

# Immobilization of Glucose Oxidase in Anthracene-Based Semi-Conducting Polymer: Application on Glucose Biosensing

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## Abstract

A glucose biosensor based on the immobilization of the enzyme glucose oxidase (GOx) onto an anthracene-based polymer analogue of poly (phenylene sulfide) (PAnS)/Gold electrode was elaborated. The building of the bridge system was evaluated by electrochemical impedance spectroscopy measurement (EIS) and cyclic voltammetry (CV). The PAnS semi-conducting polymer exhibits a good film forming, stability and adhesion on gold Electrode. The choice of the immobilization of GOx in thin film polymer is essentially based on the facility of the synthesis of the polymer also on the surface properties. The immobilized GOx was proved by cyclic voltammetry, contact angle and UV-visible spectroscopy. The GOx/PAnS/Au bioelectrode showed a linear response to glucose in the concentration ranging from  $10^{-8}$  M to  $10^{-3}$  M with a correlation coefficient of 0.98 and a sensitivity of  $2.66$  ( $k\Omega M^{-1}cm^{-2}$ ). Indeed, The GOx/PAnS/Au structure could make it a promising bioelectrode for detection of glucose in the biological samples.

**Keywords:** Semi-conducting polymer; Anthracene; Thin film; Glucose oxidase immobilization

## Introduction

A biosensor defined as a compact analytical device incorporating a biological or biologically derived sensitive element integrated or associated with a physio-chemical transducer [1]. Clark et al. was the first to develop glucose oxidase (GOx) immobilized electrode to quantify the glucose concentration [2]. Since then, the detection of biomolecules has become important in a variety of areas including bioreactors, medical diagnostics, veterinary medicine and viral diagnostic and bacterial diagnostic [3,4]. Electrochemical glucose biosensors based on biomolecules immobilized on an electrode surface play a leading role in this direction specially that the diabetes mellitus is one of the great modern health issues. Several studies have been carried out in order to improve performance of this type of sensor. Sensors continue to have an important impact in everyday life. There has been a strong demand to produce highly, sensitive, selective, responsive, and cost effective sensors [5-7].

Great efforts have been devoted to immobilization of glucose oxidase (GOx) on an electrode surface, which is one of the main factors that affect the performance of glucose biosensors. In order to have a stable, selective and sensitive response, the surface of the electrode has been modified [8]. This can be realized by conductive polymers such as polypyrrole [9], polythiophene [10], and polyaniline [11], because of a number of favorable characteristics, they afforded stable surface for the immobilization of the biocomponent and play the role of a transducers to convert a chemical signal into an electrical one [12,13]. Also, conducting polymers are known for their ability to be compatible with biomolecule in neutral aqueous solutions [14]. Biosensors, prepared using conducting polymers as a support material, have a high storage, a fast response time with an operational stability [15]. In this context, we have used a new semi-conducting polymer based on anthracene analogue of poly(phenylene sulfide) PPS as support of enzyme. This polymer was reported in our previously work [16].

Anthracene presented a good photophysical properties and it is the first aromatic material used in organic light emitting diode (OLEDs). The first experiments were carried out by Pope in the early 1960s [17]. Soon after, several reports on single-crystal anthracene-based OLEDs were published and good quantum yields were obtained (up to 5%).

Nevertheless, such devices are thick therefore they require very high operating voltage (over 100 V) and also semi-conductor layer in OFET [18]. However, the anthracene tends to recrystallize with diode operating time, which led to a degradation of device performance. But, actually, the anthracene based-polymers show the most greatly reported in organic electronics [19-23].

