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Direct Electrochemical DNA Sensor based on a new redox oligomer modified with ferrocene and carboxylic acid: Application to the detection of *Mycobacterium tuberculosis* mutant strain

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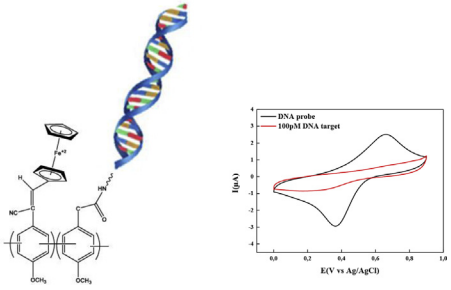
HIGHLIGHTS

- Synthesis of new redox polymer modified with ferrocene groups and carboxylic acids as functional groups in side chains.
- The functional groups give the flexibility for direct covalent attachment of biomolecules.
- The redox indicator was used for monitoring the electrochemical behavior of DNA detection.
- Biosensor has showed a good sensitivity to DNA probe of Hepatitis C and PCR samples of DNA from *Mycobacterium tuberculosis*.

GRAPHICAL ABSTRACT



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ABSTRACT

A new redox oligomer "oligo-methoxy-phenyl-acetonitrile" (Fc-acid-OMPA) modified with ferrocene groups and carboxylic acids as functional groups in side chains, has been synthesized. The redox indicator has been used for monitoring the electrochemical behavior. The functional groups give the flexibility for direct covalent attachment of biomolecules. The electrochemical properties of the redox oligomer film deposited on gold electrodes have been studied by cyclic voltammetry (CV), which showed a rate of electron transfer of 6.43 s^{-1}. The oligomer has been studied as a transducer for electrochemical DNA sensing and for this purpose the acid functional group of Fc-acid-OMPA was attached with the DNA probe of hepatitis C bearing amino group in 5′ position through amid link. The efficiency of DNA attachment on the oligomer film has been analyzed by X-Ray Photoelectron Spectrometry (XPS) and FT-IR spectroscopy. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) have been used to analyze the biosensor construction and DNA detection. A wide linear range of detection from 1 fM to 100 pM have been demonstrated with a limit of detection (LOD) of 0.2 fM. The biosensor has showed an appreciated sensitivity to PCR samples of genomic DNA from *Mycobacterium tuberculosis*, and has been able to detect a single mutation which confers resistance of *M. tuberculosis* to rifampicin drug.

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1. Introduction

The detection of specific DNA sequences is important in the fields of biochemistry and life science. Electrochemical biosensors [1,2], are among the methods for DNA detection. They combine the analytical ability of electrochemical methods with the specificity of the biological recognition process. Thanks to their remarkable sensitivity, compatibility, inherent miniaturization and low cost, modern electrochemical DNA biosensors are extremely attractive for obtaining the sequence specific information in a simpler, faster, and cheaper manner than traditional methods [3]. Electrochemical DNA biosensors are based on the immobilization of an oligonucleotide on the electrode surface and the detection through hybridization process. The viability of DNA biosensors requires an intimate connection between the nucleic acid system and the electronic transducer. Several methods of biolayer immobilization on the transducer surface have been used for the preparation of DNA sensors, including chemical adsorption [4,5] and covalent bonding [6–8]. In this respect, the modified transducer with polymer can provide a suitable interface for DNA immobilization. Searching for simpler and faster methods of immobilization, researchers focused their attention on the use of electroactive polymers as a transduction element for DNA hybridization [9–12]. The use of these redox polymers does not require the addition of extra redox indicators because it can directly report the hybridization event with improved sensitivity [13–15]. In fact, the incorporation of inorganic components into the organic polymeric structure has shown promising applications in various fields of chemistry such as catalysis, biotechnology, organic synthesis, electronics and polymers [16]. By incorporating metal complexes into a polymer backbone, the physical and chemical properties of the resulting material may be modified by the metal complexes, while the film forming properties exhibited by the polymer can be retained. Especially, polymers containing ferrocene have been used in chemically modified electrodes, to elaborate electrochemical sensors and other electronic materials [17–19]. Redox active Fc-containing conducting polymers are good candidates to play a key role as multi-electron transfer mediators in the electrochemical response processes of biological and industrial importance [20].

In our previous work [21], we studied a redox polymer, the poly(*p*-phenylene) ferrocene (Fc-PPP), which contains, in side chain, ferrocene centers that are covalently bonded to the poly(*p*-phenylene) skeleton. Through the electrochemical deposition of a linker positively charged on polymer, it has been found that this redox polymer can be used as a DNA biosensor. With the aim of making sensitive biosensors that are easy to implement, the original idea of this current work is to synthesize a new redox oligomer with ferrocene groups and also functional groups in the backbone, such as carboxylic acid groups. This new redox oligomer would allow flexibility for the direct covalent attachment of biomolecules without prior chemical modification as used in the case of the Fc-PPP polymer. The advantage of carboxylic acids functionalization is to simplify the structure of biosensors and ameliorate their selectivity. Where the negative charge of non-attached carboxylate group present on the polymer chain could prevent non-specific interaction of non-complementary DNA which leads to biosensors able to detect complex anomaly such as single mutation provided in *M. tuberculosis* strand resistance to drugs.

At this stage, the new redox oligomer 'oligo-methoxy-phenyl-acetonitrile' (Fc-acid-OMPA) has been synthesized in our laboratory. Films based on this oligomer were deposited on gold electrodes and have been characterized by various techniques to highlight their properties. The Fc-ac-OMPA was then applied for synthetic *Hepatitis C* DNA sensing where sensitivity and specificity were investigated through the variation of ferrocene signal. To demonstrate the efficiency of DNA attachment on the redox oligomer film, the biosensor has been analyzed by X-Ray Photoelectron Spectrometry (XPS) and FT-IR spectroscopy. The biosensor construction and the hybridization of complementary target (c DNA) and non-complementary target (nc DNA) as well as genomic sample of *M. tuberculosis* and the mutated stand were accomplished by following redox signal of ferrocene measured by cyclic voltammetry and electrochemical impedance spectroscopy.

