Conducting polymers with attached platinum nanoparticles towards the development of DNA biosensors

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ABSTRACT

Based on the strong interaction of noble metal particles with the sulphur atoms of thiophene derivatives, platinum nanoparticles (Pt-NPs)-dispersed poly(3-methylthiophene) (P(3-MeTh)) thin films have been successfully prepared. Evidence of the Pt-NPs confinement in the polymer was provided by the electrocatalytic activity of the modified electrodes towards the dissolved oxygen reduction reaction. The efficient immobilization by self-assembly of thiolated oligonucleotides on the embedded Pt-NPs could be monitored by electrochemical quartz crystal microbalance (EQCM) as well as the subsequent hybridization with the complementary sequence. The good conducting properties and high surface area of P(3-MeTh)/Pt-NPs modified electrodes also allowed the detection of both processes by cyclic voltammetry.

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1. Introduction

Surfaces modified with single or double-stranded DNA has been intensively studied aiming biosensing applications [1–3]. The molecular self-assembly of thiolated compounds on noble metal substrates is well documented [4,5] and there is an increasing interest in exploiting this route for the immobilization of appropriately functionalized oligonucleotides (ON) [6,7]. Other appealing approaches involve the integration of functionalized nanoparticles in hybrid systems [8] for an enhanced sensing and amplification of biorecognition events [9].

The attractive features of conducting polymer (CP) films justify the considerable attention paid to the incorporation of metal nanoparticles (NPs). The authors reported recently [10] the successful attachment of Pt-NPs to poly(3-methylthiophene) (P(3-MeTh)) films, taking advantage of the spontaneous deposition of noble metal-NPs on polymers containing sulphur in their heterocyclic structures.

This work aims to contribute for the development of functional biomolecule nanoparticle hybrid systems and their application for recognition ON sequences by means of piezoelectric transduction. It presents the preliminary results of a more systematic study intended to develop the possible use of CPs films bearing noble metal NPs as support to built electrochemical devices.

The employed approach is illustrated in Fig. 1. The immersion of P(3-MeTh) thin films, in Pt colloid solution provides an uniform distribution of individualised nanoparticles ($\varnothing = 3$ nm) on the polymer surface being achieved a surface coverage of 26% [10]. This substrate is then used for the self-assembling HS–ON (1) and subsequent hybridization with the ON complementary sequence ON (2); both processes are monitored by electrochemical quartz crystal microbalance (EQCM) and cyclic voltammetry, in the presence of $Fe(CN)_6^{3-}$.

2. Materials and methods

P(3-MeTh) films were prepared from 0.6 mol dm^{-3} monomer in 0.2 mol dm^{-3} TBAClO$_4$ acetonitrile solution, potentiostatically at $E_g = 1.420$ V, with growth charge of 15 mC cm^{-2}; under these conditions the obtained thin polymer layers (oxidation charges of c.a. 1 mC cm^{-2}) present no viscoelastic effects [11]. The base substrate was an 8 MHz AT-cut quartz crystal (QC) coated with 1000 Å Pt (0.2 cm^2 geometrical area), previously cleaned with "piranha" solution and electrochemically by potential cycling in sulphuric acid solution. *Caution!* "Piranha" solutions react violently with many organic materials and should be handled with extreme care. Prior to further modification, the polymer film has been discharged by polarisation at E = 0 V for 300 s.

Small sized (3 nm) citrate/Pt-NPs have been synthesised through a sodium borohydride (NaBH$_4$) reduction method at room temperature, as reported elsewhere [10].