While, only few works were done on anthracene use in chemical or biochemical sensor [24,25]. However, Poly (*p*-phenylenesulfide) (PPS) is a high-performance thermoplastic with many desirable characteristics, such as thermal behaviour, oxidative and chemical resistance [26]. Also, it exhibits a greater electrical conductivity ( $>10$  S.cm<sup>-1</sup>), when doped with AsF<sub>5</sub> [16]. In fact, the anthracene-based polymer analogue of poly (phenylene sulfide) (PAnS) is soluble, exhibits a good thin film quality and good thermal stability. The electrochemical gap determined by cyclic voltammetry measurements was about 2.34 eV. In this contribution, we reported the glucose oxidase enzyme immobilization based on soluble semi-conducting polymer based on anthracene (PAnS). This elaborated bioelectrode was evaluated by electrochemical impedance spectroscopy measurement (EIS) and cyclic voltammetry (CV).

## Experiments

### Material and methods

The glucose oxidase (from *Aspergillus niger*, E.C. 1.1.3.4) were purchased from Sigma Aldrich France. Chemical Glucose was obtained from Beijing Chemical Reagent and the stock solution of glucose was allowed to mutarotate at room temperature overnight before every use.

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( $K_4Fe(CN)_6 \cdot 3H_2O$ ) Potassium ferrocyanidetrihydrate, ( $K_3Fe(CN)_6$ ) potassium ferricyanide disodium hydrogenorthophosphate, Chloroform was purchased from Aldrich and potassium dihydrogen orthophosphate were obtained from Sigma Aldrich France. Hydrogen peroxide (30%) was received from Prolabo France. All reagents were of analytical grade. Phosphate buffer solutions (PBS) ( $0.16 \text{ mol L}^{-1}$ , pH 7.2) prepared with  $NaH_2PO_4$  and  $Na_2HPO_4$  were used as supporting electrolyte. Pure water was employed throughout, which was obtained using a Millipore Q water purification apparatus (resistivity over  $18 \text{ M}\Omega\text{cm}$ ). Anthracene-based semi-conducting polymer analogue of poly(phenylene sulfide)(PPS) was used to elaborate a biosensor. The synthesis and chemical properties have been reported previously [16]. The chemical structures are shown in Figure 1.

Electrochemical measurements were carried out using a Volta Lab 40 (PGZ 301, Villeurbanne, France) with standard three-electrode configuration. A platinum counter electrode was used as the counter electrode, a saturated calomel electrode  $Hg/Hg_2Cl_2/KCl(+0.248 \text{ V vs. SHE})$  as the reference electrode, and the gold electrode as the working electrode, placed in a Faraday cage at room temperature. Two electrochemical techniques were applied:

- Cyclic voltammetry (CV) performed in  $5 \text{ mM } [Fe(CN)_6^{3-/4-}]$  redox probe in PBS solution ( $0.16 \text{ M}$ , pH 7.2) at a scan-rate of  $50 \text{ mV s}^{-1}$ .
- Electrochemical impedance spectroscopy (EIS) based upon inducing a disturbance in the electrochemical reaction from its steady-state by applying an alternating potential with amplitude of  $\sim 450 \text{ mV}$  at the frequency range from  $0.1 \text{ Hz}$  to  $100 \text{ kHz}$ .

The impedance data were represented in the complex impedance plot (Nyquist plot), taking into account the electrode surface ( $0.07 \text{ cm}^2$ ), and the fitting program of Z view was employed to analyze the impedance spectra using an appropriate Randles equivalent electrical circuit.

- Contact angle measurements were carried out by a GBX-Digidrop Scientific Instrument (Romans, France) to examine the hydrophilic/hydrophobic nature of the various modification steps of the surface by the static sessile drop method.

