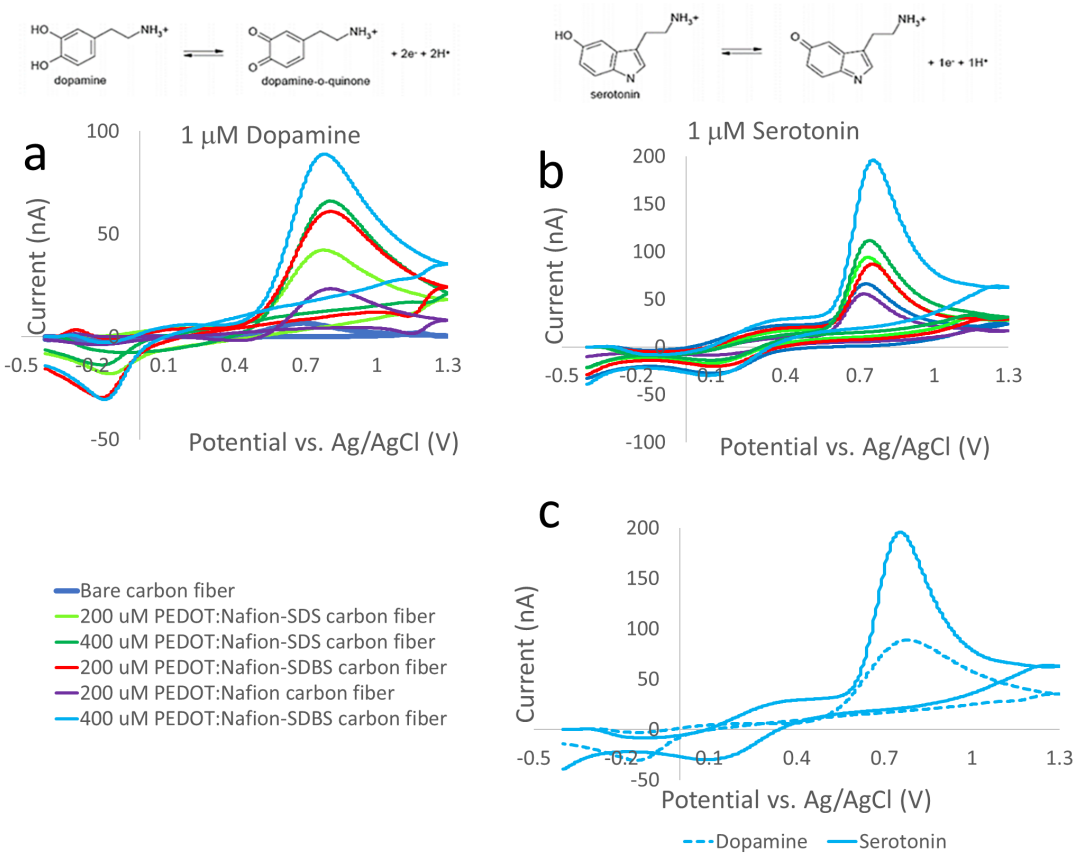

Figure 4.9: *Dopamine signal (a) and serotonin signal (b) for 1 µM with the different coatings. Signal at 400 V/s in pH 7.4 TRIS buffer. The bare carbon fiber signal is shown in blue. PEDOT:Nafion without surfactant from a 200 µM EDOT containing solution, PEDOT:Nafion-SDS from 200 µM EDOT in purple, PEDOT:Nafion-SDS from a 400 µM EDOT in dark green. Also, PEDOT:Nafion-SDBS with 200 µM EDOT in red and PEDOT:Nafion-SDBS with 400 µM EDOT in light blue. The dopamine and serotonin signals can be deconvoluted through the reduction potential for the two neurotransmitters (c). This is the case for all the coatings.*

4.2.4 Langmuir model of dopamine and dopamine-o-quinone adsorption with Nafion and with different surfactants

The Langmuir isotherm has been used to model the adsorption and desorption of dopamine on the carbon surface. [16] The desorption of dopamine is considered studying the behavior of dopamine-o-quinone. In this way we can further analyze the function of SDS and SDBS in the coating. In this model, it is considered that there is a limited amount of adsorption sites in the carbon surface. The Equation 1 describe the process:

$$\frac{\Gamma_{DA-DOQ}}{\Gamma_s} = \frac{\beta_{DA-DOQ}\ a^b_{DA-DOQ}}{1+\beta_{DA-DOQ}\ a^b_{DA-DOQ}} \tag{1}$$

Γ_{DA-DOQ} is the amount of adsorbed dopamine or dopamine-o-quinone. Γ_s is the saturated amount of dopamine or dopamine-o-quinone on the surface, meaning that it has been reached the maximum number of occupied sites on the carbon surface. β_{DA-DOQ} is the thermodynamic equilibrium constant (unitless) corresponding to dopamine or dopamine-o-quinone adsorption. a^b_{DA-DOQ} is the activity coefficient in bulk solution at equilibrium.

This last value is related to the molar concentration (C_{DA}) with the Equation 2:

$$a_{DA-DOQ} = \left(\gamma_{DA-DOQ} \cdot C_{DA}\right) \cdot \left(1L \cdot mol^{-1}\right) \tag{2}$$

where a_{DA-DOQ} is the activity coefficient for dopamine or dopamine-o-quinone in bulk solution at the adsorption equilibrium, and this coefficient is equal to 0.63 at room temperature.

The value for β_{DA} is extracted from the oxidation current for dopamine, and the value for $\beta_{DOQ}$from the reduction current. All the coefficients are reported in Table 4.3.

