Modified graphite biosensor with polypyrrole (PPy) and MWCNT-NH₂

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Abstract. The development of electrodes for biosensors with new materials is one of the alternatives for the increase of the long-term performance of these devices. Electrodes are used in the detection of the biological process related to the regeneration of muscles, tissues and bones, because they identify physiological substances that intervene in this process. The aim of this research was the development of a biosensor through the growth thin films of polypyrrole/multi-walled carbon nanotubes functionalized with NH₂ over a graphite electrode (electrodes were called PPy/MWCNTs-NH₂/EG). Different electrodes were developed varying the concentration of MWCNTs (2mg, 4mg, 6mg, 8mg). The films were electropolymerized by of cyclic voltammetry in a conventional three-electrode cell (Ag/AgCl3MKCl) as reference electrode, a graphite rod as auxiliary electrode and to 98% pure graphite electrode as working electrode. The characterization was performed by scanning electron microscopy, confocal Raman spectroscopy and electrochemical impedance spectroscopy.

1. Introduction
The composite materials with carbon nanotubes and conductive polymers are of great interest due to the properties of each component and its mixture, such as electronic transfer capacity, surface area and synergistic effects [1,2]. In several research have been reported the potential of these materials for the development of organic electronic devices, such as photovoltaic cells, fuel cells, biological sensors and flexible light emitting diodes [3]. Several polymers have been used for this purpose, such as EDOT, aniline, ethylene glycol and pyrrole (Py). Within these polymers the Py has shown remarkable properties of electroactivity, ion exchange, chemical stability, and association with inorganic or organic conductive materials [4], with great affinity to medical applications because it is naturally present in the Hemo group and Vitamin B₁₂. However, the morphology of Polypyrrole (PPy) is not the most suitable for conductivity in biomedical applications, for this reason the mix with functionalized carbon nanostructures provide an interconnection in its structure with more active contact points for the interaction with biological media [5]. For the synthesis of composite material of PPy with carbon nanotubes, the technique most used is in situ electropolymerization [6]. In this technique the carbon nanostructures are incorporated into a pyrrole solution in the form of a monomer, since it provides an adequate dispersion for the nanostructures during the reaction and helps to polymerize the PPy. An advantage of this procedure is the obtention of a nanocomposite with a large number of nanostructure. Although hereby is necessary for the monomer or the carbon nanostructures be superficially modified, functionalized with NH₂ before polymerization [7]. This research presents a simple method for the development of a graphite electrode with PPy and MWCNTs with the purpose of being used as a
biosensor. The biosensors were characterized and the electrochemical behavior of these were assessed in a simulated physiological medium that resembles the extracellular fluid environment [8].

2. Materials and methods
A graphite cylinder with a diameter of 5mm and a length of 8.5mm at 98% purity was used as a substrate for the bioelectric system. Pyrrole (Py) potassium chloride (KCl) and multi-walled carbon nanotubes functionalized with NH$_2$ were employed as conductive components. The surfaces of the cylinders were polished to mirror finished leaving exposed the surface for electropolymerization in situ. Then deionized water was used to rinse the electrodes with ultrasound for 2 minutes. 6 electrodes were developed. One target coated with PPy alone and 5 electrodes including different concentrations of MWCNTs-NH$_2$ (2mg, 4mg, 6mg (with and without magnetic stirring) and 8mg with magnetic stirring). The solutions used in the electropolymerization were prepared with deionized water, Py, KCl, and MWCNTs-NH$_2$. In this procedure, the pure Py was electropolymerized in a solution with initial concentrations of (0.25mol/LPy 0.5mol/LKCl) without carbon nanostructures, later the MWCNTs were added in it [7]. This mixture was called electrolytic solution, each electrolytic solution was subjected to ultrasound for 30 minutes before the electropolymerization, to increase the dispersion between the carbon nanostructures and the monomer to ensure adequate electropolymerization.