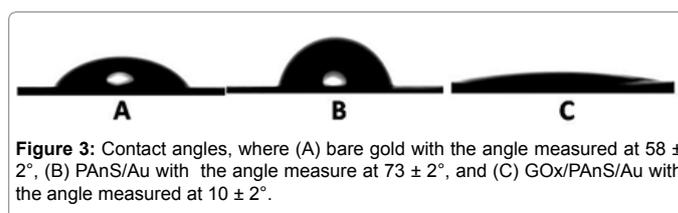
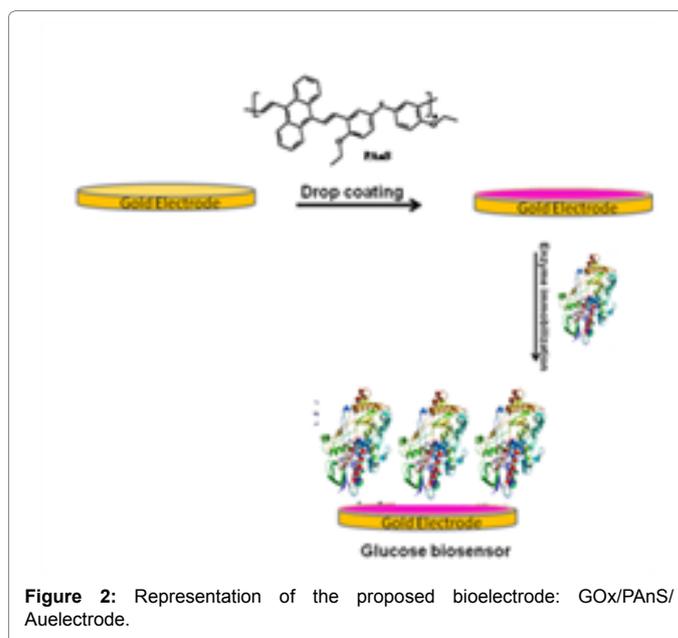
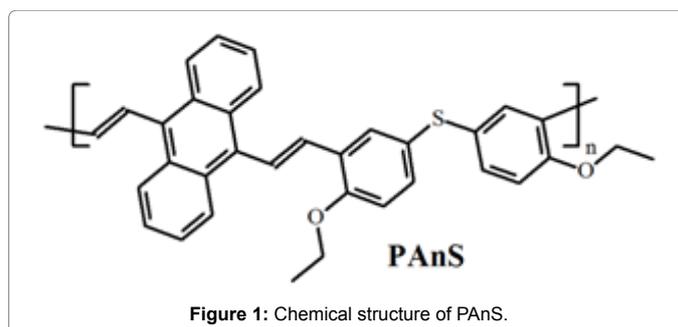
### Electrode modification and immobilization of the enzyme glucose oxidase

As a first step of experiment, the working gold electrode was ultrasonically cleaned by acetone, ethanol, and distilled water for 20 min, respectively. Then, this electrode was put it in Piranha solution ( $3:7 \text{ v/v}$  of  $H_2O_2$  and  $H_2SO_4$ ) during 3 min. Finally, the gold surface were washed extensively with water, absolute ethanol and dried under a stream of nitrogen. The ( $2.10^{-2} \text{ M}$ ) PAnS solution was prepared in chloroform; the film was spin-coated onto a gold substrate and using  $3000 \text{ tr.min}^{-1}$  speed. Then, the GOx enzyme ( $1 \text{ mg/mL PBS}$ ) was deposited by drop coating in prepared surface (PAnS/Au electrode) (Figure 2).

## Results and Discussion

### Enzyme immobilization in a PAnS film

**Wetting properties:** The wettability of the bare gold and modified electrodes (PAnS/Au and GOx/PAnS/Au) was evaluated using contact angle measurements. Figure 3 shows the increase in the contact angle for PAnS/Au ( $73^\circ$ ) in comparison with the bare gold electrode ( $58^\circ$ ). This result indicates the hydrophobe property of thin film polymer. On the other hand, we noted the decrease in contact angle ( $10^\circ$ ) following the modification of the surface by the enzyme glucose oxidase. This result



proves that the surface became more hydrophilic by immobilization of GOx in the modified surface. In fact, we can suggest that semi-conducting polymer film provides an attractive matrix for effective immobilization of glucose oxidase.