Electrode	β_{DA}x10^3	β_{DOQ}x10^3	β_{DA}/β_{DOQ}
CF Bare	54±20	54 ± 30	1
CF 200M PEDOT:Nafion	48 ± 18	6 ± 1.7	8
CF 200M PEDOT:Nafion-SDS	77 ± 7	66 ± 11	1.2
CF 200M PEDOT:Nafion-SDBS	87 ± 30	71 ± 38	1.2

Table 4.3: *Dopamine adsorption coefficients β_{DA} and dopamine-o-quinone adsorption coefficients (β_{DOQ}) for bare carbon and all the coating conditions. The ratio between the two coefficients is an indication of the reversibility of the electrochemical reactions. All the coatings were from a solution containing 200 µM EDOT.*

The saturated values are obtained from the plateau of the fitting curve at very high dopamine concentrations, considered equal to 100 μM in this work (see **Figure 4.10**b,c.). The values for very low concentration of dopamine (250 nM - 500 nM - 1 μM) can be better seen in the **Figure 4.10**a. The linearity is very good for the bare carbon and for the PEDOT:Nafion-SDS (R^2=0.999 and R^2=1 respectively). The variability in the other cases is due to a less uniform coating, that arbitrarily changes the absorption of dopamine on the surface (R^2=0.9824 for PEDOT:Nafion and R^2=0.9723 for PEDOT: Nafion-SDBS). For the calculation of the limit of the detection, we consider a signal to noise ratio equal to 3 and a noise level up to 100±20 pA (no analyte in the system). From the calibration curves we have a slope of 34.357 nA/μM with SDBS, 23.686 nA/μM with SDS, 23.591 nA/μM without surfactants and 7.1482 nA/μM for bare carbon (**Figure 4.10**a). The limit of the detection is: 41±6 nM for bare carbon, 13±3 nM for PEDOT:Nafion, 12±2.4 nM with SDS and finally 9±1.8 nM with SDBS. From Table 3.3 it can be seen that the DOQ absorption for PEDOT:Nafion without surfactants is very little, meaning that the reaction is strongly irreversible. Moreover, we can reasonably confirm that with SDS the dopamine adsorption is increased (from 54 x 10^3 to 77 x 10^3) with a small error. This is the reason of the higher signal for dopamine. Nevertheless, the process is more irreversible than the one on bare carbon (β_{DA}/β_{DOQ} >1). It seems that with SDBS and SDS as surfactant absorption of DA and DOQ are very similar. From a first glance, it appears that the adsorption coefficients of dopamine and dopamine-o-quinone are quite similar, both coatings favor the adsorption of dopamine versus dopamine-o-quinone and that the coefficient are quite similar in value. However, the calculated error of the coefficient with SDBS coating is quite high and this suggests that the coating is more non-uniform than the coating with SDS. This is evident from the SEM in Figure 4.3 and most

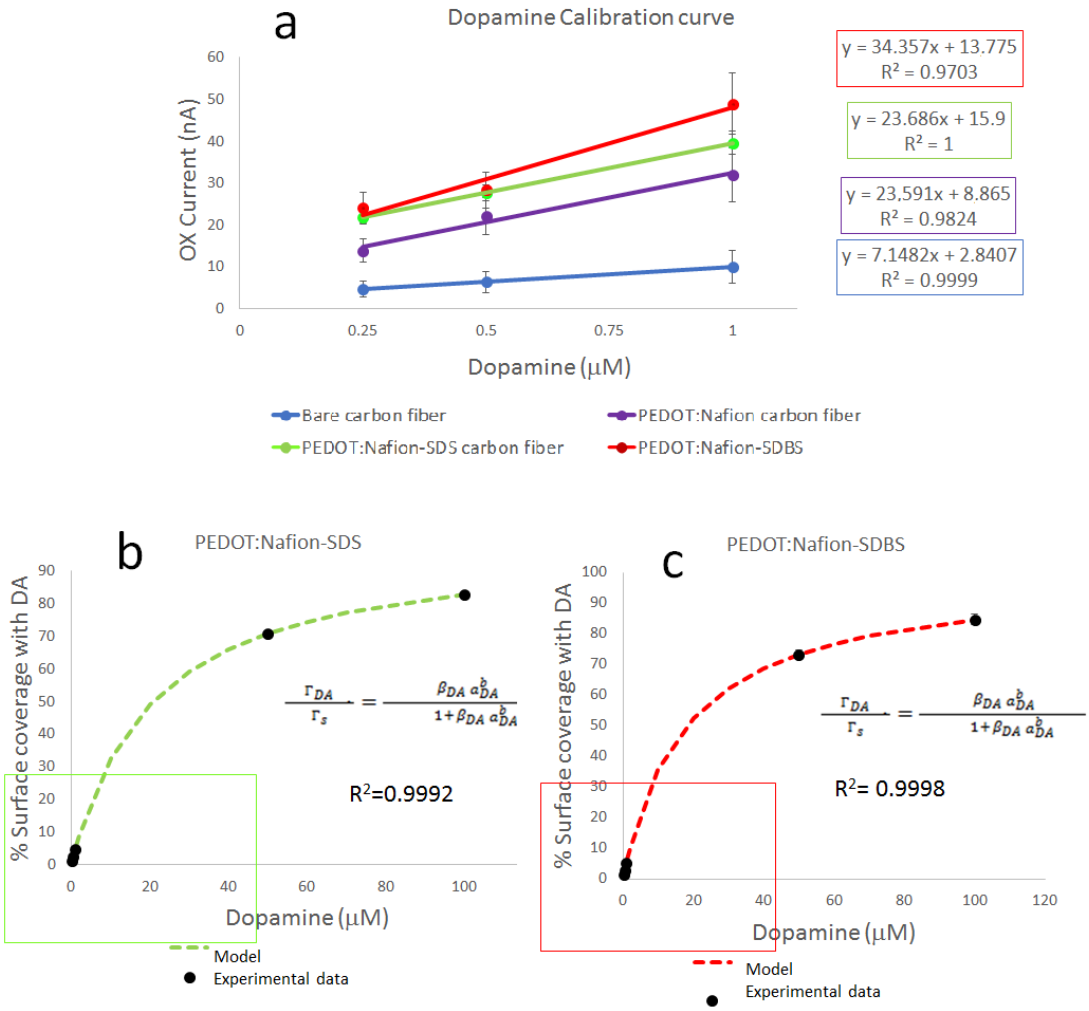

Figure 4.10: *Linear response for the carbon fiber electrodes for low DA concentrations (0.25 – 1 µM) (a). Response for the carbon fiber electrodes for different concentrations (0.25 – 0.5 - 1 - 50 – 100 µM) PEDOT:Nafion-SDS (b), PEDOT:Nafion-SDBS (c) carbon fibers fitted with the Langmuir model for dopamine adsorption. All the coatings are with 200 µM EDOT. Scan rate 400 V/s. Tested in TRIS buffer pH 7.4.*

