

ELECTRODE MODIFIED WITH A POLYMER OF ANILINE AND 3-HEXYLTHIOPHENE TO BE ASSAYED IN THE SELECTIVE DETERMINATION OF NITRATE

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ABSTRACT

In this work, platinum electrodes are modified with poly[4-(3-hexyl-2-thiophene)-aniline], PTANIr2, and characterized by cyclic voltammetry. In the presence of nitrate in the electrolytic medium, an oxidation signal at 0.6 V vs. SCE is recorded, so the response of this modified electrode, Pt|PTANIr2, is evaluated to determine its relationship with the concentration of the anion. Thus, it is verified that the best response is achieved by chrono-amperometry, obtaining a directly proportional relationship between the charge and the concentration, in an interval between 5 and 100 mg L⁻¹ of the anion, with $r^2 = 0.996$. Furthermore, it is found that the response of Pt|PTANIr2 is quite selective for nitrate, since there is no response for anions such as HPO₄⁻, PO₄²⁻, ClO₄⁻, F⁻, Cl⁻, Br⁻, I⁻, and NO₂⁻; thereby contributing significantly to solving the main problem of devices of this type proposed so far. Finally, the surfaces were characterized by atomic force microscopy, showing a strong anion-polymer layer interaction. Thus, this material can be proposed to be tested as a coulombimetric nitrate sensor, highlighting the possibility of varying the area of the electrode and/or the thickness of the deposited polymeric layer and with it, the sensitivity of the device.

Keywords: Conducting polymer; ion selective electrode; nitrate detection; nitrate sensor; polyaniline; polythiophene.

INTRODUCTION

One of the major contaminants present in food is nitrate ion, which is used in a wide range of industrial processes, such as the manufacture of fireworks, the production of fertilizers, as corrosion inhibitors and even for the meat preservation [1-3] and, because of that ability, they are considered of anthropogenic activities in the ecosystems surrounding agricultural activities [4-6].

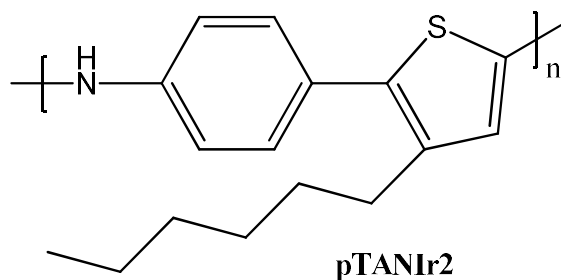
However, excess within the body can generate a highly adverse effect, since nitrate and/or nitrite may react with proteins to form N-nitrosamines, compounds known for their cancerogenic, mutagenic and teratogenic nature [2, 7-10].

This is why new strategies for early detection are evaluated every day [11-25]. Among them, electrochemical sensors allow *in situ* analysis, are fast and, in comparison, cheaper than other techniques for the determination of nitrate ions. One of the most used materials currently for the development of sensors are conducting polymers (CPs), polyaniline (PANI) and polypyrrole (PPy) and their derivatives, the most studied. These CPs can have different applications in the development of sensors, depending on the function they fulfill within the device: they can be receptors, transducers, immobilization supports, among others, although its exact role is not always clear [26, 27].

On the other hand, there are different classifications for CP-based sensors, according to the response mechanism, the detection method and the type of analyte to be evaluated. When CPs are used as transducers of the electrical signal, they can be selected according to the type of signal they transmit: potentiometric sensors, measure the cell's potential under equilibrium conditions and circulating current is zero; amperometric sensors, present a signal proportional to the concentration of the analyte, when a step potential is imposed. This phenomenon is given by the ability of the analyte and the polymer to become oxidized and/or reduced and to generate charge carriers throughout the polymer, which increase the electric current that passes through the system [27]. The study of CP-based nitrate sensors has also been developed by different authors. Despite the thermodynamic viability of the reduction of these species, the kinetics of the charge transfer is slow and the direct reduction of the nitrate ion is characterized by its low selectivity and reproducibility, generally due to passivation of the electrodes and the use of overpotentials, which tend to affect their durability [1].