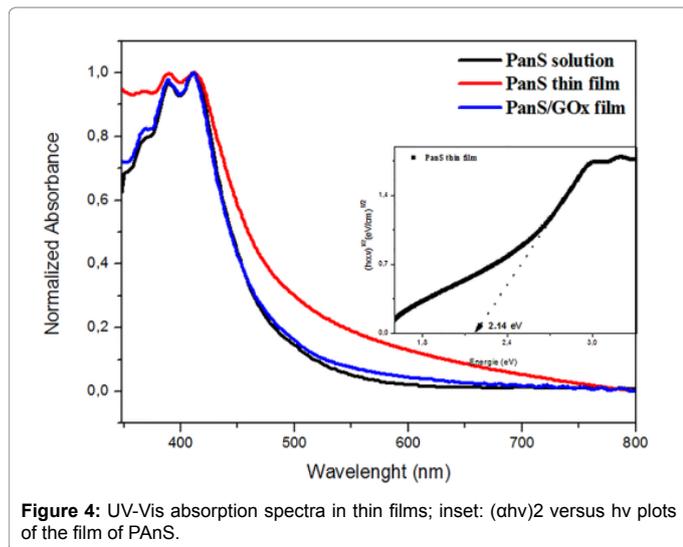
**UV-Vis absorption:** The optical characteristic of PAnS and PAnS/GOx was investigated by spectroscopy UV-vis absorption. This analysis was determined in dilute solution ( $5.10^{-5} \text{ mol.L}^{-1}$ ) and in thin film layer. The Table 1 summarizes the obtained optical data. The optical band gaps ( $E_g$ ) of PAnS film was estimated from the absorption edges, using the Tauc relation [27].

$$(\alpha hv) = A (hv - E_g)^{1/2}$$

Where  $hv$  is the absorbed photon energy and  $\alpha$  is the corresponding absorption coefficient. The  $E_g$  value was obtained also from extrapolation to  $\alpha = 0$  of the straight line portion in the  $(\alpha hv)^2$  versus  $hv$  plot (Figure 4); it was found  $2.14 \text{ eV}$ . In solid state, the PAnS and PAnS/GOx spectra showed the same feature with one shoulder and

	Dilute solution				Thin Film		
	$\lambda_{max}$ (nm)	$\epsilon_{max}$ ( $10^4 \cdot M^{-1} \cdot cm^{-1}$ )	FWHM <sup>(b)</sup> (nm)	$\lambda_{seuil}$	$\lambda_{max}$	FWHM <sup>(b)</sup> (nm)	$\lambda_{seuil}$ (nm)
PAnS	364 <sup>a</sup> ; 386; 409	0.10; 0.16; 0.18	106	482	368 <sup>a</sup> ; 389; 411	149	507
PAnS/GOx					364 <sup>a</sup> ; 386; 409	106	482

**Table 1:** UV-visible absorption data for PAnS and PAnS/GOx (a) Shoulder (b) Spectrum full width at half maximum.

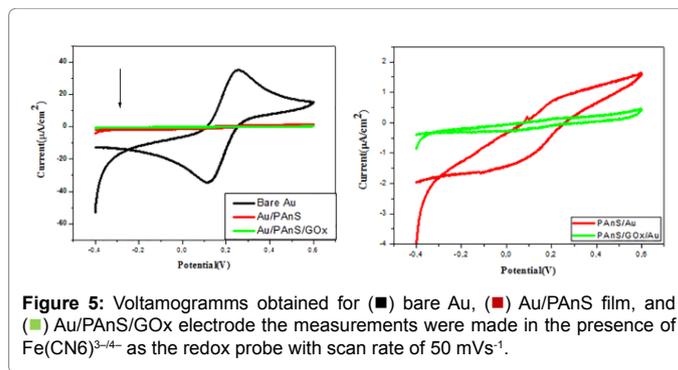


**Figure 4:** UV-Vis absorption spectra in thin films; inset:  $(nh\nu)^2$  versus  $h\nu$  plots of the film of PAnS.

tow maxima. Nevertheless, a narrower absorption spectrum was obtained for PAnS/GOx, in comparison with PAnS (Figure 4). In fact, this behaviour can be explained mainly to the minimization of  $\pi$ - $\pi$  interactions between conjugated systems in free polymer (PAnS). For this result, we can confirm the incorporation of enzymes between the macromolecular structures; which limits the implement of conjugated segments. The thin film absorption spectrum of PAnS/GOx is almost identical with that of PAnS in dilute solution (Figure 4), indicating that the GOx group removes the interchain interactions by solvent effect.