4.2.5 Serotonin and Adenosine detection

With all the different coatings with SDS and SDBS, it is possible to detect serotonin as shown in Figure 4.9b and adenosine in Figure 4.11. The analytes without surfactants are shown in Figure 4.4. Serotonin is increased up to 4X with the coatings, arriving always to an oxidation signal higher

than dopamine. Nevertheless, it is possible to distinguish dopamine versus serotonin from the difference in the reduction potential as shown in **Figure 4.9**c. Adenosine is a molecule that undergoes three different oxidation stages. The first two oxidations are irreversible while the third reaction is reversible (see reactions in **Figure 4.11**). It is possible to detect with fast scan cyclic voltammetry the first 2 irreversible oxidations, one close to +1.4 V vs. Ag/AgCl and the second one around +1 V vs. Ag/AgCl. [31] The signal of this neurotransmitter is the highest with 400 μM PEDOT:Nafion-SDBS (up to 3X for the primary oxidation). In particular, previous studies have been reported in order to decrease the oxidation potential for this analyte. [32] In this work we have a primary oxidation at +1.3 V for 400 μM PEDOT:Nafion-SDS and at +1.33 V with 400 μM PEDOT:Nafion-SDBS (**Figure 4.11**) , while it is close to 1.4 V for all the other cases.

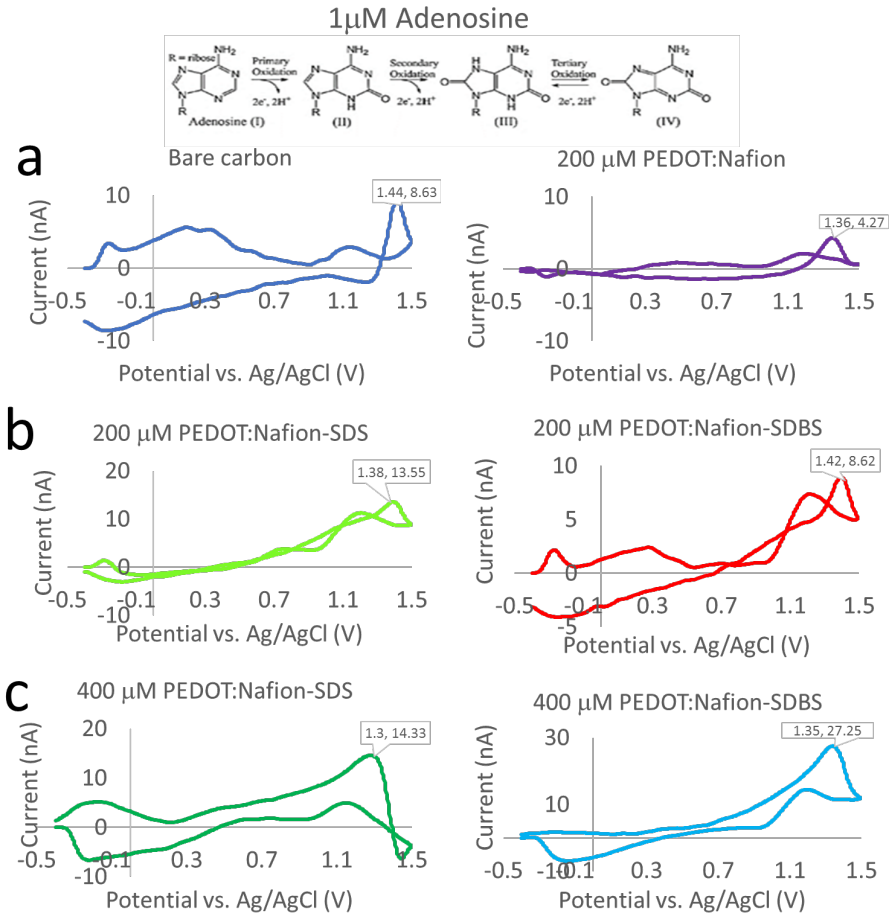

Figure 4.11: *Adenosine signals for a Bare Carbon fiber and PEDOT:Nafion (a), PEDOT:Nafion-SDS and PEDOT:Nafion-SDBS deposited from a 200 µM EDOT containing solution (b), PEDOT:Nafion-SDS and PEDOT:Nafion-SDBS from a 400 µM EDOT containing solution (c) carbon fibers. Scan rate 400 V/s.*

Sensitivity and Selectivity of all coating

The dopamine signal increased up to 4-5 times versus the bare carbon with 200 μM EDOT and the two-different surfactant, respectively. Dopamine increased up to 7-9 times with 400 μM EDOT and the surfactants. More specifically, we increased the sensitivity value from 7$\pm$3 nA/μM with bare carbon to 23.7$\pm$1 nA/μM (3.4X) with SDS and 34.4$\pm$14 nA/μM with SDBS

(5X) using 200 μM EDOT. Ascorbic Acid decreases 3 times in all the cases except with SDBS. We explain this by hypothesizing that Nafion is not incorporated into the polymer matrix with SDBS. The DOPAC signal is increased with each of the coatings, especially with SDBS as a surfactant. However, considering the peak position for oxidation and reduction of dopamine and ascorbic acid in the case of SDBS coating (**Figure 4.12**a and **Figure 4.12**b), good selectivity can be achieved by deconvoluting the dopamine and AA signals using principal component regression algorithms. The oxidation and reduction of AA is almost reversible with the reduction taking place at positive potentials. Typically, it is difficult to deconvolute dopa-mine and DOPAC signals because the cyclic voltammograms almost overlap (see Figure 4.12). However, based on the relative ratio of the oxidation currents in particular using SDS as surfactant (Figure 4.12c and Figure 4.12d), an effort to apply principal component regression algorithms or other machine learning algorithms may be worthwhile. To distinguish serotonin from dopamine, we propose to use the entire FSCV curve and the difference in the reduction potential. With the coatings that have the SDS and SDBS surfactants, dopamine is reduced close to -0.1 V vs. Ag/AgCl and serotonin at +0.1 V vs. Ag/AgCl. The only case where we cannot detect clearly dopamine versus serotonin is with the PEDOT:Nafion coating, since the reduction current for dopamine is very small due to the irreversible dopamine reaction. In Table 4.4, we provide a summary of dopamine sensitivity measurements obtained from the carbon fibers with different coatings and compared these with previously reported values. It can be seen from Table 4.4, that we have obtained the highest increase in sensitivity from bare carbon for dopamine detection, both for the PEDOT:Nafion–SDS and for the PEDOT:Nafion–SDBS coated carbon fibers.