The objective of this work is to evaluate the behavior of a modified electrode with a CP based on aniline and 3-hexylthiophene (poly[4-(3-hexyl-2-thiophene)-aniline], PTANIr2, scheme 1) to detect and quantify nitrate, taking into account

that the process that would be carried out would be the oxidation of the polymer (*p*-doping) and not the reduction of the anions, which allows to propose that this strategy would give more reproducible results, since no overpotentials are used. The selected polymer belongs to a series of analogues previously synthesized and characterized, with the aim of the preparation of modified electrodes and their possible use in various applications [28, 29].



Scheme 1. Structural formula of poly[4-(3-hexyl-2-thiophene)-aniline], PTANIr2.

EXPERIMENTAL

Electrolyte salts NaHPO₄, Na₂PO₄, LiClO₄, NaF, NaCl, NaBr, KI, NaNO₃ and NaNO₂ (Merck, > 99 %) were oven dried at 120 °C before its usage. Milli-Q grade water was used to prepare all electrolytes. I₂ (Merck, > 99 %) and tetrahydrofuran (Aldrich) were used as received. Synthesis and characterization of poly[4-(3-hexyl-2-thiophene)-aniline], PTANIr2, has been described elsewhere [28]. This polymer was doped with iodide to a constant weight and employed to modify the surface of the platinum working electrode (discs of 0.07 cm² geometric area).

A suspension of *ca.* 10 mg of polymer in 1.0 mL of tetrahydrofuran was filtered through a 60 μm pore size filter and the soluble fraction reserved. Four 5 μL drops of the soluble fraction of PTANIr2 was deposited on the Pt surface, allowing the solvent to evaporate between each drop, until the whole surface was coated producing the Pt|PTANIr2 electrode.

Voltamperometric characterization was performed on a CH Instruments Model CHI750D potentiostat. All analyzes were conducted using an anchor-type three compartment cells. Three electrode arrangement considered a Ag|AgCl, KCl

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electrode was the reference electrode, while a Pt coil of large surface area was the counter electrode, and the abovementioned Pt disc being the working electrode. As supporting electrolyte, a pH 7.00 phosphate buffer (PBS), with a phosphate concentration of 0.05 mol L^{-1} was used. Other solutions (NaF, NaCl, NaBr, KI, NaNO_3 and NaNO_2) were prepared in concentration $1.0 \times 10^{-3} \text{ mol L}^{-1}$ in the same PBS solution.

The chrono-amperometric study is carried out using the system described above, for NO_3^- concentrations between 5 and 100 mg L^{-1} . After data acquisition, currents were normalized to current densities using geometric area of electrodes. Charge calculation were made by integration of the under the area of current vs. time transients, subtracting the calculated charge from blank experiments (without NO_3^-). Chrono-amperometric measurements were made in triplicate for each point averaging the obtained charge for the construction of the calibration curve.

Morphological characterization of the modified electrodes was performed by atomic force microscopy, AFM, using an Asylum Research, MFP-3D-SA model, microscope in tapping mode.

RESULTS AND DISCUSSION

Figure 1 shows the response corresponding to the stable voltamperometric profile (5th successive cycle, that is, $n = 5$) of the modified electrode Pt|PTANIr2 against different anion solutions in a potential range of -0.9 to 1.1 V. It is clearly observed that the electrode only shows a *p*-doping response to nitrate ions. For all the other anions tested, only residual current is observed in the entire useful potential window for this electrode in the electrolytic medium used.

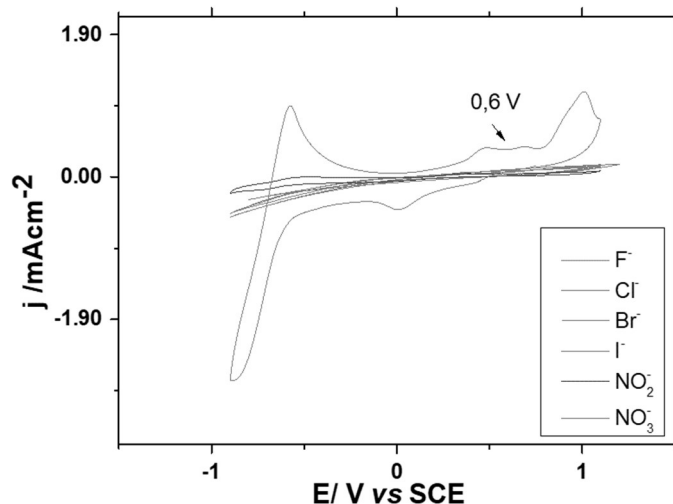


Figure 1. CV profiles of Pt|PTANIr2-doped electrode in 1.0 mmol L^{-1} F^- , Cl^- , Br^- , I^- , NO_2^- or NO_3^- . Support electrolyte: 0.05 mol L^{-1} PBS, $v = 0.1 \text{ V s}^{-1}$, $n = 5$.