2. Materials and methods

2.1. Reagents

The oligomer Fc-acid-OMPA (Fc-ac-OMPA) has been prepared by the alkaline hydrolysis of the Fc-OMPA (Scheme 1). The latter (Fc-OMPA) has been synthesized according to reference [22]. The corresponding average chain length was about 5–6 units. The general procedure of the hydrolysis can be summarized as follows: in a 25 ml flask, equipped with refrigerant, 100 mg of Fc-OMPA was placed in ethanol (Sigma Aldrich, 99.8%), and then 2 ml of a freshly-prepared concentrated solution of sodium hydroxide (Sigma Aldrich, 98%) was added. The reaction medium has been maintained under stirring and has been refluxed for 24 Hours. Then, the mixture has been acidified with a concentrated HCl solution (Sigma Aldrich, 37%). A ratio of 65% of the desired product was obtained. The hydrolysis was proved by FTIR spectra given in Fig. 1, where we could observe the appearance of a new large peak at 3378 cm^{-1} corresponding to OH-acid stretching.

All synthetic DNA sequences of *Hepatitis C* were provided by Eurogentec Company (Eurogentec, Liége, Belgium). The DNA probe has 18-bases sequences with six carbon chains (C6) as spacer and with amine group on its 5′ phosphoryl terminus: NH_2-$(CH_2)_6$-5′GAT-ACT-TCT-ATC-ACC-CAT3′.

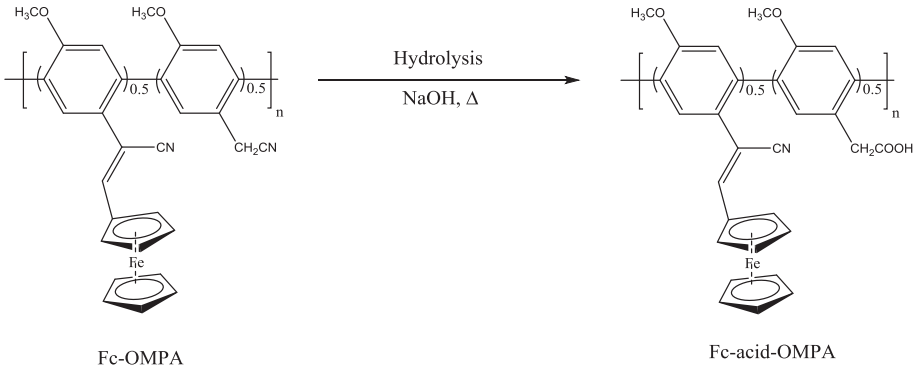
The complementary target oligonucleotides (c DNA): 5′CTA-TGA-AGA-TAG-TGG-GTA3′ The non-complementary target oligonucleotides (nc DNA): 5′ATG-TGG-TAG-AGA-TGA-CTA3′.

The genomic DNA is obtained from Pakistan patients carrying wild and mutant viruses of *Mycobacterium Tuberculosis*. The PCR samples are obtained from "laboratory I2BC" of Prof C. Sola team from the University of Paris-Sud. The DNA probe, specific to the wild strand and mutant of *Mycobacterium tuberculosis* PCR, containing 18 bases with 5′ terminal amino group modification uses a twelve carbonyl chain spacer (C12) with the sequences for a wild type are 5′-CCGACTG(T**C**G)GCGCT-GGG-3′ and the mutated type are 5′-CCGACTG(T**T**G)GCGCTGGG-3′ [23].

These probes for *M. Tuberculosis* targets specifically the wild type and the mutant strand with single nucleotide polymorphism (SNP) T (TCG/TTG) in the codon position 531 of rpoB gene conferring resistance of *M. tuberculosis* to rifampicin drug. The amplification, by PCR, of wild and mutant *M. tuberculosis* has been made as well by the team of Prof C. Sola. This preparation method extracts the bacterium by the lyse. A stage of 35 cycles PCR has been made on the DNA crud to amplify the sequences which correspond to the rpob gene using various cycles of temperatures and two primers: a forward primer with TR1 (5′TACGGTCGGCGAGCTGATCC3′) and a reverse primer TR2 (5′TACGGCGTTTCGATGAACC3′) [24]. The obtained DNA from PCR contains 411 bases. The concentration has been measured following absorbance by Nanodrop 1000. All the solutions have been stored at −20 °C until use.

2.2. Instrumentation

Electrochemical Impedance Spectroscopy (EIS) and cyclic voltammetry (CV) have been used via a Metrohm potensiostat Autolab (PGSTAT 20) supported by Nova software. The measurements were



Scheme 1. Synthesis of the oligomer Fc-acid-OMPA.

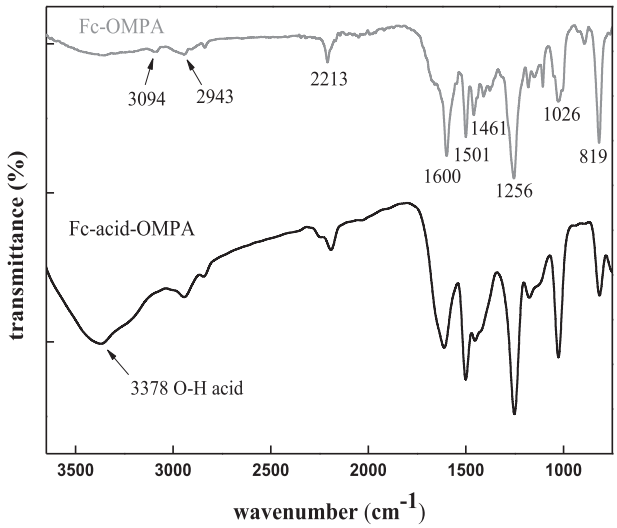


Fig. 1. FTIR spectra of (a) Fc-OMPA and (b) Fc-acid-OMPA.

carried out in an electrochemical cell involving a three-electrode system: a gold disc (surface 2.01 × 10^{-2} cm^2) as a working electrode, a platinum mesh as a counter electrode and an Ag/AgCl as a reference electrode. The electrolyte used for all electrochemical measurements was an aqueous solution containing 0.1 M $LiClO_4$ (pH = 7) at room temperature. CV have been performed with the scan rate of 50 mVs^{-1} by cycling the potential from 0 V to 0.9 V. EIS measurements have been realized with a sinusoidal excitation signal of 10 mV amplitude and a frequency range of 100 kHz to 100 mHz at 0.45 V vs Ag/AgCl.

FT-IR characterizations were obtained using a Bruker IFS66 FT-IR spectrometer (Bruker, Germany) equipped with a Mercury-cadmium-telluride (MCT) detector and an attenuated total reflectance (ATR) germanium crystal.

X-Ray photoelectron spectrometry (XPS) measurements were performed on a K Alpha spectrometer from Thermofisher, using a monochromated X-ray Source (Al Kα, 1486.6 eV). The spectrums obtained were treated by means of the "Avantage" software provided by Thermofisher. Shirley type background subtractions were used.