The oligonucleotide (HS–ON (1): HOC$_6$SSC$_6$-5′-TCG TAC CAT CTA TCC-3′; M$_W$ = 4615.8 g mol^{-1}) modified with a –HS group to favour its interaction with Pt surface, and the complementary 38-base sequence (ON (2): 5′-CGC GGT CTC AGG ATA TTT TTT TTG GAT AGA

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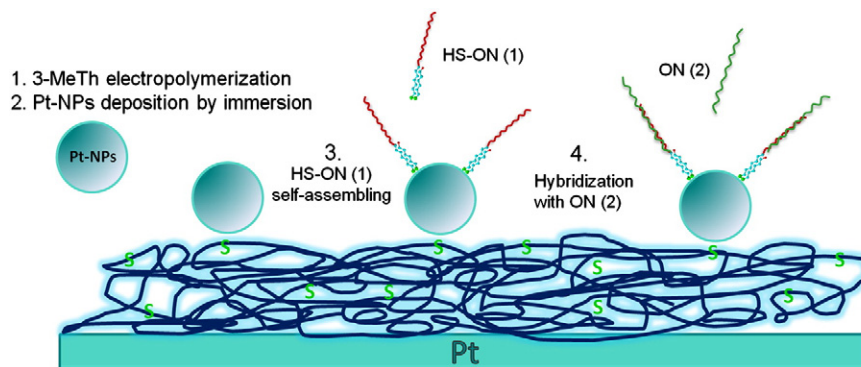*E-mail address:* luisa.abrantes@fc.ul.pt (L.M. Abrantes).

Fig. 1. Schematic representation of the methodology employed to develop a DNA biosensor.

TGG TAC GA-3′; $M_W = 11538.6$ g mol^{-1}) were provided by Friz Biochem (Neuried, Germany). HS–ON (1) 0.2 μmol dm^{-3} in 10 mmol dm^{-3} Tris–HCl (pH 7) + 0.1 mol dm^{-3} NaCl and ON (2) 1.0 μmol dm^{-3} in 10 mmol dm^{-3} TF (pH 7.4) + 0.25 mol dm^{-3} NaCl solutions were used for the immobilization and hybridization, respectively.

The confinement of the NPs on the polymers was achieved by dipping the films in the NPs suspensions for 16 h.

A frequency analyser (CH Instruments model 420) was used to estimate the amount of immobilised oligonucleotides; the experiments were performed in a single compartment cell, with the modified QC as working electrode, a Pt wire and a SCE as counter and reference electrodes, respectively.

The electrochemical experiments were performed with an IMT Electrochemical Interface and a DEA332 Digital Electrochemical Analyser connected to a computer for data acquisition (VoltaMaster2 software).

The morphological characterization of the P(3-MeTh) modified electrode was performed by atomic force microscopy (AFM) *ex situ*, in air, tapping mode, in a Nanoscope IIIa multimode microscope (Digital Instruments, Veeco).

3. Results and discussion

The potentiostatically prepared P(3-MeTh) films have been analysed by AFM, Fig. 2. An homogeneous globular morphology is clearly revealed by phase imaging (Fig. 2C), being 20 ± 2 nm the layer thickness (Fig. 2D). As reported elsewhere [10], the amount of Pt-NPs that can be appended to the polymer, estimated by EQCM, is 7.3×10^{11} NPs cm^{-2}.

The successful attachment of Pt-NPs to P(3-MeTh) can be easily reclaimed from the electrocatalytic behaviour towards the dissolved oxygen reduction (ORR), illustrated in Fig. 3. The reaction onset occurs at about 200 mV vs SCE and the current peak is observed at 55 mV (Fig. 3B), values close to those observed for a bulk platinum electrode (inset) and in fair agreement with that reported for Pt-NPs deposited on poly(o-phenylenediamine) [12].