Electrode	Sensitivity (nA/μM)	x bare carbon
CF CNTs [15]	12	x2
CF 200μM PEDOT:Nafion [11]	31	x2
CF 200μM PEDOT:Nafion-SDS	24	x3.4
CF 200μM PEDOT:Nafion-SDBS	34	x5

Table 4.4: *Dopamine signal sensitivity values. Comparison of different coatings on carbon fibers from this work and reported values from other publications. The effect of the SDS-SDBS doping can be clearly seen (this work).*

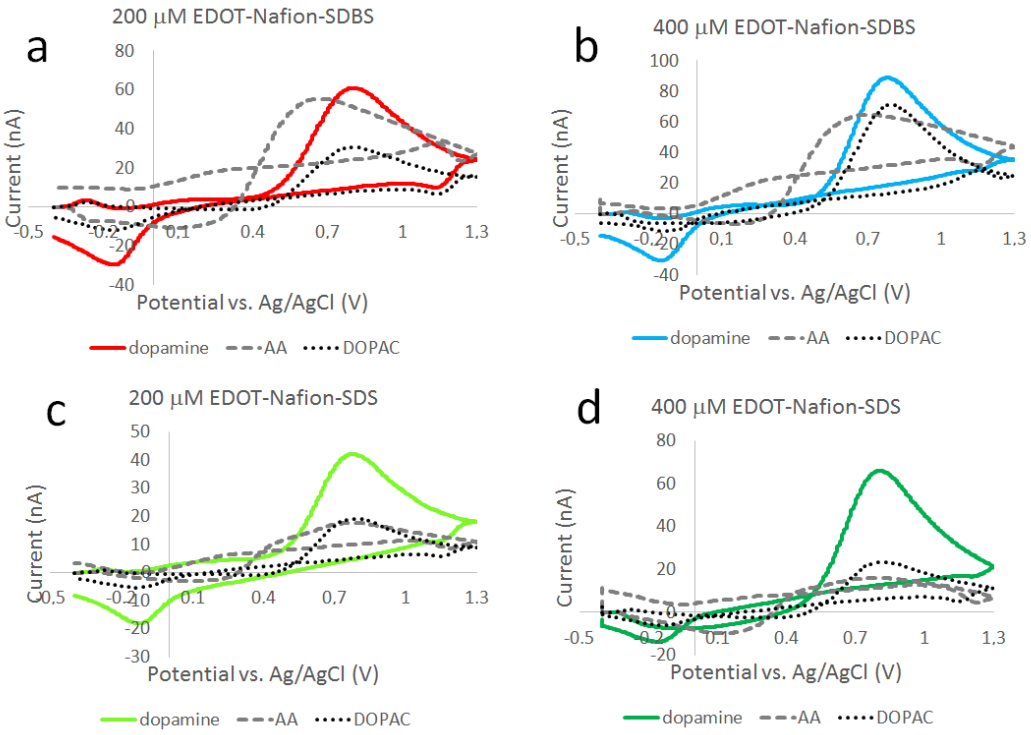

Figure 4.12: *Selectivity analysis between AA (200 µM), DOPAC (10 µM) and dopamine (1 µM). The signals are for 200 µM PEDOT:Nafion-SDBS (a), 400 µM PEDOT:Nafion-SDBS (b), 200 µM PEDOT:Nafion-SDS (c), 400 µM PEDOT:Nafion-SDS (d). Scan rate 400 V/s in pH 7.4 TRIS buffer.*

Chapter 5

Carbon nano-rods electrodes

5.1 Electrode fabrication

An array of carbon nanorods was subtractively patterned on a silicon substrate through the following series of steps: a silicon substrate was spin-coated with 1 micron of a phenolic polymer from solvent solution and baked to cure into a physically stable cross-linked polymer network. A hard mask was deposited over the polymer, 20 nm thickness, to serve as an etch mask for the cross-linked carbon polymer. On top of this hard mask, a pattern of photoresist pillars was formed, with a pillar dimension of 150 nm at a 300 nm pitch. The pattern was formed by lithographic exposure with an ArF (193 nm) ASML 1100 patterning tool, with .75 numerical aperture and conventional illumination. The photoresist was developed with .26 N TMAH, and then the resist image was transferred into the hard mask with Reactive Ion Etch (RIE) processing, using chlorine plasma. After RIE of the hard mask, the process chemistry of the etch tool was changed to an oxygen/nitrogen plasma, and the organic underlayer was partially etched to a depth of 600 nm, creating an array of pillars over the remnant underlayer. The remaining underlayer served as an electrical connection for the pillars to the output electrode. After the RIE, the initial photoresist was consumed, and the hard mask material remaining on top of the pillars was removed with dilute HF. The etched organic material was then annealed under Ar atmosphere at 900^{o}C for 10 hours to generate a glassy carbon material from the phenolic polymer. After this anneal, the carbon became conductive and was tested as an electrode material. A flowchart of the process is shown in **Figure 5.1**. Also, SEM images after the hard mask removal (**Figure5.1**g) and after annealing (**Figure5.1**h) are presented.