Obviously, this result is very relevant, as it makes this modified electrode an excellent candidate as a nitrate ion selective electrode, for two fundamental reasons: first, there is no significant response to any anion studied in equivalent concentration and, secondly, even more important, it is based on the partial oxidation process of the polymeric film (*p*-doping) and not on the reduction of the nitrate ion. This last reaction is the one that is mainly used in the development of selective ion sensors to this anion [30], which, in general, requires high values of potential, generating damage to the material and, therefore, low reproducibility and repeatability of the sensor response [1].

For the determination of nitrate in solution, a chrono-amperometric study was performed evaluating the response of the modified electrode in 1.0 mmol L^{-1} NaNO_3 solution, always using PBS 0.05 mol L^{-1} as the supporting electrolyte. For this, an oxidation potential of 0.6 V is imposed and thus the sample is subjected to the *p*-doping process for 30 s (potential step of 0.6 V during 30 s). Subsequently, undoping is carried out, applying a perturbation corresponding to a potential step of 0.45 V for 60 s, to assess the reversibility of the doping/undoping process during 4 successive cycles. This process is repeated 3 times, without changing the electrode, to establish, in addition, the stability of the deposited film under the evaluated conditions, Figure 2.

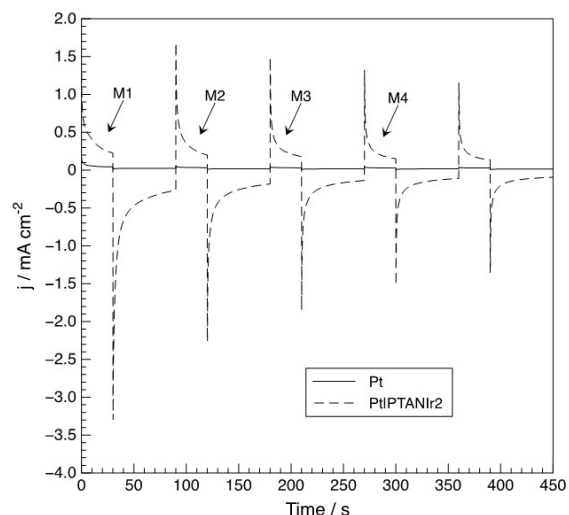


Figure 2. Response of Pt|PTANIr2 electrode to the *p*-doping/undoping process in 1.0 mmol L^{-1} NaNO_3 . Support electrolyte: 0.05 mol L^{-1} PBS; oxidation (*p*-doping) potential = 0.6 V ; undoping potential = 0.45 V .

From the results represented in Figure 2, it is verified that between the first cycle of *p*-doping/undoping (M1) and the second cycle (M2), there is a loss of around 11 % of the peak current, which accounts for that the *p*-doping/undoping process is not being fully reversible. However, this behavior is reproduced 100 % every time a new modified electrode is used, which is consistent with an excellent repeatability of the system, therefore its use is proposed to determine nitrate in solution, in the form of a disposable electrode.

Figure 3 shows the graphs that correspond, respectively, to calibration curves as a function of the peak current density (*J*) and of the charge of the response transient (*Q*) of nitrate on the modified electrode. It can be verified that the current response of the modified electrode for different concentrations of nitrate ion has a low linearity, much less than that obtained as a function of the charge. This is reflected in the parameters calculated and summarized in Table 1.