**Electrochemical characterization:** The electrochemical measurement of materials was carried out by cyclic voltammetry (CV) mainly at scan rate of  $50 \text{ mVs}^{-1}$ , in three electrodes set up (platinum sheet counter electrode and Hg/Hg<sub>2</sub>Cl<sub>2</sub>/KCl reference electrode, and Au as work electrode). The surface characterisation was investigated in aqueous solution of 0.16 M PBS (pH 7.2) containing 5 mM Fe(CN)<sub>6</sub><sup>3-/4-</sup>. The measurements were performed within the potential range of -0.4 V to 0.6 V. The electrolyte was degassed with N<sub>2</sub> for 9 minutes, in order to saturate the solution. All measurements were performed at room temperature. The superposed voltammograms of (bare Au, PAnS/Au film, and GOx/PAnS/Au electrode) were shown in Figure 5. In comparison with bare electrode voltammogram, the peak current of polymer modified gold electrode was considerably decreased (34.24  $\mu\text{A}$ ). This behaviour can be attributed to the increase of effective surface area, which indicates the surface modification by PAnS film. The Figure 5 shows an attenuation of the peak current of modified electrode by enzyme GOx compared by their analogue not modified (PAnS/Au). This decrease of the current confirms the immobilization of biomolecule (GOx) on the surface.

Electrochemical impedance spectroscopy (EIS) values were performed using the standard Randles' equivalent circuit.  $R_s$ ,  $C_{dl}$ ,  $R_{CT}$



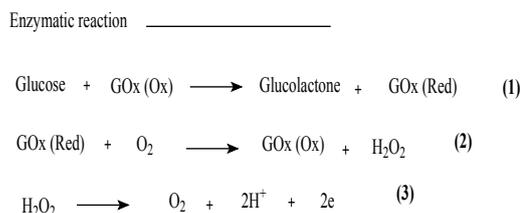
**Figure 5:** Voltammograms obtained for (■) bare Au, (■) Au/PAnS film, and (■) Au/PAnS/GOx electrode the measurements were made in the presence of Fe(CN)<sub>6</sub><sup>3-/4-</sup> as the redox probe with scan rate of  $50 \text{ mVs}^{-1}$ .

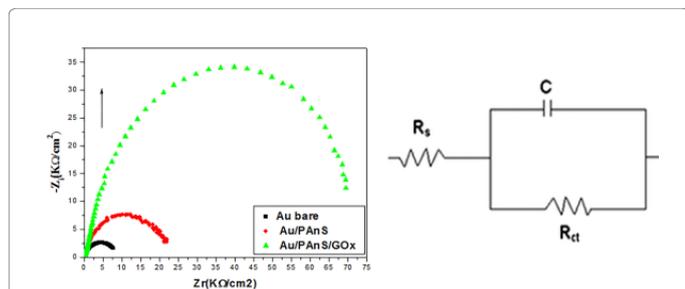
represent, respectively, the ohmic resistance of electrolyte, the double layer capacitance, and the charge-transfer resistance of the redox probe. The Faradic impedance measurements are in good agreement with CV measurements. Figure 6 shows in the impedance spectra Nyquist plots, the semi-circle portion corresponds to the electron transfer resistance at the higher frequency range which controls the electron transfer kinetics of the redox probe at the electrode surface decrease after each modification. All values of  $R_{CT}$  are given after taking the electrode surface ( $0.07 \text{ cm}^2$ ) into account.