The evaluation of the precursors and the subsequent structural chemical characterization of the electropolymerized samples confocal micro Raman spectroscopy was used in a Horiba LabRam spectrometer with a 532nm laser, a grid of 1800gr/mm, time acquisition of 15s and 20s accumulations.

The morphological characterization was performed by scanning electron microscope EVO MA10, Carl Zeiss with detection by secondary electrons and backscattered electrons.

The electrochemical experiments were carried out using Gamry instruments interface1000E potentiostat/galvanostat with a commercial acquisition, data processing, and reporting system Echem Analyst, in a three-electrode electrochemical cell, at room temperature. An Ag/AgCl was employed as reference electrode, a graphite rod as counter electrode, and a graphite cylinder were used as the working electrode. The EIS tests were performed for the electrochemical characterization of the electrodes using as electrolytic solution extracellular fluid simulated with 1X saline buffer solution (PBS) according to the Dulbecco formulation at an ambient temperature of approximately 29°C and pH 7.4. A disturbance voltage of 10mV in AC and a sweep of frequencies between 100,000Hz and 0.01Hz was performed. The results obtained were mathematically treated, using the Zview® software, to adjust the spectra to a proposed equivalent electrical circuit model. Adjustments were made to the proposed model for chi-square values ($\chi^2$) less than $10^{-4}$. For the manufacture of the electrodes, MWCNTs-NH$_2$ and a PPy conducting polymer was electrochemically deposited simultaneously. For this end, the PPy/MWCNTs-NH$_2$ compound was uniformly bound to the electrode surface. With this mixture the best electrodeposition was reached with the 4mg electrode, observing nanostructures better dispersed throughout the volume of the solution used. For the other mixtures, an adjustment was made to the initial experimental assembly, using a magnetic stirring plate during the electropolymerization process, to guarantee a homogeneous dispersion.

3. Results and discussions
By means of the morphological analysis carried out by SEM, a characteristic structure of PPy in the form of cauliflower was identified, the samples containing nanostructures are modified according to the elongated morphology of the MWCNTs-NH$_2$. Dispersion of carbon nanostructures after electropolymerization was observed as shown in Figure 1 shows the voltamograms of PPy electropolymerization in the presence of nanostructures with different concentrations (2mg, 4mg, 6mg, 6gm with stirring, 8mg with stirring) %w/w of MWCNTs-NH$_2$, identifying the positive going potential in approximately 0.6V. This value was obtained with a scan rate of 20 mV/s in a potential window where oxidation was generated like that reported by other researchers [8,9]. Then, 50 cycles of cyclic voltammetry were performed for each one of the electrodes, with a scan rate of 50 mV/s to obtain the electropolymerization of PPy films on the EG graphite electrode, being these films of PPy, the target
for the comparison in the characterization and evaluation of the composite PPy/MWCNTs-NH₂/EG. In this process the nanotubes generate on the electrode radical cations by the current flow and by their functional group of cationic character. The radical cations are generated from the monomer of PPy and by the presence of nanotubes, in the first case it is given: direct electropolymerization and in the other there is an indirect electro initiation. The mechanism will be given by means of oxidation-reduction processes of the respective constituents, monomers of PPy and MWCNTs-NH₂ depending on the state. The cationic nature of the amino functional group in nanotubes other than serving as a nucleophile, can also serve as a transporter in the encapsulation of drugs for other applications. In this case they serve as charging points to generate the next reaction and subsequent synthesis of the pyrrole monomers. In addition, the addition of nanotubes is done in order to increase the conductive nature of the polymer matrix of PPy evidenced in the EIS tests [10,11]. The voltammogram of oxidation and growth of PPy (Figure 1) was analogous to that reported by other investigations [2,8,9]. Sweeps from 0.2V to 0.9V were performed, the orientation of the arrows indicates the progress of oxidation and reduction of electropolymerization, respectively. This showed an increase in the current and the area between consecutive cycles, which represents the growth of the polymer [12].