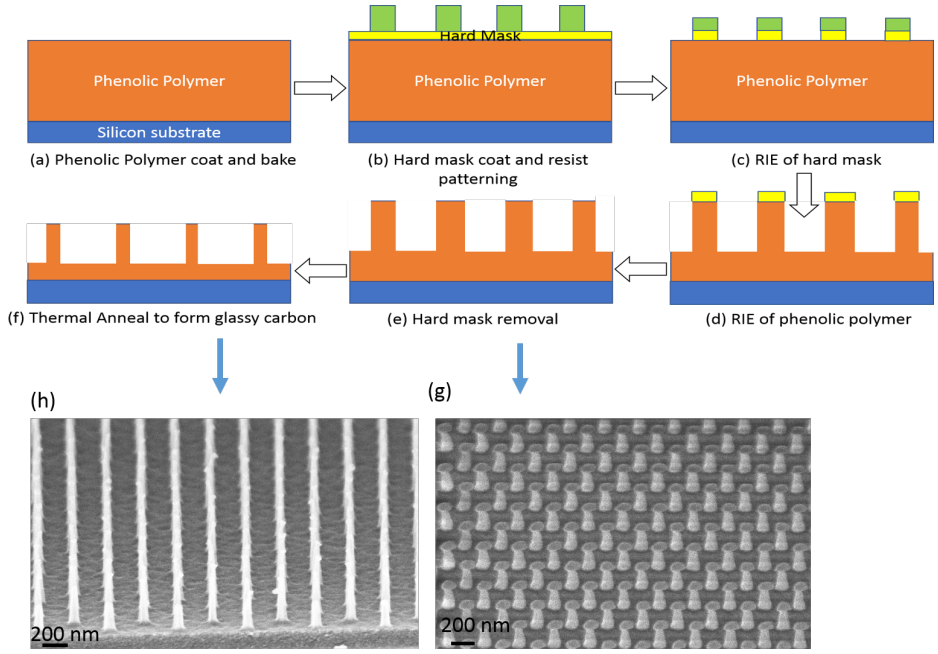

Figure 5.1: *a) Phenolic Polymer spin coating and baking. b) Hard mask deposition and deep UV lithography to define the nanopillars. c) RIE to pattern the hard mask. d) Oxygen/Nitrogen RIE to partially etch the Phenolic Polymer. e) HF hard mask removal. f) 900°C thermal annealing for 10h. g) SEM image after hard mask removal. h) SEM image after annealing.*

5.2 Electrode insulation

In order to have a small nano-rod electrode area (patterned from a Si wafer) active for the electrochemical characterization, an insulation is needed. In order to obtain this, the electrode insulation is performed through an image reversal lithographic process. The photoresist (AZ 5214) is spin-coated at 4000 rpm for 60 s, yielding a 1.5 μm thick layer. The opening defined is 30 x 20 μm. The wafer is then sliced in 1 mm wide strips to allow the immersion in the flow cell aperture. While the top sensor area is defined by the photoresist, also the Si bottom surface and sides should be isolated to prevent the charging of the solution. In the absence of this step, the signal immediately saturates. An effective isolation is achieved by covering the bottom surface and the sides with a thick (1 mm) 10:1 PDMS

(Sylgard) layer. Lastly, the top part of the strip is coated with silver paste to provide an electrical contact between the carbon rods layer and the potentiostat electrode. The total active surface should also take into account the lateral surface of the nanorods. This is evaluated approximating each rod as a truncated cone having larger base radius 42.5 nm, smaller base radius 15 nm and height 240 nm with a cylinder (radius 15 nm, height 60 nm) on top (see **Figure 5.2b**). Adding the lateral surfaces of the nanorods to the actual opening area, we have an area of around 900 μm^2.

5.3 Nanostructures geometry before and post pyrolysis

SEM figures of the pillar array are shown in Figure 5.2a prior to the wet strip and Figure 5.2b post-Ti strip and anneal. It was observed that the diameter of the polymer pillar underneath the Ti mask tapered from 100nm at the base to 60nm at the top. The lateral etching of the polymer layer was attributed to radical species from the plasma discharge. After annealing at 900^{o}C for 10 hours, slight shrinkage of the polymer layer was observed, with the base and top diameters measured as 85 and 30nm respectively.

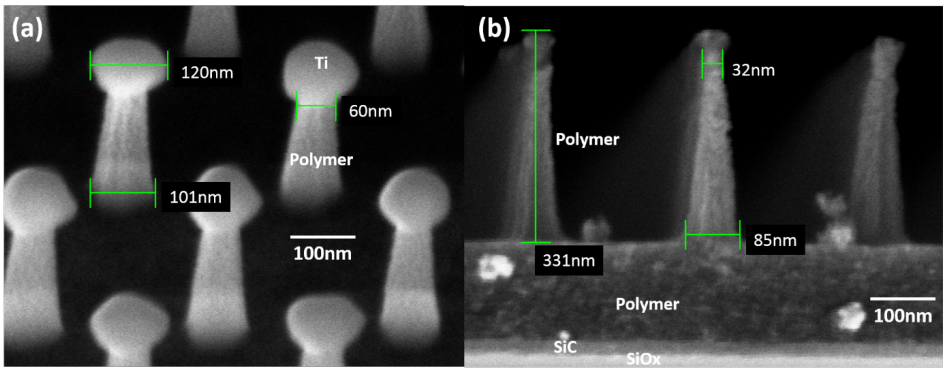

Figure 5.2: *(a) Tilt-SEM of carbon nanorod electrodes after Ti mask open and partial carbon etch. (b) x-SEM of nanorod array after Ti strip and annealing at 900^o for 10 hours.*

5.4 Characterization of nanocarbon materials

5.4.1 Resistivity and contact angle

The sheet resistance has been measured with a four-point probe system. The sheet resistance before the pyrolysis of the resist material was very high and difficult to be measured (>106 Ω/sq), with a film thickness 0.5 μm. The sheet resistance of the material after pyrolysis at 900^{o}C for 1 hour was 315 Ω/sq with a thickness 0.25 μm, leading to a resistivity of 7825-8000 μ Ω cm. After 10 hours pyrolysis at 900^{o}C, the sheet resistance of the material was 190 Ω/sq with a thickness 0.23 μm, leading to a resistivity of 4400 μ cm, similar to the one of a glassy carbon material. The contact angle after pyrolysis at 900^{o}C for 10 hours is equal to 54 deg.

5.4.2 XPS

X-Ray Photoelectron Spectroscopy (XPS) analysis of the phenolic polymer film was conducted to assess any shift in chemical bonding that occurred as a result of the annealing process. Blanket coupons were used to obtain the measurements. Survey spectra of the pre- and post-annealed films are shown in **Figure 5.3c**. As a result of annealing, a slight shift in binding energy was observed, as well as a change in the relative intensities of the C 1s and O 1s peaks. The changes were observed more readily by plotting the detailed spectra as shown in **Figure 5.3a** and **Figure 5.3b**. Post-annealing, the FWHM of the fitted peaks for both C and O decreased, which was attributed to a shift in surface charging of the annealed film. Annealing resulted in a significant increase in the relative percentage of C-C bonding in the C 1s spectra (from 24% to 57%), as calculated from the areas of the fitted peaks normalized to the total area. Conversely, the C-O bonding percentage decreased from 66% to 35% upon annealing. The percentage of C=O bonds decreased slightly (10% to 7%), however it should be noted that the fitting of this peak may have been affected by the presence of trace amounts of F contamination (either from the plasma reactor or fluoroware containers), as evidenced by the slight downward shift in binding energy. The overall composition of the film and C:O ratio are plotted in **Figure 5.3d**. The oxygen content of the film decreased by roughly half upon annealing, which corresponded well to the bonding changes observed from the detailed spectra.