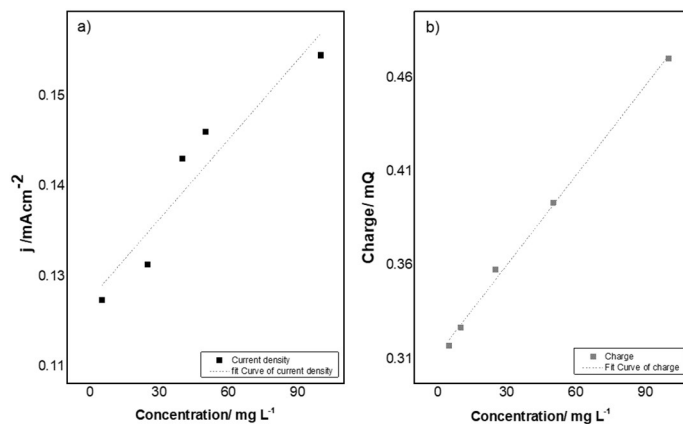


Figure 3. Nitrate calibration curves using Pt|PTANIr2-doped modified electrode: (A) Current density vs. concentration curve. (B) Charge vs. concentration curve.

Table 1. Parameters of calibration curves obtained with Pt|PTANIr2 electrode.

Current density			Charge		
Slope $\text{mA cm}^{-2} \text{ ppm}^{-1}$	Intercept mA cm^{-2}	R^2	Slope mC ppm^{-1}	Intercept mC	R^2
4.0×10^{-4}	1.23×10^{-1}	0.864	1.6×10^{-3}	3.08×10^{-1}	0.996

Considering that a new or “fresh” electrode was used for each measurement, it can be said that the system is highly sensitive to changes in concentration and that, possibly, by automating the electrode manufacturing process, even higher correlation coefficients could be achieved.

The values of the parameters in Table 1 are practically repeated when the corresponding nitrate solutions are measured now in the simultaneous presence of all the other anions analyzed (in this case, the current or charge, as appropriate, of the blank solution is the response of an all-anion solution, in the absence of nitrate). This confirms the selectivity of the electrode tested here.

To determine the linearity range of the response-concentration relationship, the range was decreased to 2.5 mg L^{-1} and increased to 500 mg L^{-1} (data not shown). The results obtained indicate that for concentrations above 50 mg L^{-1} and below 5.0 mg L^{-1} there is no linear relationship between the concentration and the amperometric response. In the case of concentrations higher to 50 mg L^{-1} , possibly to polymer saturation. In the case of concentrations below 5.0 mg L^{-1} , it is possible to affirm that the low concentration of the analyte makes the ion-film interaction insufficient, since the process is carried out without agitation, giving current values and/or charge that escape the linear regression determined above. Thus, the tested here, is between the concentrations of 5.0 and 50 mg L^{-1} , and in some cases it is possible to adequately measure up to 100 ppm .

Finally, the morphological analysis through AFM imaging of the modified electrodes is presented in (Figure 4). The modified surface, before and after the *p*-doping process, suffers a decrease in its thickness, attributable to a "rearrangement" or to a partial detachment of the film. However, full and stable surface coating is maintained.

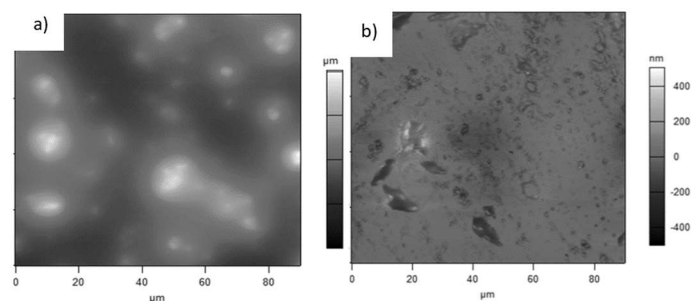


Figure 4. AFM images of PTANIr2-modified platinum electrodes: (a) Before the *p*-doping perturbation; (b) After the *p*-doping perturbation.

In addition, it is seen that the formed film is porous and after the *p*-doping process, the filling of the pores occurs, which accounts for the strong interaction between the analyte and the polymer matrix. This confirms what is deduced by the voltamperometric response, where the current increase is associated with the *p*-doping process and not with the oxidation or reduction of the nitrate ion on the electrode. Unfortunately, the high viscosity of the sample prevents obtaining images with better resolution.

Present outcomes can be compared by those reported by other authors, reviewing Table 2.

Table 2. Nitrate electrochemical sensors based on CPs.