The different processes occurring in the electrode/electrolyte interface can be modeled by the building of an equivalent electrical circuit; the recovery rate proves the effectiveness of the amendment procedure since the conducting polymer with the enzyme covering almost the entire surface of the electrode. Without the polymer layer, enzyme molecules could not be fixed onto the gold electrode surface, hence no current values could be recorded.

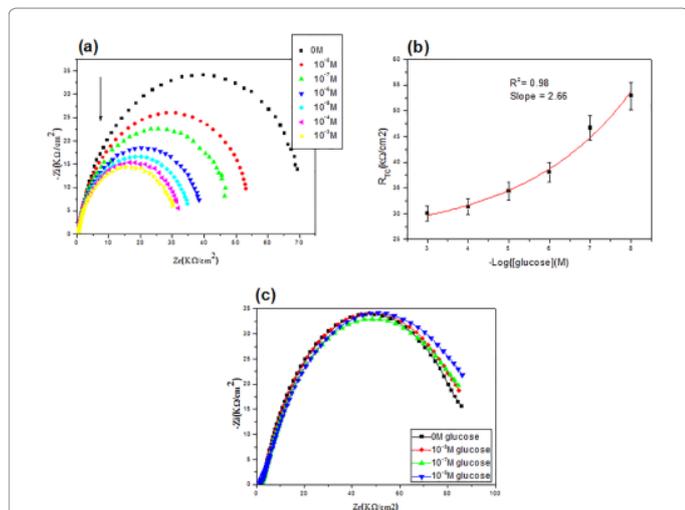
### Effect of glucose on PAnS/GOx/Au biosensor

The electrochemical measurement of materials was carried out by Electrochemical Impedance Spectroscopy (EIS) at potential (-450 mV). The surface characterization was investigated in aqueous solution of 0.16 M PBS (pH 7.2). Figure 7a shows in the impedance spectra Nyquist plots the relationship between the real and imaginary components of complex impedance of the GOx-PAnS modified electrode after adding the different glucose concentrations. The spectra are dominated by the presence of capacitive lines. They comprise a high frequency intercept on the real  $Z_r$  axis and the beginning of a semicircular arc across the high to low frequency range. The high frequency intercept is representative of a combination of electrolyte ionic resistance and the resistance of the contacts to the potentiostat. This decrease in  $-Z_i$  is attributed to the transmission of the glucose to gluconic acid and H<sub>2</sub>O<sub>2</sub> in the presence of GOx enzyme. To more explain that, glucose oxidase (GOx) is a type of oxydo-reductase enzyme that catalysis the oxidation of glucose to hydrogen peroxide and D-glucono-d-lactone, [28,29] as described by the reaction given in the following equations: GOx enzyme used with a conductive material as sensing electrode can detect glucose through the reduction/oxidation of electrode by H<sub>2</sub>O<sub>2</sub> (Eq. (3)) formed during the reaction given in Eqs. 1 and 2 [30].





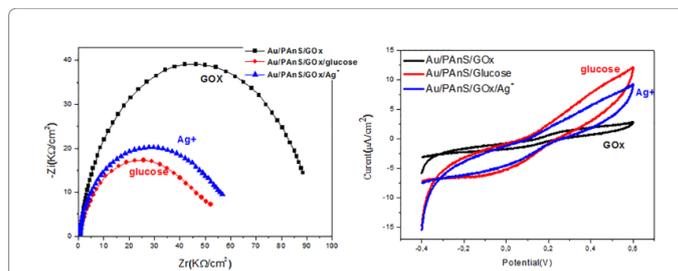
**Figure 6:** Nyquist plots obtained for (■) bare Au, (■) Au/PANs and (■) Au/PANs/GOx electrodes. The inset scheme shows the standard Randles' equivalent circuit.