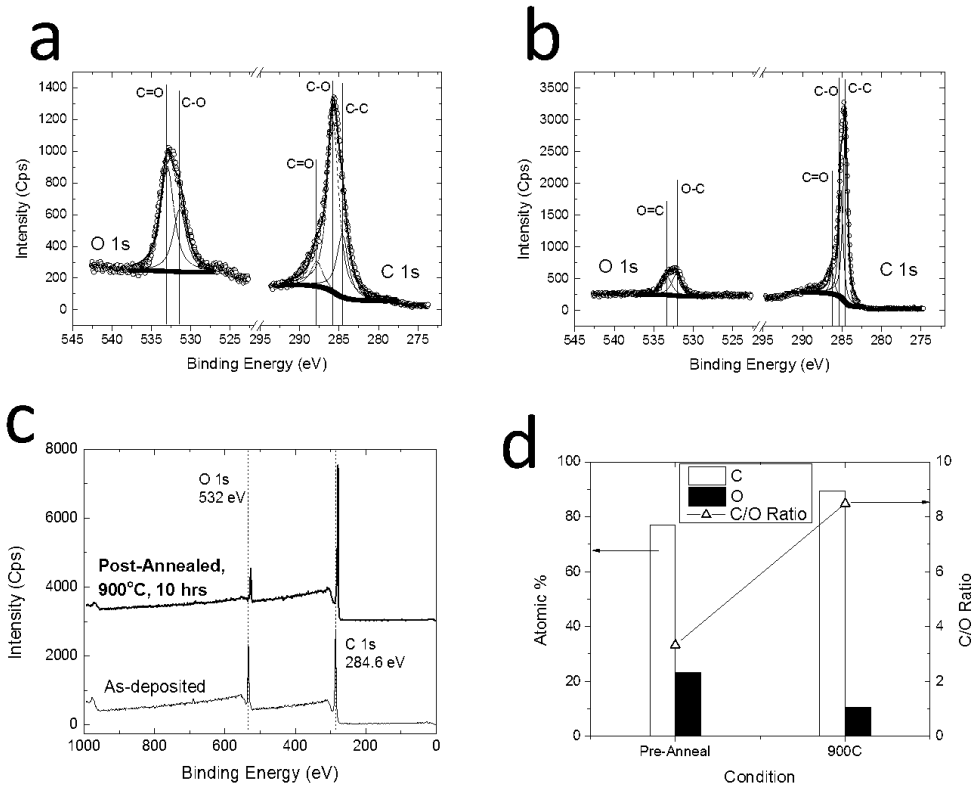

Figure 5.3: *Detailed C 1s and O 1s spectra for (a) as-deposited polymer film and (b) after 900°C annealing for 10 hours. (c) XPS survey scans of the as-deposited and post-annealed polymer (d). Atomic composition and C/O ratio of the pre- and post-annealed polymer film.*

5.4.3 Raman

In **Figure 5.4**a, the Raman spectra of the resist material before and after annealing at different conditions. In **Figure 5.4**b we compare also the nanorods spectra with a standard carbon fiber electrode.

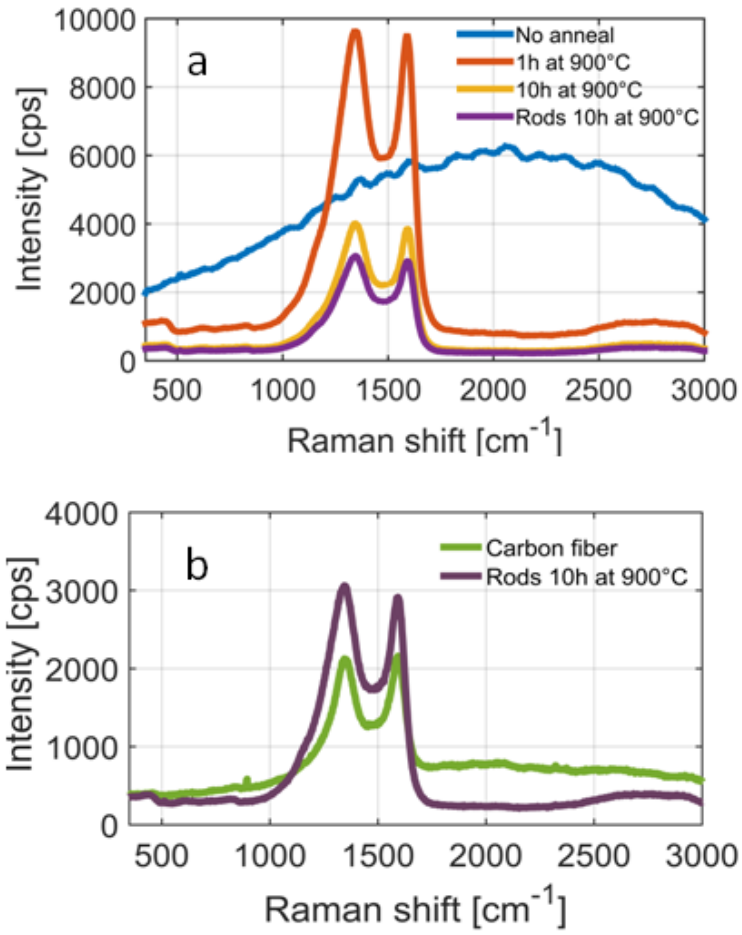

Figure 5.4: *Raman spectra as-deposited polymer film, after 900°C annealing for 1 hour, after 900°C annealing for 10 hours, after 900°C annealing for 10 hours and nanostructures pattern(a). Comparison of the nanorods spectra with a standard carbon fiber electrode (b).*

Raman spectra for the polymer before annealing is very broad. In all the other cases PPF reveal two characteristic bands at 1350 ("D" band) and 1600 ("G" band) cm^1 with intensity ratio "D"/ "G" 1.1. Similar spectra are reported also for glassy carbon material. The ratio between "D" and "G" bands correlates with the extent of microstructural disorder. The ratio for glassy carbon is in the range 1.2-1.5. The intensity ratio "D"/"G" of our PPF was slightly lower (1.1). It can be explained that the microstructure of the film is less disordered and that it contains more graphitic structure. [24]