Electrode active layers	Work mode	LOD $\mu\text{mol L}^{-1}$	Linear range $\mu\text{mol L}^{-1}$	Ref.
Graphite powder/doped-PPy	Potentiometric	53.7	--	[31]
PPy/Ag-nanoparticle	Voltammetric	5	1-10	[32]
Cu-Zn/Enzime/PPy/carbon nanotubes	Voltammetric	0.2	0.5-1000	[33]
Cu-microspheres/PANI	Voltammetric	8	20-6000	[34]
Cu-nanoparticles/PANI	Voltammetric	31	1-100000	[35]
Poly(N-methylpyrrole)	Potentiometric	5	5.6-100000	[36]
PPy/Graphene/Glassy-carbon	Potentiometric	6.3	10-100000	[37]
PPy-coated Pd nanocluster	Voltammetric	0.74	0.74-2.48	[38]
PPY-nanorod	Voltammetric	50	100-5000	[39]
PTANIr2	Voltammetric	45	80 - 800	This work

Nitrate sensor from this work shows a linear range between 80- 800 and a limit of detection (LOD) of $45 \mu\text{mol L}^{-1}$. Considering different electrode active layer designs, work modes (potentiometric or voltametric mostly) other authors have good limits of detection (LOD) and wider linear response/concentration ranges. Nevertheless, it must be pointed out that most of reviewed works reported are complex, hard to replicate and probably most expensive electrode configuration compared to present report. Finally, very few of this works makes use of doping properties of CPs layers, and most work consider the conducting polymer layer a simple support. Therefore, on most of the reviewed works sensor stability is not reported or discussed. Nitrate sensors are widely used to check concentrations in food and natural waters considering a broad concentration range. Therefore, the development of novel low-cost electrodes for the detection of nitrate is important for different industrial and environmental application.

Summarizing, electrodes modified with PTANIr2 are good candidates for the development of amperometric sensors for quantification of nitrate in aqueous solutions. This device uses the *p*-doping/undoping process from the polymeric layer, selectively up-taking nitrate ions from solution. The selectivity was proven against several other anions like F^- , Cl^- , Br^- , I^- and NO_2^- . Repeatability of results was best when using new electrodes after each measurement and to improve electrode performance, it is essential to use only partial oxidation of the polymeric film instead of full oxidation. After charge-concentration calibration curves, a linear relationship was found between oxidation charge and nitrate concentration. This relationship was maintained over a broad concentration window ($5.0 - 50.0 \text{ mg L}^{-1}$).

To improve LOD, rather than varying potential or oxidation times, it would be appropriate to increase the thickness of the polymer layer or the area of the support electrode. This is precisely what gives great versatility to this type of modified electrode, although it requires optimization of these variables depending on the analyte and the concentration of the sample to be analyzed. Future studies will consider evaluation of the effect of the area of the support electrode and the thickness of the polymeric deposit to modulate these linearity ranges and detection limits. In addition, it would be important to analyze the effect that the agitation of the solution can have on these variables during the process of reading the current or the charge generated.

CONCLUSIONS

This work shows that electrodes modified with conducting polymers can be used to detect or sense analytes of interest, in this case, the poly [4-(3-hexyl-2-thiophene)-aniline]polymer, PTANIr2, was shown to have a response to nitrate ions in the evaluated conditions, showing to be a suitable candidate to develop a selective sensor of this anion.

In any case, the repeatability of the amperometric response means that these electrodes are proposed as disposable devices, with which good reproducibility is achieved and the charge/concentration ratio is not altered, maintaining the linearity of the response in the interval informed here.

Furthermore, it is verified that the response of Pt|PTANIr2 is quite selective for nitrate in the presence of anions like HPO_4^- , PO_4^{2-} , ClO_4^- , F^- , Cl^- , Br^- , I^- , and NO_2^- . This makes this device very relevant, since this solves one of the main limitations that exist in the devices of this type proposed to date to determine nitrate.

Under the conditions tested here, the concentration range in which this sensor of nitrate would be useful is between 5.0 and 50 mg L^{-1} . However, it is modulable both as a function of the thickness of the polymeric deposit layer to prepare the modified electrode, as well as the area of the support electrode or the agitation of the solution during the measurement.

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