2.3. Biolayer construction

The gold surface, after polishing steps, has been patterned with 5 μL of the $CHCl_3$ solution of the redox oligomer (concentration: 5 mg/1.5 mL). The redox polymer was patterned onto the gold disc surface by physical adsorption (Scheme 2). The acid functions available on the surface of the oligomer layer allow ssDNA attachment. The electrode has been dipped during 45 min in the solution formed by EDC/NHS (20 mM) and DNA probe (10 μM) in PBS buffer pH = 7.4 at room temperature. In the aim to saturate the non-bonded activated carboxylic acid groups, the electrode has been immersed in 1 μM of ethanolamine (EtOH) solution in a PBS buffer for 30 min at room temperature. Then, this formed biosensor was stored in PBS buffer at 4 °C until use. The hybridization step was performed by the incubation of the biosensor in a range of DNA target concentrations from 1 fM to 100 pM in PBS buffer solution at 40 °C for 1 h.

After these three steps of the biolayer construction the modified electrode was rinsed with double-distilled water. The construction of the biosensor was followed by EIS and CV measurement. The different steps of the biolayer construction and DNA interactions are summarized in Scheme 2.

3. Results and discussion

3.1. Electrochemical properties

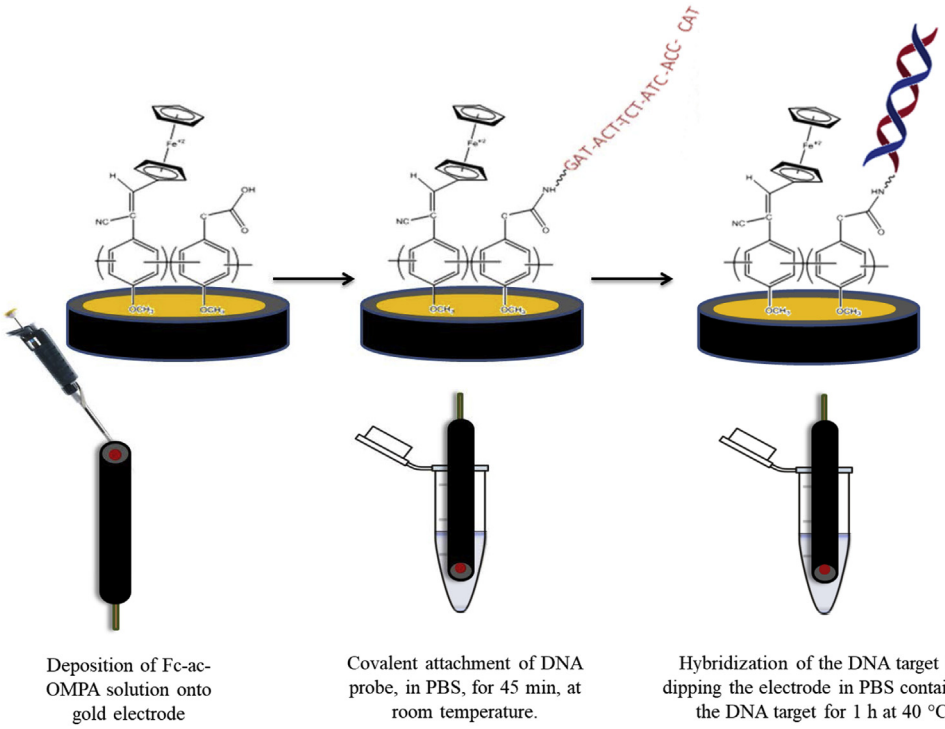
Fig. 2 shows the CV obtained with Fc-ac-OMPA oligomer layer at a scan rate of 50 mVs^{-1}. This figure shows a redox system where the anodic peak potential is obtained at 0.59 V and the cathodic peak obtained at 0.4 V. The ferrocene center attached to the oligomer is related to this redox process. The peak-to-peak separation between anodic and cathodic peaks shows a quasi-reversible behavior of the redox oligomer which is generally obtained in the case of ferrocene attached to a conducting layer [21,25].

The surface coverage (Γ) from CV measurements is calculated following the equation:

$$\Gamma = \frac{Q}{nFA}$$

where Q is the charge under the cathodic or anodic peaks, n is the number of electrons involved in the redox reaction ($n = 1$), A is the gold electrode effective area and F is the Faraday constant (96485 $Cmol^{-1}$). The number of electroactive sites on the oligomer surface has been calculated to be $\Gamma = 4.55 \pm 0.12\ 10^{-7}$ $mol.cm^{-2}$. We can conclude that this new Fc-acid-OMPA oligomer presents a higher density of ferrocene behavior compared to the redox polymer (Fc-PPP) [21].

The kinetics of charge transfer of the redox process on the oligomer membrane has been studied by sweeping the potential from 0 V to 0.9 V and varying the scan rate from 10 mVs^{-1} to 500 mVs^{-1} (Fig. 3). The variation of the peak versus the scan rate or



Scheme 2. Scheme of the biolayer formation: first covalent attachment of DNA through amide link, next hybridization with DNA complementary target.

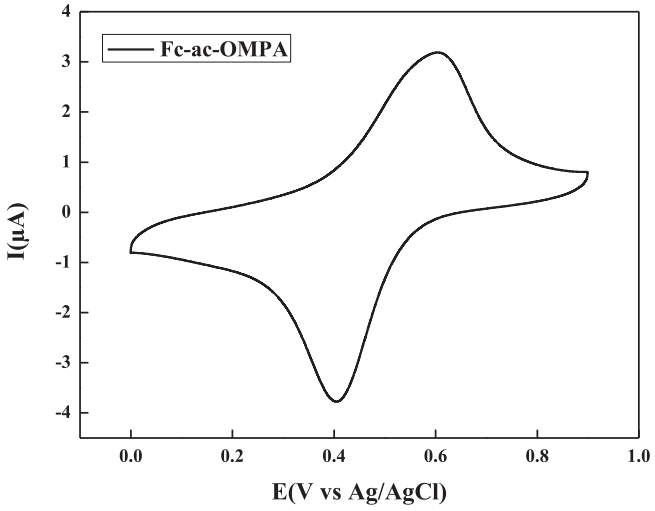


Fig. 2. Cyclic voltammetry of the gold electrode modified with oligomer layer (Fc-acid-OMPA) recorded in solution $LiClO_4$ (0.1 M, pH = 7) at scan rate of 50 mVs^{-1}.

the root of the scan rate gives some insights into the kinetic behavior and the kinetics controlling the electron transfer. Peak current varies linearly with the scan rate at lower speed and then linearly with the root of scan rate at high speed, which underlines that both electron transfer and ionic transfer related to the diffusion of ions through the oligomer control the electrochemical process (Fig. 3 upper inset). This behavior was observed in our previous works [21,26,27].