Fig. 4A shows the change in frequency during the immersion of the modified QC with the pristine P(3-MeTh) film and with the polymer layer bearing Pt-NPs in the HS–ON(1) containing solution. Using the Sauerbrey equation [13] to analyse the data it is possible to access the amount of appended oligonucleotide and to retrieve the surface coverage, Γ, Table 1 (to a negative frequency shift of 1 kHz
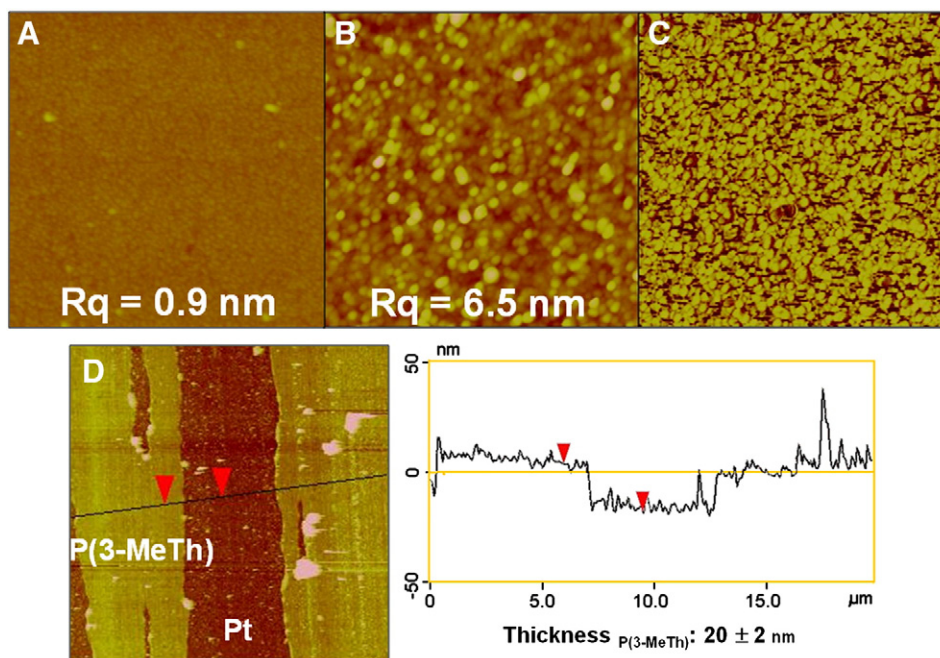

Fig. 2. Tapping mode *ex situ* AFM images (1.6×1.6 μm^2) of the (A) platinum substrate and (B and C) P(3-MeTh) film; (A and B) topographic images, Z = 80 nm; (C) phase image, Z = 85°. (D) Topographic image (20×20 μm^2) and corresponding profile of P(3-MeTh), Z = 50 nm.
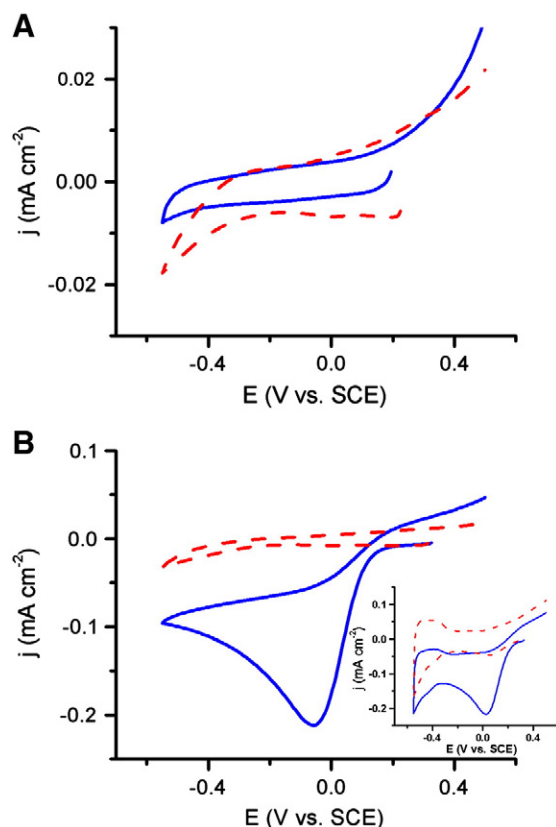
Fig. 3. Cyclic voltammograms of P(3-MeTh) (red dashed line) and P(3-MeTh)/Pt-NPs (blue solid line) modified electrodes in deaerated (A), and in aerated (B) 0.1 M phosphate buffer solution (pH 7.2); the inset shows the behaviour of a bulk platinum electrode in aerated (blue solid line) and deoxygenated (red dashed line) PB; $\nu = 50$ mV s^{-1}.