**Figure 7:** (a) Nyquist plots obtained for increasing concentrations of glucose; without glucose (■),  $10^{-8}$  M (■),  $10^{-7}$  M (■),  $10^{-6}$  M (■),  $10^{-5}$  M (■), and  $10^{-4}$  M (■),  $10^{-3}$  M (■). (b) Relative variation in  $R_{CT}$  with logarithmic concentration of glucose. (c) Diagram of Nyquist at  $-450$  mV potential in PBS solution (0.1 M) in the absence of GOx.

The impedimetric responses are presented in a linear relationship between the relative variation of  $R_{CT}$  and the logarithmic value of glucose concentrations in the range of  $10^{-8}$  M to  $10^{-3}$  M. The slope of the averaged dataset was determined to be  $2.66$  ( $K\Omega \cdot M^{-1} \cdot cm^2$ ) of glucose. This relationship has an associated R-squared value of 0.98. In order to ensure that the decrease of the  $R_{TC}$  in Figure 7a due to the enzymatic reaction of glucose and glucose oxidase, we repeated the same experiment in the absence of the enzyme (PANs/Au electrode), the Nyquist impedance spectra Figure 7b indicated the absence of the variation of  $R_{TC}$  value after adding different glucose concentrations, which indicate that the glucose was not interacting with the polymer. As shown in Figure 7c, the device has a linear current response to the logarithm of glucose concentration from  $10^{-8}$  M to  $10^{-3}$  M. In the other hand, the typical saliva glucose levels are in the range of  $8.10^{-6}$  M to  $0.21 \cdot 10^{-3}$  M, [31] which indicate that our sensor is effectively and reliably determine glucose levels in saliva.

**Inhibition study:** The activity of GOx enzyme can be inhibited by the binding of specific small molecules and ions such as ( $Ag^+$ ,  $Cd^{2+}$ ) [32]. In the first step, following stabilization of the baseline, a defined concentration ( $10^{-3}$  M) of glucose was added. The choice of the glucose concentration was determined by the calibration curve between the electron transfer resistance and different concentrations of glucose before adding the inhibitor. Inhibitor solution of  $Ag^+$  ( $1 \mu M$ ) is then



**Figure 8:** Diagram of Nyquist at  $-450$  mV potential, Voltammograms at  $50$  mVs $^{-1}$  for Au/PANs/GOx electrode (■); Au/PANs/GOx/glucose electrode (■); Au/PANs/GOx/ $Ag^+$  electrode (■), in PBS solution (0.1 M).

injected (second step) to evaluate the decrease of enzymatic activity. The surface characterisation was investigated by electrochemical measurements (CV) and (EIS) in aqueous solution of 0.1 M PBS (pH 7.2). As shown in Figure 8 the decrease of oxidation peak of current proves the decrease of quantity of detected substrate by the enzyme after injection of the inhibitor. This results confirms the activity of the enzyme glucose oxidase immobilized on the surface of the polymer PANs.

## Conclusion

The elaboration of glucose biosensor based on the immobilization of the enzyme glucose oxidase (GOx) onto an anthracene-based polymer analogue of poly (phenylene sulfide) (PANs)/Gold electrode was evaluated. The hydrophobicity of the bare gold and modified electrodes (PANs/Au and GOx/PANs/Au) was investigated using contact angle measurements. The optical characteristic of the modified surfaces was examined by spectroscopy UV-vis absorption. Results clearly suggest that semi-conducting polymer film (PANs) provides an attractive matrix for effective immobilization of glucose oxidase GOx. The GOx/PANs/Au bioelectrode showed a linear response to glucose in the concentration ranging from  $10^{-8}$  M to  $10^{-3}$  M with a correlation coefficient of 0.98 and a sensitivity of  $2.66$  ( $K\Omega \cdot M^{-1} \cdot cm^2$ ). The fabrication of the glucose sensor based on (PANs) is inexpensive, fast and simple. The biosensor seems promising for being applied in environmental analysis.

## Acknowledgments

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