In addition, to understand how the redox oligomer layer has an impact on the electron transfer process, we have calculated the electron transfer rate constants from the peak-to-peak separation of CVs at different scan rates, *v,* using Laviron's method. This later method used a numerical integration of the rate equations to correlate the standard electron transfer rate constant *ks* with the peak-to-peak separation $\Delta Ep = |E_{pc,a}\text{-}E^0|$ with $E^0=(E_{pc} + E_{pa})/2$. This method uses a dimensionless rate constant *m*. The electron transfer rate constants *ks* can be determined using the equation developed by Laviron [28]:

$$ks = \frac{mn\nu F}{RT}$$

where *R* is the universal gas constant (8.31 J mol^{-1} K^{-1}), *T* is absolute temperature (298 K), *n* is the number of electrons involved in the redox reaction ($n = 1$), *F* is the Faraday constant (96485 C mol^{-1}), and *v* is the scan rate.

This method gives some clues about the effective rate constant of charge transfer as it does take into account some behavior occur during redox process in complex redox system.

The variation of the potentials at anodic peaks with the scan rate from 10 mV s^{-1} to 500 mV s^{-1} was below 200 mV. Since the rate constants are insensitive to α (the charge transfer coefficient) *ks* were calculated directly from Laviron's working curves of m^{-1} versus $n\Delta Ep$ [29]. Using the values measured at 500 mVs^{-1} and the adjustment of the curve $\Delta Ep = f(m^{-1})$ [28], the effective ks value obtained for Fc-acid-OMPA was calculated as 6.43 ± 0.19 s^{-1}. Comparing the ks obtained with that of another redox polymer studied previously (Fc-PPP), the ks was around 5.45 ± 0.39 s^{-1} [21]. This improvement could be explained by the fact that the oligomer Fc-acid-OMPA presents more conjugation due to the substituents on their aromatic chain such as methoxy as well as the substituted groups in side chain.

3.2. Construction and detection of synthetic DNA

Biolayer construction involves covalent attachment of single stranded DNA probes (ssDNA) of *Hepatitis C*. The DNA probe was covalently bonded to the oligomer through a covalent link. The

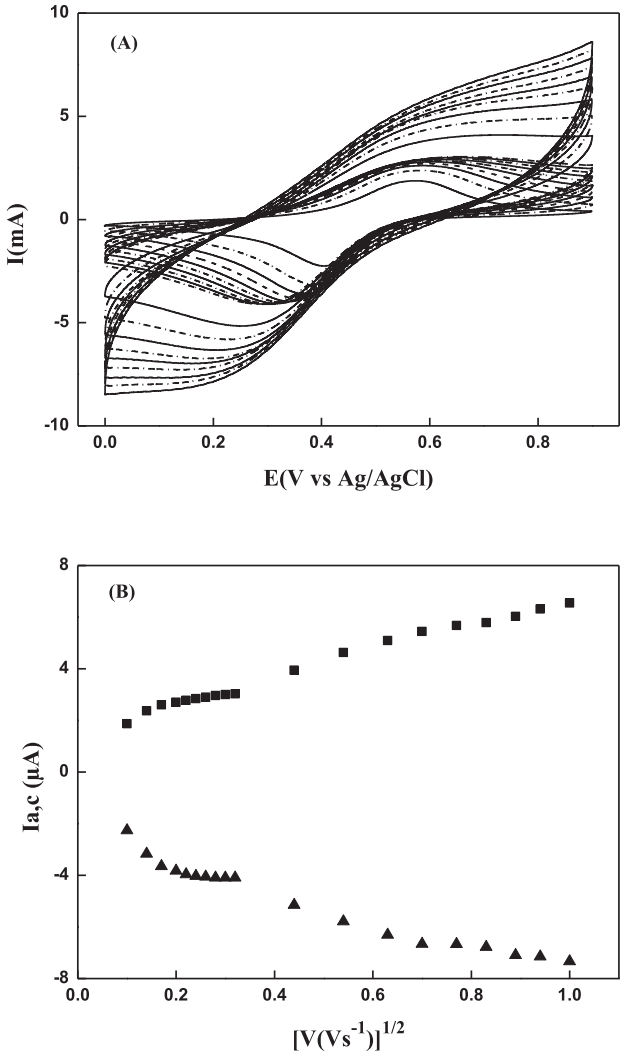


Fig. 3. (A) Cyclic voltammetry of modified gold electrode with Fc-acid-OMPA at various scans in the range of 10 m Vs^{-1} to 1 Vs^{-1}, (B) Variation of anodic and cathodic current peaks vs. root of scan rate.

formation of the electrochemical biosensor was monitored by both cyclic voltammetry (CV) and impedance spectroscopy (EIS) in $LiClO_4$ (0.1 M, pH 7). The CVs performed after each step of biosensor construction show a decrease of current after DNA attachment (Fig. 4A). This is related to the decrease of the charge transfer of the redox centers indicating the immobilization of the molecule of DNA on the surface [30,31]. This slowing down of the electrons transfer is connected to the steric and the electrostatic repulsion between the negatively charged DNA and the conjugated polymer. We also observed a shift of the anodic peak towards the positive potential after the grafting of the biolayer. This could confirm the interaction of the DNA sequence with the polymeric layer by forming a barrier to the transfer of electrons to the electrode [32]. We also calculated the surface coverage of the biolayer through the charge under the cathodic or the anodic peaks. We have obtained a value of $\Gamma = 3.83 \pm 0.08\ 10^{-7}$ mol.cm^{-2}. This value is slightly lower than that of the oligomer alone. This could be related to the negative charge of DNA that could disturb electron transfer during the redox process of ferrocene.