Fig. 4. Resonant frequency changes (A) during the immobilization of HS–ON (1) on P(3-MeTh)/Pt-NPs, from HS–ON (1) 0.2 μmol dm^{-3} (10 mmol dm^{-3} Tris–HCl (pH 7) + 0.1 mol dm^{-3} NaCl) and (B) upon hybridization from ON (2) 1.0 μmol dm^{-3} (10 mmol dm^{-3} PB (pH 7.4) + 0.25 mol dm^{-3} NaCl). In A, the grey line shows the response of pristine P(3-MeTh).

corresponds a mass increase of 7 μg cm^{-2}). Taking into account that the non-specific adsorption observed for the neat P(3-MeTh) is responsible for about 21% of total frequency variation (Fig. 4A — grey line), $\Gamma = 4.7 \times 10^{-11}$ mol cm^{-2} is obtained for the HS–ON(1) immobilised through the Pt-S interaction, estimated from the difference between the total surface coverage on the P(3-MeTh)/Pt-NPs modified electrode and that corresponding to the non-specific adsorption on P(3-MeTh) (Table 1). This value is higher than that reported for Au surfaces [6] likely due to the high surface area of the Pt-NPs.

After immobilization of the thiolated oligonucleotide on the Pt-NPs surface through Pt–S bonding, the modified electrode (P(3-MeTh)/Pt-NPs/HS–ON (1)) has been thoroughly washed to remove any non-specifically deposited molecules; the hybridization was achieved by immersion in a solution with ON (2) oligonucleotide, that contains at the 3′-end the base sequence which is complementary to the HS–ON (1). The frequency change recorded in the presence of the complementary sequence ON (2), Fig. 4B and Table 1, provides 1.3×10^{-11} mol cm^{-2} captured by hybridization (still considering that 21% are due to non specific adsorption).

The above mentioned results reveal that, under the employed conditions, the modified electrode is appropriate for the development of piezoelectric DNA biosensors. Notwithstanding, such application represents several research efforts namely towards the hybridization efficiency (optimization of the amount of immobilised thiolated oligonucleotide, HS–ON (1), selection of ON (2) concentration and reaction time). Being the frequency change the crucial measurement and the concentration of the complementary oligonucleotide the final target, it will be also necessary to establish the range of concentrations where an appropriate relation with the frequency changes is observed
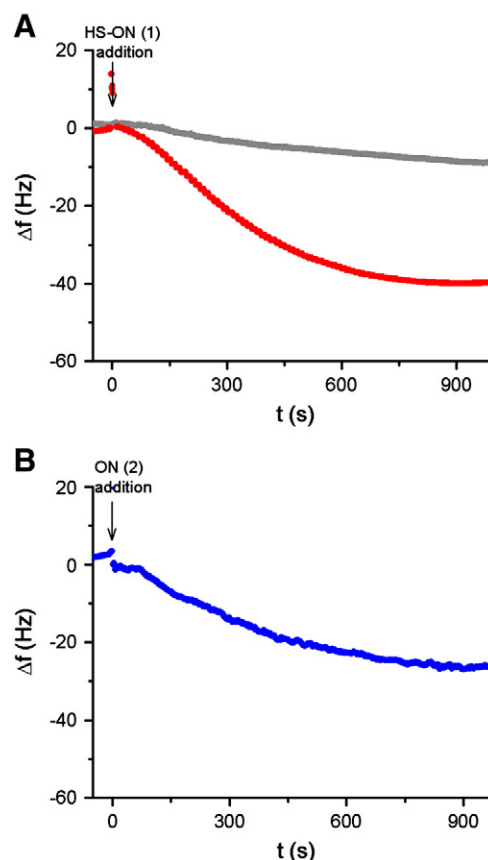
(calibration). In addition, to evaluate biosensor selectivity, non-complementary sequences and/or with one or more point-mismatch must be used. Taking into account the results obtained with sensors prepared by electropolymerization of pyrrole in the presence of oligonucleotides and on the employment of CdS-NPs [14], the present novel system clearly deserves those research efforts.