5.4.4 Electrochemical characterization

Fast Scan Cyclic Voltammetry has been used for the detection of dopamine at the electrode surface. The experiments are performed with a scan-

rate of 100 V/s. To avoid the potentiostat saturation (maximum current $\pm$2000 nA), small nanorods regions should be defined. The isolation was performed through an image reversal lithographic process and the opening defined is 30 x 20 μm. The Si bottom surface and sides were isolated through a 1 mm thick Polydimethylsiloxane (PDMS) layer. Since the sensor surface is composed of a dense matrix of nanorods, the total active are should take into account also the pillars lateral surface. The resulting total area is 900 μm^2 (see section 5.2). Since it has been shown in previous works that oxygen plasma can improve dopamine sensitivity[33, 27, 28], a 45 s treatment (50 W) is carried out before the measurement. Moreover, 30 minutes pre-treatment in TRIS buffer pH 7.4 with triangular scans up to 1.4 V at 60 Hz is performed to improve the carbon surface reactivity[34, 29]. SEM image of the defined opening with nanorods after surface treatments can be seen in Figure 5.5e. Three different dopamine concentrations are tested, namely 500 nM, 5 μM, 10 μM (**Figure 5.5a,b,c**).

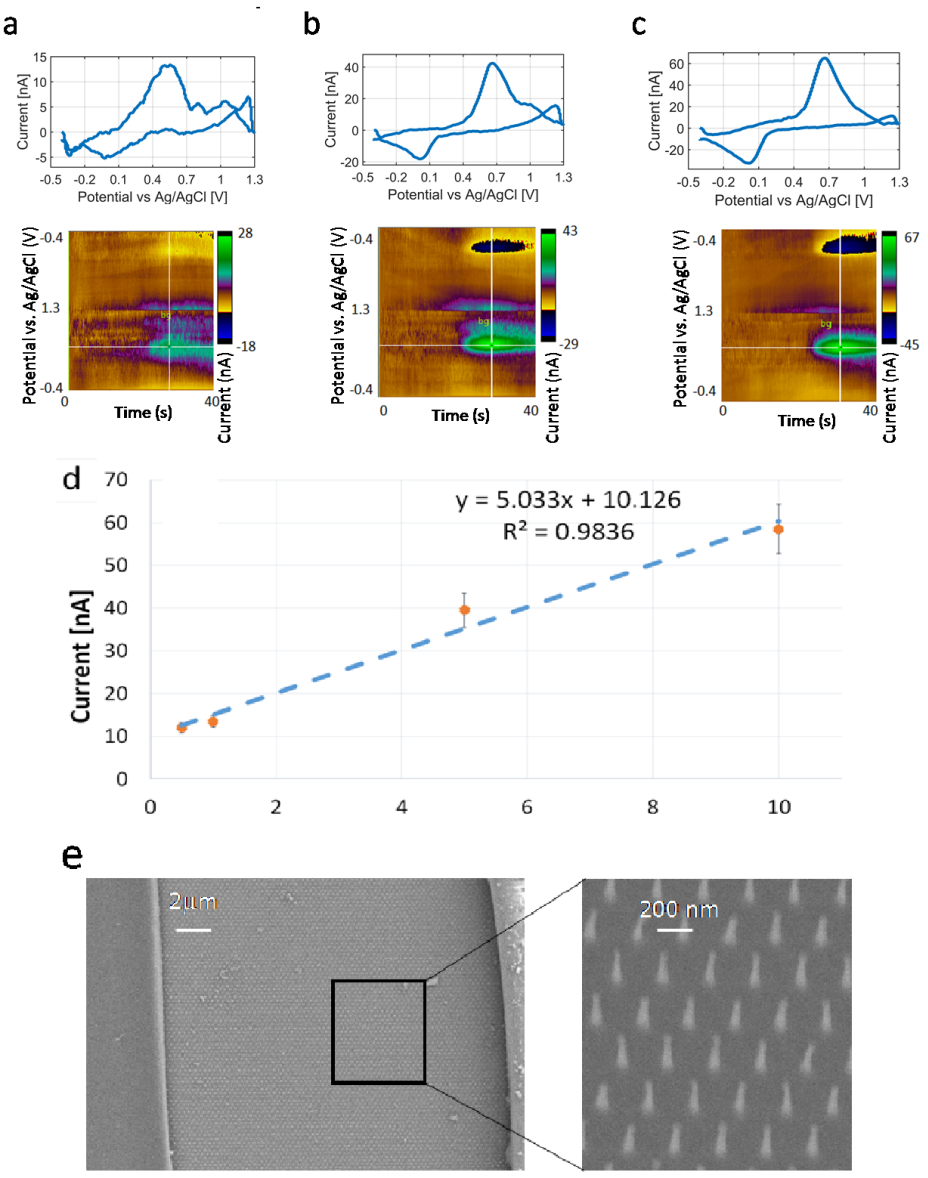

Figure 5.5: *Dopamine signal with carbon nanorods 20 µm x 30 µm for 500 nM dopamine (a), 5 µM dopamine (b), 10 µM dopamine (c). The color plots represent also the variation of the signal in time. Calibration plot with the previous oxidation signals (d). Experiment performed in TRIS buffer pH 7.4 at 100 V/s. SEM image of the electrode used for dopamine testing (e).*

Figure 5.5d reports the value of the dopamine oxidation peak as a function of the dopamine concentration. The calibration curve is linear (R^2= 0.9867) with a slope of 5±4 nA/μM (n=4 concentrations). For a signal to noise ratio equal to 3 and the noise level of the FSCV system, we have a limit of detection (LOD) of 60±5 nM. The sensitivity of a commercially available

Electrode	CD (pA/μM.μm^2)	CD for 500nM (μm^2)
PPF Microelectrode [6]	9	4
Carbon Fiber (this work)	3	2.7
Carbon Nanorods (this work)	5.6	14.9

Table 5.1: *Figures of merit of carbon electrodes tested with fast scan cyclic voltammetry. Current density (CD) values are reported based on the calibration curve and for low dopamine concentrations. The area of the PPF electrode is 500 μm^2, the area of the Carbon Fiber is 2236.5 μm^2, the area of the Carbon Nanorods (considering the nanorods area and the opening area)is 900 μm^2.*

carbon fiber with 4X larger dimension and 4X larger scan-rate tested with our system has shown a sensitivity of 7$\pm$3 nA/μM (m=7 electrodes, n=5 concentrations) and a LOD of 41$\pm$6 nM (see previous results with carbon fibers). **Table 5.1** shows figures of merit of different carbon electrodes reported previously and a comparison with this work.