EIS measurements, performed in the frequency range of 100 kHz to 100 mHz at 0.45 V, presented an increase of the impedance after DNA attachment (Fig. 4B). The experimental results were fitted by

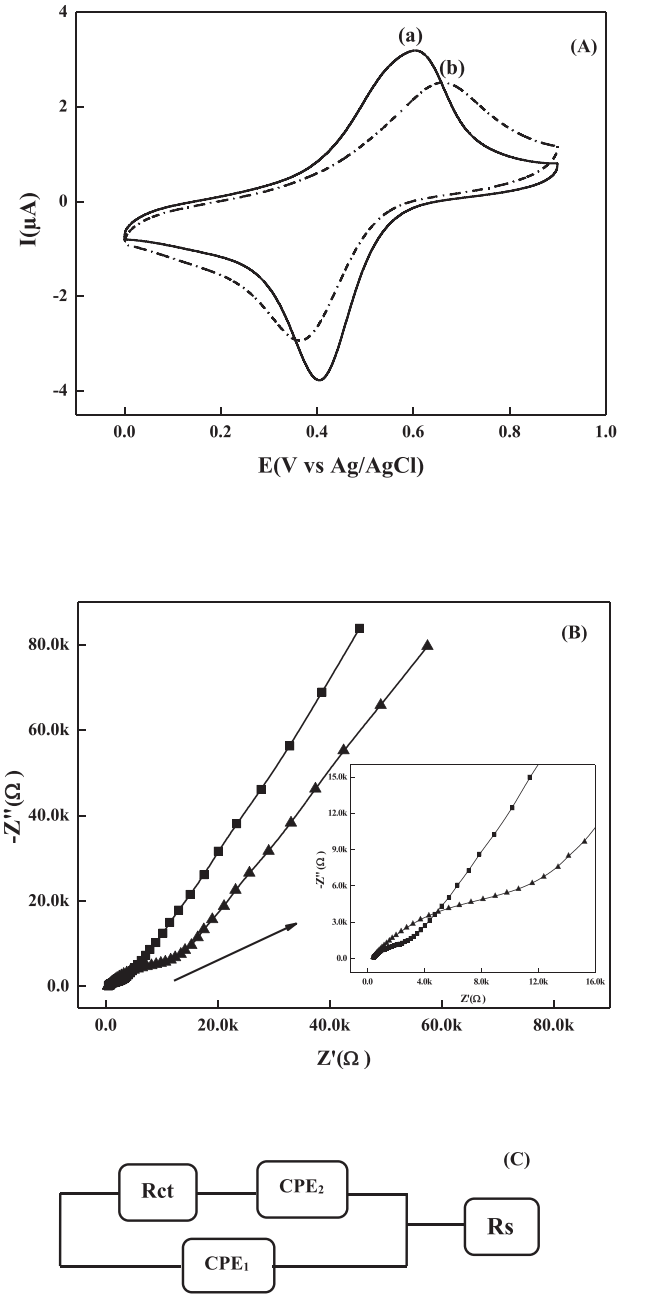


Fig. 4. (A) CV data obtained after each step of biosensor formation: curve a, Fc-acid-OMPA, curve b, Fc-acid-OMPA + DNA. The immobilization of DNA was performed in PBS (pH = 7.4) during 45 min at room temperature. The CVs are recorded in 0.1 M $LiClO_4$ (pH = 7) with scan rate of 50 mVs^{-1}.(B) Nyquist plots of the biolayer construction recorded at 0.45 V vs Ag/AgCl at Dc potential of 10 mV in the frequency range of 100 kHz to 100 mHz. (■) Fc-acid-OMPA, (▲) Fc-acid-OMPA + DNA. Inset is the zoom of diagram at lower frequency range. The points presenting the experimental data and the line is the fit obtained with the equivalent circuit shown below. (C) Randles equivalent circuit.

Randles equivalent circuit [33] presented in Fig. 4C, formed by the electrolyte resistance (R_s), the charge transfer resistance at the membrane/electrode interface (R_{ct}), the constant phase element (CPE_1) represents the double layer capacitance and the constant phase element (CPE_2) is related to the space charge capacitance in the polymer membrane. The fit results were presented in Table 1. The R_{ct} value after biolayer construction shows an increase after DNA attachment. This may be due to the biofilm blocking effect.

Table 1
Fitting parameters circuit of Fc-acid-OMPA membrane and biolayer construction.

	Fc-ac-OMPA	Fc-ac-OMPA +DNA	+100pM cDNA	+100pM ncDNA
Rs (Ω)	324.7	345.8	338.2	322.8
(RDS%)	(0.38%)	(0.38%)	(0.24%)	(0.32%)
CPE_1(μF)	Q = 1.5	Q = 3.036	Q = 1.317	Q = 3.21
(RDS%)	(5.6%)	(4.2%)	(4.6%)	(1.5%)
	n = 0.72	n = 0.60	n = 0.81	n = 0.62
	(0.5%)	(0.5%)	(0.5%)	(0.5%)
R_{ct} (kΩ)	6.05	16.82	60.19	48.21
(RDS%)	(5.44%)	(10.24%)	(3.81%)	(11.59%)
CPE_2(μF)	Q = 11.32	Q = 11.9	Q = 11.46	Q = 10.53
(RDS%)	(5.7%)	(4.6%)	(7.5%)	(1.8%)
	n = 0.67	n = 0.67	n = 0.15	n = 0.7
	(0.5%)	(0.5%)	(0.5%)	(0.5%)
χ^2	0.040	0.052	0.33	0.075

Indeed, when a large molecule (biofilm) is immobilized on the surface, it affects the permeability of the film and changes the electronic properties of the transducer in our case the charge transfer of ferrocene [34].

Molecular characterization of the biolayer construction was performed following FT-IR and XPS analysis in the aim to confirm the immobilization of the DNA probe on the polymeric surface by identifying the nature of chemical links. Fig. 5A shows the grafted DNA spectrum on the oligomer and highlights the existence of five major peaks corresponding to the following elements: Fe at 709 eV, C at 287 eV, O at 537 eV, N at 400 eV and P at 136 eV. After the grafting of DNA probe, we observe the broadening of Nitrogen (N) peak located at 400 eV (Fig. 5B). This is due to the grafting of DNA which is rich in amino groups in its chemical structure [35]. Additionally, Fig. 5C shows the appearance of a new peak at 136eV corresponding to the phosphorus (P) which is present only in DNA. The presence of these two main peaks indicates the good grafting of DNA on the polymeric membrane. The infrared spectrum of Fc-acid-OMPA oligomer deposited on a gold electrode before and after the grafting of DNA is presented in Fig. 6. The spectrum shows a refinement of the peak at 1700 cm^{-1} which is attributed to a change in the stretching of the carbonyl (C═O). The spectrum also shows the emergence of a new intense peak at 3500 cm^{-1} (NH-stretching) attributed to the DNA.