It is worth noting that rapid qualitative information on the performance of the modified electrodes can be obtained by cyclic voltammetry. In the presence of Fe(CN)$_6^{3-}$ (1 mmol dm^{-3}, aqueous solution), the responses of the P(3-MeTh) films subsequent to each modification are illustrated in Fig. 5. While the pristine polymer is electroinactive in the potential region under analysis, a well defined signal is recorded when the film is loaded with Pt-NPs and after immobilization of HS–ON (1) there is a significant decrease in the current assigned to the oxidation/reduction of the electroactive species. This effect is expected and usually attributed to the

Table 1
Resonant frequency changes of the modified QC, Δf, mass variations Δm, and surface coverage, Γ, for the immobilization of HS–ON (1) and hybridization with the complementary sequences ON (2); t = 900 s.

Substrate	HS–ON (1)			ON (2)		
	Δf (± 0.1)/ Hz	Δm/ ng cm^{-2}	10^{11} Γ/ mol cm^{-2}	Δf/Hz	Δm/ ng cm^{-2}	10^{11} Γ/ mol cm^{-2}
P(3-MeTh)/ Pt-NPs	−39.9	276.0	6,0 (4,7)*	−26.7	184.7	1.6
P(3-MeTh)	−8.6	59.7	1.3	–	–	–

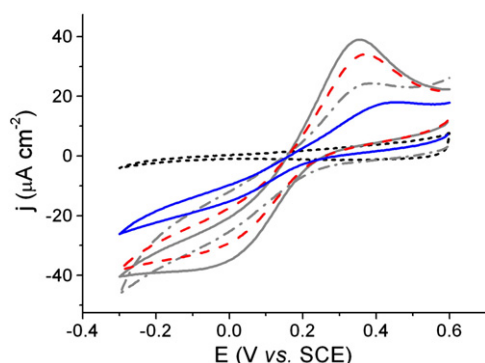* - $\Gamma_{P(3-MeTh)/Pt-NPs}$ - $\Gamma_{P(3-MeTh)}$.

Fig. 5. Cyclic voltammograms of QC/P(3-MeTh) (—·—) and P(3-MeTh)/Pt-NPs, before (—) and after (— —) immobilization of HS-ON (1) and after hybridization with the complementary sequence ON (2) (—) in 1 mmol dm^{-3} Fe(CN)$_6^{3-}$ + 10 mmol dm^{-3} Tris–HCl supporting electrolyte and comparison with the response of P(3-MeTh) (— —-) in blank solution; $v = 50$ mV s^{-1}.

electrostatic repulsion exerted by the phosphate groups of the HS–ON (1). Under these conditions the electrochemical detection of the hybridization process is possible; as can also be seen in Fig. 5, there is a further fall in the anodic and cathodic currents indicating that the electronic transfer is hindered due to the increase in the amount of negatively charged groups immobilised in the electrode.

4. Conclusions

Taking advantage of the spontaneous deposition of noble metals on polymers containing sulphur, platinum nanoparticles with 3 nm diameter have been incorporated in potentiostatically prepared poly (3-methylthiophene) (P(3-MeTh)) films, just by immersion of the polymer in Pt-NPs containing colloidal solution. A quick test to the presence of Pt-NPs is the ORR to which pristine P(3-MeTh) is inactive but P(3-MeTh)/Pt-NPs displays an interesting electrocatalytic activity.

On such electrodes, the immobilization by self-assembly of thiolated oligonucleotides is easily monitored by EQCM; under the conditions employed in this work, a relatively high surface coverage, 4.7×10^{-11} mol cm^{-2}, (when compared with those reported for gold surfaces) is observed. Also, detectable by the QC frequency changes is the hybridization process, revealing that in the present case 1.3×10^{-11} mol cm^{-2} have been confined. Voltammetric information using Fe(CN)$_6^{3-}$ as electroactive probe reinforces the well succeeded consecutive surface modifications.

As far as the authors are aware this is the first time that such attractive route is exploited to built electrochemical and piezoelectric transducers for the detection of ON sequences. Further work is in progress to optimize the preparative conditions namely polymer thickness, Pt-NPs size and dispersion.

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