The current density derived from the slope of the calibration curve of the carbon nanorod electrode considering the nanorod active area (900 μm^2) is equal to 5.6$\pm$4 pA/(pA/μM.μm^2). On the other hand, the current density of the carbon fiber (area 2236.5 μm^2) is equal to 31 pA/(μM.μm^2). Hence, we have 2X current density improvement from the bare carbon fiber that has a 4X bigger open area. Moreover, a relatively slow scan rate of 100-250 V/s was used due to the high reactivity of the surface that resulted in the background current reaching the current limit of the FSCV apparatus. Even higher current densities could be achieved with faster scan rates for smaller electrode openings since the signal is linearly proportional to the scan rate.[18] Moreover, we focus on the signal obtained for 500nM dopamine, that represents a concentration suitable for in vivo applications. It has been shown previously that dopamine transients are on the order of 50-100 nM in rats, and reached values higher than 200 nM with the use of drugs. In awake monkeys with electrical stimulation, dopamine transients reached 850 nM dopamine. The oxidation current of the nanostructured carbon electrodes (85 nm pillar base in a 30 μm x 20 μm opening) for 500 nM dopamine is 13.4 nA. The current density value (14.9 pA/μm^2 vs. 2.7 pA/μm^2) is more than 5X higher than the carbon fiber electrode at the same concentration of dopamine (results of a carbon fiber electrode shown in **Table 5.1**). Considering the state of the art electrodes for dopamine detection, the oxidation current for the nanorods at the low dopamine concentration

is higher than that of a carbon fiber microelectrode (dopamine oxidation current of 6±3 nA) with 4X larger dimensions and 4X higher scan rate, proving the effectiveness of the nanostructure to obtain very good signals at relatively low concentrations. The nanostructures increase the sensitivity per unit area of the detection by 2X while yielding an LOD of 60±5 nM. This is comparable to the LOD of the bare carbon fibers, with the same time response and with 4X smaller open area. As we and others have shown previously, for in vivo applications, one must use Nafion-based polymeric coatings in order achieve the desired selectivity versus interferents such as Ascorbic Acid and DOPAC. Also, a smaller open area and improvement in the choice of the insulated substrate will enable a decrease of the noise level, provide a lower LOD, and allow the integration of multiple carbon electrodes onto a smaller footprint.

Chapter 6

Conclusions

Carbon fiber coatings made of Poly(3,4-ethylenedioxythiophene) (PEDOT) have been synthetized using chemical oxidative polymerization of EDOT monomers in an aqueous medium containing Nafion. We analyzed the influence of SDS and SDBS as surfactants on the electro-polymerization. The degree of uniformity of the coating has been assessed with SEM and it has been shown to be very uniform when SDS was used as a surfactant during electro-polymerization. The Nafion incorporation has been demonstrated with fast scan cyclic voltammetry for SDS as surfactant with the decrease of the anionic compound ascorbic acid with the coating, but not for SDBS as a surfactant. The dopamine signal is increased up to 5X from bare carbon with 200 μM EDOT and SDBS as surfactant and up to 9X with 400 μM EDOT and SDBS. Using SDS as surfactant, the dopamine signal is increased up to 4X from bare carbon with 200μM EDOT and up to 6X from bare carbon with 400 μM EDOT- Furthermore, we increased the sensitivity value from 7$\pm$3 nA/μM with bare carbon to 23.7$\pm$1 nA/μM (3.4X) with SDS and 34.4$\pm$14 nA/μM with SDBS (5X) in the solution containing 200 μM EDOT and Nafion. Dopamine selectivity can be achieved using the full voltammetry curves with respect to ascorbic acid, serotonin and adenosine using distinguishing features of the voltammograms, such as differences in reduction or oxidation potentials and with respect to DOPAC using the lower signals. Serotonin selectivity versus dopamine can be achieved using the full voltammogram and distinguishing the different reduction potentials. An interesting shift towards less positive potentials for adenosine has been observed with the high EDOT concentration and the surfactants. Overall, the PEDOT:Nafion-SDS and the PEDOT:Nafion-SDBS are one of the best coatings ever reported for dopamine detection versus ascor-

bic acid, DOPAC, serotonin and adenosine. The coatings, especially with SDS as surfactant, are reproducible, uniform, with fast kinetics and strong dopamine and dopamine-o-quinone absorption on the surface. The possibility to perform the deposition in aqueous solutions and to use surfactants may be useful also for further studies with the addition of other materials into the polymer matrix. Further evaluation, such as stability of the coating in vivo, will open many possibilities for neurotransmitters detection with fast scan cyclic voltammetry.
Also, we have demonstrated for the first time a scalable fabrication of a nanostructured (less than 100 nm in size) glassy carbon electrode array (with over 6000 electrodes) and the detection of low concentrations of dopamine with fast scan cyclic voltammetry (FSCV). This nanostructured glassy carbon electrode array exhibits 2X higher sensitivity per unit area for dopamine sensing and more than 5X higher current density for low dopamine concentration compared to a micron-sized carbon fiber (4X bigger open area) with comparable LOD and time response. Using the same nano - lithographic technique already employed in this work, an array of nanostructured electrodes to combine neuronal firing and neurotransmitter detection in multiple locations along a long needle type implant can be realized in the future. Furthermore, neurotransmitter measurements within a single synapse could be possibly envisioned. In addition to the miniaturization, the carbon nanorod electrodes were fabricated on a CMOS compatible silicon platform which can be readily coupled to the back-side with CMOS electronics to drive the measurements and sophisticated multiplexing of signals with few wires. Further improvements on the substrate insulation may allow a decrease of the LOD and the noise level of the sensor. Furthermore, combining electro-physiological and neurochemical measurements will lead to a better understanding of the human brain and its pathologies.

6.1 Next steps

Future developments of the work include:

- animal testing of the PEDOT:Nafion-SDS/SDBS carbon microfibers for neurotransmitters detection;
- coating of the carbon nanorods electrode with the improved PEDOT: Nafion solution for increasing the selectivity;
- animal testing of the coated carbon nanorods electrode for neurotransmitters detection.

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