3.3. Hybridization and specificity

Hybridization reactions have been performed with a complementary target (c DNA) with 18 bases. The electrochemical detection of DNA targets was directly monitored by the ferrocene redox signal. Fig. 7A exhibits the CV plots after hybridization with the c DNA in a wide concentrations range from 1 fM to 100 pM. The redox current decreases while increasing the DNA target concentrations. In fact, hybridization reaction leads to significant change in the rigidity of the polymer, leading to a decrease of intrinsic conjugation. This change of structural conformation of the polymer backbone should modify its electrical parameters such as the resistance

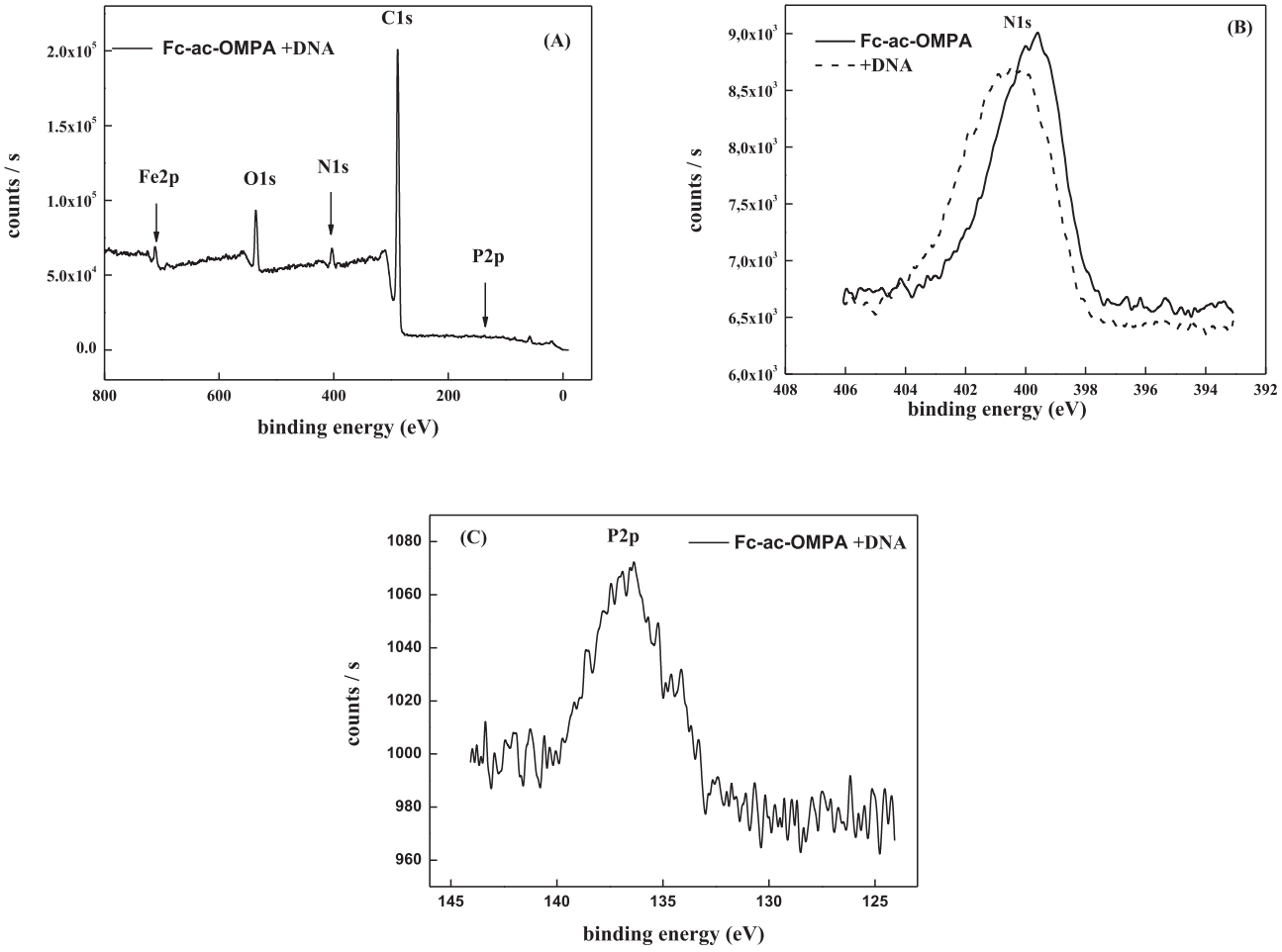


Fig. 5. XPS spectra of (A) full spectrum of Fc-acid-OMPA + DNA, (B) N1s spectra, curve a, Fc-acid-OMPA, curve b, Fc-acid-OMPA + DNA, (C) P2p spectra.

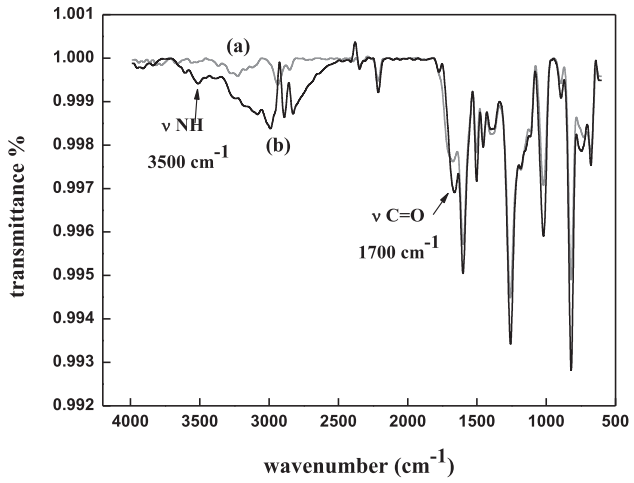


Fig. 6. FTIR spectra of (a) Fc-acid-OMPA and (b) Fc-acid-OMPA + DNA.