In all the voltammograms, the increase in the anodic slope of each curve cycle after cycle, is an indication of the decrease in the impedance of the EG samples with the PPy/ MWCNTs-NH₂coating. For the electrode with 2mg MWCNTs the anodic current (Iₐ) increased until a maximum anodic current of Iₐ = 10.49mA. While for the 4mg electrode was Iₐ = 22.19mA. For the 6mg electrodes without and with stirring, Iₐ was 17mA and 26mA, respectively. For the 8mg given that it was performed in the presence of higher magnetic agitation, Iₐ decreased to 2.45mA with a deficient deposition in the EG. The evaluation of the precursors and electropolymerization products was carried out by means of micro Raman spectroscopy, in which the characteristic pyrrole (Py) peaks were obtained according to what was reported in other investigations [4,12]. In Figure 2(a). The most characteristic peaks of this monomer are observed in the Raman shift 935 cm⁻¹ and 989 cm⁻¹ corresponding to the deformation of the ring associated with the di-cation (dipolaron) and the cation (polaron) radical, respectively. The peak 1415 cm⁻¹ can be attributed to the C − N stretch [7], and at 1049 cm⁻¹ and 1078 cm⁻¹, to the vibration in the C − H plane. In the spectrum of Py (liquid) monomer Figure 2(a), a characteristic peak was observed (gray line) at 144 cm⁻¹ this disappeared after the electropolymerization [9], as can be corroborated in Polypyrrole/EG. In the spectrum of the EG modified with PPy, characteristic peaks of the PPy were differentiated in 974 cm⁻¹ and 1049 cm⁻¹, those peaks represented the reduced state of
the polymer (benzoin form), as well as a decrease in the peak intensity of 935 cm\(^{-1}\) which is characteristic of the oxidized state of the polymer (quinoid form) [6]. For the samples with nanostructures of MWCNTs-NH\(_2\), Raman spectra were obtained, where the structure and electropolymerization of the PPy were verified, which was also corroborated with SEM. The results for the solution with 2mg, 4mg, 6mg (without stirring), 6mg and 8mg with stirring of MWCNTs-NH\(_2\) in %w/w, are shown in Figure 2(b) and Figure 2(c), where a comparative was made of spectra of the samples prepared and it was found spectra with characteristic peaks of PPy and MWCNTs-NH\(_2\).

Figure 2. Raman spectra (a) Monomer of Py (liquid) and electrodeposited on EG, (b) samples with 2mg and 6mg, (c) samples with 4mg, 6mg and 8mg with stirring.

Figure 2(a) corresponds to the spectrum of the monomer and polymer, in Figure 2(b) the spectra of the samples of 2mg and 6mg without stirring, in the Figure 2(c) the spectra of the samples of 6mg and 8mg with stirring are presented, as well as that of 4mg without stirring and the spectrum of MWCNTs-NH\(_2\). According to the literature, the characteristic peaks of MWCNTs-NH\(_2\) are present at 1535 – 1575 cm\(^{-1}\) and those corresponding to the PPy between 932 – 1048 cm\(^{-1}\) [12]. In this investigation peaks were obtained at (935, 936, 988, 989) cm\(^{-1}\) and 1053 cm\(^{-1}\). In Figure 2(c) the characteristic peaks of the MWCNTs-NH\(_2\), at 1346 cm\(^{-1}\) and 1587 cm\(^{-1}\), confirm the presence of carbon nanostructures. Figure 3 shows the images obtained in SEM for the morphology after the electrodeposition.

The films prepared with Py without MWCNTs-NH\(_2\) were more compact in morphology, compared to the other conditions presented. The films prepared with the solution Py and MWCNTs-NH\(_2\) showed the carbon nanostructures incorporated within the PPy matrix, observing the film in a porous zone with elongated morphologies, compared with films with only PPy. In these samples there is an interaction in
these linear patterns, between the matrix and the carbon nanotubes present (indicated with white arrows). The patterns were different for 4mg and 8mg images; being more noticeable the presence of MWCNTs-NH\textsubscript{2} with respect to the other solutions where the number of nanostructures in the films is lower, also they present more porous. With the Raman spectra (Figure 2) and the SEM images (Figure 3), the presence of carbon nanotubes and PPy was verified.