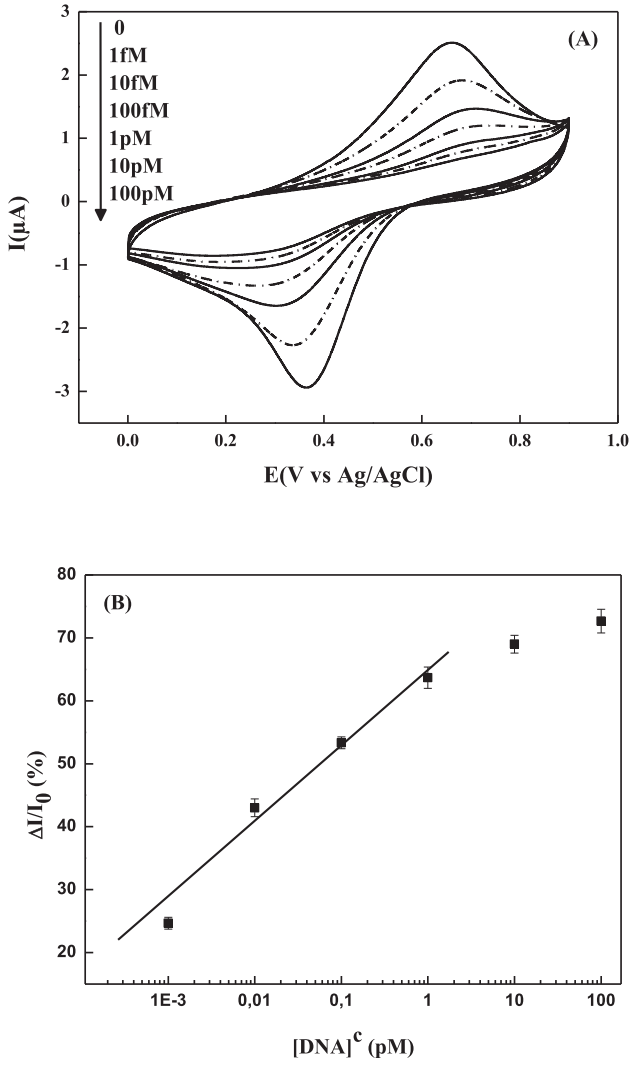


Fig. 7. (A) CV of biosensors obtained after DNA target hybridization at various concentrations from 1 fM to 100 pM, performed in PBS (pH = 7.4) during 1 h at 40 °C and recorded in 0.1 M $LiClO_4$ (pH = 7) with scan rate of 50 mVs^{-1}. (B) Plot of the calibration curve presenting the relative variation of the current peak versus concentration of DNA target at logarithm scale. I_0 is the peak current corresponding to DNA probe response and ΔI is the response of each incubation of DNA target concentration.

of electron transfer and the conductivity [36]. An attenuation of 24% on the intensity of oxidative current is obtained after hybridization with 1 fM of the DNA target, which demonstrates the high sensitivity of our biosensor.

On the basis of the changes of the current signal obtained by CV during the detection of DNA targets, the calibration curves represented in logarithmic scale were plotted (Fig. 7B). Calibration curves plotted from these results exhibit a dynamic range of detection from 1 fM to 1 pM. The limit of detection (LOD = 0.2 fM) is determined by basing on signal-to-noise ratio (S/N) of 3. The biosensor reproducibility was tested by three independent measurements. The standard deviation for these three independent measurements was calculated as 0.94–1.88% demonstrating a high reproducibility of the biosensors. The value of LOD seems promising compared to other biosensors based on redox polymers where they obtained a limit of detection about 0.78 mM [14] and 1pM [37]. Also this LOD is better compared to the LOD value obtained for biosensors using conducting polymer without redox mediator on its chain (LOD = 0.2 pM) [38]. This result accentuates the novelty of this Fc-ac-OMPA polymer which is synthesized directly with the ferrocene mediator that will directly read the electrical signal of the system. In addition, the carboxylic acid groups substituted on the polymer chain allow the detection of the DNA without any further steps as indicated in other redox polymer or conducting polymer.

The nonspecific interactions have been studied by incubating the biosensor in a non complementary DNA (nc DNA) targets bearing 18 bases. Fig. 8A and B shows the results of CV and EIS analysis obtained after the incubation of the sensor in a solution of c DNA and nc DNA targets with the same concentration of 100 pM. Fig. 8A shows a slight variation in current 12% after reaction with nc DNA target, while the incubation with complementary DNA caused a large variation of 70%. This demonstrates the high specificity of the biosensors. We have also observed through EIS measurement an increase of the charge transfer resistance value in the case of hybridization with the complementary DNA (Rct = 60.19 kΩ) while the resistance of the non-complementary (nc DNA) was lower (Rct = 48.21 kΩ) (Fig. 8B). These later results confirm cyclic voltammetry results and prove the specificity of our biosensor based on a new redox oligomer Fc-ac-OMPA.

3.4. Test of clinical samples of drug resistant *Mycobacterium tuberculosis* detection

This biosensor has been also applied for the detection of DNA from the pathogen agent of *Mycobacterium tuberculosis*. The same detection conditions as the synthetic hepatitis C DNA were used for *M. Tuberculosis* samples. Firstly, the *M. Tuberculosis* probe (1 μM) was denaturized by being heated at 95 °C for 4 min. Then the DNA was anchored on the modified redox oligomer electrode. This anchorage step was also followed by a blocking step of the non-active sites with ethanolamine. The hybridization reaction with either wild sequence (c M.T) or with the mutant sequence (nc M.T) has been performed separately with the concentration of 10 fM. The biosensors have been then analyzed using CV and EIS techniques and the results were shown in Fig. 9A and B. Hybridization reaction with c M.T sample has led to a change in the ferrocene redox current with a variation of 33%. The resulting Nyquist of c M.T, after fitting with Randles circuit, present a charge transfer resistance of about 148.80 kΩ. Incubation with the mutant nc M.T sample, the redox current has shown a smaller variation of 20% and a charge transfer resistance having a value of Rct = 111.53 kΩ. This signal decreases after the *Mycobacterium tuberculosis* strain hybridization and shows that our biosensor based on the new redox oligomer Fc-ac-OMPA is able to discriminate the hybridization of wild samples and mutant samples which differ by a single mutation (T**C**G/T**T**G).

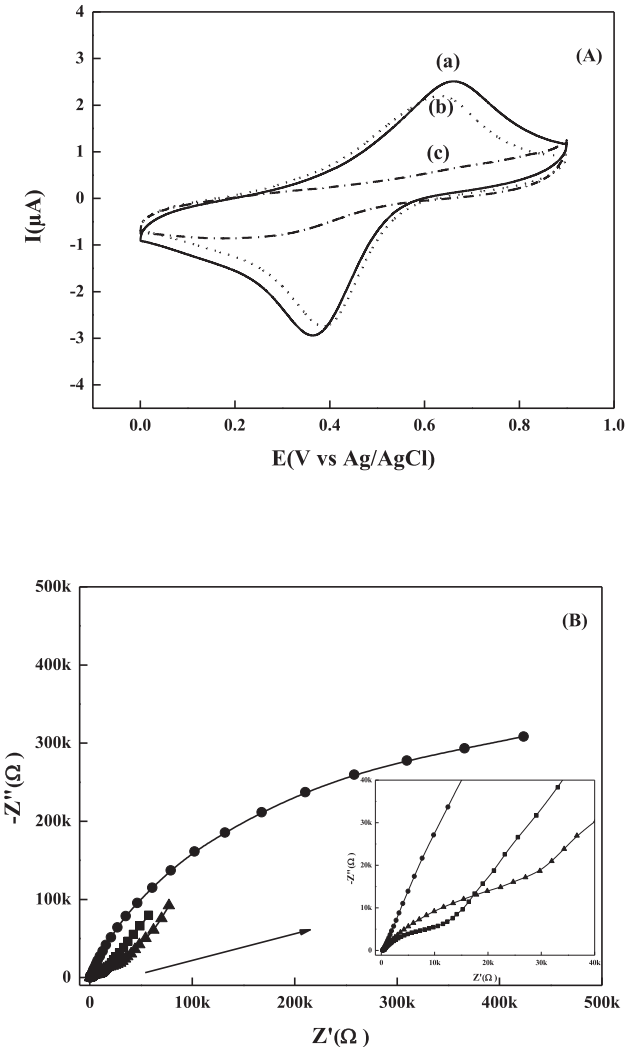


Fig. 8. (A) CV presents the results obtained for non-specific interactions compared to specific one. Curve (a) Fc-acid-OMPA + DNA, curve (b) Fc-acid-OMPA + DNA after incubation in solution of 100 pM of non-specific DNA, and curve (c) Fc-acid-OMPA + DNA after incubation in 100 pM of DNA target. CVs are recorded in 0.1 M $LiClO_4$ (pH = 7) with scan rate of 50 mVs^{-1}. (B) Nyquist plots recorded at 0.45 V vs Ag/AgCl in the frequency range of 100 kHz to 100 mHz. (■) Fc-acid-OMPA + DNA, (▲) Fc-acid-OMPA + DNA+100pM ncDNA, (●) Fc-acid-OMPA + DNA+100pM cDNA. Inset is the zoom of diagram at lower frequency range. The points presenting the experimental data and the line is the fit obtained with the equivalent circuit shown below.

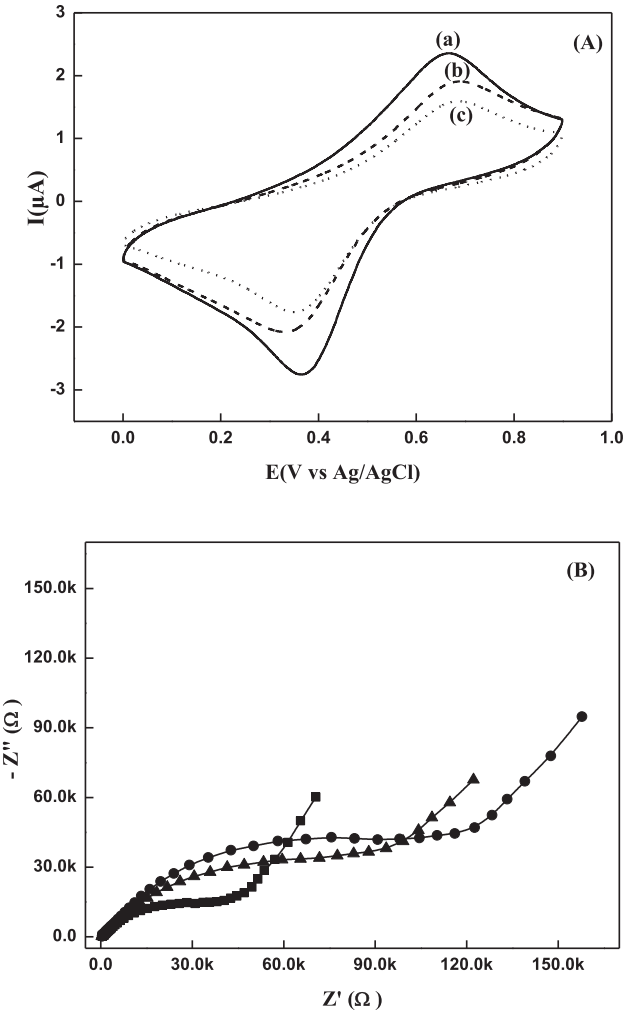


Fig. 9. (A) CV presents the results obtained for mutant and wild M.T interactions. Curve (a) Fc-acid-OMPA + M.T probe, curve (b) Fc-acid-OMPA + probe + ncM.T after incubation in solution of 10 fM of mutant M.T, and curve (c) Fc-acid-OMPA + probe + cM.T after incubation in 10 fM of wild M.T. CVs are recorded in 0.1 M $LiClO_4$ (pH = 7) with scan rate of 50 mVs^{-1}. (B) Nyquist plots recorded at 0.45V vs Ag/AgCl in the frequency range of 100 kHz to 100 mHz. (■) Fc-acid-OMPA + M.T probe, (▲) Fc-acid-OMPA + probe+10fM ncM.T, (●) Fc-acid-OMPA + probe+10fM cM.T. The points presenting the experimental data and the line is the fit obtained with the equivalent circuit shown below.

These results confirm the sensibility and the selectivity of our biosensor. This kind of redox oligomer structure allows us to obtain good performance regarding DNA sensing from a PCR amplification. Although some sensors have shown an efficient detection of DNA from PCR samples, this is the first time that a biosensor based on direct patterning with redox oligomer has revealed the pathogenic DNA detection through direct electrochemical measurement without further treatment. The easy construction of this biosensor, added to its good reproducibility and very low time consuming make this redox oligomer Fc-acid-OMPA highly competitive for DNA sensing.

4. Conclusion

In this work, we have outlined a new approach to DNA electrochemical detection with a new redox oligomer (Fc-acid-OMPA) as an efficient platform for biosensors. The presence of carboxylic groups on the oligomer chain has allowed the direct anchoring of the biolayer which was confirmed by XPS and FTIR. We have also shown that the binding of a wide range of 1 fM to 100 pM concentration of DNA targets to the surface resulted in a significant variation of redox currents and a low detection limit of 0.2 fM. The biosensor has shown appreciable sensitivity to real samples of DNA from *Mycobacterium tuberculosis*, by demonstrating a potential in its application in DNA sensing. These biosensors are able to discriminate SNP mutation coming from strand with drug resistance. The obtained specificity is provided by the structure of the oligomer where the carboxylic group which allows the DNA attachment whereas the methoxy group substituted on aromatic ring prevents non-specific DNA attachments. This type of biosensor based on redox oligomer could have a potential application in drug resistance detection for therapeutic purpose and pathogenic diagnosis.

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