The interaction between these two components can cause the characteristic peaks of the MWCNTs-NH\textsubscript{2} to change their intensity, which is presented in the Raman spectra of Figure 2(b) acquired on the sample with 6mg of MWCNTs-NH\textsubscript{2}. In these spectra, changes are observed in the intensity of the characteristic peaks, with respect to the Raman spectrum of only MWCNTs-NH\textsubscript{2}, where the higher intensity occurred at the 1338cm\textsuperscript{-1} peak and the lower intensity at 1575cm\textsuperscript{-1}. After the electropolymerization the intensity values increased for the peak at 1575cm\textsuperscript{-1}, indicating a possible change in the morphology of the nanotubes.

![Figure 3. SEM Images of EG surfaces modified with films composed of PPy/MWCNTs-NH\textsubscript{2} (a) PPy (b) 2mg (c) 4mg (d) 6mg with stirring, (e) 8mg with stirring.](image)

The electrochemical characterization by EIE of the PPy electrodes and PPy/MWCNTs-NH\textsubscript{2}/EG compounds obtained with different concentrations in \%p/p MWCNTs-NH\textsubscript{2} are shown in Figure 4, where the Bode diagram is presented in phase values against sweep frequency (Figure 4(a)), Bode in magnitude of the impedance (Figure 4(b)) and the Nyquist diagram (Figure 4(c) and 4(d)). In the Bode-phase diagram of Figure 4(a), three processes that occur at the electrode were observed. The first at high frequencies, the second at the phase angle close to 0\degree and at small impedance values. At medium and low frequencies, the impedance increases being associated with the capacitance of the electrochemical double layer in the EG interface/film composed of PPy/MWCNTs-NH\textsubscript{2} and the \( R_t \) in the EG surface. A third process was observed with an increase in the phase angle from 20\degree to 50\degree, which is associated with a diffusive phenomenon. In some samples, a decrease in the phase angle is observed at medium frequencies, reaching up to 10\degree.

In Figure 4, the solid lines indicate the fitting of the equivalent circuit model to the impedance measurements from the experimental data. In these diagrams, two electrode processes that occur during the exposure time in PBS were identified. The first is due to the electrical characteristics of the film composed of PPy and PPy/MWCNTs-NH\textsubscript{2}/EG, identified by the formation of a semicircle at high frequencies as seen in Figure 5(c) and Figure 5(d) in detail (0 to 1200ohm). The semicircle corresponds to a process limited by charge transfer at high frequencies and a second process related to mass transfer, followed by a diffusive process which was manifested by a straight line with an angle close to 45\degree at
very low frequencies. This diffusion is made from the electrolyte ions through the polymer matrix composed of PPy and MWCNTs-NH₂. The diameter of the semicircle is equal to the electron transfer resistance (Rₑ). The electrode modified with only PPy shows a large semicircle, while for the electrodes with PPy/MWCNTs-NH₂, the semicircles were small being almost a straight line in the samples with 8mg with agitation and 6mg without stirring. For the samples of 4mg without stirring and 6mg with stirring, it was observed a small semicircle more extended than the other three EG coated with the compound PPy/MWCNTs-NH₂, indicating for all a smaller Rₑ. The Rₑ of EG modified with PPy/MWCNTs-NH₂ was lower compared to EG modified only with PPy, implying that the nanocomposite film PPy/MWCNTs-NH₂ plays a key role in the conduction of electrons. The real impedance at low frequencies where the capacitive behavior was dominant, was an indicator of the combined electrolyte and film resistance including both electronic and anionic contributions [6,9]. Carbon nanotubes within the polymer matrix can lead in faster electron transfer at the EG/interface/PPy film, and load transfer at the corresponding PPy/solution film interfaces and MWCNTs-NH₂/solution unlike film PPy/solution [5].

Figure 4. Impedance spectroscopy EIE in PBS (pH 7.4), graphite electrodes modified with PPy/MWCNTs-NH₂/EG. (a) Phase (°) vs Frequency (Hz), (b) Ẑmod(ohn) vs Frequency (Hz), (c)-(d) Diagram Nyquist Ẑimg(ohn) vs Ẑreal(ohn).
Figure 5 shows the models of equivalent electrical circuits (CEE) proposed to describe the electrochemical processes that occur in the different samples, according to the quality of the impedance spectra adjustment.

These models allowed obtaining parameters related to possible electrodes mechanisms of the modified EG. The equivalent circuit was composed of a resistance $R_{\text{pbs}}$ that corresponds to the resistance of the PBS solution, followed by two RC circuits in parallel, which represented each one of the time constants identified in the Bode diagrams. $C_{\text{ppy, cnt}}$ is a constant-phase element of the composite film PPy/MWCNTs-NH2/electrolyte, $R_{\text{ppy, cnt}}$ is the load-carrying resistance to transport of the film PPy/MWCNTs-NH2, and in the internal circuit $C_{d}$ is a constant-phase element associated with the double electrochemical layer loaded with the electrons coming from the PPy/MWCNTs-NH2, and $R_{t}$ the resistance to charge transfer through the EG/film interface and $W_{t}$ the Warburg impedance of the polymer. The parameters obtained are shown in Table 1.

According to Table 1, the $R_{t}$ decreased with the increase in the concentration of carbon nanostructures, except for the 8mg sample. Possibly by the saturation of nanotubes in solution. A lower value of $R_{t}$ suggests a more reactive surface compared to the sample with the lowest number of nanostructures (2mg). It was also evidenced from the value of phase $\chi$ that this value increases when the number of carbon nanotubes increased, and at the same time it was related to a more porous morphology.

**Table 1.** Capacitance and resistance values of the interfaces of the modified graphite electrode with PPy/MWCNTs-NH2/EG composite material.

| Parameter | PPy | 2mg | 4mg | 6mg | 6mgS | 8mgS |
|-----------|-----|-----|-----|-----|------|------|
| $R_{t}$ (Ωcm$^2$) | 585.20 | 412.20 | 23.28 | 25.77 | 28.03 | 40.17 |
| $C_{\text{ppy}}$ (Fcm$^2$) | 3.55E-07 | 4.11E-04 | 4.66E-12 | 1.44E-06 | 3.10E-04 | 1.02E-03 |
| $R_{\text{ppy}}$ (Ωcm$^2$) | 63.10 | 32.45 | 23.40 | 48.88 | 3.86E-06 | 1.34E-08 |
| $N$ | 0.56 | 0.89 | 0.64 | 0.62 | 0.65 | 0.49 |
| $C_{d}$ (Fcm$^2$) | NA | 3586.50E-04 | 1.55E-04 | 4.62E-04 | 2.25E-04 | 1.14E-04 |
| $\chi$ | NA | 0.61 | 0.60 | 0.40 | 0.27 | 0.58 |
| $R_{t}$ (Ωcm$^2$) | NA | 300.60 | 239.70 | 228.40 | 111.50 | 1E20 |
| $W_{t}$ (Ωcm$^2$) | NA | NA | 2593 | 6119 | 94.20 | NA |
| $\chi^2$ | 7.98E-05 | 1.29E-04 | 7.69E-05 | 2.15E-05 | 4.66E-05 | 8.51E-05 |

4. Conclusions

Different electrodes of composite material were obtained through electropolymerization of conductive polymer and multi-walled carbon nanotubes functionalized with NH2. According to the characterization by Raman and SEM, this material presented characteristic properties of the individual components PPy and MWCNTs-NH2, as well as synergistic effects. The electrodes of 4mg, 6mg without stirring and 6mg with stirring presented the best characteristics of electrochemical stability and conduction for applications as biosensors.
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